CHAPTER II

LITERATURE REVIEW

Alpinia galanga Linn., one of the members in Zingiberaceae family, is known commonly as giant galanga or greater galangal also known in Thai as Kha. This galanga and similar forms derive from the Arabic names khulendjan or khalangian, which themselves are probably a distortion of Chinese liang-kiang "mild ginger". The genus name *Alpinia* is in memory of an Italian Botanist (Prospero Alpina, 1533-1617), and the alternative name *Languas* is based on the Malay name lengkuas, which in turn may relate to the former mentioned Chinese term. It had originated in South East Asia, probably southern China; it is now cultivated in Indochina, Thailand, Malaysia and Indonesia.

Giant galanga or greater galangale, mostly referred to simply as galanga or galangale, is perennial and growing in tropical climate. Botanical characteristics of the plant were described as follows (Srisornkampol, 1995): the stem is 2.0-2.5 m high. This galanga-like rootstock (rhizome) that built up from cylindrical subunits (circular cross-section), whose pale-reddish surface is characteristically cross-striped by reddish-brown, small rings. The interior has about the same color as the skin and is hard and woody in texture. Leaves is alternate single, acuminate tip blade with few hairy, 20-25 cm long and 7-11 cm wide. It has the outer layers which can be pealed from the soft herbacous stem of a plant. Although galanga leaves are aromatic, they are not often used for flavored purposes. Inflorescence is a panicle, 15-30 cm long. Flower has small size and white blooms. It normally blooms in mid summer. Fruit is roundish or oblong shape, 1 cm in diameter, orange-reddish but black color in mature fruit, 2-3 seeds. This is Asian spice plant cultivated for its rhizomes. The odor is aromatic, and the taste pungent. Its rhizome is used not only as a common spice for flavor soups and many other dishes but also as a medicinal and aromatic plant. It is a very popular spice in whole South East Asia and especially typicalness for the cuisine of Thailand. This plant is also known and used in Malaysia, Indonesia, Cambodia, Vietnam and Southern China.

Galanga may be used fresh or dried, which makes a great difference in flavor. Fresh galanga has a pure and refreshing odor and a mildly spicy flavor. It is the galanga of choice for all Thai foods, where it is often added to soups in form of thin slices. The ground fresh galanga rhizome is an essential ingredient in most curry pastes. Giant galanga is like ginger, its aroma merges well with garlic. Dried and powdered galanga is less fresh but more spicy, something in between of ginger and cinnamon. Dried galanga is also sold in the form of slices that must be reconstituted in warm water and come closer to fresh galanga in their flavor. In most South East Asian countries dried galanga is employed only whenever fresh galanga is not available. Indonesians, for example, frequently use slices or powder of the fresh or dried rhizome for the characteristically sweet Jawanese curries.

Giant galanga is widely used in traditional medicines in many countries. Phytochemical study revealed that the compounds in *Alpinia galanga* are resisted and non-resisted fungal substance. The resisted fungal substances are (E)-8 (17), 12-labddiene-15, 16-dial and (E)-8β (17)-epoxylabd-12-ene-15, 16-dial, and galanal A and B, and 1′-acetoxychavicol acetate and 1′-acetoxyeugenol acetate. The others were shown in Table 2.1 as follows (Morita and Itokawa, 1987; Itokawa *et al.*, 1987; De Pooter *et al.*, 1985; Barik *et al.*, 1987):

Table 2.1 The substance in rhizome and seed of *Alpinia galanga.*

Part of plant	Substance
Rhizome	1′-Acetoxychavicol acetate
	1′-Acetoxyeugenol acetate
	α-Bergamotene
	β-Bisabolene
	Borneol
	Bornyl acetate
	Camphene
	Caryophyllene oxide
	Chavicol
	Chavicol acetate
	1,8-Cineole

Table 2.1(continued)

Part of plant	Substance
	p-Cymene
	p-Cymeol
	Citronellyl acetate
	ar-Curcumene
	[di-(p-hydroxy-cis-styryl)] methane
	p-hydroxycinnamaldehyde
	Eugenyl acetate
	trans-β-Farnesene
	Geranyl acetate
	α-Humulene
	Limonene
	Linalool
	Methyleugenol
	2-Methylpropyl acetate
	Neryl acetate
	Pentadecane
	α-Pinene
	β-Pinene
	Sabinene
	Santalene
	γ-Terpinene
	α-Terpineol
	4-Terpineol
	Terpinolene
	Tridecane
	Unidentified [M] + at m/z 134
	Unidentified [M] + at m/z 134
	Unidentified [M] + at m/z 136

Table 2.1(continued)

Part of plant	Substance
Seed	Galanal A and B
	Galanolactone
	(E)-8(17), 12-Labddiene-15,16-dial
	(E)-8β(17)-Epoxylabd-12-ene-15,16-dial

Classical methods for genetic variation studies

Classification, variation and evolution of various species have been investigated by several different biological methods such as morphology, physiology and ecology. However, each method has several limitations. Systematic studies based on morphometric analysis require a large number of samples and experienced scientists to decide whether investigated characters are informative (Rinderer, 1986). More importantly, some morphometric characters are often environmentally influenced therefore populations of a particular species may be misclassified due to ecological variants. Accordingly, the classical method has been increasingly confirmed by molecular techniques, based on protein or DNA polymorphisms (Weising *et al.*, 1995).

Molecular markers for population genetic analysis

Traditional methods such as comparative morphology and physiology have been used for evaluation of genetic variability of various taxa and these methods are not adequate for study genetic variation. During the past decade, traditional methods have been increasing complemented by molecular techniques, the development of so-called "molecular markers" which are based on polymorphisms found in proteins or DNA.

Protein markers

For the generation of molecular markers based on protein polymorphisms, this method involves separation of native proteins by net charge under influence of an electricurrent, followed by application of histochemical stains to reveal the enzyme or other protein products of particular, specifiable gene. The majority of protein markers are represented by allozymes.

Allozyme

Allozyme electrophoresis has been the predominant tool used for studying genetic variation, although its preeminence is being increasingly challenged by direct DNA analysis. Some studies used seed protein patterns as the markers in plants, the majority of protein markers are represented by allozymes. Allozyme electrophoresis has been successfully applied to many organisms from bacteria to animals and plants since the 1960s. This approach is relatively straightforward and easy to carry out. A tissue extract is prepared and electrophoresed on the supporting media (usually starch or polyacrylamide gels) according to the net charges and sizes. The protein band can then be visualized by specific histochemical stains of investigated enzymes. Once the electrophoresed gel are stained, the status of homo- or heterozygosity at such a locus can be examined. The number of band is reflected from configuration of the enzyme molecule (mono, di or tetramers coupling with homo or heterozygotic states). The positions of polymorphic bands are genetically informative (Weising *et al.*, 1995)

The advantage of allozyme electrophoresis primarily relates to its speed and relatively low cost: data on hundreds of individuals at several loci can be amassed within a short period of time. Equipment demands are modest and personnel can be trained quickly although interpreting gel patterns sometimes requires considerable experience. Disadvantages are the strict requirement of fresh or frozen tissue, the need for more material than DNA methods, protein loci evolve more slowly than non-coding DNA sequences. Theoretically, the allozyme approach is a reasonably powerful technique as a large number of individuals can be determined in a limited period of time. However, it has some limitations. For instance, synonymous mutation is not able to be detected. Likewise, nucleotide substitutions changing one non-polar amino acid to another do not alter the electrophoretic mobility of the protein. As described previously, allozymes underestimate levels of genetic variation due to its low ability to detect polymorphic loci. Therefore, allozyme analysis may not be an appropriate technique for evalution of genetic variability.

DNA marker

Analysis of polymorphisms at the DNA level is the direct approach to study genetic variation at both inter- and intraspecific levels. Theoretically, various DNA-based techniques, having different sensitivity of detection are available but the most important factor is to select the most appropriate technique (e.g. reasonable sensitivity, cost-effective, less time consuming) to answer a particular problem.

Nuclear DNA, mitochondrial DNA, and nuclear ribosomal DNA have been commonly employed in genetic variation studies. The following discussion describes some of the attributes of mitochondrial and nuclear ribosomal DNA.

A. Mitochondrial DNA

Genetic polymorphisms using analysis of mitochondrial DNA (mtDNA) have been employed. Since 1979 in most cases, it is more powerful than that of allozymes for determination of population structure, biogeography and phylogenetic relationships (Avise *et al.*, 1987). Due mainly to its small genome size, rapid rate of evolutionary changes and maternal inheritance, mtDNA is also suitable for examining history and evolution among closely related taxa (Hoy, 1994). Studies of mtDNA polymorphisms have been reported using RFLP and/or DNA sequencing (Navajas *et al.*, 1996).

MtDNA has a number of positive properties which is suitable for evolutionary and systematic studies. These are (1) maternal inheritance, (2) general conservation of gene order and composition within the same division, (3) a rapid rate of sequence divergence, and (4) small size and abundance resulting in its easy isolation (Hoy, 1994). Analysis of mtDNA in various taxa causes better understanding of this extrachromosomal DNA. It was subsequently found that the universal genetic code system is not valid for some genetic codes.

B. Nuclear ribosomal DNA

Ribosomes are a major component of cells involving in translation of messenger RNAs into proteins. Ribosomes consist of ribosomal RNA (rRNA) and proteins and can be dissociated into a large and a small subunits. The ribosomal DNA is frequently used to examine interspecifically evolutionary relationships among various taxa because they are universally

present in all organisms having the protein synthesising system. The rDNA is versatile to be used for detection of polymorphisms at different levels because this moderately repeated region contains both conserved (e.g. 18S and 28S) and more variable regions (e.g. ITS, IGS).

In eukaryotes, the nuclear ribosomal genes encoding the 18S (small subunit) and 28S (large subunit) rRNAs are clustered as arrays of tandem repeats located in the nucleolar organising regions of the chromosomes (Fig. 2.1). There are approximately 100 to 500 copies of rDNA repeated transcription units found in most animals (Hoy, 1994). The repeated transcription unit is composed of part of the promoter region, external transcribed spacer (ETS), an 18S rDNA coding region, an internal noncoding transcribed spacer (ITS), a 28S rRNA coding region, and an intergenic nontranscribed spacer (IGS).

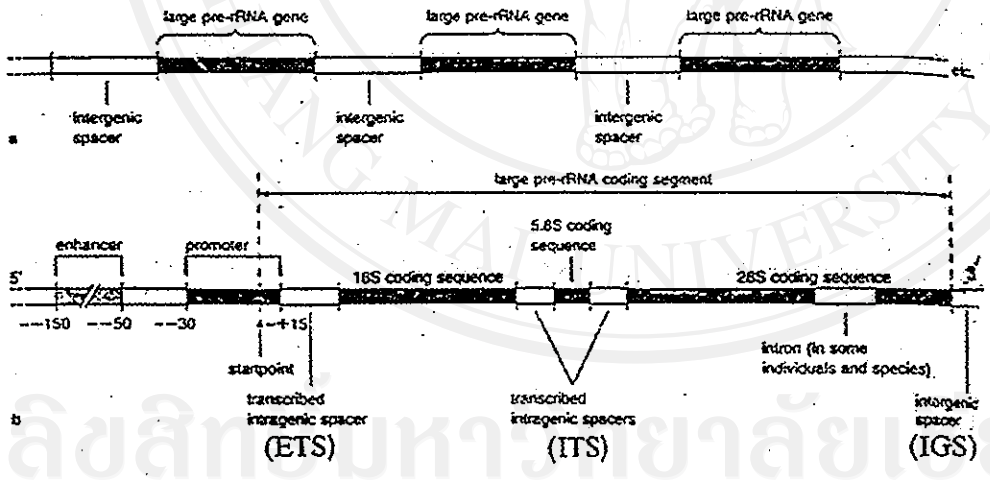

Figure 2.1 Large pre-rRNA genes and their spacer: (a) The pattern of tandemly repetitive repeats of the large pre-rRNA genes, (b) the arrangement of coding sequences and internal transcribed spacer (Wolfe, 1993).

Different portions of the rDNA repeated unit evolve with significantly different rates (Sappal *et al.*, 1995). Thus, evolutionary studies may employ different segments of the unit depending on the taxonomic levels of organisms under investigation. In general, a high degree of polymorphism has been found in the noncoding segments of the repeat unit (ETS, ITS, IGS). Thus, population and species diagnostic markers in sibling species have been studied using these segments (McLain *et al.*, 1995; Norris *et al.*, 1996; Porter and Collins,1991).

Molecular techniques based on DNA analysis

A. Restriction fragment length polymorphism analysis (RFLP)

Restriction fragment length polymorphism (RFLP) is one of several techniques used to determine DNA variation based on the assumption that digested DNA fragments illustrating identical length are similar in sequences and are from the same evolutionary origin. Technically, the target DNA digested with restriction endonucleases are size-fractionated by agarose gel electrophoresis and transferred onto a membrane. The investigated fragment(s) is identified by hybridization with the specific radiolabeled probe (Davis *et al.*, 1994). The example of RFLPs, Smith and Smith (1991) used them in hybrid corn to confirm that RFLP is a powerful tool in molecular systematics with numerous successful applications in crop species. However, RFLPs is complicated and difficult as the technique requires radioactivity which is very dangerous (Auttatinpahorkun and Auttatinpahorkun, 1993). To save time and cost of RFLP, Polymerase Chain Reaction (PCR) technique was applied to use in Randomly Amplified Polymorphic DNA (RAPD) (Williams *et al.*, 1990).

B. Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is an approach applied for population genetic and systematic studies. This technique allows short DNA fragment (usually smaller than 2 kb) to be amplified *in vitro*. To amplify a particular DNA fragment, two single-stranded complementary primers are designed to a specific motif of the DNA template. The activity of thermostable DNA polymerase in the suitable buffer system and thermo-cycling (denaturation, annealing and polymerization steps) results in exponential amplification of a given DNA fragment between the

primer sites (Weising et al., 1995).

The ability to amplify interested DNA (RT-PCR for RNAs) through PCR opens the new approach for various biological disciplines (e.g. systematics, evolution, ecology).

C. PCR-RFLPs

This technique has been, and still is, the common approach used for determination of genetic diversity at various taxonomic levels. After the DNA of interest is amplified by PCR, an aliquot of the amplification reaction is then simply digested with each restriction endonuclease. The resulting DNA fragments are electrophoresed through the appropriate gel medium (agarose or polyacrylamide) and visualized under the UV light after ethidium bromide staining. The most important advantage of this technique is that hybridization of labeled DNA probes to the target restricted DNA is obviated. Furthermore, the technique *per se* is much simpler than conventional RFLP approach.

D. Randomly amplified polymorphic DNA (RAPD) PCR.

RAPD-PCR was developed by Williams *et al.* (1990) who demonstrated that genomic DNA from a distantly related group of organisms could be amplified using a single short primer (9 or 10 nucleotides long) composed of an arbitrary oligonucleotide sequence. The primer can be randomly designed without any prior knowledge of the sequence information of organisms under investigation. The only limitation is that the primers should have at least 50% G+C content and should not contain palindromic sequences. Different random primers used with the same genomic DNA produce make the different numbers and sizes of DNA fragments (Ellsworth *et al.*, 1993). After amplification, the amplified DNA patterns can be conveniently determined by agarose gel electrophoresis. RAPD-PCR is particularly useful for species having limited genetic information or for organisms which have not been genetically investigated before.

Randomly amplified polymorphic DNA (RAPD), also known as arbitrarily primed PCR, allows the detection of polymorphisms without prior knowledge of nucleotide sequence. The polymorphisms may be used as genetic markers and may also be used for the construction of genetic maps. The method utilizes short primers of arbitrary nucleotide sequence that are

annealed in the first few cycles of PCR at low stringency. The low stringency of the early cycles ensures the generation of products by allowing priming with mismatches between primers and template. The subsequent PCR cycles are performed at a higher stringency after the generation of some initial products that now have ends complementary to the primers (Figure 2.2). Alternatively, an intermediate stringency primer annealing step may be used throughout the PCR to achieve the same outcome.

The polymerase chain reaction (PCR) was invented in 1985 by Kary Mullis, is an *in vitro* technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of known DNA sequence. PCR amplification of DNA is achieved by using oligonucleotide primers, also known as amplimers. These are short, single-stranded DNA molecules which are complementary to the ends of a defined sequence of DNA template. The primers are extended on single-stranded denatured DNA (template) by a DNA polymerase, in the presence of deoxynucleoside triphosphates (dNTPs) under suitable reaction conditions. This results in the synthesis of new DNA strands complementary to the template strands. These strands exist at this stage as double-stranded DNA molecules. Strand synthesis can be repeated by heat denaturation of the double-stranded DNA, annealing of primers by cooling the mixture and primer extension by DNA polymerase at a temperature suitable for the enzyme reaction. Each repetition of strand synthesis comprises a cycle of amplification. Each new DNA strand synthesized becomes a template for any further cycle of amplification and so the amplified target DNA sequence is selectively amplified cycle after cycle. Figure 2.2 shows the first few cycles of PCR. The first extension products result from DNA synthesis on the original template and these do not have a distinct length as the DNA polymerase will continue to synthesize new DNA until it either stops or is interrupted by the start of the next cycle. The second cycle extension products are also of indeterminate length; however, at the third cycle, fragments of 'target' sequence are synthesized which are of defined length corresponding to the positions of the primers on the original template. From the fourth cycle onwards the target sequence is amplified exponentially. Thus, amplification, as the final number of copies of the target sequence, is expressed by the formula, $(2^n-2n)x$, where:

- n = number of cycles;
- $2n$ = first product obtained after cycle 1 and second products obtained after cycle 2 with undefined length;
- x = number of copies of the original template.

Potentially, after 20 cycles of PCR there will be 2^{20}-fold amplification, assuming 100% efficiency during each cycle. The target sequence product (also known as an amplicon) which is obtained contains the oligonucleotide primer sequences at its ends. Although extremely efficient, amplification of target sequences in an exponential manner is not an unlimited process. A number of factors act against the process being 100% efficient at each cycle. Their effect is more pronounced in the later cycles of PCR. Normally, the amount of enzyme becomes limiting after 25-30 cycles of PCR, which corresponds to about 10^6-fold amplification, due to molar target excess. The enzyme activity also becomes limiting due to thermal denaturation of the enzyme during the process. Another factor that will reduce the efficiency is the reannealing of target strands as their concentration increases. The reannealing of target strands then competes with primer annealing. It is important to minimize any variance in the PCR procedure and strive to obtain cycle efficiencies of 100%.

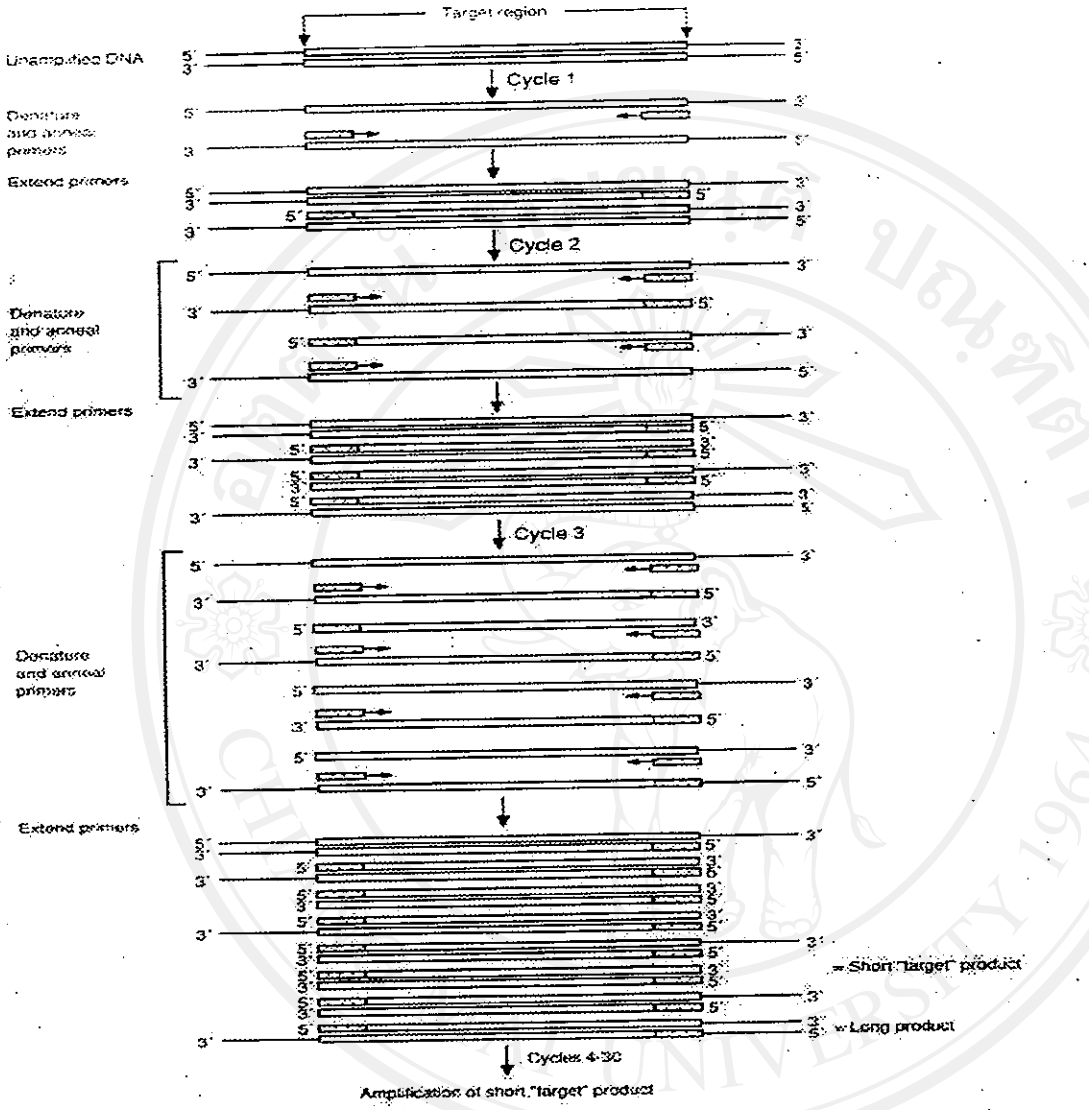

Figure 2.2 The polymerase chain reaction. PCR is a cycling process; with each cycle the number of DNA targets doubles. The strands in the targeted DNA are separated by thermal denaturation and then cooled to allow primers to anneal specifically to the target region. DNA polymerase is then used to extend the primers in the presence of the four dNTPs and suitable buffer. In this way duplicates of the original target region are produced and this 'cycle' is normally repeated for 20-40 cycles. The short 'target' products, which increase exponentially after the fourth cycle, and the long products, which increase linearly, are shown (Newton and Graham, 1994).

The basic components of a typical PCR are shown in more detail following:

- Template DNA , the size of the DNA is generally not a prime factor, if high molecular weight genomic DNAs are being used then the amplification is improved if the DNAs are digested with a rare-cutting restriction enzyme. Often the concentration of the target sequence in the template DNA is not known and it is useful to optimize the PCR with positive control DNA. Normally subnanogram quantities of a cloned template and submicrogram quantities of genomic DNA are used for PCR and optimization trials. Potentially, PCR can be used to amplify as little as a single molecule of template, although great care has to be taken when performing such experiments, particularly in the avoidance of contamination.
- Primers are designed to be exactly complementary to the template DNA. The design of the oligonucleotides is generally carried out using some simple guidelines, although several computer programs have been devised to aid primer design. In general, the primers used in PCR are between 10 and 30 nucleotides in length which allows a reasonably high annealing temperature to be used. There is no increase in specificity with primers longer than 30 nucleotides. The primers should, if possible, be made with an approximately equal number of each of the four bases, avoiding regions of unusual sequence such as stretches of polypurines, polypyrimidines or repetitive motifs. Sequences possessing significant secondary structure should also be avoided. Primer pairs should also be designed so that there is no complementarity of their $3'$ ends either *inter* or *intra* individual primers. This precaution will reduce the incidence of 'primer-dimer' formation which is an amplification artifact caused when one primer is extended by the polymerase using the other primer or itself as a template, resulting in a short incorrect product. Primers may be diluted to give a 20 μM working solution (it is often convenient when the same primer combinations are routinely used, to combine the primers in a mix where each primer is 20 μM within the mix, for subsequent dilution to up to 1 μM in the reaction mixture).
- *Taq* DNA polymerase has a $5'$ to $3'$ exonuclease activity which removes nucleotides ahead of the growing chain. It has an optimal extension rate (polymerization rate) of 35-100 nucleotides per second at 70-80 °C which is the optimum temperature range for the enzyme. Processivity, which is the average number of nucleotides incorporated before the enzyme dissociates from the DNA template, is relatively high for *Taq* DNA polymerase.

- Deoxynucleoside triphosphates (dNTPs), high-purity dNTPs, are supplied by several manufacturers either as four individual stock solutions or as a mixture of all four dNTPs. Many stock solutions are now supplied already adjusted to pH 7.5 with NaOH. PCR is normally performed with dNTP concentrations around 100 μM, although at lower dNTP concentrations (10-100 μM) *Taq* DNA polymerase has a higher fidelity. However, the optimal concentration of dNTPs depends on:
  - the $MgCl_2$ concentration
  - the reaction stringency
  - the primer concentration
  - the length of the amplified product
  - the number of cycles of PCR.

  For optimization of a particular PCR it may be necessary to empirically determine the best dNTP concentration.
- Buffers and $MgCl_2$,there are several buffers available for PCR. The most common buffer used with *Taq* DNA polymerase has the following components in a 10 times concentrated buffer and must be diluted 1:10 (v/v) prior to use:
  - 100 mM Tris-HCl, pH 8.3 at room temperature
  - 500 mM KCl
  - 15 mM $MgCl_2$
  - 0.1 % (w/v) gelatin.

  Buffer compositions for use with other thermostable polymerases may differ; however, most suppliers usually provide a 10x buffer for use with the respective enzyme.

  The $MgCl_2$ concentration in the final reaction mixture can be varied, usually within the range 0.5-5.0 mM in order to find the optimum. Mg^{2+} ions form a soluble complex with dNTPs which is essential for dNTP incorporation; they also stimulate the polymerase activity and increase the T_m of the double-stranded DNA and primer/template interaction. The concentration of $MgCl_2$ can have a dramatic effect on the specificity and yield in a PCR. Concentrations of 1.0-1.5 mM $MgCl_2$ are usually optimal, but in some cases, different amounts of Mg^{2+} may prove to be necessary. Generally, insufficient Mg^{2+} leads to low yields and excess Mg^{2+} will result in the accumulation of nonspecific products.

PCR can be inhibited or enhanced by many different substances over and above those described previously. Many factors may lead to inhibition of PCR and are focused here on those that are likely to be experienced frequently when setting up PCRs. These may be due to the nature of the native biological specimens and the method and reagents used to extract the DNA.

RAPD is also a PCR technique ideally suited to fingerprinting applications because it is fast, requires little material and is technically easy. It should be noted, however, that some workers have experienced the production of nonparental PCR bands in the offspring of known pedigrees. For this reason, RAPD would not be the method of choice for applications such as human paternity testing and pedigree analysis, where absolutely unequivocal results are essential. Where results are not required to be as stringent, RAPD certainly has an important role. In this context, RAPD has been used to fingerprint strains of serovars of *Bacillus thuringiensis*, the most commonly used biological insecticide. Similarly, the technique has been used to examine clinically important strains of other bacterial species. Plants are particularly suited to RAPD analysis. Thus, the technique has been used extensively in plant breeding studies, and applications to strawberries, wheat, oat, barley, soybean, tomato, potato and corn have all been reported (Newton and Graham, 1994).

For classification of plant by RAPD-PCR, it has been used to identify the 37 varieties of Australian rice (*Oryza sativa* L.) by 22 primers (10 nucleotides). The result revealed that total amplified DNAs were 144 bands of which only 67% can be used to classify rice varieties. Comparison of dendrograms between Australian rice and American rice showed very close relationships (Ko *et al.*, 1994). In addition, Yu and Nguyen (1994) used RAPD techniques for genetic classification of 9 spicies of upland rice and 4 species of lowland rice by 42 primers. It was found that the primers could amplify the DNA to a total of 260 bands of which eigthty percent could be used to separate the genetic species and showed highly differential DNA bands between japonica rice and indica rice. The others, upland rice and lowland rice showed a few difference of DNA bands. The study revealed that RAPD is suitable to classify the rice. RAPD technique showed high power to check the relationships between the variety of hybrid rice and parental rice (Wang *et al.*, 1994).

Identification of 14 varieties of broccoli and 12 varieties of cauliflower by RAPD technique was studied. It was found that 4 primers; PA01, PA02, PA03 and PA04, were able to amplify DNA that sizes ranged from 300 to 2000 base pairs. The data analysed by computer program showed that these plants could be clearly classified (Hu and Quiros, 1991). In genus *Allium*, Wilkie *et al.* (1993) used RAPD technique to study the types of *Allium cepa* by 20 arbitrary primers. It was found that 7 primers generated the polymorphic bands which can be used to classify this *Allium* group. RAPD is a powerful tool in molecular systematics with numerous successful applications in crop species (Irwin *et al.*, 1998).

The other application was genetic analysis of 15 cultivars of wheat by RAPD technique. It was found that 109 bands were amplified by 40 primers. Seventy-one bands (65 %) were polymorphic bands that can classify wheats (Joshi and Nguyen, 1993). Likewise, Marson *et al.* (1993) used RAPD technique for genetic analysis of hybrid maize (B73 x A7). The result was found that thirty-five of 47 primers amplified DNA products. Only ten primers showed polymorphisms that were used to identify the varieties B73, A7 and hybrid maize. Furthermore, classification of intraspecific Populus family was studied by Castiglione *et al.* (1993) using RAPD technique. Out of eighteen primers, 4 primers could be used to classify 32 clones of poplar (*Populus spp.*). The result showed that ninety-two percent of the total bands could be used to analyse the genetic relationships of Populus family .

Based on RAPD analysis, Munthali *et al.* (1996) used RAPD techniques to study somaclonal variation of the young sugar beet which were induced by tissue culture. Polymorphic DNAs were analysed in 3 generations from F_1 to F_3. It was revealed that the frequency of somaclonal variation was about 0.05%. This result was similar to the other methods such as isozyme and RFLP techniques. While Adam *et al.* (1993) used RAPD techniques with terpenoid study to compare juniper from Abha town in Saudi Arabia , *Juniperus excelsa* from Greek; and *J. procera* from Addis Ababa town in Ethiopia and showed that both methods could separate the difference of juniper accessions clearly. Kamaley and Carey (1995) used RAPD to select the variety of American elm (*Ulmus americana* L.). It was found that the result of this study could follow Mendel's Laws. The fingerprint of American elm DNA had always been specific regardless of DNA sources or fingerprinting techniques. RAPD was therefore chosen because the technique was easy and more appropriate for selection and analysis of plant genetics.

In Thailand, Apisittiwanid *et al.* (1995) used RAPD techniques to analise the diversity of 5 species of rice (*Oryza* spp.). Seven of 48 primers amplified PCR DNA products which were 68 polymorphic bands. The sizes ranged between 300 and 1700 bp. The rice could be divided into 4 groups which were consistent with the characteristics of species.

Genetic analysis of 10 species of *Curcuma* spp. was investigated at species and clonal levels using RAPD technique. Forty-eight random primers were used to amplify DNA fragments. Three primers designated OPA20, OPD11 and OPAB04 produced 37 polymorphic DNA bands ranged in sizes from 200 to 1700 bp. These DNA fingerprint patterns were able to distinguish and devided all the 10 species into two groups, early and medium flowering groups (Arunyawat, 1997). While, Chiangda (1998) reported that analysis of genetic relationship among twenty cultivars of Litchi (*Litchi chinensis* Sonn.) was investigated by RAPD technique. Sixty-nine arbitrary primers were applied to amplify DNA products by PCR. Five primers (OPAK10, OPAQ12, OPAS10, OPB18 and OPC09) were able to produce 60 polymorphic DNA bands with sizes ranged from 200 to 2000 bp. RAPD was a powerful technique to distinguish among Litchi cultivars. To identify the genetics of other crops, Sakdren *et al.* (1994) reported the using RAPD techniques to identify the genetics of the 10 varieties of elephant grass (*Typha elephantina*). One-hundred and fourteen primers were screened for the genetic analysis and 14 were selected providing 158 polymorphic bands which ranged in size from 150 to 2300 bp. This fingerprint was useful as genetic marker that could separate 10 varieties of elephant grass clearly. As Jamjunta (1996) studied the differentiation of 5 *Pueraria* species by RAPDs. It was found that four of 30 primers provided DNA fingerprints that could be used to separate 5 species of *Pueraria* spp. and each primer revealed the specific DNA fingerprint patterns for each of the sample. Moreover, Temeesak (1993) used RAPD techniques for classification of Brassica family. This study was focused on the relationships of interspecific hybrid and checked the differentiation of hybrid and parental turnips. It was found that four of 60 primers showed the difference of F1 hybrid. RAPD techniques could be used to save time in plant improvement.

Aims of this thesis

Prior to the present research, there have been insufficient reports on genetic variation studies concerning *Alpinia* spp. Thus, the objective of this thesis was to utilize randomly amplified polymorphic DNA (RAPD) markers in separating *Alpinia* spp. accessions from different geographic locations in Thailand for classification and selection of giant galanga cultivars which produce anti-fungal agent.