



# Genetic Relatedness of *Dendrobium* Orchids in Myanmar



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# Outline

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- Introduction
- Objective
- Materials and methods
- Result and discussion



# Background and rationale

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- Orchid flora of Myanmar is highly diverse
- Over 800 orchid species are distributed in Myanmar (Kurzweil and Lwin 2014)
- Plentiful in many parts of Myanmar both terrestrial and epiphytic
- *Dendrobium* is one of the largest genera of orchid family, approximately 800-1500 species and mainly distributed in South, East and South-east Asia and Australia (Wood 2006; Zhu et al., 2009)



# Background and rationale

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- Around 129 species were recorded from Myanmar (Kurzweil and Lwin, 2014; Govaerts et al., 2017)
- *Dendrobiums* are one of the most popular orchids for their medicinal and commercial cut flowers and potted plants around the world
- Some species are large-flowered and brightly colored



# Background and rationale

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- Commonly used as decorations at convocation ceremonies, weddings and other festivals or offered to Buddha images
- orchid flowers sold on the domestic market are grown locally, while some are imported from Thailand



# Uses of medicinal orchids

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- Many *Dendrobium species* have been used in traditional medicine for many centuries in Asian countries
- to clear away toxic materials accumulated in human tissues, and as health care products to improve the immune system
- to decrease the growth of cancer & blood sugar level, enhance blood flow, and strengthen the body's resistance
- Myanmar orchid species have medicinal properties, nine species of *Dendrobium* have tonic (San and Myint, 2009)



# Background and rationale

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- Most *Dendrobium* speices only survive in certain habitats and
- Susceptible to the deterioration and fragmentation of natural ecosystems
- Nowadays, due to over-collection and habitat destruction, natural *Dendrobium* populations are under severe threat of extinction



# Background and rationale

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- due to the increasing demand for cut flowers in the market, a large number of novel *Dendrobium* cultivars are continually being imported for commercial cultivation, leading to a narrow genetic base for cultivar improvements.
- characterization of the genetic diversity and examination of the genetic relationship among *Dendrobium* species are important for the sustainable conservation and increased use of plant genetic resources.
- For genetic resource conservation of *Dendrobium* species, as well as identification for guaranteeing their quality and safety in practical use of wild and cultivated plants for medicinal purposes, different molecular markers system were developed.

# Objectives

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- i. To calculate the genetic relatedness of *Dendrobium species*
- ii. To document the DNA fingerprint of these species for the conservation and further research purposes



# Materials and Methods

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- A total of 25 *Dendrobium* species (17 species from Minglardon orchid farm, Yangon and 8 species from Doekwin farm, Pyin Oo Lwin)
- Sampling and DNA Extraction (CTAB method)
- Genotyping with molecular markers



Table 1. List of *Dendrobium species*

No	Common Name and Code	Scientific name	No	Common Name and Code	Scientific name
1	Mya-sit-kyo, မြေခဲကြိုး (MSK)	<i>Dendrobium pendulum</i> Roxb	14	Kein-na-yi/Kadaw-sut, ကိန်နီ (KNY)	<i>Dendrobium draconis</i> Rchb.f
2	Daung-myi-thitkwa, သေဒီးခြံ (DMTK)	<i>Dendrobium nobile</i> Lindl	15	Swe Ku Let tan do Arme , အာမဲလက်တံတို (AMLTD)	<i>Dendrobium fanbriatum</i>
3	Tagunlonshwe, တပိုင်လုံးရွှေ (TGLS)	<i>Dendrobium densiflorum</i> Lindl	16	Arme-Mauk-khan-wa , အာမဲစောက်ခင်းဝါ (AMKW)	<i>Dendrobium suavissimum</i> Rchb.f
4	Tagunlon-ngwe/Kyet-u-hnit, တပိုင်လုံးငွေ (GLN)	<i>Dendrobium thyrsiflorum</i> Rchb.f.	17	Ka-yan-yaung-lwin-pyin/ ခရိုင်ရောင်လွင်ပြင် (Tagunlon-gyi, (KYLP)	<i>Dendrobium parishii</i> Rchb.f
5	Thi-kyu-kyu, သင်းကြဲကြဲ (TKK)	<i>Dendrobium primulinum</i> Lindl	18	Ah-sein-nu-yaungthitkwa, အစိမ်းရောင်သစ်ရွှေ, (ASNY)	<i>Dendrobium porphyrochilum</i> Lindl
6	Kha-mauk-kyo , ခစောက်ကြိုး (KMK)	<i>Dendrobium aphyllum</i> Roxb.	19	Yone-mhwe-thitkwa , ယုန်ရွှေ (YM)	<i>Dendrobium senile</i>
7	Shwetu/Mout khan war, ရွှေတူစောက်ခင်းဝါ (STMKW)	<i>Dendrobium chrysotaxum</i> Lindl	20	Ngwe-tu , ငွေတူ (NT)	<i>Dendrobium formosum</i> Roxb
8	Sinma-myet-kwin, ဆင်မချက်တွင်း (SMMK)	<i>Dendrobium pulchellum</i> Roxb	21	Myet-me-lay, မျက်မဲလေး (MML)	<i>Dendrobium gibsonii</i> Lindl
9	Waso-pan/, ဝါခိုဝန်း (WSP)	<i>Dendrobium moschatum</i>	22	Pan- mwe-thitkwa , ဝန်းရွှေ (PM)	<i>Dendrobium heterocarpum</i>
10	Swe Ku Let tan shay Arme, အာမဲလက်တံရှည် (AMLTS)	<i>Dendrobium chrysanthum</i>	23	Yadanar-shwe-khet/Shwe-wa-galay , ရတနာရွှေစက်, (YDNSK)	<i>Dendrobium aggregatum</i> Roxb
11	Mahar-dewi/ Payaung-setku-pan, မဟာဒေဝီ, (MHDW)	<i>Dendrobium cariniferum</i> Rchb.	24	Lwoa-thwarthitkwa, လွှာသွား (LTTW)	<i>Dendrobium keithii</i> Sidl
12	Kong-lon-phyu , ခေါင်းလောင်းပြူ (KLP)	<i>Dendrobium cretaceum</i> Lindl.	25	Pa Zum , ပုခံ (PZ)	<i>Dendrobium incurvum</i>
13	Shwe-wa-galay, ရွှေဝါကလေး (SWGL)	<i>Dendrobium Aphrodite</i> Rchb.f			

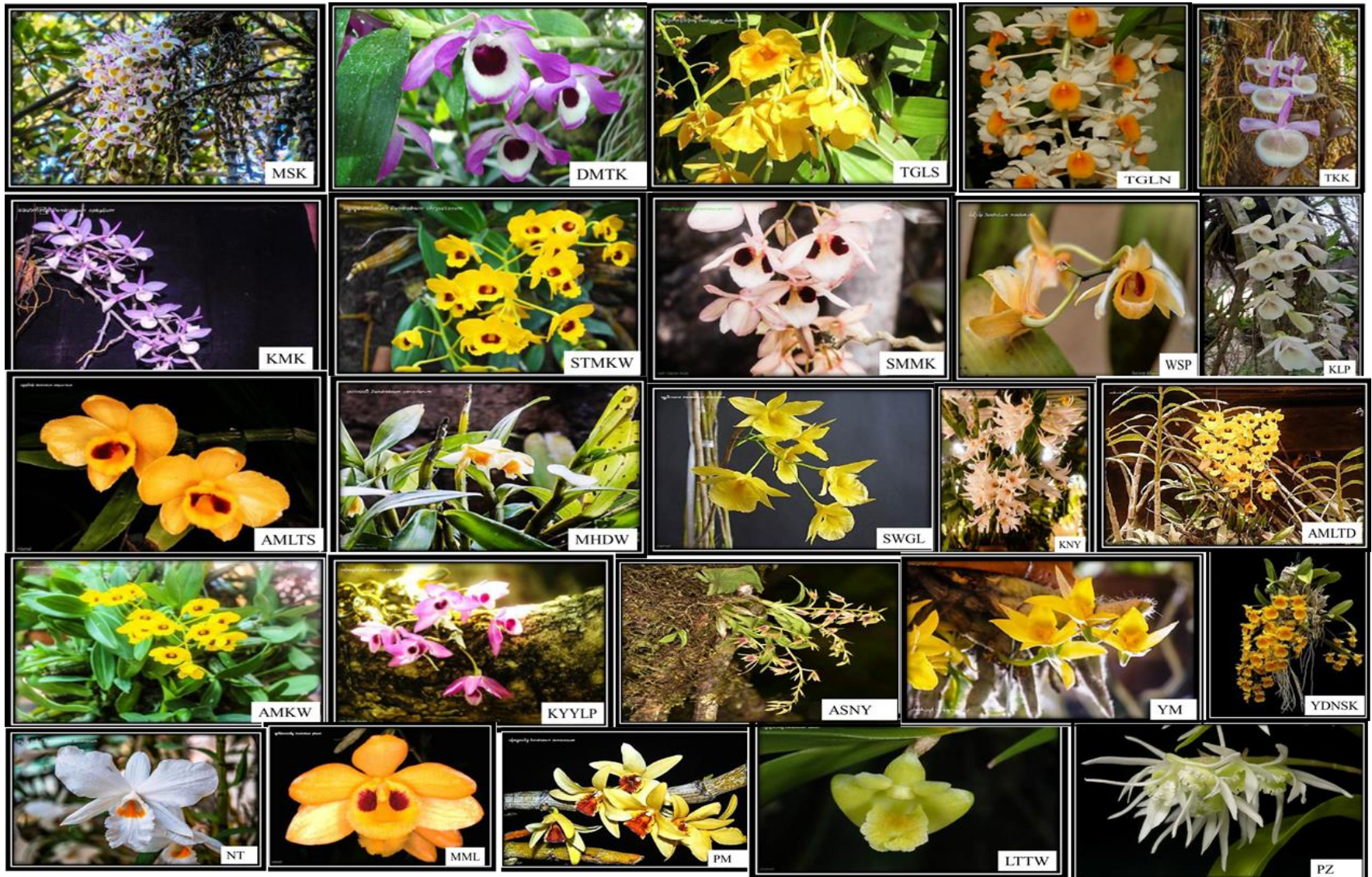
## Table 2. Uses of medicinal orchids

No	Orchid species	Part used and medicinal usage
1.	<i>Dendrobium pendulum</i> (Mya-sit-kyo)	Leaves-Tonic for liver
2.	<i>D. nobile</i> (Daung-myi-thitkwa)	stem-To relief pain and treat stomachache , hasten recovery after an illness
3.	<i>D. densiflorum</i> (Tagunlon-Shwe)	Leaves- boils in Nepal, to help set bones
4.	<i>D. thyrsiflorum</i> (Tagunlon-ngwe)	Leaves- for stomach and promotes production of body fluids
5.	<i>D. primulinum</i> (Thi-kyu-kyu)	Stem-to treat burns, eczema and paralysis
6	<i>D. aphyllum</i> (Kha-mauk-kyo)	stem- Tonic, earache
7	<i>D. crysotoxum</i> (Shwetu/Mout khan war)	Stem/Fruit- Tonic, Flowers - tea in China
8.	<i>D. pulchellum</i> (Sinma-myet-kwin)	Tonic
9.	<i>D. moschatum</i> (Waso-pan)	Leaves- Tonic for the liver, to cure ear pain, Pseudobulbs- to treat fractures and dislocations
10.	<i>D. chrysanthum</i> (Swe Ku Let tan shay Arme)	Tonic
11.	<i>D. cariniferum</i> (Mahar-dewi)	Stem/Fruit- Tonic
12.	<i>D. draconis</i> (Kein-na-yi)	Tonic, to treat fractures and dislocations in Nepal
13.	<i>D. fimbriatum</i> (Swe Ku Let tan do Arme)	Stem-Tonic, to improve eyesight
14.	<i>D. parishii</i> (Ka-yan-yaung-lwin-pyin)	Stem- Tonic, to stop thirst
15	<i>D. Cretaceum lindle</i> (kong lon phyu)	Tonic
16	<i>D. aphrodite</i> (shwe wa galay)	Tonic
17	<i>D. suavissimum</i> (Arme mauk khan wa)	Tonic

# Orchid species



# Fig.1. Flowers of *Dendrobium* species



Adopted from (Worldview International Foundation, final report,2016)

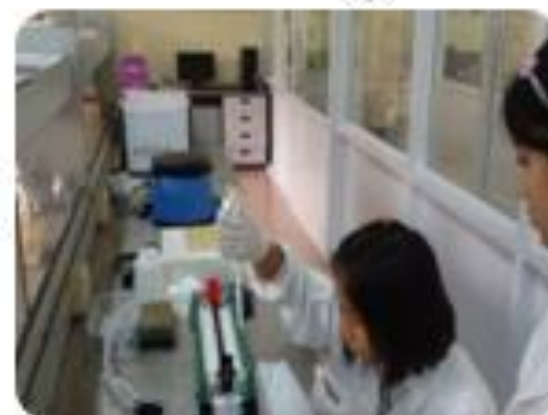
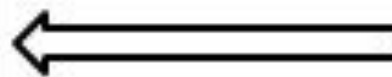
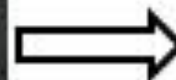
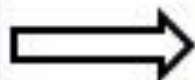
# Table 3 .SSR markers used in this study

No.	Name	Primer sequence (5' to 3')	Motif	Annealing Temp °C	Size (bp)
1	DNeSSR9	F:AAGAGATGGGTTCTCTTTAC R:CAGCTCAAGAACATGACTTTT	(CTT)7	55	89-190
2	DNeSSR24	F:GGACGTGTGCAATATCATAAC R:GGTACAGGTGGTTGTTGAC	(GTG)4 (TGGCCA)3	55	120-245
3	DNeSSR25	F:GCTCAATTGGTGAAATGGT R:CTAGCTCTGATGAGCTTCTTG	(TGA)10	55	95-150
4	DNeSSR27	F:AAAGCGTCAATAAGGGAAC R:AGCAATGGAGCGAAGATAG	(AAG)10	53	224-265
5	DNeSSR58	F:ACTACGCGATCAGGTCTGTG R:CTCTGGTGCTTACTACAATGCT	(CTT)7	55	107-141
6	DNeSSR85	F:TCTGGAGGAAGCGAGAAAGC R:TACAACAGCGTAGCGTCTGC	(TG)6(TGA)4	58	182-247
7	DNeSSR86	F:AAGCAGCGGGAGGAGCAGAA R:ACATCGCAGTCATCAATGTAA	(TC)7	58	237-292
8	DNeSSR92	F:ACTTCTAGGGTTAGGGTTTGTG R:AAGGTCTTGAAACAGGCATC	(CGGCAA)3	58	234-295
9	DNeSSR97	F:GGCTACTGTTCCCGATGCTC R:GGCTGAATGGATACGGTCTT	(TTCA)3	58	166-279
10	DNeSSR99	F:GATTTGAGGGATTGAGATG R:AGTTCATTCAGGGAACCATA	(TGCT)3	53	213-247
11	DNeSSR114	F:TCAACTCCCGAAGAAAAGTC R:GCACATAGTTCCAACAAATAA	(TA)7	55	225-314
12	DNeSSR164	F:CAGGATGCTCTAAGCCAGTA R:AAAGAACAATGAAAGGCAAG	(TC)8	55	112-192
13	DNeSSR166	F:TCCGTGTCCTTGTCTTAC R:TCTTCCCTTTTCTTCT	(AG)8	53	143-237
14	DNeSSR170	F:AGAAGCTCGCTGTCTCCATC R:TGAAGTTGCTGCTGTTGTTGT	(GCC)4-(CAA)4	55	170-300
15	DNeSSR178	F:TGGATACAGTTCCCATCACCT R:AACATACTTCCCAATACCAACG	(CGG)5	57	200-400

\*adopted from Lu et al., 2014

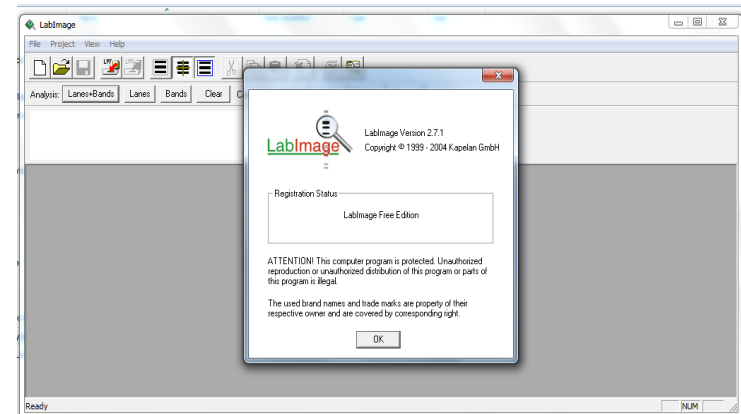
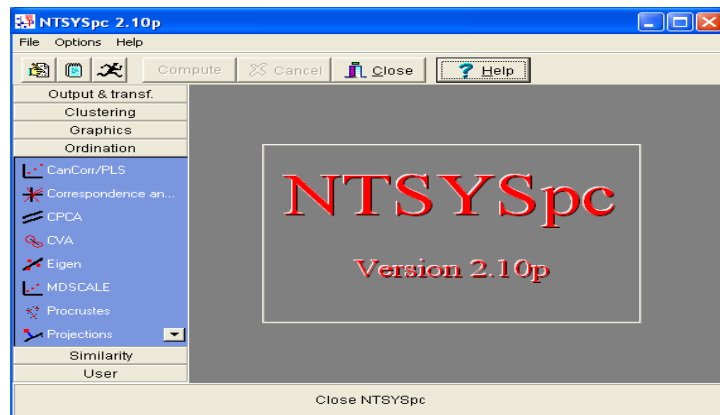
# Sampling, DNA Extraction and Genotyping

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# Analysis

- LabImage2.7 software programme(to estimate the size of products)
- scored as 1 (present) or 0 (absent) for data analysis
- NTSYS Pc.2.1



# Results and Discussion

Table 4. Primers, amplification conditions and polymorphism of SSR markers used among 25 *Dendrobium species*.

No.	Primer	No. of loci	No. of polymorphic loci	Polymorphic loci (%)	PIC
1	DNeSSR9	28	18	64.29	0.926
2	DNeSSR24	27	21	77.78	0.943
3	DNeSSR25	23	20	86.96	0.945
4	DNeSSR27	6	6	100	0.833
5	DNeSSR58	19	12	63.16	0.898
6	DNeSSR85	19	16	84.21	0.931
7	DNeSSR86	20	15	75	0.916
8	DNeSSR92	20	13	65	0.905
9	DNeSSR97	23	10	43.48	0.880
10	DNeSSR99	15	11	73.33	0.889
11	DNeSSR114	20	19	95	0.939
12	DNeSSR164	19	16	84.21	0.920
13	DNeSSR166	20	14	70	0.917
14	DNeSSR170	19	13	68.42	0.900
15	DNeSSR178	18	8	44.44	0.805
Mean		<b>19.73</b>	<b>14.13</b>	<b>73.02</b>	<b>0.903</b>
Total		296	212		

(Bostein et al., 1980)

# Banding pattern information

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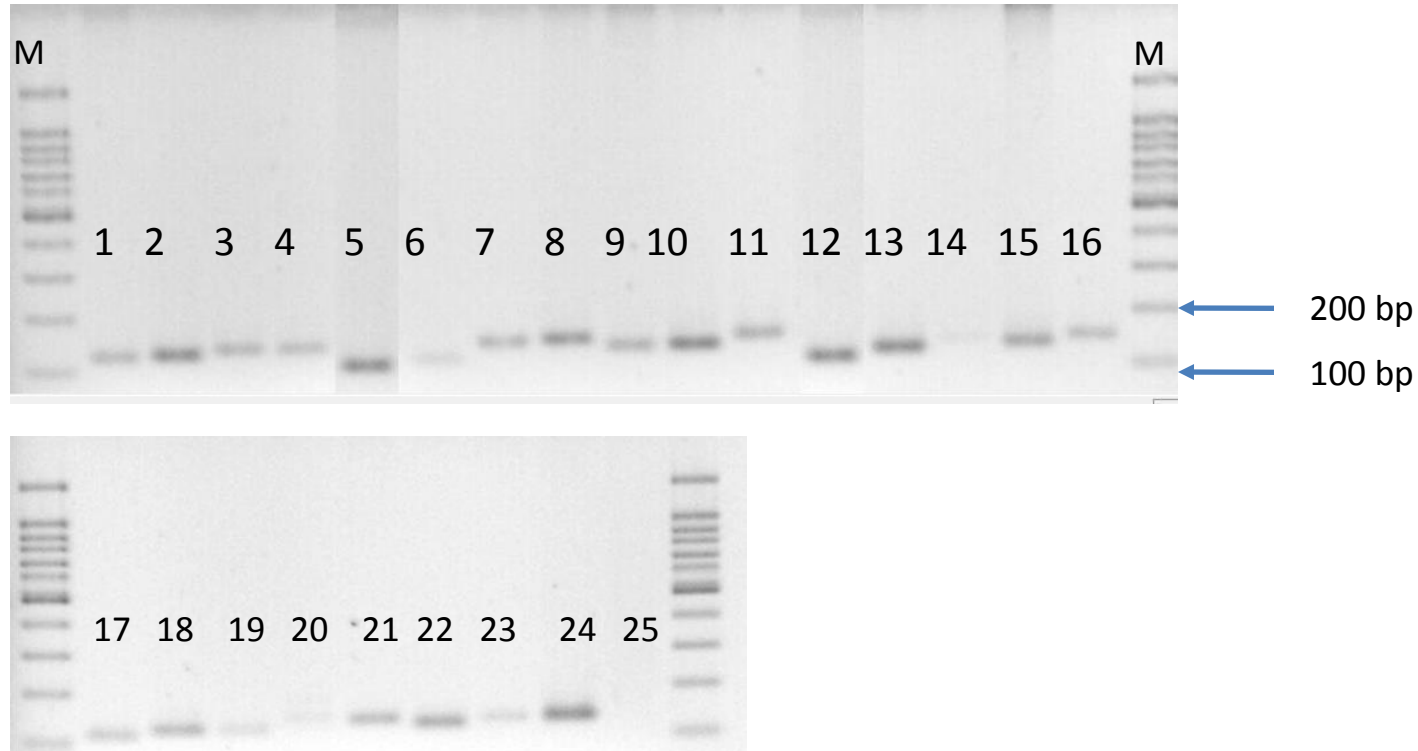
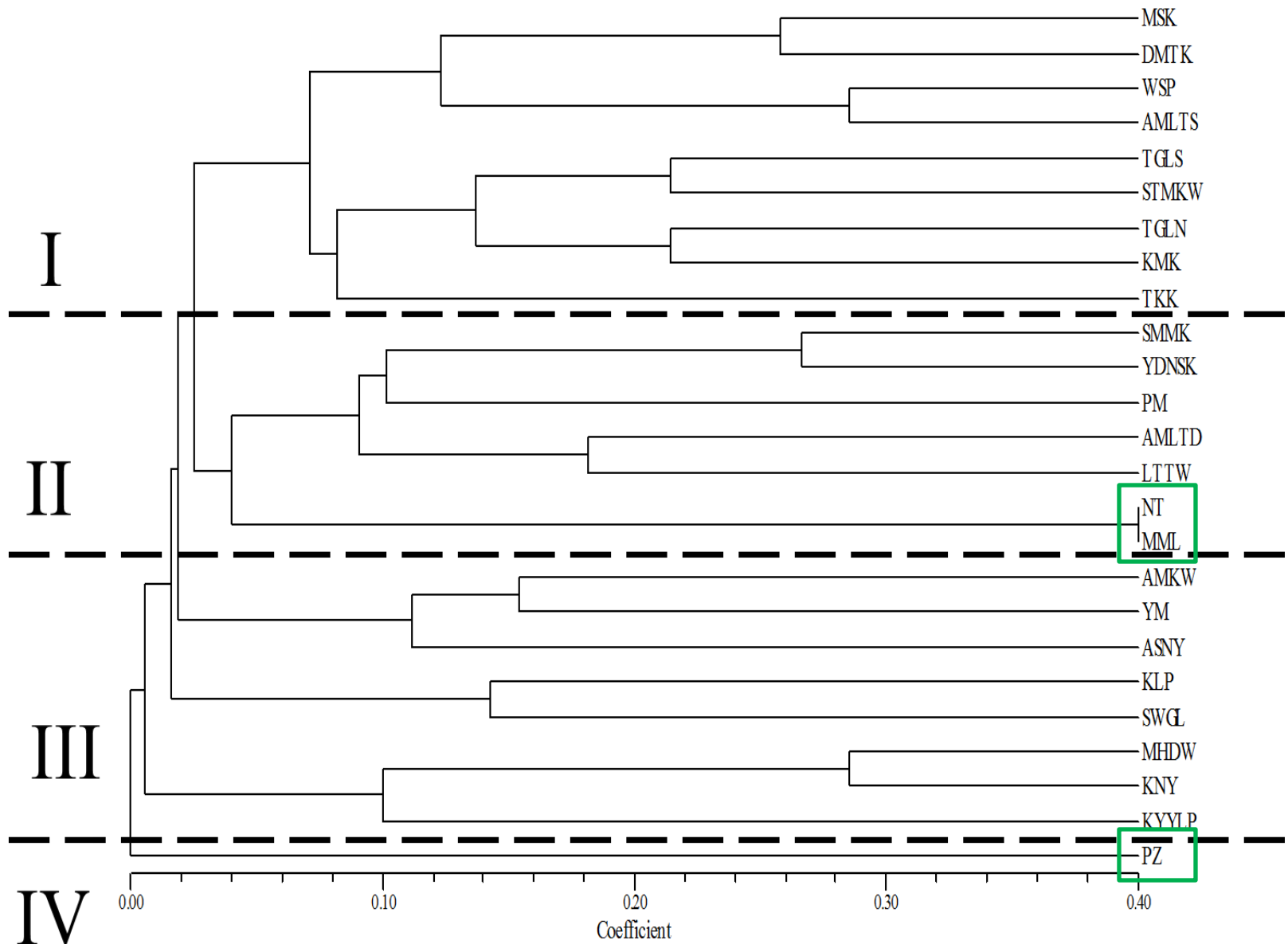


Fig. 2. Agarose gel photographs of the PCR products amplified by DNeSSR25 primer. M: DNA molecular standards with lengths (bp) as shown at the right side. Lanes 1–25: genomic DNA samples from 25 *Dendrobium* species (1–25) as listed in Table 2.

# Fig. 2. Genetic Relationships among species



# Summary

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- Our study demonstrated that SSR markers have been successfully used to fingerprint and assess the extent of genetic variation among *Dendrobium* species.
- Our current work provided useful genetic information about *Dendrobium* species, which will be useful for germplasm management and for breeding in supporting the selection of parental genotypes.
- Extensive research on morphology and molecular characterization of more orchid species from different regions is necessary to be able to find out more genetic information in detail.

# Acknowledgement





**Thank You For Your Attention**