Original article

Genetic Diversity of *Cinnamomum porrectum* (Roxb.) Kosterm. in Southern Thailand Detected by Inter Simple Sequence Repeat (ISSR) Analysis

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ABSTRACT

Genetic diversity of *Cinnamomum porrectum* (Roxb.) Kosterm. ('Thep tharo') in southern Thailand was assessed by analyzing molecular genetic markers at the population level. Inter simple sequence repeat (ISSR) markers were used to analyze the genetic diversity of six populations in Phangnga, Phuket, Phatthalung, Satun, Krabi, and Songkhla provinces. In total, 73 amplified bands were obtained from 117 individuals of all sampled populations using seven primers. At the species level, the percentage of polymorphic loci (*P*), the mean expected hetero zygosity (H_e), and the Shannon's information index of diversity (*I*) were high (87.67%, 0.2883, and 0.4343, respectively). However, *P,* H_e, and *I* were relatively low at the population level with averages of 47.03%, 0.1689, and 0.2508, respectively. Among the six populations investigated, the Songkhla and Phangnga populations revealed the highest genetic diversity, followed by Satun, Phatthalung, Phuket and Krabi populations, respectively. Based on Nei's genetic differentiation (G_{st}) value, 35.51% of genetic differentiation existed among populations. The gene flow (N_m) was 0.9081. UPGMA cluster analysis divided the populations into two main groups.One consisted of the populations of Satun, Songkhla, Phangnga, and Phatthalung, while the other was composed of the Phuket and Krabi populations. Based on the results of the present study and field survey, it could be suggested that in situ gene conservation is very important for this species, especially in the Songkhla and Phangnga populations due to their higher genetic diversity and larger population sizes. At the same time, ex situ gene conservation should be established as an option to conserve the genetic material of the Satun, Phatthalung, Phuket, and Krabi populations that showed low genetic diversity and small population sizes.

Keywords: *Cinnamomum porrectum,* 'Thep tharo', genetic diversity, ISSR, conservation

INTRODUCTION

Cinnamomum porrectum (Roxb.) Kosterm., local name 'Thep tharo', is a medicinal and aromatic tree belonging to the family Lauraceae. It is mostly distributed throughout southern Thailand. All plant parts of this species provide various benefits to local people including medicine, food, and wood construction, as well as for uses in religious and cultural ceremonies (Chayamarit, 1997;

Palanuvej *et al.*, 2006; Phongpaichit *et al*., 2006; Denrungruang, 2007; Plansangkate *et al.,* 2007). In particular, its pleasant-smelling roots have been carved into various types of products such as Buddha images, souvenirs, tea sets, candlesticks, etc. *C. porrectum* is becoming one of the important economic tree species in southern Thailand. At the same time, the increasing demand for raw materials for wood carving and other products has contributed to the destruction of *C. porrectum* populations in natural forest. Moreover, the habitats of this species are also facing severe threats imposed by human activities, especially the expansion of rubber and oil palm plantations. This action has decreased the distribution of *C. porrectum* and consequently, it may lead to a more restricted genetic resource. Currently, the remaining populations of *C. porrectum* are rather small and fragmented; *C. porrectum* will become extinct in the near future. It is well known that the stability and the evolutionary potential of a species depend on its genetic diversity. Therefore, it is important to obtain knowledge of the amount of genetic diversity to provide information for the development of strategies for the conservation and sustainable utilization of a species.

Inter simple sequence repeat (ISSR) is a newly developed modification of simple sequence repeat (SSR) based marker systems (Zietkiewicz *et al.,* 1994). It has advantages over other DNA polymorphism analysis techniques, such as low quantities of template DNA required, no need of sequence data for primer construction, random distribution throughout the genome, generation of many informative bands per reaction, low development costs, etc (Li and Chen, 2004; Ci *et al.,* 2008). Therefore, the ISSR technique has been broadly and successfully used in studies of the genetic diversity of many plant species (Li and Chen, 2004; Ho, 2006; Lu *et al.,* 2006; Xiao *et al.,* 2006; Ju and Wang, 2008; Li and Chen, 2009, He *et al.,* 2010). In the present study, the ISSR technique was used to investigate the genetic diversity of *C. porrectum* from six populations in southern Thailand in order to provide scientific evidence for future genetic resource conservation programs.

MATERIALS AND METHODS

Sampling

A total of 117 individuals of *C. porrectum* from six populations were sampled (Table 1). The very young leaves were collected in each population and stored in an ice box in the field and transferred to a freezer (-60˚C) in the laboratory until DNA extraction was carried out.

DNA Extraction and PCR Amplification

Total DNA was extracted using the CTAB method (Doyle and Doyle, 1987) with some modification. Seven ISSR primers showing clear and reproducible band patterns were used to provide polymorphic markers for genetic diversity. The PCR amplifications were performed in a 10 µL reaction volume containing 1.5 µL of 30 ng of template DNA, 1 µL of 2 mM dNTPs, 1 µL of 10 × PCR buffer, 0.4 µL of 50 mM MgCl2, 0.1 µL of 5 unit/µL Taq polymerase (RBC bioscience), 0.8 µL of 10 pmole/µL primer, and 5.2 µL of pure water. Amplification was performed in a T-Gradient thermocycler (Biometa) under the following cycle profile: 5 min

at 94 ˚C, followed by 30 s at 94 ˚C, 45 s annealing temperature of each primer (Table 2), and 2 min extension at 72˚C for 29 cycles, and 7 min at 72 ˚C for a final extension. The amplification products were analyzed on 1.5% agarose gels in 1 × TBE buffer and detected by staining with ethidium bromide. Band size was estimated from a 100 bp DNA ladder (Fermentas).

Data Analysis

ISSR bands were used to assign loci for each primer and scored as presence (1) and absence (0). The band presence or absence data matrix was analyzed by POPGENE (Yeh *et al.,* 1999), making the assumption that the populations are in Hardy-Weinberg equilibrium at these ISSR marker loci. The following indices were used to quantify the amount of genetic diversity within each population examined: the percentage of polymorphic loci (*P*), the mean expected heterozygosity (H_e) (Nei, 1973), and Shannon's information index of diversity (*I*). Genetic diversity parameters (*P,* H_e, and *I*) were also calculated at the species level.

Genetic differentiation among populations was estimated by Nei's gene diversity statistic (Nei, 1973). The amount of gene flow among these populations was estimated as $Nm = 0.5(1-G_{st})/G_{st}$ (McDermott and McDonald, 1993). The pairwise genetic distance between populations (Nei, 1972) was calculated to construct a UPGMA dendrogram.

Table 1 Population codes and number of samples of *Cinnamomum porrectum* used for inter simple sequence repeat (ISSR) analysis.

Population code	Population location	Latitude	Longitude	No. of samples
PNG	Thai Mueang, Phangnga	8˚ 23' 21" N	98˚ 15' 35" E	55
PK	Thalang, Phuket	8˚ 05' 07" N	98˚ 19' 43" E	4
PTL	Tamot, Phatthalung	7˚ 16' 14" N	100˚ 01' 32" E	7
ST	Khuan Don, Satun	6˚ 45' 39" N	100˚ 09' 27" E	9
KB	Mueang Krabi, Krabi	8˚ 09' 10" N	98˚ 51' 09" E	3
SK	Hat Yai, Songkhla	7˚ 01' 08" N	100˚ 17' 32" E	39
Total				**117**

Table 2 List of selected inter simple sequence repeat (ISSR) primers, sequence, annealing temperature, approximate size range (in base pairs) of the bands, number of monomorphic and polymorphic bands.

Primer	Sequence (5'---3')	Annealing Temperature (˚C)	Size range of bands (bp)	Total number of band scored	Number of monomorphic bands	Number of polymorphic bands	% PPB
UBC815	$(CT)_8G$	60	500-2 500	9	1	8	88.89
UBC827	$(AC)_8G$	55	480-1 700	8	1	7	87.50
UBC834	$(AG)_8(CT)T$	53	360-1 500	9	1	8	88.89
UBC841	$(GA)_8(CT)C$	60	150-1 750	13	3	10	76.92
UBC842	$(GA)_8(CT)G$	58	300-1 600	12	1	11	91.67
UBC855	$(AC)_8(CT)T$	55	300-3 000	12	1	11	91.67
TH37643	$(ACTG)_4$	50	280-1 750	10	1	9	90.00
	Total			73.00	9.00	64.00	
	Average			10.43	1.29	9.14	87.67
	SD			1.90	0.76	1.57	

Note: % PPB = percentage of polymorphic loci

RESULTS AND DISCUSSION

ISSR Polymorphism

For the 117 individuals of *C. porrectum* from the six populations, seven primers produced a total of 73 amplified bands, of which 64 (87.67 %) were polymorphic and 9 (12.33 %) were monomorphic. The bands per primer ranged from 8 to 13, with an average of 10.43 ± 1.90. The size of the amplified bands ranged from 150 to 3,000 bp (Table 2). Each of 117 individuals presented a unique ISSR genotype, indicating extensive genetic variation in the populations studied.

Genetic Diversity and Differentiation

At the species level, the percentage of polymorphic loci (*P*), the mean expected heterozygosity (H_e), and Shannon's information index of diversity (*I*) were 87.67 %, 0.2883, and 0.4343, respectively. At the population level, all populations had a wide range of genetic variation. The value of P per population ranged from 16.44 to 78.08 %, with an average of 47.03 %. He ranged from 0.0699 to 0.2681, with an average of 0.1689, and I ranged from 0.1008 to 0.3947, with an average of 0.2508 (Table 3). Among the six populations investigated, the Songkhla and Phangnga populations revealed the highest genetic diversity, followed by the Satun, Phatthalung, Phuket and Krabi populations, respectively.

The value of Nei's genetic differentiation (G_{st}) was 0.3551, indicating that about 35.51 % of the genetic variation was among populations. The level of gene flow (N_m), which was the number of migrating individuals among populations per generation, was calculated to be 0.9081 (Table 3).

Table 3 Genetic diversity parameters of *Cinnamomum porrectum* from six populations based on inter simple sequence repeat (ISSR) markers.

Population	*P* (%)	H_e	*I*	G_{st}	N_m
Phangnga	78.08	0.2524	0.3817		
Phuket	28.77	0.1097	0.1624		
Phatthalung	36.99	0.1282	0.1927		
Satun	49.32	0.1850	0.2724		
Krabi	16.44	0.0699	0.1008		
Songkhla	72.60	0.2681	0.3947		
Population level	47.03	0.1689	0.2508		
Species level	87.67	0.2883	0.4343	0.3551	0.9081

Notes: *P* = percentage of polymorphic loci; H_e = the mean expected heterozygosity (Nei, 1973); *I* = Shannon's information index (Lewontin, 1972); G_{st} = Nei's genetic differentiation; N_m = Gene flow

According to the remaining populations of *C. porrectum* in southern Thailand, which are rather small and fragmented, it could be predicted that they should have be low genetic diversity. However, the results for the ISSR marker in this study showed that the genetic diversity of *C. porrectum* at the species level was relatively high (H_e = 0.2883 and *I* = 0.4343) when compared with other species in the same genus also using ISSR markers, such as *C. osmophloeum* (H_e = 0.2213 and *I* = 0.5746), *C. macrostemon*

(H_e = 0.2205 and I = 0.3313), *C. insulari-montanum* (H_e = 0.1639 and I = 0.2393) (Ho, 2006), and *C. camphora* (H_e = 0.2564 and I = 0.4003) (Ju and Wang, 2008). Hamrick and Loveless (1989) found that tropical trees tended to present high levels of genetic diversity which was related to their life history and ecological characteristics such as a wide geographical range, outcrossing, and biotic dispersal. On the basis of life history characteristics, *C. porrectum* is a long-lived, woody and perennial tree species. In addition, the mating system can influence the levels of genetic diversity. Outcrossing species commonly have considerably higher levels of genetic diversity than selfing species (Hamrick and Godt, 1989). Uthairatsamee (2011) concluded that *C. porrectum* was 'facultative outcrossing'. The combination of these life history traits should enable the species to maintain a high level of genetic diversity.

The Songkhla and Phangnga populations had more genetic variation, followed by the Satun, Phatthalung, Phuket, and Krabi populations, respectively. This result was related to population size. Nevertheless, the population size was not correlated with genetic diversity in the cases of the Songkhla and Phangnga populations. The population size of Songkhla was smaller than for the Phangnga population but it showed higher genetic diversity. This is agreed with the studies of many plants (Schmidt and Jensen, 2000; Tero *et al.,* 2003; Kang *et al.,* 2005). The results demonstrated that the uneven distribution of genetic diversity wais substantially and apparently related to the habitat (Lu *et al.,* 2006). The genetic diversity at the population level of this species was very low in the Krabi and Phuket populations (H_e = 0.0699 and 0.1097, respectively). Masayuki (2003) stated that a critical factors affecting low genetic diversity within a population was the number of remnant individuals. Based on the field survey, *C. porrectum* in the Krabi and Phuket populations had few remnant individuals.

Based on the Gst value, most of the genetic differentiation of *C. porrectum* was within a population (64.49 %) which was similar to some other species in the same genus using ISSR markers, such as *C. osmophloeum* (71.63 %), *C. macrostemon* (57.81 %), *C. insulari-montanum* (50.12 %) (Ho, 2006), and *C. camphora* (51.19 %) (Ju and Wang, 2008). Besides depending very much on the gene pool and geographic range of the species as a whole, the situation of population differentiation was related to life history traits. In addition,the mating system played a critical role for the population genetic structure. In outcrossing species, genetic differentiation occurred less than 20% among populations (Hogbin and Peakall, 1999). Although outcrossing species generally have lower levels of population differentiation (Hamrick and Godt, 1989; Hogbin and Peakall, 1999), a higher level of genetic differentiation was detected among populations of some endemic and outcrossing species such as *Litsea szemaois* (Ci *et al.,* 2008) and, *C. porrectum*. Based on the field observation, there were numerous seedlings close to their presumed maternal trees. This suggested that fruit dispersal of *C. porrectum* was mainly by gravity and such limited dispersal could strongly influence the genetic diversity within and among populations. Although there was no direct report on pollination in *C. porrectum,* it probably relied on small g eneralist insects, based on pollination studies of other species in *Cinnamomum* spp. with similar floral structure and habitat (Fan *et al.,* 2006). Pollen dispersal was limited in insect pollinated plants compared to the distances travelleds by wind- dispersed pollen and this tends to increase population differentiation (Hamrick and Loveless, 1989; Hamrick and Godt, 1989).

The indirect estimation of the level of gene flow of *C. porrectum* was moderate

(N_m = 0.9081), which meant that the numbers of migrants per generation were greaterthan one successful migrant, and the level of genetic diversity maintained within a population was less susceptible to genetic drift. A migration rate of 0.5 was considered sufficient to overcome the diversifying effects of random drift (Ellstrand and Elam, 1993). However, compared with the most widely distributed *Cinnamomum* species, *C. macrostemon, C. insulari-montanum,* and *C. camphora* (N_m = 0.6533, 0.5024, and 0.5244, respectively), the gene flow of *C. porrectum* was high (N_m = 0.9081). The reasons for the higher gene flow value detected here were the topography and climatic conditions in southern Thailand which were uniform landscape increased gene flow, since dispersal between populations was likely to be easier.

Genetic Identity and Genetic Distances

The Nei's genetic identity value varied from 0.8108 to 0.9367, and the pair wise genetic distance was between 0.0654 and 0.2097 (Table 4). Among the six populations, the genetic distance between the Satun and Songkhla populations was 0.0654, so their genetic relationship was thenearest. However, the genetic relationship between the populations of Phangnga and Krabi was 0.2097 which was showing the farthest genetic distance in the populations. The relatively low values of pairwise wise genetic distance between some populations was likely due to their genetic relatedness. On the other hand, a high value of pair wise genetic distance revealed that the two populations were genetically distant.

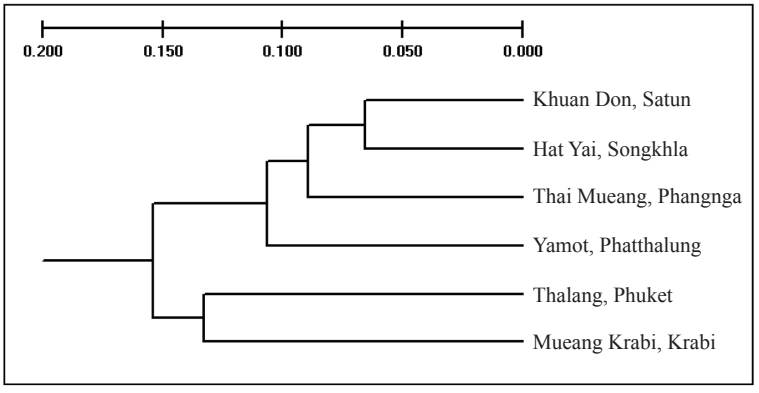

Figure 1 UPGMA dendrogram representing the genetic distances among six populations of *Cinnamomum porrectum* based on Nei's (1978) genetic distance.

Table 4 Genetic identity and genetic distance among six populations of *Cinnamomum porrectum* based on inter simple sequence repeat (ISSR) data.

Populations	Phangnga	Phuket	Phatthalung	Satun	Krabi	Songkhla
Phangnga	****	0.8697	0.8949	0.8981	0.8108	0.9314
Phuket	0.1397	****	0.8539	0.8716	0.8756	0.8948
Phatthalung	0.1111	0.1580	****	0.9041	0.8618	0.8986
Satun	0.1075	0.1374	0.1008	****	0.8300	0.9367
Krabi	0.2097	0.1329	0.1487	0.1864	****	0.8677
Songkhla	0.0711	0.1111	0.1069	0.0654	0.1419	****

Notes: above diagonal = genetic identity; below diagonal = genetic distance

On the basis of Nei's genetic distance (Nei, 1978), a dendrogram of the six populations was generated using UPGMA cluster analysis. The six populations were divided into two main groups (Figure 1). In the first group, the populations from Phuket and Krabi were clearly distinguished from all other populations, implying that these populations were genetically more distinct from the other populations. The second group contained the remaining four populations of Satun, Songkhla, Phangnga and Phatthalung. In the dendrogram, populations of the second group had considerably smaller genetic distances and were clustered together. Within this group, the population from Satun was closer to Songkhla and more distant from Phangnga and Phatthalung, respectively.

The ISSR data results in the present study did not provide a clear-cut separation among the *C. porrectum* populations in relation to the origin of their respective geographical region although there was an irregular trend that accessions from the same region were clustered together. It could be that the long history of the habit of this species in each population might have contributed to the dispersion of alleles throughout the population, lessening the influence of geography on the pattern of variation among them.

Conservation Implications

Information on genetic diversity could provide a basis for conservation and utilization of *C. porrectum*. The results of this study showed that the genetic diversity at the species level of *C. porrectum* was relatively high when compared with other species in the same genus and it was not regarded as endangered by the International Union for the Conservation of Nature (IUCN, 2010). Nonetheless, the habitats are facing severe threats imposed by human activities, especially the expansion of rubber and oil palm plantations. Moreover, there are high demands for raw materials for wood carving and other products. These factors can lead to illegal cutting of *C. porrectum* in natural forest. In addition, the results of the present study showed that there was very low genetic diversity at the population level, especially in the case of the Krabi and Phuket populations. If some conservation measures are not adopted, *C. porrectum* will become extinct in the near future. Although in recent years, the Royal Forest Department has begun to carry out a conservation program for this species, due to the difficulty of seed availability, the problem of a narrow genetic base could occur in the future. Thus, an in situ gene conservation area for *C. porrectum* will be established to maintain genetic diversity and also supply seeds and seedlings for the restoration program in natural forest and for

cultivation in tree farms. Based on the results obtained in the present study, the Songkhla and Phangnga populations that showed high genetic diversity and large population sizes are suitable populations for the establishment of in situ gene conservation areas. Besides in situ gene conservation measures, ex situ gene conservation may be the only option when in situ gene conservation measures are not applicable; for example, the stands are too small, fragmented, and threatened by encroachment. Based on the field survey of the six populations, the habitats of *C. porrectum* in the Satun, Phatthalung, Phuket, and Krabi populations have been destroyed by extensive deforestation. In addition, according to the results of the present study, these populations showed low genetic diversity. Thus, ex situ gene conservation will be considered as an option to conserve the genetic material from these populations that cannot be conserved using in situ gene conservation.

CONCLUSION

Inter simple sequence repeat markers were successfully applied to study the genetic diversity of *C. porrectum* from six populations in southern Thailand. The result showed that the genetic diversity of this species was high at the species level. However, it was relatively low at the population level. Among the six populations, the Songkhla and Phangnga populations revealed the highest genetic diversity, followed by the Satun, Phatthalung, Phuket and Krabi populations, respectively. UPGMA cluster analysis divided the populations into two main groups. The Phuket and Krabi populations formed one group, while the. Sa tun, Songkhla, Phangnga, and Phatthalung populations were in the other group. This is the first report concerning the genetic diversity of *C. porrectum* and provides basic genetic information which should acilitate attempts to conserve this species.

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REFERENCES

Chayamarit, K. 1997. **Thai Medicinal Plant.** Vol. 6. Forest Herbarium, Royal Forest Department,Bangkok. (in Thai)

Ci, X.-Q., J.-Q. Chen, Q.-M. Li and J. Li. 2008.AFLP and ISSR analysis reveals high genetic variation and inter-population differen tiation in fragmented populations of the endangered Litsea szemaois (Lauraceae) from Southwest China.**Plant Syst Evol** 273: 237-246

Denrungruang, P. 2007. Preliminary assay on antioxidative activity of some Lauraceae barks. **Thai J. Biotechnol.** 8 (1): 49-54.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. **Phytochemical Bulletin** 19: 11-15.

Ellstrand, N.C. and D.R. Elam. 1993. Population genetic consequences of small population size: Implications for plant conservation. A**nnu. Rev. Ecol. Syst.** 24: 217-242.

Fan, Y.B., R.M. Wang, F.J. Pan and P.S Yang. 2006. Study of the floral and pollination biology of *Cinnamomum camphora* and Litsea cubeba (Lauraceae). **Journal of the National Taiwan Museum** 59 (1): 75-90.

Hamrick, J.L. and M.D. Loveless. 1989. The genetic structure of tropical tree populations: associations with reproductive biology, pp. 129-146. *In* J.H.Bock and Y.B. Linhart, eds. **The Evolutionary Ecology of Plants.** Westview Press, Boulder.

_____ and M.J.W. Godt. 1989. Allozyme diversity in plant species, pp. 43-63. *In* A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S. Weir, eds. **Plant Population Genetics, Breeding, and Genetic Resources.** Sinauer, Sunder land Massachusetts.

He, X.H., H. Pan, L.B. Deng, J.C. Pan, F. Li and Y.R. Li. 2010. Genetic diversity of Natural *Myrica rubra* Sieb. et Zucc populations in Guangxi revealed by ISSR markers. **Agricultural Sciences in China** 9 (5): 626-632.

Ho, K.Y. 2006. Genetic variation and taxonomic relationship of *Cinnamomum osmophloeum, C. macrostemon and C. insulari-montanum* (Lauraceae) in Taiwan. **Bio Formosa** 41 (2): 93-102.

Hogbin, P.M. and R. Peakall.1999. Evaluation of the contribution of genetic research to the management of the endangered plant Zieria prostrate. **Conserv Biol** 13: 514-522.

IUCN. 2010. **IUCN Red List of Threatened Species**. Version 2010.4. Available source: http://www.iucnredlist.org, accessed: March 3, 2011.

Ju, L.P. and Y.N. Wang. 2008. Genetic variation of *Cinnamomum camphora* populations of Taiwan and surrounding areas. **Quarterly Journal of Chinese Forestry** 41 (4): 437-447.

Kang, M., Q.G. Ye and H.W. Huang. 2005. Genetic consequence of restricted habitat and population decline in endangered Isoetes sinensis (Isoetaceae). **Ann Bot** 96: 1265-1274.

Lewontin, R.C. 1972. Testing the theory of natural selection. **Nature** 236: 181-182.

Li, H.S. and G.Z. Chen. 2004. Genetic diversity of *Sonneratia alba* in China detected by inter-simple sequence repeats (ISSR) analysis. **Acta Botanica Sinica** 46 (5): 515-521.

_____2009. Genetic variation within the endangered mangrove species *Sonneratia paracaseolaris* (Sonneratiaceae) in China detected by inter-simple sequence repeats analysis. **Biochemical Systematics and Ecology** 37: 260-265.

Lu, Z., Y. Wang, Y. Peng, H. Korpelaine and C. Li. 2006. Genetic diversity of *Populus cathayana* Rehd populations in southwestern China revealed by ISSR markers. **Plant Science** 170: 407-412.

Masayuki, M. 2003. Population genetics of threatened wild plants in Japan. **J. Pl Res** 116: 169-174.

McDermott, J.M. and B.A. McDonald.1993. Gene flow in plant pathosystems. **Annu. Rev. Phytopathol.** 31: 353-373.

Nei, M. 1972. Genetic distance between populations. **American Naturalist** 106: 283-292.

_____. 1973. Analysis of gene diversity in subdivided populations. **Proc. Natl. Acad. Sci. USA** 70: 3321- 3323.

_____. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. **Genetics** 89: 583-590.

Palanuvej, C., P. Werawatganone, V. Lipipun and N. Ruangrungsi. 2006. Chemical composition and antimicrobial activity against *Candida albicans* of essential oil from leaves of *Cinnamomum porrectum*. **Thai J. Health Res** 20 (1): 69-76.

Phongpaichit, S., S. Kummee, L. Nilrat and A. Itharat. 2006. Antimicrobial activity of oil from the root of *Cinnamomum porrectum*. **Songkla nakarin J. Sci. Technol.** 29 (1): 11-16.

Plansangkate, W., N. Sirinupong,R.Chirunthorn, K. Tunsuwan, T. Supavita, A.Itharat and P. Leesurapong. 2007. A study of people's utility of Teptaro (*Cinnamomum porrectum* Kosterm) through local wisdom. *In* A. Itharat, ed. **Cycle of Product Development from *Cinnamomum porrectum*.** Prince of Songkhla University, Hat Yai, Songkhla . (in Thai)

Schmidt, K. and K. Jensen. 2000. Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris*(Scroph ulariaceae) and its relation to population size and reproductive components. **Amer J. Bot** 87: 678-689.

Tero, N., J. Aspi, P. Siikamäki, A. Jäkäläniemi and J. Tuomi. 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species, Silene tatarica. **Molecular Ecology** 12: 2073-2085.

Uthairatsamee, S. 2011. **Morphological, Phytochemical, and Genetic Characteristics of *Cinnamomum porrectum* (Roxb.) Kosterm. for In Situ Gene Conservation in Thai Mueang District, Phangnga Province, Thailand.** Ph.D. Thesis, Kasetsart University .

Xiao, M., Q. Li, L. Wang, L. Guo, J. Li, L. Tang and F. Chen. 2006. ISSR analysis of the genetic diversity of the endangered species Sinopodo phyllum hexandrum (Royle) Ying from western Sichuan province, China. **J. Integr Plant** Biol 48: 1140-1146.

Yeh, F.C., R.C. Yang and T. Boyle. 1999. **POPGENE version 1.31.** University of Alberta, Canada.

Zietkiewicz, E., A.Rafalski and D.Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. **Genomics** 20: 176-183