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STUDY OF THE GENETIC DIVERSITY AND CONSERVATION OF DENDROBIUM SPECIES

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Abstract

Genetic diversity, which shows potential of evolution of a species is an important parameter in the proper management and conservation of the species. Therefore, the preservation of the genetic variation is necessary for carrying out the species conservation and choosing and breeding different varieties of new plants which are appropriate for a variety of environment conditions. The dendrobium is one of the biggest groups in the family of the Orchidaceae consisting nearly 1500 species all around the world and it is mostly found in the equatorial and surrounding areas of Asia, Europe and Australia and the surrounding islands of Oceania. In the last few years, different types of molecular methods such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and amplified fragment length polymorphism (AFLP) are employed to study the genetic diversity and structure of endangered plants which are within species and among species, allowing the researchers to find the reasons behind the dangers and also to find possible conservation strategies. Tissue culture, gives us a method by which we can produce a huge amount of genetically similar plants, healthy and physiologically superior quality plantlets in a short amount of time. In the production of the plants by this method, there is a very high amount of survival percentage, which is accompanied by a good quality of acclimatized plantlets, which are in great demand in commercial levels and orchid production by the process of micropropagation.

Keywords: genetic diversity, orchidaceae, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), micropropagation

Introduction

Genetic diversity is considered as a key of biological diversity, and it shows the ability, a given species can undergo so as to adjust to the surroundings and the ability of the species to survive for the long-term and undergo evolution as the changes occurs in the environment constantly (Tripathi et al., 2012;Chen et al., 2013). Normally, the population mean genetic diversity, mean fitness, and the size of the population are related to each other positively (Rao and Hodgkin, 2002). But, orchids are known to show a distinct nature in relation with these characteristics. For instance, under normal conditions, a rare and endangered plants normally shows little amount of genetic diversity (Swensen et al., 1995), however some of the rare orchids such as *D. nobile* (Bhattacharyya et al., 2013), *D. officinale* (Ding et al., 2008; Li et al., 2008), and *Calanthe tsoongiana* T. Tang et F.T. Wang (Qian et al., 2013) shows higher amount of genetic diversity. Generally, a common species shows high genetic diversity (Yu et al., 2011).

The Orchidaceae family constitutes about 10% of all flower plants, it contains a big reserve of 25,000 species (Dressler, 1993), and so the biologists and the people are very interested in these plants (Cozzolino and Widmer, 2005). Unfortunately, many of these species which possess great conservation and economic value are threatened to extinction because of the excessive exploitation, and therefore proper study and conservation of these species are very. The genetic diversity and phylogenetic study of Orchidaceae are directly connected to the effective study and proper use of the available resources and also more important to the production of improved-quality new varieties and therefore growth of the ornamental industry. Morphological and anatomical analysis are normally used to study of the relationship among orchid species. However, these methods are often afflicted by plant variability and growth habitats (Morris et al., 1996; Stern, 1997a,b; Stern and Whitten,1999). In the last few years, a quick improvement in the biotechnology field at the molecular level has equipped modern plant taxonomists with important tools to trace out accurate evolutionary history Orchidaceae (Cozzolino and Widmer, 2005; Hsiao et al., 2011). In the last few years, the fast improvement of biotechnology at the molecular level has provided the plant taxonomists with important resources to study the precise evolutionary trends of the Orchidaceae (Cozzolino and Widmer, 2005; Hsiao et al., 2011).

Microsatellites also known as simple sequence repeats are short, DNA tandem repeats (2–6 bp motifs), and they are mostly dinucleotide or trinucleotide repeats spread all over the plant genomes. Microsatellite markers are multiple allelic, they are co-dominant, which are highly reproducible and polymorphic. They are known to be used in a large amount of fields, which includes identification of the individual, research of the genetic diversity, structure of population and mapping of genome (Riaz et al., 2004; Song et al., 2004; This et al., 2004; Silfverberg et al., 2006; Gu et al., 2007; Ma et al., 2009; Zhang et al., 2009;). In Dendrobium genus, microsatellite markers are developed in the species Dendrobium officinale and D. fimbriatum (Gu et al., 2007; Fan et al., 2009; Xie et al., 2010). But, there are no materials available in relation to the isolation and growth of microsatellite markers for D. loddigesii.

Genetic diversity

Genetic diversity, which shows potential of evolution of a species is an important parameter in the proper management and conservation of the species. Therefore, the preservation of the genetic variation is necessary for carrying out the species conservation programs (Avise and Hamrick, 1996) and choosing and breeding different varieties of new plants which are appropriate for a variety of environment conditions (Rice and Emery, 2003). The Dendrobium is one of the biggest groups in the family of the Orchidaceae consisting nearly 1500 species all around the world and it is mostly found in the tropical and subtropical areas of Asia, Europe and Australia (Burke et al., 2008). The Dendrobium species can produce pollen and dust-like seeds which can travel a very long distance by various pollinators and the winds, and as a result is generally believed to help the regular gene flow among the populations (Arditti and Ghani, 2000a,b). In China, the genus Dendrobium comprised of 74 species and two varieties (Tsi, 1999), and about 50 species are used as traditional medicines for hundreds of years because of their pharmacological properties, which includes nourishing yin, purifying the “evilheat,”, improving immune system, lowering blood sugar levels, checking cancer, and elongating life (Bao et al., 2001; Bulpitt etal., 2007). For example, “Tongpi Fengdou,” a popular traditional Chinese tonic medicine, is prepared from the stems of *Dendrobium moniliforme* (Linnaeus) Swartz (Hu, 1970; Zhang et al., 2011). Because of the large amount of uses of the flower in horticulture and traditional medicine, all Dendrobium species are classified as critically endangered species in China, on the other hand the information of the genetic diversity of the Dendrobium genus is very little and the measures taken to conserve are limited. Until now, the genetic diversity has been studied for conservation purposes in a few Dendrobium species, such as *Dendrobium officinale* Kimura et Migo (Ding et al.,2008, 2009; Li et al., 2008; Hou et al., 2012), *Dendrobium loddigesii* Rolfe (Cai et al., 2011, 2012), and *Dendrobium nobile* Lindley(Bhattacharyya et al., 2013); but the study has not been done not yet in *D. moniliforme*, a popular traditional Chinese medicinal plant.

Because of the habitat loss, overexploitation, pollution, and climate change, a large number of species has become extinct in the last few decades (Frankham et al., 2014). The conservation genetics studies rare and endangered species which has important genetic resources and they have become very important and extensive (Al-Qurainy et al., 2013). An important reason for the conservation of threatened and endangered species is to preserve the genetic diversity of these species, an important factor which is crucial for their adaptation to changing environments, survive long-term, and evolution (Avise and Hamrick, 1996; Swarts et al.,2009; Munoz et al., 2010; Frankham, 2012). Therefore, the study of the genetic diversity in endangered species is very important for building an effective and efficient conservation ways (Geet al., 1998; Gale et al., 2010). For example, by studying the population genetics of the endangered plant *Narcissus pseudonarcissus L.*, Colling et al. (2010) suggested that many of its populations in the different regions should be preserved to save the overall genetic variation. Chen et al. (2013) established that the current genetic content of the natural populations of *Dipsacus chinensis* (Miq.)Maxim was strongly affected by harvesting and habitat fragmentation. These studies gave us useful knowledge for protecting critically endangered species.

Molecular Markers

The research instruments used by the plant taxonomists to study the precise evolutionary trends of the Orchidaceae are biochemical marker isozymes, plastid and nuclear sequences of ITS, matK, rbcL, psbA-trnH (Cameronet al., 1999; Chemisquy and Morrone, 2012; Cisternas et al., 2012;Górniak et al., 2010; Lau et al., 2001; Sharma et al., 2012; Xianget al., 2013), DNA markers such as RFLP are also used (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), ISSR (inter-simple sequence repeat) and AFLP(amplified fragment length polymorphism) markers. Microsatellites, which are also called as simple sequence repeats (SSRs), are repetitive DNA sequences with repeat motifs of 1–6 nucleotides at a given locus. Although other DNA markers are sensitive to PCR amplification environments, or requires a large amount of labor with difficult procedures, SSR markers are multi-allelic, co-dominantly inherited, found commonly, spread around the genome, and they are simpleand even automatically scored (Powell et al., 1996). SSR genotyping are commonly used for genetic identification and to assess the parentage (Buteler et al., 2002; Malysheva et al., 2003), study of the genetic diversity and phylogenetic studies (McCouch et al.,2000; Zhu et al., 2000), building of the genetic maps (Temnykhet al., 2000), etc.. Therefore, when compared to the other ways, competitive genomic mining of SSR markers between the closely related species is considered as one of the most efficient and useful way for the discovery of novel DNA marker. Sequence data that we obtain from several crop plant species that are studied shows that enough homology are seen among genomes of two or more closely related genera/species. Hence, the primer pairs obtained from a species can be used to sense the SSRs in similar species and sometimes among other genera which are of the same family (Kalia et al., 2010).

On the contrary, if little amount of SSR marker resources is available, it delays the study of genetics in the Orchidaceae family. Until now, a proper study on the detailed phylogenetic relationships of the orchids in China at molecular level is not available. A large amount of SSR markers are found in several orchid genus such as Phalaenop-sis (Hsu et al., 2011), Vanda (Teh et al., 2011), Doritis (Jantasuriyaratet al., 2012), and Cymbidium (Huang et al., 2010), and the cross-taxon transferability of SSR markers are commonly seen in the genus Dendrobium (Gu et al., 2007; Lu et al., 2012a,b, 2013; Xie et al.,2010). As Dendrobium is one of the biggest genera in the Orchidaceae family(Baker and Baker, 1996), exchange of the Dendrobium SSR markers to the other Orchidaceae plants are possible with a large potential in both basic and applied orchid studies.

Out of all the molecular tools, RFLP needs a large amounts of DNA, however it produces little information; RAPD results are most of the times very hard to reproduce; and SSRs results are known to produce medium amount of results both in abundance and reproducibility (Garcia et al., 2004;Agarwal et al., 2008). However, AFLP is a highly powerful and consistent technique that can be used for finding the polymorphisms and study of genetic diversity of species, especially when used together with fluorescent DNA sequencing equipment (Huangand Sun, 1999; Bonin et al., 2007). Therefore, AFLP markers are often used to find the genetic diversity of rare and endangered plant species and they are used to design effective ways for protection and conservation (Damasceno et al., 2011; Vanden-Broeck et al., 2011).

Sequence-related amplified polymorphism (SRAP), is a recent molecular marker first showed by Li and Quiros (2001), which targets to amplifying of open reading frames (ORFs). SRAP are known to have many advantages such as simplicity, reproducibility, high throughput rate, easy to isolate the bands for sequencing and it aims at open reading frames (ORFs). SRAP are used often in molecular identification, construction of the genetic linkage map, gene tagging, genomic and cDNA fingerprinting, analysis of genetic diversity and comparative genetics of different species (Wang et al., 2009a,b; Li and Quiros, 2001; Ferriol et al., 2003; Budak et al., 2004; Ding et al., 2008; Li et al., 2010).

Recently, many types of new marker techniques are developed along with the fast growing genomic research (Gupta and Rustgi, 2004). Because of the tremendous growth in public biological databases, the growth of the functional markers which are found in or near the candidate genes are very easy (Andersen and Lubberstedt, 2003). Starting a way, away from random DNA markers towards gene-targeted markers, a novel marker system known as Start Codon Targeted (SCoT) Polymorphism (Collard and Mackill, 2009) was built which is based on the short conserved region flanking, the ATG start codon in plant genes. SCoT markers are primarily reproducible, and is known that the primer length and annealing temperature are not the only factors used to determine the reproducibility. They are major markers like random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) and can be used for genetic analysis, quantitative trait loci (QTL) mapping and bulk segregation analysis (Collard and Mackill, 2009). The different uses of these markers such as in the diversity analysis and diagnostic fingerprinting are successfully showed in peanut, potato and grape (Gorji et al., 2011; Guo et al., 2012; Xiong et al., 2011).

Here, we make three tables on genetic diversity of dendrobium species using various marker genes.

Table 1

Species	DNA Markers	Development Methods	Potential Applications	References
Dendrobium chrysotoxum Lindl.	ISSR Markers, SCoT markers	using 20 different inter-simple sequence repeats (ISSR) and 9 start codon targeted (SCoT) primers	different conservation strategies suited for effectively managing the endangered orchid	Leimapokpam Tikendra et al. (2021)
44 species of 15 Orchidaceae genera	simple sequence repeat (SSR) markers	SSR markerswere applied to test their transferability and polymorphism	microsatellite markers enrich the available resource ofmolecular markers, which would facilitate further evolution and genetic diversity studies, germplasmappraisal and molecular breeding of Orchidaceae species	Jie Yu Kang et al. (2015)
Dendrobium moniliforme	fluorescent amplified fragment length polymorphism (AFLP)	Dendrobium moniliforme (Orchidaceae) from different regions of Asia were collected to investigate itsgenetic diversity, using fluorescent amplified fragment length polymorphism (AFLP)	isolation by distance (IBD) did exist in D. moniliforme and some useful conservation strategies were proposed for theeffective and sustainable exploitation of D. moniliforme.	Meirong Ye et al. (2015)
Dendrobium loddigesii Rolfe	simple sequence repeats (SSR)	12 new microsatellite markers of D. loddigesii were developed	will contribute to research on individual identification, genetic diversity, population structure, genome mapping and conservation biology of D. loddigesii.	Xiaoyan Cai et al. (2012)

Dendrobium loddigesii Rolfe	sequence-related amplified polymorphism (SRAP) markers	SRAP markers were applied to assess the level and pattern of genetic diversity in seven populations of D. loddigesii	Recommendations for conservation of the endangered species resources are proposed.	Xiaoyan Cai et al. (2011)
Dendrobium nobile Lindl.	Start Codon Targeted (SCoT) marker	sixty individuals comprising of six natural populations were investigated for the existing natural genetic diversity.	it can be seen as a preliminary point for future research on the population and evolutionary genetics of this endangered orchid species of medicinal importance	Paromik Bhattacharyya et al. (2013)
31 Dendrobium species	inter-simple sequence repeats (ISSRs)	inter-simple sequence repeats (ISSRs) to evaluate genetic diversity and phylogenetic relationship among 31 Dendrobium species	demonstrated the utility of ISSR marker for species diagnosis and genetic diversity study of the genus Dendrobium	Hui-Zhong Wang et al. (2009)
Dendrobium thyrsiflorum	AFLP markers	phenolics, flavonoids and alkaloid contents alongwith antioxidant activity measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays revealed	can be utilized for conservation strategy formulation of this important medicinal orchid with high antioxidant activity	Paromik Bhattacharyya et al. (2017)
Dendrobium plants	single nucleotide polymorphism (SNP) markers	investigated genetic diversity and variations among 7 Dendrobium mutant genotypes and 11 commercial Dendrobium cultivars using single nucleotide polymorphism (SNP) markers	the SNPs and KASP assay sets are an economically efficient tool for mutant screening and for selection of elite genotypes in Dendrobium breeding programs.	Jaihyunk Ryu et al. (2018)
D. catenatum	simple sequence repeats (SSR)	simple sequence repeats (SSR) were identified from 3814 genomic scaffolds in D. catenatum	valuable for genetic studies and molecular breeding in Dendrobium	T.M. Zhao et al. (2019)
Dendrobium species.	SCoT and TRAP markers	start codon targeted (SCoT) and target region amplification	SCoT and TRAPmarkers are informative	Shangguo Feng et al. (2015)

		polymorphism (TRAP), were used for genetic relationship analysis of 36 Dendrobium species	and can be used to evaluate genetic relationships between Dendrobium species	
Dendrobium nobile Lindl.	EST-SSR markers	generate new EST-SSR markers and to evaluate their potential for cross-species utilization in phylogeny study of genus Dendrobium	would facilitate further evolution and genetic diversity studies, germplasm appraisal, genetic mapping, and molecular breeding of D. nobile and other congeneric species.	Jiang-Jie Lu et al.(2013)
Dendrobium moniliforme (L.) Sw. and *Dendrobium loddigesii Rolfe*	polymorphic chloroplast microsatellite primers	polymorphism of the nine chloroplast microsatellite primers was tested across Dendrobium moniliforme (L.) Sw. and Dendrobium loddigesii Rolfe	useful tool for the study of genetic diversity, population genetic structure, evolution of D. officinale and establishment of effective conservation strategies	Wen Xu et al (2011)
Dendrobium nobile	ISSR and DAMD markers	genetic and chemical diversity existing amongst 6 natural populations of D. nobile were assessed using molecular markers	the cumulative marker approach could be the best suited for assessing the genetic relationships with high accuracy amongst distinct D. nobile accessions	Paromik Bhattacharyya et al. (2015)
Dendrobium speciosum complex (Orchidaceae)	Amplified Fragment Length Polymorphism (AFLP)	analyses of Amplified Fragment Length Polymorphism (AFLP) profiles for D. speciosum sampled from across its distribution showed that the complex consists of two highly supported main groups	contributes to our understanding of the factors shaping biodiversity patterns in Australia's mesic biome.	Lalita Simpson et al. (2017)
Dendrobium species of "Fengdou" (DSFs)	single nucleotide polymorphisms (SNPs) and insertions/ deletions (indels)	Plastomewide comparison showed the co-occurrence of single nucleotide polymorphisms (SNPs) and insertions/ deletions (indels), which can be explained by both the repeat-associated and indel-associated mutation	we recommend using large single-copy (LSC)for accurate authentication of DSFs	Ludan Li et al. (2020)

		hypotheses.		

Table 2

Species	DNA Markers	Development Methods	Potential Applications	References
Dendrobium officinale Kimura et Migo (D. officinale)	start codon targeted (SCoT) polymorphism molecular markers	Using 13 selected SCoT primers, 181 bands were generated, 157 (86.86%) of which were polymorphic	provides guidelines for D. officinale 'Ruishen No.2' identification, for the breeding of D. officinale plants with a high content of active ingredients, and for industrial production	Qingguo Liet al. (2018)
Dendrobium officinale	inter-simple sequence repeat (ISSR) and random amplified polymorphic (RAPD)	Genetic diversity was examined within and among nine natural populations using inter-simple sequence repeat (ISSR) and random amplified polymorphic (RAPD)	a novel evolutionary unit should also be paid more attention to during D. officinale conservation practice	G. Ding et al. (2009)
Dendrobium officinale	amplified fragment length polymorphism (AFLP)	Data of 12 populations were used to assess its genetic diversity and population structure, employing the method of amplified fragment length polymorphism (AFLP)	Keeping a stable environment is critical for the in situ conservation and management of this rare and endangered plant, and for ex situ conservation it is important to design an integrated germplasm bank	Xuexia Li et al (2007)
Dendrobium officinale	15 polymorphic trinucleotide microsatellite loci	15 polymorphic trinucleotide microsatellite loci of D. officinale were developed to examine the genetic diversity and structure of three D. officinale germplasm collections	The 15 new microsatellite loci may be used as a powerful tool for further evaluation and conservation of the genetic diversity of D. officinale germplasm resources	Beiwei Hou et al. (2012)
Dendrobium officinale	sequence-related amplified polymorphis	84 individuals from nine wild populations of D. officinale were analyzed using the method of	In situ conservation is the first advocated and ex situ should be proposed at the same	Ge Ding et al. (2009)

	m (SRAP)	sequence-related amplified polymorphism	time to protect the endangered plant and to preserve germplasm resources	
Dendrobium nobile	SSR primers	Seven pairs of genomic SSR primers were newly designed, and two pairs were chosen from the EST-SSRs	seven new microsatellite loci may be informative for further evaluation and conservation of the genetic diversity of D. nobile	Wenjin Yan et al. (2015)
Dendrobium nobile Lindl.	randomly amplified polymorphic DNA (RAPD)	The genetic structure of D. nobile from Northeast India was investigated using randomly amplified polymorphic DNA (RAPD)	The present findings are useful outcomes for germplasm conservation and formulation of new breeding strategies for this extremely important medicinal orchid species	Paromik Bhattacharyya et al. (2014)
Dendrobium plants	sequence-related amplified polymorphism (SRAP) markers	sequence-related amplified polymorphism (SRAP) markers were applied to molecular phylogeny analysis and species identification of 31 Chinese Dendrobium species	the SRAP marker system is informative and would facilitate further application in germplasm appraisal, evolution, and genetic diversity studies in the genus Dendrobium	Shang-Guo Feng et al. (2013)
Dendrobium officinale K. Kimura et Migo	75 EST-SSRs	81 D. officinale individuals including wild-collected, market-collected, and two germplasm resources from Zhejiang and Jiangxi province was scanned with 75 EST-SSRs	the D. officinale germplasm resources from Jiangxi was recommended for hybridization in order to develop superior cultivars	Xiankun Xie et al. (2020)
D. nobile and D. moniliforme	random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR)	genetic linkage maps were constructed using 90 F1 progeny individuals derived from an interspecific cross between D. nobile and D. moniliforme using RAPD and ISSR	provide an important basis for genetic studies and further medicinal and horticultural traits mapping and marker-assisted selection in Dendrobium breeding programmes	Shangguo Feng et al. (2013)
Dendrobium, Geodorum, Cy	NGS based ddRAD	Genetic diversity was assessed in the four	signifying that selection might have	Subhas Chandra

mbidium and Rhynchostylis	sequencing	orchid species using NGS based ddRAD sequencing data	played a role in evolution of these genes in these four groups of orchids	Ray et al. (2016)
Dendrobium nobile	ten novel polymorphic microsatellite markers	ten novel polymorphic microsatellite markers were isolated by a modified biotin-streptavidin capture method	highly informative for further genetic diversity studies and could be used to evaluate the conservation of D. nobile efficiently	Wenjin Yan et al. (2014)
Dendrobium officinale	13 novel microsatellite makers	isolated and developed 13 novel microsatellite makers from expressed sequence tag sequences of endangered Chinese endemic herb Dendrobium officinale	potential for application in germplasm appraisal, genetic diversity study, genetic mapping, and molecular breeding in D. officinale and other congeneric species	Jiang-Jie Lu et al. (2012)
Dendrobium species	rDNA ITS region sequence analysis	the rDNA ITS region sequence analysis was developed for rapid and accurate identification of thirteen wild and cultivated Dendrobium species belonging to two sections Formosae and Chrysotoxae	can be used as an effective tool for molecular identification and classification, as well as the reconstruction of the phylogeny of wild and cultivated Dendrobium species belonging to different sections	Hongmei Liu et al. (2019)
36 Dendrobium species	sequence analysis of the internal transcribed spacer (ITS) region of ribosomal DNA	sequences of the complete ITS region obtained from the 36 Dendrobium species and 2 outgroup species by using PCR amplification and direct DNA sequencing	phylogenetic relationships revealed by ITS DNA analysis partially supported previously published morphological data	Z-Q Yuan et al. (2009)
Dendrobium officinale	allele-specific PCR primers based on SNPs	Two pairs of allele-specific PCR primers based on SNPs were designed to authenticate two genuine populations	methods based on SNPs of rDNA ITS region and AS-PCR are simple, practical and effective for genuine germplasm authentication of D. officinale during the process of GAP and hygienic food quality control	Ge Ding et al. (2008)

Dendrobium officinale	simple sequence repeats (SSRs)	8527 potential genic simple sequence repeats (SSRs) were identified from 7332 (18.15%) unigene sequences, of which 1023 (2.53%) unigenes contained more than one SSR	these genic SSR markers are valuable tools not only for germplasm conservation of this species but also for phylogenetic studies of Dendrobium	Meng Xu et al. (2017)
Limonium species (Limonium sinense, L. bicolor, L. aureum and L. wrightii)	single nucleotide polymorphism (SNP), amplification refractory mutation system (ARMS), Inter simple sequence repeat (ISSR)	single nucleotide polymorphism (SNP) and amplification refractory mutation system (ARMS) have been applied to authenticate Limonium species, Inter simple sequence repeat (ISSR) was used to assess genetic diversity and population structure of L. sinense and a high level of genetic diversity was detected	SNP and ARMS could be used to authenticate not only Limonium species but related herbs on rDNA internal transcribed spacer region	G. Ding et al. (2013)
Dendrobium sonia-28	protocorm-like bodies (PLBs)	selection of F. proliferatum-tolerant protocorm-like bodies (PLBs) was carried out by assessing the effects of differing concentrations of fusaric acid (FA)	showing different banding patterns for each FA concentration and specific bands for selected and control plants	Rahaleh Dehgahi et al. (2015)
Phytophthora palmivora	rep-PCR (BOX, ERIC, REP and M13) and microsatellite markers	genetic diversity among 81 isolates of P. palmivora from various host plants and geographical regions in Indonesia and Japan was evaluated using rep-PCR (BOX, ERIC, REP and M13) and microsatellite markers	possible explanations for the results and suggested strategies for disease management are discussed	Masanto et al. (2019)
Habenaria edgeworthii Hook. f. ex Collett	Inter-simple sequence repeat (ISSR)	variations among morphological, phytochemical and molecular markers were assessed	can be useful for breeding programme of the species when no other genetic information, such as linkage maps and quantitative trait loci, is available	Lalit Giri et al. (2016)

Table 3

Species	DNA Marker types/ Methods used	Development Methods	Potential Applications	References
Dendrobium moniliforme	DNA (cpDNA) markers (*trn*C-*pet*N and *trn*E-*trn*T)	One hundred and thirty-five samples were collected from 18 natural populations of *D. moniliforme* covering the entire range of the Sino-Japanese Floristic Region (SJFR) of East Asia	results supported the hypothesis that glacial refugia were maintained on different spatial-temporal scales in the SJFR during the last glacial maximum or earlier cold periods, suggesting that Quaternary refugial isolation promoted allopatric speciation of *D. moniliforme* in East Asia	Meirong Ye et al. (2016)
D. officinale	Near-infrared spectroscopy coupled to chemometrics	Near-infrared spectroscopy coupled to chemometrics was used to develop a method to discriminate *D. officinale*, from non-*D. officinale*	the results show that coupling of near-infrared spectroscopy to chemometric techniques is a valuable tool for rapid, inexpensive, and non-invasive authentication of *D. officinale*	Yangchao Wei et al. (2014)
Dendrobium officinale	Infrared and Ultraviolet-Visible Spectroscopies with Data Visualization and Mining	two types of spectra combined with unsupervised and supervised pattern recognition were investigated for authentication of 17 Dendrobium species	the data visualization and mining strategy are effective approaches for original plant authentication of Fengdou materials in the herbal market	Ye Wang et al. (2020)
D. naungmungense	used the next-generation sequencing technology and assembled a complete plastid genome	A total of 123 genes were predicted, including 38 tRNA genes, 8 rRNA genes, and 77 protein-coding genes	suggested *D. naungmungense* to be sister to *Dendrobium wardianum*	Min-Hua Wang et al. (2019)
Dendrobium hancockii Rolfe	the first complete chloroplast genome sequence of *D. hancockii* was reported and characterized	It encodes 106 genes, consisting of 72 unique protein-coding genes, 30 unique tRNA gene, and 4 unique rRNA genes	The phylogenetic analysis indicated that *D. hancockii* is basal-most species for the sect. *Dendrobium*	Zhenyu Hou et al. (2019)

Dendrobium wattii	The complete chloroplast (cp) genome sequence and the genome features of *D. wattii* were reported for new data on the phylogeny of *Dendrobium*	The complete cp genome sequence of *D. wattii* is 159,366 bp in length, including a large single-copy region (LSC, 87,192 bp), a small single-copy region (SSC, 18,422 bp), and two inverted repeat sequences (IRs, 26,876 bp, each)	suggested that *D. wattii* be closely related to other species of *Dendrobium*	Yan-Ping Wang et al. (2020)
Dendrobium pseudotenellum	we report the complete chloroplast (cp) genome sequence and its genome features of *D. pseudotenellum*	The cp genome encoded 125 genes, including 80 protein-coding genes, 37 tRNAs, and 8 rRNAs	helpful for the phylogeny and conservation of *Dendrobium*	Quing-Qin Tan et al. (2020)
Dendrobium harveyanum	we report the complete chloroplast (cp) genome sequence and the cp genome features of *D. harveyanum*	The cp genome encoded 138 genes, of which 120 were unique genes	show that *D. harveyanum* is closely related to other species in *Dendrobium*	Zhi-Cong Huang et al. (2019)
Dendrobium strongylanthum	the complete chloroplast genome was constructed from whole-genome Illumina sequencing data	A total of 130 chloroplast genes were successfully annotated, including 84 protein coding genes, 38 tRNA genes, and eight rRNA genes	showed that the chloroplast genome of *Dendrobium strongylanthum* is related to that of the *Dendrobium officinal*	Jing Li et al. (2015)
Dendrobium chrysocrepis	we report the complete chloroplast (cp) genome sequence and the cp genome features of *D. chrysocrepis*	The cp genome encoded 130 genes, of which 115 were unique genes	indicated that *D. chrysocrepis* was closely related to other species in *Dendrobium*	Jin Zhang et al. (2010)
Dendrobium wangliangii	the complete chloroplast (cp) genome sequence and the genome features of *D. wangliangii* were analyzed	The cp genome contains 129 genes, consisting of 124 unique genes (78 protein-coding genes, 38 tRNAs, and 8 rRNAs)	showed that *D. wangliangii* nested with other *Dendrobium* s pp. and was closely related to *D. ellipsophyllum*, *D. wattii* and *D. longicornu*	Shi-Cheng Shao et al. (2010)
Dendrobium longicornu Lind	we report the first complete chloroplast genome of *D. longicornu*	The cp genome encoded 142 genes, of which 110 were unique genes (80	showed that *D. longicornu* clustered together with *D. ellipsophy*	Xin-Yi Wu et al. (2019)

		protein-coding genes, 26 tRNAs and 4 rRNAs)		
D. densiflorum	the first complete chloroplast genome sequence of *D. densiflorum* was reported and characterized	The complete cpDNA of *D. densiflorum* is a circular molecule of 153,122 bp, which contains 76 protein-coding genes, 30 tRNA genes, and four rRNA genes	indicated that the newly sequenced cpDNA of *D. densiflorum* could be used for the phylogenetic study of *Dendrobium* speci es	Liu Wei et al. (2020)
Dendrobium nobile	The complete chloroplast (cp) genome sequence of *Dendrobium nobile* is studied	130 unique genes were annotated and they were consisted of 76 protein-coding genes, 30 tRNA genes and 4 rRNA genes	Fourteen genes contained one or two introns are shown	Wenjin Yan et al. (2015)
Dendrobium thyrsiflorum Rchb.f.	the complete chloroplast (cp) genome of *D. thyrsiflorum* was deciphered by high-throughput sequencing	A total of 126 genes were *de novo* assembled in this cp genome, including 78 protein genes, 40 tRNA genes and 8 rRNA genes	provides molecular information for future evolution, genetic and molecular biology studies of *Dendrobium*	Bin Zhu et al. (2019)
Dendrobium zhenghuoens e	we reported the complete chloroplast genome of *D. zhenghuoense*	The genome contained 130 genes, including 75 protein-coding genes, 38 tRNA genes and 8 rRNA genes	indicated that *D. zhenghuoense* was the sister to the rest 11 species of *Dendrobium* teste d	Yuan-Zhen Huang et al. (2019)
Dendrobium officinale	The complete chloroplast sequence of *Dendrobium officin ale* was reported and characterized	The complete cp DNA contains 83 protein-coding genes, 39 tRNA genes and 8 rRNA genes	Fourteen genes contained one or two introns	Pei Yang et al. (2014)
Dendrobium bellatulum R olfe	we reported the complete chloroplast (cp) genome sequence and the cp genomic features of *D. bellatulum*	The genome was 152,107 bp long with 129 genes comprising 83 protein-coding genes, 40 tRNA genes, and 6 rRNA genes	indicated that *D. bellatulum* is clustered with other species in *Dendrobium*	Yun-Jiao Zhang et al. (2018)
Dendrobium officinale‘ zhong ke IV hao	we reported and characterized the complete chloroplast (cp) genome sequence of *Dendrobium officinale*‘ zhong ke	the complete cp DNA contains 89 protein-coding genes, 30 tRNA genes, and 8 rRNA genes	showed that the chloroplast genome of *D officinale ‘zhong ke IV hao’* is related to that of the traditional *D.officina*	Zhimin Zhong (2016)

	IV hao', a new variety from self-cross plants of imported Sichuan *D. officinale*		*le*	

Tissue Culture

Even though there is a very big demands in the market, propagation of Dendrobium orchids by means of sexual hybridization are not used for commercial purposes as there is not much profit because of the long amount of time in the generation period which normally takes 3 years to produce the seeds of the flowers and also because of the absence of functonal genetic variability (Kuehnle and Sugii, 1992; Yin et al., 2011; Poobathy et al., 2012). The production of the Dendrobium orchids by means of tissue culture propagation by both in-vitro and agricultural cultivation methods are developed for mass production to control this problem. Hence it is a key factor to build an efficient way in preservation of Dendrobium orchids (Kuehnle and Sugii, 1992; Bustam et al., 2013). There are two methods by which the endangered plant species are preserved, in situ conservation method, where the plants are conserved in their natural habitat, and by the method of ex situ conservation, in which the plants are kept in an artificial surrounding (Hirano et al., 2005; Bustam et al., 2013).

Tissue culture is also used as a method to get new *Dendrobium* cultivars (Cardoso, 2012; Teixeira da Silva et al., 2015a) and the process of the production of the *in vitro* environment gives us a chance for producing seedlings obtained by symbiotic method (Teixeira da Silva et al., 2015b) or asymbiotic (Teixeira da Silva et al., 2015c) growing in an aseptic surrounding following the proper instructions (Teixeira da Silva et al., 2016a). The plants that are grown by the method of tissue culture needs a great amount of treatments to harden the plants and also to avoid the plant death after the are brought in the *ex vitro* conditions (Pospišilová et al., 1999; Ziv and Chen, 2008). Plantlets which are grown in *in vitro* are sometimes not able to produce resistance for some small or big microbial pathogens or stresses both biotic and abiotic as a result of the *in vitro* controlled environment, they are recognized by an aseptic conditions with little differnces in the temperature, the high amount of the air humidity, high nutrients available and a little amount of light intensity and carbon dioxide (CO2) (Teixeira da Silva et al., 2015b). The conditions mentioned give a photomixotrophic growth and the requirement of the carbohydrate to be used in the culture medium. After the survival, they are then transferred to greenhouse or the artificial environment, and it greatly affected by physiological and anatomical requirements. There are no studies available that links the responses that are produced by *Dendrobium* as a result of the biotic stresses. Some of the pests that are known to feed on *Dendrobium* plants during acclimatization are slugs, snails, *Dendrobium* beetles, thrips, mealybugs, and many others during acclimatization (MM Hossain, personal observation).

Dendrobium in Traditional Chinese Medicine (TCM)

A huge number of species with its origin in China of Dendrobium genus are known to use as a traditional medicine for the last 2300 years. The different parts of the plants can be used as a very high quality tonic in Traditional Chinese Medicine (TCM). These plants carry different types of medicinal qualities such as maintaining a healthy kidney, lungs, enhance the stomach, maintaining the production of various body fluids.

About 40 species of Dendrobium genus are known to be used in TCM. And the development of the Dendrobium industry can be described by the three main phases. (a) the phase in which the Dendrobium is collected from the wild, (b) the phase of large scale commercial cultivation in an artificial-sheltered environment, (c) the phase in which the cultivation is done in an ecologically-friendly way. Advancement in the field of seed technology helps in improving the quality of the products and the involvement of the farming technology helps in increased production of the Dendrobium in TCM industry. Both methods of cultivation i.e. cultivation in an artificial-sheltered environment and cultivation in an ecologically-friendly environment helps in conserving the dendrobium species. They use the seedlings that are grown from the seeds which are produced by sexual reproduction and clonal propagation based on meristematic are not used.

The development fields of the Dendrobium industry

The advancements in the technology has improved the Dendrobium industry. Technologies such as the mycorrhizal technology and fertilizers by using microorganism eases the production of seed and also helps in germination of the seed. Its survival and the growth rates (Teixeira et al. 2015). Technology such as sowing directly along with symbiotic fungi are known to use ecological cultivation,

and the resulting culture substrates which are mixed along with fungi are known to develop stronger root systems, thicker stems and improve the yield (Xu et al., 2014).

And these methods are low cost and can be easily operated and the technique fully relies on natural conditions from the very beginning i.e. sowing to harvest and there is no need for chemical fertilizer or pesticides (Zhang et al.,2012).

Big improvements are seen in the pharmacological research of the Dendrobium species. Polysaccharides, the substanceThe main substance that we get from the Dendrobium, polysaccharides shows the properties of resistance to oxidization, tumor resistance and increased immunity (Kang et al., 2011). Recently, the polysaccharide of *D. officinale* is known to decrease the blood glucose (Sun et al., 2016). But, a lot research is very much needed to properly know the pharmacologic effect, action mechanism and the extreme effect of the Dendrobium species for medicinal uses.

The relationship between Dendrobium industry and species conservation

The demand for the Dendrobium species is rising rapidly because of its distinct medicinal properties of the medicinal Dendrobium species, and it has caused a big pressure on the wild population (Hinsley et al., 2018). However, to conserve and revive the wild resources to prevent species extinction, and at the same time continue giving the opportunities, especially in regions of low economic development is a very big problem (Ding et al., 2009; Hinsley et al., 2019). It is said that the cultivation of the Dendrobium provides the opportunity to connect both the commercial TCM industry as well as develop the steps for biodiversity conservation, and also to provide the sustainable development of the Dendrobium industry can be achieved in the very nar future.

Conclusion

The main function of the conservation genetics is to study the variation in the genetics and structure of population of the vulnerable and high risk species to obtain the knowledge on genetics critical for developing useful conservation strategies. Maintaining the genetic diversity is very important in making sure of the continued survival of the species and continue to keep the evolutionary potential (Zhang et al., 2005; Wu et al., 2006). Both in-situ and ex-situ conservation methods can be used to conserve the orchids properly. The in-situ conservation way is mostly used for preserving the total gene pool of those particular species in the original habitats for these organisms and they are most useful in preserving the vulnerable and endangered species (Zhao et al., 2017). Habitat conservation is very important as orchids normally have particular habitats that they thrive and also depend a lot on the pollination, and hence these species these species exists together with other organisms like fungi and various pollinating agents and their life cycle cannot be complete without these organisms (Li and Ge, 2006). Habitat destruction and decrease in the population of a species highly increases the chance of extinction. The reason is because of the loss of genetic diversity due to random genetic drift and inbreeding depression (Reed and Frankham, 2003; Li and Ge, 2006). Hence, the main aim of conservation should be immediate preservation of the natural habitats consisting genetically different orchid species which are very sensitive to the destruction of the habitat and exploitation of the germplasm by both humans and animals.

Keeping a high population genetic diversity is very important for the survival as every population is its own distinct gene pool, and destruction of the population may cause irreparable loss of genetic diversity (Pinheiro et al., 2012). We can increase the genetic diversity by transferring the pollen or mature seeds taken from other populations. We can also get genetically stable orchids that are micropropagated obtained from healthy orchid populations can be introduced to enhance genetic diversity and conserve diverse gene pools. In situ conservation plan should be implemented with a prime focus on protecting the core areas with vulnerable populations to preserve and maintain the prevailing genetic diversity. The ex-situ approach of conservation should involve the establishment of germplasm or seed bank for D. chrysotoxum.
Orchid seed collection, plant transfer to different suitable habitats, and micropropagated plants re-introduction to increase population size and genetic heterozygosity level can be some of the crucial works associated with ex situ orchid conservation.

References

1. Bai, M.F., Wu, T.L., Huang, M., Zhao, T.G., 2004. Rapid propagation of Dendrobum loddigesii Rolfe by tissue culture. Seed 23, 44–45.
2. Benbouza, H., Jacquemin, J.M., Baudoin, J.P., Mergeai, G., 2006. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR makers in polyacrylamide gels. Biotechnol. Agron. Soc. Environ. 10, 77–81.
3. Cai, X.Y., Feng, Z.Y., Zhang, X.X., Xu, W., Hou, B.W., Ding, X.Y., 2011. Genetic diversity and population structure of an endangered Orchid (Dendrobium loddigesii Rolfe) from China revealed by SRAP markers. Sci. Hortic. 129, 877–881.
4. Chen, X.Q., Luo, Y.B.,Wood, J.J., 2009. Dendrobium. In:Wu, Z.Y., Raven, P.H., Hong, D.Y. (Eds.), Flora of China, vol. 25 (Orchidaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, pp. 372–379.
5. Chinese Pharmacopoeia Editorial Committee, 2010. The Pharmacopoeia of the People's Republic of China: I. Chemical Industry Press, Beijing.
6. Chun, Z., 2005. Resource crisis of Dendrobium and its protective countermeasure. Resour. Dev. Mark 21, 139–140.
7. Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. 1, 47–50.
8. Fan, W.J., Luo, Y.M., Li, X.X., Gu, S., Xie, M.L., He, J., Cai, W.T., Ding, X.Y., 2009. Development of microsatellite markers in Dendrobium fimbriatum Hook, an endangered Chinese endemic herb. Mol. Ecol. Res. 9, 373–375.
9. Glenn, T.C., Schable, N.A., 2005. Isolating microsatellite DNA loci. Methods Enzymol. 395, 202–222.
10. Gu, S., Ding, X.Y., Wang, Y., Zhou, Q., Ding, G., Li, X.X., Qian, L., 2007. Isolation and characterization of microsatellite of markers in Dendrobium officinale, an endangered herb endemic to China. Mol. Ecol. Notes 7, 1166–1168.
11. Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106.
12. Kendall, M.D., 1970. Rank Correlation Methods, third ed. London.
13. Konishi, T., Iwata, H., Yashiro, K., Tsumura, Y., Ohsawa, R., Yasui, Y., Ohnishi, O., 2006. Development and characterization of microsatellite markers for common buckwheat. Breed Sci. 56, 277–285.
14. Lalitha, S., 2000. Primer premier 5. Biotech. Software Internet Rep. 1, 270–272.
15. Ma, K.H., Kim, N.S., Lee, G.A., Lee, S.K., Lee, J.K., Yi, J.Y., Park, Y.J., Kim, T.S., Gwag, J.G., Kwon, S.J., 2009. Development of SSR markers for studies of diversity in the genus Fagopyrum. Theor. Appl. Genet. 119, 1247–1254.
16. Michalakis, Y., Excoffier, L., 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 142, 1061–1064.
17. Miller, M.P., 1997. Tools for Population Genetic Analysis (TFPGA)1.3: A Windows Program for the Analysis of Allozyme and Molecular Population Genetic Data. Department of Biological Sciences, Northern Arizona University, Arizona, USA.
18. Qian, L., Ding, G., Zhou, Q., Feng, Z.Y., Ding, X.Y., Gu, S., Wang, Y., Li, X.X., Chu, B.H., 2008. Molecular authentication of Dendrobium loddigesii Rolfe by amplification refractory mutation system (ARMS). Planta Med. 74, 470–473.
19. Riaz, S., Dangl, G.S., Edwards, K.J., Meredith, C.P., 2004. A microsatellite marker based framework linkage map of Vitis vinifera L. Theor. Appl. Genet. 108, 864–872.
20. Rice, K.J., Emery, N.C., 2003. Managing microevolution: restoration in the face of global change. Front. Ecol. Environ. 1, 469–478.
21. Rohlf, F.J., 2000. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.1. Exeter Software, Setauket, New York.
22. Silfverberg, S.D., Matasci, C.L., Van deWeg,W.E., Van Kaauwen, M.P.V.,Walser, M., Kodde, L.P., Soglio, V., Gianfranceschi, L., Durel, C.E., Costa, F., Yamamoto, T., Koller, B., Gessler, C., Patocchi, A., 2006.

Microsatellite markers spanning the apple (Malus _ domestica Borkh.) genome. Tree Genet. Genomes 2, 202–224.
23. Sneath, P.H.A., Sokal, R.R., 1973. Numerical Taxonomy. Freeman, San Francisco, CA.
24. Song, Q.J., Marek, L.F., Shoemaker, R.C., Lark, K.G., Concibido, V.C., Delannay, X., Specht, J.E., Cregan, P.B., 2004. A new integrated genetic linkage map of the soybean. Theor. Appl. Genet. 109, 122–128.
25. Takezaki, N., Nei, M., 1999. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144, 389–399
26. Agarwal, M., Shrivastava, N., Padh, H., 2008. Advances in molecular marker tech-niques and their applications in plant sciences. Plant Cell Rep. 27, 617–631.
27. Akkaya, M.S., Bhagwat, A.A., Cregan, P.B., 1992. Length polymorphisms of simplesequence repeat DNA in soybean. Genetics 132, 1131–1139.
28. Al-Qurainy, F., Khan, S., Nadeem, M., Tarroum, M., Alaklabi, A., 2013. Assessmentof phylogenetic relationship of rare plant species collected from Saudi Arabiausing internal transcribed spacer sequences of nuclear ribosomal DNA. Genet.Mol. Res. 12, 723–730.
29. Amat, M.E., Silvertown, J., Vargas, P., 2013. Strong spatial genetic structure reduces reproductive success in the critically endangered plant genus Pseudomisopates.J. Hered. 104, 692–703.
30. Arditti, J., Ghani, A.K.A., 2000a. Tansley review No. 110 – numerical and physicalproperties of orchid seeds and their biological implications. New Phytol. 145,367–421.
31. Arditti, J., Ghani, A.K.A., 2000b. Numerical and physical properties of orchid seedsand their biological implications (vol 145, pg 367, 2000). New Phytol. 146, 569.
32. Arduino, P., Verra, F., Cianchi, R., Rossi, W., Corrias, B., Bullini, L., 1996. Geneticvariation and natural hybridization between Orchis laxiflora and Orchis palustris(Orchidaceae). Plant Syst. Evol. 202, 87–109.
33. Arias, D.M., Albarran-Lara, A.L., Gonzalez-Rodriguez, A., Penaloza-Ramirez, J.,Dorado, O., Leyva, E., 2012. Genetic diversity and structure of wild populationsof the tropical dry forest tree Jacaratia mexicana (Brassicales: Caricaceae) at alocal scale in Mexico.
34. Rev. Biol. Trop. 60, 1–10.Avise, J.C., Hamrick, J.L., 1996. Conservation Genetics: Case Histories from Nature.Chapman and Hall, New York.
35. Bao, X.S., Shun, Q.S., Chen, L.Z., 2001. The plants of Dendrobium (Shi-hu) in China.Shanghai Medicinal University Press and Fudan University Press, Shanghai.
36. Bhattacharyya, P., Kumaria, S., Kumar, S., Tandon, P., 2013. Start codon targeted(SCoT) marker reveals genetic diversity of Dendrobium nobile Lindl., an endangered medicinal orchid species. Gene 529, 21–26.
37. Bohonak, A.J., 2002. IBD (isolation by distance): a program for analyses of isolationby distance. J. Hered. 93, 153–154.
38. Bonin, A., Bellemain, E., Eidesen, P.B., Pompanon, F., Brochmann, C., Taberlet, P., 2004.How to track and assess genotyping errors in population genetics studies. Mol.Ecol. 13, 3261–3273.
39. Bonin, A., Ehrich, D., Manel, S., 2007. Statistical analysis of amplified fragment lengthpolymorphism data: a toolbox for molecular ecologists and evolutionists. Mol.Ecol. 16, 3737–3758.
40. Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980. Construction of a geneticlinkage map in man using restriction fragment length polymorphisms. Am. J.Hum. Genet. 32, 314–331.
41. Bulpitt, C.J., Li, Y., Bulpitt, P.F., Wang, J., 2007. The use of orchids in Chinese medicine.J. R. Soc. Med. 100, 558–563.
42. Burke, J.M., Bayly, M.J., Adams, P.B., Ladiges, P.Y., 2008. Molecular phylogeneticanalysis of Dendrobium (Orchidaceae), with emphasis on the Australian sec-tion Dendrocoryne, and implications for generic classification. Aust. Syst. Bot.21, 1–14.
43. Cai, X.Y., Feng, Z.Y., Beiwei Hou, B.W., Wenrui Xing, W.R., Xiaoyu Ding, X.Y., 2012.Development of microsatellite markers for genetic diversity analysis of Dendro-bium loddigesii Rolfe, an endangered orchid in China. Biochem. Syst. Ecol. 43,42–47.
44. Cai, X.Y., Feng, Z.Y., Zhang, X.X., Xu, W., Hou, B.W., Ding, X.Y., 2011. Genetic diversityand population structure of an endangered Orchid (Dendrobium loddigesii Rolfe) from China revealed by SRAP markers. Sci. Hortic. – Amst. 129, 877–881.

45. Chen, X.H., Guan, J.J., Ding, R., Zhang, Q., Ling, X.Z., Qu, B., Zhang, L.J., 2013. Conserva-tion genetics of the endangered terrestrial orchid Liparis japonica in NortheastChina based on AFLP markers. Plant Syst. Evol. 299, 691–698.
46. Cipollini, K., Millam, K.C., Burks, D., Cipollini, D., Girod, S., VanGundy, Z., Peters,J.L., 2013. Genetic structure of the endangered northeastern bulrush (Scirpusancistrochaetus) in Pennsylvania, USA, using information from RAPD and SNPs.Biochem. Genet. 51, 686–697.
47. Colling, G., Hemmer, P., Bonniot, A., Hermant, S., Matthies, D., 2010. Populationgenetic structure of wild daffodils (Narcissus pseudonarcissus L.) at differentspatial scales. Plant Syst. Evol. 287, 99–111.
48. Cota, L.G., Vieira, F.A., Melo Junior, A.F., Brandao, M.M., Santana, K.N., Guedes, M.L.,Oliveira, D.A., 2011. Genetic diversity of Annona crassiflora (Annonaceae) innorthern Minas Gerais State. Genet. Mol. Res. 10, 2172–2180.
49. Cozzolino, S., Cafasso, D., Pellegrino, G., Musacchio, A., Widmer, A., 2003. Fine-scale phylogeographical analysis of Mediterranean Anacamptis palustris (Orchi-daceae) populations based on chloroplast minisatellite and microsatellitevariation. Mol. Ecol. 12, 2783–2792.
50. Crispo, E., Hendry, A.P., 2005. Does time since colonization influence isolation bydistance? A meta-analysis. Conserv. Genet. 6, 665–682.
51. Damasceno, J.O., Ruas, E.A., Rodrigues, L.A., Ruas, C.F., Bianchini, E., Pimenta,J.A., Ruas, P.M., 2011. Genetic differentiation in Aspidosperma polyneuron(Apocynaceae) over a short geographic distance as assessed by AFLP markers.Genet. Mol. Res. 10, 1180–1187.
52. de Menezes, I.P., Gaiotto, F.A., Hoffmann, L.V., Ciampi, A.Y., Barroso, P.A., 2014.Genetic diversity and structure of natural populations of Gossypium mustelinum,a wild relative of cotton, in the basin of the De Contas River in Bahia, Brazil.Genetica 142, 99–108.
53. Ding, G., Li, X., Ding, X., Qian, L., 2009. Genetic diversity across natural popula-tions of Dendrobium officinale, the endangered medicinal herb endemic to China,revealed by ISSR and RAPD markers. Genetika 45, 375–382.
54. Ding, G., Zhang, D.Z., Ding, X.Y., Zhou, Q., Zhang, W.C., Li, X.X., 2008. Genetic variationand conservation of the endangered Chinese endemic herb Dendrobium officinalebased on SRAP analysis. Plant Syst. Evol. 276, 149–156.
55. Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of indi-viduals using the software STRUCTURE: a simulation study. Mol. Ecol. 14,2611–2620.
56. Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin, version 3.0: an integrated soft-ware package for population genetics data analysis. Evol. Bioinf. Online 1, 47–50,
57. L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferredfrom metric distances among DNA haplotypes: application to human mitochon-drial DNA restriction data. Genetics 131, 479–491.
58. Ferreira, R.C., Piredda, R., Bagnoli, F., Bellarosa, R., Attimonelli, M., Fineschi, S.,Schirone, B., Simeone, M.C., 2011. Phylogeography and conservation perspec-tives of an endangered macaronesian endemic: Picconia azorica (Tutin) Knobl.(Oleaceae). Eur. J. Forest Res. 130, 181–195.
59. Frankham, R., 2012. How closely does genetic diversity in finite populations conformto predictions of neutral theory? Large deficits in regions of low recombination.Heredity 108, 167–178.
60. Frankham, R., Bradshaw, C.J.A., Brook, B.W., 2014. Genetics in conservation man-agement: revised recommendations for the 50/500 rules, Red List criteria andpopulation viability analyses. Biol. Conserv. 170, 53–63.
61. Gale, S.W., Maeda, A., Chen, C.I., Yukawa, T., 2010. Inter-specific relationships andhierarchical spatial genetic structuring in Nervilia nipponica, an endangeredorchid in Japan. J. Plant Res. 123, 625–637.
62. Garcia, A.A.F., Benchimol, L.L., Barbosa, A.M.M., Geraldi, I.O., Souza, C.L., de Souza, A.P.,2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies intropical maize inbred lines. Genet. Mol. Biol. 27, 579–588.
63. Ge, S., Hong, D.Y., Wang, H.Q., Liu, Z.Y., Zhang, C.M., 1998. Population genetic struc-ture and conservation of an endangered conifer, Cathaya argyrophylla (Pinaceae).Int. J. Plant Sci. 159, 351–357.
64. George, S., Sharma, J., Yadon, V.L., 2009. Genetic diversity of the endangered and nar-row endemic Piperia yadonii (Orchidaceae) assessed with Issr Polymorphisms.Am. J. Bot. 96, 2022–2030.

65. Hou, B.W., Tian, M., Luo, J., Ji, Y., Xue, Q.Y., Ding, X.Y., 2012. Genetic diversity assess-ment and ex situ conservation strategy of the endangered Dendrobium officinale(Orchidaceae) using new trinucleotide microsatellite markers. Plant Syst. Evol.298, 1483–1491.
66. Hu, S.Y., 1970. Dendrobium in Chinese medicine. Econ. Bot. 24, 165–174.
67. Huang, J.C., Sun, M., 1999. A modified AFLP with fluorescence-labelled primersand automated DNA sequencer detection for efficient fingerprinting analysisin plants. Biotechnol. Tech. 13, 277–278.
68. Hutchinson, D.W., Templeton, A.R., 1999. Correlation of pairwise genetic andgeographic distance measures: inferring the relative influences of geneflow and drift on the distribution of genetic variability. Evolution 53,1898–1914.
69. Jacquemyn, H., Brys, R., Adriaens, D., Honnay, O., Roldán-Ruiz, I., 2009. Effects ofpopulation size and forest management on genetic diversity and structure ofthe tuberous orchid Orchis mascula. Conserv. Genet. 10, 161–168.
70. Jacquemyn, H., Vandepitte, K., Brys, R., Honnay, O., Roldán-Ruiz, I., 2007. Fitness vari-ation and genetic diversity in small, remnant populations of the food deceptiveorchid Orchis purpurea. Biol. Conserv. 139, 203–210.
71. Jadwiszczak, K.A., Banaszek, A., Jablonska, E., Sozinov, O.V., 2012. Chloroplast DNAvariation of Betula humilis Schrk. in Poland and Belarus. Tree Genet. Genomes 8,1017–1030.
72. Jensen, J.L., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. BMC Genet. 6, 13.
73. Li, X., Ding, X., Chu, B., Zhou, Q., Ding, G., Gu, S., 2008. Genetic diversity analysis andconservation of the endangered Chinese endemic herb Dendrobium officinaleKimura et Migo (Orchidaceae) based on AFLP. Genetica 133, 159–166.
74. Lopez, L., Barreiro, R., 2013. Genetic guidelines for the conservation of the endan-gered polyploid Centaurea borjae (Asteraceae). J. Plant Res. 126, 81–93.
75. Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPDmarkers. Mol. Ecol. 3, 91–99.
76. Munoz, M., Warner, J., Albertazzi, F.J., 2010. Genetic diversity analysis of the endan-gered slipper orchid Phragmipedium longifolium in Costa Rica. Plant Syst. Evol.290, 217–223.
77. Nei, M., Li, W.H., 1979. Mathematical model for studying genetic variation in termsof restriction endonucleases. Proc. Natl. Acad. Sci. U. S. A. 76, 5269–5273.
78. Nordstrom, S., Hedren, M., 2009. Genetic diversity and differentiation of allopoly-ploid Dactylorhiza (Orchidaceae) with particular focus on the Dactylorhizamajalis ssp traunsteineri/ lapponica complex. Biol. J. Linn. Soc. 97, 52–67.
79. Nybom, H., 2004. Comparison of different nuclear DNA markers for estimatingintraspecific genetic diversity in plants. Mol. Ecol. 13, 1143–1155.
80. Phillips, R.D., Dixon, K.W., Peakall, R., 2012. Low population genetic differentiationin the Orchidaceae: implications for the diversification of the family. Mol. Ecol.21, 5208–5220.
81. Pinheiro, F., Cozzolino, S., de Barros, F., Gouveia, T.M.Z.M., Suzuki, R.M., Fay, M.F.,Palma-Silva, C., 2013. Phylogeographic structure and outbreeding depressionreveal early stages of reproductive isolation in the Neotropical Orchid Epiden-drum Denticulatum. Evolution 67, 2024–2039.
82. Pinheiro, F., de Barros, F., Palma-Silva, C., Fay, M.F., Lexer, C., Cozzolino, S., 2011. Phylogeography and genetic differentiation along the distributional range ofthe orchid Epidendrum fulgens: a neotropical coastal species not restricted toglacial refugia. J. Biogeogr. 38, 1923–1935.
83. Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structureusing multilocus genotype data. Genetics 155, 945–959.
84. Qian, X., Wang, C.X., Tian, M., 2013. Genetic diversity and population differentiationof Calanthe tsoongiana, a rare and endemic orchid in China. Int. J. Mol. Sci. 14,20399–20413.
85. Qu, R.Z., Hou, L., Lü, H.L., Li, H.Y., 2004. The gene flow of population genetic structure.Heredity 26, 377–382 (Chinese edition).
86. Rao, V.R., Hodgkin, T., 2002. Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell 68, 1–19.

87. Rousset, F., 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145, 1219–1228.
88. Swarts, N.D., Dixon, K.W., 2009. Terrestrial orchid conservation in the age of extinc-tion. Ann. Bot. 104, 543–556.
89. Swarts, N.D., Sinclair, E.A., Krauss, S.L., Dixon, K.W., 2009. Genetic diversity infragmented populations of the critically endangered spider orchid Caladeniahuegelii: implications for conservation. Conserv. Genet. 10, 1199–1208.
90. Swensen, S.M., Allan, G.J., Howe, M., Elisens, W.J., Junak, S.A., Rieseberg, L.H., 1995.Genetic analysis of the endangered island endemic Malacothamnus fascicula-tus (Nutt.) Greene var. nesioticus (Rob.) Kearn. (Malvaceae). Conserv. Biol. 9,404–415.
91. Tripathi, N., Saini, N., Nair, P., Tiwari, S., 2012. Lack of genetic diversity of a criticallyendangered important medicinal plant Chlorophytum borivilianum in CentralIndia revealed by AFLP markers. Physiol. Mol. Biol. Plants 18, 161–167.
92. Tsi, Z.H., 1999. Flora of China. Science Press, Beijing (Chinese edition).
93. Vekemans, X., 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoirede Genetique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.
94. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J.,Peleman, J., Kuiper, M., et al., 1995. AFLP: a new technique for DNA fingerprinting.Nucleic Acids Res. 23, 4407–4414.
95. Wichmann, M.C., Alexander, M.J., Soons, M.B., Galsworthy, S., Dunne, L., Gould, R.,Fairfax, C., Niggemann, M., Hails, R.S., Bullock, J.M., 2009. Human-mediated dis-persal of seeds over long distances. Proc. R. Soc. B 276, 523–532.
96. Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA poly-morphisms amplified by arbitrary primers are useful as genetic markers. NucleicAcids Res. 18, 6531–6535.
97. Wright, S., 1951. The genetical structure of populations. Ann. Eugen. 15,323–354.
98. Xiang, X.G., Hu, H., Wang, W., Jin, X.H., 2011. DNA barcoding of the recently evolvedgenus Holcoglossum (Orchidaceae: Aeridinae): a test of DNA barcode candi-dates. Mol. Ecol. Resour. 11, 1012–1021.
99. Xiang, X.G., Schuiteman, A., Li, D.Z., Huang, W.C., Chung, S.W., Li, J.W., Zhou, H.L.,Jin, W.T., Lai, Y.J., Li, Z.Y., Jin, X.H., 2013. Molecular systematics of Dendrobium(Orchidaceae, Dendrobieae) from mainland Asia based on plastid and nuclearsequences. Mol. Phylogenet. Evol. 69, 950–960.
100. Yu, H.H., Yang, Z.L., Sun, B., Liu, R.N., 2011. Genetic diversity and relationship ofendangered plant Magnolia officinalis (Magnoliaceae) assessed with ISSR poly-morphisms. Biochem. Syst. Ecol. 39, 71–78.
101. Zhang, Q.Q., Liu, S.J., Fang, C.W., He, X.L., 2011. Analysis of chemical constituents ofessential oil from flowers of Dendrobium moniliforme (L.) Sw. by GC-MS. Mod.Chin. Med. 13, 34–36 (Chinese edition).
102. Zhivotovsky, L.A., 1999. Estimating population structure in diploids with multilocusdominant DNA markers. Mol. Ecol. 8, 907–913.Zhu, G.H., Ji, Z.H., Wood, J.J., Wood, H.P., 2009. Dendrobium. In: Wu, C.Y., Raven, P.H.,Hong, D.Y. (Eds.), Flora of China. Scientific Press, Beijing (Chinese edition).
103. Bai, M.F., Wu, T.L., Huang, M., Zhao, T.G., 2004. Rapid propagation of *Dendrobum loddigesii Rolfe* by tissue culture. Seed 23, 44–45.
104. Ahmad, F., Ahmad, I., Khan, M.S., 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res, 163: 173–181.
105. An, Y.F., Zhang, Y.Q., Zhou, L.M., Feng, D.Q., 2014. The research progress of cultivation matrixes of *Dendrobium candidum*. China Pharmacy, 25: 2581–2583. (in Chinese)
106. Assis, A.M., Faria, R.T., Colombo, L.A., de Carvalho, J.F.R.P., 2005. Utilização de substratos à base de coco no cultivo de *Dendrobiumnobile* Lindl. (Orchidaceae). Acta Scient Agron, 27: 255–259. (inPortuguese)
107. Atwell, B.J., Kriedemann, P.E., Turnbull, C.G.N., Eamus, D., Bieleski, R.L. (Eds.), 2010. Epiphytes, in: Plants in Action.
108. Bhattacharjee, D.K., 2003. Effects of plant growth regulators and explants on the tissue culture of a monopodial and a sympodialorchid species [M.D. dissertation]. University of Chittagong,
109. Bangladesh.
110. Bhattacharyya, P.N., Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World J Microbiol Biotechnol, 28: 1327–1350.

111. Cardoso, J.C., 2012. *Dendrobium* 'Brazilian Fire 101' – New option of color of flowers for the orchid market. Hortic Bras, 30: 561–564.

112. Cardoso, J.C., Ono, E.O., 2011. *In vitro* growth of *Brassocattleya* orchid hybrid in different concentrations of KNO3, NH4NO3 and benzylaminopurine. Hortic Bras, 29: 359–363.

113. Cardoso, J.C., Rossi, M.L., Rosalem, I.B., Teixeira da Silva, J.A., 2013. Pre-acclimatization in the greenhouse: An alternative to optimizing the micropropagation of gerbera. Sci Hortic, 164: 616–624.

114. Cha-um, S., Ulziibat, B., Kirdmanee, C., 2010. Effects of temperature and relative humidity during *in vitro* acclimatization, on physiological changes and growth characters of *Phalaenopsis* adapted to *in vivo*. Aus J Crop Sci, 4: 750–756.

115. da Silva, F.F., Wallach, R., Chen, Y., 1993. Hydraulic properties of *Sphagnum* peat moss and tuff (scoria) and their potential effects on water availability. Plant Soil, 154: 119–126.

116. de Moraes, L.M., Cavalcante, L.C.D., Faria, R.T., 2002. Substratos para aclimatização de plântulas de *Dendrobium nobile* Lindl. (Orchidaceae) propagadas *in vitro*. Acta Scient, 24: 1397–1400. (in Portuguese)

117. Dan,Y.,Meng,Z.X., Guo,S.X., 2012a. Effects of forty strains of Orchidaceae

118. mycorrhizal fungi on growth of protocorms and plantlets of *Dendrobium candidum* and *D. nobile*. Afr J Microbiol Res, 6: 34–39.

119. Dan, Y., Yu, X., Guo, S.X., Meng, Z., 2012b. Effects of forty-two strains of orchid mycorrhizal fungi on growth of plantlets of *Anoectochilus roxburghii*. Afr J Microbiol Res, 6: 1411–1416.

120. Das, A.K., Das, J., Gogoi, H.K., Srivastava, R.B., 2008. Mass propagation of orchids through *in vitro* seed culture technology. J Cell Tiss Res, 8: 1585–1588.

121. Deb, C.R., Imchen, T., 2010. An efficient *in vitro* hardening of tissue culture raised plants. Biotechnology, 9: 79–83.

122. Di Benedetto, A., 2007. Alternative substrates for potted ornamental plants based on Argentinean peat and Argentinean river waste: A review. Floriculture Ornamental Biotechnol, 7: 90–101.

123. Dobránszki, J., Mendler-Drienyovszki, N., 2014a. Cytokinins affect the stomatal conductance and CO2 exchange of *in vitro* apple leaves. Int J Hortic Sci, 20: 25–28.

124. Dobránszki, J., Mendler-Drienyovszki, N., 2014b. Cytokinin-induced changes in the chlorophyll content and fluorescence of *in vitro* apple leaves. J Plant Physiol, 171: 1472–1478.

125. Dohling, S., Kumaria, S., Tandon, P., 2012. Multiple shoot induction from axillary bud cultures of the medicinal orchid, *Dendrobium longicornu*. AoB Plants, 2012: pls032.

126. Drew, A.P., Kavanagh, K.L., Maynard, C.A., 1992. Acclimatizing micropropagated black cherry by comparison with half-sib seedlings. Physiol Plant, 86: 459–464.

127. Duan, J., Duan, Y.P., 2013. The High Efficient Cultivation Techniques of *Dendrobium*. Fujian Science and Technology Press, Fuzhou.

128. Evans, M.R., Smith, J.N., Cloyd, R.A., 1998. Fungus gnat population development in coconut coir and *Sphagnum* peat-based substrates. Horttechnology, 8: 406–409.

129. Faria, D.C., Dias, A.C., Melo, I.S., de Carvalho Costa, F.E., 2013a. Endophytic bacteria isolated from orchid and their potential to promote plant growth.World J Microbiol Biotechnol, 29: 217–221.

130. Faria, R.T., Rodrigues, F.N., Oliveira, L.V.R., Müller, C., 2004. *In vitro Dendrobium nobile* plant growth and rooting in different sucrose concentrations. Hortic Bras, 22: 780–783. (in Portuguese)

131. Faria, R.T., Takahashi, L.S.A., Lone, A.B., Barbosa, C.M., Takahashi, A., Silva, G.L., 2011. UEL7: Nova cultivar de *Dendrobium*. Hortic Bras, 29: 441–442. (in Portuguese)

132. Faria, R.T., Takahashi, L.S.A., Lone, A.B., de Souza, G.R.B., da Silva, G.L., Hoshino, R.T., 2013b. UEL 8: Nova cultivar de *Dendrobium*. Hortic Bras, 31: 509–511. (in Portuguese)

133. Gangaprasad, A., 1996. *In vitro* culture of selected rare and endangered exquisite orchids of Western Ghat [Ph.D. dissertation]. Kerala University, Thiruvananthapurum.

134. Grout, B.W.W., Millam, S., 1985. Photosynthetic development of micropropagated strawberry plantlets following transplanting. Ann Bot, 55: 129–131.

135. Guo, S., Xu, J., 1990. Effects of fungi and its liquid extract on seed germination of *Dendrobium hancockii* Rolf. China J Chinese Materia Medica, 15: 7397–7399. (in Chinese)

136. Hajong, S., Kumaria, S., Tandon, P., 2010. *In vitro* propagation of the medicinal orchid *Dendrobium chrysanthum*. Proc Indian Natl Sci Acad, 76: 1–6.
137. Han, X.H., Sun, J.L., Duan, C.H., 2013. Rooting medium optimization and transplanting matrix selection of *Dendrobium candidum*. Guangdong Agric Sci, 2: 16–19. (in Chinese)
138. Hazarika, B.N., 2006. Morpho-physiological disorders in *in vitro* culture of plants. Sci Hortic, 108: 105–120.
139. Hazarika, B.N., Sarma, C.M., 1995. *In vitro* germination and regeneration of *Dendrobium transparens* Lindl. J Orchid Soc India, 9: 51–54.
140. He, X.H., Duan, Y.H., Chen, Y.L., Xu, M.G., 2010. A 60-year journey of mycorrhizal research in China: Past, present and future directions. Sci China Life Sci, 53: 1374–1398.
141. Hoagland, D.R., Arnon, D.I., 1950. The water-culturemethod for growing plants without soil. Calif Agr Exp Sta Circ, 347: 1–32.
142. Hossain, M.M., 2013. *In vitro* embryo morphogenesis and micropropagation of *Dendrobium aggregatum* Roxb. Plant Tissue Cult Biotechnol, 23: 241–249.
143. Hossain, M.M., Sharma, M., Pathak, P., 2013. *In vitro* propagation of *Dendrobium aphyllum* (Orchidaceae)—Seed germination to flowering. J Plant Biochem Biotechnol, 22: 157–167.
144. Hou, X.Q., Guo, S.X., 2009. Interaction between a dark septate endophytic isolate from *Dendrobium* sp. and roots of *D. nobile* seedlings. J Integr Plant Biol, 51: 374–381.
145. Hronková, M., Zahradnic˘ková, H., Šimkova, M., Šimek, P., Heydová, A., 2003. The role of abscisic acid in acclimatization of plants cultivated *in vitro* to *ex vitro* conditions. Biol Plant, 46: 535–541.
146. Indhumathi, K., Kannan, M., Jawaharlal, M., Amarnath, V., 2003. Standardization of pre-hardening and hardening techniques in *in vitro* derived plantlets of *Dendrobium* orchid hybrid Sonia-17. J Ornament Hort, 6: 212–216.
147. Jeon, M.W., Ali, M.B., Hahn, E.J., Paek, K.Y., 2005. Effects of photon flux density on the morphology, photosynthesis and growth of a CAM orchid *Doritaenopsis* during post-micropropagation acclimatization. Plant Growth Regul, 45: 139–147.
148. Jeon, M.W., Ali, M.B., Hahn, E.J., Paek, K.Y., 2006. Photosynthetic pigments, morphology and leaf gas exchange during *ex vitro* acclimatization of micropropagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature. Environ Exp Bot, 55: 183–194.
149. Jeong, B.R., Fujiwara, K., Kozai, T., 1995. Environmental control and photoautotrophic micropropagation. Hort Rev, 17: 125–172.
150. Kabir, M.F., Rahman, M.S., Jamal, A., Rahman, M., Khalekuzzaman, M., 2013. Multiple shoot regeneration in *Dendrobium fimbriatum* Hook —An ornamental orchid. J Animal Plant Sci, 23: 1140–1145.
151. Kadlec˘ ek, P., Tichá, I., Haisel, D., C˘ apková, V., Schäfer, C., 2001.
152. Importance of *in vitro* pretreatment for *ex vitro* acclimatization and
153. growth. Plant Sci, 161: 695–701.
154. Khosravi, A.R., Kadir, M.A., Kazemin, S.B., Zaman, F.Q., de Silva, A.E., 2008. Establishment of a plant regeneration system from callus of *Dendrobium* cv. Serdang Beauty. Afr J Biotechnol, 7: 4093–4099.
155. Knudson, C., 1946. A nutrient for germination of orchids. An Orchid Soc Bull, 15: 214–217.
156. Kolomeitseva, G.L., Tsavkelova, E.A., Gusev, E.M., Malina, N.E., 2002. On symbiosis of orchids and active isolate of the bacterium *Bacillus pumilus* in culture *in vitro*. Bull GBS Russian Acad Sci, 183: 117–126. (in Russian)
157. Kong, Q., Yuan, S.Y., Végvári, G.Y., 2007. Micropropagation of an orchid *Dendrobium strongylanthum* Rchb. f Int J Hortic Sci, 13: 61–64.
158. Kumar, K., Rao, I.U., 2012. Morphophysiologicals [sic] problems in acclimatization of micropropagated plants in *ex vitro* conditions — A review. J Ornament Hort Plants, 2: 271–283.
159. Kumari, I.P., George, T.S., Rajmohan, K., 2013. Influence of plant growth regulators on *in vitro* clonal propagation of *Dendrobium* Sonia 'Earsakul'. J Biol Innov, 2: 51–58.
160. Limpanavech, P., Chaiyasuta, S., Vongpromek, R., Pichyangkura, R., Khunwasi, C., Chadchawan, S., Lotrakul, P., Bunjongrat, R., Chaidee, A., Bangyeekhun, T., 2008. Chitosan effects on floral production, gene expression and anatomical changes in the *Dendrobium* orchid.

161. Sci Hortic, 116: 65–72.
162. Lo, S.F., Nalawade, S.M.,Kuo, C.L., Chen, C.L., Tsay, H.S., 2004. Asymbiotic germination of immature seeds, plantlet development and *ex vitro* establishment of plants of *Dendrobium tosaense* Makino — A medicinally important orchid. InVitro Cell Dev Biol Plant, 40: 528–535.
163. Lone, A.B., Barbosa, C.M., Faria, R.T., Takahashi, L.S.A., Fonseca, I.C.B., 2008. Seleção de genótipos de *Dendrobium phalaenopsis* (Orchidaceae) nas fases de propagação *in vitro* e aclimatização. Semina: Ciências Agrárias, 29: 755–760. (in Portuguese)
164. Luo, J.P., Wang, Y., Zha, X.Q., Huang, L., 2008. Micropropagation of *Dendrobium densiflorum* Lindl exWall. through protocorm-like bodies: Effects of plant growth regulators and lanthanoids. Plant Cell Tiss Organ Cult, 93: 333–340.
165. Luo, J.P.,Wawrosch, C., Kopp, B., 2009. Enhanced micropropagation of *Dendrobium huoshanense* C.Z.Tang et S.J. Cheng through protocormlike bodies: the effects of cytokinins, carbohydrate sources and cold pretreatment. Sci Hortic, 123: 258–262.
166. Malabadi, R.B., Mulgund, G.S., Kallappa, N., 2005. Micropropagation of *Dendrobium nobile* from shoot tip sections. J Plant Physiol, 162: 473–478.
167. Maridass, M., Mahesh, R., Raju, G., Benniamin, A., Muthuchelian, K., 2010. *In vitro* propagation of *Dendrobium nanum* through rhizome bud culture. Int J Biol Technol, 1: 50–54.
168. Martin, K.P., Geevarghese, J., Joseph, D., Madassery, J., 2005. *In vitro* propagation of *Dendrobium* hybrids using flower stalk node explants. Indian J Exp Biol, 43: 280–285.
169. Martin, K.P., Madassery, J., 2006. Rapid *in-vitro* propagation of *Dendrobium* hybrids through direct shoot formation from foliar explants, and protocorm-like bodies. Sci Hortic, 108: 95–99.
170. McCormick, M.K., Taylor, D.L., Juhaszova, K., Burnett, R.K., Jr., Whigham, C.F., O'Neill, J.F., 2012. Limitations on orchid recruitment: not a simple picture. Mol Ecol, 21: 1511–1523.
171. Meerow, A.W., 1994. Growth of two subtropical ornamentals using coir (coconut mesocarp pith) as a peat substitute. Hort Sci, 29: 1484–1486.
172. Mitra, A., Dey, S., Sawarkar, S.K., 1998. Photoautotrophic *in vitro* multiplication of the orchid *Dendrobium* under CO2 enrichment. Biol Plant, 41: 145–148.
173. Mitra, G.C., Prasad, R.N., Roychowdary, A., 1976. Inorganic salts and differentiation of protocorms in seed-callus of an orchid and correlated changes in its free amino acid content. Indian J Exp Biol, 14: 350–351.
174. MMA. 1992. Lista oficial da flora ameaçada de extinção. Portaria IBAMA N°06-N, de 15 de janeiro de 1992 (in Portuguese).
175. MMA, 2008. Instrução Normativa n° 6, de 23 de Setembro de 2008. Lista oficial das espécies da flora brasileira ameaçadas de extinção. Diário Oficial [da República Federativa do Brasil] 145(185), sect, 1: 75–83.
176. Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15: 473–497.
177. Nandy, P., 2003. Studies on *in vitro* culture of six epiphytic orchids of Bangladesh [M.D. dissertation]. University of Chittagong, Bangladesh.
178. Nayak, N.R., Sahoo, S., Patnaik, S., Rath, S.P., 2002. Establishment of thin cross section (TCS) culture method for rapid micropropagation of *Cymbidium aloifolium* (L.) Sw. and *Dendrobium nobile* Lindl. (Orchidaceae). Sci Hortic, 94: 107–116.
179. Nguyen, Q.T., Hoang, T.V., Nguyen, H.N., Nguyen, S.D., Huynh, D.H., 2010. Photoautotrophic growth of *Dendrobium* 'Burana white' under different light and ventilation conditions. Prop Ornament Plants, 10: 227–236.
180. Noguera, P., Abad, M., Noguera, V., Puchades, R., Maquieira, A., 2000. Coconut coir waste, a new and viable ecologically-friendly peat substitute. Acta Hortic, 517: 279–286.
181. Nongdam, P., Tikendra, L., 2014. Establishment of an efficient *in vitro* regeneration protocol for rapid and mass propagation of *Dendrobium chrysotoxum* Lindl. using seed culture. ScientificWorld J, 740150: 1–8.
182. Nowak, J., Pruski, K., 2004. Priming tissue cultured propagules, in: Low cost options for tissue culture technology in developing countries. Proceedings of a Technical Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna,
183. 26–30 August 2002, pp. 68–81.

184. Oliveira, L.C., Geisel, A., Marx, A., 2005. Use of Vime's bark as substrate component to grow ornamental plants. Rev Ciências Agrovet, 4: 126–132. (in Portuguese)
185. Overvoorde, P., Fukaki, H., Beeckman, T., 2010. Auxin control of root development. Cold Spring Harb Perspect Biol, 2: a001537.
186. Pan, M.J., Van Staden, J., 1998. The use of charcoal in *in vitro* culture
187. —A review. Plant Growth Regul, 26: 155–163.
188. Pant, B., Thapa, D., 2012. *In vitro* mass propagation of an epiphytic orchid, *Dendrobium primulinum* Lindl. through shoot tip culture. Afr J Biotechnol, 11: 9970–9974.
189. Parthibhan, S., Franklin Benjamin, J.H., Muthukumar, M., Ahamed Sherif, N., Senthil Kumar, T., Rao, M.V., 2012. Influence of nutritional media and photoperiods on *in vitro* asymbiotic seed germination and seedling development of *Dendrobium aqueum* Lindley. Afr J Plant
190. Sci, 6: 383–393.
191. Parthibhan, S., Rao, M.V., Kumar, T.S., 2015. *In vitro* regeneration from protocorms in *Dendrobium aqueum* Lindley —An imperiled orchid. J Genetic Eng Biotechnol, 17: 227–233.
192. Patten, C.L., Glick, B.R., 2002. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl Environ Microbiol, 68: 3795–3801.
193. Paul, S., Kumaria, S., Tandon, P., 2012. An effective nutrient medium for asymbiotic seed germination and large-scale *in vitro* regeneration of *Dendrobium hookerianum*, a threatened orchid of northeast India. AoB Plants, 2012: 462–465.
194. Pospišilová, J., Ticha, I., Kadlecˇek, P., Haisel, D., Plzakova, Š., 1999. Acclimatization of micropropagated plants to *ex-vitro* conditions. Biol Plant, 42: 481–497.
195. Pradhan, S., Paudel, Y.P., Pant, B., 2013. Efficient regeneration of plants from shoot tip explants of *Dendrobium densiflorum* Lindl., a medicinal orchid. Afr J Biotech, 12: 1378–1383.
196. Pridgeon, A., Morrison, A., 2006. The Illustrated Encyclopedia of Orchids — Over 1100 Species Illustrated and Identified. Timber Press Ltd, Portland, pp. 87–103.
197. Puchooa, D., 2004. Comparison of different culture media for the *in vitro* culture of *Dendrobium* (Orchidaceae). Intl J Agric Biol, 6: 884–888.
198. Pyati, A.N., Murthy, H.N., Hahn, E.J., Paek, Y.K., 2002. *In vitro* propagation of *Dendrobium macrostachyum* Lindl. Indian J Exp Biol, 40: 620–623.
199. Qian,W.L., Zhang, J.X.,Wu, K.L., Zeng, S.J., 2013. Study on propagation and cultivation technique of *Dendrobium huoshanense* seedlings. J Tropical Subtropical Bot, 21: 240–246. (in Chinese)
200. Radojevic, L., Djordjevic, N., Petrovic, J., 1990. *In vitro* culture technique for carnation breeding. Acta Hortic, 280: 163–168.
201. Rangsayatorn, N., 2009. Micropropagation of *Dendrobium draconis* Rchb. f. from thin cross-section culture. Sci Hortic, 122: 662–665.
202. Rani, C.L., Vidya, C., Mercy, S.T., 2006. *Ex vitro* establishment of *Dendrobium* hybrids as influenced by potting media. J Orchid Soc India, 20: 31–35.
203. Rao, S., Barman, B., 2014. *In vitro* micropropagation of *Dendrobium chrysanthum*Wall. Ex Lindl. – a threatened orchid. Scholars Acad J Biosci, 2: 39–42. Rout, G.R., Debata, B.K., Das, P., 1989a. *In vitro* mass-scale propagation of *Rosa hybrida* cv Landora. Curr Sci, 58: 876–878.
204. Rout, G.R., Debata, B.K., Das, P., 1989b. Micropropagation of *Rosa hybrid* cv. Queen Elizabeth through *in vitro* culture of axillary buds. Orissa J Hortic, 16: 1–9.
205. Roy, J., Banerjee, N., 2003. Induction of callus and plant regeneration from shoot-tip explants of *Dendrobium fimbriatum* Lindl. var. oculatum Hk. F. Sci Hortic, 97: 333–340.
206. Roy, J., Naha, S., Majumdar, M., Banerjee, N., 2007. Direct and callusmediated protocorm-like body induction from shoot-tips of *Dendrobium chrysotoxum* Lindl. (Orchidaceae). Plant Cell Tiss Org Cult, 90: 31–39.
207. Sagawa, Y., Shoji, T., 1967. Clonal propagation of Dendrobiums through shoot meristem culture. Amer Ochid Soc Bull, 36: 856–859.
208. Sharma, A., Tandon, P., 1992. *In vitro* culture of *Dendrobium wardianum* Warner. morphogenetic effects of some nitrogenous adjuvants. Indian J Plant Physiol, 35: 80–83.

209. Sharma, J., Chauhan, Y.S., 1995. Establishment of *in vitro* raised seedlings of *Dendrobium chrysanthum* and *Paphiopedilum spicerianum*. J Orchid Soc India, 9: 37–41.
210. Sharma, U., Rao, V.R., Mohan, J.S.S., Reddy, A.S., 2007. *In vitro* propagation of *Dendrobium microbulbon* A. Rich. — A rare ethnomedicinal herb. Indian J Biotechnol, 6: 381–384.
211. Sorgato, J.S., Rosa, Y.B.C.J., Soares, J.S., Pinto, J.V.C., de Sousa, G.G., Rosa, D.B.C.J., 2016. Luminosity and water immersion in the intermediate acclimatization of *Dendrobium phalaenopsis*. Hortic Bras, 34: 80–85.
212. Sorgato, J.S., Rosa, Y.B.C.J., Soares, J.S., Soares, C.R.L., de Sousa, G.G., 2015a. Light in intermediate acclimatization of *in vitro* germinated seedlings of *Dendrobium phalaenopsis* Deang Suree. Ciência Rural, 45: 231–237. (in Portuguese)
213. Sorgato, J.S., Soares, J.S., Rosa, Y.B.C.J., Soares, C.R.L., Pereira, S.T.S., Rezende, L.S., 2015b. Immersion in nutrient solution and gibberellic acid promote intermediate acclimatization in *Dendrobium phalaenopsis* Deang Suree. Braz J Biosc, 13: 176–180. (in Portuguese)
214. Spaepen, S., Vanderleyden, J., Remans, R., 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. Microbiol Rev, 31: 425–448.
215. Spomer, L.A., Berry, W.L., Tibbitts, T.W., 1997. Plant culture in solid media, in: Langhans, R.W., Tibbitts, T.W. (Eds.), Plant Growth Chamber Handbook. Iowa State University of Science and Technology, Ames, pp. 105–118.
216. Sujjaritthurakarn, P., Kanchanapoom, K., 2011. Efficient direct protocorm-like bodies induction of dwarf *Dendrobium* using thidiazuron. Not Sci Biol, 3: 88–92.
217. Sunitibala, H., Kishor, R., 2009. Micropropagation of *Dendrobium transparens* L. from axenic pseudobulb segments. Indian J Biotech, 8: 448–452.
218. Teixeira da Silva, J.A., 2014. Photoauto-, photohetero- and photomixotrophic *in vitro* propagation of papaya (*Carica papaya* L.) and response of seed and seedlings to light-emitting diodes. Thammasat Int J Sci Technol, 19: 57–71.
219. Teixeira da Silva, J.A., Dobránszki, J., Cardoso, J.C., Chandler, S.F., Zeng, S.J., 2016c. Review: methods for genetic transformation in *Dendrobium*. Plant Cell Rep, 35: 483–504.
220. Teixeira da Silva, J.A., Dobránszki, J., Cardoso, J.C., Zeng, S.J., 2015a Micropropagation of *Dendrobium*: A review. Plant Cell Rep, 34: 671–704.
221. Teixeira da Silva, J.A., Giang, D.T.T., Tanaka, M., 2006. Photoautotrophic micropropagation of *Spathiphyllum*. Photosynthetica, 44: 53–61.
222. Teixeira da Silva, J.A., Jin, X.H., Dobránszki, J., Lu, J.J.,Wang, H.Z., Zotz, G., Cardoso, J.C., Zeng, S.J., 2016b. Advances in *Dendrobium* molecular research: applications in genetic variation, identification and breeding. Mol Phylogenet Evol, 95: 196–216.
223. Teixeira da Silva, J.A., Tsavkelova, E., Ng, T.B., Dobránszki, J., Parthibhan, S., Cardoso, J.C., Rao, M.V., Zeng, S.J., 2015c. Asymbiotic *in vitro* seed propagation of *Dendrobium*. Plant Cell Rep, 34: 1685–1706.
224. Teixeira da Silva, J.A., Tsavkelova, E., Zeng, S.J., Ng, T.B., Dobránszki, J., Parthibhan, S., Cardoso, J.C., Rao, M.V., 2015b. Symbiotic *in vitro* seed propagation of *Dendrobium*: fungal and bacterial partners and their influence on plant growth and development. Planta, 242: 1–22.
225. Teixeira da Silva, J.A., Winarto, B., Dobránszki, J., Cardoso, J.C., Zeng, S.J., 2016a. Tissue disinfection for preparation of *Dendrobium in vitro* culture. Folia Hortic, 28: 57–75.
226. Thimijan, R.W., Heins, R.D., 1983. Photometric, radiometric, and quantum light units of measure: A review of procedures for interconversion. Hortscience, 18: 818–822.
227. Thomale, H., 1954. Die Orchideen. Eugen Ulmer, Stuttgart, pp. 62–88.
228. Tsavkelova, E.A., 2011. Bacteria associated with orchid roots, in: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Plant Growth Responses. Springer, Haridwar, pp. 221–259.
229. Tsavkelova, E.A., Cherdyntseva, T.A., Klimova, S.Y., Shestakov, A.I., Botina, S.G., Netrusov, A.I., 2007. Orchid-associated bacteria produce indole-3-acetic acid, promote seed germination, and increase their microbial yield in response to exogenous auxin. Arch Microbiol, 188:
230. 655–664.

231. Tsavkelova, E.A., Cherdyntseva, T.A., Netrusov, A.I., 2003. Phytohormones production by the fungi associated with orchids. Mycol Phytopath, 37: 75–83. (in Russian) Vacin, E.,Went, F.W., 1949. Some pH changes in nutrient solutions. Bot Gaz, 110: 605–613.

232. Van Staden, J., Zazimalova, E., George, E.F., 2008. Plant growth regulators II: cytokinins, their analogues and antagonists, in: George, E.F., Hall, M.A., De Klerk, G.J. (Eds.), Plant Propagation by Tissue Culture, vol. 1, 3rd ed. The Background. Springer, Dordrecht, pp. 205–226.

233. Venturieri, G.A., Pickscius, F.J., 2013. Propagation of noble dendrobium (*Dendrobium nobile* Lindl.) by cutting. Acta Sci, 35: 501–504.

234. Vijayakumar, S., Rajalkshmi, G., Kalimuthu, K., 2012. Propagation of *Dendrobium aggregatum* through the culture of immature seeds from green capsules. Lankesteriana, 12: 131–135.

235. Vyas, S., Kapoor-Pandey, P., Guha, S., Rao, I.U., 2011. Synchronous plantlet formation by using banana extract and *in vitro* hardening in orchid, *Dendrobium lituiflorum* Lindl. J Ornament Hort Plants, 1:175–184.

236. Wang, H., Fang, H.,Wang, Y., Duan, L., Guo, S., 2011. *In situ* seed baiting techniques in *Dendrobium officinale* Kimuraet Migo and *Dendrobium nobile* Lindl.: The endangered Chinese endemic *Dendrobium* (Orchidaceae).World J Microbiol Biotechnol, 27: 2051–2059.

237. Wang, Q.X., Yan, N., Ji, D.G., Li, S.Y., Hu, H., 2013. *In vitro* growth and carbon utilization of the green-leaved orchid *Dendrobium officinale* are promoted by mycorrhizal associations. Bot Studies, 54: 23.

238. Winarto, B., Rachmawati, F., 2013. *In vitro* propagation protocol of *Dendrobium* 'Gradita 31' via protocorm like bodies. Thammasat Int J Sci Technol, 18: 54–68.

239. Winarto, B., Rachmawati, F., Santi, A., Teixeira da Silva, J.A., 2013. Mass propagation of *Dendrobium* 'Zahra FR 62', a new hybrid used for cut flowers, using bioreactor culture. Sci Hortic, 161: 170–180.

240. Winarto, B., Teixeira da Silva, J.A., 2015. Use of coconut water and fertilizer for *in vitro* proliferation and plantlet production of *Dendrobium* 'Gradita 31'. In Vitro Cell Dev Biol Plant, 51: 303–314.

241. Wood, H.P., 2006. The Dendrobiums. AR G Gantner Verlag, Ruggell, Leichenstein.

242. Wu, H.F., Song, X.Q., Liu, H.X., 2012. *Ex-situ* symbiotic seed germination of *Dendrobium catenatum*. Acta Ecol Sin, 32: 2491–2497. (in Chinese)

243. Wu, Z.Y., Raven, P.H., Hong, D.Y., 2009. Flora of China (Vol. 25, Orchidaceae). Science Press, Missouri Botanical Garden Press, Beijing, St. Louis, pp. 367–397.

244. Xiao, Y., Zhang, Y.Z., 2013. The effect of media and plant training and season on the pre-planting of the tissue culture shoots of *Dendrobium candidum* Wall. ex Lindl. J Xinyang Agric College, 23: 93–94. (in Chinese)

245. Yoon, Y.J., Mobin, M., Hahn, E.J., Paek, K.Y., 2009. Impact of *in vitro* CO2 enrichment and sugar deprivation on acclamatory responses of *Phalaenopsis* plantlets to *ex vitro* conditions. Environ Exp Bot, 65: 183–188.

246. Zeng, S.J., Cheng, S.J., 1996. Tissue culture and rapid propagation of *Dendrobium*. J Chin Med Mater, 19: 490–491.

247. Zeng, S.J., Cheng, S.J., Zhang, J.L., Zhao, F.P., 1998. Embryo culture and propagation of *Dendrobium in vitro*. Acta Hortic Sin, 25: 75–80. (in Chinese)

248. Zeng, S.J., Hu, S.H., 2004. The Dendrobium Orchids. Guangdong Science and Technology Press, Guangzhou. Zhan, Q.C., Li, X., Qi, Y., Yang, S.X., Ye, Q.M., Jian, L.G., 2010. The rapid

249. propagation of *Dendrobium wilsonii* Rolfe and its transplanting. Northern Hortic, 16: 137–139. (in Chinese)

250. Zhang, L., Chen, J., Lv, Y., Gao, C., Guo, S., 2011. *Mycena* sp., amycorrhizal fungus of the orchid *Dendrobium officinale*. Mycol Progress, 11: 395–401.

251. Zhang, N.G., Yong, J.W.H., Hew, C.S., Zhu, X., 1995. The production of cytokine, abscissic acid and auxin by CAM orchid aerial roots. J Plant Physiol, 147: 371–377.

252. Zhao, M.M., Zhang, G., Zhang, D.W., Hsiao, Y.Y., Guo, S.X., 2013. ESTs analysis reveals putative genes involved in symbiotic seed germination in *Dendrobium officinale*. PLoS ONE, 8:

253. e72705.

254. Zhao, P.,Wu, F., Feng, F.S.,Wang,W.J., 2008. Protocorm-like body (PLB) formation and plant regeneration from the callus culture of *Dendrobium candidum*Wall ex Lindl. In Vitro Cell Dev Biol Plant, 44: 178–185.

255. Zhao, Y.D., 2011. Auxin biosynthesis and its role in plant development . Ann Rev Plant Biol, 61: 49–64.

256. Zhou, Y.J., Yang, F.S., Song, X.Q., Zhu, G.P., Hu, M.J., 2009. Effects of mycorrhizal fungi on seedling's growth and photosynthetic capability of *Dendrobium sinense*, endemic to Hainan. North Hortic, 12: 11–15.

257. Ziv, M., 1986. *In vitro* hardening and acclimatization of tissue culture plants, in: Withers, L.A., Alderson, P.G. (Eds.), Plant Tissue Culture and its Agricultural Applications. Butterworths, London, pp. 187– 203.

258. Ziv, M., Chen, J., 2008. The anatomy and morphology of tissue cultured plants, in: George, E.F., Hall, M.A., De Klerk, G.J. (Eds.), Plant Propagation by Tissue Culture, vol. 1, 3rd ed. The Background. Springer, Dordrecht, pp. 465–477.