Genetic relationships of 62 *Dendrobium* species base on RAPD analysis

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ABSTRACT

The objective of this study was used to investigate the genetic relationships of Native orchid (*Dendrobium*) base on Randomly Amplified Polymorphic DNA (RAPD) analysis. The sixty-two sample of *Dendrobium* species were collected from Sakon Nakhon, Nong Khai, Nakhon Phanom and Mugdahan province, Thailand. Orchids in *Dendrobium* species were very similar and can't be separated by the morphology appearance. The RAPD technique was used to determine the genetic relationships of them. The data was analyzed by Quality one (BioRad, USA) program. Then DNA fingerprints were analyzed by N-TSYSpc2.1 and produced phylogenetic trees. The results showed that RAPD technique can be used to identify all of samples. Six primers; M13F, S7, S18, S36, S39, and S55 could generate different bands of all samples and got 2,555 bands. The phylogenetic tree can showed the genetic relationships of all sample.

Keywords: Native Orchid, *Dendrobium* species, Randomly Amplified Polymorphic DNA (RAPD), genetic relationships.

INTRODUCTION

In Thailand, *Dendrobium* is the largest orchid genus with more than 150 native species classified into 14 sections (Seidenfaden. 1985). It is also one of the most popular orchids for commercial production with the drastic increase of demand for cut flower and pot plants over the years. Several of native *Dendrobium* species were morphologically similarity, thereby identify them through vegetative anatomy very difficult except during flowering period. Identification of *Dendrobium* species becomes necessary for sustainable use and conservation of the plant genetic resources. The development of a simple and reliable approach to identify these plant species is therefore needed. A number of investigations have shown genetic polymorphism in many plants by the use of various fingerprinting techniques such as isozymes and RFLP, AFLP, SSRs markers or RAPD techniques (Peyachokragul *et al*. 2014; Khosravi. 2009; Zhu1 and Li. 2011; Chattopadhyay *et al*. 2012). RAPD developed by Williams *et al* (1990) can reveal high levels of polymorphism and has the advantages of speed, low cost and requirement for only minute amounts of plant

materials. This method has been successfully used in identification of plants, insect and fungi (Govarthanan *et al*. 2011; González *et al*. 2007; Aufave-Brown *et al*. 1992), and can be applied for differentiation of orchid (Antony *et al*. 2012; Pinheiro. 2012). The aims of this study were investigated of DNA fingerprint and genetic relationships of 62 *Dendrobium* species collecting from Sakon Nakhon and neighboring provinces, Thailand.

MATERIALS AND METHOD

The chemicals used for DNA extraction consists of CTAB-analysis buffer, polyvinylpyrolidone (AppliChem), citric acid monohydrate (MERCK), phenol: chloroform Kit (Thermo Sciencetific), chloroform (BDH), iso amyl alcohol (Ajax Finechem Pty Ltd.), 2-mercaptoethanol (Sigma-Aldrich), 2-propanol (Schalau).

The chemicals used for PCR including a 10X buffer, $MgCl_2$, Taq polymerase 10 mM, dNTP (Vivantis), Primer (Biodesign), bromophenol blue, Xylene, glycerol (Ajax Finechem Pty Ltd.), tris Hydrocloride, ethidium bromide, agarose powder (Vivantis), boric acid (MERCK), EDTA disodium salt (BDH), 1kb Molecular ruler (BIO-RAD).

Sixty-two of *Dendrobium* species was selected from Sakon Nakhon province, and Thai-Laos territory trading at Pak-Khat and Rattanawapi districts of Nong Khai provinces, Tha Uthen district of Nakhon Phanom province and, Mungdahan province, Thailand during 2007-2010. They were planted in faculty of Natural resources, Rajamangala University of Technology I-san Sakon Nakhon Campus. Morphology (leaf, stem and flower) was recorded. Flower is the best characteristic that used to identify them (Fig. 1) but almost of native orchid was flowering once for year. The RAPD were investigated to identify and got relationships of the collecting *Dendrobium* species in this study. Genomic DNA extraction of the sample was performed by using a modified CTAB (Doyle and Doyle (1987) refer to Padmalatha *et al*. 2006). Fresh leaf samples were collected from inner leaf for genomic DNA extraction.

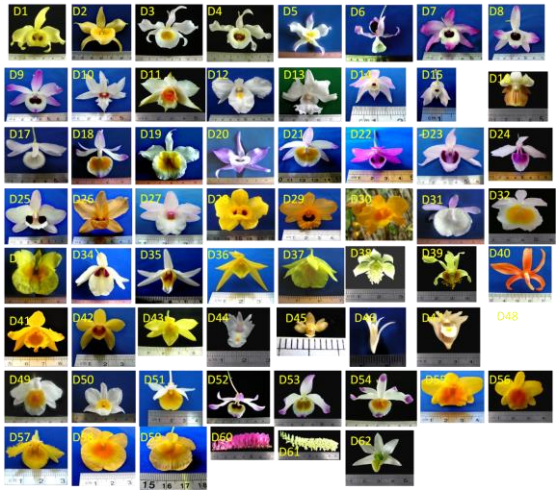

Fig. 1 Characteristic of 62 *Dendrobium* species flower of orchid in this study.

For RAPD, 120 primers synthesis from Biodesign Co.,Ltd, Thailand were used to screen for polymorphisms. Polymerase chain reaction (PCR) amplifications were performed using a Life Express Thermal Cycler Model TC-96/G/H(b), with reactions consisting of 3 min initial denaturation at 95 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 36 °C for 15 s and extension at 72 °C for 15 s, and one final extension step of 72 °C for 5 min. RAPD amplification products were visualized under ultraviolet light using a Gel DocTH XR imaging system (BIO-RAD, USA).

RAPD bands were individually scored and statistically analyzed by following the assumption that fragment size as a locus was considered as either present (1) or absent (0) and finally constructed a binary matrix. Only fragments that had a molecular weight greater than 50 bps and high intensity were

considered for data analysis. The Dice's similarity coefficient matrix was calculated using NTSYSpc package (Version 2.1) (Rohlf, 2000). The percent polymorphism was calculated as the ratio of total number of polymorphic bands.

RESULTS AND DISCUSSION

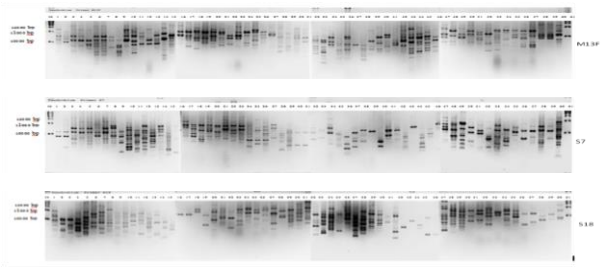

Fig. 2 Random Amplified polymorphic DNA (RAPD) fingerprints of *Dendrobium* species using M13F, S7 and S18 primers. DNA aliquots from 62 *Dendrobium* species (Lends 1-62).

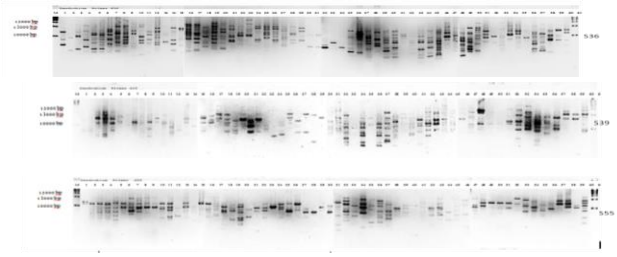

Fig. 3 Random Amplified polymorphic DNA (RAPD) fingerprints of *Dendrobium sp*. using S36, S39 and S55 primers. DNA aliquots from 62 *Dendrobium* species (Lends 1-62). 1= *Dendrobium friedericksianum* Rchb.f.,

The DNA of 62 *Dendrobium* species were extracted by modified CTAB protocol described by Doyle and Doyle (1987) refer to Padmalatha *et al*. 2006. Some of DNA samples were not clean enough for PCR process, the citric acid was added into the extraction buffer. And then, washed with phenol:iso amyl alcohol:chloroform (24:1:24). The cleaning DNA was used for Polymerase Chain Reaction (PCR). Cool conditions were needed all time of DNA extraction. DNA samples were evaluated to amplify by 120 primer from Biodesign CO.,Ltd. Six primers (M13F, S7, S18, S36, S39 and S55) gave clearly bands in all samples. The sequences of 6 primers show in Table 1.

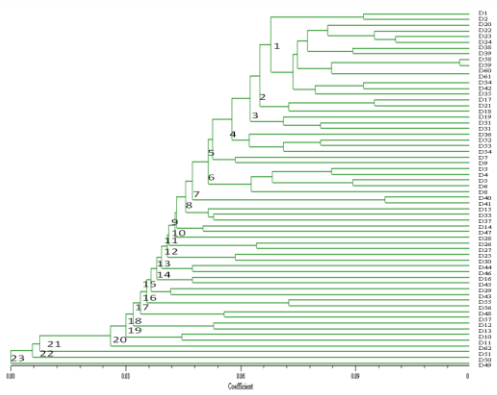

Fig. 4. Dendrogram from RAPD profile, showing the genetic relationships among 62 *Dendrobium* species (r=0.80458)

RAPD profile and analysis, the 6 selected primers that used in this study was generated 2,555 recordable bands. The RAPD bands were calculated by Quanlity One (BioRad, USA). The polymorphic of all samples were generated unique pattern of the samples (Fig. 2-3). The genetic relationships of 62 samples were showed dendrogram in Fig. 4. According to the similarity matrix of samples were grouped into twenty-three clusters (1 to 23) with a similarity level of 0.80 (Fig. 4). For example, cluster I consisted of 15 samples

Table 1 List of 6 primers were selected and used in this study.

Primer	Sequence				
M13F	TGT	AAA	ACG	GCC	ACT
S7	GGT	GAC	GCA	G	
S18	CCA	CAG	CAG	T	
S36	AGC	CAG	CGA	A	
S39	CAA	CGT	TCG	G	
S55	CAT	CCG	TGC	T	

Table 2 List of scientific name of 62 *Dendrobium* species in this study

No	scientific name	No.	scientific name
1	*Dendrobium friedericksianum* Rchb.f.	2	*D. Friedericksianum* Rchb.f.
3	*D. signatum* Rchb.f.	4	*D. signatum* Rchb.f.
5	*D. nobile* Lindl.	6	*D. tortile* Lindl.
7	*D. nobile* Lindl.	8	*D.nobile* Lindl.
9	*D. nobile* Lindl.	10	*D. Draconis* Rchb.f.
11	*D. Cariniferum* Rchb.f.	12	*Formosum* Roxb.ex Lindl.
13	*D. virgineum* Rchb.f.	14	*Hercoglossum* Rchb.f.
15	*D. linguella* Rchb.f.,	16	*D. Ellipsophyllum* Tang&Wang.
17	*D. aphyllum*(Roxb)	18	*D. Crystallinum* Rchb.f.
19	*D. Crystallinum* Rchb.f.	20	*D. lituiflorum* Lindl.
21	*Gratiossimum* Rchb.f.	22	*D. parishii* Rchb.f.
23	*D. anosmum* Lindl.	24	*D. anosmum* Lindl.
25	*D. puchellum* Lindl	26	*D. moschatum*(Buch.-Ham.) Sw.
27	*D. cumulatum* Lindl.	28	*D. Chrysanthum* Lindl.
29	*D. Fimbriatum* Hook.	30	*D. Fimbriatum* Hook.
31	*D. primulinum* Lindl.	32	*D. primulinum* Lindl.
33	*D. Capillipes* Rchb.f.	34	*D. Albosanguinium* Lindl.
35	*D. Heterocarpum* Lindl.	36	*D. Trigonopus* Rchb.f.
37	*D. Dixanthum* Rchb.f.	38	*D. Delacourii*.
39	*D. venutum* Teijsm.&Binnend.	40	*D. Unicum* Seidenf.
41	*D. Henryi* Schltr.	42	*D. Sulcatum* Lindl.
43	*D. Senile* Par.&Rchb.f.	44	*D. acerosum.*
45	*D. Keithii* Ridl.	46	*D. Parcum* Rchb.f.
47	*D. Pachyphyllum* (Kze.) Bakh.f.,	48	*Dendrobium pachyphyllum* (Kze.) Bakh.f.
49	*D. Farmeri* Paxt.	50	*D. Palpebrae*
51	*D. Thyrsiflorum* Rchb.f.	52	*D. Findlayanum* Par.&Rchb.f.
53	*D. Findlayanum* Par.&Rchb.f.,	54	*D. Pendulum* Roxb.
55	*D. Chrysotoxum* Lindl.	56	*D. Chrysotoxum* Lindl.
57	*D. Harveyanum* Rchb.f.	58	*D. Lindleyi* Steud.
59	*D. Jenkensii* Wall.ex lindl.	60	*D. secundum* (Bl.) Lindl.
61	*D. secundum* “Alba”,	62	*D. Scarbrilingue* Lindl.

and was further divided into five subclusters

with a similarity level of 0.87 (Fig. 4).

CONCLUSION

From this study, we were collected 62 *Dendrobium* species from Sakon Nakhon, Nong Khai, Nakhon Phanom and Mugdahan province, Thailand. Flower is the best characteristic that used to identify them but almost of native orchid was flowering once for year. The RAPD technique was performed for analyzed genetic relationships. The result showed that RAPD technique were identified 62 of *Dendrobium* species by using 6 primers (M13F, S7, S18, S36, S39 and S55) and gave clearly bands of all samples. Example, *D. secundum*. and *D. secundum* "Alba" were vary similarity on morphology only flower color was difference and the relationships showed similarity level more than 92%. However, the DNA fingerprints were found that the fingerprint showed the difference patterns on 5 primers from 6 primers. So that, other *Dendrobium* species were less closely relationshohip than these. Thus, the polymorphic will be generated unique pattern and clearly to separate them.

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