CHAPTER I

INTRODUCTION

1.1 Introduction

Rice equals wheat in importance as a staple food for mankind. Ninety percent of rice is grown and consumed in Asia. Although China is the leading producer, Thailand, Vietnam and the United States of America are the world's leading rice exporters.

Rice can be grown from tropical Asia where it originated to latitude 35°S and 53 ° N (Chang, 1979). It is grown at sea level as well as at elevations of 2,500 meters or even higher (Khush, 1975). The typical rice crop is not only grown under irrigated or rainfed, puddled, lowland conditions, but also grows in deep water area where the water reach up to 4 meters. Upland rice can be grown without standing water. These diverse cultural geographic conditions along with the force of natural selection have brought about various diversity in rice. There are approximately 120,000 cultivars (IRRI, 1980) present throughout the world.

1.2 Taxonomy and Breeding behavior

Rice has been classified into the genus *Oryza* under family Poaceae. This genus is highly variable and widely distributed throughout the world. There are about 20 well defined species, two of which the Asian rice (*Oryza sativa* L.) and African rice (*O. glaberrima*) are cultivated.

Members of the *Oryza* are characterized by 6 stamens, spikelets with a single flower and the suppression of glumes. Different authors have proposed at least 75 specific names for it various entities. Only 20-25 species are recognized by modern taxonomists (Khush, 1975). Recent taxonomic revisions have reduced the number into 20 species, although some workers do not endorse all revisions. Chang, 1979 has listed 20 species and their distributions. It can be noted that the cultivated types and their wild

relatives are diploid (2n=24) while more than half of the wild species are tetraploid (2n=48). For some species, their genomes have not been verified.

Since the *Oryza* genus contains a species complex, it has received a diversity of taxonomic and nomenclatural treatments creating confusion in the rice literature.

The genus *Oryza* is also characterized by sterility barriers and the members are grouped into species complex. The "sativa complex " includes *O.sativa, O. glaberrima* and their wild relatives. Members of this complex carries the A genome which is subdivided into A,A^b ,A^{Cu} and A^g to indicate partial sterility and minor pairing aberrations in hybrids. The "officinalis complex " are those that posses C, CD,BC or E genomes. These included *O. officinalis, O. minuta, O. eichingeri, O. punctata, O. alta, O. grandiglumis O. latiforia* and *O. australiensis.* The last complex includes *O. brachyanta* (F genome) and taxa of undetermined genome(s) such as *O. ridleyi* and *O. granulata* (Chang, 1979).

1.3 Origin and Distribution

Roschevicz first postulated in 1931 that the center of origin of genus *Oryza* was in Africa. However, a recent survey and re-examination of the geographic distribution of this genus and some possibilities of phylogeny (Oka, 1974) suggested that the valid 20 species originated from the Gonwanaland continent (Chang, 1976a, 1976b) before it drifted into separated continents about 230-600 million years ago.

The two cultigens of *O. sativa* and *O. glaberrima* are thought to represent two independent and parallel domestication (Chang, 1979). The Asia rice is believed to be domesticated 9,000 years ago and could have been cultivated independently and concurrently at many sites within a broad belt that extends from the foothill of the Himalayas to South East Asia and Southwest China. Chang (1976) strongly suggested that *O. sativa* has a diffuse origin because of the continuous distribution of *O.rufipogon,O. nivara* and the spontanea forms of *O.sativa* present in these area.

Once it was domesticated, rice was most probably introduced from the Nepal, Assam, Myanmar and Yunan area into the Yellow River Valley, and from Indochina via a coastal route into the lower Yangtze River basin where the temperate race (cool-tolerant)

became established (Chang, 1976). From China it was introduced to Japan in the third century. The short-grained cool-tolerant and low amylose race was later designated as Japonica or Sinica rice(Chang, 1976b).

The tropical race (*Indica*) spread southward into Sri Lanka and the Malay Archipelago and northward into Central and South China. The *Indica* race of China was initially grown in the middle Yangtze Valley basin probably before 200AD.From ancient India, men carried the *Indica* type westward to the Middle East, Europe and Africa (Chang, 1976).

The tall, large and bold grained *Javanica* race (bulu or gundil) of Indonesia appeared to be a more recent product of selection from the *Indica* race. Rice was being cultivated in Indonesia around 1084 BC. From these, the *Javanica* race spreaded to the Philippines, Taiwan and Japan.

Asian rice was introduced from Europe to South, Central and North America and was cultivated in the U.S.A. during the seventeenth century (Anishetty, 1983). It is believed that rice reached East Africa along Sabaer Lane more than 1,000 years ago. However, its introduction into West Africa by the Portuguese was more recent perhaps 450 years ago (Anishetty, 1983).

The cultivated African rice *O. glaberrima* exhibits less diversity than its Asian counterpart. Also its distribution is limited to tropical West Africa (Chang, 1979). *O. glaberrima* probably originated in the inland delta of the Niger river in Mali about 3500 years ago (Anishetty, 1983). Its primary center of diversity is in the swampy area of the upper Niger, and it has two secondary centers which lie to the southwest of the Guinea coast (Chang, 1979).

1.4 Evolution and Geographical Distribution

Many workers (Chatterjee, 1948,Sampath, 1962 and Oka, 1974) considered that *O. perennis* Moench was the common progenitor of both the Asian and African rice. Porters (1956) suggested that the common progenitor was a rhizomatous and floating form, but he did not name it. Chang (1976) considered the name *O. perennis* was an ambiguous designation used differently by various rice workers and which also included

the annual weed races. He further classified *O. perennis* as a wild perennial which refers to *O.rufipogon* in Asia and *O. longistaminata* in Africa. For wild annual forms he refers to *O. nivara* or *O. barthii* in Asia and Africa respectively.

There are some hypothesis which have been considered for rice evolution as follows.

1.4.1 Asian rice

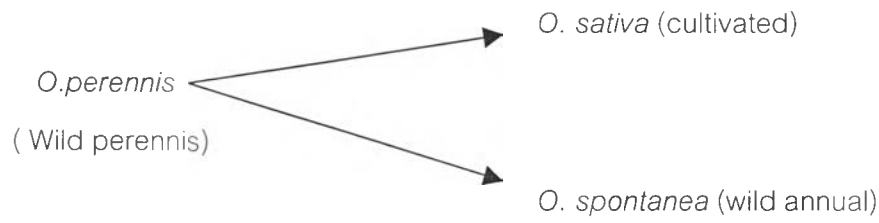
Both Asian and African rice posses the A genome (Sharma and Shastry, 1965). Ng, 1978 reviewed literatures about the ancestor of Asian rice and found many authors postulated that *O. sativa* evolved from *O. rufipogon O. officinalis O. nivara, O. minuta* and *O. australiensis* by examining their morphology and distribution. However, previous cytological works did not support this hypothesis. So he believed that *O. minuta* and *O. officinalis* could not be ancestors of *O. sativa* although they were found near the field of *O. sativa*. He had stated that *O.australiensis* which had a different genome and distribution could not really be the ancestor. Two species,*O.rufipogon and O. nivara*, which posses the A genome are most probably the ancestor of *O. sativa*. Both Oka and Chang believed these two species are the ancestor of Asian rice. However, their hypothesis differed as follows :

Chang Hypothesis

Wild perennial		Wild annual		Cultivated
O. rufipogon	⟶	*O. nivara*	⟶	*O. sativa*

(Chang 1976a, 1976b)

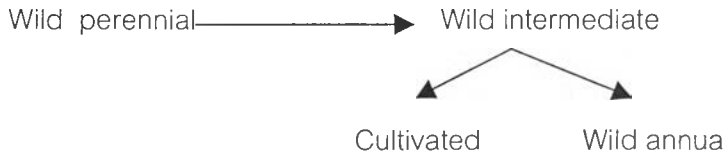
Oka Hypothesis



(Oka,1964,1974,1975,1977)

In the first hypothesis, it was assumed that domestication of *O. sativa* came by way of the annual species *O. nivara* and that the ultimate progenitor was the perennial with the general concept of the evolution of cereal crops. The second hypothesis, assumed that the wild perennial species gave rise to both the wild annual and cultivated forms.

Recently, Sano *et al.*, 1980 had proposed a new hypothesis. They considered high evolutionary potentiality, adaptability in disturbed habitat and high seed productivity. These seemed to assume that the intermediate perennial-annual populations were more likely to be the wild progenitors of *O. sativa* than perennial ones.



1.4.2 African rice

Studies of Ogbe and Williams (Anishetty, 1983) indicated that *O. barthii* was the progenitor of African rice. *O. barthii* itself was derived from *O. longistaminata* which was very variable. *O. longistaminata* grows widely throughout tropical and southern Africa. It is a perennial with extensive creeping underground rhizomes with long ligules and long slender spikelets. .

Very often *O. glaberrima* is grown mixed with annual weed races which is sometimes called *O. staphii* and considered to be an intermediate in the evolution of *O. glaberrima* from *O. barthii.*

1.4.3 Thai rice

Thai rices including wild and cultivated rice are grown in irrigated or rain-fed, paddy lowland soils, but it also grows in water as deep as 4 meters. Upland forms are grown without standing water. These diverse cultural geographical conditions along with the force of natural selection have brought about various diversity in rice. There are approximately 850 wild rices and 17,127 cultivars present in Thailand (Songkarn Chitrakon, 2001).

Wild rices in Thailand comprised of *O. rufipon* Griff (*O. perennis* Moench.) possessing diploid and A genome, *O. nivara* Sharma *et* Shastry possessing diploid and A genome,*O. Officinalis* Wall *ex* Watt possessing diploid and CC genome,*O. ridleyi* Hook possessing tetraploid and *O. granulata* Nees *et* Arn. *ex* Watt possessing tetraploid (Songkarn Chitrakon,2001).

Cultivated rice cultivars differentiated from wild rice about 7,000 years ago and are of the *indica* subspecies possess diploid AA genome. It has been recorded in the Thai history that *O. sativa* has been cultivated in northern part of Thailand not less than 5,000 years ago (Charnvit Kasetsiri, 1999). Based on archaeological evidences found, glutaneous rice and upland rice cultivation were the major practice during the 6-11th century of the Dhawaravadi Period (Charnvit Kasetsiri ,1999). It was in Ayutthaya dynasty in the 15th century, that the lowland rice cultivation and non-glutinous rice had become dominant in the central plain to promote export of rice to China in 1722-1751 (Charnvit Kasetsiri , 1999)

During these 500 years of lowland rice evolution more than 10,000 cultivars were established by farmers in this country. Native cultivars of Thai lowland rice are the precious living fossils to study evolution at the molecular level, how the alteration at the genome level made these cultivars adaptable to submergence and abiotic stress such

as saline and acid soils, adverse climatic condition such as warm and humid tropical weather.

Among these abiotic stress saline soil is the most important problem, since almost 20 % of total area (17.8 million rai) in the Northeastern part is considered saline soil. In the south about 2.3 million rai in the coastal area is known as saline soil, which show electrical conductivity (EC) between 4-6 dS m^{-1}.

A rice variety is defined as salt tolerant rice when it can grow in liquid medium containing 0.3 % NaCl (EC 6 dS m^{-1}).

From the list of lowland rice cultivars recommended by the Rice Division, Department of Agriculture during the past 50 years, 10 high quality indigenous cultivars recommended for planting in the north, central, northeast and south Thailand with known character of salt tolerance were selected (Table 1.1).

Khao Dawk Mali 105(KDML 105) is one of the salt tolerant rice that can be referred to as "the genetic wonder of Thai Rice". The Selection Committee of the Department of Agriculture has announced and recommended KDML 105 as a high-quality rice in May 1959. This cultivar is best known for its soft white, long, slender grains with sweet pandanus-leaf fragrance. In addition, KDML 105 is resistant to drought, and acid soil but not resistant to blast, bacterial blight and brown plant hopper. For more than 40 years seeds of KDML105 have been distributed to farmers throughout Thailand as one of the recommended cultivar (Yaowanuj Vejpada, 2000).

Since the central plain and northern regions are the main rice growing area of Thailand, there are many high-quality cultivars which were selected besides KDML 105, for planting in these two regions (Suthep Limthongkul, 1988).

Khao Tah Haeng 17 is another indigenous rice that was awarded a prize in 1950, and announced as a recommended cultivar in 1956 and 1965. The grain of Khao Tah Haeng 17 is pure white and strong, which is good for milling. Khao Tah Haeng 17 is a recommended cultivar both in central and northern regions of Thailand, and is known as one of the salt tolerant rice.

Leuang Pratew 123 is the cultivar introduced by the Department of Agriculture in 1955. Leuang Pratew 123 has high grain strength, high 100-grain weight and high

filled grains/panicle. Leuang Pratew 123 is tolerant to saline and acid soils but not resistant to drought.

Gow Ruang 88 is the non-glutinous rice that was introduced in 1962. Gow Ruang 88 has high 100 grain weight and resistant to saline soil and bacterial blight but not resistant to blast.

Muey Nawng 62 M is a glutinous rice originated in Ubolrajthani. This cultivar is salt sensitive, but resistant to gall midge. In 1955, this cultivar was recommended for growing in north and northeast regions of Thailand.

Five cultivars of indigenous rice were representative of the Southern Region. The breeder seeds of these five cultivars were kindly provided by Pattalung Rice Research Center, and their salt tolerance or susceptibility characteristics were provided by Mr. Samreuang Ton (personal communication).

Nahng Pa-yah 132 is a salt tolerant rice introduced by the Department of Agriculture in 1962.

Look Daeng Pattani and Leb Nok Pattani are indigenous rice of Pattani, which were both introduced in 1994. Look Daeng Pattani is salt tolerant while Leb Nok Pattani is not resistant to saline soil.

Yah Yaw and Foi Tawng are also native cultivars of southern origin, which are both salt sensitive cultivars.

1.4.4 Exotic rice

Pokkali is a tall native cultivar from Sri Lanka, which is known as a standard salt tolerant indica rice (Ahmed and Gupta,1991; Akbar, 1986).

IR 28 is a semi-dwarf cultivar bred at the International Rice Research Institute, which is known as salt sensitive cultivar (Akbar and Senadhira, 1988).

Table1.1 Common name, characteristics, recommended year, and region and country of origin of rice cultivars.

Cultivar	Type	Harvesting day	Height (cm)	Abiotic stress			Region and Country (Rec.year)	Reference
				Saline soil	Drought stress	Acid Soil		
Pokkali	non glutinous	-	-	T	-	-	Sri Lanka	Ahmed and Gupta,1991; Akbar, 1986.
Khao Dawk Mali 105	non glutinous	20 Nov.	130	T	T	T	All regions, Thailand (1959)	TRRI, Eksngwon Chuwisitkul, 2000
Khao Tah Haeng 17	non glutinous	20 Dec.	150	T	-	-	C,N, Thailand (1956, 1965)	TRRI, Eksngwon Chuwisitkul, 2000
Leuang Pratew 123	non glutinous	19 Dec.	150	T	S	T	C, N, Thailand (1965)	TRRI, Eksngwon Chuwisitkul, 2000
Look Daeng Pattani	non glutinous	Jan. –Feb.	160	T	-	T	ST, Thailand (1994)	TRRI, Eksngwon Chuwisitkul, 2000
Gow Ruang 88	non glutinous	21 Nov.	140	T	-	-	C,N, Thailand (1962)	TRRI, Eksngwon Chuwisitkul, 2000
IR 28	non glutinous	-	-	S	-	-	IRRI, The Philippines	Akbar and Senadhira, 1988.
Muey Nawng 62 M	glutinous	20 Nov.	140	S	S	-	N, Thailand (1959)	TRRI, Eksngwon Chuwisitkul, 2000
Nahng Pa-yah 132	non glutinous	16 Feb.	175	S	-	-	ST, Thailand (1962)	TRRI, Eksngwon Chuwisitkul, 2000
Yah Yaw	non glutinous	-	-	S	-	-	ST, Thailand	Pattalung Rice Research center, Samreuang Ton,2000
Foi Tawng	non glutinous	-	-	S	-	-	ST, Thailand	Pattalung Rice Research center, Samreuang Ton,2000
Leb Nok Pattani	non glutinous	Feb.	170	S	-	-	ST, Thailand (1994),	TRRI, Eksngwon Chuwisitkul, 2000

- = No information T = Resistant S = Sensitive C = Central N = Northern ST = Southern
Rec. year = Recommended year TRRI = Thai Rice Research Institute IRRI = The International Rice Research Institute

1.5 Molecular Markers

Various molecular markers can be used to study the genetic diversity and genetic similarities associated with specific phenotypes. The DNA probes obtained from digestion of DNA or cDNA are used when a specific gene or gene products are known to associate with a specific character.

1.5.1 Restriction fragment length polymorphism (RFLP)

RFLP involves preparation of DNA probes, from either genomic or cDNA libraries after digestion target DNAs with restriction enzymes, labeling of the restriction fragment with ^{32}P or biotin, gel electrophoresis, and Southern blot hybridization. Wang and Tanksley (1989) analyzed 70 cultivars of rice, representing the breadth of the species *Oryza sativa*, by using 10 rice RFLP markers. Polymorphism was detected for all probes, and 58 of 70 cultivars tested could be uniquely distinguished from one another by combining all probe-enzyme combinations. Within-population variation, usually in the form of homozygous, variant alleles was found for 26% of the rice cultivars. Based on genetic distance calculations, the ratio of the genetic variation between versus within rice cultivars was estimated to be around 12 to 1. An RFLP based on dendrogram was constructed depicting genetic distances among these rice cultivars.

Started in 1992 Tanksley *et al.* have developed a molecular linkage map for rice at Cornell University through the support of the Rockefeller foundation. The purpose of the project was to develop a complete molecular linkage map (based mainly on RFLPs) that could be used by rice scientists throughout the world for a cultivar of applications in breeding and genetics. More than 600 RFLP markers have been localized on this rice map with an average spacing of approximately 1 marker every 2 cM. The markers localized on the map are derived from both genomic and cDNA libraries from several sources: from the *indica* rice species, IR 36 (both genomic and cDNA clones), from oat, barley (cDNA's only), and maize (genomic clones).

Claes *et al.* (1990) found eight proteins to be induced in rice plant under salt stress. Partial amino acid sequences of one protein of 15 kDa, and an isoelectric point of 5.5, were determined. Based on the amino acid information, an oligonucleotide probe was synthesized. Using this probe, a cDNA clone, *Sal*T, was obtained and found to contain an open reading frame coding for a protein of 145 amino acid residues. Using the cDNA as a probe, mRNA hybridization analysis was carried out. The *Sal*T mRNA was found to accumulate very rapidly in sheaths and roots from mature plants and seedlings upon treatment with sodium chloride(1%), potassium chloride (1%), air drying, ABA (20μM) and polyethylene glycol (5%). Generally, no induction was observed in the leaf lamina even when the stress should affect all parts of the plant uniformly. The organ-specific response of *Sal*T is correlated with the pattern of Na^+ accumulation during salt stress.

1.5.2 Randomly Amplified Polymorphic DNAs (RAPD)

Much interest has recently arisen in the PCR-based RAPD method of DNA finger printing (Williams *et al*, 1990) because of its simplicity. Since it only involves PCR and agarose gel electrophoresis. In addition, RAPD polymorphisms are usually visualized without the need of labeled radioisotopes, which can be a significant handling and disposal concern. Reproducibility of the RAPD makers is, however, dependent upon the annealing of 9- to 10- base arbitrary sequences of the primers. Primers of a higher AT composition anneal at a low temperature than do those with higher GC composition. It becomes more difficult, therefore, to obtain repeatable results using standard PCR conditions as the number of primers screened increases. In addition, subtle differences in Mg^{++} concentration, dNTP concentration, cycling parameters, and other conditions significantly affect performance of the PCR reaction, affecting the reproducibility of some RAPD analyses and the transfer of RAPD identities among laboratories (Wolff *et al* , 1993).

Zheng *et al.* (1991) used RAPD markers to detect DNA polymorphism among 19 rice cultivars of different types and origins, including Wulinai, a dwarf mutant among somaclones of Taizhongyn 39, and ITA 234, an upland cultivar from Nigeria with 2

primers : AP8a and AP8b. The result showed that primers could amplify segments of genomic DNA of unidentified origin. Amplification products show polymorphisms among different cultivars.

Dawson *et al.* (1993) used RAPD markers to analyze genetic diversity within and between *Hordeum spontaneum* populations sampled from Israel. Nei 's index of genetic differentiation was used to partition diversity into within and between population components. Fifty seven per cent of the variation detected was partitioned within 10*H. spontaneum* populations. Using principal component and multiple regression analysis, part of the variation detected between populations was seen to be associated with certain ecogeographical factors. Fifty eight per cent of the distribution of the phenotypic frequencies of three RAPD phenotypes detected using a single primer in 20*H. spontaneum* populations could be accounted for by four ecogeographical variables, suggesting adaptive variation at certain RAPD loci.

Rice RAPD markers have been reported by Monna *et al.*(1994) in Rice Genome Newsletter (Vol.3, No.2) to be very useful tool in the construction a rice physical map for map-based cloning of important genes by tagging RAPD markers to sequence tagged site (STSs),using bulked segregation strategy, in combination with RFLP markers.

By using sixty 10-nucleotides long random primers containing 40-70% GC content to generate RAPD markers in DNAs from F2 populations of cultivars Nipponbare (*japonica*) and Kasalath (*indica*) single cross, Monna *et al.* had pointed out the advantage of random pairing of primers : in PCR by using double primer RAPD (DP-RAPD). Amplification with two random primers resulted in 102 markers in the range of <100 bp to 2 kb that can be separated on 2% agarose gel and 4.5% polyacrylamide gel. The map positions of RAPD markers were determined by placing them onto a map of approximately 1000 RFLP markers established by the Rice Genome Research Program (Kurata *et al.*, 1994) and 102 RAPD markers generated by this study were reported to be fairly distributed in all 12 chromosomes of rice.

Yu and Nguyen (1994) analyzed genetic variation in 13 indigenous rice cultivars in the Philippines, in which 7 cultivars are *japonica* subspecies and 6 cultivars are *indica* subspecies that can be divided into nine upland and four lowland rice cultivars by RAPD method using 42 random primers. They reported 260 PCR products

of which 208 PCR products (80%) were polymorphic. All 42 primers used in the experiment were amplified and typically generated one-to-four major bands. Only two primers showed no polymorphisms. In general, a higher level of polymorphism was found between *japonica* and *indica* subspecies while fewer polymorphisms were found between upland and lowland cultivars within the *indica* subspecies. A dendrogram that showed the genetic distances of 13 rice cultivars was constructed based on their DNA polymorphisms in the similarity coefficient of 0.2-0.8.

Katiyar *et al.* (1994) used 10-mer oligonucleotide primer (Operon Technology) Bulk Segregant Analysis developed by Michelmone et al, 1991 to identify a RAPD marker linked to a gall midge resistant gene in cultivar Duokang #1 from China. Only RAPD marker OPM 6 was tightly linked to gall midge resistantce gene of Duokang #1.

Ko *et al.* (1994) used RAPD to study the genetic relationships among 37 rice cultivars by using the polymerase chain reaction (PCR) with 27 arbitrary oligonucleotide primers. There were 144 amplification products, of which 67% were polymorphism and 7 selected primers gave sufficient polymorphism to identify individual cultivar. RAPD polymorphism patterns confirmed that commercial Australian and USA lines and their relatives were very closely related with similarity indices of 88-97%. Three cultivars originating from more distant geographical centers were easily distinquished, producing cultivar-specific amplification profiles and expressing a lower similarity index of 80% to all other cultivars tested.

Godwin *et al.* (1997) performed RAPD analysis among eight rice somaclonal families known to vary for specific characters and four somaclonal families which were phenotypically normal. The parental cultivar, *indica* rice cv. FR 13 A, was found to be homogeneous and homozygous at all but one of the 45 RAPD loci. Polymorphisms were found at 28 of the 45 bands among the somaclonal families, including both loss of parental bands, and the appearance of novel non-parental bands. Segregation data revealed both heterozygous and homozygous mutation events, with recessive mutations more prevalent than dominant. All somaclonal families differed significantly from the parental material, indicating that genomic alterations occurred in all families regardless of phenotype. None of the variant families could be regarded as isogenic lines of FR

13A at the DNA level. However, some of the DNA level variation may be in highly repeated sequences with no phenotypic effects.

Verma *et al.* (1999) analyzed genetic variation among the 17 scented basmati rice accessions collected from the farmers' fields located at different places in India using 10 primers by RAPD method. The number of polymorphic / monomorphic bands among the pair wise combinations of the accessions and the total number of bands were determined to categorize all the accessions in different groups of similarity and dissimilarity at the genetic level. The procedure described also allows the identification and discrimination of the individual genotypes of basmati rice including the identification of the duplicates in genetic resource collections.

Baishya *et al.* (2000) investigated genetic variation in nine aromatic and four nonaromatic rice cultivars (*Oryza sativa* L.) at the DNA level using RAPD technique. Twenty six random primers were used to amplify DNA segments and 177 PCR products were obtained of which 98 were polymorphic. One primer did not show polymorphism. A dendrogram showing the genetic distances of 13 rice cultivars was constructed based on RAPD data.

1.5.3 Amplified fragment length polymorphism (AFLP)

AFLP which combines the desirable characteristics of RFLP and RAPD was developed by Vos *et al.* in 1995. AFLP defined sequences as adapters onto enzyme-digested genomic DNA. The restriction enzymes selected for the digestion (such as *Eco* RI and *Mse* I) are particularly stable and well suited for non-GC rich regions of DNA. The desired DNA fragments are selectively amplified by PCR using primers of defined sequences, resulting in highly reproducible DNA fragment patterns using standard PCR conditions. Use of a high-resolution sequencing gel allows large numbers of amplified DNA fragments to be separated for detection. The percentage of reactions yielding useful polymorphisms is very high, and the presence of multiple polymorphic bands/reaction add to the efficiency of the technique.

High reproducibility, rapid generation, and high frequency of identifiable AFLP polymorphisms make AFLP DNA analysis an attractive technique for identifying

polymorphisms and for determining linkages by analyzing individuals from a segregation population. AFLP analysis does require technical expertise for pouring sequencing gels and using radioactive label to detect the polymorphisms on an autoradiogram, AFLP markers were readily scored in segregating populations to determine inheritance of the polymorphism. Because multiple polymorphic bands were resolved for each segregating sibling in a single gel lane, multiple loci could be mapped at the same time, increasing the efficiency of determining linkages from a map. Additional developments of AFLP DNA mapping procedures will enhance the efficiency and ease of the technique, ensuring its wider use in genome mapping programs.

Huh and Huh (2001) used AFLP to study genetic diversity of seven wild radish populations in Korea. On average 58.4 % polymorphic markers were generated using eight primer pairs. Seongsanpo population at Cheiu-do showed the highest within population variability, whereas Kuryongpo population at Gyeongsangbukdo exhibited the lowest genetic diversity of all the Korean wild radish populations. The majority of genetic variance (96.7%) resided within populations. The average number of individuals exchanged between populations per generation was very high. These estimates are considerably higher than those from species with similar life history and ecological characteristics. The wild radish populations of Korea were separated by the PCR, showing genetic differentiation between population locations.

1.5.4 Microsatellites or Simple Sequence Length Polymorphism (SSLP)

Microsatellites consist of short, tandem repeats, typically1 to 10 nucleotides of simple DNA sequence which is inherited in a Mendelian fashion. They are abundant, widely spread throughout the chromosome and are highly polymorphic in eukaryotic genomes (Tautz, 1989). Arrays of the repeat have been found to vary dramatically in length, from several to hundreds of base pairs, providing a new and plentiful source of allelic polymorphisms (Tautz, 1989., Weber and May,1989., Litt and Luty,1989). Microsattellite arrays are embedded in unique DNA sequence, sometimes within the coding region of genes, but more commonly in the untranslated regions of the genome (Valdes *et al.*, 1993).

The most common repeats are the dinucleotides $(GT/AC)_n$,$(CT/AG)_n$, and $(TA/AT)_n$ (Tautz and Renz,1984).The $(GT/AC)_n$ repeats are the most abundant and informative in vertebrates, whereas in plants it is the $(TA/AT)_n$ repeats(Lagercrantz *et al.*,1993)

Recently microsatellites have been increasingly used as the markers of choice. There are some advantages of utilizing microsatellites over other markers, which make them desirable. First, microsatellite loci have been found in large numbers and relatively evenly spaced throughout the genome. Second, a small quantity of DNA is used in an analysis of microsatellites. Third, these microsatellite arrays are highly susceptible to length mutation. Finally, microsatellite alleles are codominent markers inherited in a Mendelian fashion.

Wannawichitra *et al.* (2001) used SSLP to study the genetic relationships among 48 rice cultivars by using PCR with 64 SSLP markers. Rice could be distinguished into main 3 groups: (1) local Thai rices and the hybrid RD group, (2) IR group and (3) exotic group. A dendrogram showed that the genetic distance of 48 rice cultivars constructed based on their DNA polymorphisms were in the similarity coefficient range of 0.09-0.90.

1.6 Correlation between genetic variation and morphological index of salt sensitive and tolerant rice cultivars

1.6.1 Why should salt tolerant/sensitive rice be studied?

Rice is a staple food for millions of people in the world. To sustain the food supply for an increasing population of rice eaters, the world rice production must be increased further. Unfortunately, several biotic and abiotic factors lower rice production. Among them, soil salinity is one of the most widespread soil problems. In Thailand, salinity is a major abiotic constraint lowering rice production. Particularly in Northeast Thailand, out of 17% of the land, is salt-affected to various degrees (Arunin 1984). The ultimate source of the salt is rock salt in Mahasarakham Formation (Kohyama *et*

*al.*1993). Water affected with the salt, transports salt to the soil surface by capillary rise and/or seepage through the soil layers (Kohyama *et al.*1993).

The salt-affected lands are classified according to percentage of area of salt patch on the soil surface (denuded patch mainly due to high salinity) into 5 categories (Arunin 1984): non salt-affected, potentially salt-affected, slightly salt-affected, moderately salt-affected and strongly salt-affected.

The salt-affected areas are considered expanding due to human activities such as deforestation, salt-making and construction of dams, roads and water reservoirs (Mitsuchi *et al.*1989). All of these human activities except salt-making have been believed to promote salinization through rising levels of the saline groundwater (Mitsuchi *et al.*1989).

The present extant of the salt-affected soil in the Northeast already substantially constricts rice production. The rapid expansion of the salt-affected soil will make the constraints much more serious in the near future. To improve rice yield in such environments, it is important to breed and select rice cultivars adaptable to salinity. Many studies have been conducted to identify the characteristics which lead to rice tolerance to salinity and to improve the salinity tolerance for maintaining yield at a sufficient level.

Rice is one of the grain crops sensitive to soil salinity and the sensitivity to salinity varies with the growth stages. In general, rice is most sensitive at the seedling stage and becomes less sensitive as it grows and develops. There have been, however, conflicting results about whether plant sensitivity at the reproductive stage is similar to that at the seedling stage. Kaddah and Fakhry (1961) observed that the sensitivity decreased as plants grew and developed. On the other hand, Iwaki (1956) reported that the reproductive growth represented by the number of spikelets and fertility was more affected than the vegetative growth, suggesting that plants at the reproductive stage were more sensitive. These conflicting observation may be due to the differences in the cultivars studied, since there was a significant interaction in yield response to salinity between cultivars and developmental stages at which plants were subjected salinity. A positive relation between salinity tolerance at the seedling stage and reproductive stage seems to exist, depending on the cultivars observed.

Varietal differences in salinity tolerance at the seedling stage have been well documented (Flowers and Yeo, 1981). The tolerance was often related to the low sodium concentration cf leaf, shoot and tissues in both greenhouse and field. Therefore, some studies were centered on the root ability to exclude sodium, the low transport of sodium to the shoot and the vigor of plants. Tolerance to high tissue sodium concentration also plays a role in seedling tolerance.

On the other hand, few studies (Bohra and Doerffling, 1993) have been carried out on the tolerance at the reproductive stage. A tolerant cultivar at the seedling stage due to low sodium concentration had a low sodium content at maturity and gave a high yield, suggesting that a low sodium concentration may also be beneficial at the reproductive stage. There was a relationship between seedling vigor under salinity and yield with some exceptions. In general, considering that the relation between salinity tolerance at the seedling stage and yield under salinity depends on the cultivars observed, it is unlikely that a low sodium concentration would play an important role in tolerance at the reproductive stage. It should be determined whether a low sodium concentration in plant could be related to the ability to maintain yield at a sufficient level under salinity among a wider range of cultivars.

1.6.2 Information about Thai rice in terms of salt tolerance and sensitivity.

In Thailand, varietal differences in salinity tolerance at many stage have been studied such as germination rate, morphology of leaves and roots at seedling stage and anthesis stage.

1.6.2.1 Germination stage

Rice is tolerant at the germination stage. Germination rate was delayed under low salinity while decreased to 50% under high salinity (20-30 dS/m). In the Northeast Saline Soil Development Project germination rate of 84 salt tolerant rice cultivars were examined in saline solution ranging from 1-25 dS/m. It was found that most of them germinated well in $\leq$ 14 dS/m salinity. Germination rate and strength decreased when

the salinity level was increased. Some rice cultivars germinate well under high salinity level such as Gow Ruang 88 and RD 3 germinated at 25 dS/m and KDML 105 germinated at 20 dS/m respectively.

1.6.2.2 Seedling stage

Rice seedling with a few leaves (2^{nd} and 3^{rd} leaf stage) is most sensitive to salinity and show symptoms such as thin-shallow roots and burned leaf margin. Seedlings' growth measured as plant height and tillering decreased to 50% at 6 dS/m.

1.6.2.3 Anthesis stage

Rice plant is sensitive at this stage and the phenology related are: decrease of number of panicle/tiller, panicle exertion, number of grains/panicle and thousand grains weight.

1.7 Rationale of this research

As reviewed previously, very scant information is available about the salt tolerance/sensitivity in Thail rices especially at the genetic level by any type of molecular markers within indigenous salt tolerant cultivars, and between salt tolerant and salt sensitive cultivars. To understand the linkage between saline vulnerability at different developmental stages of lowland *indica* rice cultivars recommended for planting in various ecogeographical regions, the same set of seed population should be used for both genetic variation study and phenological indicators for saline vulnerability.

RAPD method via PCR was selected although it has several disadvantages because of its simplicity, require small amount of DNA, and consume less cost and time comparing to RFLP, AFLP and SSLP, so that the genetic diversity experiment can be done in parallel with saline vulnerability assessment in 12 cultivars at all 3 stages of development under both control and salt stress conditions all together 24 treatments at each stage.

The hardest part at the beginning of this research was to get enough amount of high uniformity breeder seeds for native salt susceptible cultivars. Without the assistance form Mr. Samreung Ton, Phattalung Rice Research Center, this project could not be conducted.

It is hoped that this research can provide linkage between saline vulnerability phenology and genetic diversity among 12 lowland rice cultivars, of which 6 cultivars are known as salt tolerant rice and 6 cultivars are known as salt sensitive rice.

In this research, the 10-nucleotide random primers designed and purchased from Operon Technologies were based on the published sequences of the top 20 random RAPD primers recommended for distinguishing *japonica* and *indica* subspecies reported by the Rice Genome Research Project led by Sasaki *et al.*, Tsukaba Ibaraki 305 Japan (Monna *et al.* 1994).

The expected outcome of this project is to understand genetic variation of lowland Thai rices in two aspects : (1) genetic diversity in relation to geodistribution (2) identify standard salt tolerant and salt sensitive Thai cultivars by RAPD DNA finger print and their relationships with salt tolerance phenology usually used in the conventional breeding program for better salt tolerant cultivar.

1.8 Objectives

1. To understand genetic variation among native lowland rice cultivars of known saline vulnerability by using random RAPD primers to identify individual cultivar by RAPD-DNA fingerprint.

2. To link the genetic index with the phenological parameters used by conventional breeders in the improvement of salt tolerant rice cultivars.