PSU PRINCE OF SONGKLA UNIVERSITY

Songklanakarin J. Sci. Technol.
42 (5), 1065-1070, Sep. - Oct. 2020

SJST
http://www.sjst.psu.ac.th

Original Article

Population analysis of *Epipactis flava* Seidenf. in Thailand using SRAP and RAPD markers

Waroon Suwankitti, Sirinat Wankaew, Boonsita La-ongdet, Surin Peyachoknagul, Somjit Homchan, Anupan Kongbangkerd, and Maliwan Nakkuntod*

Department of Biology, Faculty of Science,
Naresuan University, Mueang, Phitsanulok, 65000 Thailand

Received: 6 March 2019; Accepted: 9 July 2019

Abstract

Epipactis flava Seidenf. is the only rheophytic orchid found in Thailand. Specific habitat requirements and environmental changes of the growing area have now resulted in *E. flava* becoming an endangered species and appropriate management protocol is essential. Genetic diversity and population structure of *E. flava* were evaluated using RAPD and SRAP markers. Nine RAPD primers were selected with 95.93% polymorphism and eight SRAP primer combinations showing 92.29% polymorphism. Nei's genetic diversity (*H*) within populations from RAPD and SRAP was 0.2303 and 0.2016 and the Shannon diversity index (*I*) was 0.3380 and 0.2970, respectively. Among populations, *H* (0.3450 and 0.3237) and *I* (0.5156 and 0.4852) were also surveyed. Overall genetic diversity between and within populations was high. Results suggest that *in situ* conservation together with the introduction of plants from different locations is recommended to increase genetic variation.

Keywords: *Epipactis flava*, rheophytic orchid, DNA marker, diversity, population

1. Introduction

Orchidaceae is the largest family of flowering plants containing approximately 25,000 species (Cribb, Dixon, Kell, & Barrett, 2003; Dressler, 1993). However, plant numbers have decreased as a result of over-collection for ornamental or medicinal purposes (Qian, Wang, & Tian, 2013) and through deforestation. Orchids are classified into five types by habitat as terrestrial, lithophytic, epiphytic, saprophytic and rheophytic (Pedersen, Watthana, & Srimuang, 2013; Pridgeon, Cribb, Chase, & Rasmussen, 1999). Rheophytic orchids are rare because they require a specific environment of fast-flowing streams and/or a flooding period of 4-5 months to complete their life cycle (Pedersen *et al.*, 2013). In Thailand, *Epipactis flava* is found in limestone areas along fast-flowing streams or waterfalls in Tak, Kanchanaburi and Nan Provinces (Pedersen *et al.*, 2013). During the dry season from October to January the water level drops and *E. flava* produces vegetative growth as stems and leaves, while from February to March its flowers bloom and produce seeds. During the rainy season from May to September, water levels rise and the leaves and flowers become detached with only the rhizomes remaining on rocks under the water (Borja *et al.*, 2009; Vermulen & Tsu kaya, 2011).

E. flava Seidenf. is a rheophytic orchid found in Thailand, Laos and Vietnam (Pedersen *et al.*, 2013). Specific habitat requirements and environmental changes of the growing area have resulted in *E. flava* becoming an endangered species and appropriate management protocol is needed. Genetic diversity and population structure of *E. flava* require urgent evaluation. The genetic diversity of this species has not been recently studied. Various types of DNA markers are useful for this purpose because they are not affected by the environment and provide large amounts of information within a short time period. Sequence related amplified polymorphism (SRAP) developed by Li & Quiros (2001) amplifies open reading frames in genomic DNA; and random amplified polymorphic DNA (RAPD; Williams, Kubelik, Livak, Rafal ski, & Tingey, 1990) using short random primers are markers of choice for species that have not been previously studied for

*Corresponding author
Email address: lotharmali@yahoo.com

genetic diversity. Both techniques require no genetic information from samples and results can be attained through a significantly less technically demanding process. Three marker systems were compared in basil (genus *Ocimum*) and results showed the estimates of genetic diversity ranking as SRAP >RAPD >ISSR (Chen *et al.*, 2013). The goal of genetic resource conservation is to maintain high levels of genetic diversity and to develop tools for population study that can be used for future conservation plans.

Here, genetic diversity and population structure of *E. flava* were evaluated in different locations using RAPD and SRAP markers. The results provide useful information for conservation planning of this endangered species.

2. Materials and Methods

2.1 Plant materials and DNA extraction

One hundred and forty-four *E. flava* samples were collected from Ban Huai Tham Suea (HS1-HS45), Mae Sot District, Huai Hin Dang (HD1-HD30), Thee Lo Su Waterfall (TS1-TS15), Pa La Ta Waterfall (PT1-PT25), Umphang District, Tak Province and Takien Thong Waterfall (TT1-TT29), Sangkla Buri District, and Kanchanaburi Province, Thailand (Figure 1). Genomic DNA was extracted from fresh leaves using a modified CTAB method (Doyle & Doyle, 1987) and adjusted to a concentration of 25 ng/µl for the amplification steps.

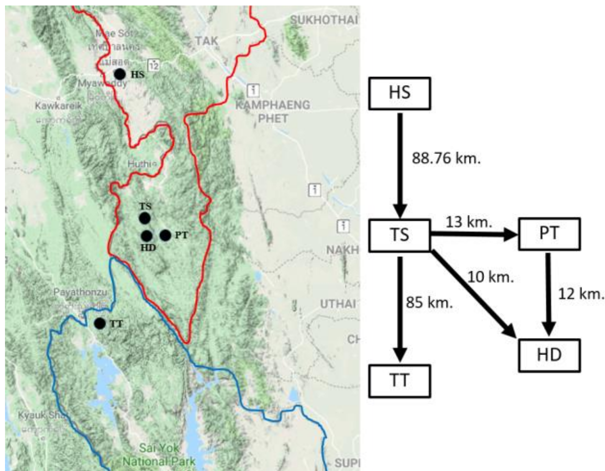

Figure 1. Locations of collected *E. flava* populations.

2.2 RAPD analysis

One hundred and eight RAPD primers were screened using three DNA samples from different populations and nine polymorphic primers were selected for analysis. PCR reactions consisted of 1X PCR buffer (including 3.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.25 µM primer, 25 ng DNA, and 1 unit of *Taq* polymerase (Intron Biotechnology Co., Ltd., South Korea) in a total volume of 20 µl. PCR reaction conditions were initial denaturation at 94ºC for 5 min, 40 cycles of denaturation at 94ºC for 1 min, annealing at 35ºC for 1 min, extension at 72ºC for 2 min and final extension at 72ºC for 5 min. All PCR products were stored at 4ºC until visualized on 1.5% agarose gel prepared in 1X TAE buffer containing 0.5 µg/ml ethidium bromide (EtBr).

2.3 SRAP analysis

Three DNA samples from different populations were screened with 100 SRAP primer combinations (Li & Quiros, 2001) and the polymorphic ones were selected. Eight selected primer pairs were used to analyze all samples. PCR reactions consisted of 1X PCR buffer (including 3.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.25 µM of each forward and reverse primer, 25 ng DNA and 1 unit of *Taq* polymerase (Intron Biotechnology Co., Ltd., South Korea) in a total volume of 20 µl. PCR reaction conditions were initial denaturation at 94ºC for 5 min, 5 cycles of denaturation at 94ºC for 1 min, annealing at 35ºC for 1 min, extension at 72ºC for 1 min, followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 52ºC for 1 min, extension at 72ºC for 1 min and final extension at 72ºC for 5 min. All PCR products were stored at 4ºC until visualized on 1.7% agarose gel prepared in 1X TAE buffer containing 0.5 µg/ml ethidium bromide (EtBr).

2.4 Data analysis

RAPD and SRAP bands were recorded as present or absent for each individual in a binary matrix. Clustering analysis was conducted using the unweighted pair group method with arithmetic mean (UPGMA). Mean Shannon diversity index (*I*), mean Nei's (1973) genetic diversity (*H*), mean number of alleles per locus (*Na*), and the effective number of alleles per locus (*Ne*) were estimated by POPGENE version 1.32 (Yeh, Yang, Boyle, Ye, & Mao, 1997).

3. Results and Discussion

3.1 Genetic assessment using DNA markers

Nine polymorphic RAPD primers were selected from 108 primers and these were used to amplify all samples. Fifty-three bands were generated with an average of 5.89 bands per primer. Size of PCR products ranged from 300 to 2,000 bp. Number of total bands produced by each primer ranged from five to seven containing four to seven polymorphic bands at an average of 95.93% polymorphism.

Eight SRAP primer combinations showing polymorphic patterns generated 38 bands with an average of 4.75 bands per primer pair. Size of PCR products from each primer combination varied from 200 bp to 700 bp. Number of total bands produced by each primer combination ranged from four to six containing three to five polymorphic bands. Percentage of SRAP polymorphism (92.29%) was slightly lower than for RAPD markers (95.93%). High polymorphism produced by RAPD and SRAP markers has been reported in buffalo grass (Budak *et al.*, 2004) and in *Cucurbita pepo* (Ferriol, Pic, & Nuez, 2003). Furthermore, SRAP markers were shown to be very effective tools for analyses in populations with low genetic variability such as watermelon and Turkish olive varieties (Isik, Doganlar, & Frary, 2011). Previous studies suggested that SRAP markers have higher performance than other tested molecular marker systems (Ferriol *et al.*, 2003; Ruiz, Garcia-Martinez, Picó, Gao, & Quiros, 2005). By con-

trast, RAPD markers showed higher potential for evaluation of genetic diversity in *E. flava* in our study. However, both marker types have proved effective systems for genomic research (Xu & Zhao, 2009). Here, SRAP showed lower polymorphism than RAPD but the SRAP technique gave higher stability (Liu, Gong, Huang, & Zhu, 2004).

3.2 Population analysis

Analysis of genetic diversity within and among populations is the first step towards conservation of plants species (Govindaraj, Vetriventhan, & Srinivasan, 2015). RAPD and SRAP are random markers which do not require any sequence information. Therefore, they have been widely used in many rare or less studied species such as pepper (Jiang & Liu, 2011).

In this study, genetic diversity parameters both within and among populations were calculated based on RAPD and SRAP data (Table 1). Within each population, percentage of polymorphism loci (PPB) ranged from 45.28% to 71.70% and from 39.47% to 71.05%, Nei's genetic diversity (*H*) ranged from 0.1907 to 0.3034 and from 0.1698 to 0.2950, Shannon diversity index (*I*) showed similar trends, ranging from 0.2755 to 0.4386 and from 0.2425 to 0.4286, observed number of alleles (*Na*) ranged from 1.4528 to 1.7170 and from 1.3947 to 1.7105, with effective number of alleles ranging from 1.3462 to 1.5458 and from 1.2884 to 1.5247 for RAPD and SRAP markers, respectively. Mean values within populations for RAPD and SRAP markers were *H* (0.2303 and 0.2016), *I* (0.3380 and 0.2970), *Na* (1.5962 and 1.5368), and *Ne* (1.4054 and 1.3537), compared with values among populations as *H* (0.3450 and 0.3237), *I* (0.5156 and 0.4852), *Na* (1.9623 and 1.9211), and *Ne* (1.5848 and 1.5494) indicating that level of genetic diversity within populations was lower than among populations. Genetic variations of the five populations showed that effective number of alleles, Nei's genetic diversity and the Shannon diversity index from Pa La Ta Waterfall were the highest. All parameters in each population and averages within and among populations gave higher results from RAPD than SRAP markers. This indicates that the RAPD markers were more effective for genetic diversity assessment in *E. flava*; however, both markers provided results in the same direction. A combined analysis of RAPD and SRAP markers is shown in Table 2. The results indicate that genetic diversity of *E. flava* by both RAPD and SRAP markers was the highest in the PT population, while TS and HD populations were the lowest. Comparison of genetic distances and genetic identity between all pairs of populations is shown in Table 3. The TT population showed the highest genetic distance from other populations, in agreement with its location in Kanchanaburi Province. A previous study concerning genetic diversity found that plant species which were obstructed by geographical environment maintain lower genetic diversity than widespread species (Nybom, 2004). However, genetic diversity of *Magnolia officinalis*, a traditional tree found in many regions of China, was low as revealed by ISSR marker (Yu, Yan, Sun, & Liu, 2011). In this study, *E. flava*, an endangered species found only in a specific habitat surrounded by mountain ranges, had high genetic diversity within populations (PPB: 57.14%; *H*: 0.2183; *I*: 0.3209; *Na*: 1.5715; *Ne*: 1.3838) and among populations (PPB: 94.51%; *H*: 0.3361; *I*: 0.5029; *Na*: 1.9451; *Ne*: 1.5700). Therefore, the level of genetic diversity may not be directly correlated to species status or geographical barriers. For *E. flava*, the reproductive system favors a cross-pollination pathway (Pedersen *et al.*, 2018), thus maintaining its high genetic diversity, and this suggests that the genetic drift is not currently of great concern for this species.

A dendrogram was constructed for the five populations from Ban Huai Tham Suea (HS), Huai Hin Dang (HD), Thee Lo Su Waterfall (TS), Pa La Ta Waterfall (PT) and Takien Thong Waterfall (TT) using UPGMA analysis. Single markers, RAPD, SRAP and a combination of both markers generated similar dendrograms and the combined result is shown as Figure 2. The five populations clearly grouped into two major clusters. Cluster I included four populations (HS, HD, TS, PT) from Tak Province. HD and TS populations clustered because they were located closest together, while Cluster II included only the TT population from Kanchanaburi Province as the most distant from the other populations. In previous studies, RAPD has shown different clustering in a dendrogram than SRAP (Budak *et al.*, 2004; Liu *et al.*, 2008). This suggests that the DNA regions targeted by SRAP are different from those targeted by the RAPD markers. ISSR and RAPD target noncoding regions (Parsons, Newbury, Jackson, Ford, & Loyd, 1997; William *et al.*, 1990) while SRAP has been reported to target mainly open reading frame (ORF) regions (Li & Quiros, 2001). Therefore, combining DNA marker systems which target different regions should improve genome information. Our results, using a combination of data sets, and several previous studies including genetic diversity analysis in rice (Davier wala *et al.*, 2000), radish (Liu *et al.*,

Table 1. Genetic diversity parameters in five populations, Ban Huai Tham Suea (HS), Huai Hin Dang (HD), Thee Lo Su Waterfall (TS), Pa La Ta Waterfall (PT) and Takien Thong Waterfall (TT) compared between RAPD and SRAP markers.

Code	Individual code	Sample size	PPB[a]		*H*[b]		*I*[c]		*Na*[d]		*Ne*[e]	
			RAPD	SRAP	RAPD	SRAP	RAPD	SRAP	RAPD	SRAP	RAPD	SRAP
HS	1-45	45	67.92	47.37	0.2399	0.1759	0.3631	0.2633	1.6792	1.4737	1.3890	1.2884
HD	1-30	30	45.28	42.11	0.1907	0.1752	0.2755	0.2527	1.4528	1.4211	1.3462	1.3211
TS	1-15	15	47.17	39.47	0.2113	0.1698	0.3009	0.2425	1.4717	1.3947	1.3942	1.3164
PT	1-25	25	71.70	71.05	0.3034	0.2950	0.4386	0.4286	1.7170	1.7105	1.5458	1.5247
TT	1-29	29	66.04	68.42	0.2060	0.1923	0.3119	0.2977	1.6604	1.6842	1.3517	1.3177
Average within population			59.62	53.68	0.2303	0.2016	0.3380	0.2970	1.5962	1.5368	1.4054	1.3537
Average among population			96.23	92.11	0.3450	0.3237	0.5156	0.4852	1.9623	1.9211	1.5848	1.5494

[a] Percentage of polymorphism loci, [b] Nei's (1973) genetic diversity, [c] Shannon's diversity index, [d] Observed number of alleles, [e] Effective number of alleles.

Table 2. Genetic diversity parameters in five populations at Ban Huai Thum Suea (HS), Huai Hin Dang (HD), Thee Lo Su Waterfall (TS), Pa La Ta Waterfall (PT) and Takien Thong Waterfall (TT) from combined RAPD and SRAP markers.

Population code	Individual code	Sample size	PPB[a]	*H*[b]	*I*[c]	*Na*[d]	*Ne*[e]
HS	1-45	45	59.34	0.2132	0.3214	1.5934	1.347
HD	1-30	30	43.96	0.1842	0.266	1.4396	1.3357
TS	1-15	15	43.96	0.194	0.2765	1.4396	1.3617
PT	1-25	25	71.43	0.2999	0.4345	1.7143	1.537
TT	1-29	29	67.03	0.2003	0.3059	1.6703	1.3375
	Average within population		57.14	0.2183	0.3209	1.5714	1.3838
	Average among population		94.51	0.3361	0.5029	1.9451	1.57

[a] Percentage of polymorphism loci, [b] Nei's (1973) genetic diversity, [c] Shannon's diversity index, [d] Observed number of alleles, [e] Effective number of alleles.

Table 3. Nei's genetic distance (below diagonal) and genetic identity (above diagonal) in five populations at Ban Huai Thum Suea (HS), Huai Hin Dang (HD), Thee Lo Su Waterfall (TS), Pa La Ta Waterfall (PT) and Takien Thong Waterfall (TT) analyzed by combined RAPD and SRAP markers.

Location	HS	HD	TS	TT	PT
HS	-	0.9408	0.9318	0.628	0.9147
HD	0.061	-	0.9897	0.6021	0.9033
TS	0.0706	0.0104	-	0.573	0.8896
TT	0.4652	0.5074	0.5569	-	0.7271
PT	0.0892	0.1017	0.117	0.3187	-

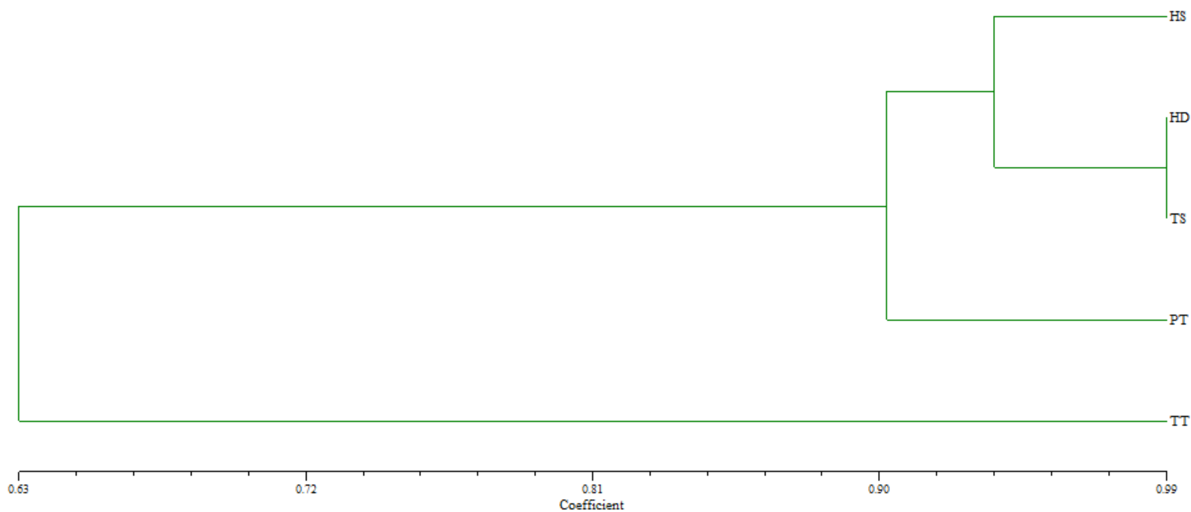

Figure 2. Unweighted pair-group method with arithmetic mean (UPGMA) dendrogram of genetic relationships among five populations at Ban Huai Thum Suea (HS), Huai Hin Dang (HD), Thee Lo Su Waterfall (TS), Pa La Ta Waterfall (PT) and Takien Thong Waterfall (TT).

2008), mushroom (Fu *et al.*, 2010), buffalo grasses (Budak *et al.*, 2004), Jerusalem artichoke (Wangsomnuk *et al.*, 2011), and Turkish melons (Yildiz, Ekbic, Keles, Sensoy, & Abak, 2011) confirmed this supposition.

E. flava populations were collected in western areas of Thailand, which may be obstructed by the Tanaosri Mountain Range. Pedersen *et al.* (2013) reported that this orchid was formerly found in the northern area of Nan but seems now to be extinct (Sittisudjathum pers. comm). Population reduction was caused by low germination rate, habitat fragmentation and human overexploitation. Seeds of *E. flava* are lacking in endosperm and can only germinate with symbiotic fungi in natural conditions (Pedersen *et al.*, 2013). Excessive exploitation can also cause destruction of species resources through environmental deterioration (Bao, Shun, Ye, & Gu, 1999).

4. Conclusions

This is the first study concerning the genetic diversity of *E. flava* by RAPD and SRAP markers. RAPD markers showed the highest Nei's genetic diversity (0.3450) while SRAP and a combination of both marker data sets gave lower results (0.3237, 0.3361). However, both marker types can

provide more accurate information on population genetic diversity than traditional methods, with simple and reproducible results. The five populations were shown to be divided into two groups. Takien Thong Waterfall population from Kanchanaburi Province was clearly separated from the other populations in Tak Province by the mountain range between them. Overall genetic diversity both between and within populations was high when compared to other reported plants. Thus, *in situ* conservation is recommended for *E. flava* together with introduction of plants from different locations to increase genetic variation.

Acknowledgements

This study was financially supported by grant from The Research Fund, Naresuan University, Thailand. We are also grateful to Suchat Chanhomhuan and Salil Sittisudjathum for *E. flava* locations information.

References

Bao, X. S., Shun, Q. S., Ye, Y. Q., & Gu, H. F., (1999). History and current status of Dendrobium medicinal “fengdou”. *Chinese Traditional and Herbal Drugs*, *22*, 540–542.

Borja, A., Bricker, S. B., Dauer, D. M., Demetriades, N. T., Ferreira, J. G., Forbes, A. T., . . . Zhu, C., (2009). Ecological integrity assessment ecosystem-based approach and integrative methodologies are these concepts equivalent? *Marine Pollution Bulletin*, *58*, 457–458.

Budak, H., Shearman, R. C., Parmaksiz, I., Gaussoin, R. E., Riordan, T. P., & Dweikat, I., (2004). Molecular characterization of Buffalo grass germplasm using sequence related amplified polymorphism markers. *Theoretical and Applied Genetics*, *108*, 28–334.

Chen, S. Y., Dai, T. X., Chang, Y. T., Wang, S. S., Ou, S. L., Chuang, W. L., . . . Ku, H. M., (2013). Genetic diversity among *Ocimum* species based on ISSR, RAPD and SRAP markers. *Australian Journal of Crop Science*, *7*, 1463–471.

Cribb, P. J., Dixon, K. W., Kell, S. P. & Barrett, R. L., (2003). *Orchid conservation*. Kota Kinabaru, Sabah: Natural History Publications.

Davierwala, A. P., Chowdari, K. V., Kumar, S., Reddy, A. P. K., Ranjekar, P. K. & Gupta, V. S., (2000). Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. *Genetica*, *108*, 269–284.

Doyle, J., & Doyle, J., (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, *19*, 11–15.

Dressler, R. L., (1993). *Phylogeny and classification of the orchid family*. Portland, OR: Portland Dioscorides Press.

Ferriol, M., Pic, B., & Nuez, F., (2003). Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP marker. *Theoretical and Applied Genetics*, *107*, 271–282.

Fu, L. Z., Hong-Yu, Z., Xue-Qian, W., Hai-Bo, L., Hai-Long, W., Qing-Qi, W., & Li-An, W., (2010). Evaluation of genetic diversity in *Lentinula edodes* strains using RAPD, ISSR and SRAP markers. *World Journal Microbiology and Biotechnology*, *26*, 709–716.

Govindaraj, M., Vetriventhan, M. & Srinivasan, M., (2015). Importance of genetic diversity assessment in crop plants and iIts recent advance: An overview of its analytical perspectives. *Genetics Research International*, *2015*, 1–14. doi:10.1155/2015/431487

Isik, N., Doganlar, S., & Frary, A., (2011). Genetic diversity of Turkish olive varieties assessed by simple sequence repeat and sequence-related amplified polymorphism markers. *Crop Science*, *51*, 1646–1654

Jiang, Y. & Liu, J. P., (2011). Evaluation of genetic diversity in *Piper* spp. using RAPD and SRAP markers. *Genetic and Molecular Research*, *10*, 2934–2943. doi:10.4238/2011.November.29.4

Li, G., & Quiros, C.F., (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theoretical and Applied Genetics*, *103*, 455–461.

Liu, L. W., Zhao, L. P., Gong, Y. Q., Wang, M. X., Chen, L. M., Yang, J. L., . . . Wang, L. Z. (2008) DNA fingerprinting and genetic diversity analysis of late-bolting radish cultivars with RAPD, ISSR and SRAP markers. *Scientia Horticulturae*, *116*, 240–247.

Liu, L. W., Gong, Y. Q., Huang, H., & Zhu, X. W., (2004) Novel molecular marker systems—SRAP and TRAP and their application. *Hereditas*, *26*, 777–781

Nybom, H., (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, *13*, 1143–1155. doi:10.1111/j.1365-294X.2004.02141.x

Parsons, B. J., Newbury, J. H., Jackson, M. T., Ford, L., & Loyd, B. V., (1997). Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. *Molecular Breeding*, *3*, 115–125

Pedersen, H. Æ., Srimuang, K., Bänziger, H., & Watthana, S., (2018). Pollination system diversity in *Epipactis* (Orchidaceae): New insights from studies of *E. flava* in Thailand. *Plant Systematics and Evolution*, *304*, 895–909. doi:10.1007/s00606-018-1520-5

Pedersen, H. Æ., Watthana, S., & Srimuang, K., (2013). Orchids in the torrent: On the circumscription, conservation and rheophytic habit of *Epipactis flava*. *Botanical Journal of the Linnean Soc*iety, *172*, 358–370

Pridgeon, A. M., Cribb, P. J., Chase, M. W., & Rasmussen, F. N., (1999). *Genera Orchidacearum*. Oxford, England: Oxford University Press.

Qian, X., Wang, C., & Tian, M., (2013). Genetic diversity and population differentiation of *Calanthe tsoongiana*, a rare and endemic orchid in China. *International Journal of Molecular Sciences*, *14*, 20399–20413

Ruiz, J. J., Garcia-Martinez, S., Picó, B., Gao, M. Q., & Quiros, C. F., (2005). Genetic variability and relationship of closely related Spanish traditional cultivars of tomato as detected by SRAP and SSR markers. *Journal of American Society for Horticultural Science*, *130*, 8–94

Vermeulen, J. J., & Tsukaya, H., (2011). An assumed rheophytic orchid *Bulbophyllum rheophyton* from Borneo. *Plant Systematic and Evolution*, *293*, 71–73

Wangsomnuk, P. P., Khampa, S., Wangsomnuk, P., Jogloy, S., Mornkham, T., Ruttawat, B., Patanothai, A., & Fu, Y.B., (2011). Genetic diversity of worldwide Jerusalem artichoke (*Helianthus tuberosus*) germplasm as revealed by RAPD markers. *Genetics and Molecular Research*, *10*, 4012–4025. doi:10.4238/2011

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V., (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, *18*, 6531–6535

Xu, C., & Zhao, B. H., (2009). The development and application of SRAP molecular markers. *Life Science Instruments*, *7*, 24–27

Yeh, F. C., Yang, R. C., Boyle, T. B. J., Ye, Z. H., & Mao, J. X., (1997). *POPGENE, the user-friendly shareware for population genetic analysis*. Alberta, Canada: Molecular Biology and Biotechnology Center, University of Alberta.

Yildiz, M., Ekbic, E., Keles, D., Sensoy, S., & Abak, K., (2011). Use of ISSR, SRAP, and RAPD markers to assess genetic diversity in Turkish melons. *Scientia Horticulturae*, *130*, 349–353.

Yu, H. H., Yan, Z. L., Sun, B., & Liu, R. N., (2011). Genetic diversity and relationship of endangered plant *Magnolia officinalis* (Magnoliaceae) assessed with ISSR polymorphisms. *Biochemical Systematics and Ecology*, *39*, 71–78. doi:10.1016/j.bse.2010.12.003