Random Amplified Polymorphic DNA Analysis of Galanga (*Alpinia* spp.) Accessions

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ABSTRACT

Thirty-seven galanga (Alpinia spp.) accessions, 31 cultivated and 6 wild landraces from different areas in Thailand were evaluated for genetic diversity, using random amplified polymorphic DNA (RAPD) primers. Out of 22 random primers used in this study, eight primers (OPA20, OPB18, OPC09, OPD02, OPD11, OPG13, OPK12 and OPAX17) produced a total of 73 polymorphic bands. UPGMA cluster analysis of genetic similarity estimates (Jaccard's coefficient) separated the accessions into 5 major clusters. The dendrogram showed no relation with their morphological characters such as type, color of rhizome and collection sites which were indicated by the regions of Thailand. However, this study illustrated that RAPD analysis could be a useful tool to evaluate genetic diversity in galanga accessions. The highly- informative primers identified in this study would be available for further genetic analysis of galanga for plant selection and improvement.

Key words: Galanga, *Alpinia* spp., RAPD, Fingerprint

INTRODUCTION

Galanga (*Alpinia* spp.) is a member of Zingiberaceae family. It has originated in South East Asia, probably southern China and is now cultivated in Indochina, Thailand, Malaysia and Indonesia. Galanga is a very popular spice in South East Asia and especially typical for the cuisine of Thailand. Its rhizome is used not only as a common spice to flavor soups and many other dishes but also as a medicinal and aromatic plant.

Srisornkampol (1996) reported the test of extract from galanga by TLC-bioassay with the fungi *Cladosporium cladosporioides.* The active part was then purified by preparative chromotography and the structural elucidation identified by GC-MS spectroscopy method was confirmed as 1´- acetoxychavicol acetate. The study of Bhasabuttra (1997) found that galanga extracts could inhibit the growth of *Colletotrichum gloeosporioides* (Penz.) Sacc. The extract purified with ethylacetate by column chromatography technique showed the efficiency to control the anthracnose postharvest disease on the surface of mango's fruit (Jariyanusorn, 2002). Itokawa et al., (1987) also revealed that 1´- acetoxychavicol acetate and 1´- acetoxyeugenol acetate from galanga had the power to inhibit cancer Sarcoma 180 ascites in mouse. Consequently, galanga shows potentials for future benefit.

Galanga in Thailand consists of many local varieties, showing wide variations in morphological characteristics. Classification of galanga varieties using only morphological characteristics of the plant can not identify the differences due to its close relationships. Phenotypes of galanga may also be influenced or modified by environmental conditions (Sakdren et al., 1994), thereby confusing the classification based only on morphology.

At present, among several molecular approaches employed to assess genetic diversity and relationship in plant species, RAPD (random amplified polymorphic DNA) analysis is the simplest and least laborious method. The information on genetic diversity and relationship within and among crop species is essential for the efficient utilization of plant genetic resource collections (Irwin et al., 1998). The evaluation of galanga genetic diversity and relationship is still insufficiently carried out. Therefore, this study is concerned with the evaluation of the genetic diversity and relationship of galanga varieties by RAPD analysis.

MATERIALS AND METHODS

Plant material and DNA extraction

Thirty-seven galanga accessions were collected from different areas of Thailand (Table 1). Total DNA was extracted from 50 mg of young leaf tissue, using the SDS extraction procedure (Kuntapanom and Ikeda, 1998).

Table 1. Accessions of galanga used in the variation study

	Name (colored-sized rhizome, type)	Collection site
1	KhaDang (red-medium, cultivated)	Khugtaphao, Uttaradit
2	KhaYuek (white-large, cultivated)	Khugtaphao, Uttaradit
3	KhaLeang (yellow-medium, wild)	Khugtaphao, Uttaradit
4	KhaSaku (red-small, wild)	Khugtaphao, Uttaradit
5	KhaYuek (white-large, cultivated)	Banhuahad, Uttaradit
6	KhaDang (red-medium, cultivated)	Banhuahad, Uttaradit
7	KhaPar (red-medium, wild)	Banhiha, Uttaradit
8	KhaDang (red-medium, cultivated)	Tron, Uttaradit
9	KhaDang (red-medium, cultivated)	Kosumpee, Kamphaengphet
10	KhaYuek (white-medium, cultivated)	Bansakaew3, Kamphaengphet
11	KhaYuek (white-large, cultivated)	Bankang9, Nakhon Sawan
12	KhaYuek (white-large, cultivated)	Bankang3, Nakhon Sawan
13	KhaYai (white-large, cultivated)	Banyantar, Nakhon Sawan
14	KhaLing (red-medium, wild)	Banyantar, Nakhon Sawan
15	KhaTadang (red-large, cultivated)	Bankardan, Nakhon Sawan
16	KhaDang (red-medium, cultivated)	Bansalaloi, Lop Buri
17	KhaLing (red-medium, wild)	Khon Kaen
18	KhaYuek (white-medium, cultivated)	Bansakaew, Kamphaengphet
19	KhaTadang (red-medium, cultivated)	Banaoyklongthakhuy, Lop Buri
20	Kha (red-medium, cultivated)	Takai, Chachoengsao
21	KhaKaw (red-medium, cultivated)	Bankrokanya, Samut Prakan
22	KhaLeang (yellow-medium, wild)	Bankrokanya, Samut Prakan
23	Kha (red-medium, cultivated)	Barpar, Samut Prakan
24	Kha (red-large, cultivated)	Barpar, Samut Prakan
25	KhaDang (red-medium, cultivated)	Panumsarakam, Chachoengsao
26	KhaNoldang (red-large, cultivated)	Nayararm, Rayong
27	Kha (red-medium, cultivated)	Lapsing, Chanthaburi
28	KhaDang (red-medium, cultivated)	Nongbon, Srakaew
29	KhaKut (white-large, wild)	Bantayag, Srakaew
30	KhaLuang (white-small, cultivated)	Taham, Prachin Buri
31	KhaTadang (red-medium, cultivated)	Banna, Nakhon Nayok
32	KhaNol (red-medium, cultivated)	Banna, Nakhon Nayok
33	KhaYai (white-large, cultivated)	Banna, Nakhon Nayok
34	KhaTadang (red-medium, cultivated)	Jombuo, Rachaburi
35	KhaTadang (red-medium, cultivated)	Danmakhamthia, Kanchanaburi
36	KhaDang (red-medium, cultivated)	Uthong, Suphan Buri
37	KhaDang (red-medium, cultivated)	Pothong, Angthong

Note: Rhizome diameter; large 4-6 cm, medium 2-4 cm, small 1-2 cm.

PCR amplification for RAPDs

PCR amplification was performed according to Arunyawat (1997), using primers synthesized by Operon Technologies (Alameda, USA). The reaction mixture (20 µl) consisted of 1x Reaction buffer (QIAGEN), 2 mM $MgCl_2$, 150 µM dNTPs, 40 ng of each primer, 1 U *Taq* DNA Polymerase (Promega) and 30 ng template DNA. PCR was carried out in a thermal cycler: GeneAmp PCR system (MJ Research model PTC 100) under the following conditions (Table 2). The amplified products were electrophoresed in a 2% agarose gel, stained with ethidium bromide and photographed by UV transilluminator (Syngene) for data analysis.

Table 2. PCR condition.

Temperature (˚C)	:	93	34	72	;	93	36	72	;	93	37	72	;	72
Time (minutes)	:	1	1	2	;	1	1	2	;	1	1	2	;	5
No. of cycle (cycle)	:		2		;		2		;		36		;	1

Statistical analysis of genetic variation

The reproducible and well-resolvable bands were scored from the photographs. The presence of the band was coded as 1 whereas the absence was coded as 0. The data matrices were analysed by the SIMQUAL program of NTSYS-pc (Version 1.8) and similarities between accessions were estimated, using the Jaccard coefficient. Dendrogram was produced from the resultant similarity matrices, using the UPGMA method.

RESULTS

Thirty-seven galanga accessions, 30 cultivated and 7 wild landraces from different areas in Thailand were used for RAPD analysis. Out of 22 random primers, eight primers (OPA20, OPB18, OPC09, OPD02, OPD11, OPG13, OPK12 and OPAX17) produced a total of 73 polymorphic bands. Band sizes ranged from 0.75 to 2.5 kb. An example of the amplification patterns obtained with one RAPD primer is presented in Figure 1.

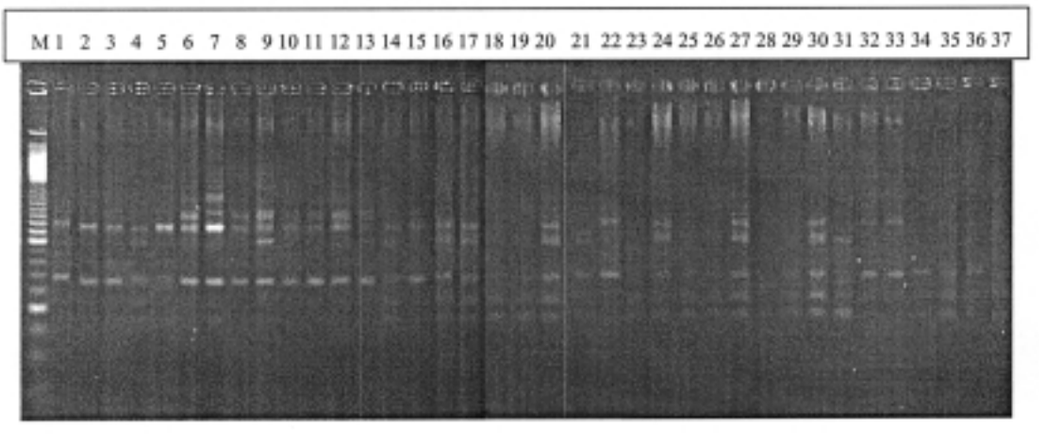

Figure 1. Amplification patterns obtained from 37 galanga accessions using RAPD primer OPAX17. Numbers of lanes represent galanga accessions as listed in Table 1. M: 100 bp DNA Step Ladder size marker.

Based on RAPD bands amplified by 8 primers, genetic distances among the 37 accessions were calculated and a dendrogram was constructed by UPGMA method (Figure 2). The accessions were divided into five major clusters in which common characteristics such as collection sites and morphological characteristics among members were not separated. The first cluster consisted of two red-medium cultivated rhizome accessions. The second cluster consisted of one red-medium cultivated and one red-medium wild rhizome accessions. The third cluster included two red-medium cultivated and one white-medium cultivated rhizome accessions. The fourth was the largest cluster, including thirteen cultivated- and four wild accessions, most of which had red-medium rhizome. The fifth cluster included two wild- and eleven cultivated accessions that were of six white-, six red- and one yellow rhizomes.

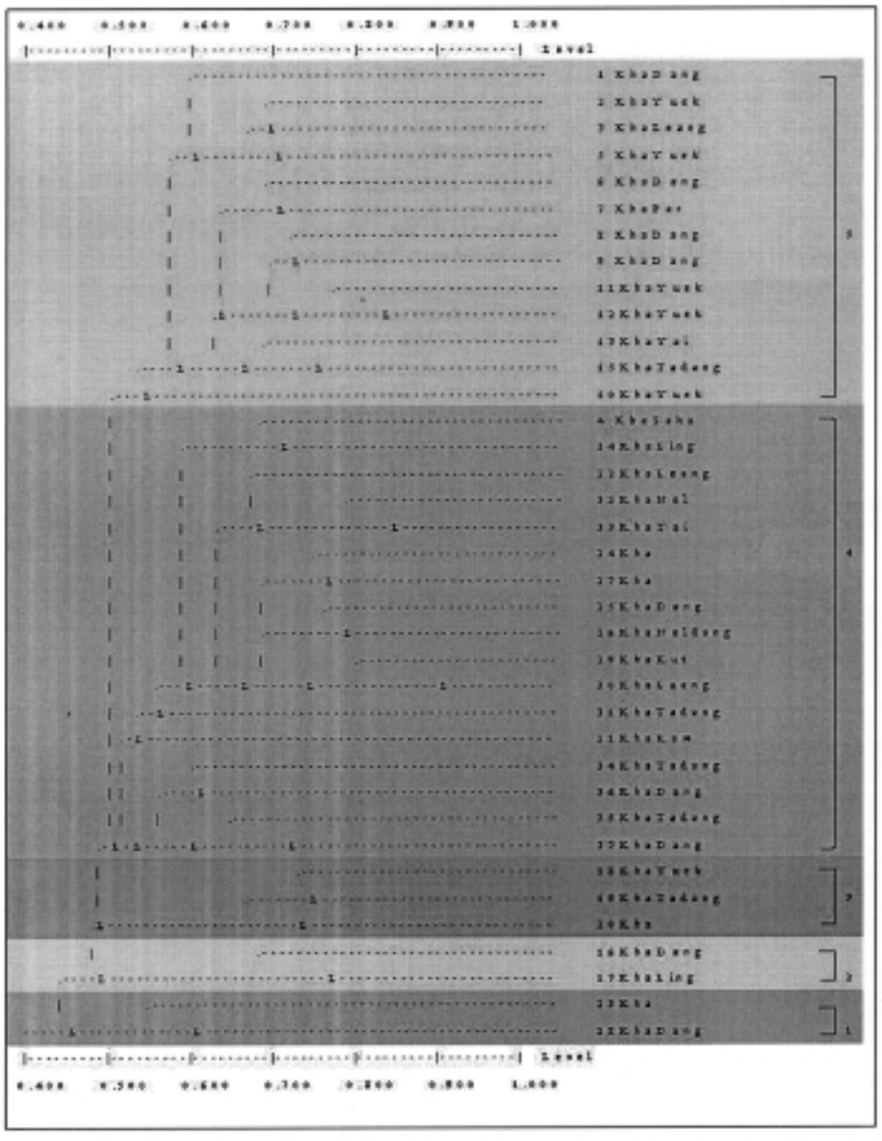

Figure 2. A dendrogram of 37 galanga accessions from Thailand based on RAPDs using UPGMA method.

DISCUSSION AND CONCLUSIONS

Random amplified polymorphic DNA (RAPD) technique has been increasingly used for the determination of genetic variability in various taxa. RAPD is particularly useful for rapid detection of divergence and for the identification of DNA markers between investigated taxa (Hadrys et al., 1992). Ochiai et al., (2001) revealed that RAPDs were able to distinguish taro accessions of Hawaiian origin and between triploid/diploid accessions which were found to be monomorphic with isozymes. These studies show that molecular markers can readily dissect genetic differences between closely-related genotypes as compared to isozymes.

In this study, RAPD technique using 8 RAPD primers (OPA20, OPB18, OPC09, OPD02, OPD11, OPG13, OPK12 and OPAX17) was able to separate 37 accessions of galanga into 5 clusters. The wild accessions did not make an independent cluster. Though four of seven wild accessions (# 4, 14, 22 and 29) adjoined one another in the fourth cluster, others spread over three clusters. This suggests that the wild type may have been developed to the cultivated type. Most of cultivated accessions have been improved from local varieties by conscious breeding. As for cultivated accessions, they were classified into five clusters without any relation with the morphological characteristics or collection sites.

The patterns were not geographically specific. It could be explained that galanga has been concurrently moved across vast geographic locations within Thailand, therefore, genetic differentiation of *Alpinia* spp. has been affected. In a review of taro (Irwin et al., 1998), it was thought that taro grown in Hawaii were introduced by Polynesian immigrants. It was suggested that the large number of varieties had been derived from crosses made by old Hawaiians and/or selection and propagation of mutant accessions. However, the DNA fingerprints indicated significant genetic diversity between them. Therefore, RAPDs were used routinely by plant breeders to identify genetic variation.

In this galanga study, the available information on classification could not be drawn from the constructed dendrogram due to lack of relationship between the clusters, the morphological characters and the collection sites in each regions of Thailand. It was possible that galanga collected from different areas were of similar varieties. More importantly, nomenclature of galanga varies in different areas and therefore complicates the classification system.

In studying the genetic relationship of galanga, RAPD data together with morphological and physiological data were required. When combined, the data could be used to select the galanga of interest. Genetic improvement of galanga should be based on molecular variation as well as morphological differences. Although the markers from this study could not provide an accurate estimate of genetic relationships among accessions with morphological data, the technique could be conducted to randomly screen a large number of galanga and thus provide a more reliable sample of data for estimation of genetic relationship. This study illustrated that RAPD analysis is a useful tool to evaluate genetic diversity in galanga accessions. The primers identified in this study will be used for further genetic analysis and breeding program of galanga.

ACKNOWLEDGEMENTS

This study was supported by the Subproject: Graduate Study and Research in Ag-biotech, Faculty of Agriculture, Chiang Mai University. The authors also express sincere thanks to Dr. Weenun Bundithya for comments on the manuscript and to Shinshu University in AIEJ Short-term Student Exchange Promotion Program for training support to Orapin Saritnum.

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