Kasetsart J. (Nat. Sci.) 40 : 456 - 461 (2006)

Analysis of Intersectional Hybrids of *Dendrobium* by RAPD Technique

Sirilak Inthawong[1]*, Weenun Bundithya[2], Nuttha Kuanprasert [2] and Pimchai Apavatjrut[2]

ABSTRACT

Thirty interspecific hybridizations of inter- or intra-section of *Dendrobium* were made. Five crosses showed good compatibility and could yield hybrids as follows: 2 intersectional hybridizations, 1) section *Phalaenanthe*: *Den. phalaenopsis* (D017) × section *Formosae*: *Den. draconis* (D022) and 2) section *Dendrobium*: *Den. finlayanum* (D030) × section *Formosae*: *Den. cariniferum* (D018); and 3 intrasectional hybridizations, 1) section *Dendrobium*: D030 × *Den. nobile* (D031), 2) section *Formosae*: *Den. trigonopus* (D037) × D022, and 3) D037 × *Den. infundibulum* (D034). The greatest number of plantlets (1,250 plantlets) was obtained from cross D037 × D022 whereas the least number (3 plantlets) was found in cross D037 × D034. After transplanting for 6 months, survival rate of D037 × D034 was 100% whereas no survival plant was found in cross D030 × D018.

Parents and hybrids of 5 crosses were analyzed by RAPD using 21 decamer primers and the number of primers generating polymorphic patterns of each cross varied. RAPD markers were able to differentiate parents in inter- and intra-sectional crosses and these markers could confirm the interspecific hybrids.

Key words: *Dendrobium*, interspecific hybridization, RAPD technique

INTRODUCTION

Dendrobium is one of the largest genera in the family Orchidaceae. It comprises over 1,000 species distributed from the foothills of the Himalayas through Southeast Asia to Japan, Australia and the Pacific Islands. Botanists divided this genus into 41 sections (Baker *et al*., 1996).

About 80% of world's dendrobium production is from Thailand. Commercial growers put major efforts on breeding for novel *Dendrobiums* with desirable traits. Traditional breeding is limited by time and cross-incompatibility among inter- and intra-section (Chia *et al*., 2001). From an interesting study of sexual compatibility in *Dendrobium*, Wilfret and Kamemoto (1969) made a systematic study utilizing 37 species in 10 of Schlecter (1912) 41 sections for 721 pollinations, 5 intrasectional and 20 intersectional combinations resulted in successful hybrids. By making crosses among 8 horticultural important sections, Kamemoto and Wilfret (1980) could predict obtainable hybrid percentage and observe relationship among those species. Furthermore, Kamemoto (1987) found possible intersectional crosses from 4 sections,

[1] Development of Economic Flower Crop : Orchid (DEFCO) Project, Faculty of Agriculture, Chiang Mai University, Chiangmai 50200, Thailand.

[2] Department of Horticulture, Faculty of Agriculture, Chiang Mai University, Chiangmai 50200, Thailand.

* Corresponding author, e-mail: Jupiter496@yahoo.com

Received date : 16/07/05 Accepted date : 30/01/06

Phalaenanthe, *Ceratobium*, *Eleutheroglossum* and *Latourea*. This finding has given numerous impact on dendrobium breeding program, especially for commercial potted cultivars. However, novel characteristics in terms of shapes, colors, growth and yields are still desirable. Several Thai *Dendrobium* orchid species in sections *Callista*, *Dendrobium* and *Formosae* show some unique characteristics for example sweet scent of *Den. scabrilinque*, glossy and fragrant flower of *Den. trigonopus*, bright orange color of *Den. unicum* and compact plant and attractive lip of *Den. cruentum*. They are good sources for such special characters that can possibly be introduced to new commercial hybrids.

The development of molecular markers for identification of genotypes and early detection of inter-specific hybrids may be important for breeding programs and in protecting plant breeders' rights (Benedetti *et al*., 1998). RAPD (Random Amplified Polymorphic DNA) technique provides a useful tool for breeding applications. This technique followed a simple and fast procedure, required a low quantity of DNA and was easily automated (Ballester and Vicent, 1998).

In some recent publications, RAPD technique has been used widely for identification of interspecific hybrids in several kinds of plants, such as *Cattleya* (Benner *et al*., 1995), *Phalaenopsis* (Chen *et al*., 2001), *Anthurium* (Ranamukhaarachchi *et al*., 2001), *Lilium* (Wiejacha *et al*., 2001), asparagus (Caporali *et al*., 1996), pepper (Balleter and Vicente, 1998), carrot (Grzebelus *et al*., 2001), blueberry (Levi and Rowland, 1997), almond (Bartolozzi *et al*., 1998) and pear (Sharifani and Jackson, 2000).

In this study, RAPD technique was used to trace some markers among inter- or intra-sectional hybrids of *Dendrobium* and their parental lines.

MATERIALS AND METHODS

Plant materials

Eleven species of Thai *Dendrobium* orchid were obtained from 4 sections, 1) section *Callista*: *Den. chrysotoxum* (D026), 2) section *Dendrobium*: *Den. crystallinum* (D044), *Den. findlayanum* (D030), *Den. nobile* (D031) and *Den. unicum* (D015), 3) section *Formosae*: *Den. cariniferum* (D018), *Den. draconis* (D022), *Den. infundibulum* (D034), *Den. scabrilinque* (D012) and *Den. trigonopus* (D037) and 4) section *Phalaenanthe*: *Den. phalaenopsis* (D017).

Testing for cross compatibility

Thirty interspecific hybridizations of inter- or intra-section were studied. The result of cross compatibility and number of capsules were recorded. Three months after pollination, capsule of each cross was harvested and then germinated *in vitro* using Vacin and Went (1949) agar medium. A number of plantlets and survival rate after 6-month transplanting were recorded.

RAPD analysis

DNA was extracted from young leaves of 5 selected hybrids and their parents using a 2x CTAB (hexadecyltrimethyl ammonium bromide) procedure (Doyle and Doyle, 1990). Polymerase chain reaction (PCR) was carried out in a 20 μl reaction mixture containing 5 ng of DNA template, 1x PCR buffer (20mM Tris-HCl pH 8.0, 0.1mM EDTA, 1mM DTT, 50% glycerol), 1.5 mM $MgCl_2$, 100 μM dNTPs, 100 ng primer, 0.8 unit *Taq* DNA polymerase and dH_2O. Twenty-one decamer primers, OPF01-20 and OPD03 (Operon Technologies Inc.) were used for PCR amplification. The DNA was amplified in the Perkin Elmer Gene Amp PCR System 2400 (Perkin-Elmer Cetus Co.). The PCR program was modified from Chen *et al*. (1998) as follows: 2 cycles of 94 °C 60 s, 36 °C 10 s and 72 °C 70 s; 30 cycles of 94 °C 60 s, 42 °C 45 s and 72 °C 70 s;

and 1 cycle at 72 °C for 240 s. The PCR products were kept at 4 °C prior to analysis.

The PCR products were separated by electrophoresis on 1.8% agarose gels in 1x TBE buffer. The amplified DNA bands were stained in 0.1 μg/ml ethidium bromide and then photographed under UV light using Gel Documentation (Lab Focus. Co., Ltd.).

RESULTS

Testing for cross compatibility

Thirty interspecific hybridizations, 21 intersectional and 9 intrasectional crosses were made. Compatibility was found and capsules were set in the following crosses: 2 intersectional hybridizations (9.52%), 1) section *Phalaenanthe*: *Den. phalaenopsis* (D017) × section *Formosae*: *Den. draconis* (D022) and 2) section *Dendrobium*: *Den. findlayanum* (D030) × section *Formosae*: *Den. cariniferum* (D018) and 3 intrasectional hybridizations (33.3%), 1) section *Dendrobium*: D030 × *Den. nobile* (D031), 2) section *Formosae*: *Den. trigonopus* (D037) × D022 and 3) D037 × *Den. infundibulum* (D034). After culturing in modified Vacin and Went (1949)(CMU1) agar medium for 8 months, the greatest number of hybrid seedlings was 1,250 plantlets obtained from D037 × D022 while other crosses, D030 × D031, D017 × D022, D030 × D018, and D037 × D034, showed various numbers of hybrid seedlings, i.e. 600, 125, 100, and 3 plantlets, respectively (Table 1).

Eight-month old hybrid seedlings were transplanted from *in vitro* to shadehouse condition. After six-month period, seedlings of D037 × D034 showed the greatest survival rate (100%) whereas those of crosses D017 × D022, D030 × D031 and D037 × D022 yielded only 55.6, 26.7 and 19.4% survival rate, respectively. Hybrid seedlings of crosses D030 × D018 were not viable (Table 1).

RAPD analysis

The 21 decamer primers were evaluated for amplification of 4 compatible crosses and their hybrids. The number of primers giving polymorphic pattern varied among crosses. Suitable primers for each cross could be described as follows: 7 primers (OPF01, 02, 03, 04, 05, 06 and OPD03) for cross D017 × D022; 6 primers (OPF01, 02, 03, 04, 06 and 20) for cross D037 × D022; 5 primers (OPF01, 02, 04, 05 and 14) for cross D030 × D031; and 5 primers (OPF01, 04, 06, 14 and OPD03) for cross D037 × D034. Selected DNA fingerprints (Figure 1-4) were presented showing polymorphic RAPD markers from either parents that appeared in hybrid banding. Two markers from cross D017 × D022, 1040 bp. and 739 bp., represented those derived from D017 (female parent) and D022 (male parent) respectively (Figure 1). For cross D037 × D022, the 1239 bp. and 1219 bp. from D037 (female

Table 1 Number of seedlings per pod obtained *in vitro*, Number of transplanted seedlings and Number of survival plantlets after 6-month transplanting.

Crosses	No. of seedlings per pod obtained *in vitro*	No. of transplanted seedlings	No. of survival plantlets after 6-month transplanting
D017 × D022	125	18	10
D018 × D030	no viable seedling	-	-
D030 × D018	100	60	0
D030 × D031	600	390	104
D037 × D034	3	3	3
D037 × D022	1,250	350	68
D034 × D022	no viable seedling	-	-

parent) and D022 (male parent), respectively, appeared in hybrids (Figure 2). Three markers were found in cross D030 × D031 of which the 1182 bp. marker was derived from D030 (female parent) while the 831 bp. and 750 bp. derived from D031 (male parent) (Figure 3). Four markers were found in cross D037 × D034 of which the 564 bp., 490 bp. and 273 bp. Markers was derived from D037 (female parent) while the 476 bp. from D034 (male parent) (Figure 4).

DISCUSSION

Testing for cross compatibility

Thirty interspecific hybridizations of inter- or intra-section were done. Two intersectional hybridizations, D017 × D022 and D030 × D018; and 3 intrasectional hybridizations, D030 × D031, D037 × D022 and D037 × D034 showed good compatibility and could yield hybrids but in the other crosses were incompatibility. Wilfret and Kamemoto (1969) reported that failure in producing successful intra- and inter-sectional crosses could show some

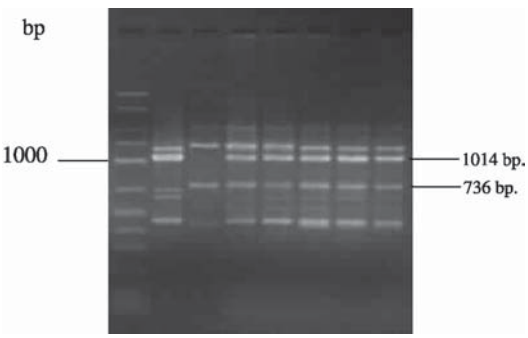

Figure 1 RAPD banding pattern of cross D017 × D022 generated by primer OPF03: M: 50-2500 bp. marker; P1: D017; P2: D022 and 1-5: F_1 hybrids. 1014 and 736 bp. are RAPD markers.

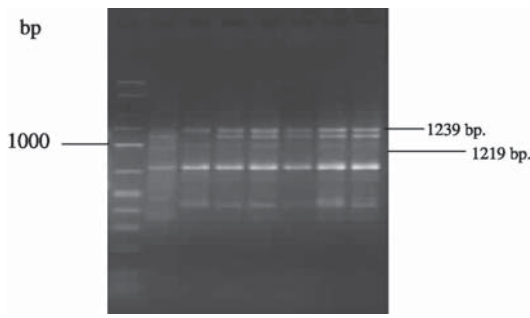

Figure 2 RAPD banding pattern of cross D037 × D022 generated by primer OPF03: M: 50-2500 bp. marker; P1: D037; P2: D022 and 1-5: F_1 hybrids. 1239 and 1219 bp. are RAPD markers.

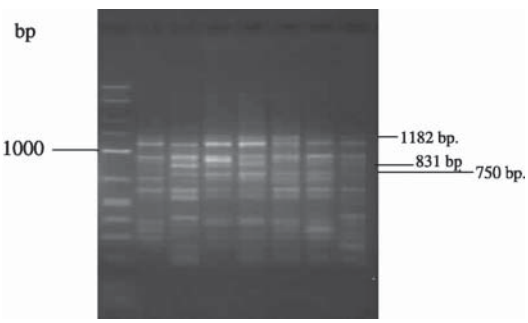

Figure 3 RAPD banding pattern of cross D030 × D031 generated by primer OPF14: M: 50-2500 bp. marker; P1: D037; P2: D022 and 1-5: F_1 hybrids. 1182, 831 and 750 bp. are RAPD markers.

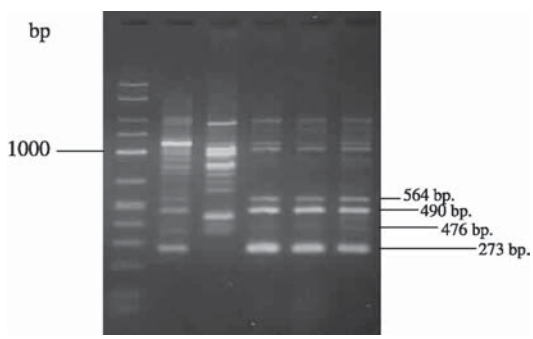

Figure 4 RAPD banding pattern of cross D037 × D034 generated by primer OPF06: M: 50-2500 bp. marker; P1: D037; P2: D034 and 1-3: F_1 hybrids. 564, 490, 476 and 273 bp. are RAPD markers.

degrees of relationship of species, success in obtaining interspecific hybrids usually indicated a close relationship. One of the main problems in making new hybrid cultivar in *Dendrobium* was difficulty of fruit setting. In this study, when reciprocal crosses were made of all compatible crosses, it was found that none of them could produce hybrid. It showed that certain species could be used as only mother plant and could not be used as pollen donor. This is similar to report by Reed *et al.* (2002) in *Clethra* that reciprocal crosses failed to set fruit. Wilfret and Kamemoto (1969) reported that the *Dendrobium* breeding program needs time to test the compatibility of interspecific crosses and study on the factors of incompatible crosses.

RAPD analysis

Confirmation of the interspecific hybrid relied on molecular comparisons of the hybrids with their parents (Hansen, 1998). Twenty-one decamer primers were used for amplification of the hybrids which were obtained in this study and their parents, and the number of primers giving polymorphic patterns varied among crosses. Two of these primers namely OPF01 and OPF04 (data not shown) could produce different DNA fingerprint patterns that enabled differentiation among the parental cultivars of all intrasectional crosses. It indicated that any *Dendrobium* cultivars had a unique genetics (Ranamukhaarachchi *et al*., 2001).

Table 2 List of 9 decamer primers and their sequences giving polymorphic patterns among crosses.

Primer code	Sequence (5′ - 3′)
OPF01	ACGGATCCTG
OPF02	GAGGATCCCT
OPF03	CCTGATCACC
OPF04	GGTGATCAGG
OPF05	CCGAATTCCC
OPF06	GGGAATTCCC
OPF14	TGCTGCAGGT
OPF20	GGTCTAGAGG
OPD03	GTCGCCGTCA

It was able to prove that the hybrids were the result of genetic combination of their parents. RAPD bandings of hybrids when using suitable primers for each cross, clearly indicated that the DNA came from the 2 parents by the appearance of RAPD markers i.e. $OPF03_{736,\ 1040}$ in cross D017 × D022, $OPF03_{1219,\ 1239}$ in cross D037 × D022, $OPF14_{750,\ 831,\ 1182}$ in cross D030 × D031 and $OPF06_{273,\ 476,\ 490,\ 564}$ in cross D037 × D034. The same results were also reported in the other orchids, such as *Cattleya* by Benner *et al*. (1995) and *Phalaenopsis* by Chen *et al*. (2001).

The establishment of polymorphic bands in the parental genotypes of *Dendrobium* was relatively easy, although not all of the markers were useful for the detection of hybrids. Selected bands could be used for further hybridizations. Due to high heterozygosity of the cultivars, some polymorphic bands could not be used for hybrid identification (Wiejacha *et al*., 2001).

In summary, RAPD marker could be used for identification of *Dendrobium* cultivars as shown by different DNA fingerprinting from each plant. These polymorphisms could be used for detection of inter- or intra-sectional hybrid.

ACKNOWLEDGEMENTS

This work was financially supported by the Development of Economic Flower Crop : Orchid (DEFCO) Project, Faculty of Agriculture, Chiang Mai University. We thank for the use of equipments from ‘ H.R.H. Princess Maha Chakri Sirindhorn Plant Germplasm Project’ (RSPG Project), Chiang Mai, Thailand.

LITERATURE CITED

Baker, L. M. and C. O. Baker. 1996. **Orchid Species Culture *Dendrobium***. Timber Press,

Portland, Oregon. 852 p.

Ballester, J. and M. C. de Vicent. 1998. Determination of F_1 hybrid seed purity in pepper using PCR-based markers. **Euphytica** 103: 223-226.

Bartolozzi, F., M. L. Warburton, S. Arulsekar and T. M. Gradiziel. 1998. Genetic characterization and relatedness among California almond cultivars and breeding lines detected by random amplified polymorphic DNA (RAPD) analysis. **J. Amer. Soc. Hort. Sci.** 123: 381-387.

Benedetti, L. D., G. Burchi, A. Mercuri and T. Schiva. 1998. Use of RAPD analysis for genotype identification in *Alstroemeria*. **Acta Hort.** 461: 277-279.

Benner, M. S., M. D. Braunstein and M. U. Weisberg. 1995. Detection of DNA polymorphisms within the genus *Cattleya* (Orchidaceae). **Plant Mol. Biol. Sci.** 13: 147-155.

Caporali, E., A. Carboni, A. Spada, G. P. Marziani Longo, R. Biffi, F. Restivo and F. Tassi. 1996. Construction of a linkage map in *Asparagus officinalis* through RFLP and RAPD analysis. **Acta Hort.** 415: 435-440.

Chen, W. H., T. M. Chen., Y. M. Fu, R. M. Hsieh and W. S. Chen. 1998. Studies on somaclonal variation in *Phalaenopsis.* **Plant Cell Rep.** 18: 7-13.

Chen, W. H., Y. M. Fu, W. T. Tsai, M. S. Chyou, R. M. Hsieh, C. C. Wu and W. S. Lin. 2001. Identification and phylogenetic relationship of *Phalaenopsis* species *via* molecular analysis. **Taiwan Sugar Res.** 48: 23-39.

Chia, T. F., A. Y. H. Lim, Y. Luan and I. Ng. 2001. Transgenic *Dendrobium* (Orchid). **Biotechnol. in Agri. and Forestry** 48: 95-106.

Doyle, L. J. and J. J. Doyle. 1990. Isolation of plant DNA from fresh tissue. **Focus** 12: 13-14.

Grzebelus, D., R. Baranski, B. Michalik and P. W. Simon. 2001. Comparison of RAPD and AFLP techniques used for the evolution of genetic diversity of carrot breeding materials. **Acta Hort** 546: 413-416.

Hansen, L. N. 1998. Intertribal somatic hybridization between rapid cycling *Brassica oleracea* (L.) and *Camelina sativa* (L.) Crantz. **Euphytica** 104: 173-179.

Kamemoto, H. 1987. **The Breeding of Horticultural Crop**. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei.

Kamemoto, H. and G. J. Wilfret. 1980. Inter- and multi- sectional *Dendrobium* hybrids, pp. 255-261. *In* **Proc. of the 9th World Orchid Conf.,** Bangkok.

Levi, A. and L. J. Rowland. 1997. Identificating blueberry cultivars and evaluating their genetic relationships using random amplified polymorphic DNA (RAPD) and simple sequence repeat- (SSR-) anchored primers. **J. Amer. Soc. Hort. Sci.** 122: 74-78.

Ranamukhaarachchi, D. G., R. J. Henny, C. L. Guy and Q. B. Li. 2001. DNA fingerprinting to identify nine *Anthurium* pot plant cultivars and examine their relationship. **HortScience** 36: 758-760.

Reed, S. M., Y. Joung and M. Roh. 2002. Interspecific hybridization in *Clethra*. **HortScience** 37: 393-397.

Sharifani, M. M. and J. F. Jackson. 2000. Characterization of pear species and cultivars using RAPD primers. **Acta Hort.** 538: 499-504.

Wiejacha, K., A. Marasek, I. Sabala and T. Orlikowska. 2001. Molecular markers in detection of distant hybrids in *Lilium*. **Acta Hort.** 546: 281-285.

Wilfret, G. J. and H. Kamemoto. 1969. Genome and karyotype relationships in the genus *Dendrobium* (Orchidaceae), pp. 18-47. *In* H. Kamemoto, T. D. Amore and A. R. Kuehnle (eds.). **Breeding Dendrobium Orchids in Hawaii.** University of Hawaii Press, Hawaii.