CHAPTER V

DISCUSSION

Basically, genetic studies at DNA level were limited due mainly to insufficient DNA amount required by classical molecular approaches. Therefore genetic analysis such as hybridization-based RFLP was not practised in this study. After the polymerase chain reaction (PCR) has been introduced, it is much more convenient to study other genetic markers (PCR-RFLP, RAPD, microsatellite markers) which could be analysed without the requirement for large amount of DNA.

The samples representing accurate geographic origins were required. The problem of this study, however, was resulted from sampling strategy. It was difficult to collect *Alpinia* spp. throughout the geographic ranges of the origin. Difficulties for sampling of giant galanga were that it was introduced and exchanged among areas, therefore geographic origin of *Alpinia* spp. in Thailand is not accurate.

In the present study, genomic DNA of *Alpinia* spp. was extracted using SDS extraction procedure. This DNA extraction was simple and rapid. Moreover, the quality of DNA was good. The amount of extracted DNA was enough to use for PCR amplification.

On the basis of randomly amplified polymorphic DNA (RAPD), this technique has been increasingly used for determination of genetic variability in various taxa. RAPD is particularly useful for rapid detection of divergence and for identification of DNA markers between investigated taxa (Hadrys *et al.*, 1992). As Swoboda and Bhalla (1997) used this technique to determine inter- and intraspecific variation of wild and cultivated forms of fan flower, *Scaevola* spp.. Large genetic differences among these species were found from RAPD analysis suggesting the possibility to apply this approach for breeding programs of these taxa at both intra- and interspecific levels.

On the other application, RAPDs were able to distinguish among taro accessions of Hawaiian origin, and between triploid/diploid accessions which were found to be monomorphic with isozymes. These studies confirm evidence by others which show that molecular markers can

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more readily dissect genetic differences between closely related genotypes as compared to isozymes (Ochiai *et al.*, 2001).

Evidence from recent analysis of marker data from other crops has shown that isozyme agreement with pedigree data is poor (Dudley, 1994). This is due to the low number of isozyme markers available to obtain adequate representation of the genome.

Comparisons between molecular markers have indicated that marker type (RFLP, AFLP, RAPD) was unimportant in separating genetic relationships among germplasm. RAPDs would be the marker of choice, since they offer the advantage of being technically undemanding, use no radioactivity or polyacrylamide, and they are relatively cost effective as compared with the other procedures. Further, RAPD markers tend to reside in regions with many repeated sequences and therefore in non-coding regions which are more susceptible to mutation. Consequently, they usually reveal more polymorphisms compared with isozymes or RFLP which are mostly representative of conserved genome regions.

As for RAPDs, The use of this marker to genetically fingerprint plants which are morphologically similar or indistinguishable has been established as a reliable, efficient and very informative tool.

In this study, randomly amplified polymorphic DNA (RAPD) technique has been able to separate 37 accessions of giant galanga into 5 clusters (Figure 4.11) using 8 RAPD primers (OPA20, OPB18, OPC09, OPD02, OPD11, OPG13, OPK12 and OPAX17). These 5 major clusters revealed as follows:

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 13 cultivated- and 4 wild accessions, most of which had red-medium rhizome.

The fifth cluster included two wild- and eleven cultivated accessions that six white-, six red- and one yellow rhizome accessions.

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As these results, though four of seven wild accessions (# 4, 14, 22 and 29) adjoined one another in the fourth cluster, other wild accessions spread over three clusters and did not make an independent cluster (Figure4.11.1). This suggests that the wild type was 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 their morphological characters. In addition, giant galangas had no relationships between the clusters and the color of rhizomes (Figure 4.11.2) or the collection sites which were indicated by the regions of Thailand (Figure 4.11.3).

The mean genetic distances of overall primers for all possible pairwise comparisons were subjected to classical reconstruction (using UPGMA). It indicated large genetic differences between congenital species like *Alpinia* spp. Nevertheless, non-geographically specific patterns were observed among all samples of giant galanga. The reason to explain this was that they have been concurrently moved across vast geographic locations within Thailand, genetic differentiation of *Alpinia* spp. has been affected. In a review of taro (Irwin *et al.*, 1998), it revealed that taro grown in Hawaii was thought to have been introduced by Polynesian immigrants. Although it was thought that the Polynesians brought only a few varieties, approximately 300 have been reported to have existed in Hawaii at one time (Handy and Handy, 1972; Krauss, 1993). It had been suggested that the large number of varieties had been derived from crosses made by old Hawaiians and/or selection and propagation of mutant accessions. The phylogenetic tree shows that Hawaiian accessions were separated into 6 branches. One branch contained the majority of the Hawaiian accessions, which were closely related with about 80 % similarity. This group consisted of accessions that were the more important commercial ones, and suggested that the Hawaiians had favored them.

In this giant galanga study, the available information on classification could not be drawn from the constructed dendrogram (Figure 4.11) because of no relationships between the clusters and the morphological characters and the collection sites in each regions of Thailand. All this could be due to giant galangas in the same group that were collected from different areas, were similar varieties. More importantly, nomenclature of galanga was varied in different areas and therefore complicated the classification system. Even though the RAPD markers were not related to the phenotype and collection sites, they may possibly be concerned with the gene

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expression involving anti fungal agent synthesis in giant galanga on which the environment has some effects.

In studying, the genetic relationships of giant galanga, RAPD data together with morphological and physiological data were required. Overall data suggested that in combined, the data could be used to select the high potential giant galanga. Of interest was the fourth cluster being observed as the largest cluster, including 13 cultivated- and 4 wild accessions, most of which had red-medium rhizome. Included were the high potential anti-fungal agent accessions from the East site such as accession No. 25, 27 and 30. The accession No. 21, another accession with high potential, was also in this cluster. Though it was collected from the Central, the site was close to the East. So, it would be possible for the accession to spread around the areas.

Regarding the genetic information and the genetic similarity (or distance), it was found that some RAPD markers were probably linked to the high potential anti-fungal agent from giant galanga. The 950 bp (using OPB18), 1300 bp (using OPC09), 1300 bp, 1400 bp, 1900 bp (using OPK12), and 950 bp, 1000 bp (using OPAX17) fragments were present and specific to the high potential accession. While, only the 1300 bp (using OPK12) fragment was present in all of low potential accession. These fragments may be useful as RAPD markers for plant improvement.

Genetic improvement of giant galanga should be based on molecular variation as well as morphological differences. Although, this study showed the markers which cannot provide an accurate estimate of genetic relationships among accessions within morphological data. However, the technique could be conducted to screen galanga at random and thus provide a more reliable sample of data for estimation of relationships which concern to selection of giant galanga producing anti-fungal agent.

The DNA fingerprints indicated significant genetic diversity among galanga. This argues that crosses based on morphology alone can be limiting and misleading to the breeder. The current study provided database for giant galanga breeders to make informed choices in selection of parental accessions to use in a breeding program based upon genetic diversity.

RAPD markers appear to be a good choice for assessing genetic relationships in giant galanga with polymorphism levels sufficiently high to establish informative fingerprints with relatively few markers. The highly informative primers identified in our fingerprinting studies could be useful in future genetic analysis to establish evolutionary and genetic relationships.

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RAPDs are currently used routinely by plant breeders to identify genetic variation (Keil and Griffin, 1994; Lashermes *et al.*, 1996; Perron *et al.*, 1995; Fico *et al.*, 2003), locate regions of the genome linked to agronomically important genes (Reiter *et al.*, 1992; Martin *et al.*, 1991; Michelmore *et al.*, 1991; Pillay and Kenny, 1996; Ochiai *et al.*, 2001), and facilitate introgression of desirable genes into commercial accessions (Stuber, 1992; Lavi *et al.*, 1994).

RAPD are useful not only for determination of genetic variation and population structure in various organisms but also for identification of markers linked to biologically important phenotypes such as resistance to diseases (Irwin *et al.*, 1998; Tangjingjai, 1998). Bai *et al.* (1997) screened seven hundred and fifty-six arbitrary primers for identification of RAPD markers linked to common bacterial resistance genes in a bean plant (*Phaseolus vulgaris*) while introgression of nematode (*Meloidogyne arenaria*) resistance genes in *Arachis hypogaea* from *A. cardenasii* can be identified by RAPD analysis of a F_2 population derived from the cross between these two species (Garcia *et al.*, 1996).

When the reference population of a species under investigation is available, RAPD is also an important approach that can be used for construction of a genome map. This application is widely used for mapping of plant genomes at present (Marillia and Scoles, 1996).

Regarding the convenience and flexibility of RAPD, it can be concluded that RAPD is suitable to be used for population genetic studies of various taxa (Tangjingjai, 1998; Songram, 1997). In the present study, it suggested that this technique may be able to detect the genetic variation of *Alpinia* spp. for selection of the levels of anti-fungal substance in giant galanga. These markers are being developed to identify anti-fungal (1'-acetoxychavicol acetate) gene(s) of gaint galanga in the future.

However, this study illustrated that RAPD analysis is a useful tool to evaluate genetic diversity in giant galanga accessions. The highly informative primers identified in this study will be available in future genetic analysis and breeding program of giant galanga.

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