THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Tropical Agriculture)

DEGREE

Tropical Agriculture	Agriculture
FIELD	**FACULTY**

TITLE: Genetic Diversity of Rice (*Oryza sativa* L.) Based on Blast Disease Resistance in Thailand

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THESIS

GENETIC DIVERSITY OF RICE (*Oryza sativa* L.) BASED ON BLAST DISEASE RESISTANCE IN THAILAND

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Tropical Agriculture)
Graduate School, Kasetsart University
2013

Abdu Salih Ali 2013: Genetic Diversity of Rice (*Oryza sativa L.*) Based on Blast Disease Resistance in Thailand. Master of Science (Tropical Agriculture), Major Field: Tropical Agriculture, Faculty of Agriculture. Thesis Advisor: Assistant Professor Tanee Sreewongchai, Ph.D. 83 pages.

Blast is a fungal disease that affects rice plants in most rice growing provinces of Thailand. This study was carried out with the identification of blast disease resistant rice varieties using mixed and individual isolates. In first phase, three hundred eleven genetically diversified varieties/accessions which include 263 landrace, 43 improved and 5 wild rice varieties/accessions were obtained by the National Rice Gene Bank of Thailand. The screening for blast resistance at seedling stage was done using 63 mixed diversified blast isolates followed by 29 individual isolates. The blast isolates were collected from seven provinces of Thailand. The result indicated that a total of 35 varieties/accession were found to be resistant and out of these 35 only 9 genotypes were highly resistant to all tested blast isolates. While in the second phase, 30 rice genotypes composed of Indica, Japonica and Tropical Japonica categories that include resistant and susceptible check varieties were used to evaluate the resistance ability against 23 individual blast isolates. The result revealed that only RD41 of the indica category was found highly resistant to all 23 individual blast isolates with Resistance Index (RI) value of one. The resistance check IR64 had RI value of 0.86 and this showed reduced resistance level. PSL2 and JHN genotypes were resistant against all blast isolates with RI value of 0.95 except to isolate P7.1 and P40.4. Among the 23 blast isolates studied, only P3.2 collected from Wong Thong district of Phitsanulok province was the most virulent pathogen in over 33% of the total inoculated rice plants. The resistant genotypes identified in this study could be used in future breeding programs in order to come up with agronomically important and blast disease resistant rice genotypes.

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Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

First and foremost I would thank the Almighty Allah for his indispensable gift, strength, health and providence throughout my life.

I would like to express my deepest respect and most sincere gratitude to my Advisor, Assistant Professor Dr. Tanee Sreewongchai, for his guidance and encouragement at all stages of my work. I am greatly indebted to his assistance and understanding in matters of non academic concern which have helped me endure some difficult times during my study period. I am also extremely indebted to my co-advisor Associate Professor Dr. Prapa Srippichitt for sharing me her invaluable experience and knowledge. I am thankful to her for picking me up as a student at the decisive stage of my MSc study. The seed for the experiment was obtained through Dr. Nonglak Parinthawong from the national rice gene bank of Thailand. I am greatly indebted to Dr. Nonglak Parinthawong for her support and cooperation.

I am also grateful to my employer, Afar Pastoral and Agro-Pastoral Research Institute (APARI), Samara, Ethiopia, for granting me a study leave in order to pursue my study and the Rural Capacity Building Project of the Ministry of Agriculture and Rural Development of the Federal Democratic Republic of Ethiopia for the financial support of my study. A very special appreciation is due to my beloved wife Semira Zubeyr not only for her constant encouragement but also for her patience and understanding throughout. May Allah bless her in all her endeavours because without her tolerance, completion of this study would not have been possible. I also wish to express my heartfelt thanks to Dr. Sarbast for his uncountable support and encouragement in difficult times. My thanks also go to Ethiopian and Thai friends at Kasetsart University for their good friendship. I am also greatly indebted to my lovely Mother Ma'aro Humed for her love and encouragement throughout.

Abdu Salih Ali

March 2013

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LIST OF ABBREVIATIONS

^{o}C	=	Degree Celsius
Baht	=	Thailand Currency
CNT1	=	Chainate1
CNRRI	=	China National Rice Research Institute
g	=	Gram
h	=	hour
IRRI	=	International Rice Research Institute
JHN	=	Jao Hom Nin
MPa	=	Mega pascal
KDML105	=	Kow dock Mali105
NERICA	=	New Rice for Africa
OAE	=	Office of Agricultural Economics
p.oryzae	=	*Pyricularia oryzae*
PTT	=	Pathumthani
RD	=	Research Development
RFA	=	rice flour agar
SPR	=	Suphanburi
UPGMA	=	Unwaited Paired Group Method with Artimetic Average
WARDA	=	West African Rice Development Association

GENETIC DIVERSITY OF RICE (*Oryza sativa* L.) BASED ON BLAST DISEASE RESISTANCE IN THAILAND

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population, influences the livelihoods and economies of several billion people and for hundreds of millions it is the only thing between them and starvation (IRRI, 2006). It is one of the most important cereal crops in the world and provides more than 50% of the calories consumed by humans in Asia (Khush, 1997). The two cultivated rice species are *O. sativa*, widely grown in Asia and other countries, and *O. glaberrima*, grown in Africa only. *O. sativa* evolved from perennial or annual types of *O. rufipogon* and diversified into two subspecies, indica and japonica (Oka, 1974; Chang, 1985). Many rice genotypes have evolved to adapt to various environments, including irrigated, rain-fed lowland, and upland ecosystems between 55 N° and 36 S° latitude (Khush, 1997). More than 780,000 varieties have been collected worldwide; 109,136 varieties are deposited in the International Rice Genebank of the International Rice Research Institute (IRRI), and more than 80,000 and 70,000 varieties have been collected in India and China (FAO, 2009).

Rice is also one of the most important staple food crops of the increasing world population and is the leading cereal crop of South-East Asia, which is the highly populated region of the world. It is the only major food crop that can be grown in standing water of vast areas in tropic and sub tropics (Pennisi, 2010). It is an important culinary and cultural component throughout East Asia. Glutinous rice is generally reserved for use in festival foods and desserts such as, popped snacks in Cambodia, China, Indonesia, Japan, Korea, Myanmar, Philippines, and Vietnam. It also serves as the staple food in Laos and Northeast of Thailand (Ronald and Leung, 2002).

Similarly, rice is one of the major stable and foreign currency earning crops of Thailand. The total area of land under rice cultivation in this country is estimated to be about 11 million hectare. This area accounts for approximately 40 percent of the total area cover under crop production (Kupkanchanakul, 2000).

Diseases are among the most significant limiting factors that affect its production, causing annual yield losses conservatively estimated at 5% (Song and Goodman, 2001). More than 70 diseases caused by fungi, bacteria, viruses or nematodes have been recorded on rice (Manandhar *et al.*, 1998), among which rice blast caused by the fungus *Pyricularia oryzae* is the most serious yield limiting disease (Song and Goodman, 2001). It is one of the most serious and destructive fungal diseases of rice production worldwide (Ou, 1985). Rice seedlings or plants at the tillering stage are often completely killed. It is highly adaptable to different environmental conditions and can be found in irrigated lowland, rain-fed upland or deepwater rice fields (Ou, 1985; Latif *et al*., 2011). In favorable environmental conditions, this disease completely destroys seedlings in nurseries and crops in the tillering stage. Leaf blast stunts plant height, reduces the number of bearing panicles and weight of individual grains (Thruston, 1998). It also increases plant respiration and reduces the maximum photosynthetic rate at light saturation and initial light use efficiency (Pinnschmidt *et al*., 1994). The economic loss caused by rice blast was reported to be nearly US$ 5 billion per year.

In Thailand, rice blast disease outbreak in 1992 brought about over 1.25 million rai (0.2 million hectar) damage that incurred a loss of over 1 billion Baht* (Song and Aree, 2001). Later in 1995, the second wide spread of the disease occurrence called for serious attention to prevent such outbreak. The blast disease (due to *Pyricularia oryzae*) could be normally observed in most rice production areas and in many rice varieties, including glutinous and non glutinous, in both native and improved varieties (Song and Aree, 2001).

Generally, this disease attacks all parts of the rice plant causing losses upwards of hundreds of millions of tons of rice grain annually. Such losses have lead to rice shortages in many developing countries and could have an influence on the country's economy. Effective control of this devastating disease is imperative for global food security and social well-being. Identification of resistant varieties is one of the most effective, economic and safe ways to control rice blast in the field. Thus, this study was proposed to know the genetic diversity of rice genotypes based on blast disease resistance and selection of suitable parents for rice breeding programs to develop varieties with durable resistance.

OBJECTIVES

Over all objectives

To study the genetic diversity of rice (*Oryzea sativa* L.) varieties based on blast disease resistance

Specific objectives

1. To identify rice varieties resistant to blast disease from landrace, improved and wild rice (*Oryzae spp.*) in Thailand.

2. To evaluate the genetic diversity of the three groups of improved rice varieties based on blast disease resistance.

LITERATURE REVIEW

Importance of rice

Rice is the main sources of food for 50% of world's population and so may be considered the most important plant on earth (Shimamoto, 1995). It is an important agricultural commodity that supplies approximately 23 % of the per capita energy for six billion people worldwide (Maclean, 1997).

Africa has become a big player in international rice market, accounting for about 32% of global imports in 2006, at record level of 9 million tones that year. Africa's emergence as a big rice importer is explained by the fact that during the last decade rice has become the most rapidly growing food source in sub Saharan African (Sohl, 2005). WARDA breakthrough in producing the New Rice for Africa (NERICA) and offers welcome relief to Africa's rice farmers. It is new and unique opportunity for sustainable agricultural development in the rainfed environments where most of Africa's rice farmers earn living (Somado *et al*., 2008).

The term NERICA stands for 'New Rice for Africa'. It is used to refer to genetic material derived from the successful crossing of the two species of cultivated rice, the African rice (*O. glaberrima* Steud) and the Asian rice (*O. sativa* L) to produce progeny (known as Interspecifc) that combine the best traits of both parents. These include high yields from the Asian parent and the ability from the African parent to thrive in harsh environments (Somado *et al*., 2008).

Importance of rice in Thailand

Rice farmers generally work at the subsistence level, selling only their excess production. Thai rice farms are larger than the average Asian rice farm, 3-4 hectares compared with the average of 1-2 hectare (Kupkanchanakul, 2000). Because of its importance, rice is considered a 'strategic resource' in Thailand and has been assigned

as one of the high priority topic in the National Research Strategic Plan (Office of the National Research Council of Thailand, 2008). According to Thailand's agricultural research, the largest share of government budget was for crop research with relatively small budgets for livestock, forestry and fisheries (Fan *et al.,* 2004). Of these crop researches, the rice research continuously received the highest priority. From 1960 to 2000, Rice Research Institute was the most funded government agency for rice research with an average budget of 111.20 million Baht at 1988 price or 35.74% of all agricultural research budgets. Thus, these funding of several research activities lead to technological development in rice production (Jaroensathapornkul, 2007).

Thailand is the biggest exporter with 38% of the total worldwide rice export (Dooren, 2005). Since 1998 Thailand has enjoyed fast growth in export value of high quality rice (Jasmine). The export value of jasmine rice increased from 1,358 million baht in 1988 to 27,252 million baht in 1997. Production of jasmine rice expanded rapidly in the Northeast as well as the North where over 70 percent of blast disease took place in 1992 (Song and Aree, 2001). The export value of rice is around 100,000 million baht (OAE, 2008). Rice farmers are also one of the major employers with more than 26% of the population (17 million people) involved in rice farming (OAE, 2008).

Blast is known to cause severe yield losses in rice production systems with high inputs of nitrogen fertilizer and favorable climatic conditions (Yashida and Parao, 1976; Ou, 1985). Blast epidemics are commonly known to be affected by variation in climate (Suzuki, 1975), varietal susceptibility, and crop management practices such as nitrogen application and availability of water supply (Ou, 1985). Yield loss caused by blast epidemics has been previously studied (Pinnschmidt, 1989; Pinnschmidt *et al*., 1994). Although there are some resistant strains of rice, the disease persists wherever rice is grown.

Constraints of Rice production

A crop failure, for any reason, poses a real threat of starvation (TeBeest *et al.*, 2007). A number of fungus, bacteria, virus, nematode and mycoplasma-like organisms cause disease to rice plants. Among these the fungal diseases *viz*. blast (*Pyricularia grisea*), brown spot (*Bipolaris oryzae*), stem rot (*Sclerotium oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*), bacterial disease such as bacterial blight (*Xanthomonas oryzae pv. oryzae*) and viral disease such as tungro (rice tungro virus) are most important. These diseases are considered as a serious constraint for rice production (Vasudevan *et al*., 2002). Every year the amount of crops lost to rice blast could feed 60 million people. Although there are some resistant strains of rice, the disease persists wherever rice is grown. The disease has never been eradicated from a region.

Rice blast disease

Economic importance of blast

Blast is considered a major disease of rice because of its wide distribution and destructiveness under favorable conditions. Although blast is capable of causing very severe losses of up to 100%, little information exists on the extent and intensity of actual losses in farmers' fields. Losses of 5 to 10%, 8%, and 14% were recorded in India (1960-1961), Korea (mid-1970s), and in China (1980-1981), respectively. In the Philippines, yield losses ranging from 50 to 85% were reported (IRRI, 2009). (Detailed Table 1).

Table 1 Yield losses due to blast disease in different countries

%Yield loss	Country	Year
5-10	India	1960-‘61
50-60	Philippines	1963
70-85	Philippines	1969-‘70
8	Korea	Mid-70s
14	China	1980-‘81
60	Thailand	1992

Source: IRRI, (2009).

Rice blast is a problem almost everywhere that rice is grown. This fungal disease is estimated to cause production losses of US$55 million each year in South and Southeast Asia (Robert, 1991). The losses are even higher in East Asia and other more temperate rice growing regions around the world (Robert, 1991). Rice blast is a widespread and damaging disease of cultivated rice caused by the fungus *P. oryza* (Rossman *et al*., 1990). It is the most destructive pathogen of rice worldwide; around 50% of production may be lost in a field moderately affected by the fungus. Each year the fungus destroys rice enough to feed an estimated 60 million people (Zeigler *et al*., 1994). Thus, blast is the most devastating disease and occurs worldwide and is capable of causing severe losses of up to 85% in many countries (Teng and Revilla, 1996). Yield loss caused by blast varies depending on growing season and production level. For instance, in southern China, the yield loss caused by blast was estimated of about 39.4% of total loss from disease during early season and 19.8% during late season (Shen and Lin, 1994). The fungus produces lesions on leaves, nodes and different parts of panicles and grains at different developmental stages, resulting in leaf blast and neck blast.

The neck blast makes more significant yield and quality losses than leaf blast (Katsube and Koshimizu, 1970). Outbreak of neck blast in northern Thailand was reported in 1992 that damaged two hundred thousand hectares of rice growing areas

(Disthaporn, 1994). The resistances at seedling and neck stages may differ for some varieties. It was suggested that blast resistances of breeding lines and varieties should be periodically screened at both stages (Jennings *et al*, 1979*)*. The high-yielding indica variety Zhongl56 developed in China National Rice Research Institute (CNRRI) was resistant to certain isolates of the blast fungus at seedling stage, but susceptible to neck blast with the same isolates (Zhuang *et al.*, 2002).

According to Ou (1985), in Thailand, serious attention to blast disease has been given since 1959. Numerous cultivars have been tested for resistance and hybridization and selection have begun. Hundreds of crosses have been made and progenies tested in various regions. And vigorous programs are in progress but no new cultivars have yet been released. However, susceptible old cultivars have gradually been eliminated and more resistant ones are in wider use.

Rice blast is caused by the two generic causal organisms namely *Pyricularia* and *Dactylaria*. Both *Pyricularia* and *Dactylaria* were found by Saccarde in 1880. The names *Pyricularia grisea* and *Dactylaria grisea*, *P. oryzea* and *D. orizea* have all been used interchangeably by different workers. Asuyama (1965) concluded that the blast fungus on rice and cub grass is included in the genus Pyricularia (Ou, 1985).

Blast epidemics are commonly known to be affected by climate, varietal susceptibility and crop management practices such as nitrogen inputs and availability of water supply (Suzuki, 1975; Ou, 1985). Air and dew temperature significantly affect blast infection processes including infection, latency, lesion growth and sporulation, and play important roles in blast epidemics (Ou, 1985; Teng, 1994).

Taxonomy and nomenclature

Alexopolos *et al*. (1996) reported the taxonomic definition of the anamorph state of blast fungus is as follows:

Division: *Eumycota*

Subdivision: *Ascomycotina*

Class: *Pyronomycetes*

Order: *Diaporthales*

Family: *Magnaporthaceae*

Genus: *Magnaporthe*

Infection and Symptoms

Rice infection by *P. grisea* is initiated when three-celled, teardrop shaped conidia land on the surface of a rice leaf. These spores germinate immediately on contact with the rice leaf, and adhere tightly to the hydrophobic surface by means of spore tip mucilage that is released from the apex of the spore (Hamer *et al*, 1988). Germination proceeds by extension of a narrow germ tube that emerges from the conidium within an hour of its landing on the leaf surface (Hamer *et al*, 1988; Talbot, 2003). Within 4 h, the germ tube starts to swell at its apex, and flattens against the surface of the rice leaf. The germ tube apex then develops into a swollen dome-shaped cell, called the appressorium (Howard *et al*., 1991; Valent *et al*., 1991; Talbot, 2003).

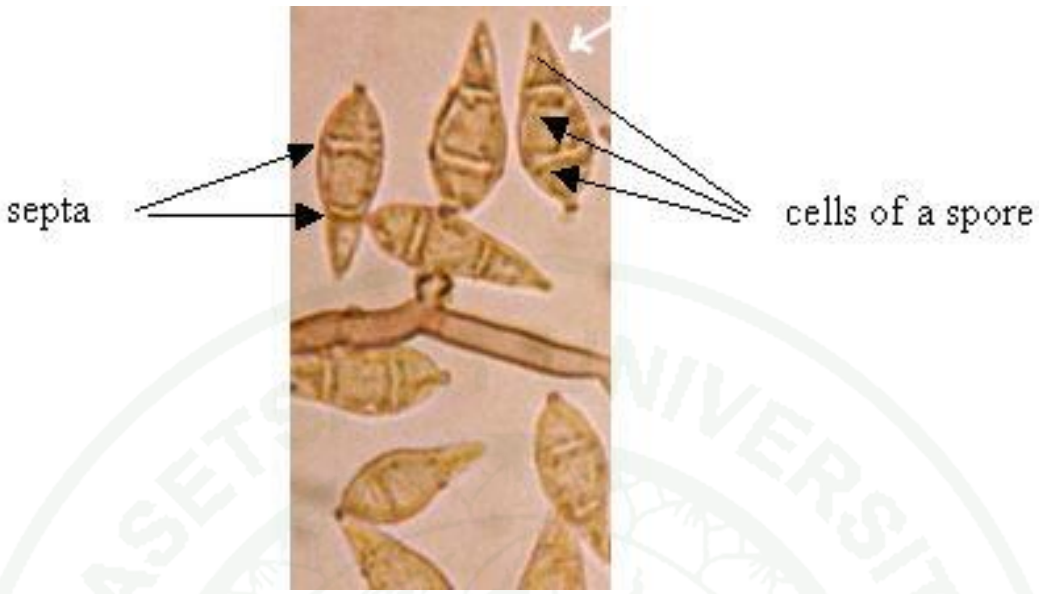

Figure 1 Rice causal organism mature conidia of p. oryzae with three or two separate celled:

Source: IRRI (2009)

The fungus produces spots or lesions on leaves, nodes, and different parts of the panicles and the grains, but seldom, if ever, on the leaf sheath in nature (Ou, 1985). This pathogen can cause symptoms of brown chlorotic lesions on leaf, stem, collar, neck and panicle of rice at different growing stages. Severe blast can cause significant yield losses (Pinnschmidt, 1989). Leaf blast lesions reduce the net photosynthetic rate of individual leaves to an extent far beyond the visible diseased leaf fraction (Bastiaan, 1991). The predominant symptoms of blast disease in any given area depend up on the climatic conditions. In temperate regions, where long periods of drizzle or light rain occur, leaf blast at the tillering stage is often severe and may kill the plants completely. In the tropics, seedlings in nurseries are often vulnerable, but after transplanting severe infections are seldom found (Ou, 1985).

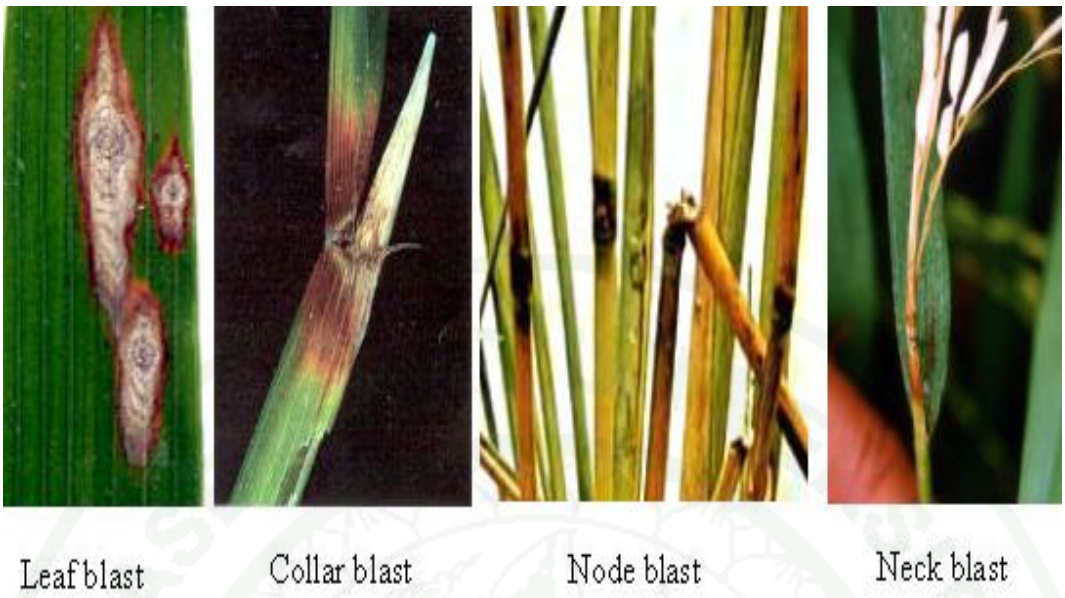

Figure 2 Symptoms of above ground part of the rice plant attacked by the fungus (*P. oryzae*):

Source: IRRI (2009)

Mechanism of blast infection

To bring about rice blast disease, *M. grisea* has evolved a remarkable mechanism involving production of a cell that is required for attachment to the rice leaf surface and for generation of mechanical force to penetrate the rice leaf cuticle. To bring about rice blast disease, *M. grisea* undergoes a series of defined morphogenetic developmental steps, leading to the production of a specialized infection structure called the appressorium (Talbot, 2003). These cells are produced on the surface of rice leaves, and bring about plant infection primarily by physical breakage of the leaf cuticle. Experiments performed in the early 1990s demonstrated that appressoria of the rice blast fungus generate substantial turgor. The turgor pressure in appressoria can be as high as 8 MPa (Howard *et al.*, 1991) and is essential for the mechanical penetration of the rice leaf cuticle.

Disease Cycle and Epidemiology

Plant diseases are often severe during periods of warm temperatures and high moisture. Throughout the world, rice is normally grown partially submerged in water in paddies, although in some regions rice is grown as upland rice in much the same way as wheat. Generally, rice blast is favored by moderate temperatures (24 °C) and periods of high moisture that are 12 hours or longer, conditions readily attainable in flooded rice fields. Spores produced as the primary inoculums on the overwintering tissues produce the initial infections on young seedlings when the spores that are deposited on leaves, germinate and invade leaf tissues (TeBeest *et al.*, 2007).

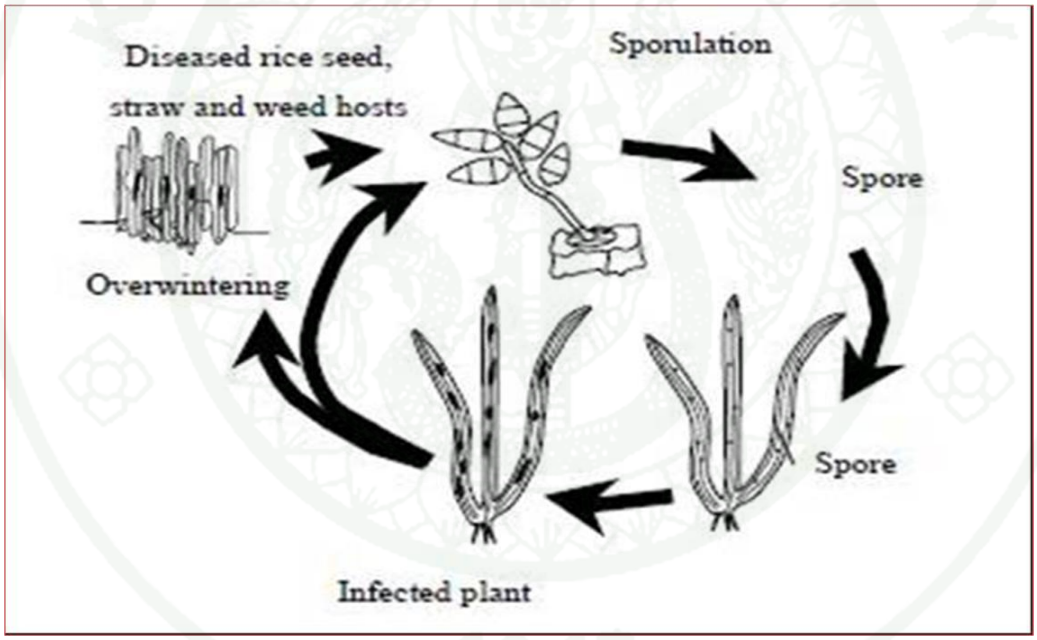

Figure 3 Disease cycle of rice blast

Source: Borromeo et al., (1993)

Conidia are produced on lesions on the rice plant about 6 days after inoculation. The rate of sporulation increases with increase in relative humidity; below 93% RH no conidia are produced (Hemmi and Imura, 1939). Kato and Kozak (1974) and El Refaei (1977) in their study to see the effect of temperature on lesion

enlargement and sporulation potential reported that sporulation reached a peak earlier at higher temperatures, but maximum potential was at medium temperatures. Toyoda and Suzuki (1952) reported on spore production from different types of lesions in a moist chamber and found that small lesions produced fewer spores and took longer to do so than large lesions.

Inoculation of the pathogen

The hemibiotrophic pathogen *Pyricularia oryzae* causes the rice blast disease and the interaction of rice with *P. oryzae* has been a subject under intensive studies and currently, rice blast system is a well-established model for studying how each plant cell responds to an invading pathogen (Silue *et al.*, 1992; Valent 1997). The resistant and susceptible reactions to *P. oryzae* are typically determined several days after infection (Valent *et al.*, 1997; Jia *et al.*, 2003). In fact, *M. grisea* conidia germinate within 30 minutes of attachment upon receiving host inductive signals, appressoria form between 4-8 h and penetration pegs form approximately 24 h after inoculation (Talbot, 2003). To determine the reaction of rice plants to specific races of *P. oryzae*, and for environmental, nutritional and other specific studies, artificial inoculations are necessary (Andersen *et al., 1947*; Panzer and Beier, 1958; Latterell *et al*., 1965).The most conventional method is to spray a conidial suspension in water on to the leaves, keeping the necessary control of the environmental conditions under which the plants are kept (Ou, 1985).

The density or concentration of conidia in the water suspension used for artificial inoculation should be regulated in comparative determination of varietal reaction. Kobayashi and Abumiya (1960) found that the density of conidia affects not only the number but also the type of lesion. Ordinary, concentrations of $2x10^4$-$5x10^4$ spores/ml were used. Kiyosawa and Fujimaki (1967) showed that among the concentrations tested ($1x10^4$ –$4x10^4$), $3x10^4$ spores/ml was the optimum concentration for the development of maximum number of lesions on the leaves. Goto *et al*. (1961) found that when plants were inoculated, usually the upper three leaves were infected,

and the number and size of the lesions were greater on younger than the older leaves. On single leaves maximum infection occurred when the leaves were fully expanded. Sprinkling with water, two or three times each day, depending on the weather, can easily maintain the necessary moisture. Infection takes place in the range 16–35 °C but the optimum temperature is between 24 °C and 28 °C (Ou, 1985).

Many workers also inject a spore suspension into the leaf sheaths of seedlings and lesions appear on the young leaves which unfold in a few days (Kuribayash and Terazawa, 1953). When necessary, two or more separate injection, about one week apart, may be made on the same seedlings. Hashioka (1963) tried the "coleoptiles test" by smearing a conidial smearing seeds. Ohata and Kozaka (1967) inoculated by punching holes in rice leaves. For neck blast inoculation, Ou and Nuque (1963) injected about 1ml of spore suspension into the leaf sheaths of emerging panicles (about half way emerged) with an automatic syringe. Using this technique, a large number of panicles could be inoculated and often 100% infection occurred. Sakomoto (1949, 1951) introduced the leaf sheath inoculation method of determining varietal resistance. Leaf sheaths are cut into 7–10 cm pieces filled inside with spore suspension and incubated for about 40 h at 24–28 °C. Ou (1985) suggested that sprinkling with water, two or three times each day depending on the weather, can easily maintain the necessary moisture. He also explains that infection takes place in the range of 16–35 °C but the optimum temperature is between 24 and 28°C. In most of temperate regions, tests may be conducted from June to September. In the tropics, temperature is more or less favorable throughout the year and the test may be made at any time, provided that high moisture is maintained.

Rice blast management strategies

Chemical control

Rice blast disease control is afforded by an array of fungicides and its effectiveness is determined by the compound, timing and method of application.

Chemicals have been used extensively in Japan for controlling blast disease and virtually all rice fields have been treated with chemicals in recent years. Certain fungicides target specific stages in the *M. oryzae* life-cycle; for example, the melanin biosynthetic inhibitors (MBIs) interfere with formation of the melanized appressorium (Iwata, 2001; Kurahashi, 2001).

Cultural systems

Besides the use of chemicals and resistant cultivars, the control of blast disease in Japan also relies on adjusting cultural practices (Ou, 1985). Time of planting has been demonstrated to be an important factor in blast disease development, early plantings in Japan usually having less disease than later plantings (Kuribayashi and Ichikawa, 1952). Seedlings raised in upland nurseries are more susceptible to blast even after they are transplanted. This is explained by the lower silicon content of the epidermal cells. Yamada and Ota (1956) showed that up land seedlings had higher physiological activities, producing more roots and absorbing more nitrogen than seedlings grown in wet nurseries.

Biological control

Most approaches to biological control have been focused on primarily soil-borne diseases (Sanford and Broadfoot, 1931; Kloepper and Schroth, 1979). In contrast little information has been reported about biological control for foliar airborne diseases because many effective chemical pesticides have been developed. Recently, plant pathologists and farmers have paid more attention to biological control as an alternative measure to using chemical pesticides, in response to consumer demand for safe farm products and concern about environmental pollution. Several successful reports about biological control for airborne diseases have been published, *e.g.*, chitinolytic activity of Serratia marcescens for rice blast disease (Someya *et al*., 2002) and antimicrobial compounds produced by *Bacillus licheniformis* for tomato

gray mold disease (Lee *et al*., 2006). Most biological control agents protect against disease utilizing antagonistic or killing effects against pathogens.

Resistant cultivars

The use of resistant cultivars has been the preferred method for controlling this disease and considerable effort has been directed toward the understanding of genetic resistance mechanisms. However, blast resistance is rarely effective for more than 2-3 years (Correa-Victoria and Zeigler, 1993). It is not known whether the ability of the pathogen to overcome resistant cultivars reflects shifts in the frequency of formerly rare pathotypes, the frequent occurrence of genetic changes to new forms of virulence or a combination of both phenomena (Correa-Victoria, and Zeigler 1993). Consequently, detailed genetic information on population structure is essential for understanding the virulence dynamics of the pathogen and devising more effective strategies to reduce the impact of rice blast disease.

Evaluation of varietal resistance

Varietal differences in resistance can be and often have been observed in ordinary fields. For instance, during 1900-1910, cultivars Kameji and Aikkoku were found to be highly resistant in Japan while Asahi, Omachi and Shinriki were slightly, moderately and very susceptible respectively (Ito, 1965). Rice leaves infected by the blast fungus show various types of reaction depending up on varietal resistance or susceptibility. Resistance cultivars show no symptoms or very minute brown specks to larger brown spots about 1mm in diameter. Intermediate groups show more or less roundish, restricted lesions, 2–3mm in diameter, with a grey center and brown margin (Ou, 1985). He also suggested that susceptible cultivars produce large elliptical lesions 1–2cm long with grey centre and brown brawn margin and, in case of very susceptible cultivars, large broadly elliptical lesions.

Relationship between leaf blast and neck blast

Testing neck blast by means of field experiment is usually difficult because of the great variation in result from test to test (Ou and Nuque, 1963), but tests may be conducted where natural conditions are favorable. It has been reported that certain cultivars resistant to blast in the leaf stage were observed to be susceptible to neck rot at later stage of growth and, conversely, cultivars susceptible to leaf blast showed little or no neck blast (Hashioka, 1950a; Ito, 1965; Chang, *et al*., 1965). Such observations have led to the belief that resistance to leaf blast and neck rot are not correlated and that different genes.

Scales of resistance or susceptibility

Based up on the type, color and number of lesions, as well as the amount of stunting, workers have designed various scales to classify degrees of resistance or types of reactions (Ou, 1985). Hashioka (1950b) recognized five classes of lesions, which He designated A, B, C, D and E. In addition He used A_1, A_2 and A_3 etc to indicate few, several and many lesions respectively. Sasaki (1923) classified plants in to susceptible and resistance groups based on the presence or absence of lesions. It is obvious that there was no uniform basis for comparison and that, since the strain of fungus in use was unknown, exact information cannot be derived from these early experiments. But nowadays there is Standard Evaluation System (SES) for rice published by International Rice Research Institute (IRRI) and it is given in Table 2.

Table 2 The description of severity index of blast disease with the seven (0-6) lesion type categories.

Severity Index	Description
0	no evidence of infection
1	brown, pinpoint smaller than 0.5 mm without sporulation
2	brown, pinpoint smaller than 0.5–1mm without sporulation
3	small eyespots about 1–3 mm with gray center, lesion capable of sporulation
4	small eyespots about 3 mm or more with gray center and dark margin, lesion capable of sporulation
5	susceptible sporulating type, coalescence lesion without dark margin
6	susceptible sporulating type, lesion without dark margin

Source: Rouman *et al*., (1997)

Lee *et al.* (2009) also evaluated rice blast severity on test entries growing in inoculated upland blast nurseries using the standard visual 0 to 9 scale where a 0 rating indicates complete disease immunity and the 9 rating indicates complete disease susceptibility usually ending with total yield loss and/or plant death.

Ratings are often summarized as visual ratings of:

0 to 3 = R (resistant),

3 to 4 = MR (moderately resistant),

5 to 6 = MS (moderately susceptible to susceptible)

7 = S (susceptible), and

8 to 9 = VS (very susceptible)

Table 3 Scales of resistance and susceptibility

Scale	Description
0	No lesions
1	Small brown specks of pinhead size
2	Large brown specks
3	Small roundish to slightly elongated necrotic gray spots, about 1–2 mm in diameter
4	Typical blast lesion, elliptical 1–2 cm long, infecting less than 2% of leaf area
5	Typical blast lesions, infecting less than 10 % of the leaf area
6	Typical blast lesions, infecting 10–25 % of the leaf area
7	Typical blast lesions, infecting 26–50 % of the leaf area
8	Typical blast lesion, infecting 51–75 % of the leaf area
9	All leaves dead

Source: IRRI (1980)

Genetics of resistance

Yamasaki and Kiyosawa (1966) carried out a serious of experiment on inheritance of resistance to blast. Gene analysis were made using some cultivars belonging to the Aichi-asahi, Kanto 51, To-to and Ishikari-Shiroka types, and the applicability of the gene-for-gene hypothesis was proposed (Yamasaki and Kiyosawa, 1966). The cultivars of the Aichi-asahi type carry one dominant gene Pi-a controlling resistance to the fungus strains Ina-72 and Ina 168. Kanto 51 has one gene Pi-k controlling medium resistance to the fungus strain P-26 and high resistance to strains Hoku 1, Ken 54-20, Ken54-04 and Ina 168. The Ishikari Shiroka types, carries one gene Pi-I controlling medium resistance to strains P-2b, Ina 72, Ken 54-04 and Ina 168. Genes Pi-k and Pi-I showed variation from complete to in complete dominance depending on environmental conditions. Gene pi-a showed complete dominance. The three genes behaved independently (Yamasaki and Kiyosawa, 1966).

Ezuka *et al*. (1969) tested 373 rice cultivars in Japan with seven strains of the fungus and found 11 reaction types. Cultivars of the so called Kanto 51 type of Kiyosawa were further divided in to three genotypes: (1) Pi-k; (2) Pi-I and Pi-k; and (3) Pi-I and Pi-m. The To-to type was also divided in three genotypes: (1) Pi-a and Pi-k; (2) Pi-a, Pi-k and Pi-I; and (3) Pi-a, Pi-i and Pi-m. Inheritance studies were also made in India Padmanabhan *et al*. (1974) reported that resistance in Zenith, Te-tep and Tadukan was controlled by three dominant genes and that the presence of any two of the three genes might confer resistance. In the cross Zenith x S.67, it was controlled by one gene, in S.67 x CO13 by two. The presence of the inhibitory genes in C15309 was indicated. A possible influence of cytoplasm was inferred in the cross between CO4xS.67.

Genetic Diversity

Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species and it serves as a way for populations adapt to changing environments. It is the essential to meet the diverse goals of plant breeding such as producing cultivars with increasing yield, genetic adoption, desirable quantity, pest and disease resistant (Nevo *et al*., 1982). Specific simplicity and genetic uniformity of modern agricultural plant communities offered advantages to farmers, as well as to consumers and processors. The genotypic and phenotypic uniformity of modern cultivars permit farmers to fully exploit the increase in efficiency in terms of yield per unit area *vis-à-vis* mechanization, fertilization, irrigation, harvesting and storage, and other land- and labor productivity-enhancing activities. Quantitative classification offers a quantified degree of divergence among genotypes or populations, this serve as a sound basis of grouping any two or more genotypes based on minimum divergence between them (Sharma, 1997).

Genetic diversity in rice

Geographic and crop diversity, coupled with diverse traditional agricultural systems, contributes to the diversity of crop genetic resources. Molecular markers and more recently, high throughput genome sequence efforts, have dramatically increased the capability to characterize genetic diversity and population structure in plant germplasm pools (Joshi *et al*., 2000). Increasing plant biodiversity in cropping systems has been considered a strategy to enhance the resilience of the ecosystem to withstand epidemics (Browning and Frey, 1969; Wolfe, 1985; 2000). Research by Yunnan Agricultural University in China, in collaboration with IRRI, has provided evidence that crop diversity is a possible strategy to manage blast disease in cropping systems that contain the blast-susceptible glutinous rice cultivars. Zhu *et al.* (2000) have shown that genetic diversification of the rice crop provides an ecological approach to disease management that can be highly effective in large-scale rice production systems. Although, resistance to blast is often short-lived, some cultivars are considered to possess durable resistance (Johnson, 1981). Durable resistance is thought to be associated with partial resistance, that is, in many cases under polygenic control (Wang *et al.*, 1994).

The success of the breeding strategies relies heavily on the genetic diversity of the crop. Rice gene banks around the world exhibit a very large amount of genetic diversity present in farmers' cultivars, landraces, as well as in the genetic makeup of the 22 Oryzea species. At the IRRI, in Mani la, Philippines, there are more than108,000 accessions conserved (Jackson and Lettington, 2003); in addition, there are hundreds of rice accessions held in trust in other CGIA R centers; WARDA ; CIAT; and International Institute for Tropical Agriculture (IITA) . Almost as many accessions are preserved in gene banks in other Asian countries such as China, India, Indonesia, Philippines, and Thailand (Jackson *et al*., 1997). Furthermore, considering that the International Rice Genome Sequencing Project has identified more than 80,000 genes in the rice genome and that each gene has an unknown n number of

alleles, the conclusion is that breeders will continue to have useful genetic diversity to draw on for many generations to come as long as there is a good choice germplasm.

MATERIALS AND METHODS

Experiment 1

Identification of landrace, improved and wild species of rice resistance to mixed and individual isolates of blast pathogen

Plant materials

Seeds of all collected rice varieties/accessions were sown on moist tissue paper for about 3-5 days and then the seedlings were transferred to plastic tray containing soil. Each variety/accession was prepared in three replications. A total of 311 rice varieties/accessions, including resistant and susceptible controls, were planted and used to screen for blast disease. All rice materials were provided by the National Rice Genebank of Thailand. These varieties/accessions were grouped into three *i.e*. landrace (263 genotypes), improved genotypes (43 varieties) and wild rice species (5 accessions). Out of the 263 landrace rice varieties 69, 88, 67 and 39 varieties were collected from the Central, Northeastern, Northern and Southern regions of Thailand, respectively (Figure 4, Table 3, and Appendex 1). The improved lines were cultivars with high yielding and/or cultivar with agronomic desirable traits. While the landraces were local lines which have not been planted for (commercially) but they had different special traits. The cultivars were used to identify compatible host-pathogen interaction among various Thailand blast fungus isolates.

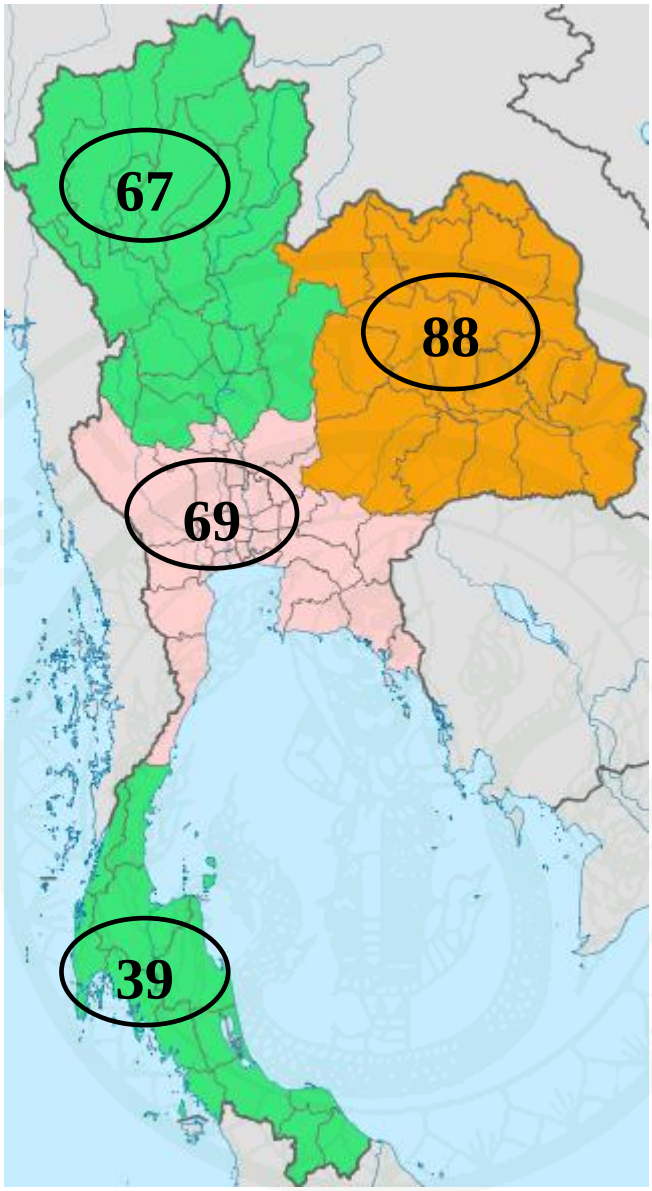

Figure 4 Regions of Thailand where local cultivars were collected: northern (67), north-eastern (88), central (69) and southern (39)

Table 4 Sources and types of rice varieties/accessations used in experiment I

Type of rice	Source(location)	Total
Landrace	Central	69
Landrace	Northeast	88
Landrace	Northern	67
Landrace	South	39
Improved varieties	-	43
Wild species	-	5
Total		311

Blast isolates

A total of ninety three blast isolates were used in this experiment. All these diversified blast isolates were collected from 7 provinces (Phitsanulok, Ubon Ratchathani, Khon Kean, Chaing Rai, Nong Khai, Chaiyaphum and Udon Thani) of Thailand (Figure. 5). These provinces were known for widespread distribution of rice blast disease occurrence and hence selected to capture the available pathogenic diversity as a hotspot areas. They did also represent the major rice growing areas of the seven provinces (Uckarach *et al*., 2011).

In this study, the utilization of ninety three isolates was held in two Phases. The first 64 fungal isolates were used as a mixed isolates, while the rest of 29 isolates were used as individual (Tables 4 and 5). Samples were collected randomly at 30-40 days after transplanting the rice following disease development at tillering stage. For each sample, blast isolates were obtained from collected samples. In each blast disease symptom sample, the location, the date, the plant's parts from which sample were obtained, and the host-cultivar were be noted. The samples were given the number,

followed by the type of lesions that were sampled as leaf, collar, neck or seed. The samples were then taken to laboratory and stored in desiccators for further processing following the method described by Uckarach *et al.* (2011).

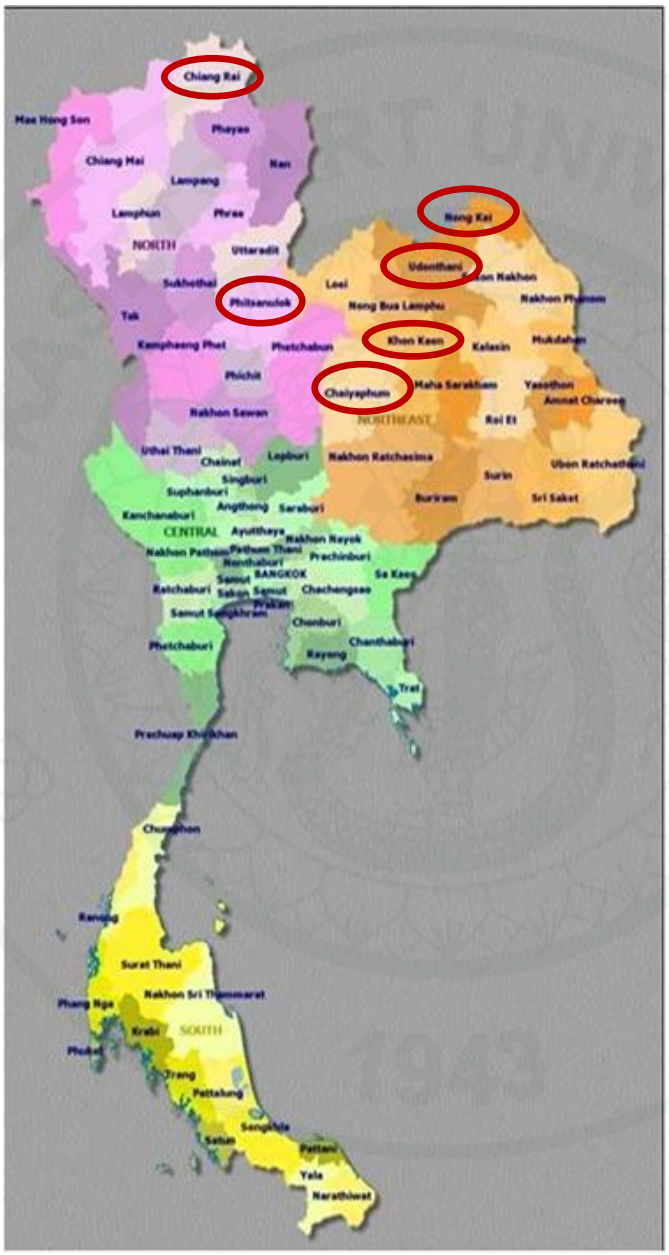

Figure 5 Provinces of Thailand where samples of the fungus were collected

Table 5 Blast fungus isolates used as mixed isolates in Experiment I

Entry	Isolate code	Location	Varieties	Infected plant part
1	BAG25.1	Kumohawapi, Udon Thani	KDML105	Leaf
2	BAG1.2	Wang Thong, Phitsanulok	KDML105	Leaf
3	BAG19.4	Kut Chap, Udon Thani	KDML105	Leaf
4	BAG20.1	Nong Han, Udon Thani	RD10	Leaf
5	BAG1.5	Wang Thong, Phitsanulok	KDML105	Leaf
6	BAG1.6	Wang Thong, Phitsanulok	KDML105	Leaf
7	BAG2.1	Mueang, Ubon Ratchathani	KDML105	Leaf
8	BAG2.2	Mueang, Ubon Ratchathani	KDML105	Leaf
9	BAG29.1	Nong Wua So, Udon Thani	RD6	Leaf
10	BAG29.2	Nong Wua So, Udon Thani	RD6	Leaf
11	BAG3.1	Wang Thong, Phitsanulok	KDML105	Leaf
12	BAG3.2	Wang Thong, Phitsanulok	KDML105	Leaf
13	BAG30.2	Nong Saeng, Udon Thani	RD6	Leaf
14	BAG3.4	Wang Thong, Phitsanulok	KDML105	Leaf
15	BAG26.1	So Phisai, Nong Khai	RD10	Leaf
16	BAG4.1	Mueang, Phitsanulok	KDML105	PN
17	BAG4.2	Mueang, Phitsanulok	KDML105	PN
18	BAG4.3	Mueang, Phitsanulok	KDML105	PN
19	BAG33.2	Bueng Khong Long, Nong Khai	RD6	Leaf
20	BAG4.5	Mueang, Phitsanulok	KDML105	PN
21	BAG4.6	Mueang, Phitsanulok	KDML105	PN
22	BAG4.7	Mueang, Phitsanulok	KDML105	PN
23	BAG5.1	Mueang, Khon Kaen	1034N.110	PN

Table 5 (Continued.)

Entry	Isolate code	Location	Varieties	Infected plant part
24	BAG5.2	Mueang, Khon Kaen	1034N.110	PN
25	BAG6.2	Mueang, Khon Kaen	1030N.8	PN
26	BAG6.3	Mueang, Khon Kaen	1030N.8	PN
27	BAG27.2	SaKhrai, Nong Khai	RD10	Leaf
28	BAG6.2	Mueang, Khon Kaen	1030N.8	PN
29	BAG6.3	Mueang, Khon Kaen	1030N.8	PN
30	BAG6.4	Mueang, Khon Kaen	1030N.8	PN
31	BAG6.5	Mueang, Khon Kaen	1030N.8	PN
32	BAG6.6	Mueang, Khon Kaen	1030N.8	PN
33	BAG11.3	Tha Bo, Nong Khai	RD6	Leaf
34	BAG11.4	Tha Bo, Nong Khai	RD6	Leaf
35	BAG7.3	Mueang, Phitsanulok	KDML105	LC
36	BAG8.1	Mueang, Ubon Ratchathani	KDML105	Leaf
37	BAG22.1	Thung Fon, Udon Thani	RD10	leaf
38	BAG8.3	Mueang, Ubon Ratchathani	KDML105	Leaf
39	BAG8.4	Mueang, Ubon Ratchathani	KDML105	Leaf
40	BAG21.1	Wang Sam Mo, Udon Thani	RD6	Leaf
41	BAG9.1	Mueang, Chiang Rai	KDML105	PN
42	BAG25.1	Kumohawapi, Udon Thani	KDML105	Leaf
43	BAG9.3	Mueang, Chiang Rai	KDML105	PN
44	BAG9.4	Mueang, Chiang Rai	KDML105	PN
45	BAG13.1	Sangkhom, Nong Khai	RD6	Leaf
46	BAG13.2	Sangkhom, Nong Khai	RD6	Leaf
47	BAG10.1	Mueang, Nong Khai	RD6	Leaf

Table 5 (Continued.)

Entry	Isolate code	Location	Varieties	Infected Plant part
48	BAG10.2	Mueang, Nong Khai	RD6	Leaf
49	BAG10.3	Mueang, Nong Khai	RD6	Leaf
50	BAG11.1	Tha Bo, Nong Khai	RD6	Leaf
51	BAG19.4	Kut Chap, Udon Thani	KDML105	Leaf
52	BAG11.3	Tha Bo, Nong Khai	RD6	Leaf
53	BAG11.4	Tha Bo, Nong Khai	RD6	Leaf
54	BAG11.5	Tha Bo, Nong Khai	RD6	Leaf
55	BAG12.1	Phon Phisai, Nong Khai	RD6	Leaf
56	BAG12.2	Phon Phisai, Nong Khai	RD6	Leaf
57	BAG12.3	Phon Phisai, Nong Khai	RD6	Leaf
58	BAG20.2	Nong Han, Udon Thani	RD10	Leaf
59	BAG12.5	Phon Phisai, Nong Khai	RD6	Leaf
60	BAG13.1	Sangkhom, Nong Khai	RD6	Leaf
61	BAG13.2	Sangkhom, Nong Khai	RD6	Leaf
62	BAG13.3	Sangkhom, Nong Khai	RD6	Leaf
63	BAG14.1	Si Chiang Mai, Nong Khai	KDML105	Leaf
64	BAG14.2	Si Chiang Mai, Nong Khai	KDML105	Leaf

LC = Leaf collar, PN = Panicle neck

Table 6 Blast fungus isolates used as individual in Experiment I

Isolate code	Rice variety isolated	Organ Infected	Location collected
Bag 1.1	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 1.3	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 1.4	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 2.3	KDML105	Leaf	Mueang, Ubon Ratchathani
Bag 2.4	KDML105	Leaf	Mueang, Ubon Ratchathani
Bag 3.3	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 3.5	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 4.4	KDML105	PN	Mueang, Phitsanulok
Bag 5.3	1034N.110	PN	Mueang, Khon Kaen
Bag 5.4	1034N.110	PN	Mueang, Khon Kaen
Bag 6.1	1030N.8	PN	Mueang, Khon Kaen
Bag 7.1	KDML105	LC	Mueang, Phitsanulok
Bag 7.2	KDML105	LC	Mueang, Phitsanulok
Bag 8.2	KDML105	Leaf	Mueang, UbonRatchathani
Bag 8.5	KDML105	Leaf	Mueang, UbonRatchathani
Bag 9.2	KDML105	PN	Mueang, Chiang Rai
Bag 9.5	KDML105	PN	Mueang, Chiang Rai
Bag 9.6	KDML105	PN	Mueang, Chiang Rai
Bag 11.2	RD6	Leaf	Tha Bo, Nong Khai
Bag 12.4	RD6	Leaf	Phon Phisai, Nong Khai
Bag 14.3	KDML105	Leaf	Si Chiang Mai, Nong Khai
Bag 15.1	KDML105	Leaf	Mueang, Udon Thani
Bag 16.1	RD6	Leaf	Phen, Udon Thani
Bag 17.2	KDML105	Leaf	Chatturat, Chaiyaphum
Bag 19.2	KDML105	Leaf	Kut Chap, Udon Thani
Bag 24.1	KDML105	Leaf	Mueang, Chaiyaphum
Bag 24.2	KDML105	Leaf	Mueang, Chaiyaphum
Bag 28.2	RD6	Leaf	Bueng Kan, Nong Khai
Bag 31.1	RD6	Leaf	Ban Dung, Udon Thani

Experimental Equipment

Petri dish, glass, plastic bottles, flasks, filter papers, Pot, balance, desiccators, microscope, autoclave, Sprayer, cutter, paper bags, tissue (soft paper), Rice flour agar (RFA) medium, ampcillin, and Water agar medium

Isolation of *P. oryzae* from infected rice samples

Infected leaves and necks of the rice plants were taken out from the storage. Each diseased leaf samples were cut into small pieces about 1 cm in length and then kept on plates in sterile distilled water. Single typical lesions that subsequently developed were taken from each sample and then placed on sterilized glass slides in Petri dishes with filter paper and incubated at 25–28^{o}C for about four days until adequate sporulation. The sporulating lesions were examined under stereoscopic microscope. After three or four days, the young colonies observed and or single conidium was identified under stereo microscopic. Blast pathogen isolates were typically gray or white colonies. Clusters of conidia then aseptically transferred with a pointed capillary tube on a thin layer of rice flour agar medium (RFA). And then a single colony was cut using a sterile scalpel into a small piece from the colony edge. Using the new RFA medium Petri dish with filter paper, transfer the cut out pieces onto the filter paper. The dried mycelium's in filter papers in the sealed bags were stored at –20^{o}C and can be kept for at least 10 years. (Sriboonjit, 2000).

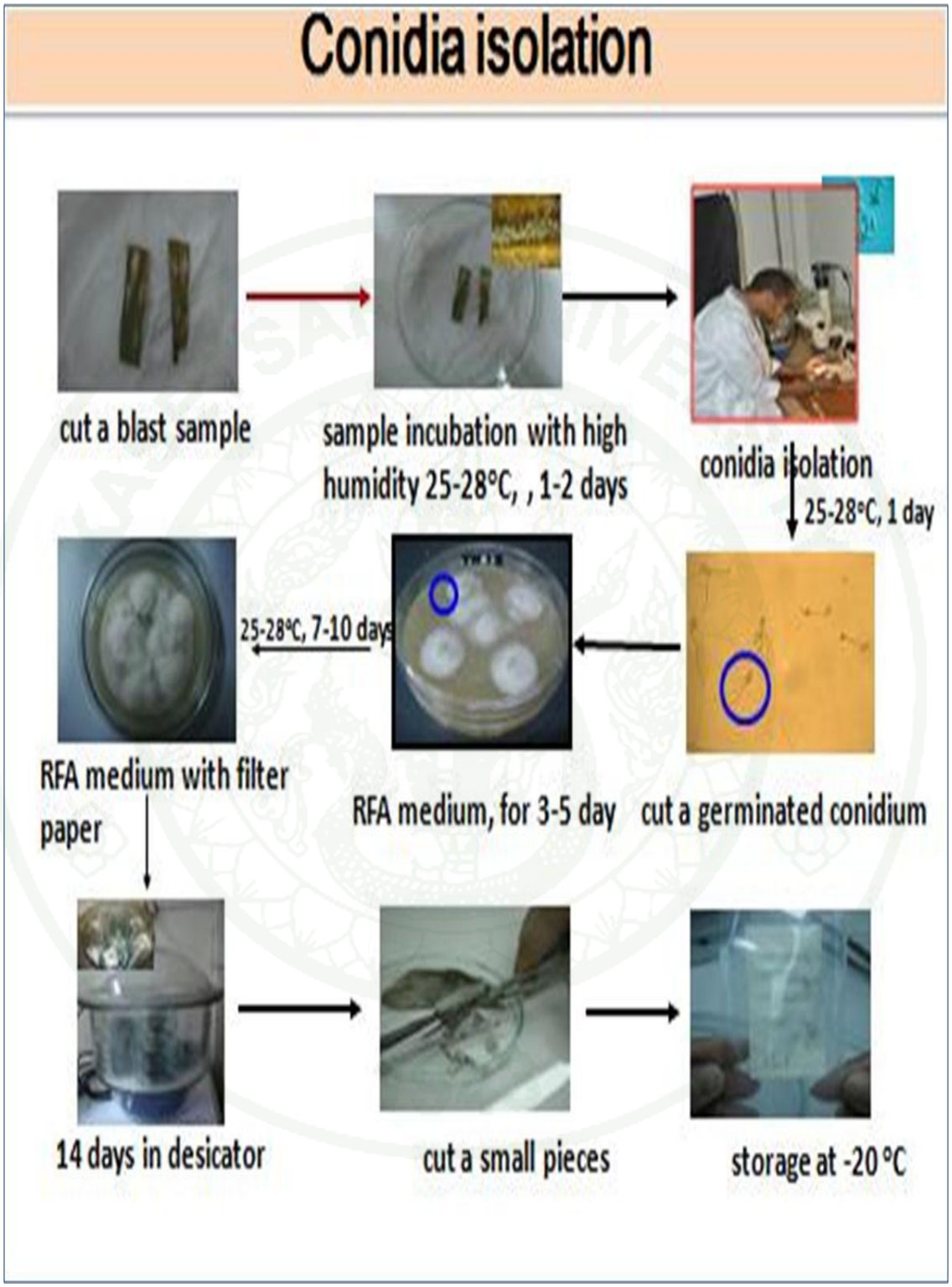

Figure 6 Schematic flows of single conidia isolation of blast pathogen.

Evaluation of leaf blast disease resistance

A total of 116 isolates were used in experiments I and II to identify compatible host-plant interaction using 311 rice genotypes in the first experiment; and 30 improved lines in the second experiment. These improved lines were high yielding cultivars with agronomic desirable traits. The experimental design was randomized complete block in 3 replications. Rice plants were grown in plastic trays and each variety was planted in one row per replication. JHN and IR64 were used as resistant and KML105 as susceptible check varieties. The Urea (46-0-0) was applied at 20 g per plastic tray. The seedlings were kept outdoors in the nylon net for 21 days before inoculation.

Fungal inoculation

Each of the blast isolates were cultured on rice flour agar media (RFA) and incubated at 25^{o}C with 12 hours, fluorescent light per day for about 8-10 days (Figure 7). After 10 days of culture, fungal conidia were scraped out from the surface under room temperature for further multiplication (Figure 8). The enhanced sporulation was adjusted to concentration of 5x10^{4} conidia /ml using sterilized distilled water. The prepared conidial suspensions of the fungus with the addition of 0.5 % gelatin were sprayed or inoculated at fourth leaf stage on each rice plant seedlings using the sprayer machine (Figure 9 and 10). After inoculation, the seedlings were kept in a dew chamber of plastic cover at 25 ^{o}C and above 95 % relative humidity for about 16 hours to create conducive environment for the penetration of the conidia and disease development (Figure 11). Then seedlings were transferred to the growth room for additional 7–10 days under high humid condition which was maintained by mechanical sprayer every three hours or three to four times per day. The leaf blast was recorded 7 days after inoculation. Disease development was monitored daily, and plants were maintained in a greenhouse at 24 to 30 °C (Figure 12).

Figure 7 Conidium culture of germinated at 28^{o}C for 10 days.

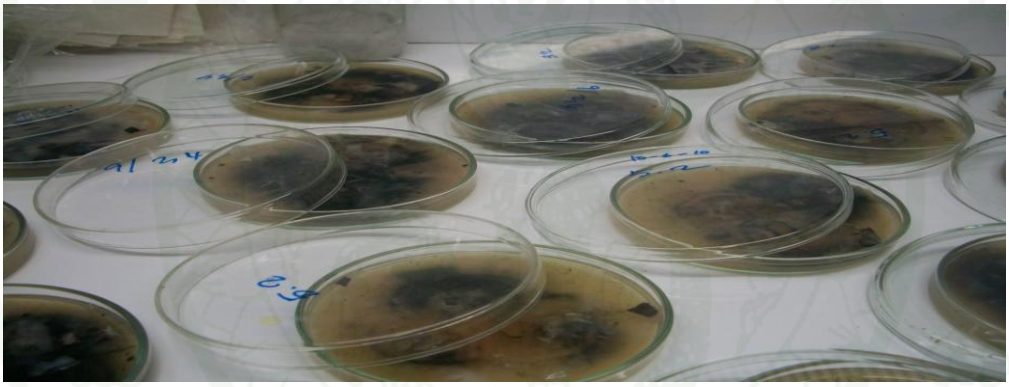

Figure 8 Scraped spore keep at 28^{o}C for 1–2 days.

Figure 9 Fourteen days old rice seedlings

Figure 10 Inoculation of Leaf at 3–5 stage after seeding

Figure 11 The dew chamber incubation at 28^{o}C for 16-18 hrs.

Figure 12 Schematic flows of blast pathogen inoculation.

Scoring of symptoms

Infection of the *P. oryzae* was scored and evaluated seven to ten days after inoculation. Each seedling was examined and rated using a classification of an international standard scale with 7 lesion type categories (Figure 13). Taking into consideration only two types of reaction of the host, compatible or susceptible (S) and incompatible or resistant reaction (R) was occurred. The varieties were considered most susceptible if it is most common and the majority of the lesions developed were of types 5 or 6. Where no sporulation developed, the disease severity indexes were types 0-2. The types 3-4 were considered as resistant and intermediate (Roumen *et al*., 1997).

Figure 13 The 7 lesion type scales for the assessment of symptoms induced by the Blast pathogen on rice leaves.

The data were classified into 2 groups, resistant (R = 0, 1, 2, 3) and susceptible (S = 4, 5, 6) reactions. Resistance index (RI) formula modified from Ahn 1994 (*Sirithunya et al.,* 2002) was used to assess the resistance index.

RI = R/T

R = Number of isolates giving resistance reaction

T = Total number of isolates used for screening

The RI value ranged from 0 to 1 where RI value equal to 0 or 1 indicated that the rice cultivar or line was considered as susceptible or resistant to all isolates, respectively.

Statistical analysis

The data on disease severity score of 30 parental lines screened against 23 blast isolates were analyzed using the average of 3 replications. The resistance index (RI) was analyzed using the mean and most of the data that analyzed phylogenic of blast isolates used NTSYS-pc Version 2.0. For pathotypic similarities among the samples based on the CANBERRA coefficients were calculated using the Simlnt module of NTSYS-pc. Cluster analysis was performed on similarity matrices with the SAHN module of NTSYS-pc using UPGMA algorithm. All dendrogams were generated from the Graphics and Tree plot module of NTSYS-pc.

Experiment 2

Evaluation of improved varieties of rice for resistance to individual isolates of blast pathogen

Plant material

A total of 30 genotypes composed of indica, japonica and tropical japonica categories including blast resistant and susceptible control were used in this study. Descriptions including names, sources and cultivars group of the genotypes were shown in Table 7.

These varieties were selected to evaluate the resistance of the rice genotypes against 23 individual blast isolates and to study the genetic diversity of the 23 blast pathogen races as well. Ten isolates namely P1.1, P1.2, P3.2, P2.4, P3.4, P3.5, P5.1, P8.2, P20.4 and P8.3 were isolated from the leaves of the rice varieties KDML105 and RD10 from different locations. Four isolates P6.4, P4.7, P4.6 and P9.2 were isolated from the neck of two different rice varieties KDML105 and 1030N.8 (Details of the 23 blast pathogens were given in Table 8).

Methodology

The methodology described in the first experiment was also used in this experiment.

Table 7 Names, origin and cultivar group of rice cultivars used in the Experiment II.

Entry	Cultivar name	Country of origin	Cultivar group
1	KDML105	Thailand	Indica
2	PTT1	Thailand	Indica
3	CNT1	Thailand	Indica
4	RD6	Thailand	Indica
5	RD7	Thailand	Indica
6	RD29	Thailand	Indica
7	RD41	Thailand	Indica
8	RD31	Thailand	Indica
9	RD43	Thailand	Indica
10	PSL2	Thailand	Indica
11	SKN1	Thailand	Indica
12	SPR1	Thailand	Indica
13	SPR2	Thailand	Indica
14	SPR3	Thailand	Indica
15	SPR80	Thailand	Indica
16	SPR90	Thailand	Indica
17	JHN	Thailand	Indica
18	Homcholalist	Thailand	Indica
19	IR64	Philippines	Indica
20	IR80151B	Philippines	Indica
21	CH1	China	Indica
22	CH2	China	Indica
23	Nerica3	Africa	Indica
24	Basmeti1	India	Indica
25	CO39	Philippines	Indica
26	KOH2	Japan	Japonica
27	Chainate1	Japan	Japonica
28	Japonica1	Japan	Japonica
29	Japonica2	Japan	Japonica
30	Azucena	Philippines	Tropical Japonica

Table 8 Twenty-three isolates of rice blast fungus isolates used in Experiment II

Isolate code	Rice variety isolated	Organ infected	Location collected
Bag 1.1	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 1.2	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 3.2	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 6.4	1030N.8	Neck	Mueang, Khon Kean
Bag 10.4	RD6	leaf	Mueang, Nong Khai
Bag 2.4	KDML105	Leaf	Mueang, Ubon Ratchathani
Bag 3.4	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 3.5	KDML105	Leaf	Wang Thong, Phitsanulok
Bag 34.1	Not identified	Not identified	Colleagues
Bag 4.7	KDML105	Neck	Mueang, Phitsanulok
Bag 7.3	KDML105	Leaf Collar	Mueang, Phitsanulok
Bag 7.1	KDML105	Leaf Collar	Mueang, Phitsanulok
Bag 5.1	KDML105	Leaf	Khon Kean
Bag 8.2	KDML105	Leaf	Mueang, UbonRatchathani
THL64	Not identified	Not identified	Colleagues
Bag 9.2	KDML105	Neck	Mueang, Chiang Rai
Bag 20.4	RD10	Leaf	NongHan, Udon Thani
Bag 36.5	Not identified	Not identified	Colleagues
Bag 4.6	KDML105	Neck	Mueang, Phitsanulok
Bag 8.3	KDML105	Leaf	Mueang, UbonRatchathani
THL196	Not identified	Not identified	Colleagues
Bag 40.3	Not identified	Not identified	Colleagues
Bag 40.4	Not identified	Not identified	Colleagues

RESULTS AND DISSCUSSIONS

Experiment 1

Identification of landrace, improved and wild species of rice resistance to mixed and individual isolates of blast pathogen

The analysis was based on the Resistance Index (RI) methodology (Sirithunya *et al*., 2002) of disease data analysis to determine the available genetic variation in disease resistance. Accordingly, out of the 311 varieties/accessions compared a total of 35, 142, and 134 cultivars were found resistant, intermediate and susceptible respectively when tested against the 64 mixed blast isolates (Table 9). The 35 resistant genotypes (25 landraces, 9 improved and 1 wild rice) were also tested in a follow-up experiment against 29 individual blast isolates and the result showed that 9 were highly resistant to all isolates of blast with no symptom of the disease (0 score). Out of the 9 highly resistant genotypes, 4 (GS23107, GS19769, GS20874 and GS23774) were from landraces, while 5 (Chinate1, Suphanburi1, Suphanburi60, Suphanburi90 and JHN) were from the improved varieties.

Subsequently, the 9 highly resistant rice varieties were tested together with IR64 (resistant check) and KDML105 (susceptible check) to confirm resistance of the genotypes against the 29 individual isolates. As expected, the RI value of all the tested genotypes were found to be 1 and KDML105 was susceptible to all isolates with RI value of 0 (Table 10). While the resistances check IR64 had RI value of 0.86 and this showed reduced resistance level.

Comparisons among the regions indicated that the landraces which were collected from central region were susceptible, while highest percentage of those landraces from the southern region showed resistance against all blast isolates. The detailed descriptions of the landrace rice varieties were indicated in Table 11.

The results from this study indicated the existence of genetic diversity among the rice varieties collected from the different regions of Thailand against blast pathogens and their interaction. It was clear that the blast disease caused by *P. oryzae* was one of the destructive diseases of rice and could cause severe damage and yield reduction with favorable environmental conditions and susceptible varieties. This experiment showed differences in the resistance to blast among cultivars collected from different regions in Thailand. These differences are in agreement with the report of Ou (1985) who recorded variability in resistance from region to region or from country to country. The differences could probably be related to the availability of predisposing factors that favor disease development (Babujee and Gnanamanickam, 2000). Artificial inoculation conducted in the greenhouse showed that 80% of the local cultivars tested against blast showed either susceptible or intermediate resistance to the 29 isolates of *P. oryzae*. 4 (GS23107, GS19769, GS20874 and GS23774) out of the 263 local cultivars were highly resistant to all 29 isolates of *P. grisea*. Similar screening trials were carried out in other rice growing countries; such as in Bangladesh, Mohanta *et al*. (2003) reported that among 28 restorer lines and 3 standard checks, 3 were highly resistant, 12 were resistant and 16 were moderately susceptible. Similarly Dissanayake (1995), in Sri Lanka, revealed that out of 22 cultivated rice varieties used, only 6 varieties were resistant to blast at two sites, with this difference attributed to parental sources.

A comparison of the regions in the current study showed that all resistant varieties were obtained from the southern, northern and northeastern provinces of Thailand. However, the results from this inoculation study need to be verified with field-based observations. The susceptibility of most of the varieties clearly suggests the need for the development and promotion of blast-resistant varieties.

Table 9 Reaction of 311 rice varieties using 64 mixed blast pathogen isolates

Variety group	Range of score (0-6)	Number of varieties	Total variety tested (%)
Resistance	0-2	35	11
Intermediate	3-4	142	46
Susceptible	5-6	134	43
Total		311	100

Table 10 Name, resistance level, type of cultivar and origin of 9 highly resistant cultivars plus susceptible and resistance check.

Code/Name	Resistance level	Type of cultivar	Origin	Score	RI Value
GS23107	Resistant	Landrace	Thailand	0	1
GS19769	Resistant	Landrace	Thailand	0	1
GS20874	Resistant	Landrace	Thailand	0	1
GS23774	Resistant	Landrace	Thailand	0	1
Chinate1	Resistant	Improved	Thailand	0	1
Suphan Buri1	Resistant	Improved	Thailand	0	1
Suphan Buri60	Resistant	Improved	Thailand	0	1
Suphan Buri90	Resistant	Improved	Thailand	0	1
JHN	Resistant	Improved	Thailand	0	1
IR64	Resistant	Resistant check	IRRI	0	1
KDML105	Susceptible	Susceptible check	Thailand	6	0

IRRI = International Rice Research Institute

RI = Resistance index

RI = R/T

Table 11 Reaction of different category of rice species against blast isolates across regions.

Category	Region	Susceptible	Intermediate	Resistance	Total
Landrace	Central	51.00%	49.00%	0	69
	Northeast	48.85%	39.80%	11.35%	88
	Northern	40.3%	46.27%	13.43%	67
	Southern	20.52%	64.10%	15.38%	39
Improved	---	44.18%	34.88%	20.93%	43
Wild species	---	40.00%	40.00%	20.00%	5

The numbers in parenthesis showed the percentage of their degree level of disease incidence against blast isolates

Experiment 2

Evaluation of improved varieties of rice for resistance to individual isolates of blast pathogens

Genotype reaction against blast pathogen

A diversified set of 30 cultivars were used to identify the resistant cultivars and to characterize isolates for virulence diversity under greenhouse conditions. These cultivars were from 3 diversified groups (indica, japonica and tropical japonica) of 5 different countries (China, India, Japan, Philippines, and Thailand) and only 1variety from Africa.

The resistance index (RI) was used to identify the percentage resistance ability of the rice genotypes against blast isolates. The 1.0 RI value indicates that rice variety could resist to all tested-isolates. On the other hand, the 0 RI valued indicates that rice variety could not resist to any tested blast isolates. The standard resistant check JHN variety was resistant to all blast isolates except to the isolate P40.4. While the standard susceptible check (KDML105) expressed the most susceptible reaction of all the 30 varieties tested. The screening of 30 rice cultivars against 23 individual rice blast isolates also revealed that only 1variety namely RD41 of the indica category from Thailand was found resistant to all 23 individual blast isolates with RI value of 1 followed by PSL2, and JHN. These varieties were also resistant against all individual blast isolates used in experiment 2 except for the isolates P7.1 and P40.4 respectively and displayed intermediate response toward the blast disease. The RI value of both PSL2 and JHN was 0.95. In addition, genotype RD41 could be grow in all locations of Thailand that the pathogenic blast fungus isolates were collected for this study (Table 12).

On the other hand, 3 varieties (KDML105, SKN1 and Azucena) showed susceptible reaction with the lowest RI values of 0.21, 0.52 and 0.56, respectively.

The RI value of RD41 is 79%, 48% and 44% higher than KDML105, SKN1 and Azucena, respectively as shown in Figure 14. Among the indica groups, 65% of the total varieties had RI value range between 0.65 up to 0.86. Generally in the present study based on the value of resistance index the interaction of these genotypes was grouped in to four. These genotypes having RI value of greater than 9 were belongs resistant; genotypes with RI value 7.6-8.9 were intermediate resistant; genotypes with RI value equals to 6-7.5 were belongs to moderately susceptible and while these genotypes with RI value of less than 6 were belongs to susceptible group.

About 70% of the genotypes from japonica category showed resistant to moderate response and the resistance index value of this group were in the range of 0.73-0.91. Cultivar Japonica2 was the most resistant variety of the group followed by Japonica1 and Chinate1. Japonica2 was resistant to all blast isolates except isolate P40.3 and P40.4 and displayed susceptible and intermediate response toward the blast disease respectively. The result of Azucena, which is the only rice genotype belongs to the tropical japonica group and originated from Philippines, showed less broad spectrum resistance index than Japonica group (Table 13).

Similar results were also reported by Haq *et al*. (2002) in a screening of 25 rice germplasm, and found that 2 lines of KSK-282 and IRRI-6 were highly resistant. Field screening of 40 entries/varieties during 2005-2006 against the blast disease revealed that only 1 entry of 99513 from PARC, 1 entry of KSK-10 from Rice Research Institute, Kala Shah Kaku and DM-2-25-9-02 from NIAB, showed resistant response (Arshad *et al*., 2008). Castano *et al*. (1990) also developed methods for screening of 437 upland genotypes from Indonesia, Colombia and IRRI (Philippines) for resistance to *P. oryzae* 6 times within 2 years and found that 176 genotypes were highly resistant while others had low to high susceptibility to rice blast disease.

Table 12 Response to reaction of the rice genotypes against blast isolates

Rice variety	Isolate number																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	RI
KDML105	S	I	I	S	S	S	I	I	R	S	R	I	R	S	S	S	S	I	S	S	I	R	R	0.21
PTT1	R	R	R	R	R	I	R	I	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	0.86
CNT1	S	I	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	0.78
RD6	S	S	I	R	S	I	R	I	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	0.65
RD7	S	I	I	S	I	R	R	I	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R	0.69
RD29	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	I	R	R	0.86
RD41	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	1.00
RD31	I	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.91
RD43	S	I	R	I	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.82
PSL2	R	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.95
SKN1	S	I	R	I	I	R	R	I	R	R	R	I	R	S	R	I	S	R	I	S	R	R	R	0.52
SPR1	I	S	I	R	I	R	I	I	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	0.69
SPR2	S	I	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.78
SPR3	I	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	0.86

Table 12 (Continued)

	Isolate number																						
Rice variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	RI
SPR80	I	I	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.86
SPR90	S	I	I	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.82
IR64	S	R	I	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.86
IR10151	R	R	R	I	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	0.86
Basmati1	R	R	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	I	0.78
Japonica2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	I	0.91
JHN	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	0.95
CO39	R	R	S	I	R	R	R	R	R	R	R	R	R	R	I	R	S	S	I	S	R	R	I	0.65
Homchilalist	I	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	0.73
KOH2	I	R	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	S	0.73
Chainate1	R	I	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	S	0.78
Nerica3	S	I	R	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.82
Japonica1	R	S	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	0.82
CH1	R	R	I	I	R	R	R	R	R	R	R	R	R	R	R	R	I	R	I	R	R	R	R	0.82
CH2	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0.91
Azucena	I	R	R	R	R	R	I	R	R	R	R	R	R	I	I	I	S	I	I	S	R	R	S	0.56

1: P3.2, 2: P8.2, 3: P6.4, 4: P10.4, 5: P9.2, 6: P3.4, 7: P2.4, 8: P7.1, 9: P7.3, 10: P1.2, 11: P5.1, 12: P36.5, 13: P20.4, 14: P1.1, 15: P4.7, 16: P34.1, 17: P3.5, 18: P40.3, 19: P8.3, 20:PTHL196, 21: P4.6, 22: PTHL64, 23:P40.4

R = Resistant response, I = Intermediate response, S = Susceptible response RI = Resistant index

Table 13 Number of different category of rice genotypes that react to blast isolates

	Category of rice genotypes								
	Indica(25)			Japonica(4)			Tropical Japonica (1)		
Name of Isolate	R	I	S	R	I	S	R	I	S
P3.2	9	6	10	3	1	0	0	1	0
P8.2	12	11	2	2	1	1	1	0	0
P6.4	12	12	1	1	3	0	1	0	0
P10.4	11	10	4	1	3	0	1	0	0
P9.2	15	8	2	3	1	0	1	0	0
P3.4	20	4	1	4	0	0	1	0	0
P2.4	23	2	0	4	0	0	0	1	0
P7.1	17	8	0	4	0	0	1	0	0
P7.3	25	0	0	4	0	0	1	0	0
P1.2	24	0	1	4	0	0	1	0	0
P5.1	25	0	0	4	0	0	1	0	0
P36.5	23	2	0	4	0	0	1	0	0
P20.4	25	0	0	4	0	0	1	0	0
P1.1	22	1	2	4	0	0	0	1	0
P4.7	23	1	1	4	0	0	0	1	0
P34.1	23	1	1	4	0	0	0	1	0
P3.5	18	3	5	3	0	1	0	0	1
P40.3	20	3	2	0	3	1	0	1	0
P8.3	19	4	2	4	0	0	0	0	1
PTHL196	20	1	4	4	0	0	0	0	1
P4.6	22	3	0	4	0	0	1	0	0
PTHL64	25	0	0	4	0	0	1	0	0
P40.4	22	3	0	1	1	2	0	0	1

R = Resistant response I = Intermediate response S = Susceptible response

Numbers in parenthesis indicate total number of genotypes from each group.

RD41, JHN and IR64 rice genotypes had a broad spectrum resistance. The resistances of all genotypes were still stable for most of the isolates used in this study. However, it was observed that lesion type score 5 and 3 was recorded for IR64 and JHN, respectively against the blast isolate P3.2 for IR64 and blast fungus P40.4 for JHN. Many researchers (Sirithunya *et al*., 2004, Noenplab *et al*., 2006) have been

using these genotypes as donors for blast resistance in breeding programs and to improve blast resistance of target or new rice varieties. But the present result indicates the need for alternative resistant genotypes that can replace both JHN and IR64. Variety RD41 showed the only broad spectrum resistant to all isolates and for this reason it can replace to JHN and IR64 as a donor of resistant gene.

Jao Hom Nin (JHN) rice variety has been demonstrated broad spectrum resistance to Thai blast pathogen populations. It had been identified that the position of resistance genes were located on chromosome 1 and 11 (Noenplab *et al*., 2006). The resistance gene on chromosome 11 showed major effect while the gene on chromosome 1 showed minor effect. Plant breeders achieved considerable early success in disease control through using new resistant cultivars. However, they often found that within a few years of the introduction of an R (Resistant) gene into commercial production, the gene ceased to protect the crop from disease, and severe epidemics occurred (Buchanan *et al*., 2000). And this indicated that resistance is gradually reduced over time and this also refers to the gene for gene model that predicts plant resistance would occur only when a plant possesses a dominant resistance gene (R) and the pathogen expressed the complementary dominant a virulence gene (Avr) (Buchanan *et al*., 2000).

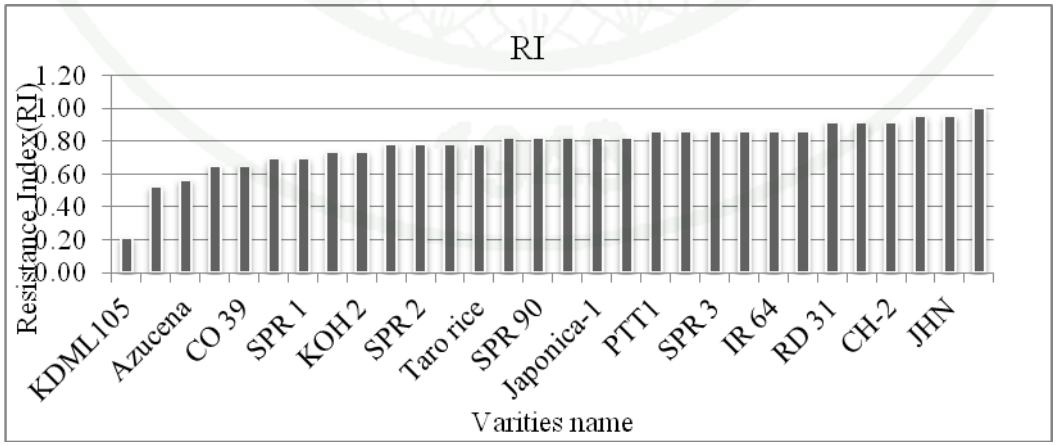

Figure 14 Resistance index (RI) of 30 test cultivars screened for blast resistance using 23 isolates.

Pathogenecity analysis

Pathotypic similarity and cluster analysis of 23 blast isolate

Dendrogram was derived from the virulence pattern of 23 blast isolates using 30 genotypes as shown in Figure 15. The 23 isolates used to screen the genotypes showed similar pathotypic. The isolates were grouped as 1 using similarity index of 69% and grouped into two using similarity index of 65%. While the rice genotypes were grouped into one using the similarity index of 59% and into three using similarity index of 54%.

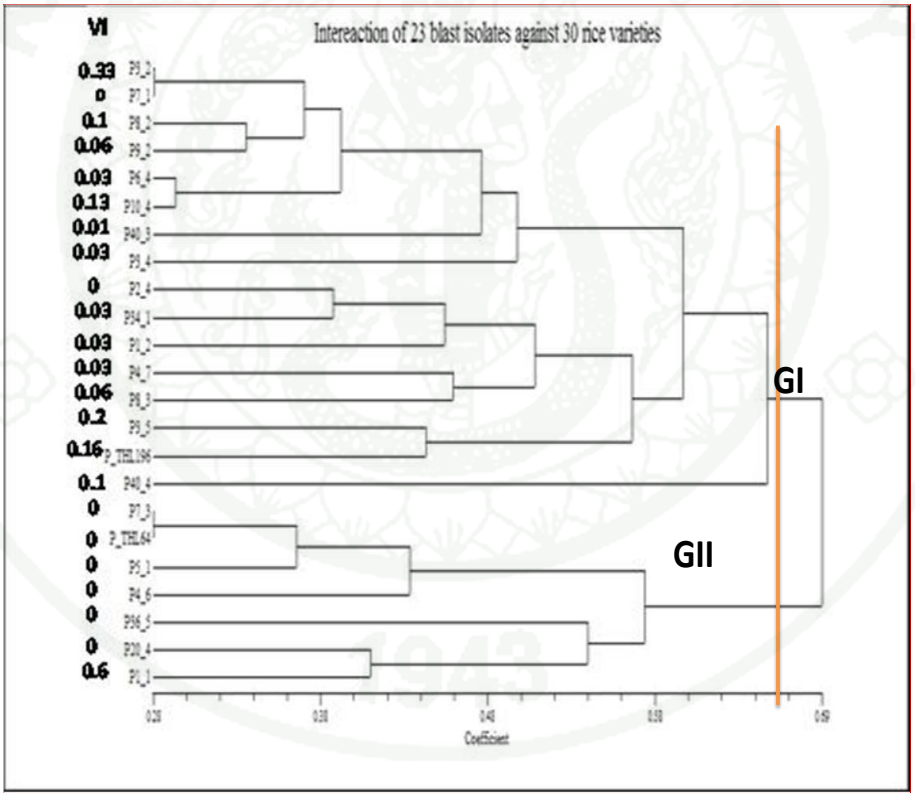

Figure 15 Dendogram derived from virulence pattern of 23 blast isolates using 30 caltivars by simple maching followed by UPGMA NTSYS-pc.

Clustering analysis of the 23 isolates based on their virulence pattern showed that 15 isolates belongs to group I that is virulent (more aggressive). While 7 isolates belongs to group II and that is avirulent at similarity index of 65% (Figure 15).

The similarity and clustering analysis of the 30 rice genotypes based on the intereaction of the 23 blast isoltes showed that, the genotypes were grouped into two main groups at similarity index of 57% that is susceptible genotypes belongs to group I, resistant and intermediate belongs to group II. In addition to this, group two also divided into two sub-groups at similarity index of 54%. Sub-group I belongs to resistant and sub-group II belongs to resistant to Intermediate (Figure 16).

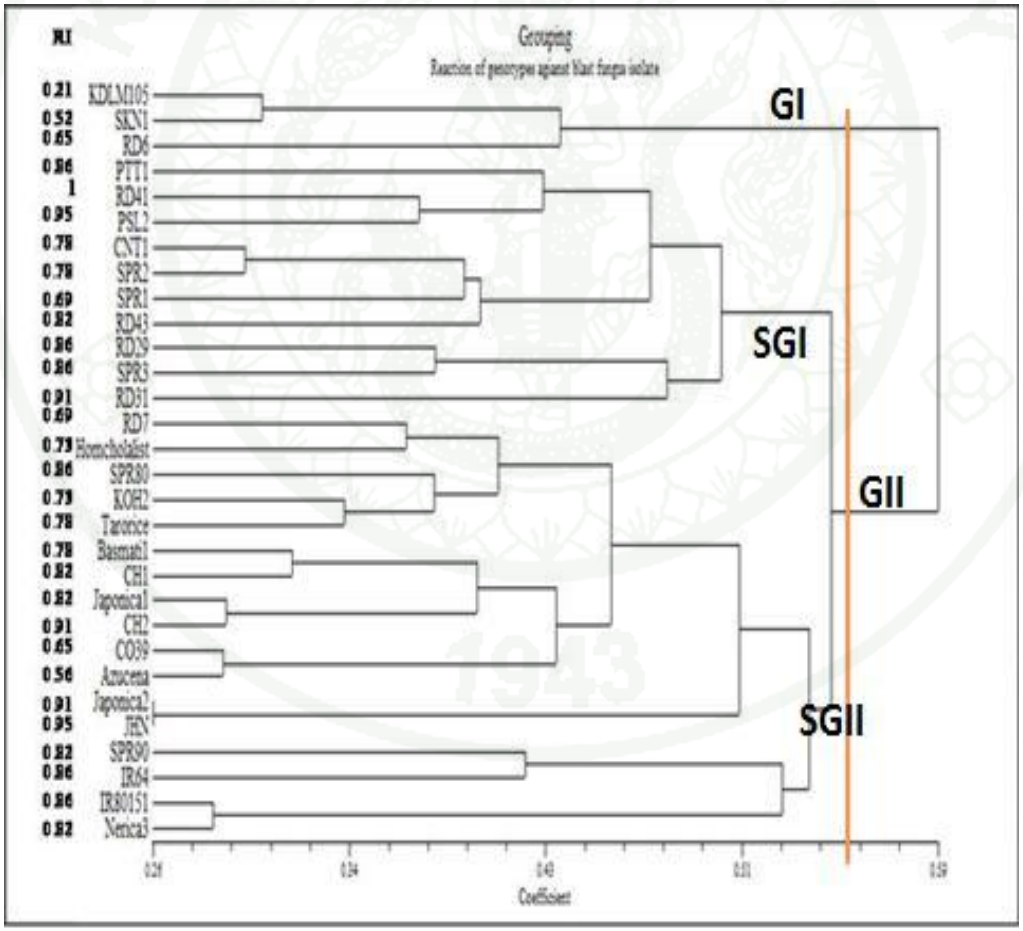

Figure 16 Dendogram of 30 test varieties screened for blast resistance using 23 isolates

Virulence Analysis

The term virulence is used to differentiate the ability of blast fungus strain to overcome the resistance gene of a particular rice variety. A diversified set of 30 cultivars were used to characterize isolates for virulence diversity under greenhouse conditions. These cultivars were collections from three diversified groups (Indica, Japonica and Tropical japonica) from five different countries (China, India, Japan, Philippines and Thailand) and only 1 variety from Africa.

The result of virulence test of 23 isolates with 30 test varieties is summarized in Table 13. The data was classified into 2 groups, Avirulent (0, 1, 2 & 3) and Virulent (4, 5 & 6). The relationship among different fungi isolates collected from different organ of rice varieties and different locations greatly varied with rice cultivars. Hence, inoculated healthy rice plants of 30 varieties using different blast isolates produced typical symptoms of the disease. Among the 23 blast fungus isolates studied, only the blast fungus P3.2 obtained from the leaf organ of KDML105, Wong Thong, Phitsanulok province was the most predominant, virulent and aggressive pathogen in over 33% of the total inoculated rice plants followed by isolate P3.5 and it was virulent for about 20% of the total inoculated rice plants. Both isolates P3.2 and P3.5 were collected from areas well known for widespread rice blast disease occurrence and they do also represent the major rice growing areas of Thailand.

Blast fungus isolates harvested from neck organ of different rice varieties showed that, P4.7, P4.6, and P9.2 were avirulent for about 94% to all rice genotypes tested in this study. While three blast isolates (P2.4, P5.1, and P20.4) harvested from leaf organ, two isolates (P7.1 and P7.3) from leaf collar, one isolate (P4.6) from neck organ and two isolates (P36.5 and PTHL64) collected from the unknown organ and location showed that 100% avirulent to all 30 rice genotypes tested (Figure 17).

In the present studies, the result showed differences in virulence test of 23 blast isolates. These differences are in agreement to those reported by other workers

such as Jamaluddin, *et al*., (2011) reported that among the seven rice blast fungus, *M. oryzae* was significantly high and appeared as the pre-dominant fungus. Similarly, Naeem *et al*., (2001) also recorded *Pyricularia oryzae, Alternaria padwickii, Curvularia sp., Fusarium moniliforme* and *Bipolaris oryzae* from seeds, shoot and root of different rice varieties.

Table 14 Summary of 23 *Pyricuria oryza* isolates virulence reaction on 30 parental lines and test varieties.

No.	Blast Isolate	Avirulence	Virulent	Virulence (%)
1	P3.2	20	10	33
2	P8.2	27	3	10
3	P6.4	29	1	3
4	P10.4	26	4	13
5	P9.2	28	2	7
6	P3.4	29	1	3
7	P2.4	30	0	0
8	P7.1	30	0	0
9	P7.3	30	0	0
10	P1.2	29	1	3
11	P5.1	30	0	0
12	P36.5	30	0	0
13	P20.4	30	0	0
14	P1.1	28	2	7
15	P4.7	29	1	3
16	P34.1	29	1	3
17	P3.5	24	6	20
18	P40.3	27	3	10
19	P8.3	28	2	7
20	PTHL196	25	5	17
21	P4.6	30	0	0
22	PTHL64	30	0	0
23	P40.4	27	3	10

The result could indicate that, out of 30 diversified genotypes tested, RD41, PSL2 and JHN rice genotypes that had the highest RI value 1 and 0.95 could be grown in all locations of Mueang, Ubon Racthathani, Mueang, Phitsanulok, Nong Han, Udon Thani and Khon Kean provinces of Thailand because of their resistance to blast isolates collected from these locations. While rice blast pathogenic fungus harvested from Wang Thong and Phitsanulok province were the most virulent and aggressive pathogen to most of the rice genotypes tested against the blast.

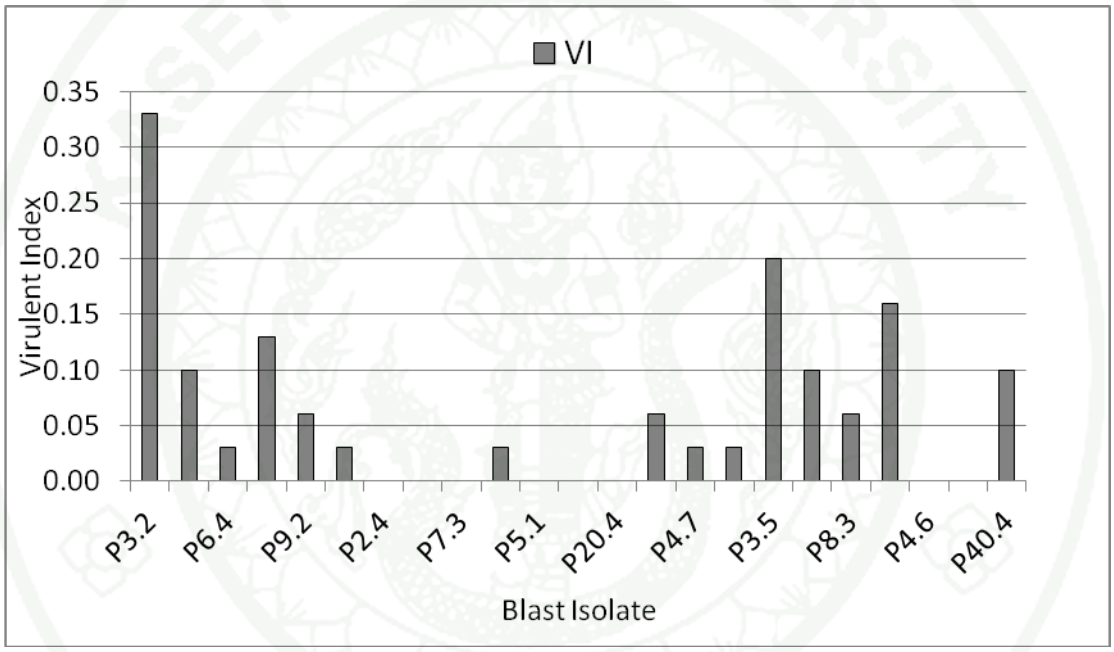

Figure 17 Virulent index (VI) of 23 blast fungus isolates against 30 test varieties.

CONCLUSION AND RECOMMENDATION

Conclusion

This study showed that there was variability in resistance among the different rice genotypes evaluated. All resistant genotypes were obtained from the Southern, Northern and Northeastern regions of Thailand. These areas could be used as a potential source of blast disease resistant genotypes.

Inclusion of blast disease resistance as criteria in rice breeding is feasible as it has no effect on the environment and it is relatively cheap as compared to chemicals. Nine highly resistant genotypes namely, GS23107, GS19769, GS20874, GS23774, Nat1, Suphanburi1, Suphanburi60, Suphanburi90, JHN and IR64 were identified in the first experiment. Out of these 9 genotypes, 4 genotypes GS23107 (from Northeastern), GS23774 (from South), and GS19769, GS20874 (from North) of Thailand were obtained from the landraces and 5 genotypes were obtained from improved.

Among the seven provinces where the 23 blast fungus isolates were collected, the isolates from Wong Thong district at the Phitsanulok province were the most virulent and aggressive pathogen in over 33% of the total inoculated rice plants.

Recommendation

Rice genotypes GS23107, GS19769, GS20874, GS23774, Nat1, Suphanburi1, Suphanburi60, Suphanburi90, JHN and IR64 from Experiment I and RD41, PSL2 and JHN from Experiment II could be used in future breeding programs in order to come up with agronomically important and blast disease resistant rice genotypes.

In addition to this, RD41 genotype can grow in all locations of Thailand that the pathogenic blast fungus isolates were collected for this study. However, the results

from this inoculation study need to be verified with field-based observations. The susceptibility of most of the varieties clearly suggests the need for the development and promotion of blast-resistant varieties.

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APPENDIX

APPENDIX

Appendix Table 1 Code of 311 rice genotypes used in the Experiment 1

R.no	GS.No	R.No	GS.No	R.No	GS.No	R.No	GS.No	R.No	GS.No
1	829	26	5382	51	9601	76	1625	101	5778
2	962	27	5422	52	10623	77	2686	102	5779
3	1218	28	5431	53	10624	78	2762	103	5801
4	1315	29	5552	54	10628	79	2937	104	5802
5	1321	30	6179	55	10654	80	2942	105	5836
6	1626	31	6191	56	11049	81	3238	106	7620
7	2054	32	6543	57	11057	82	3241	107	7691
8	2073	33	6564	58	11070	83	3278	108	7692
9	2094	34	6565	59	11081	84	3279	109	7693
10	2954	35	6746	60	11097	85	3321	110	7772
11	2960	36	6750	61	11098	86	3379	111	7972
12	3043	37	6754	62	11104	87	4047	112	7980
13	3095	38	7150	63	11108	88	4052	113	7987
14	3695	39	6797	64	11117	89	4090	114	8178
15	3772	40	7536	65	12906	90	4373	115	10154
16	3777	41	7601	66	10810	91	5565	116	10185
17	3803	42	8013	67	10977	92	5612	117	10159
18	3815	43	8188	68	11041	93	5647	118	10164
19	4077	44	8205	69	11044	94	5655	119	10165
20	5335	45	8211	70	11045	95	5661	120	10670
21	5342	46	7549	71	12924	96	5722	121	10689
22	5353	47	7553	72	14161	97	5749	122	12160
23	5355	48	7574	73	12944	98	5765	123	12510
24	5357	49	7590	74	14170	99	5772	124	13202
25	5359	50	7592	75	13197	100	5773	125	13207

Appendix Table1 (Continued.)

R.No	GS.No	R.No	GS.No	R.No	GS.No	R.No	GS.No	R.No	GS.No
126	13224	151	21610	176	101	201	5999	226	16236
127	13228	152	21611	177	102	202	3000	227	16238
128	13722	153	21650	178	1416	203	6001	228	18442
129	13738	154	21656	179	1445	204	6004	229	19002
130	13937	155	21689	180	1856	205	6008	230	19055
131	13955	156	21702	181	2715	206	6327	231	19683
132	13956	157	21789	182	3057	207	6473	232	19685
133	13957	158	21898	183	3058	208	6503	233	19700
134	13972	159	21907	184	3437	209	7331	234	19725
135	13995	160	21931	185	3447	210	7528	235	19728
136	14042	161	21947	186	3578	211	7731	236	19769
137	14055	162	22214	187	3594	212	7753	237	19817
138	14069	163	22220	188	3597	123	8322	238	20874
139	14086	164	22299	189	3671	214	8900	239	20881
140	15894	165	22718	190	4419	215	9047	240	21855
141	15895	166	22721	191	4448	216	9250	241	21865
142	15905	167	22764	192	1942	217	9264	242	22330
143	18425	168	22773	193	5250	218	10870	243	22588
144	19877	169	22820	194	5264	219	10916	244	22645
145	19878	170	22841	195	5315	220	10917	245	23007
146	19961	171	22848	196	5324	221	11828	246	23035
147	19967	172	23107	197	5450	222	11835	247	23163
148	20282	173	23181	198	5996	223	11839	248	23543
149	21601	174	23275	199	5963	224	11903	249	23570
150	21608	175	23279	200	5998	225	11919	250	23649

Appendix Table1 (Continued.)

R.No	GS.No	R.No	GS.No	R.no	GS.No
251	1771	276	11179	301	20712
252	1985	277	13451	302	23898
325	4029	278	14314	303	24392
254	4031	279	14321	304	16234
255	4037	280	14328	305	13745
256	4106	281	14429	306	13746
257	4215	282	15565	307	15188
258	4238	283	15583	308	15136
259	4275	284	15587	309	16119
260	4393	285	15667	310	16181
261	4517	286	15668	311	18745
262	4522	287	15672		
326	4641	288	16618		
264	6996	289	16747		
265	7045	290	17367		
266	7062	291	19528		
267	7087	292	21973		
268	9655	293	23502		
269	9667	294	23511		
270	9741	295	23774		
271	9954	296	23834		
272	9978	297	23835		
273	10333	298	23842		
274	10350	299	16240		
275	10398	300	19869		

Appendix Table 2 Average of three replication of the 30 genotypes used in the second experiment

Variety	P3_2	P8_2	P6_4	P10_4	P9_2	P3_4	P2_4	P7_1
KDLM105	5.3	4.0	3.7	5.0	4.7	5.0	3.7	2.7
PTT1	3.0	0.7	0.3	0.3	1.0	2.3	1.0	2.3
CNT1	4.7	3.0	2.0	2.3	2.3	1.7	0.3	1.0
RD6	5.3	4.7	3.0	2.0	5.3	2.3	0.3	3.0
RD7	5.3	2.7	2.7	4.3	3.7	0.7	0.0	2.3
RD29	1.3	2.0	2.3	0.0	0.7	0.7	0.3	1.7
RD41	1.3	0.3	0.7	1.0	0.7	0.3	0.3	2.0
RD31	2.7	2.0	1.3	0.0	2.3	0.0	0.7	2.0
RD43	5.0	3.7	1.7	3.0	1.0	0.3	2.0	3.7
PSL2	1.3	0.7	0.7	0.7	1.0	0.3	0.7	2.3
SKN1	5.3	3.7	0.0	3.3	3.0	2.3	1.7	3.7
SPR1	3.7	4.7	3.7	1.7	4.0	0.7	3.0	3.7
SPR2	5.0	4.0	3.7	3.3	2.7	1.7	1.3	1.0
SPR3	3.7	0.7	3.0	1.3	0.0	0.0	0.3	1.3
SPR80	2.7	2.7	1.7	3.0	1.0	0.0	0.0	1.0
SPR90	5.0	4.0	4.0	5.7	0.0	1.0	0.5	1.3
IR64	5.3	0.0	2.3	5.3	0.0	0.0	0.0	1.0
IR80151	1.7	0.0	1.5	3.0	0.3	2.3	0.0	1.3
Basmati1	1.0	1.0	3.3	2.7	2.3	0.7	1.0	1.0
Japonica2	0.3	0.7	0.0	1.0	1.0	1.0	0.0	1.0
JHN	1.0	0.3	0.0	0.0	0.7	1.7	0.0	1.0
CO39	1.0	0.7	5.3	3.3	0.5	0.7	1.0	1.0
Homcholalist	2.3	2.7	2.7	2.3	1.0	2.0	0.7	1.0
KOH2	2.7	1.5	2.3	3.0	2.7	1.5	2.0	0.3
Chainate1	1.7	3.3	4.0	2.3	0.0	0.7	0.0	1.0
Nerica3	4.3	3.3	1.5	2.0	3.0	2.3	0.0	2.0
Japonica1	1.0	4.3	2.3	2.7	0.3	0.0	1.0	1.3
CH1	1.0	2.0	3.0	1.3	1.7	0.7	0.3	1.7
CH2	1.0	2.3	1.7	1.3	2.0	2.0	1.0	1.3
Azucena	1.3	2.0	2.0	1.7	2.0	0.0	2.3	1.0

Appendix Table 2 (Continued)

Variety	P7_3	P1_2	P5_1	P36_5	P20_4	P1_1	P4_7	P34_1
KDLM105	0.0	4.7	0.3	3.0	1.0	5.7	5.7	5.0
PTT1	0.3	0.3	0.7	0.0	1.7	3.3	0.0	0.7
CNT1	0.0	0.7	0.7	0.7	0.0	0.0	0.7	0.3
RD6	2.0	1.0	0.7	0.7	0.3	0.3	1.3	0.5
RD7	0.0	1.0	0.3	0.0	0.0	1.3	0.0	0.3
RD29	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0
RD41	0.7	0.3	0.7	0.0	0.0	0.0	0.3	0.3
RD31	1.3	0.3	0.3	0.0	0.0	0.3	0.0	0.0
RD43	0.7	1.0	0.7	0.7	0.3	0.3	0.0	1.3
PSL2	0.0	1.3	0.3	0.0	1.0	0.3	0.3	0.3
SKN1	0.0	0.5	0.3	2.7	1.0	4.7	1.3	2.7
SPR1	0.7	1.0	0.5	0.0	0.0	0.0	0.3	0.7
SPR2	0.3	1.0	0.3	0.3	0.0	0.0	0.0	0.3
SPR3	0.0	0.7	0.0	0.0	0.0	0.0	0.3	0.3
SPR80	0.0	0.3	0.0	0.3	0.0	1.3	0.0	0.3
SPR90	0.3	0.5	0.0	0.3	0.3	0.0	0.3	0.7
IR64	0.0	0.7	0.0	0.0	0.3	0.0	0.0	0.0
IR80151	0.0	0.0	0.0	0.5	0.3	0.0	0.0	0.3
Basmati1	0.0	0.0	0.0	0.0	0.3	0.7	0.0	1.3
Japonica2	0.0	0.0	0.0	0.3	0.0	0.0	0.3	0.0
JHN	0.3	0.3	0.0	0.0	0.0	0.0	0.3	0.0
CO39	0.0	0.3	0.0	1.3	0.3	1.7	3.0	1.3
Homcholalist	0.0	0.5	0.0	0.0	0.0	1.3	0.3	2.0
KOH2	0.0	1.5	0.0	0.2	0.0	0.0	0.7	1.5
Chainate1	0.0	0.5	0.0	0.0	0.0	0.0	0.3	0.3
Nerica3	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0
Japonica1	0.0	0.3	0.0	0.3	0.7	0.0	0.0	0.0
CH1	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3
CH2	0.0	0.0	0.0	0.3	1.3	0.7	0.0	0.0
Azucena	0.0	1.3	0.0	0.7	0.3	3.0	4.0	2.3

Appendix Table 2 (Continued.)

Variety	P3_5	P40_3	P8_3	P_THL196	P4_6	P_THL64	P40_4
KDLM105	6.0	3.7	4.7	6.0	3.0	0.0	0.3
PTT1	1.0	0.3	0.0	0.0	1.0	0.0	0.3
CNT1	0.3	2.3	0.0	0.7	1.0	0.0	0.0
RD6	6.0	0.0	6.0	1.0	1.7	0.0	0.3
RD7	3.0	0.3	0.3	1.3	0.0	0.0	0.7
RD29	1.0	1.3	2.3	0.3	3.0	0.0	0.3
RD41	0.3	0.7	0.3	0.0	2.0	0.3	0.0
RD31	0.3	0.0	0.0	0.0	0.0	0.0	0.3
RD43	1.3	0.3	0.7	0.0	1.3	0.0	0.0
PSL2	1.0	0.7	0.3	0.0	0.0	0.0	0.3
SKN1	6.0	0.3	3.3	6.0	0.3	0.0	0.0
SPR1	1.3	3.3	0.3	0.0	0.3	0.0	0.7
SPR2	0.3	0.7	0.3	0.0	1.3	0.0	0.0
SPR3	0.3	1.0	0.7	0.3	3.0	0.0	0.3
SPR80	0.0	2.0	0.7	0.0	0.0	0.0	1.7
SPR90	0.0	0.3	0.0	0.0	0.3	0.0	0.3
IR64	0.0	0.0	0.0	0.0	0.0	0.0	0.3
IR80151	1.3	0.7	0.0	4.0	0.7	0.0	0.0
Basmati1	0.0	4.5	0.3	0.0	0.0	0.0	3.3
Japonica2	0.0	5.3	0.3	0.0	0.0	0.0	2.3
JHN	0.0	1.7	0.3	0.0	0.0	0.0	3.3
CO39	6.0	5.3	4.0	5.7	1.7	0.0	4.0
Homcholalist	5.7	0.3	0.7	5.3	0.0	0.0	0.0
KOH2	0.3	4.0	0.3	0.0	0.0	0.0	5.0
Chainate1	2.0	3.7	1.5	0.0	0.0	0.0	5.7
Nerica3	1.0	1.3	0.0	0.3	0.7	0.0	0.0
Japonica1	0.7	4.0	0.7	0.7	0.0	0.0	1.3
CH1	4.0	1.0	3.0	0.3	0.0	0.0	1.7
CH2	1.0	0.7	0.7	0.3	0.0	0.0	0.3
Azucena	5.0	3.0	3.3	5.0	0.0	0.0	4.7

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