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Orchid Biology: Recent Trends & Challenges

Springer

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Editors
Shaik Mahammad Khasim
Department of Botany and Microbiology
Acharya Nagarjuna University
Guntur, Andhra Pradesh, India

María Teresa González-Arnao
Facultad de Ciencias Químicas, Laboratory of Plant Biotechnology and Cryobiology
Universidad Veracruzana
Orizaba, Veracruz, Mexico

Sadanand Nagesh Hegde
Former Director, Orchid Research and Development Center
State Forest Research Institute
Itanagar, Arunachal Pradesh, India

Kanchit Thammasiri
Department of Plant Science
Faculty of Science
Mahidol University
Bangkok, Thailand

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Foreword

The family Orchidaceae is the largest family of flowering plants next only to Asteraceae. A great majority of orchids are epiphytic herbs. Although orchids are familiar to the general public due to their ornamental value, they also represent one of the most interesting groups of plants in a number of areas – ecology, morphology, physiology and embryology. Flowers of no other groups of plants show such diversity as orchids; apart from their variations in size, shape and colour, many of them show amazing resemblance to various animals including humans. Also, the flowers of orchids remain fresh for several weeks both on the plants and as cut inflorescences. As it is comparatively easy to raise interspecific and even intergeneric hybrids in orchids, a large number of hybrids, showing unparalleled diversity of flowers, are available in the market. Because of these desirable ornamental features, there is a great demand for orchid plants as well as cut flowers. Unfortunately, though India has great diversity of orchids, our floriculture industry of orchids has remained poor when compared to many Southeast Asian countries such as Singapore, Philippines and Thailand. As orchids are generally slow-growing, their multiplication through conventional means is very time-consuming. Orchids were the first to be commercially propagated using the technique of micropropagation. Now, most of the standard nurseries dealing with orchids use this technology routinely.

Pollination biology of orchids is fascinating and highly variable. Extensive studies have been carried out on pollination of orchids since the time of Darwin. Some species exhibit typical entomophily and a good number of them have also evolved autogamous self-pollination. However, a large number of species exhibit highly specialized pollination system – each orchid species is pollinated by just one specific pollinator. Many of them have evolved deceptive pollination syndrome; they attract animal pollinators by falsely exhibiting the presence of rewards, but do not provide any rewards. Sexual deception is the extreme form of pollination deception evolved to attract species-specific male pollinators. In all the species of *Ophrys*, for example, the flower not only resembles the female of the pollinating insect but also secretes species-specific pheromone to attract male pollinator. The male insect attracted by these features lands on the flower and tries to mate; this is termed as pseudo-copulation, during which it brings about pollination. Seeds of orchids are very small, almost microscopic, and are produced in large numbers. In some species the number of seeds per fruit is reported to be over a million!

In recent decades, overexploitation, human-induced environmental changes, biological invasions and climate change are creating havoc to the sustainability of our biodiversity. These changes have initiated the ‘sixth mass species extinction crisis’ in which a large proportion of species would become extinct in a geologically short time. As orchids require special habitat, they are highly vulnerable to these changes and require urgent conservation efforts using all available means. Apart from their horticultural importance, in recent years bioactive compounds from plant sources including orchids are gaining importance throughout the world. This again leads to overexploitation of wild species. Many of the overexploited orchid species have already been included in Appendices I and II of CITES. One of the basic requirements for effective conservation is the availability of data on the biology of the species. There is an urgent need to generate such data on most of the wild species of orchids.

Because of their ornamental and other biological importance, orchids have been favorite materials for research for centuries. Extensive studies are being carried out on both fundamental and applied areas of orchids. However, most of the recent literature is scattered in a large number of research papers and reviews. Although there are several books on horticultural aspects of orchids, there are no recent books bringing together the exciting details on other areas of orchid biology in recent years. The present book on *The Orchid Biology: Recent Trends and Challenges* edited by Professor S. M. Khasim and his coeditors from India, Mexico and Thailand is most welcome. This book is the outcome of the proceedings of an international symposium on ‘Biodiversity of Medicinal Plants and Orchids: Emerging Trends and Challenges’ held during 9–11 February 2018 at Acharya Nagarjuna University, Guntur, India, and also some invited chapters by experts. All the chapters have been grouped under five relevant sections: Cryopreservation and Biotechnology, Orchid Biodiversity and Conservation, Anatomy and Physiology, Pollination Biology and Orchid Chemicals and Bioactive Compounds. I understand that all the chapters have been peer-reviewed before sending to the press. I congratulate the editors for undertaking this compilation. I am confident that various chapters would provide a critical account of the past, present and future trends in diverse areas of orchid biology. Studies on the biology of orchids of India are very limited in spite of the vast diversity it has. Apart from providing consolidated data on various areas of orchid biology, I hope the book would encourage young researchers to take up studies on orchids, particularly of Indian biodiversity hotspots such as North-East Himalaya and the Western Ghats.

K. R. Shivanna, FNA, FASc, FNAAS, FNASc

INSA Honorary Scientist
Ashoka Trust for Research
in Ecology and the Environment
Bangalore, India
Formerly Professor and Head,
Department of Botany
University of Delhi
New Delhi, India
15 April 2019

Preface

The Orchidaceae constitutes one of the largest families of flowering plants comprising about 28,484 species. It contributes about 40 percent of monocotyledons. In India, it represents the second largest flowering plant family with 1,141 species in 166 genera and contributes about 10% Indian flora. Orchids comprise a unique group of plants, and their flowers are the most enchanting and exquisite creation of nature. Phylogenetically and taxonomically, the Orchidaceae has been considered as a highly evolved family amongst angiosperms. Orchids show the incredible range of diversity in shape, size and colour of flowers. Orchids with the most attractive and bewitchingly beautiful flowers have commercial importance in floriculture market around the globe. Millions of cut flowers of *Cymbidium*, *Dendrobium*, *Cattleya*, *Paphiopedilum*, *Phalaenopsis*, *Vanda*, etc. besides pot plants of orchids are sold in Western countries, and thus, orchid cut flower industry has now become a multimillion-dollar business in Europe, USA and Southeast Asia.

Besides ornamental value, orchids have got immense pharmaceutical potential. Root tubers of *Habenaria edgeworthii* form an important composition of 'Astavarga' group of drugs in Ayurvedic medicines. It is an established fact that tubers of some terrestrial orchids have been used for treatment of diarrhoea, dysentery, intestinal disorders, cough, cold and tuberculosis. Some orchids, particularly belonging to the genera such as *Aerides*, *Arachnis*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Epidendrum*, *Oncidium*, *Paphiopedilum*, *Phalaenopsis*, *Renanthera*, *Vanda*, etc., have been extensively used to produce the internationally acclaimed hybrids. The Indian orchids are paradoxically victims of their own beauty and popularity. As a result, their natural populations have been declining rapidly because of unbridled commercial exploitation in India and abroad as well. Further this situation has led the orchids to the verge of extinction, e.g. *Renanthera imschootiana*, *Diplomeris hirsuta*, *Paphiopedilum fairrieanum* (already extinct), *Cypripedium elegans*, *Taeniophyllum andamanicum*, etc.

An edited book titled *The Orchid Biology: Recent Trends and Challenges* is the outcome of the proceedings of an international symposium on 'Biodiversity of Medicinal Plants and Orchids: Emerging Trends and Challenges' held during 9–11 February 2018 at Acharya Nagarjuna University, Guntur, India. Besides that, we also invited eminent orchid experts across the globe to contribute to this book, so as to enable us to report on state of the art of scientific investigations that have been going on for the last several decades on orchid biology. All papers contained in this book were peer-reviewed by international experts. Further, the manuscripts were reviewed by editors and those papers that were judged as suitable for publication following the authors' considerations of reviewer suggestions appeared in this edited book.

In view of the importance of orchids globally for their large-scale production and exploitation for the human health and wealth, we felt that the comprehensive compilation by international experts is the need of the hour.

The present book contains five sections: (I) Cryopreservation and Biotechnology, (II) Orchid Biodiversity and Conservation, (III) Anatomy and Physiology, (IV) Pollination Biology and (V) Orchid Chemicals and Bioactive Compounds. All five sections contain 28 papers authored by eminent orchid experts/professors across the globe. This book serves as a reference book for researchers, teachers, orchid enthusiasts, orchid growers and students of biotechnology, botany, pharmaceutical sciences and ethnomedicine. It would be of equal interest to horticultural industry especially orchid industry, agricultural scientists and policy makers.

We would like to express our sense of gratitude to all contributors from India and abroad for accepting our invitation to contribute chapters and for not only sharing their knowledge but also for admirably integrating expertise in composing the chapters of the various aspects of orchid biology. We greatly acknowledge Dr. So-Young Park (Chungbuk, Republic of Korea), Dr. Apiradee (Bangkok, Thailand), Prof. M. M. Hossain and Prof. M. K. Huda (Chittagong, Bangladesh), Prof. P. Kaushik (Haridwar, India), Prof. S. N. Sinha (Kalyani, India), Dr. A. N. Rao (ORDC, Manipur, India), Prof. Navdeep Shekhar (Faridkot, India) and Dr. M. M. Hoque (Chittagong, Bangladesh) for their commitment and dedication for bringing the final shape of this edited book. I am very much indebted to my professor, guide and philosopher, Dr. P. R. Mohana Rao, an eminent orchid embryologist for his invaluable support all throughout my academic journey.

We are also thankful to our colleagues and research scholars at the Department of Botany and Microbiology, Acharya Nagarjuna University, India, for the preparation of the manuscript. We profusely thank Ms. Aakanksha Tyagi, associate editor, Springer Science, India, and her staff for their unstinted support and very effective execution of this project.

Guntur, Andhra Pradesh, India — S. M. Khasim
Itanagar, Arunachal Pradesh, India — Sadanand N. Hegde
Orizaba, Veracruz, Mexico — María Teresa González-Arnao
Bangkok, Thailand — K. Thammasiri

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About the Editors

Shaik Mahammad Khasim is head of the Department of Botany and Microbiology, Acharya Nagarjuna University, India, and former chairman, Board of Studies in Biotechnology. He has made significant contribution in the field of orchid biology and conservation. He has been working on molecular characterization, biodiversity, micropropagation, ethnomedicine, and phytochemistry of orchids. He published good number of research papers in national and international journals. He is the recipient of the *Usha Vij Memorial Award-2018* for his outstanding contribution in orchid biology conferred by the Orchid Society of India (TOSI), Chandigarh. He is an associate fellow of A.P. Academy of Science (India); life member of TOSI, Indian Science Congress Association (Calcutta), and member of the American Orchid Society (Florida) and the International Society for Horticultural Science (ISHS), Belgium.

Sadanand N. Hegde, former Director of Orchid Research and Development Center, State Forest Research Institute, Arunachal Pradesh, India, has made significant contribution in orchid taxonomy and conservation. His initial works were on the cyto-taxonomic studies on the orchids of Western Ghats of India at the University of Agricultural Sciences, Bangalore, and subsequently at the Karnataka University, Dharwad, on cyto- and chemotaxonomic studies in the tribe Epidendreae of Orchidaceae. He explored 600 orchid species from Arunachal Pradesh and registered 8 new species as well. During his tenure as Director, he developed orchids as a supplemental crop for the tribal farmers of Arunachal Pradesh and other Northeastern states of India. He registered 6 new hybrid orchids and bred 16 new hybrids. He is recipient of Dr. TN Khooshoo Memorial Environment Award (2004) conferred by the Orchid Society of India (TOSI). He is also the founder member of TOSI, Chandigarh.

María Teresa González-Arnao is a professor in Universidad Veracruzana, Veracruz, México, since 2004. She has been working on plant biotechnology especially in plant cryopreservation techniques for last several decades. She has published research papers in national and international journals such as *Acta Horticulturae*, *European Food Research and Technology*, *HortScience*, *Cryobiology*, *Revista Advances en Investigación Agropecuria* (AIA), and *Journal of Plant Biochemistry and Biotechnology*. She also contributed several chapters in technical books published by Springer-Verlag. She is the member of the International Society for Horticultural Science (ISHS), Belgium.

Kanchit Thammasiri is an associate professor and head of the Department of Plant Science, Mahidol University, Bangkok (Thailand). He has made significant contribution in the field of cryopreservation of Thai orchid seeds and their micropropagation. His current research involves the orchid breeding and micropropagation of selected genera, such as cattleya alliance, dendrobiums, phalaenopsis and vandaceous-type orchids, and cryopreservation of Thai orchids. He published his research papers in national and international journals, such as *CryoLetters*, *Acta Horticulturae*, *ScienceAsia*, *Richardia*, *Lindleyana*, and *Plant Cell, Tissue and Organ Culture*. He is member of the American Orchid Society, Florida, and also International Society for Horticultural Science (ISHS), Belgium.

Contributors

Most. Tanzila Akter Department of Botany, University of Chittagong, Chittagong, Bangladesh

S. Akter Department of Botany, University of Chittagong, Chittagong, Bangladesh

Anbarasan M. R. Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

V. P. Bhatt Department of Botany, Government Post-Graduate College, Karnaprayag, Uttarakhand, India

Karabi Biswas Environmental Microbiology Research Laboratory, Department of Botany, University of Kalyani, Kalyani, West Bengal, India

Mantana Buanong Division of Postharvest Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand
Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok, Thailand

Chandrasekaran S. Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

Carlos A. Cruz-Cruz Facultad de Ciencias Químicas, Universidad Veracruzana, Orizaba, Veracruz, México

B. T. Dangat Department of Botany, Smt. Meenalben Mehta College, Panchgani, Shivaji University, Kolhapur, Maharashtra, India

P. K. Sudha Devi Department of Floriculture and Landscaping, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Natalia Raquel Dolce Instituto de Botánica del Nordeste (IBONE), Universidad Nacional del Nordeste (UNNE), Facultad de Ciencias Agrarias, UNNE, Corrientes, Argentina

Eduardo Alberto Flachsland Instituto de Botánica del Nordeste (IBONE), Universidad Nacional del Nordeste (UNNE) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Agrarias, UNNE, Corrientes, Argentina

María Teresa González-Arnao Facultad de Ciencias Químicas, Laboratory of Plant Biotechnology and Cryobiology, Universidad Veracruzana, Orizaba, Veracruz, México

R. V. Gurav Department of Botany, Shivaji University, Kolhapur, Maharashtra, India

Sadanand N. Hegde Former Director, Orchid Research and Development Center, State Forest Research Institute, Itanagar, Arunachal Pradesh, India

Fabiola Hernández-Ramírez Facultad de Ciencias Químicas, Universidad Veracruzana, Orizaba, Veracruz, México

Mohammed Mozammel Hoque Department of Botany, University of Chittagong, Chittagong, Bangladesh

M. M. Hossain Department of Botany, University of Chittagong, Chittagong, Bangladesh

Thanh-Tam Ho Department of Horticultural Science, Division of Animal, Horticulture and Food Sciences, Chungbuk National University, Cheongju, Republic of Korea

Mohammed Kamrul Huda Department of Botany, University of Chittagong, Chittagong, Bangladesh

P. Janaki Rao Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India

K. Jhansi Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

Kamaladhasan N. Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

Velusamy Karthikeyan Department of Botany, Thiagarajar College, Madurai, Tamil Nadu, India

S. M. Khasim Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

Haeng-Hoon Kim Sunchon National University, Suncheon, Korea

Galina L. Kolomeitseva Laboratory of Tropical Plants, Tsitsin Main Botanical Garden of the Russian Academy of Sciences, Moscow, Russian Federation

Krishnankutty N. Department of Biological Sciences, ASSEFA College of Education, Madurai, India

K. Ajith Kumar Department of Fruit Science, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Ricardo Daniel Medina Instituto de Botánica del Nordeste (IBONE), Universidad Nacional del Nordeste (UNNE) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Facultad de Ciencias Agrarias, UNNE, Corrientes, Argentina

Thangavelu Muthukumar Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India

M. Raja Naik College of Horticulture (Dr.Y.S.R.H.U), Anantharajupeta, Andhra Pradesh, India

S. B. Padal Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India

Kee-Yoeup Paek Department of Horticultural Science, Division of Animal, Horticulture and Food Sciences, Chungbuk National University, Cheongju, Republic of Korea

So-Young Park Department of Horticultural Science, Division of Animal, Horticulture and Food Sciences, Chungbuk National University, Cheongju, Republic of Korea

Elena Popova K. A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia

Udai C. Pradhan U.C. Pradhan Orchid Nursery, Kalimpong, Darjeeling, West Bengal, India

R. Prameela Department of Botany, M.R. College for Women, Vizianagaram, Andhra Pradesh, India

Mohammed Rahamtulla Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

Minhajur Rahman Department of Botany, University of Chittagong, Chittagong, Bangladesh

Kuppu Rajendran Department of Botany, Thiagarajar College, Madurai, Tamil Nadu, India

Mohan Raj R. Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

M. Ramakrishna College of Horticulture (Dr.Y.S.R.H.U), Anantharajupeta, Andhra Pradesh, India

G. Ramesh K.B.N. College (Autonomous), Vijayawada, Andhra Pradesh, India

Venkatesh Rampilla Department of Botany, Dr. L.H.R Govt. Degree College, Mylavaram, Andhra Pradesh, India

J. Ramudu V.S.R. Government Degree College, Movva, Andhra Pradesh, India

J. Prakasa Rao Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India

Satyawada Rama Rao Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong, Meghalaya, India

Martha P. Rascón-Díaz Facultad de Ciencias Químicas, Universidad Veracruzana, Orizaba, Veracruz, México

Ashis Kumar Roy Buraganj Kalkut Singh High School, Darjeeling, West Bengal, India

Indhar Saidanyan R. Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

A. V. Santhoshkumar Department of Tree Physiology and Breeding, College of Forestry, Kerala Agricultural University, Thrissur, Kerala, India

Saravanan S. Department of Botany (DDE), Alagappa University, Karaikudi, India

Kullaiyan Sathiyadash Department of Botany, Thiagarajar College, Madurai, Tamil Nadu, India

Sankar Narayan Sinha Environmental Microbiology Research Laboratory, Department of Botany, University of Kalyani, Kalyani, West Bengal, India

Soundararajan N. Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

M. Tarakeswara Naidu Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India

Graciela Terada Instituto de Botánica del Nordeste (IBONE), Universidad Nacional del Nordeste (UNNE) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Ciencias Agrarias, UNNE, Corrientes, Argentina

Kanchit Thammasiri Department of Plant Science, Faculty of Science, Mahidol University, Bangkok, Thailand

S. B. Uddin Department of Botany, University of Chittagong, Chittagong, Bangladesh

Apiradee Uthairatanakij Postharvest Technology Innovation Center, Commission on Higher Education, Bangkok, Thailand

M. Venkaiah Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India

Part I

Cryopreservation and Biotechnology

Cryopreservation Development of Some Endangered Thai Orchid Species

1

Kanchit Thammasiri

Abstract

Thailand is the origin of about 1, 300 tropical orchid species and 180–190 genera. Deforestation and over-collection of wild Thai orchids for trade has placed orchid species at a risk of extinction. Therefore, the conservation as well as sustainable use is urgently needed to conserve orchids by various means. The genera *Paphiopedilum* and *Dendrobium cruentum* are listed in Appendix I of CITES. At the Department of Plant Science, Faculty of Science, Mahidol University, various methods of cryopreservation and conservation of Thai orchid species have been carried out. For cryopreservation, recent methods were used, namely, vitrification (dehydration in PVS2 solution, consisted of 30% glycerol, 15% ethylene glycol and 15% dimethyl sulfoxide, prepared in modified Vacin and Went liquid medium), encapsulation-dehydration (encapsulation in calcium alginate beads followed by air-drying in a laminar air-flow cabinet), encapsulation-vitrification (encapsulation in calcium alginate beads followed by dehydration in PVS2 solution), droplet-vitrification (fast freezing from small drops of PVS2 solution on aluminium strip) and cryo-plate (a combination of encapsulation and droplet on very fast freezing aluminium plate) dehydrated with silica gel and drying beads. Application of these methods in seeds was successful in *Dendrobium chrysotoxum* (99%, vitrification), *D. cruentum* (32%, vitrification), *D. draconis* (95%, vitrification), *D. hercoglossum* (80%, encapsulation-vitrification), *Doritis pulcherrima* (62%, vitrification), *Rhynchostylis coelestis* (85%, vitrification), *Vanda coerulea* (67%, vitrification), as well as in protocorms of *D. cruentum* (33%, vitrification; 27%, encapsulation-dehydration), *D. cariniferum* (15%, encapsulation-vitrification), *Grammaytophyllum speciosum* (14%, encapsulation-vitrification), *Rhynchostylis gigantea* (19%, vitrification), *V. coerulea* (40%, encapsulation-dehydration), *Seidenfadenia mitrata* (67%, vitrification) and *Arundina graminifolia* (76% and 74%, cryo-plate dehydrated with drying beads

K. Thammasiri (✉)
Department of Plant Science, Faculty of Science, Mahidol University, Bangkok, Thailand

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and silica gel, respectively; 33% droplet-vitrification; 64% encapsulation-dehydration with drying beads or silica gel). Cryopreserved seeds and protocorms were able to develop into normal seedlings. These techniques appear to be promising for the cryopreservation of some Thai orchid germplasm.

Keywords
Vitrification · Encapsulation-dehydration · Encapsulation-vitrification · Droplet-vitrification · Cryo-plate · Drying beads · Silica gel

1.1 Introduction

Thailand is the origin of about 1, 300 tropical orchid species and 178 genera. Many Thai orchid species have good horticultural characteristics and are used as parents for breeding, making Thailand the No.1 orchid exporter. Climate change, deforestation (habitat destruction), and over-collection of wild Thai orchids for trade has placed Thai orchids at a risk of extinction. Therefore, conservation, social awareness and consciousness, as well as sustainable use are urgently needed to conserve orchids by various means (Thammasiri 2008). At the Department of Plant Science, Faculty of Science, Mahidol University, various methods of *ex situ* conservation of Thai orchid species have been carried out, namely, cryopreservation, seed stores under Orchid Seed Stores For Sustainable Use (OSSSU) project and micropropagation.

1.2 Cryopreservation Technology

After meeting and discussing with Professor Akira Sakai at the International Workshop on *In Vitro* Conservation of Plant Genetic Resources, 4–6 July 1995 in Kuala Lumpur, Malaysia, he came to demonstrate vitrification-based methods for plant cryopreservation at my department. A little later, I started doing research on cryopreservation of jackfruit embryonic axes which was very successful and novel (Thammasiri 1999). I then shifted my interest in cryopreservation research into orchids for which I am the pioneer. I published the first paper on *Doritis pulcherrima*, a wild Thai orchid, on seed cryopreservation by vitrification with 62% (Thammasiri 2000). It was also the first paper on seed cryopreservation by vitrification [dehydration in PVS2 solution, consisting of 30% (w/v) glycerol, 15% (w/v) ethylene glycol and 15% (w/v) dimethyl sulfoxide, prepared in modified Vacin and Went liquid medium].

Later my post-graduate and doctoral students studied recent methods, namely, vitrification (dehydration in PVS2 solution), encapsulation-dehydration (encapsulation in calcium alginate beads followed by air-drying in a laminar air-flow cabinet), encapsulation-vitrification (encapsulation in calcium alginate beads followed by dehydration in PVS2 solution) and droplet-vitrification (fast freezing from small drops of PVS2 solution with plant materials inside on a 7 × 20 mm sterile aluminum foil strip). Application of these methods on seeds was successful in many Thai orchid species. Cryopreserved seeds and protocorms were able to develop into normal seedlings. These methods appear to be promising techniques for cryopreservation of many Thai orchid species.

Thammasiri (2002) presented "Preservation of Seeds of Some Thai Orchid Species by Vitrification" at the 16th World Orchid Conference. *Dendrobium chrysotoxum, D. draconis, Doritis pulcherrima and Rhynchostylis coelestis* had 99%, 95%, 62% and 85% germination, respectively, after seed cryopreservation by vitrification. Other Thai orchid seeds were later successfully cryopreserved, such as in *D. cruentum* (32% by vitrification) (Kagawa 2006), *Vanda coerulea* (67% by vitrification) (Thammasiri and Soamkul 2007), *D. hercoglossum* (80% by encapsulation-vitrification) as well as in protocorms of *D. cruentum* (33% by vitrification and 27% by encapsulation-dehydration) (Kagawa 2006), *D. cariniferum* (15% by encapsulation-vitrification) (Pornchuti and Thammasiri 2008), *Vanda coerulea* (40% by encapsulation-dehydration) (Jitsopakul et al. 2008) and *Seidenfadenia mitrata* (67% by vitrification).

Sopalun et al. (2010a, b) studied three vitrification-based methods, namely, droplet-vitrification, encapsulation-dehydration and encapsulation-vitrification, for cryopreservation of protocorms of *Grammatophyllum speciosum*, known as "Tiger orchid" or "Giant orchid". Protocorms, 0.1 cm in diameter, developed from 2-month-old germinating seeds were used. For droplet-vitrification (Fig. 1.1), protocorms were precultured on filter paper soaked in half strength Murashige and Skoog medium (½MS) containing 0.4 M sucrose at 25 ± 2 °C for 2 days, followed by soaking in loading solution (2 M glycerol and 0.4 M sucrose in ½MS liquid medium) for 20 min and then dehydrated with PVS2 solution in ½MS liquid medium containing 0.4 M sucrose at pH 5.7 for 30 min. For encapsulation-dehydration (Fig. 1.2), encapsulated protocorms were precultured in ½MS liquid medium containing 0.4M sucrose on a shaker (110 rpm) at 25 ± 2 °C for 2 days, followed by soaking in the same loading solution for 20 min and then exposed to a sterile air-flow at 2.5 inches/water column from the laminar air-flow cabinet for 8 h. For encapsulation-vitrification (Fig.1.3), encapsulated protocorms were precultured in ½MS liquid medium containing 0.4 M sucrose for 1 or 2 days, followed by soaking in the same loading solution for 20 min and then dehydrated with PVS2 solution for 60 min. For all three methods, preculturing with 0.4 M sucrose for 2 days resulted in a significant induction of dehydration and freezing tolerance. The cryopreservation results showed the highest protocorm regrowth after droplet-vitrification (38%), followed by encapsulation-dehydration (24%) and encapsulation-vitrification (14%). Plantlets developed from these three methods did not show any abnormal characteristics or ploidy level change when investigated by flow cytometry.

Cordova II and Thammasiri (2016) developed the cryo-plate method using silica gel or drying beads for dehydration (Fig. 1.4). Protocorms were placed in the preculture solution consisting of 0.7 M sucrose on a shaker (110 rpm) at 25 ± 3 °C for 1 day. After that, protocorms were placed one by one in the wells which filled before with the alginate solution containing 2% (w/v) sodium alginate in calcium-free 1/2 MS

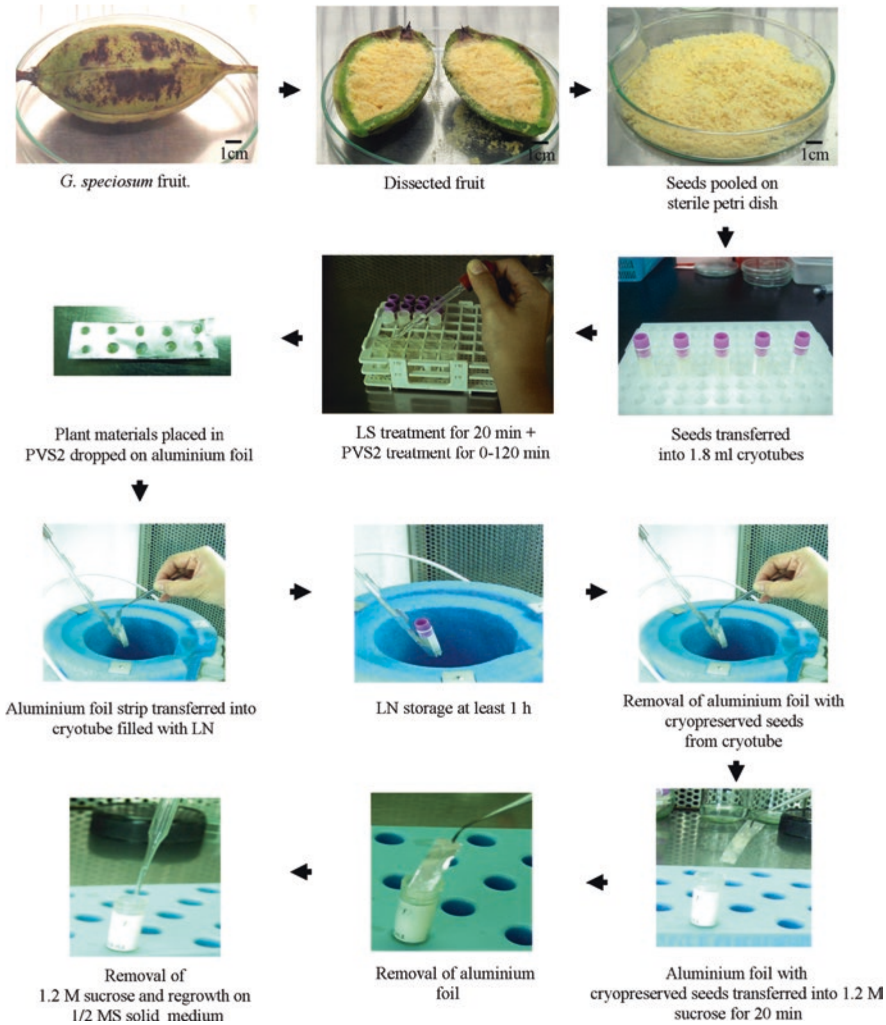

Fig. 1.1 Established protocol for cryopreservation of *G. speciosum* seeds by droplet-vitrification

basal medium with 0.4 M sucrose. The cryo-plates were hardened for 20 min by slowly dispensing the calcium chloride solution containing 0.1 M calcium chloride in 1/2 MS basal medium with 0.4 M sucrose. Then the cryo-plates were surface dried using sterile filter paper, placed in Petri dishes containing silica gel or drying beads in a laminar air-flow cabinet. Cryo-plates were dehydrated for 5 h until 25% moisture content was achieved. Dehydrated cryo-plates were placed in 2 ml cryotubes and plunged directly into liquid nitrogen for 1 day. Cryo-plates were removed from

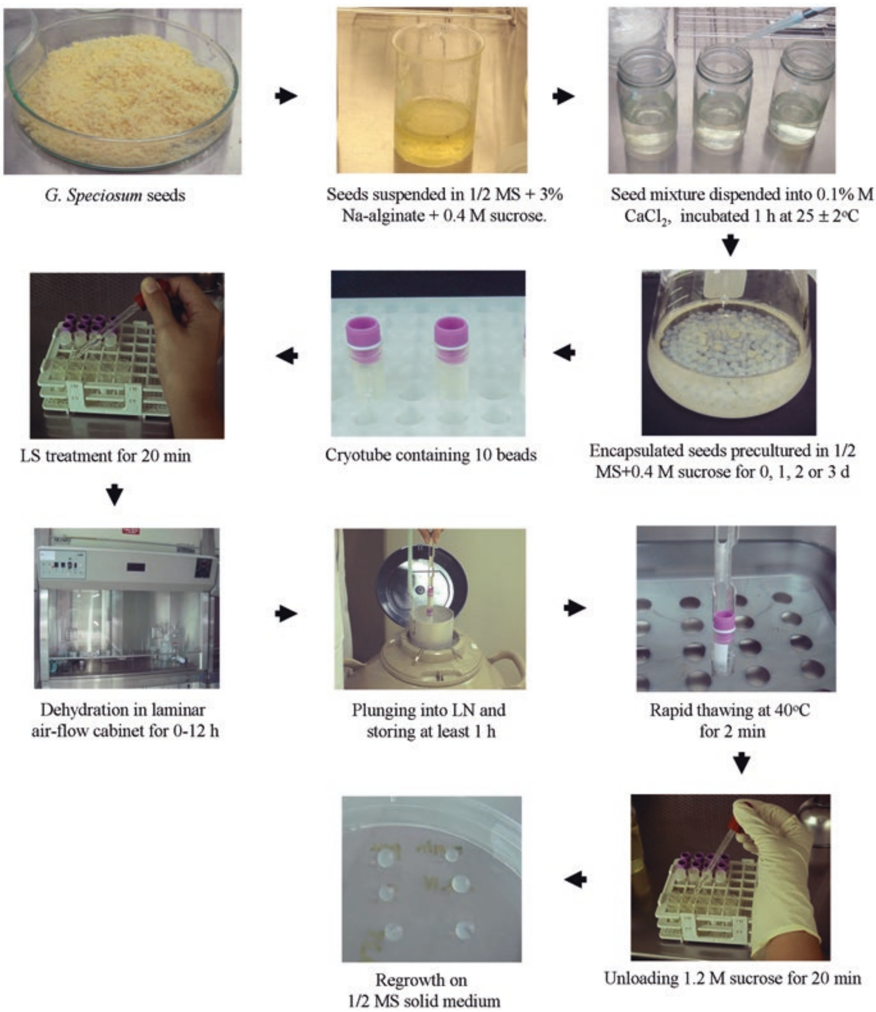

Fig. 1.2 Established protocol for cryopreservation of *G. speciosum* seeds by encapsulation-dehydration

cryotubes and warmed in unloading solution (1.2 M sucrose solution) for 20 min. Protocorms were then removed from the cryo-plate and placed on 1/2 MS agar medium for regrowth. Growth conditions were conducted using 16 h light at 25 ± 3 °C.

For effect of the cryo-plate method, regrowth of control treatments dehydrated using silica gel was observed to be 90%. Regrowth of control treatments dehydrated using drying beads was observed to be 92.1%. In all other treatments, regrowth was

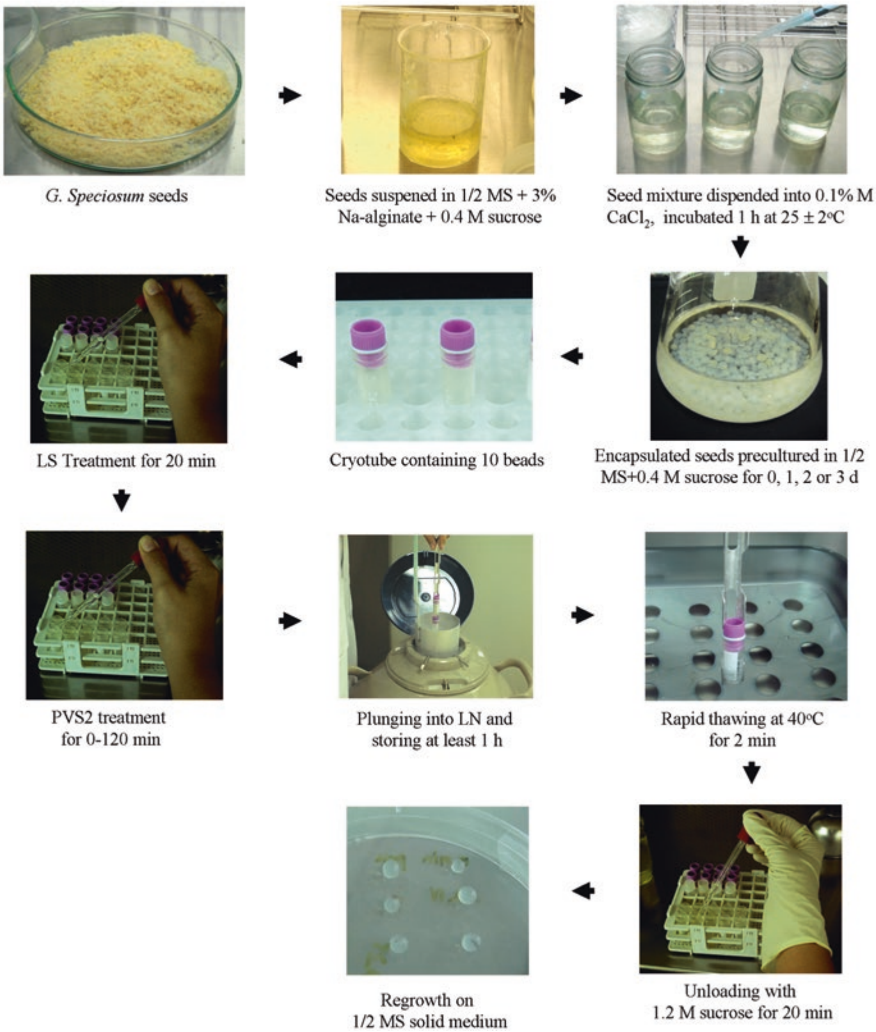

Fig. 1.3 Established protocol for cryopreservation of *G. speciosum* seeds by encapsulation-vitrification

observed to be 73.8% using silica gel for dehydration. Regrowth for all other treatments dehydrated using drying beads was observed to be 76.5%. Regrowth was observed in the second week of transfer to 1/2 MS media. Dehydration using silica gel or drying beads did not significantly affect regrowth rate. Protocorms dehydrated using silica gel or drying beads developed into normal plantlets.

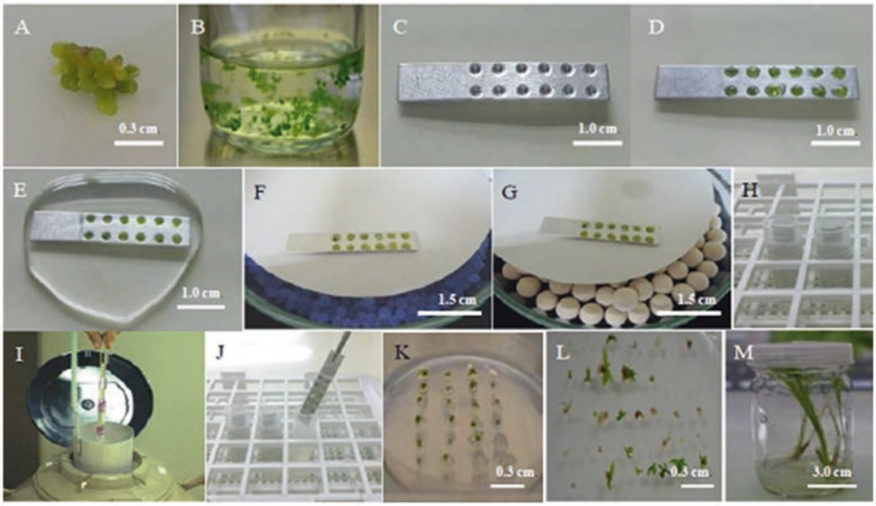

Fig. 1.4 Cryo-plate method dehydrated with silica gel or drying beads. (**a**) Protocorm development. (**b**) Preculture of protocorms in 1/2 MS liquid medium with 0.7 M sucrose for 1 day. (**c**) Pour the alginate solution containing 2% (w/v) sodium alginate in calcium-free 1/2 MS basal medium with 0.4 M sucrose in the wells. (**d**) Place the precultured protocorms in the wells one by one. (**e**) Pour the calcium chloride solution containing 0.1 M calcium chloride in 1/2 MS basal medium with 0.4 M sucrose. (**f**) Dehydration with 50 g silica gel. (**g**) Dehydration with 30 g drying beads. (**h**) Put each cryo-plate in a 2 ml cryotube. (**i**) Plunge 2 ml cryotubes into liquid nitrogen for 1 day. (**j**) Warming in 1.2 M sucrose solution for 20 min. (**k**) Plate on 1/2 MS agar medium. (**l**) Regrowth, (**m**) Regrowth after 60 days

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References

Cordova LB II, Thammasiri K (2016) Cryopreservation on a cryo-plate of *Arundina graminifolia* protocorms, dehydrated with silica gel and drying beads. *CryoLetters* 37:68–76

Jitsopakul N, Thammasiri K, Ishikawa K (2008) Cryopreservation of *Vanda coerulea* protocorms by encapsulation-dehydration method. *CryoLetters* 29:253–260

Kagawa K (2006) Cryopreservation of *Dendrobium cruentum* Rchb. f. M.Sc. Thesis, Faculty of Graduate Studies, Mahidol University, Thailand

Pornchuti W, Thammasiri K (2008) Cryopreservation of protocorms of *Dendrobium cariniferum* Rchb. f. Acta Hortic 788:63–68

Sopalun K, Thammasiri K, Ishikawa K (2010a) Micropropagation of the Thai orchid *Grammatophyllum speciosum* Blume. Plant Cells Tissue Organ Cult 101:143–150

Sopalun K, Thammasiri K, Ishikawa K (2010b) Vitrification-based cryopreservation of *Grammatophyllum speciosum* protocorms. *CryoLetters* 31:347–357
Thammasiri K (1999) Cryopreservation of embryonic axes of jackfruit. *Cryo-Letters* 20:21–28
Thammasiri K (2000) Cryopreservation of seeds of a Thai orchid (*Doritis pulcherrima* Lindl.) by vitrification. *CryoLetters* 21:237–244
Thammasiri K (2002) Preservation of seeds of some Thai orchid species by vitrification. In: Proceedings of the 16th World Orchid Conference, pp 248–251
Thammasiri K (2008) Cryopreservation of some Thai orchid species. Acta Hortic 788:53–62
Thammasiri K, Soamkul L (2007) Cryopreservation of *Vanda coerulea* Griff. Ex Lindl. Seeds by vitrification. *ScienceAsia* 33:223–227

Status of Orchid Industry in India

2

Sadanand N. Hegde

Abstract

India is known for its rich biodiversity, and one of its dominant plant families is Orchidaceae consisting of about 1350 species in 185 genera occurring in diverse phytogeographical conditions ranging from tropical, subtropical and temperate conditions with varying microclimates. However, despite the rich natural occurrence and ideal agroclimatic conditions in India, orchid-based floriculture has not been systematically developed, and the people are yet to reap the benefit out of this natural resource having the least production area and minimum contribution in the overall turnover of floriculture products. In fact, India has lagged other countries in Orchid trade. In this paper, an attempt has been made to trace the history of sustainable development of orchids in India briefly. Distributions of some of the commercially important orchid species and hybrids in various agroclimatic conditions have been provided. The role of governments, non-government organizations and individuals in promoting the development of orchid industry in India has been discussed. An assessment and analysis of commercial activities based on import and export of various orchid products such as cut flowers, tissue culture plants and flasks from major ports of the country have been carried out. The need for a coordinated effort in focused R&D programme in developing new hybrid varieties suitable for various agroclimatic conditions of this country involving various Institutions of Excellence in developing climate-specific hybrids of temperate cymbidiums and paphiopedilums, tropical dendrobiums and vandas and intermediate cattleyas and phalaenopsis, besides other ornamental native species for both cut flowers and pot plants along with their cultivation practices and packages, has been suggested. Besides, potentials of medicinally important orchids and their R&D programme in boosting commercial production have also been suggested. A strong extension programme of the

S. N. Hegde (✉)
Orchid Research and Development Center, State Forest Research Institute, Itanagar, Arunachal Pradesh, India

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technology- and market-driven approach to reach the stakeholders, farmers and growers, in villages and urban clusters involving the communities in promoting cultivation and production of the commercial orchids, has been proposed for the development of a vibrant orchid industry. Involvement of corporate sector to produce quality planting materials in large quantities, distribution to the growers/farmers in village and urban clusters and marketing of their products has been emphasized for the benefit of the society and to boost orchid industry in India.

Keywords
Orchid industry · Orchid germination · Greenhouse technology · Export and import in India

2.1 Introduction

Over the last half a century, floriculture has emerged as an export-oriented multibillion-dollar business globally. It mainly involves cut flowers, pot plants, cut foliage, bulbs, tubers, seeds, rooted cuttings, tissue culture (TC) flasks, dry flowers and leaves. It has been observed that production and trade of floricultural crops have ever increasing trend. In the international trade, major crops involved are *Alstroemeria*, carnations, chrysanthemums, gerbera, gladiolus, gypsophila, anthuriums, lilies, roses, tulips and of course orchids (*Aranda*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Oncidium*, *Paphiopedilum*, *Phalaenopsis*, *Vanda*, etc.). Orchids command high value and great in demand in the World Flower Trade. It is mainly because of attractive flower shape, size, colour and long shelf life of cut flowers and pot plants of orchids. Out of about US $21 billion floriculture trade, 8% is that of orchids and has an increasing trend of 15% annually. It is significant to note that Netherlands is the world's leading flower producer and exporter. The Dutch control the world export and auctioning of floricultural produces. Major consumers of floriculture products are Japan, European countries, South Korea, Thailand, Indonesia and Pacific countries with ever increasing demand. Major exporters of floriculture products are Holland, Columbia, Israel, Italy, Spain, Thailand, France, the USA, South America, New Zealand, Ecuador, etc. Orchids have gained importance as one of the highly priced floricultural crops across the world

2.2 Indian Scenario

India is one of the orchid-rich countries in the world with about 1350 species occurring in diverse phytogeographical conditions from warm coastal regions to the cool Himalayan ranges providing tropical, subtropical and temperate conditions with varying microclimates giving rise to rich biodiversity. Such a natural situation is congenial to grow commercial varieties of orchids to meet the world market demand. However, despite the rich natural occurrence and ideal agroclimatic conditions in

India, orchid-based floriculture has not been systematically developed, and the people are yet to reap the benefit out of this natural resource. Important floriculture crops in India are amaryllis, anthuriums, carnation, chrysanthemum, gladiolus, jasmine, marigold, petunias, roses and, of course, orchids. Total acreage of these crops is about 73,619 ha with 34,349 tons of loose flowers and 49,366 cut flowers. Orchids have the least production area and minimum contribution in the overall turnover of floriculture products. In fact, India has lagged other countries in orchid trade despite its rich natural resources, ideal agroclimate and technical know-how. Out of about Rs. 500 crore businesses in floriculture industry in India, orchids have the least contribution in our country.

2.3 Orchid Development in India

In India, for the first time, Hooker (1890) brought out "Flora of British India" describing 1200 species unravelling the richness of orchids in this country. Subsequent workers added number of species, and today we are a proud nation with about 1350 species in about 185 genera of orchids known in our country (Hegde 2014, Jain Mehrotra 1984; Misra 2007; Rao 2014). Out of them as many as 200 species are highly ornamental, about 55 species are medicinally important, and others are biological curiosities with ecological significance (Hegde 1997; Vij 2001).

Realizing the importance of orchids in floriculture especially that of the hybrid varieties developed from the native ornamental species from India and other parts of the world, the Government of India initiated developmental programmes through various government and non-government agencies to ensure conservation of native germplasm both in situ and ex situ and promoting sustainable development of orchid industry through research and development programmes through ICAR, universities and state and central government agencies (Hegde 1986, 2000, 2005, 2014). Many private players, viz. U.C. Pradhan Laboratories, Kalimpong; Indo-American Hybrid Seeds, Bangalore; and A.V. Thomas & Co, Kerala, are also involved in production of planting materials adopting tissue culture and modern biotechnological approaches and trade of planting materials. National Horticulture Board, NABARD and APEDA have been supporting entrepreneurs to undertake export-oriented orchid trade. Some NGO activities to promote research and development and creating awareness have also been undertaken by TOSI, OSA, TOSKAR and other floriculture societies in various parts of India.

Meanwhile, orchid growing as a hobby elsewhere in the world has transformed itself as a vibrant floriculture industry of commerce with multimillion-dollar business of plants and cut-flower trade. Discovery of aseptic culture of seeds by Knudson (1946) and meristem culture by Morel (1960) revolutionized orchid industry elsewhere in the world with an array of orchid hybrids and cut-flower varieties grown in modern climate-controlled polyhouses. Over the last 50 years, tissue culture technology has added dimension to the industry both in terms of quality and quantity. There are more than 1,25,000 man-made hybrids besides about 25,000 species worlds over making it highly specialized, competitive and vibrant industry of commerce. With the modern biotechnological approaches, it has become possible to produce an array of hybrids and newer clones released to the market – specializing in flower decorations, corsages, bouquet making, pot plants with amazing colour of flowers of orchids, besides various social functions (Pathak et al. 2001; Hegde 2001, 2006, 2009; Vij 2001).

It is worthy to note India so far could produce and register hardly about 200 orchid hybrids (Pradhan 2017) which need to be accelerated to compete in the world market. Meanwhile, however, our expertises in biotechnological approach have contributed in producing tissue-cultured true-to-type hybrid clones which need to be commercially exploited.

2.4 Commercial Potentials

Commercial potentials of orchids in India or its strength in orchid development and trade lies in (1) rich orchid diversity/germplasm; (2) varying and ideal agroclimate from tropical to temperate regions to grow varieties of orchids; (3) technical know-how of orchid growing, propagation technique, biotechnological backing and greenhouse technology; (4) cheap labour; and (5) evergrowing high-end consumer market.

On the other hand, our weaknesses are (1) lack of quality planting materials in adequate quantity; (2) lack of market-driven approach in the production of plants and flowers adopting modern technologies; (3) lack of production of our own hybrids that can compete in world market; (4) lack of consistent R&D backup with new hybrid varieties and technical innovations; (5) lack of production of planting material; (6) lack of quality and quantity of cut flowers to feed the market; (7) lack of training and extension programmes; and (8) lack of involvement of communities both at rural and urban levels in suitable agroclimatic zones and developed hub of activities leading to market places.

2.5 Agroclimate and Orchid Germplasm

Agroclimatically, we have mainly three broad climatic zones, (i) tropical, (ii) subtropical and (iii) temperate, for growing orchids. Depending upon rain pattern, humidity, temperature and elevation, there exist varying types of microclimatic conditions. This has in fact given rise to orchid diversity with about 1350 species in 185 genera occurring in their natural habitats of our country. About 200 species of them are ornamental which could serve as germplasm for breeding, cultivation/farming and production of quality planting materials in the respective agroclimatic zones (Hegde 2001, Pathak et al. 2001). Besides, over the last 50 years, a large number of exotic species and hybrids have been imported and grown in various institutions and by individual hobbyists. This is indeed our strength and potential that should be sustainably utilized for developing orchid industry (Hegde 2014). It is important to note humidity ranging between 50% and 80% is a common requirement for all types