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THESIS

IDENTIFICATION OF NON-AGED-RELATED BROAD SPECTRUM RESISTANCE AND MARKER ASSISTED INTROGRESSION OF MULTIPLE GENES FOR THE IMPROVEMENT OF BACTERIAL BLIGHT RESISTANCE IN RICE

KHIN MYO WIN

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Bacterial blight (BB) caused by *Xanthomonas oryzae* pv.*oryzae* is one of the most devastating rice diseases. Large-scale and long-term cultivation of rice varieties with single gene may enable the pathogen easily to overcome their resistance. Therefore, identification of excellent genetic resources and resistance genes is need for an improvement of BB resistance in rice breeding program. In the first study, we would like to identify a non age-related broad spectrum resistance to BB in the rice variety IR1188. The genes confer the non-age-related broad spectrum resistance to BB (*Xa*21 and seedling resistance genes) possessing by the IR1188 were introgressed into KDML105 through three rounds of marker assisted backcrossing (MAB) and phenotypic selection. Sixty KDML105 backcross introgression lines (KBILs), all carrying the *Xa21*, were challenged with 19 isolates. Three seedling resistance (SR) loci inherited from IR1188 were identified on rice chromosomes 1 (RM302-RM212), 8 (RM210-RM149) and 11 (RM287-RM224). Based on the data obtained from the rainfed lowland project, all KBILs and the original KDML105 were not significantly different in their agronomic performance. The information of seedling resistance identified in this experiment was lead to identify *a* non age-related broad spectrum BB resistance in KDML105.

In the second study, an improved Manawthukha rice line 'Yn 3248-2-128-76-4-3-75' (MK-75) that has high yield, good grain and cooking quality was used as recipient parent to improve its resistance to the BB. RGDU-07097-1-8M-9 (RG-9), an improved resistance line carrying the *xa5*, *Xa21* and *xa33* was used as donor parent. Three backcrosses and one selfing with the aided of marker assisted selection (MAS) were successfully transfered the resistant alleles of *xa5*, *Xa21* and *xa33* from RG-9 into the MK-75 while maintain the cooking quality of the MK-75. Twenty eight selected $BC_3F_{2:3}$ introgression lines (MK75ILs) carrying different combinations of these loci were tested for BB resistance at the seedling and tillering stages against ten Thai *Xoo* and five Myanmar *Xoo* isolates. The triple and double resistance gene-introgressed lines (*xa5Xa21xa33* or *xa5Xa21* or *xa5xa33* or *Xa21xa33)* had higher resistance and wider resistant spectrum to BB than MK-75 against both Thai and Myanmar *Xoo* isolates. The single resistance gene-introgressed lines carrying the *xa5* had higher resistance than that carrying the *Xa21* and *xa33* against Thai *Xoo* isolates, while resistance gene-introgressed lines carrying *Xa21* showed higher level of resistance to Myanmar *Xoo* isolates followed by lines with *xa5* and *xa33* respectively. These results clearly indicated that pyramiding of multiple genes is a useful approach for improving BB resistance in MK-75. All MK75ILs had physical grain quality, fragrance and intermediate amylose content similar to the MK-75.

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Khin Myo Win

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INTRODUCTION

Rice (*Oryzae sativa*.L) is one of world's most important cereals, which collectively provides the largest source of calories for human consumption. It is considered as the most staple food crop in the world especially in Asia because more than half of the world's population consumes rice (Hossain, 1997). In the world's rice production areas, rice crop has been affected by a number of biotic and abiotic stresses (Srinivasan and Gnanamanickam, 2005). Among them diseases, insect pests and weeds are major constraints limiting rice production. Rice diseases are reported to cause annual yield loss conservatively at 5% (Song *et al*., 2001). Bacterial blight disease (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most destructive diseases of rice throughout the world. Yield losses in severely infected fields ranging from 20 to 30%, but it can reach as high as 80%and grain quality (Khush *et al*., 1989; Noh *et al*., 2007). BB was reported as major disease affecting rice production in irrigated and rain-fed lowland ecosystems throughout Asia, northern Australia, mainland Africa, southern part of the United States and Latin America (Mew, 1987). It is a limiting factor to rice yield in all major rice-growing regions of the world.

Plant disease management has three approaches; agriculture management, protectant application, and resistant cultivar cultivation. Cultural control such as seed disinfection, proper drainage, removal of diseased plants, weeds and debris are important cultural practices at the nursery stage and judicious fertilization and proper plant spacing are the recommended cultural methods of control (Goto, 1992). Protectant application such as chemical control for BB used Bordeaux mixture with or

without sugar, copper–soap mixture and copper–mercury fungicides. However, effective and economical chemical control has yet to be developed for this disease. This may be because the pathogen population is highly variable in its sensitivity to the antibiotics used for control. The existence and development of drug-resistant isolates also pose serious problems in formulating fool-proof control agents (Ou, 1972; Gnanamanickam *et al*., 1999; Lee *et al*., 2003). The most effective approach to struggle with BB is the cultivation of resistant varieties (Khush *et al*., 1989). Currently, the utilization of rice varieties carrying resistance genes proves to be one of the most economical, effective, and environment-friendly strategies for the management of rice BB (Zheng *et al*., 2009). Therefore, identification of excellent genetic resources and resistance genes is need for an improvement of BB resistance in rice breeding program. So, new rice varieties must continuously be developed with improved yield, pest and disease resistance and stress tolerance in order to fill the demand of the increasing world population.

Currently, more than 38 BB resistance genes have been identified in cultivated and wild rice (Gu, *et al*., 2004; Korinsak *et al*., 2009b; Korinsak *et al*., 2009c; Guo *et al*., 2010; Miao *et al*., 2010; Bhasin *et al*., 2012) and mapped to different chromosomes. Some of BB resistance genes have been transferred to modern rice varieties through both conventional breeding (Khush *et al*., 1989) and marker assisted selection (MAS) (Sonti, 1998; Rao *et al*., 2002). Among above genes for which linked markers are available, *Xa21* is the most widely use in rice breeding program because of its broad spectrum resistance to most isolates of *Xoo* (Ikeda *et al*., 1990; Khush *et al*., 1989, 1991). However, it confers resistance at only adult plant stage (Mazzola *et al*., 1994; Century *et al*., 1999; Ponciano *et al*., 2007). Another BB resistance gene that confers resistance at all growth stages is *xa5*. *xa5* exhibited a broad spectrum of resistance to *Xoo* isolatesin Asia with an exception of South Asia (India and Nepal) (Adhikari *et al*., 1995). *xa33* was identified on long arm of the chromosome 6 from the rice cultivar Ba7. It was tightly linked with the marker RM5509 and RM7243. It showed resistance to some Thai isolates (Korinsak *et al*., 2009a). Large-scale and long-term cultivation of varieties with single gene may enable the pathogen to overcome BB resistance. There are numbers of reports on the breakdown of BB

resistance (Mew *et al*., 1992, Huang *et al.*, 1997). For example, widespread cultivation of varieties with *Xa4* has led to predominance of *Xoo* races that can overcome in Philippines India, Indonesia, and China (Mew, 1987; Huang *et al*., 1997). Goel *et al*. (1998) reported that several isolates of the pathogen from Punjab, were virulent to IRBB21 (carrying the *Xa21*). So, the deployment of rice cultivars that have multiple BB resistance genes is expected to lead to more durable resistance. Gene pyramiding has been successfully applied in several crop breeding programs, leading to the development and/or release of many varieties and lines possessing multiple attributes (Huang *et al*., 1997; Samis *et al*., 2002; Jiang *et al*., 2004; Suh *et al*., 2009). Through gene interaction and complementation, lines with pyramided genes have been found to increase resistance quantitatively and provided a wider spectrum of resistance over lines with single gene (Yoshimura *et al*., 1995; Singh *et al*., 2001; Kim *et al*., 2009). Pyramiding resistance genes can be very difficult using conventional methods of breeding due to epitasis or masking effects of other genes. However, it can be possible if DNA markers linked with the target genes are available (Huang *et al*., 1997).

Marker assisted backcrossing (MAB) has been used successfully to introgress one or several target genes from a donor into the genome of a recipient parent (Frisch *et al*., 1999; Frisch and Melchinger, 2005). Marker assisted selection (MAS) greatly increases the efficiency and effectiveness of selection for breeding program. Currently, MAS has been used extensively to increase selection efficiency for disease resistance governed by qualitative genes and quantitative trait loci in rice (Wang *et al*., 1994). MAB and MAS were successfully employed in Thailand rice breeding program to improve resistance or tolerance to biotic and abiotic stresses in Jasmine rice (Siangliw *et al*., 2003; Jairin *et al*., 2005; Toojinda *et al*., 2005 and Jairin *et al*., 2008).

In this study, we attempt to improve bacterial blight resistance in Thai jasmine rice variety, Khao Dawk Mali 105 (KDML105) and Myanmar rice variety, Aromatic Manawthukha. KDML105 is the most popular jasmine rice cultivar that broadly grows in Thailand. KDML105 has low yield and susceptible to many diseases

including BB disease. It was improved for bacterial blight resistance by marker assisted backcross breeding using IR1188 as the donor and KDML105 as a recipient parent. An indica rice variety IR1188 possesses *Xa21* and unknown seedling resistance genes. It was introduced from Cornel University. Marker assisted and phenotypic selections were carried out to facilitate the transferring of the *Xa21* and the unknown seedling resistance genes into the KDML105 genetic background. This population was developed by Rice Gene Discovery Unit via NSTDA project. In this study, backcross introgression lines of the KDML105 (KBILs) that were successfully introgressed the positive allele of *Xa21* from IR1188 were used as materials to identify resistant genes functioning at seedling stage. We reported here the discovery of seedling resistance genes in IR1188 that lead to the successive improvement of a non age-related broad spectrum BB resistance in KDML105.

In Myanmar, rice is grown throughout the country in all agro-ecological conditions. Although a large number of rice varieties have been released in Myanmar, the popular cultivar Manawthukha occupies 21.6% of total cultivated area. It is an elite non-fragrant rice cultivar and released in 1978. This variety is popular amongst rice farmers because of its high yield, high milling recovery and well adaptation. Due to the high demand for quality rice in local and international markets, the development of high yielding and quality is the top priority in Myanmar rice breeding programs. It was improved for fragrance and intermediate amylose content by marker assisted backcross breeding using Basmati 370 as the donor during 2004-2009 (Yi *et al*., 2009). Although the new improved Manawthukha lines are accepted by Myanmar farmers but they are very susceptible to BB. In this study, new improved aromatic Manawthukha rice line ‘Yn 3248-2-128-76-4-3-75’ (MK-75) was selected based on the results of yield performance and cooking quality tested in multi-locations in Myanmar. To breed the improved aromatic Manawthukha for a non age-related broad spectrum resistance, RGD07097-1-MAS-8-9 (RG-9) carrying *xa5*, *Xa21* and *xa33* genes was used as a donor parent. MAB was carried out to facilitate the transferring of *xa5*, *Xa21* and *xa33* from RG-9 into the aromatic Manawthukha genetic background.

OBJECTIVES

The major objectives of this study were:

1. To identify the new *Xa* genes conferring seedling resistance from the backcross population derived from KDML105 and IR1188.

2. To incorporate the Bacterial Blight (BB) resistant genes, *xa5*, *Xa21*, *xa33* into Basmati-like quality Manawthukha by marker assisted backcrossing (MAB) strategies.

LITERATURE REVIEW

1. Importance of rice

Rice is one of the most important staple foods for nearly half of world' population, especially in developing countries. It is grown particularly for the humid tropics across the globe. It is cultivated in Africa, America, Australia, Europe and Asia. Asia counts for 90% of the world's production of rice due to its favorable hot and humid climate (Hossain, 1997). Annually 320 million tons of rice is produced in Asia, but less than 5% of the rice produced is trade in the international market because almost 90% of it is consumed by Asian countries (http://www.rf.org.htm). Rice is grown in a wide range of environments and is productive in many situations where other crops would fail. Rice-growing environments are based on their hydrological characteristics and include irrigated, rain-fed lowland, rain-fed upland and flood-prone (IRRI, 1993). Worldwide, about 80 million ha of irrigated lowland rice provide 75% of the world's rice production. These systems remain the most important rice production systems for food security, particularly in Asian countries. About 60 million ha of rainfed lowlands supply about 20% of the world's rice production. Rainfed rice environments experience multiple abiotic stresses and high uncertainty in timing, duration, and intensity of rainfall. Rainfed lowland rice predominates in areas of greatest poverty: South Asia, parts of Southeast Asia, and essentially all of Africa. Upland rice is grown under dryland conditions in mixed farming systems without irrigation and puddling. It covers about 14 million ha and contributes only 4% of the world's total rice production. It causes low yields because of many conisolatets (typically only about 1 t/ha). In Central and West Africa, the rice belt of Africa, upland areas represent about 40% of the area under rice cultivation and employ about 70% of the region's rice farmers. Flood-prone rice ecosystems (almost 8%), subject to uncontrolled flooding, submerged for as long as five months at a time with water depth from 0.5 to 4.0 m or more, and even intermittent flooding with brackish water caused by tidal fluctuations. Included here are tidal rice lands in coastal plains. Several biotic and abiotic stresses, as well as narrow genetic diversity in modern cultivars of rice, are the major constraints to further increases in productivity.

Agriculture in Myanmar, dominated by rice cultivation, generates a direct or indirect economic livelihood for over 75% of the population. Rice is the principal human food resource and primary foreign exchange earner of Myanmar. It is an important crop for Myanmar, which has the highest per capita consumption of rice in the world: more than 210 kg per person per year. Rice cultivation covers eight million ha, 66 % of total cultivated area (http://faostat.fao.org, 2009). It is grown by resource poor rural farmers and landless agricultural labourers on small farms averaging only 2.3 ha in size (Okamoto, 2004). National average yield is 4.03 T ha^{-1} (MOAI, 2009). Myanmar is geographically diverse with four recognizable regions (the Ayeyarwady delta, a coastal strip, the central dry plain and mountain ranges), mainly irrigated lowland 18%, rainfed lowland 52.8%, upland 14.9% and flood-prone land 14.3%. Rice diseases, insect pests and weeds are major conisolatets for increasing productivity of rice. Among them, bacterial blight disease is a serious disease. Garcia *et al*. (1998) reported that bacterial blight was major disease in 80% of the township surveyed with 2.8% affected area and estimated losses of 398 kg ha^{-1}.

2. Bacterial Blight Disease in Rice

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive bacterial diseases of rice in irrigated and rain-fed lowland ecosystem. It is one of the oldest known diseases and was first reported in Fukuoka, Japan as early as 1884 in rice. Subsequently, its incidence has been reported from different parts of Asia, Northern Australia, Africa and United States of America. In 1909, masses of bacteria were isolated from the acidic turbid dewdrops of infected rice leaves, and the disease was reproduced by inoculating healthy leaves with these dewdrops. Shortly thereafter its aetiology as a bacterial disease was established, and the causal agent was isolated and classified as *Bacillus oryzae* (Mizukami and Wakimoto, 1969). The bacterium was renamed *Pseudomonas oryzae* and later *Xanthomonas oryzae* (Ishiyama, 1922). In 1978, it was reclassified as *X. campestris* pv. *oryzae* (Dye *et al*., 1978). In 1990, Swings proposed this species *X. campestris* pv. *oryzae* to change the name on the basic of phenotypic, genotypic, and

chemotaxonomic data. Finally, the causal agent of BB of rice was reclassified as *X. oryzae* pv. *oryzae* (Swings *et al*., 1990).

3. Causal Organism

The causal organism of the disease is *Xanthomonas oryzae* pv. *oryzae*, a rod-shaped with a polar flagellum, 0.4-0.8 x 1.5-2.9um occurring single or in pairs. The organism is gram negative and non-spore forming (Ishiyama, 1922). Bacterial cells are surrounded by mucous capsules. Colonies are circular, convex, whitish yellow to straw yellow later, with smooth surface and entire margin, viscid and opaque against transmitted light. The yellow pigments are soluble in water. The bacteria multiply inside the leaf tissues and invade the vascular system. Some ooze out from water pores. The bacterium has many strains with different ability (virulence) to infect rice plants. Some strains are weak and cause only small lesions while others are virulent, causing large lesions on the same variety. The bacterium infects the plants by entering leaf tissues through natural opening, growth opening at the base of leaf sheaths caused by emergence of new roots, and through leaf of root wounds caused by certain management practices during transplanting. Wounds on rice leaves are also favorable for entry of the pathogen. The infection seems more successful in the case of entry of the pathogen through wound sites than natural openings.

4. Symptom

X. oryzae pv. *oryzae* enters the plants either through wounds or hydathodes, multiplies in the epitheme and moves to the xylem vessels where active multiplication results in blight on the leaves. Cells on the leaf surface may become suspended in guttation fluid as it exudes at night and enters the plants by swimming, or passively as the fluid is withdrawn into the leaf in the morning (Figure 1A) (Curtis, 1943). *X. oryzae* pv. *oryzae* may also gain access to the xylem through wounds or opening caused by emerging roots at the base of the leaf sheath. Once in the vascular system, *X. oryzae* pv. *oryzae* continues to grow until the xylem vessels are clogged with

bacterial cells and extracellular polysaccharides (EPS or xanthan) (Shen and Ronald, 2002).

Bacterial blight is a vascular disease resulting in a systemic infection of rice (Mew, 1987). There are three types of symptoms; wilting (kreset), leaf blight, and pale yellow leaves. Kreset is the result of systemic infection that is common in the tropics in young plants and during the tillering stage of susceptible cultivars. In this stage, leaves of infected plants wilt, roll up, turn grey-green and wither, and entire plants finally die. The bacterium spreads through the vascular system to the growing point of the young plants and infects the base of other leaves. Individual tiller or plant may die. If the plants are alive from this infection, they look stunted and yellowish. The most prominent symptom of leaf blight is wavy elongated lesions, which develops along the leaf margins (Figure 1B). The lesion enlarges in length and width, and may have wavy margins. It turns a whitish-straw color from its initial water-soaked grayish or yellowish hue in 1-2 weeks. Leaf blight may occur at all growth stages, but it is common from maximum tillering until maturity. As the disease advances, the lesions may cover the entire blade which turns white and later grayish. Lesions extend to the leaf sheaths and reach the lower internodes of susceptible varieties. Drops of bacterial ooze can be seen in the morning on young lesions. Bacterial ooze may be observed in humid and warm conditions. On panicles, the disease causes grey to light brown lesions on glumes causing infertile and low quality grains. The third symptom, pale or yellow symptom is also the results of systemic infection at the tillering stage. At this infection, bacteria are found in the internodes and crowns of affected stems, but not in the leaf itself (Ou, 1985; Goto, 1992).

When the kresek type of the disease (disease of young plants) appears, the crop can suffer heavy losses. When the disease appears on adults plants, usually at or soon after maximum tillering, poor gain quality results. The earlier the attack the more severe is the yield losses.

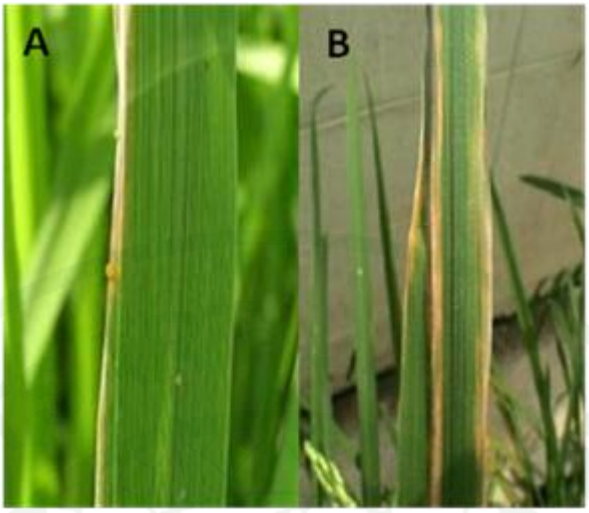

Figure 1 Droplets of bacterial exudates on young lesions observed during early morning with high dew formation (A) and Rice leaves infected with *Xanthomonas oryzae* pv. *oryzae* (B).

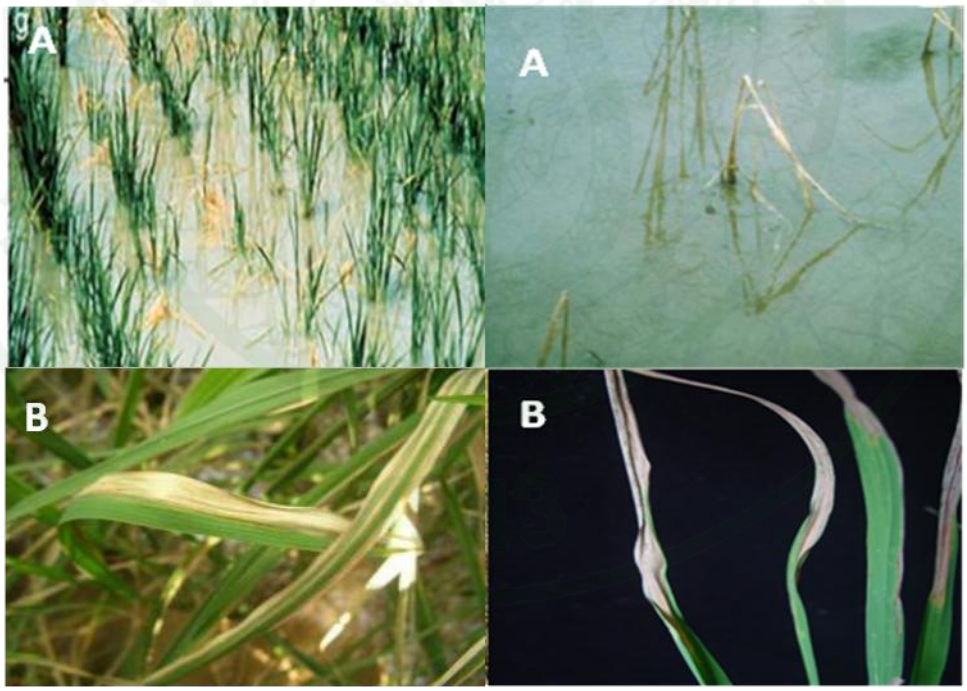

Figure 2 The BB disease symptom (A) kresek symptom occur on the seedling plant, (B) leaf blight symptom occur on adult plant

Source: www.ricethailand.go.th/rkb/data_005/Image

5. Disease Cycle

The soil is not considered as an important source of inoculum (Tagami *et al*., 1963). The bacterium can survive in soil only for one to two months (Wakimoto, 1956). It can survive in a dry form on seeds from infected plants, stored rice straw and rice stubble. This dry form of bacteria becomes activated by moisture. Growth form bacteria are normally found in stubble and in some susceptible grasses, especially *Leersia* sp., *Leptocloa chinensis*, *Cyperus rotundus* etc., which serve as alternate hosts.

The bacterial blight pathogen enters through natural openings like hydathodes and stomata as well as through wounds (Mew and Huang, 1984). Upon entry into the host, the pathogen reaches the vascular tissue, particularly the xylem, from where it multiplies and spreads throughout the plant, resulting in a systemic infection. The disease is carried from field to field by rainstorms and typhoons, irrigation water and insects. However, the role of water as a primary mode of transmission has been disputed as the pathogen survives only for 15 days in field water (Tagami *et al*., 1963). It also survives on alternate grass hosts in the absence of the rice plants.

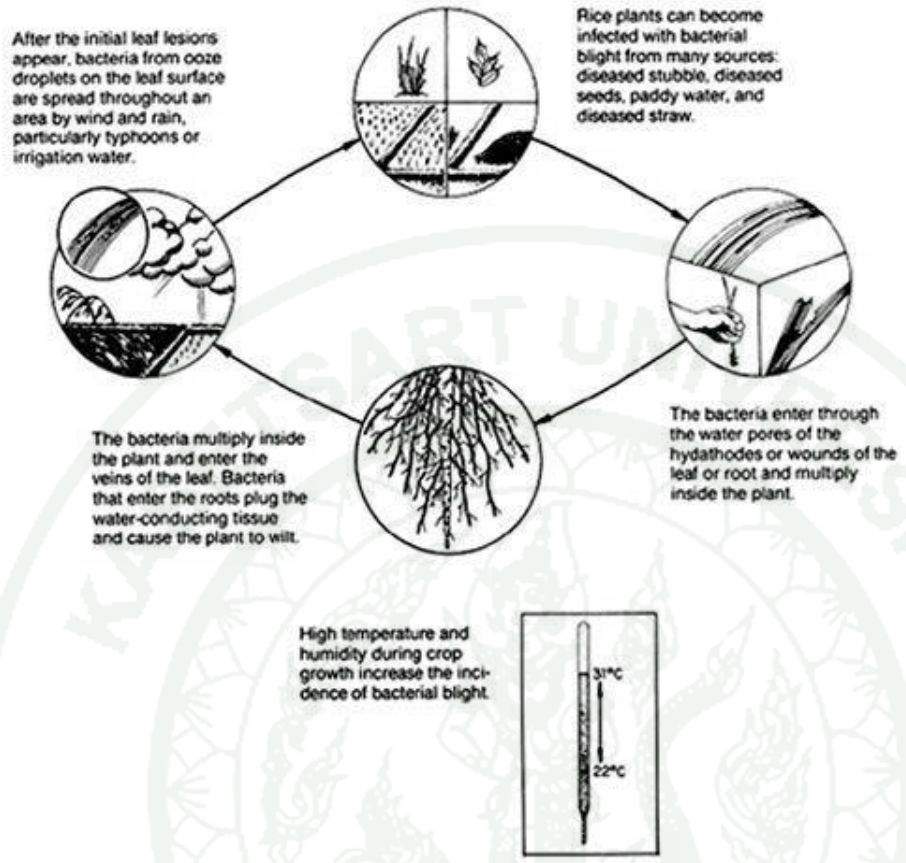

Figure 3 The life cycle of *Xanthomonas oryza* pv. *Oryzae*

Source: www.knowledgebank.irri.org

6. Factor Contributing to Infection and Development of the Disease

The incidence and severity of bacterial blight are influenced by various environmental factors. They are divided into climatic conditions, topographic and soil conditions, and cultural practices. Among them, rainfall and nitrogen fertilization are the most important factors affecting the occurrence of the disease (Ezuka and Kaku, 2000).

Bacterial blight is a typical water- born disease because the causal organism is disseminated by irrigation water. Therefore, it is natural that the disease is hardly seen in upland rice unless the land in irrigated or flooded by heavy rainfall (Kuwatsuka, 1944). It is usually prevalent during the wet season due to the strong winds and

splashing or windblown rain, which may contribute to wounding of plants and favor dissemination of the bacterium from plant to plant. The most important factors for disease epidemics are rainfall, humidity, temperature, flood and typhoon during the rice growing season (Mizukami and Wakimoto, 1969). Heavy rainfall causing flood of nurseries or fields accelerates the disease occurrence and increases it severity because the submerged rice plants may be infected with the pathogen in the water.

High humidity and warm temperature are also some of the factors favoring the disease. The optimum temperature for lesion development was reported to be 25-30º C, while the symptoms hardly appear at a low temperature of 17 ºC (Mukko *et al*., 1957; Wantanabe, 1966). However, Horino and Yamada, (1982) reported that lesion development at a constant temperature of 34ºC was greater than at 28ºC and 24ºC.

There are many sources of initial inoculums of the disease. Weeds around the field, rice straws, and ratooned plants are good sources of inoculums. Surviving bacteria may also be found in the paddy and irrigation canal. Some crop management practices also contribute to infection. Excessive use of nitrogen fertilizer increases the incidence of the disease. During transplanting, cutting the seedling tips encourages infection through the cut tissue.

7. Disease management

BB disease management centers around methods that reduce the initial inoculum and subsequent development of the pathogen on host plants and this can be accomplished through chemical protection, host plant resistance, and biological control.

(a) Chemical control

Attempts to control BB through chemicals like Bordeaux mixture with or without sugar, copper–soap mixture, copper–mercury fungicides were made. Spraying copper oxychloride and streptomycin solution at short intervals was

recommended to control this disease Mizukami and Wakimoto (1969). In several countries in tropical Asia, disinfection of rice seeds with mercuric compounds, antibiotic solutions or hot water is practiced although seed transmission of the disease is an uncertain source of primary inoculums. Synthetic organic bactericides such as nickel dimethyl dithiocarbamate, dithianone, phenazine and phenazine Noxide were also recommended. Spraying techlofthalam was more useful than soil application and it translocated readily and inhibited bacterial multiplication in rice plants. In order to inhibit the bacterial multiplication and prevent or retard the disease, the application of probenazole to the paddy water before and after transplanting the seedlings in the temperate regions have been documented (Nino-Liu *et al*., 2006). However, chemical control of BB in the tropic monsoon climate of Asia is impractical, and no truly effective bactericide is commercially available for disease control (Ou, 1972; Lee *et al*., 2003).

(b) Biological control

Biological control appears to offer an ecology-conscious and cost-effective solution to this serious threat to rice cultivation. Biological control is an environmentally friendly and cost-effective alternative to chemical control. Vasudevan *et al*. (2002) described that largely because of the rapid growth, easy handling and effective colonization of the antagonistic isolatesto the rhizosphere, bacterial antagonists of *X. oryzae* pv. *oryzae* have received particular attention as biocontrol candidates. Among those antagonists native isolatesof the a rice-associated rhizobacteria; *P. fluorescences* and *P. putida* isolatessignificantly suppressed bacterial leaf blight severity when sprayed on leaves (Sivamani *et al*., 1987). Moreover, different species of *Bacillus* have been employed as seed treatment before sowing, root dipping prior to transplanting and foliar sprays in the fields. Vasudevan *et al*. (2002) demonstrated that disease suppression ability of bacterial antagonists approximately 60% and plant height and grain yield increased by two-fold.

(c) Host resistance

Planting resistant cultivar has been the major method of BB management. In the absence of effective chemical or other control agents against the BB pathogen, host resistance has gained enormous importance in controlling this disease. In rice, the genetics of resistance to several pathogens has been well characterized. Resistance of rice plants towards *Xoo* at different growth stages varies according to host genotypes as seedling resistance (at seedling stage) and adult plant resistance (at adult stage but susceptible at seedling stage). In response to a pathogen, the host plant expresses various degrees of resistance which is usually classified into two categories namely qualitative resistance and quantitative resistance. Qualitative resistance is generally controlled by major genes while quantitative resistance is controlled by polygenic factors. Qualitative resistance is the resistance conferred by a single major gene which may be dominant or recessive. Quantitative resistance, also known as horizontal resistance is a low-level of resistance that generally shows no pathogen race specificity. This type of resistance, governed by quantitative trait loci (QTL), can prevent the breakdown of varietal resistance in a breeding program (Ogawa and Sekizawa, 1980). This type of resistance is complicated for genetic analysis because of their continuous variation with no distinct classes in a segregating population.

8. Identification and Molecular Mapping of Bacterial Blight Resistance Genes

Currently, more than 38 BB resistance genes have been identified in cultivated and wild rice and some of these have been transmitted to modern rice varieties (Gu *et al*., 2008; Korinsak *et al*., 2009b; Korinsak *et al*., 2009c; Guo *et al*., 2010; Miao *et al*., 2010; Bhasin et al., 2012). Long arm of chromosome 11 is rich in R genes for disease resistance to bacterial blight: *Xa10, Xa3, Xa 4, Xa21, Xa22, Xa23, Xa26* and *Xa1, Xa2* located together on chromosome 4 (Causse *et al*., 1994). Based on morphological and molecular markers, some of the resistance genes against bacterial blight have been mapped to rice chromosomes. Examples of the mapping include *Xa1, Xa2, Xa12* and *Xa14* on chromosome 4, *xa5*, on chromosome 5, *Xa7* on chromosome 6, *xa13* on

chromosome 8, *Xa3, Xa4, Xa10, Xa21, Xa22(t)* and *Xa23(t)* on chromosome 11 and *Xa25(t)* on chromosome 12 (Lin *et al*., 1996; Nagato and Yoshimura, 1998; Chen *et al*., 2002). The three recessive genes *xa15, xa19* and *xa20* were created by mutagenesis and each confers a wide spectrum of resistance to *Xoo* (Lee *et al*., 2003). Two genes *Xa21* and *Xa23* have been identified from two related wild species, *O. longestaminata* and *O. rufipogon*, respectively, while *Xa27* is from a distantly related species, *O.minuta* (Gu *et al*., 2004). *Xa1* and *Xa3* conferred broad spectrum resistance to multiple *Xoo* strains (Khush *et al*., 1989; Zhang *et al*., 2001). Two BB resistance genes *Xa1* and *Xa21* have isolated using map-based cloning strategies (Song *et al*., 1995; Yoshimura *et al*., 1998). At present, six R genes *Xa1, xa5, xa13, Xa21, Xa26* and *Xa27* were cloned by map-based cloning. In rice breeding programs at IRRI, and in national rice improvement programs in the Philippines, India and Indonesia, *xa5, Xa7* and *Xa21* are being transferred to commercially important rice varieties.

Many of the resistance genes to the bacterial blight have been tagged with RFLP, RAPD and microsatellite markers in genetic maps. The resistance genes, *Xa1* and *Xa2* were identified by Sakaguchi *et al*. (1967). The BB resistance gene, *Xa2*, confers specific resistance to T7147 (Japan *Xoo* race 2) and *Xa1* gene confers a high level of specific resistance to race 1 isolate of *Xoo* in Japan (Sakaguchi *et al*., 1967). Furthermore, *Xa2* was mapped on chromosome 4, linked to *Xa1* with a recombination frequency of 2-16% (Yoshimura *et al*., 1994). *Xa2* was to link to Npb 197 marker, located in chromosome 4 (Yoshimura *et al*., 1995a). *Xa1* gene was tagged with RFLP marker XNpb235 and mapped to chromosome 4 (Yoshimura *et al*., 1996).

The *Xa4* gene for BB resistance was introduced from TKM6 into improved varieties and has provided durable resistance to BB in China for 2 decades (Bonman *et al*., 1992). *Xa4* gene was mapped on chromosome 11 and found to be closely linked to Npb78 marker with the distance of 1.7 cm (Yoshimura *et al*., 1995b). The gene *Xa4* was mapped on chromosome 11 linked to the RFLP marker G181 and PCR marker M55 and co-segregated with the marker RS-13(Yoshimura *et al*., 1992). *Xa4* gene is one of the most widely exploited resistance gene in many Asian rice breeding programs and conferred durable resistance in many commercial rice cultivars (Mew *et*

al., 1992). *Xa4* gene was identified by Petpisit *et al*. (1997) and rice cultivars carrying the *Xa4* gene are resistant to bacterial blight at all stages of plant growth and it had been widely used in the breeding program (Khush, 1981).

The recessive resistance gene, *xa5* was the first to be placed into the linkage group through trisomic analysis and with the genetic studies using morphological markers (Yoshimura *et al*., 1984). RFLP markers RG556, RG207, RZ390 and SSR markers RM122 and RM390 were closely linked to *xa5* gene (Blair and McCouch, 1997). Rice cultivars containing *xa5* gene can provide resistance in parts of Southeast and Northeast Asia but not in South Asia (Aldhikari *et al*., 1995). This result indicated that the *xa5* gene might be useful for many countries in Asia. *xa5* was identified from the variety Ajaya. This variety is highly resistant to all pathotypes in India (DRR 1996). Rao *et al*. (2003) reported that *xa5(t)* was tightly linked with the two microsatellite markers RM 390 and RM 31 at a distance of 14.5 cM and 17.7 cM receptively on chromosome 5.

Xa7 is a dominant resistance gene directed against *Xoo* located on chromosome 6 and originally identified in rice cultivar DV85. The resistance gene conveys resistance at flowering stage (Sidhu *et al*., 1978a). It was the most resistant to BB in Indonesia (Kadir *et al*., 2004) and expressed low level of susceptibility to Korean BB races (Jena *et al*., 2007). This resistance gene was resistant to Philippine races 2 and 3 (Ezuka and Sakaguchi, 1978). Yoshimura *et al*. (1996) reported the positions of *Xa7* at 107.3, 103.0 and 90.5 cM using RFLP markers. Subsequently, Porter *et al*. (2003) studied about development and mapping of markers linked to the rice BB resistance gene *Xa7* and found that *Xa7* was also mapped to position 107.3 cM on RGP map with various molecular markers including AFLP, SSR and STS. Recently, the high resolution mapping and the genetic prediction of resistance gene *Xa7* have been reported (Chen *et al*., 2008). *Xa7* was mapped to the 0.21 cM interval between the STMS and SSR markers GDSSR02 and RM20593, respectively.

The recessive gene *xa8* was originally identified from a rice line PI 231129 (Sidhu *et al*., 1978b). This resistance gene conferred moderate to high level of

resistance to most of the prevalent *Xoo* isolates in Punjab, India. It was mapped with SSR marker RM 214 on chromosome 7.

The resistance R gene *Xa10* was originally identified from rice cultivar Cas 209 (Mew *et al*., 1982; Yoshimura *et al*., 1983). *Xa10* confers race- specific resistance to only a few Philippine races bacterial blight pathogen. The *Xa10* locus was initially mapped to the long arm of chromosome 11 in the region between proximal RAPD marker 007 (5.3 cM) and RFLP marker CD 0365 (16.2 cM) (Yoshimura *et al*., 1995). The R locus was later integrated to the region between RFLP markers RG 103 and RG 1109 on the rice genetic map of double haploid lines (IR 64 and Azucena) (Ramalingam *et al*., 2003). Gu *et al*. (2008) reported that *Xa10* was finely mapped at genetic distance of 0.28 cM between proximal marker 491 and distal marker M419 and co- segregated with markers S723 and M419.

Ogawa and Yamamoto (1986) identified the BB resistance gene *Xa11*. *Xa11* conferred resistance to Japanese races of *Xoo*. It was mapped by RAPD, CAPs and SSR markers at a distance of RM347 (2.0 cM) and KuX11 (1.0 cM) in the long arm of chromosome 3 (Goto *et al*., 2009).

The recessive R gene *xa13* confers resistance to the Philippine *Xoo* races that is compatible with most of the BB resistance genes. The *xa13* was first characterized in the rice variety BJ1 and mapped on the long arm of chromosome 8 (Ogawa *et al*., 1987; Sanchez *et al*., 1999). The gene was tagged with the RAPD marker OPACO5 990 and RFLP marker RG136 and mapped on chromosome 8 using the double haploid population of IR 64 and Azucena (Zhang *et al*., 1996).

The dominant resistant gene, *Xa21* was transferred from wild rice species *O. longistaminata* to IR 24 (Khush *et al*., 1989). *Xa21* confers resistance to pathotypes from the Philippines and India at post-seedling growth stage. *Xa21* located on chromosome 11, is one of the most preferred genes for improving resistance in rice against bacterial blight because it confers broad spectrum resistance to most isolates of *X. oryzae* pv. *oryzae* (Ikeda *et al*., 1990; Khush *et al*., 1989, 1991). *Xa21* gene has

been tagged with molecular markers (Ronald *et al*., 1992) and using marker-assisted breeding (Sharama *et al*., 2001; Singh *et al*., 2001) and by transformation (Song *et al.*, 1995). The gene *Xa21* was identified with RFLP marker RG 103, RAPD 248 and RAPD 818 markers. The RFLP marker RG103 was found to be tightly linked to the gene at the distance of 1.2 cM (Ronald *et al*., 1992).

xa24, a new recessive gene was identified from DV86. Wu *et al*. (2008) found that *xa24* was resisted to the Philippine *Xoo* races 4, 6, 10 and Chinese *Xoo* srtains Zhe173, JL691, and KS-1-21, and was mapped on chromosome 2 within a 0.14 cM region, and an approximately 71 kb in length between RM14222 and RM14226.

A new dominant gene, *Xa25(t)* was identified from Minghui 63, a restorer line for a number of rice hybrids that are widely cultivated in China (Chen *et al*., 2002). He reported that Minguhi 63 carried at least two bacterial blight resistance genes. The *Xa25(t)* gene conferred resistance to Philippine races of *Xoo* in both seedling and adult stages. It was mapped to the centromeric region of chromosome 12, 2.5 cM from a disease resistance gene-homologous sequence, NBS109 and 7.3 cM from RFLP marker, G 1314.

Another dominant gene *Xa26(t)* was identified from Minghui 63. It was against a Chinese *Xoo* isolate in both seedling and adult stages. The *Xa26* locus was mapped to a region of about 1.68cM. It co-segregated with marker R1506 and at a distance of 0.21cM from marker RM 224 on one side and 1.47 cM from marker Y6855RA on other side, on chromosome 11. *Xa3* had the same copy numbers of *Xa26* family members from the rice line Minghui 63 (Xiang *et al*., 2006). Many reports concluded that *Xa3*, *Xa4*, *Xa6*, *xa9* and *Xa26* were the same gene (Ogawa *et al*., 1986b; Sun *et al*., 2004; Xiang *et al*., 2006).

Xa27(t), located on the long arm of chromosome 6 was identified in wild rice *O.minuta* (Amante-Bordeos *et al*., 1992). It was mapped from a progeny of interspecific hybrids of *O. sativa* cultivar IR 31917-45-3-2 and *O. minuta*. *Xa27(t)*

was located within the a genetic interval of 0.52 cM, flanked markers M964 and M1197 and co-segregated with markers M631, M1230 and M449 (Gu *et al*., 2004).

xa32(t) was identified in wild rice *O. barthii*. It was mapped with SSR markers using bulk segregation analysis (BSA). BSA indicated presence of *O. barthii* on the terminal region of chromosome 6 at a distance of 9.3 cM proximal to RM588 (Singh *et al*., 2007).

xa33(t) was identified on long arm of the chromosome 6 from the rice cultivar Ba-7. It was tightly linked with the marker RM5509 and RM7243 (Korinsak *et al*., 2009a). The dominant gene *Xa34*(t), identified form the rice cultivar Pinkaset was located on long arm of the chromosome 11. It was linked with the marker RM224 (Korinsak *et al*., 2009b).

9. Marker assisted selection

Plant breeding describes methods for the creation, selection, and fixation of superior plant phenotypes in the development of improved cultivars suited to needs of farmers and consumers. Primary goal of plant breeding with agricultural and horticultural crops have typically aimed at improved yields, nutritional qualities, and other traits of commercial value. Predicted population growth and pressure on the environment, traits relating to yield stability and sustainability should be a major focus of plant breeding efforts. These traits include durable disease resistance, abiotic stress tolerance and nutrient and water-use efficiency (Mackill *et al*., 1999; Slafer *et al*., 2005; Trethowan *et al*., 2005). Despite optimism about continued yield improvement from conventional breeding, new technologies such as biotechnology will be needed to maximize the probability of success (Ortiz, 1998; Ruttan, 1999; Huang *et al*., 2002). DNA markers can be used to detect the presence of allelic variation in the genes for desired traits. By using DNA markers to assist in plant breeding, efficiency and precision could be greatly increased. The use of DNA markers in plant breeding is called marker-assisted selection (MAS) and is a component of the new discipline of ‘molecular breeding’. Molecular markers are

especially advantageous for agronomic traits that are otherwise difficult to tag such as resistance to pathogens, insects and nematodes, tolerance to abiotic stresses, quality parameters and quantitative traits (Collard and Mackill, 2008).

In breeding for disease and pest resistance, the segregating populations derived from crosses between the resistant sources and desirable and productive genotypes are selected either at natural disease or pest 'hot-spots' or under artificially created disease and pest nurseries or by infecting individual plants under controlled environments. Screening of plants with several different pathogens and their pathotypes or pests and their biotypes simultaneously or even sequentially is difficult. Availability of tightly linked genetic markers for resistance genes will help in identifying plants carrying these genes simultaneously without subjecting them to the pathogen or insect attack in early generations. Only the materials in the advanced generations would be required to be tested in disease and insect nurseries. Thus, with MAS, it is now possible for the breeder to conduct many rounds of selection in a year without depending on the natural occurrence of the pest or pathogen and theoretically without the pest or pathogen as well. However, the presence of different races or biotypes complicates the development and application of molecular marker assisted selection. Markers developed for one pathotype or biotype may not have application to other locations in which different pathotypes or biotypes occur unless resistance is controlled by the same gene. Pathogens and insects are known to overcome resistance provided by single gene. Durability of resistance has been increased in several crops by incorporating genetic diversity of the major resistance genes. Cultivar diversification, cultivar mixtures, multilines and pyramiding of resistance genes have been successfully used. MAS for resistance genes (R) can be useful in all these approaches. Based on host-pathogen or host-insect interaction alone it is often not possible to discriminate the presence of additional R gene(s). With MAS, new R gene segregation can be followed even in the presence of the existing R gene(s) and hence R genes from diverse sources can be incorporated in a single genotype for durable resistance (Mohan *et al*., 1997).

(a) Marker-assisted backcrossing

Backcrossing is a plant breeding method most commonly used to incorporate one or a few genes into an adapted or elite variety. In most cases, the parent used for backcrossing has a large number of desirable attributes but is deficient in only a few characteristics (Allard, 1999). The use of DNA markers in backcrossing greatly increases the efficiency of selection. Three general levels of marker-assisted backcrossing (MAB) were described by Holland (2004). In the first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is referred to as 'foreground selection' (Hospital and Charcosset, 1997). This may be particularly useful for traits that have laborious or time-consuming phenotypic screening procedures. Furthermore, recessive alleles can be selected, which is difficult to do using conventional methods. The second level involves selecting BC progeny with the target gene and recombination events between the target loci and linked flanking markers referred to as 'recombinant selection'. The purpose of recombinant selection is to reduce the size of the donor chromosome segment containing the target locus (i.e. size of the introgression). This is important because the rate of decrease of this donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent, referred to as 'linkage drag' (Hospital, 2005). Using conventional breeding methods, the donor segment can remain very large even with many BC generations (Ribaut and Hoisington, 1998; Salina *et al*., 2003). By using markers that flank a target gene, linkage drag can be minimized. Since double recombination events occurring on both sides of a target locus are extremely rare, loss of vigor of the lines. Recombinant selection is usually performed using at least two BC generations (Frisch *et al*., 1999b).

The third level of MAB involves selecting BC progeny with the greatest proportion of recurrent parent (RP) genome, using markers that are unlinked to the target locus referred to as 'background selection'. Background selection refers to the use of tightly linked flanking markers for recombinant selection and unlinked markers to select for the RP (Hospital and Charcosset, 1997; Frisch *et al*., 1999b). Background

markers are useful because the RP recovery can be greatly accelerated. With conventional backcrossing, it takes a minimum of six BC generations to recover the RP and there may still be several donor chromosome fragments unlinked to the target gene. Using markers, it can be achieved by BC_4, BC_3 or even BC_2 (Visscher *et al*., 1996; Hospital and Charcosset, 1997; Frisch *et al*., 1999 a, b), thus saving two to four BC generations.

(b) Marker-assisted pyramiding

Pyramiding is the process of combining several genes together into a single genotype. Pyramiding may be possible through conventional breeding but it is usually not easy to identify the plants containing more than one gene. Using conventional phenotypic selection, individual plants must be evaluated for all tested traits. Therefore, it may be very difficult to assess plants from certain population types (e.g. F_2) or for traits with destructive bioassays. DNA markers can greatly facilitate selection because DNA marker assays are non-destructive and markers for multiple specific genes can be tested using a single DNA sample without phenotyping. The most widespread application for pyramiding has been for combining multiple disease resistance genes (i.e. combining qualitative resistance genes together into a single genotype). The motive for this has been the development of 'durable' or stable disease resistance since pathogens frequently overcome single gene host resistance over time due to the emergence of new plant pathogen races. The combination of multiple genes (effective against specific races of a pathogen) can provide durable (broad spectrum) resistance (Kloppers and Pretorius, 1997; Shanti *et al*., 2001; Singh *et al*., 2001). The incorporation of quantitative resistance controlled by QTLs offers another promising strategy to develop durable disease resistance.

10. Pyramiding of R-Genes

In rice, single-gene resistance has been the primary means of control of BB, but unfortunately, due to continuous and large-scale use of single-gene resistance, there has been a shift in the virulence pattern of the isolates, leading to breakdown of

resistance (Mew *et al*., 1992). Hence, pyramiding of R-genes can delay the virulence shifts. According to Kinoshita (1995), the pyramiding of multiple resistance genes into rice varieties is one way to develop durable resistance to BB. Several workers have started to pyramid lines with different R-gene combinations and these lines have also been included in screening effective gene/gene combinations. Different resistance genes often confer resistance to different isolates, races or biotypes. Combining these resistances broadens the number of races or biotypes that a variety can resist (Mackill and Ni, 2001). Furthermore, combining major and minor genes resistance may lead to increase durability of resistance (Wang *et al*., 1994; Bharathkumar *et al*., 2008). The plants carrying gene combinations exhibit highly resistance phenotype (Perez *et al*., 2008). Li *et al*. (2001) reported that transmission of one high resistance gene and to collocate with 2 to 3 other resistance genes can facilitate the broad-spectrum and durable resistance.

MAS could be used to pyramid genes from multiple parents (i.e. populations derived from multiple crosses). The successful of BB resistance gene pyramiding using MAS has been reported from several rice breeding programs, for examples, Abenes *et al*. (1993) pyramided four BB resistance genes, *Xa4*, *xa5*, *xa13*, and *Xa21*, using MAS. In rice breeding programs at the International Rice Research Institute (IRRI) and national rice improvement programs in the Philippines, Indonesia and India, resistance genes *Xa4*, *xa5*, *Xa7* and *Xa21* were targeted for transfer to commercially important rice varieties (Nelson *et al*., 1996). The pyramiding lines with two, three, and four resistance genes had shown a wider spectrum and a higher-level of resistance than lines with only single gene according to Huang *et al*. (1997) and Sanchez *et al*. (2000). Jena *et al*. (2007) also reported that *Xa4*+*xa5*+*Xa21* pyramiding lines expressed high levels of resistance to all races in Korea. These three resistance genes were also introgressed into Samba Mashuri and the pyramid lines exhibited high levels of resistance to *Xoo* (Sundarum *et al*., 2008). *Xa21* and *Xa4* from IRBB60 were introgressed into a hybrid rice line, Shunhui527 using MAS. The improved lines expressed high level of resistance to the *Xoo* isolate CI-C VIII (Huang *et al*., 2003). In addition, *Xa21* and *Xa7* were pyramided into Minghui 63 background and the improved lines showed high level of resistance to BB (Huang *et al*., 2003). Moreover,

TGMS rice was introgressed with *Xa4*, *Xa7* and *Xa21* for the development of two-line hybrids. Rice cv. PR106 was introgressed with pyramided resistance genes *xa5, xa13* and *Xa21* from pyramid line IRBB62 using MAS (Singh *et al*., 2001).

Pyramiding may involve combining genes from more than two parents. Hittalmani *et al*. (2000) and Castro *et al*. (2003) combined genes originating from three parents for rice blast and stripe rust in barley, respectively. *Bph1* and *bph2* were introduced into BPH-susceptible japonica cultivars, and progenies with both gene combinations confer strong resistance to the new biotype of BPH in Japan (Sharma *et al*., 2004).

11. Cooking and eating quality

Rice grain quality includes processing quality, appearance quality, nutritional quality, cooking and eating quality, among which cooking and eating quality is higher acceptance by most consumers. The cooking and eating quality of the rice grain is one of the most serious problems in many rice producing areas of the world. Aroma is the highest desired trait of good quality rice and amylose content is important determinant of cooking and eating quality. Aromatic or scented rice fetching premium price in international market is characterized by its natural fragrance or pleasant aroma and good eating quality. Grain appearance, processing behavior, cooking and eating quality are directly related to three physico-chemical properties of rice grain starch making up 90% of total dry weight of the endosperm, namely amylose content(AC), gel consistency(GC) and gelatinization temperature(GT).

(a) Aroma

Aromatic or scented or fragrant rice, a special group of rice that emits natural fragrance in the field at flowering, harvesting, in storage, during milling, cooking and eating has been cultivated mainly in South and South-East Asian countries from ancient time. This specialty rice can be defined as those with different grain shape, size, color, chemical composition and cooking characteristics compared

with the common long-, medium- or short-grain type. Aromatic rice has a relatively diverse range of aroma that is much more dominant than in non-aromatic cultivars. The scent or natural fragrance in the kernel is the much valued quality factor. The most popular aromatic rices are Basmati rice from India and Jasmine rice from Thailand (Vanavichit, 2007; http://www.scitopics.com/Aroma_in_Rice.html).

The aroma of both aromatic and non-aromatic rice is composed of a complex mixture of odor-active compounds. Several odor active compounds in cooked aromatic rice have been determined using odor units (Buttery *et al*., 1983). Although several volatile flavor compounds have been identified to be responsible for aroma such as, Carbohydrate, Ketone, Aldehyde, Acetaldehyde, Alcohol, (Mahatheeranont *et al*., 2001), the most potent compound of aroma in Basmati and Jasmine-style fragrant rice is pop-corn like flavor compound 2-acetyl-1-pyrroline (2AP) (Buttery *et al*., 1983; Lorieux *et al*., 1996; Nadaf *et al*., 2006). In rice, 2AP has a lower odor threshold (0.02 ng/L) than many other volatile compounds and the concentration is very low (Buttery *et al*., 1983). The concentration of 2AP in milled Basmati rice showed (0.06ppm) and non-aromatic rice did not show any present of 2AP (Nadaf *et al*., 2006). Lorieux *et al*. (1996) and Bradbury *et al*. (2005a) confirmed that 2-acetyl- 1-pyrroline (2AP) and aroma were perfectly correlated and mapped at the same locus and 2AP concentration in aromatic rice is 12 times higher than that of common rice. Many researchers reported that a single nuclear recessive gene controlled aroma in Basmati and Jasmine-style aromatic rice (Ahn *et al*., 1992; Buttery *et al*., 1983; Bradbury *et al*., 2005a; Wanchana *et al*., 2005). Bradbury *et al*. (2005b) has been identified aroma is recessive traits due to an eight base pair deletion and three SNP in exon seven of the gene which encodes a putative betaine aldehyde dehydrogenase 2 (badh 2) on chromosome 8 of *Oryza sativa* largely controlled by the level of 2AP. An aromatic allele, a 3-bp insertion in exon 13 of Os2AP was found as a major allele in aromatic rice varieties from Myanmar (Myint *et al*., 2012).

(b) Amylose content

Amylose content of rice endosperm is a major determinant of cooking and eating quality (*Oryza sativa* L.) (Juliano, 1985). The ratio of amylose to total starch, measured as amylose content, varies from cultivar to cultivar; it means 18-32% in indica rice and 10-22% in japonica. The high amylose levels are usually associated with dry, fluffy, and separate cooked rice grain (Juliano, 1985). According to the proportion of amylose in total starch of endosperm, rice varieties are classified as waxy (0-2%), very low (5-12%), low (12-20%), intermediate (20-25%) and high (25- 33%) (Juliano *et al*., 1981). The inheritance of amylose content have revealed one major gene and several modifiers gene with high amylose content incompletely dominant over low and intermediate amylose content (Sano, 1984; Kumar and Khush, 1986; Kumar *et al*., 1987). It is genetically controlled by a multiple series at the *Waxy* locus in non-waxy rice isolates(Kumar *et al*., 1987) and at least two functional alleles at *Waxy* locus, Wx^a and Wx^b which controlled not only the level of the gene product but also amylase content in endosperm starch. Wx^a was predominant in *Indica* sub species, Wx^b was predominant in *Japonica* type and Wx^a produces higher level of *Waxy* protein and amylose than Wx^b (Sano, 1984).

(c) Gel-consistency (GC)

The GC is responsible for softness of cooked rice. Index of cooked rice texture is evaluated by gel-consistency. Varieties with similar amylose content softer gel-consistency are the prefer type by most consumers. The inheritance of GC was controlled by one gene and the short and hard gel consistency was dominance over long and soft gel consistency (Chen and Li, 1981). However, the major *Wx* gene plus several minor genes and/ or modifier governed the expression of GC (Lanceras *et al*., 2000; Tian *et al*., 2005). Many studies for GC showed that three QTLs on chromosome 6 and 7 with their interaction (Lanceras *et al*., 2000), three QTLs on chromosome 1, 2 and 6 (Tian *et al*., 2005) contributed for GC variation. On the other hand, many reported papers examined that *Wx* was not responsible for GC and some minor genes involved in governing GC. Their QTLs analysis for GC

indicated that two QTLs on chromosome 2 and 7 with positive additive effects (He *et al*., 1999).

(d) Gelatinization Temperature (GT)

Gelatinization temperature, time required for cooking is physical property of cooked rice. GT affects water absorption, volume expansion and kernel elongation, the quality and quantity of starch and GT strongly influence the cooking and eating quality of rice. The length elongation of cooked rice are probably inherited in a polygenic fashion and those QTLs detected on chromosome 2, 3 and 6 (Ge *et al.*, 2004) and a major and a minor on chromosome 6 (He *et al.*, 1999), three QTL on chromosome 2 and 6 (Lanceras *et al.*, 2000), two QTLs on chromosome 3 and 6. The (*alk)* gene on chromosome 6 that encodes soluble starch synthase *(SSIIa*) was responsible for the GT variation (Lanceras *et al.*, 2000; Li *et al.*, 2003; Umemeto and Terashima, 2002; Tian *etal*., 2005; He *et al.*, 2006).

MATERIALS AND METHODS

Part I: Identification of genes conferring seedling resistance to bacterial blight from KDML105 introgression lines, for a non-aged-related broad spectrum resistance

1. Plant Materials

Khao Dawk Mali 105 (KDML105), a Thai jasmine rice, is known as a premium aromatic-quality rice in the world rice market. KDML105 has been a popular crop in rainfed lowlands in the North and Northeast of Thailand for more than 40 years. KDML105 is very susceptible to BB in all growth stages. IR1188 is an indica rice variety that possesses *Xa21*. It has been introduced from Cornell University. IR1188 shows non-age-related broad-spectrum BB resistance against Thai *Xoo* isolates.

2. Development of backcross introgression lines

The breeding scheme used to develop KDML105 backcross introgression lines (KBILs) is shown in Figure 1. This population was developed by Rice Gene Discovery Unit, Thailand via NSDTA project. Rice isolate IR1188 was crossed with KDML105, and the F_1 plants were backcrossed to KDML105 to generate the BC_1F_1 generation. The BC_1F_1 seeds were germinated at Kasetsart University in 27.5 cm × 36.5 cm × 11.5 cm polyvinyl trays filled with soil from a BB-infected paddy field collected at the university. Uninfected BC_1F_1 seedlings were then genotyped with the *Xa21*-specific marker PB7-8 (Chunwongse *et al*., 1993) to identify the BC_1F_1 plants carrying the *Xa21*. Consecutive backcrossing and selection were performed until the BC_3F_1 generation. This procedure was coupled with MAS for *Xa21*. The BC_3F_1 plants were allowed to self-pollinate to develop the BC_3F_2 generation. The PB7-8 marker was again used to select the BC_3F_2 plants carrying the homozygous IR1188 allele. Finally, sixty BC_3F_2 plants carrying the IR1188 allele (*Xa21*) were selected and used

for this study. Pedigree selection within the line was applied until the BC_3F_7 generation.

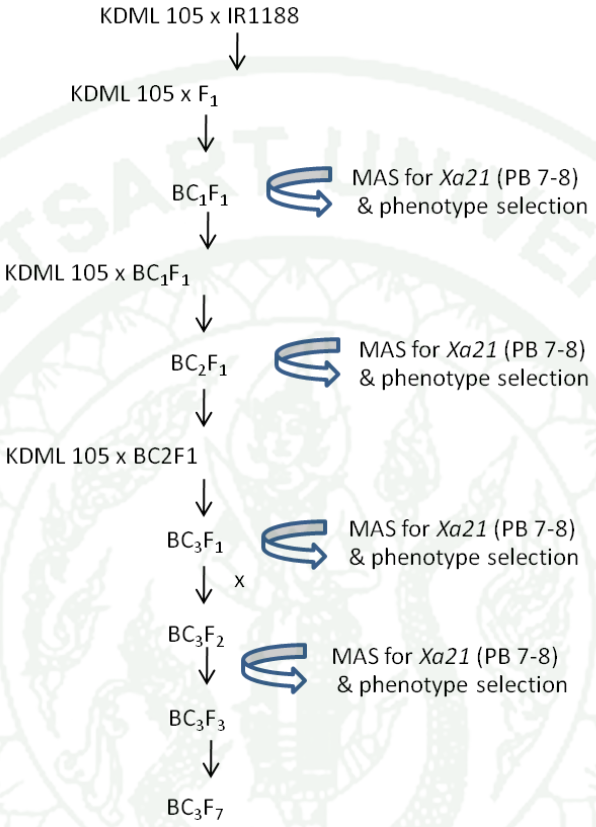

Figure 4 Schematic showing the development of KDML105 introgression lines (KBILs) with *Xa21* by integrated marker assisted and phenotypic selection in backcross breeding.

3. Background determination

DNA of the KBILs and parents was extracted with a DNA trap kit (http://dnatec.kps.ku.ac.th). Sixty-seven rice microsatellite markers (SSR) well distributed over the whole rice genome, together with markers that are associated with the cooking characteristics of KDML105, were used to determine the background genotype of the KBILs. Information on the SSR markers, including the primer

sequences and genomic locations, was obtained from the Gramene database (http://www.gramene.org/).

4. DNA extraction and PCR amplification

In each sample, leaf sample of one-month-old seedling was collected and cut into small pieces and put in 1.5 ml plastic tube. The sample was stored in container at –20°C and extracted DNA at a later date. A total genomic DNA of two parents and individual plants of progeny were isolated from 0.5 grams of leaf tissue according to the DNA trap method developed by DNA Technology Laboratory, Kasetsart University, Kamphaeng Saen, Thailand. The leaf sample was frozen with liquid nitrogen and ground into a fine powder with conical plastic grinder and the powder was then added to 1000ul of extraction buffer and incubated at 65°C for one hour. The sample was placed in ice for 5 minutes and added 100ul of neutralizer and mixed well using vortex genie. The content was spun in a centrifuge at 14000 rpm for 7 min and then aqueous solution was transferred to new 1.5ml tube. DNA was precipitated in 500ul of trapping buffer and gently mixed and spun at 2200 rpm for one minute. The supernatant was removed and the pellet was washed twice with each 500ul of washing buffer 1 and washing buffer 2, spun at 2200 rpm and 14000 rpm for one minute, receptively. The sample was dried at 65°C for one hour, after that DNA was re-hydrated with 100ul of elution buffer and incubated for 30 min at 65°C. After finished centrifugation of sample for two minutes at 14000 rpm, DNA was transferred into a new 0.2 ml tube to a final concentration of 50-100ng per ul. Genomic DNA was amplified by PCR. The PCR reaction was performed in a 10ul reaction mixture containing 2ul of template DNA (50ng), 1ul of 10xPCR buffer, 0.8 ul of 25mM $MgCl_2$ (final concentration 2mM), 2ul of 1mM dNTP (final concentration 0.2uM), 0.4 ul of 5uM forward and reverse primers (final concentration 0.2uM), 0.5 ul of 1 unit of Taq DNA polymerase (final concentration 0.5 unit). The volume was completed to 10ul with distilled water. Sample was covered with one drop of mineral oil. PCR reaction was initiated at 94°C denatured temperature for 3 min followed by 35 cycles of 94°C for 30 sec, 55°C for 30sec, 72°C for 1 min and 30sec and final 5 min incubation at 72°C was allowed for completion of primer extension. The amplified

product was electrophoresed on 4.5% denaturing silver-stained polyacrylamide gel for SSR marker and 1% agarose gel for PB7-8.

5. Testing for BB resistance at the seedling stage

Sixty BC_3F_7 KBI Lines were used to evaluate for seedling resistance against broad spectrum of *Xoo* isolates.

(a) Bacterial isolates

Nineteen isolates of *X. oryzae* pv.*oryzae*, namely, BR1-2, CRL1-3, NW3-1, SK2-3, PSL, TB9603, TXO4, TXO5, TXO32, TXO40, TXO50, TXO61, TXO80, TXO105, TXO112, TXO141, TXO142, TXO150 and TXO155 were used for disease assessment. Among the isolates, BR1-2, CRL1-3, NW3-1, SK2-3, the most virulent isolates in Thailand were kindly provided by Dr. Sujin Patrapuwadol, Plant Pathology Department, Kasetsart University. Another isolates were collected from various rice-growing provinces. These isolates represented the virulence spectrum of BB found in Thailand (Sriprakhon, 2009; Sansook *et al*., 2011). Table 1 presents detailed information on the *Xoo* isolates including location and year of the collection.

Table 1 List of *Xoo* isolates indicated by the code, locations and years of BB collection from various rice growing regions of Thailand

No.	Code	Locations	Years
1	BR1-2	Buri Ram	2009
2	CRL1-3	Ching Rai	2009
3	NW3-1	Nakhon Sawan	2009
4	SK2-3	Sukhothai	2009
5	PSL	Phitsanulok	2008
6	TB9603	Phrae	
7	TXO4	Nakhon Pathom	2002
8	TXO5	Nakhon Pathom	2002
9	TXO32	Sukhothai	2004
10	TXO40	Phitsanulok	2003
11	TXO50	Phrae	2003
12	TXO61	Ubon Ratchathani	2004
13	TXO80	Ubon Ratchathani	2005
14	TXO105	Ubon Ratchathani	2006
15	TXO112	Ubon Ratchathani	2006
16	TXO141	Nong Bua Lum Phu	2008
17	TXO142	Udon Thani	2008
18	TXO150	Chai Nat	2008
19	TXO155	Kamphang Saen	2009

(b) Rice seedling preparation

Rice seeds of KBILs and the parental lines were soaked in clean water for 72 hr and then incubated at room temperature. The germinated seeds of each line were planted in plastic trays with 6 x 12 holes (hole size 5 x 5 x 4 cm) and kept in the greenhouse under high humidity for a month. The plants were watered two times per day and fertilized weekly with 15-15-15 NPK fertilizer. During the final week, the

fertilizer was applied three days before inoculation. The experimental unit consisted of 2 trays. One hole with four plants was planted for each rice line.

(c) *Xoo* inoculation and disease scoring

All *Xoo* isolates from lyophilized cultures were revived on peptone sucrose agar (PSA: peptone 5 g, sucrose 20 g and agar 20 g adjusted to 1 liter) and incubated at 28ºC for 72 hours. A 3-day-old culture of each isolate was used to prepare inoculum suspensions. For each isolate, the bacterial colonies on the PSA were suspended with sterilized distilled water and adjusted to concentrations of approximately 10^9 cfu/ml prior to inoculation. The inoculation procedures were adapted from those described by Korinsak *et al*., (2009).

The rice plants were individually inoculated with each *Xoo* isolate at the seedling stage (30 days after sowing), following the clipping inoculation method as described by Kauffman *et al*. (1973). The inoculation was conducted in the morning, and the top two fully expanded leaves (a total of 10 leaves per rice line) were inoculated. The inoculated plants were kept in the greenhouse under high humidity to reduce the possible effects of high temperature on disease reactions. This condition also favors the entry of bacteria into infection courts in the presence of sufficient moisture on the leaf surface and maintains inoculation time consistency. The entire experiment was performed twice.

The disease was scored by measuring the lesion length (LL) of the inoculated leaves at 14 days after inoculation (DAI). The means of the LL were calculated for each KBIL and used to identify associations between LL and genotypes (DNA markers). The resistance reaction was classified as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) if the lesion length was 0-3 cm, 3.1-6 cm, 6.1-9 cm and more than 9 cm, respectively (Yang *et al*., 2003; Zhang *et al*., 2006).

6. Agronomic performance and adaptation in rainfed lowland environment

(a) Agronomic characteristics of the KBILs

The agronomic characteristics of the KBILs and KDML105 were evaluated in a replicated trial (2 replications) in which the tested varieties were arranged following a randomized complete block design (RCBD). The experiment was conducted by Rice Gene Discovery Unit at the Kamphaeng Saen campus (KPS), Kasetsart University, Thailand, from July through December, 2003. The seeds of KBILs and KDML105 were sown in a seed bed nursery. Three-week-old seedlings were then manually transplanted into the rice field, with one seedling planted per hill. The plot size was 2 m x 3 m, and each plot consisted of eight rows with twelve plants per row and a planting density of 25 cm between plants (within a row) and 25 cm between rows. Field management, including fertilizer application and weed and pest control, was performed following the protocol described by Yi *et al*. (2009). The traits measured for data collection included the days to 50% heading (DH), plant height (PH), number of effective tillers per plant (NETP), number of grains per panicle (NGP) and grain yield (GY).

(b) Yield stability of the selected KBIL lines

Seven KBI lines were selected based on marker information (carrying *Xa21* and QTL for seedling resistance) (Table 2) and based on agronomic performance at KPS (i.e., non-significant differences from KDML105). Yield stability was evaluated at 10 locations, including Chiangrai (CRI), Chiangmai (CMI), Surin (SRN), Sakon Nakhon (SKN), Udonthani (UDN), Khon Khean (KKN), Nongkai (NKI), Chumphae (CPA), Ubon Rachatani (UBN) and Nakorn Ratchasima (NRM) in North and Northeast Thailand during the wet season of 2005. In 2006, five out of seven lines were selected for the second round of the yield trial. This round was conducted in 11 locations, namely, CRI, CPA, KKN, NKI, SKN, UDN, NRM, SRN, UBN, Maehongson (MHS) and Phrae (PRE). All of the experiments followed a randomized complete block design with 3 replications. The rice varieties KDML105

and RD15 (early variety control) were used as standard controls. The planting and field management procedures were similar to those used in the experiment conducted at KPS. Four traits, including DH, PH, GY and the number of panicles per hill (NPH), were measured.

(c) Yield and agronomic performance in farmer fields

Four lines, together with KDML105 and RD15, which were used as controls, were selected and planted in farmers' fields at 1) Borabue, Mahasarakam; 2) Sri Bunrueang, Nong Bualamphu; 3) Krasang, Buriram; 4) Pimai, Nakorn Ratchasima; 5) Meuang Ubon, Ubon Ratchatani; and 6) Mahachanachai, Yasothon in 2007. The materials were planted following a randomized complete block design with three replications. The size of each plot was 1.5 x 4 m, with 6 rows of 16 hills. The distance between hills was 25 cm. Fertilizer was applied at a rate of 3-6-6 (NPK) kg/rai as the basal application and a rate of 3-0-0 (NPK) kg/rai at the late tillering stage. Data on grain yield (GY), days to 75% heading (DH), plant height (PH) and number of effective tillers per plant (NETP) were collected.

7. Evaluation of grain quality traits

Grain quality includes morphological feature of grains and cooking quality. Rice grains were harvested at maturity for each entry and dried naturally in a green house. The dried grains were stored in the room temperature for one month before evaluation of grain quality traits. A total of 100 grams sample each of all entries were randomly collected from each replication and mixed together. After mixing sample, 50 grams of each entry was randomly collected and dehulled by small machine and polished by minipolisher. Grain quality was evaluated using grain samples harvested from the UBN, SRN, KKN, NKI and UDN experiments in 2005 and from UBN in 2006. Rice grains of the KBILs and checks were harvested and naturally sun-dried in a greenhouse. The dried grains were stored at room temperature for one month prior to the evaluation of grain quality traits. Grain samples of 100 g were taken from each replicate and combined. Fifty grams of mixed samples were then used as the material

for the grain quality test. The grain samples were mechanically dehulled and polished by a mini-polisher. The evaluations of four grain quality traits were replicated three times and conducted at the Ubon Rice Research Center for both the 2005 and 2006 samples.

Evaluation for presence or absence of aroma in KDML introgression lines (KBIL) and the parents was determined by sensory method. For the simple sensory test developed from Rice Gene Discovery Unit, Kasetsart University, Thailand (Wanchana *et al*., 2005), five seeds of brown rice were placed into 1.5 ml centrifuge tube and 200 ul of distilled water was added and incubated at 65˚C for three hours with the lids on. The samples were allowed to cool and the lids were then opened one by one and the samples were smelled and scored for aroma by three panelists.

Amylose content (AC) was measured using the procedure of Juliano (1985) with minor modification. 100 mg of rice powder was incubated at room temperature for overnight in a solution of 1 ml of 95% ethanol and 9 ml of 1N sodium hydroxide to gelatinize the starch. After making up volume of the content to 100ml with distilled water, 5 ml was taken into new conical flask. The sample was added with 1ml of 1N acetic acid solution and 2ml of iodine reagent (0.2gm iodine and 2gm Potassium iodide in 100ml water) and volume is made up to 100ml with distilled water and mixed well. The absorbance was recorded at 620nm using a spectrophotometer. The AC was estimated using a standard curve developed from known quantities of purified potato amylose from Fluka Thailand.

Gel Consistency was measured by the length in a culture tube of cold gel according to the method of Cagampang *et al*., (1973). One hundred milligrams of rice powder was put in a 10mmx110mm culture tube and wetted with 0.2 ml of 95% ethanol containing 0.025% Bromothymol blue. Two milliliters of 0.2N KOH was added. The sample was mixed using vortex Genie mixer. The test tube was covered with glass marble. The sample was cooked in a boiling water bath for 8 minutes, making sure that the tube content reach 2/3 the height of the tube. The test tube was removed from the water bath and let stand at room temperature for 5 minutes. The

tube was cooled in an ice-water bath for 20 minutes and laid horizontally on a laboratory table lined with millimeter graphing paper. The total length of the gel was measured in millimeter one hour later as distance from the bottom of the tube to the front of the gel migration. The gel length thus obtained provides a measurement of the GC: the longer the distance, the softer the gel. The gel consistency value was evaluated by hard (26-40 mm), medium (41-60 mm) and soft (61-100 mm), short gel indicates hard GC and long gel represents soft GC.

Gelatinization temperature was indirectly measured by evaluating the Alkali Spreading Value (ASV) using the method of Little *et al*., (1958). Each sample was tested three times. Each time, six intact milled grains were put in a petridish, to which 10 ml of 1.7% KOH was added. The grains were carefully separated from each other using a forceps and incubated at 30˚C for 23 hours to allow spreading of the grains. The spreading value of the grains was scored on a numerical scale of 1 to 7 by visual assessment. 1, grain unaffected; 2, grain swollen; 3, grain swollen, collar incomplete and narrow; 4, grain swollen, collar complete and wide; 5, grain split , collar complete and wide; 6, grain dispersed, merging with collar and 7, grain dispersed and disappeared completely. Alkali spreading value (ASV) corresponds to GT as follows; 1-2 high (74-79°C), 3 high- intermediate, 4-5 intermediate (70-74°C) and 6-7 low (55-69°C). A larger ASV represents more spreading in alkali, indicating a lower GT and a smaller ASV indicates a higher GT.

(a) Physical grain qualities of rice

Physical grain qualities of rice, including seed length, seed shape and seed color, were also determined in 2005 and 2006. Seed length (SL) was measured with a Vernier caliper. The seed shape (SS) is the ratio of seed length to seed width. If the ratio is 3 or more, the shape is considered slender (Sl). If the ratio is between 2.1 and 3, the shape is intermediate (I). If the ratio is less than 2, the shape is bold (Bl). The seed color (SC) is scored as S (straw color) or B (brown color).

8. Statistical analysis

All traits in each experiment were subjected to statistical analysis with the CROPSTAT software program. An analysis of variance (ANOVA) was performed based on the randomized complete block design (RCBD). For multi-location trials and farmers' fields, a combined analysis of variance was performed using the mean value of the data on all traits in each experiment. The KBILs and controls were used as treatments, and the locations were used as replicates. The standard error and the probability of differentiation were calculated for all possible comparisons of the lines across the experiment with least-squares means. The least significant difference (LSD) was determined at a 5% probability level to perform the comparison between the mean values of each KBIL and the KDML105 control (recipient parent).

To identify genome regions associated with BB resistance at the seedling stage, a genotype-phenotype analysis was performed based on the information on the DNA genotype and LL. The marker genotype data were used as independent variables. Therefore, the independent variables had two levels each, with one level corresponding to the IR1188 allele and the other level corresponding to the KDML105 allele. A simple linear regression and a one-way analysis of variance (ANOVA) were performed with STATGRAPHICS version 3.0 to test the associations between the marker genotypes and the resistance phenotypes (the LL). Markers showing significant differences at a level of < 0.01 (F test) were used to identify seedling resistance.

Part II: Marker assisted Introgression of multiple genes for bacterial blight resistance in Aromatic Manawthukha, MK-75

1. Plant Materials

Rice variety Yn 3248-2-128-76-4-3-75 (MK-75) is the elite line derived from the BC_4F_4 population of the cross between Manawthukha (recurrent parent) and Basmati 370 (donor parent) (Yi *et al*., 2009). It has medium plant height, strong fragrance, and intermediate amylose content (AC) and intermediate gelatinization temperature (GT). RGD07097-1-MAS-8-9 (RG-9) is an improved line of jasmine rice. It has a jasmine cooking quality profile and BB resistance (*xa5*, *Xa21* and *xa33*). RG-9 was developed from multiple crosses among rice varieties Ba7 (donor of the *xa33*), IR1188 (donor of the *Xa21*), IR62266 (donor of the *xa5*) and KDML105 by marker assisted selection (Korinsak *et al*., 2009). RG-9 has medium plant height, non-fragrance, low AC and high GT. In this study, MK-75 and RG-9 were used as recipient and donor parents respectively to develop the backcross introgression population of the MK-75. The two parents are quite divergent in panicle type and morphological features (Figure 5).

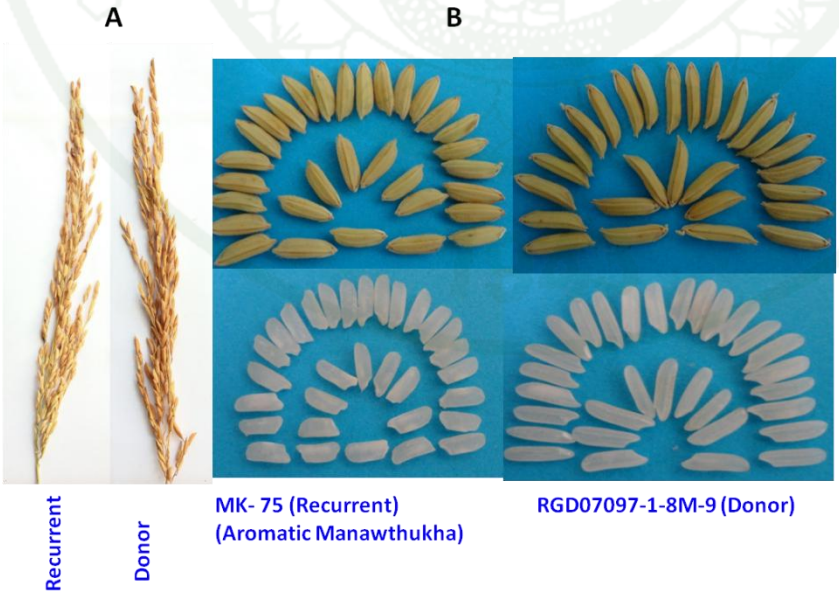

Figure 5 Characteristics of Panicle type (A), Grain sizes and Shapes (B) of two parents

2. DNA markers

Four markers including functional markers PAxa5, PB7-8 and flanking markers RM7243 and RM5509 were used for foreground selection in each of the backcross and selfing generations (Figure 1). The PAxa5 is a functional marker for the *xa5* located on chromosome 5. This marker was developed by the Rice Gene Discovery Unit to distinguish resistant and susceptible alleles of the *xa5* (unpublished paper). PB7-8 is a gene specific marker for the *Xa21* (Chunwongse *et al*., 1993). RM7243 and RM5509 were a microsatellite marker flanking the *xa33(t)* located on chromosome 6 (Korinsak *et al*., 2009). They were used to distinguish resistant and susceptible alleles of the *xa33*. All markers are co-dominance in which they can identify the heterozygous representing the presence of resistant alleles derived from the RG-9 in all backcross generations. In the BC_3F_1 generation, three markers determining cooking quality including Aromaker (fragrance), Waxy (AC) and SNP2340-41 (GT) were used to check the gaining of the cooking quality profile of the MK-75 in the selected BC_3F_1 progenies. Aromarker is developed based on 8-bps deletion in the seventh exon of the *Os2AP* gene on chromosome 8 (Vanavichit, 2007). It can be used to distinguish between fragrance and non-fragrance. Waxy (SNP) marker was developed based on single nucleotide change at the putative 5' leader intron splice site of *Wx* gene. The polymorphism was detected using restriction endonuclease *AccI* (Ayres *et al*., 1997). SNP2340-41, a fuctional marker for the *SSIIa* determining the gelatinization temperature (GT), was developed by Rice Gene Discovery Unit, Thailand. Type and chromosomal location of molecular markers were presented in Table 2.

Table 2 Type, chromosomal location and primer sequences of molecular markers for MAS of BB resistance genes

Gene	Marker	Type of marker	Chr.	Primer sequence	Remark
xa5	PAxa-5	SNP	5	F1- GGC CAC CTT CGA GCT CTA CC	RGDU
				F2- GCT CGC CAT TCA AGT TCT TGT C	
				R1- CAG ATA CCT TAT CAA ACT GCT C	
				R2- CAA CAT TGC AACTCC TGT ATA AG	
Xa21	PB7-8	STS	11	7F- AGA CGC GGA AGG GTG GTT CCC GGA	Chunwongs
				8R- AGA CGC GGT AAT CGA AAG ATG AAA	(1993)
xa33	RM5509	SSR	6	F- GAT GAT CCA TGC TTT GGC C	Korinsak et al.,
				R- TTC CAG CAG AAA GAA GAC GC	(2009)
	RM7243	SSR	6	F- TGT GGT GGA CCA CGG AAG ATG G	Korinsak et al.,
				R- GCA CTC TGC ACT GAG AGC AAC AGG	(2009)

F = forward primer, R = reward primer.

3. Development of introgression lines

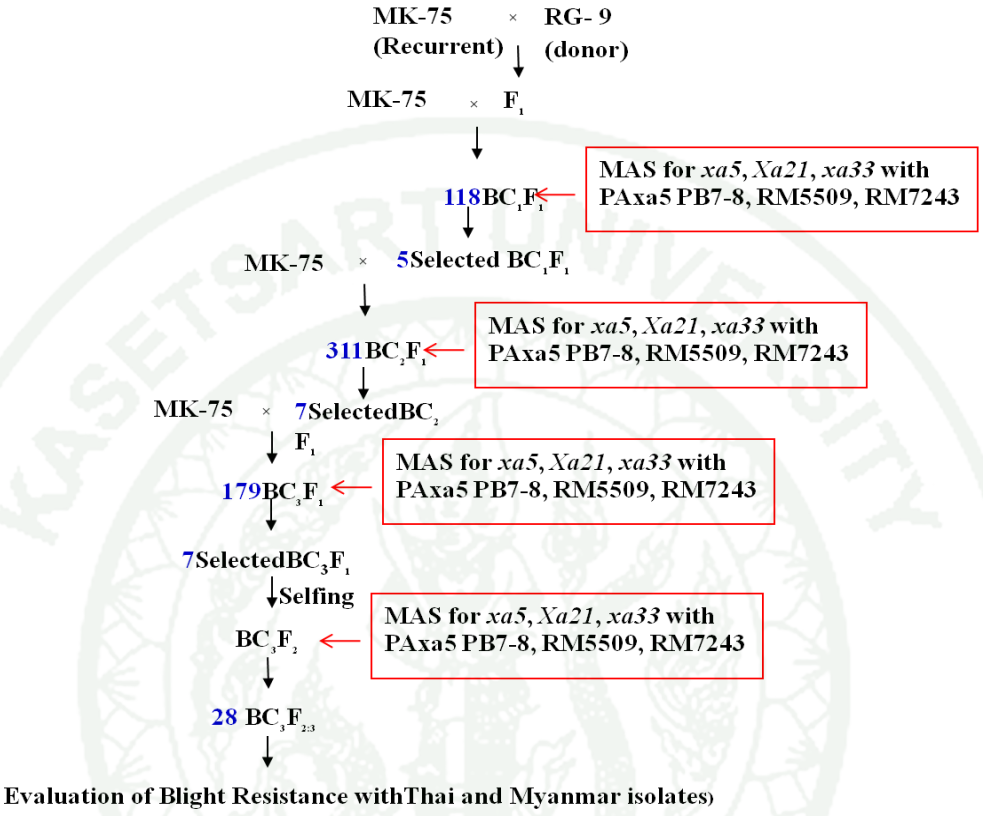

Figure 6 Schematic representation of gene introgression using marker assisted selection in backcross breeding.

As shown in Figure 1, to introgress the *xa5*, *Xa21* and *xa33* from the RG-9 into the MK-75, we carried out three cycles of backcrossing and advanced one more selfing generation as shown in Figure 1. The MK-75 was cross-pollinated with the RG-9, using the MK-75 as female parent and the RG-9 as male parent, to produce the F_1 generation. The F_1 plants were checked for the cross-pollination by using the PB7-8 marker. Five F_1 plants were then backcrossed to the MK-75 (using mixed pollen) to generate the BC_1F_1 generation. Molecular markers PAxa-5, PB7-8, RM7243 and RM5509 were used for foreground selection to identify the BC_1F_1plants that carried the target alleles (heterozygous at the four markers). Five BC_1F_1 plants were selected for further backcrossing. Repeated backcrossing and foreground selection were conducted until the BC_3F_1 generation was produced. The selected BC_3F_1 plants

carrying the three target alleles were genotyped with three markers (Aromarker, Waxy and SNP2340-41) determining the cooking quality. Seven BC_3F_1 plants showing heterozygous at PAxa-5, PB7-8, RM7243 and RM5509 and homozygous (MK-75) at Aromarker, Waxy and SNP2340-41 were self-pollinated to generate the BC_3F_2 generation. The foreground selection was carried out to identify the BC_3F_2 plants that carried the homozygous RG-9 allele of the *xa5*, *Xa21* and *xa33*. Finally twenty eight BC_3F_2 plants were selected for assessment of BB resistance in this study. Selective markers and their polymorphism information were shown in (Figure 7).

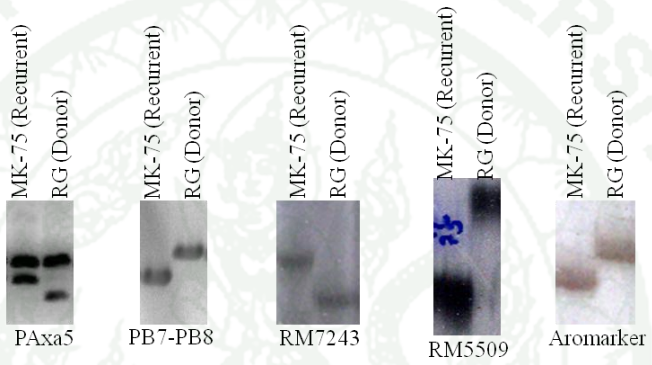

Figure 7 Selective markers and their polymorphism information between the two parents for genotypic selection of individual plant

4. DNA extraction and PCR amplification

The total genomic DNA of the parents and progenies were extracted from the young leaf tissue using DNA Trap method according to the procedure developed by DNA technology laboratory, Kasetsart University, Kamphaeng Sean, Thailand. The amplified PCR products of the PAxa-5 and PB7-8 markers were loaded into 2% and 1% agarose gel and separated by electrophoresis in 1x TBE buffer at 50^0C and 60 W. The PCR products of the PAxa5, PB7-8 were visualized using ethidium bromide isolating. The amplified PCR products of the Aromarker, Waxy, SNP2340-41, RM5509 and RM7243 were loaded into a 4.5% polyacrylamide gel and separated by electrophoresis in 1×TBE buffer at 50 ˚C and 60 W. The PCR products were visualized using silver staining. Polymorphisms in the DNA profiles were scored visually by comparison with two parents and a standard DNA ladder.

5. Assessment of Bacterial blight resistance

Bacterial isolates: Twelve Thai *Xoo* isolates comprised of BR1-2, CML3-1, CRL1-3, NH3-1, NB7-6, NB7-7, NP3-3, PSL, SK2-3, TXO85, TXO150 and TXO155, representing the genetic diversity of the groups that have been identified in Thailand were used to assess the BB resistance in the parental varieties including , KDML105 (a susceptible check for Thai *Xoo* isolates), original Manawthukha, MK-75 (recurrent parent), IR62266 (an original donor of the *xa5*), IR1188 (an original donor of the *Xa21*), IRBB21 (an isogenic line carrying the *Xa21* from IRRI), Ba7 (an original donor of the *xa33*) and RG-9 (donor parent) at seedling and maximum tillering stages. The most virulent bacterial isolates including BR1-2, CRL1-3, NPL3-3, NW3-1, SK2-3, and the weak virulent *Xoo* isolates including CML3-1, NB7-6, NB7-7 were kindly provided by Dr. Sujin Patrapuwadol, Plant Pathology Department, Kasetsart University. The *Xoo* isolates TXO85, TXO150 and TXO155 were obtained from Rice Gene Discovery Unit. These isolates were collected from various rice-growing provinces and represented a virulence spectrum of the BB found in Thailand. The genetic diversity of these isolates have been identified (Sriprakhon, 2009; Somsanook *et al*., 2011). Of the twelve *Xoo* isolates, ten *Xoo* including BR1-2, CRL1-3, NH3-1, NB7-7, NP3-3, PSL, SK2-3, TXO85, TXO150 and TXO155 which selected based on the resistant specificity to each resistant gene in parental lines were used to inoculate twenty eight $BC_3F_{2:3}$ of the Aromatic Manawthukha introgression lines (MK-75ILs) and their parents at both seedling (30 days) and tillering (60 days) stages. This experiment was conducted at Rice Gene Discovery Unit in Thailand. The MK-75ILs and parents were sent to Department of Agricultural Research (DAR, Myanmar) for assessment of BB resistance at the seedling stage. Five representative isolates of five Myanmar races of *Xanthomonas oryzae* pv. *oryzae* designated as Nankhan, Leway, Hmawbi, Thekon and Mattaya (Myint *et al*., 1995) were used to inoculate the individual MK-75ILs and their parents. Race I is the most virulent isolate in Myanmar and prevalent in four major rice growing divisions of Myanmar. List of *Xoo* isolatescollected from various rice growing regions of Thailand and Myanmar is shown in Table 3.

Table 3 List of *Xoo* isolates from various rice growing regions of Thailand and Myanmar

No.	Isolate code	Locations	Years
1	BR1-2	Buri Ram	2009
2	CML3-1	Ching Mai	2009
3	CRL1-3	Ching Rai	2009
4	NB7-6	Nontha Buri	2009
5	NB7-7	Nontha Buri	2009
6	NP3-3	Nakhon Pathom	2009
7	NW3-1	Nakhon Sawan	2009
8	SK2-3	Sukhothai	2009
9	PSL	Phitsanulok	2008
10	TXO85	Ubon Ratchathani	2006
11	TXO150	Chai Nat	2008
12	TXO155	Kamphang Sean	2009
13	Race I	Nankhan/Myanmar	2009
14	Race II	Leway/Myanmar	2009
15	Race III	Hmawbi/Myanmar	2009
16	Race IV	Thekhon/Myanmar	2009
17	Race V	Mattaya/Myanmar	2009

Rice seed preparation, inoculum preparation, inoculation and disease scoring procedure were the same as the experiment I. The disease was scored by measuring the lesion length (LL) of the inoculated leaves after inoculation about 12-18 days (DAI) depend on the susceptible control show symptoms sufficiently. The resistance reaction was classified as resistant (R), moderately resistance (MR), moderately susceptible (MS) and susceptible (S) when the lesion length was 0-3 cm, 3.1-6 cm, 6.1-9 cm and more than 9 cm, respectively (Yang *et al*., 2003; Zhang *et al*., 2006).

6. Grain quality evaluation

Grain quality was evaluated using grain samples harvested from Thailand experiment in 2012. Rice grains of the introgression lines and checks were harvested and sun-dried naturally in a greenhouse. The dried grains were stored at room temperature for one month prior to the evaluation of grain quality traits. Grain samples were mechanically dehulled and polished by a mini polisher. The evaluations of four grain quality traits were replicated three times and conducted at Rice Gene Discovery Unit in 2012.

Evaluation for presence or absence of aroma in aromatic Manawthukha introgression lines (MK-75ILs) and the parents was determined by sensory method, developed from Rice Gene Discovery Unit, Kasetsart University, Thailand.

The ratio of amylase to total starch was measured as amylase content (Juliano, 1985). The GC was measured by the length of grain starch slurry in a culture tube of cold gel; the length of the gel, the distance from the bottom of the tube to the front of the gel migration, was measured in millimeters after one hour later. The longer gel is considered to be softer than the shorter gel (Cagampang *et al*., 1973). ASV is an indicator of the time requirement for cooking (gelatinization temperature; GT). It is indirectly estimated by the GT; a larger ASV represents increased spreading in alkali and therefore a lower GT while a smaller ASV indicates a higher GT. Aroma, AC, GC and ASV were evaluated as the same procedures described in experiment I.

For physical grain quality features, the sizes and shapes of the whole grain and kernel were measured. Ten seeds of milled rice kernel were used for the measurements of length and breadth using venires calipers and the length/breadth ratios (L/B) were calculated. The measured and calculated physical grain quality traits included the length and breadth of whole grain (WGL and WGB), the length and breadth of kernel (GL and GB), the length/breadth ratios of the whole grain (WL/WB) and the length/breadth ratios of the kernel (KL/KB).

7. Statistical analysis

All traits in each experiment were subjected to statistical analysis with the CROPSTAT software program. An analysis of variance (ANOVA) was performed based on the randomized complete block design (RCBD). The least significant difference (LSD) was determined at a 5% probability level to perform the comparison between the mean values of each entry and the parents. Grain quality performances, morphological features of all entries were compared with Aromatic Manawthukha (MK-75) whereas the LL and resistance reaction were compared with the RG-9 line (donor parent).

RESULTS AND DISCUSSION

Results

Part I: Identification of genes conferring seedling resistance to bacterial blight from KDML105 introgression lines, for a non-age- related broad spectrum resistance

1. Segregation of seedling resistance in KBILs

Artificial inoculation was performed with the 19 *Xoo* isolates. The results for leaf lesions in the KBILs and the parents in the tests against the 19 *Xoo* isolates are presented in Table 4. The recipient parent, KDML105, was very susceptible (LL > 10 cm) to all *Xoo* isolates. IR1188 exhibited a higher resistance to all *Xoo* isolates than KDML105. IR1188 was highly resistant (LL < 3 cm) to *Xoo* isolates TB9603, TXO32, TXO40, TXO50, TXO61, TXO80 and TXO105, moderate resistant (LL < 6 cm) to *Xoo* isolates BR1-2, SK2-3, PSL, TXO4, TXO5 and TXO112 and moderate susceptible (6 cm < LL < 10 cm) to *Xoo* isolates NW3-1, TXO141, TXO142, TXO150 and TXO155. Significant differences in the LLs were observed between KDML105 and IR1188 against all *Xoo* isolates. Segregation of the LL against all *Xoo* isolates was observed among the KBILs (all lines carrying the *Xa21*) and showed a continuous distribution. These results indicated that *Xa21* was not fully expressed in the 30-day-old seedlings in this experiment and that a polygenic control might, therefore, be responsible for BB resistance at the seedling stage in this material. The LLs of the KBILs varied greatly, with values between those of KDML105 and IR1188 (Table 4).

Table 4 Resistance phenotypes (lesion length (cm) to nineteen *Xoo* isolates) of KBILs and parental lines inoculated on 30-days old seedlings

Xoo Isolates	KBILs			KDML105	IR1188	F test	LSD
	Min	Max	Mean				
BR1-2	3.5	29	16.4 [c]	24.1 [b]	4.5 [b]	**	2.2
CRL1-3	1.8	29.6	13.2 [b]	27.4 [c]	2.6 [b]	**	2.6
NW3-1	6.6	27.9	15.1 [c]	27.9 [c]	6.6 [b]	**	2.0
SK2-3	5.5	20.4	12.1 [b]	21.1 [b]	4.6 [b]	**	2.1
PSL	4.	27.2	12 [b]	24.4 [b]	4.1 [b]	**	2.1
TB9603	3.0	26.5	11.9 [b]	16.3 [b]	2.9 [b]	**	4.1
TXO4	3.3	21.0	9.9 [b]	22.6 [b]	3.1 [b]	**	3.2
TXO5	4.5	25.8	10.4 [b]	31.5 [c]	4.6 [b]	**	4.1
TXO32	2.3	25.5	10.9 [b]	17.4 [b]	2.4 [b]	**	4.5
TXO40	0.3	15.6	4.1 [a]	10.1 [a]	1.0 [a]	**	3.0
TXO50	1.5	8.0	3.3 [a]	10.1 [a]	2.3 [b]	**	1.8
TXO61	2.3	23.3	8.3 [b]	16.7 [b]	1.4 [b]	**	4.4
TXO80	2.0	28.1	11.3 [b]	14.7 [a]	2.2 [b]	**	4.5
TXO105	1.6	20.5	5.4 [a]	16.7 [b]	1.5 [a]	**	2.2
TXO112	4.7	27.4	15.1 [c]	22.9 [b]	4.2 [b]	**	2.1
TXO141	5.6	17.1	11.5 [b]	17.2 [b]	7.9 [c]	**	2.4
TXO142	5.8	20.9	12.9 [b]	15.5 [b]	7.3 [c]	**	2.9
TXO150	2.6	12.9	8.1 [b]	15.1 [b]	7.9 [c]	**	2.6
TXO155	6.1	25.4	12.5 [b]	24.1 [b]	8.3 [c]	**	3.4

LSD was calculated from the differences between the introgression lines based on isolate

a, b, c indicates significant difference of lesion length among the isolates

** = Significant at P< 0.01

2. Identification of the seedling resistance

A genotypic-phenotypic association analysis identified four quantitative trait loci (QTLs), *qBB1*, *qBB8.1*, *qBB8.2* and *qBB11* (at the 0.01 level of significance), mapped on rice chromosomes 1, 8 and 11. For all of the QTLs except for *qBB8.1*, resistance against each *Xoo* isolate coincidentally mapped to the same genomic locations. Locus *qBB1* was mapped to the RM302-RM212 interval on chromosome 1, and locus *qBB8.2* was mapped to the RM210-RM149 interval on chromosome 8. The locus *qBB8.1* function against *Xoo* isolates TB9603 was mapped to the RM149-RM264 interval on the same chromosome. Locus *qBB11* was mapped to the RM287-RM224 interval, located very close to *Xa21* on chromosome 11. The IR1188 alleles at all identified QTLs contributed to a shorter LL (Table 5).

3. Agronomic characteristics of the KBILs

An analysis of variance (ANOVA) for DH, PH, NEPT, NGP and GY is presented in Table 6. Significant genotype variances were observed for all traits among individuals of the KBILs and the recurrent parent, KDML105. The mean values of the KBILs ranged from 79 to 101 days (DH), 73 to 121 cm (PH), 133 to 392 tillers/m^2 (NEPT), 96 to 193 spikelets/panicle (NGP) and 444 to 881 Kg/rai (GY), whereas the values for KDML105 were 79 days, 110 cm, 156 tillers/m^2, 130 spikelets/panicle and 588 Kg/rai, respectively. Selected KBILs showing agronomic characteristics similar to those of the recipient, KDML105 (data not shown), and carrying BB resistance were chosen for the multi-location trial.

Table 5 Summary of BB resistance QTL expressed at seedling stage detected in backcross introgression lines of KDML105 (KBILs) in response to inoculation with nineteen *Xoo* isolates.

QTL	Chr.	Marker interval	Genome position (MP)	Positive allele	*Xoo* isolates
qBB1	1	RM302-RM212	36.39-36.45	IR1188	BR1-2, CRL3-1, NW3-1, SK2-3,TB9603, TXO4, TXO5,TXO32,TXO40, TXO50, TXO61,TXO80, TXO112, TXO141, TXO142, TXO150, TXO155
qBB8.1	8	RM149-RM264	26.36-29.85	IR1188	BR1-2, CRL3-1, NW3-1, SK2-3, TB9603,
qBB8.2	8	RM210-RM149	23.85-26.36	IR1188	TXO4, TXO5, TXO32, TXO40, TXO50, TXO61, TXO80, TXO105, TXO112, TXO141, TXO142, TXO150, TXO155
qBB11	11	RM287-RM224	13.74-21.84	IR1188	BR1-2,CRL3-1, NW3-1, SK2-3, TB9603, TXO4, TXO5,TXO32,TXO40,TXO50,TXO61,TXO80, TXO105, TXO112,TXO141, TXO142, TXO150, TXO155

Table 6 Comparison of yield and agronomic characters obtained at Kasetsart University between original KDML105 and KBIL using Analysis of Variance

Trait	KBIL			KDML105	F-Test	Grand mean	5% LSD	CV (%)
	Min	Max	Mean					
DF	79	101	84	79±0	**	85	5.2	3.1
PH	73	121	107	110±5	**	107	11.8	5.5
NEPT	133	392	210	156±16	**	258	101.8	19.7
NGP	96	193	136	130±15	**	137	28	10.1
GY	444	881	678	588±10	**	682	221.3	16.2

DF = Days to flowering (days); PH = Plant height (cm); NEPT = Number of effective tillers per m^2; NGP = number of grains per panicle; GY = Grain yield (Kg/rai)
** Significant at P< 0.01

4. Agronomic and yield performance of selected KBI lines in rainfed environments

In 2005, seven lines were selected based on the QTL information and agronomic characters obtained in 2003. In 2006, five lines were included in the multi-location trial (Table 7). In both years, the mean GY of the lines tested in the multi-location trial, except for RGD9904-188-8-1-23-8, was 568.3 kg/rai, compared with 473.1 kg/rai for KDML105 in 2006. However, this mean GY value did not differ significantly from KDML105 in 2005. Nevertheless, certain KBI lines showed higher GY values than KDML105 in both years, although the difference was not significant (Table 8). For traits such as DH, PH and NPH, none of the lines were observed to differ from KDML105 in 2005 or 2006. Based on the 2006 data, four lines were selected and planted in 6 farmers' fields. As also observed in the multi-location trials in 2005 and 2006, the GY, DH, PH and NPT of the test lines did not differ from the corresponding values for KDML105. The combined analysis of three years of field

trials showed that line RGD9904-104-4-1-19-14 (501.3 kg/rai) had a significantly higher GY than that of KDML105 (465.8 kg/rai), and the results showed that the yield and adaptation of selected lines were similar to those of KDML105 (Table 6). In contrast, the remaining traits did not differ from those of KDML105. This result shows that the lines developed were highly similar to KDML105 in terms of flowering and other agronomic characters. The tested lines differ from KDML105 by possessing seedling and adult plant resistance to bacterial blight disease.

Table 7 Genotypic data of the parents and selected KDML introgression lines

Lines	QTL/gene for BB resistance				Field Test		
	qBB1	***qBB8.2***	***qBB11***	***Xa21***	**2005**	**2006**	**2007**
RGD9904-104-4-1-19-14	+	+	+	+	×	×	×
RGD9904-104-4-1-19-3	+	+	+	+	×		
RGD9904-104-4-1-19-8	+	+	+	+	×	×	×
RGD9904-104-4-1-19-10	+	+	+	+	×		
RGD9904-104-4-1-19-13	+	+	+	+	×	×	×
RGD9904-188-8-1-23-8	-	-	-	+			
RGD9904-250-2-1-24-3	-	-	-	+	×	×	×
KDML105	-	-	-	-	×	×	×
IR1188	+	+	+	+			

Notes; + = carrying a resistant allele, - = carrying a susceptible allele

× = including in the experiments

5. Chemical and physical grain quality

In 2005, the chemical and physical grain qualities of the KBILs planted in the multi-location trials were taken from 5 locations, whereas these two parameters were only determined from UBN in 2006. The average amylose content (AC) in the KBI lines (15.3% and 14.5% in 2005 and 2006, respectively) tested did not differ from that of KDML105 (15.8% and 15.2% in 2005 and 2006, respectively) (Table 9). The same

outcome was obtained for GC in 2005 (the KBILs had a value of 88.8 mm, whereas KDML105 had 86.6 mm; these values were measured for the cooked gel after placement on graph paper) and for GT in 2005 and 2006 (a mean score of 6.8 for both KBILs and KDML105 in 2005, a mean score of 6.9 for the KBILs in 2006, and a mean score of 7 for KDML105 in 2006). The analysis of aroma indicated that certain lines showed a mild aroma in certain locations. This outcome could be attributed to post-harvest handling. In general, however, aroma was shown by all lines in 2005. As was the case with chemical quality, none of the physical grain qualities were found to differ from those of KDML105 (Table 10). Moreover, lines RGD9904-188-8-1-23-8 and RGD9904-250-2-1-24-3 were slightly longer than KDML105, and all lines had a slender seed shape, similar to that of KDML105, in both years.

The amylose content of RGD9904-104-4-1-19-13 was 16.5%, significantly higher than the value of 15.3% found for KDML105, based on the samples obtained from the trials conducted in the farmers' fields in 2007. Although the AC of RGD9904-104-4-1-19-13 was higher, this amount is still in the intermediate-AC range, similar to that of KDML105 (Table 9). GT did not differ between the KBILs and KDML105, and aroma was found to be present in all lines tested. The seed length, shape and color were all similar to those of KDML105 (Table 10).

Table 8 Yield and agronomic performance of KDML105 and selected KBILs in multi-location and farmer's field trials in rainfed environments

Lines	GY (Kg/rai)				DH (d)	PH (cm)	NPH
	2005	2006	2007	combined	Combined	combined	Combined
RGD9904-104-4-1-19-14	492.5	527.1	484.3	501.3	112	140	10
RGD9904-104-4-1-19-3	450.1						
RGD9904-104-4-1-19-8	472.7	503.8	439.2	471.9	111	137	9
RGD9904-104-4-1-19-10	473.7						
RGD9904-104-4-1-19-13	457.4	491.7	466.2	471.8	112	137	10
RGD9904-188-8-1-23-8	411.7	568.3					
RGD9904-250-2-1-24-3	457.8	521.5	455.0	478.1	115	142	9
KDML105	441.2	473.1	483.1	465.8	112	139	10
RD15	460.5	470.9	446.2	459.2	107	135	10
F test	ns	*	ns	ns	**	*	Ns
CV%	22	11.9	10.9	3.6	1.4	1.6	5
5 % LSD	85.3	51.4	59.4	31.2	2.9	4.0	0.9
Mean	459	507.0	464.1	475	112	138	9

** Significant at P< 0.01

* Significant at P<0.05

Table 9 Chemical characteristics of the Grain quality of original KDML105 and some KBIL lines

Lines	Chemical Characters									
	2005				2006			2007		
	AC	GC	ASV	Fragrance	AC	ASV	Fragrance	AC	ASV	Fragrance
RGD9904-104-4-1-19-14	14.9	87.0	6.8	+	14.6	7	+	15.6	7	++
RGD9904-104-4-1-19-3	14.9	88.3	6.8	++						
RGD9904-104-4-1-19-8	15.9	89.0	6.9	++	14.6	7	+	15.6	7	++
RGD9904-104-4-1-19-10	15.1	88.5	6.8	++						
RGD9904-104-4-1-19-13	15.5	88.5	7.0	++	15.6	7	++	16.5	7	++
RGD9904-188-8-1-23-8	15.5	92.5	6.6	++	16	6.9	++			
RGD9904-250-2-1-24-3	16.0	88.5	6.8	++	14.5	7	++	15.7	7	++
KDML105	15.8	86.8	6.8	++	15.2	7	++	15.3	7	++
RD15	15.1	85.0	6.7	++	11.6	6.7	++	15.6	7	++
Mean	15.3	88.8	6.8		14.5	6.9		15.6	7	
SD	0.5	2.5	0.1		1.35	0.2		0.4	0	

Notes; In 2005, AC, GC, ASV are mean from five locations.

In 2007, AC, GC, ASV are mean from two locations.

+= Mild aroma, ++=Strong aroma

Table 10 Physical characteristics of the Grain quality of the original KDML105 and some KBIL lines

Lines	Physical Grain Characters								
	2005			2006			2007		
	SL (mean)	SS	SC	SL	SS	SC	SL 9 (mean)	SS	SC
RGD9904-104-4-1-19-14	7.1	Sl	S	7.1	Sl	S	7.2	Sl	S
RGD9904-104-4-1-19-3	7.1	Sl	S						
RGD9904-104-4-1-19-8	7.1	Sl	S	7.4	Sl	S	7.3	Sl	S
RGD9904-104-4-1-19-10	7.1	Sl	S						
RGD9904-104-4-1-19-13	7.0	Sl	S	7.1	Sl	S	7.4	Sl	S
RGD9904-188-8-1-23-8	7.7	Sl	S	8.1	Sl	S			
RGD9904-250-2-1-24-3	7.4	Sl	S	8.0	Sl	S	7.9	Sl	S
KDML105	7.4	Sl	S	7.9	Sl	S	7.7	Sl	S
RD15	7.4	Sl	S	7.5	Sl	S	7.8	Sl	S
Mean	7.3			7.6			7.4		
SD	0.3			0.4			0.2		

Notes; S= straw colour, Sl= selender

In 2005, seed length(SL) is mean from five locations.

In 2007, seed length(SL) is mean from two locations.

Part II: Marker assisted Introgression of multiple genes for bacterial blight resistance in Aromatic Manawthukha rice line, MK-75

1. Marker-assisted selection

Table11 showed the result of marker assisted foreground selection in this study. In the F_1 generation, thirteen F_1 plants from the crosses between MK-75 and RG-9 were tested for hybridity using the PB7-8 marker. Five positive F_1 plants were backcrossed to generate 118 BC_1F_1 plants and were subjected to screening with PAxa5, PB7-8, RM5509, RM7243 markers. Thirteen out of 118 plants were identified carrying the positive alleles ($xa5^{RG}xa5^{MK}$/$Xa21^{RG}Xa21^{MK}$/$xa33^{RG}xa33^{MK}$) at selected markers. Based on the marker profile and agro-morphological similarity to recurrent parent, five BC_1F_1 plants were selected and used to generate 311 BC_2F_1 plants. Of these, twenty BC_2F_1 heterozygous plants ($xa5^{RG}xa5^{MK}$/$Xa21^{RG}Xa21^{MK}$/ $xa33^{RG}xa33^{MK}$) were identified and seven out of 20 BC_2F_1 plants were backcrossed to 179 BC_3F_1 plants. Fifteen BC_3F_1 heterozygous plants were identified and checked for cooking quality profile using Aromarker, Waxy and SNP2340-41. All Fifteen BC_3F_1 heterozygous plants ($xa5^{RG}xa5^{MK}$/$Xa21^{RG}Xa21^{MK}$/$xa33^{RG}xa33^{MK}$) showed a homozygous band at the three markers ($Aro^{MK}Aro^{MK}$/$Wx^{MK}Wx^{MK}$/ $SNP2340^{MK}$ $SNP2340^{MK}$). Finally, seven best plants were forwarded to generate 420 BC_3F_2 families. Six triple homozygous genes introgressed-families and fifty six homozygous genes introgressed-families were identified. To test for BB resistance in this study, 28 BC_3F_2 families consisted of triple, double and single gene combinations were selected. Six BC_3F_2 families (the triple resistance gene-introgressed lines: MK-75IL+3) have the $xa5^{RG}xa5^{RG}$/$Xa21^{RG}Xa21^{RG}$/$xa33^{RG}xa33^{RG}$ genotype. Five, four and five BC_3F_2 families (the double resistance gene-introgressed lines: MK-75IL+2) have $Xa21^{RG}Xa21^{RG}$/$xa33^{RG}xa33^{RG}$, $xa5^{RG}xa5^{RG}$/$Xa21^{RG}Xa21^{RG}$ and $xa5^{RG}xa5^{RG}$/ $xa33^{RG}xa33^{RG}$ genotypes respectively. Two, three and three BC_3F_2 families (the single resistance gene-introgressed lines: MK-75IL+1) have the $xa5^{RG}xa5^{RG}$, $Xa21^{RG}Xa21^{RG}$ and / $xa33^{RG}xa33^{RG}$ genotypes respectively (Table 12). They were advanced to generate $BC_3F_{2:3}$ generation.

.

Table 11 Marker assisted selection on different generations of (MK-75) and (RG-9)

Generation	Genotpye	No. of lines	Linked markers	Number of lines								No. of selected lines
				none	*xa5*	*Xa21*	*xa33*	*xa5+xa21*	*xa5+xa33*	*Xa21+xa33*	*xa5+Xa21+xa33*	
F_1	$xa5^{RG-9}Xa5^{MK-75}$/ $Xa21^{RG-9}xa21^{MK-75}$/ $xa33^{RG-9}Xa33$	13	PB7-8	0							13	5
BC_1F_1	$xa5^{RG-9}Xa5^{MK-75}$/$Xa21^{RG-9}xa21^{MK-75}$/$xa33^{RG-9}Xa33^{MK-75}$	118	PAxa5, PB7-8, RM5509, RM7243	21	16	24	10	13	9	12	13	5
BC_2F_1	$xa5^{RG-9}Xa5^{MK-75}$/ $Xa21^{RG-9}xa21$/ $xa33^{RG-9}Xa33^{MK-75}$	311	PAxa5, PB7-8, RM5509, RM7243	54	42	44	42	36	33	40	20	7
BC_3F_1	$xa5^{RG-9}Xa5^{MK-75}$/ $Xa21^{RG-9}xa21^{MK-75}$/ $xa33^{RG-9}Xa33^{MK-75}$	179	PAxa5, PB7-8, RM5509, RM7243	62	20	22	15	20	13	12	15	7
BC_3F_2	$xa^{RG-9}5xa5^{RG-9}$/ $Xa21^{RG-9}Xa21^{RG-9}$ /$xa33^{RG-9}xa33^{RG-9}$	420	PAxa5, PB7-8, RM5509, RM7243	208	48	31	71	20	15	21	6	28

Table 12 Marker genotypes of the twenty eight MK-75 ILs and the two parents

+ = presence of resistance gene and aroma

Sr.no	Code	*xa5*$^{RG-9}$	*Xa21* $^{RG-9}$	*xa33* $^{RG-9}$	Aromaker^{MK-75}	GT^{MK-75}
1	RGD12-79-11	+	+	+	+	+
2	RGD12-79-42	+	+	+	+	+
3	RGD12-95-38	+	+	+	+	+
4	RGD12-95-39	+	+	+	+	+
5	RGD12-95-45	+	+	+	+	+
6	RGD12-95-57	+	+	+	+	+
7	RGD12-79-5	-	+	+	+	+
8	RGD12-95-69	-	+	+	+	+
9	RGD12-111-19	-	+	+	+	+
10	RGD12-127-30	-	+	+	+	+
11	RGD12-142-49	-	+	+	+	+
12	RGD12-79-24	+	+	-	+	+
13	RGD12-95-44	+	+	-	+	+
14	RGD12-127-29	+	+	-	+	+
15	RGD12-154-34	+	+	-	+	+
16	RGD12-95-66	+	-	+	+	+
17	RGD12-104-50	+	-	+	+	+
18	RGD12-127-26	+	-	+	+	+
19	RDG12-142-8	+	-	+	+	+
20	RDG12-154-44	+	-	+	+	+
21	RGD12-79-41	-	+	-	+	+
22	RGD12-104-8	-	+	-	+	+
23	RDG12-127-27	-	+	-	+	+
24	RGD12-104-59	-	-	+	+	+
25	RGD12-111-8	-	-	+	+	+
26	RGD12-127-49	-	-	+	+	+
27	RGD12-142-6	+	-	-	+	+
28	RGD12-142-38	+	-	-	+	+
29	MK-75	-	-	-	+	+
30	RG-9	+	+	+	-	-

2. Evaluation of resistance in parental lines at the seedling and tillering stages

The resistance level of eight rice varieties to the 12 Thai *Xoo* isolates at both seedling and tillering stages are showed in Table 14 and 15. At the seedling stage, KDML105, MK and MK-75 (recipient parent) were susceptible to all isolates while the donor parent RG-9 showed highly resistance reaction. The IR62266 (the *xa5* ancestor of the RG9) were resistant or moderately resistant to all isolates except for the SK2-3. The Ba 7 (the *xa33* ancestor of the RG9) was highly resistant to 5 isolates (TXO85, TXO150, CML3-1, NB7-6 and PSL), moderately resistant to 5 isolates (TXO155, BR1-2, CRL1-3, NB7-7 and NW3-1) and susceptible to 2 isolates (NPL3-3 and SK2-3). IRBB21 (carrying the *Xa21*) was resistant to 4 isolates (TXO85, TXO150, NPL3-3 and PSL), moderately resistant to 7 isolates (BR1-2, CML3-1, CRL1-3, NB7-6, NB7-7, NW3-1 and SK2-3), moderately susceptible to 1 isolate (TXO155). IR1188 (the *Xa21* ancestor of the RG9) was resistant to 5 isolates (TXO85, TXO150, CML3-1, NPL3-3 and PSL), moderately resistant to 6 isolates (BR1-2, CRL1-3, NB7-6, NB7-7, NW3-1 and SK2-3B), moderately susceptible to 1 isolate (TXO155).

At the tillering stage, KDML105, MK and MK-75 were susceptible to all isolates while RG-9 was resistant to all isolates. IR62266 was resistant to all isolates except for the SK2-3. The Ba 7 was highly resistant to 10 isolates (TXO85, TXO150, PSL, TXO155, BR1-2, CML3-1, CRL1-3, NB7-6, NB7-7 and NW3-1) and susceptible to 2 isolates (NPL3-3 and SK2-3). IRBB21 was found resistance to 7 isolates (TXO85, TXO150, CML3-1, CRL1-3, PSL, NW3-1 and NPL3-3), moderately resistance to 5 isolates (TXO155, BR1-2, NB7-6, NB7-7 and SK2-3). The IR1188 was resistant to 8 isolates (TXO85, TXO150, CML3-1, NPL3-3, PSL, TXO155, CRL1-3 and NW3-1) and moderately resistant to 4 isolates (BR1-2, NB7-6, NB7-7and SK2-3).

Table 13 Resistance reaction of the parents against Twelve Thai isolates at the seedling stage

Sr.no	Isolates	KDML105	MNTK	MK-75	IR62266 (*xa5*)	IR1188 (*Xa21/qBB1/qBB8/qBB11*)	IRBB21 (*Xa21*)	Ba7 (*xa33*)	RG-9 (*xa5/Xa21/xa33*)
1	TXO85	S	S	S	R	R	R	R	R
2	TXO150	S	S	S	R	R	R	R	R
3	TXO155	S	S	S	MR	MS	MS	MR	R
4	BR1-2	S	S	S	R	MR	MR	MR	R
5	CRL1-3	S	S	S	R	MR	MR	MR	R
6	CML3-1	S	S	S	R	R	R	R	R
7	NB7-6	S	S	S	R	MR	MR	R	R
8	NB7-7	S	S	S	R	MR	MR	MR	R
9	NW3-1	S	S	S	MR	MR	MR	MR	R
10	NPL3-3	S	S	S	R	R	MR	S	R
11	SK2-3	S	S	S	S	MR	MR	S	R
12	PSL	S	S	S	R	R	R	R	R

R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S=susceptible

Table 14 Resistance reaction of the parents against Twelve Thai isolates at the tillering stage

Sr.no	Isolates	KDML105	MNTK	MK-75	IR62266 (*xa5*)	IR1188 (*Xa21/qBB1/qBB8/qBB11*)	IRBB21 (*Xa21*)	Ba7 ((*xa33*)	RG-9 (*xa5/Xa21/xa33*)
1	TXO85	S	S	S	R	R	R	R	R
2	TXO150	S	S	S	R	R	R	R	R
3	TXO155	S	S	S	R	R	MR	R	R
4	BR1-2	S	S	S	R	MR	MR	R	R
5	CRL1-3	S	S	S	R	R	R	R	R
6	CML3-1	S	S	S	R	R	R	R	R
7	NB7-6	S	S	S	R	MR	MR	R	R
8	NB7-7	S	S	S	R	MR	MR	R	R
9	NW3-1	S	S	S	R	R	R	R	R
10	NPL3-3	S	S	S	R	R	R	S	R
11	SK2-3	S	S	S	S	MR	MR	S	R
12	PSL	S	S	S	R	R	R	R	R

R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = susceptible

3. Evaluation of resistance in the two parents and MK-75 introgression lines

In order to validate the efficiency of MAS, twenty eight $BC_3F_{2:3}$ pyramid lines fixed as homozygous at the target allele of BB resistant genes and parents were evaluated with ten isolates at the seedling stage and tillering stage. The LL of the MK-75ILs and the two parents were presented in (Table 15 and 16). The two parents MK-75 and RG-9 were significantly different in the LL when tested against all Thai *Xoo* isolates at both seedling and tillering stage (Figure 8 and 9). The average LL was more than 10 cm and less than 2.0 cm for MK-75 and RG-9 respectively. The LL of the recipient MK-75 was not significant difference from that of the original MK. The susceptible check KDML105 had shown the most susceptible reaction (the longest LL) against all isolates. All of the MK-75ILs were found to be more resistance (shorter LL) than the recipient MK-75 against all isolates. There were varying degrees of resistance to each of the isolates, but no isolate could break the resistance of any of the three gene pyramids at both seedling and tillering stages.

At the seedling stage, the MK-75ILs+1($xa5^{RG}$) showed moderately resistance against TXO150, TXO155, BR1-2, NW3-1 and resistance reaction to TXO85, CRlL1-3, NB7-7 and PSL. It showed moderately susceptible reaction to NPL3-3 (the LL range from 6.3 to 7 cm) and susceptible reaction to SK2-3(9.7 -10.1 cm). The MK-75ILs+1 ($Xa21^{RG}$) showed resistant reaction to TXO85 and PSL and moderately resistance reaction against BR1-2, CRL1-3, NB7-7, NPL3-3 and NW3-1. These lines exhibited moderately susceptible reaction to TXO150 (the LL range from 8.6-9cm) TXO155 (the LL range from 6.7-8cm) and SK2-3 (the LL range from 6.1-8cm). The MK-75ILs+1 ($xa33^{RG}$) was susceptible to the CRL1-3 (the LL ranged from 11.4 to 12.9 cm), NPL3-3 (the LL ranged from 14.5 to 17.8 cm) and SK2-3 (the LL ranged from 10.0 to 14.1cm), TXO155 (the LL was 9.1-9.9 cm). These lines showed resistance reaction to TXO85 and PSL and moderately resistance to TXO150, BR1-2, NB7-7 and NW3-1.

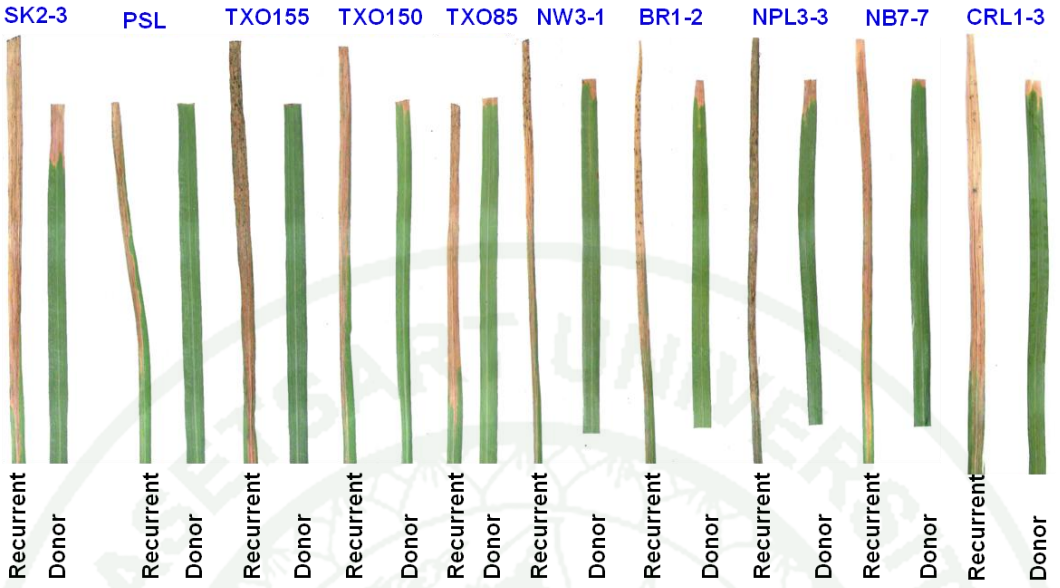

Figure 8 Resistance reaction of the two parents against 10 Thai isolates at the seedling stage

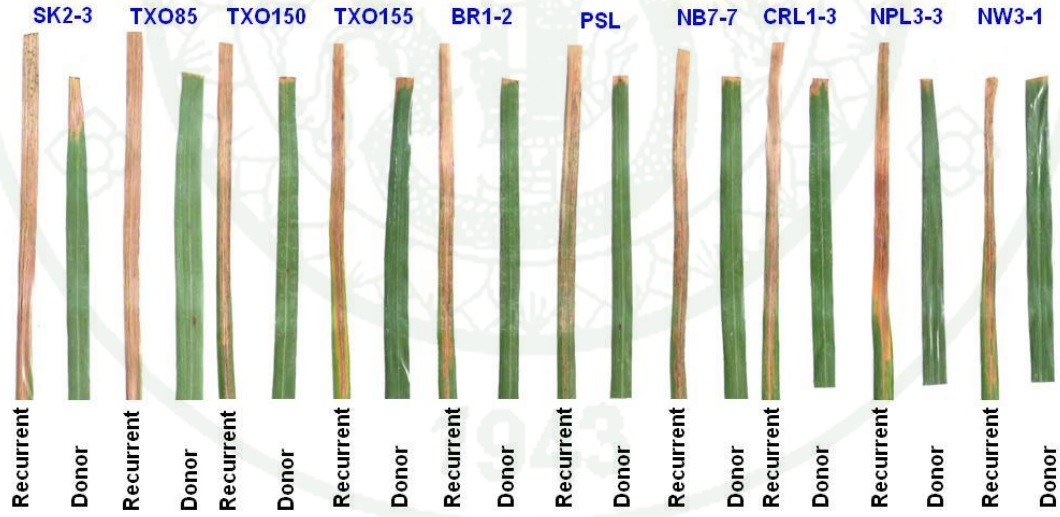

Figure 9 Resistance reaction of the two parents against 10 Thai isolates at the tillering stage

The MK-75ILs+3 and MK-75ILs+2 ($xa5^{RG}Xa21^{RG}$) had shown a high level of resistance against all isolates at seedling stage that was similar to the RG-9. Most of MK-75ILs+2 ($Xa21^{RG}xa33^{RG}$) exhibited a high level of resistance against all isolates except for against TXO150, TXO155, PSL and NW3-1. Most of the MK-75IL+2 ($xa5^{RG}xa33^{RG}$) also showed a high level of resistance against all isolates except for against the NPL3-3 and SK2-3.

At the tillering stage, the MK-75ILs+1($xa5^{RG}$) had resistance (the LL was less than 3cm) and moderately resistance with the LL of >6cm against all isolates except SK2-3. These lines exhibited susceptible reaction to SK2-3(the LL ranged from 9.4-9.9cm). The MK-75ILs+1 ($Xa21^{RG}$) was resistance reaction to TXO85, TXO155, NPL3-3 and PSL. These lines showed moderately resistance reaction to TXO150, BR1-2, CRL1-3, NB7-7 and NW3-1 and moderately susceptible reaction to SK2-3 (the LL ranged from 6.8 to 7.1cm). The MK-75IL+1 ($xa33^{RG}$) was resistance against TXO85, TXO150, BR1-2 and PSL and susceptible to the CRL1-3 (the LL ranged from12.7 to 16), NPL3-3 (the LL ranged from 9.9 to 13.8 cm) and SK2-3. These lines exhibited moderately resistance reaction to TXO155, NB7-7 and NW3-1.

The MK-75ILs+3 and MK-75ILs+2 ($xa5^{RG}Xa21^{RG}$), ($Xa21^{RG}xa33^{RG}$) exhibited a high level of resistance against all isolates that was similar to the RG-9 except NW3-1. Most of MK-75ILs+2 ($xa5^{RG}xa33^{RG}$) showed a high level of resistance against all isolates except NPL3-3 (the LL ranged from 2.0-3.1 cm) and SK2-3 (the LL ranged from 7.1 to 8.1 cm).

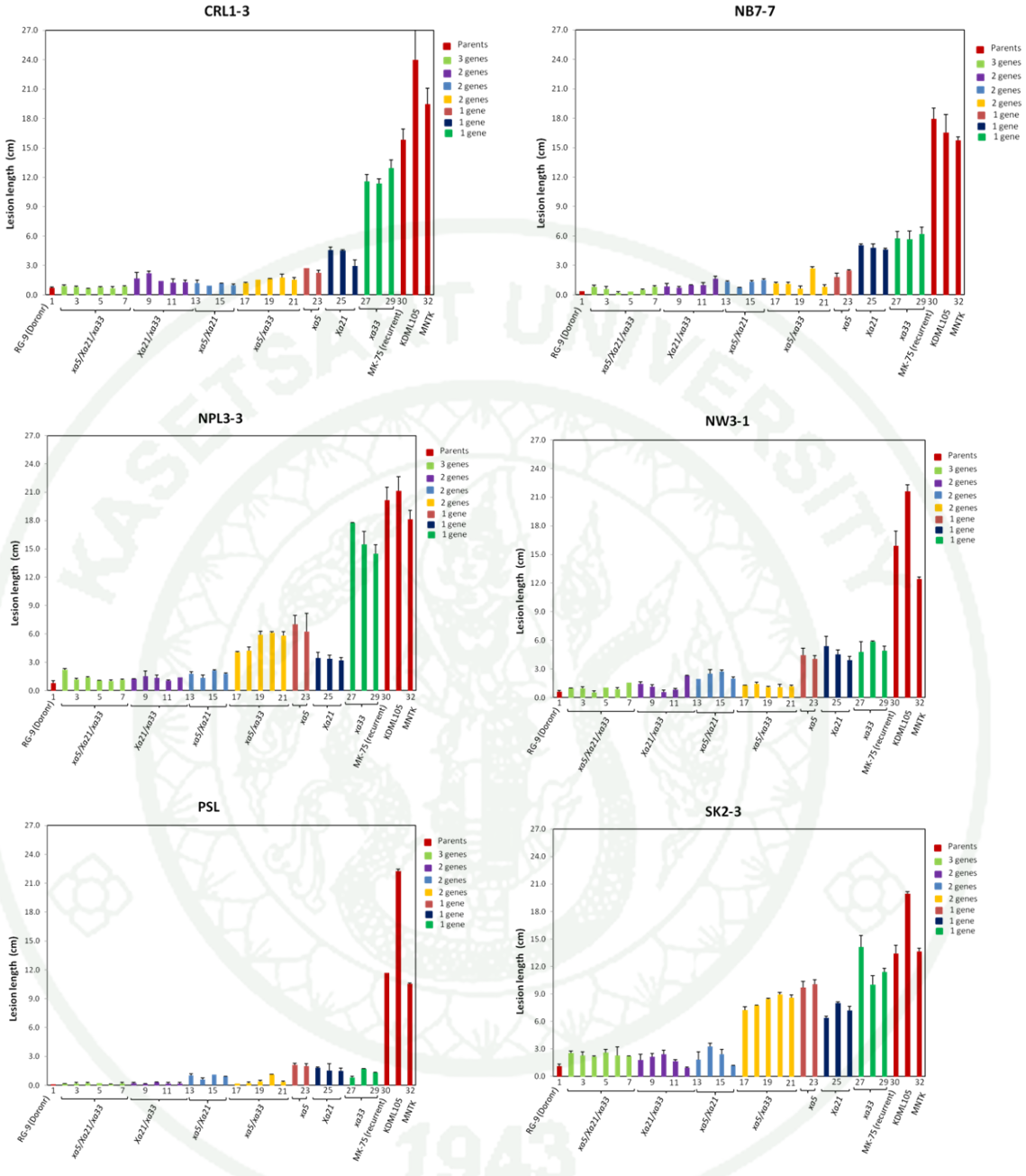

Figure 10 Lesion lengths of the two parents and MK-75ILs against 10 *Xoo* isolates at the seedling stage

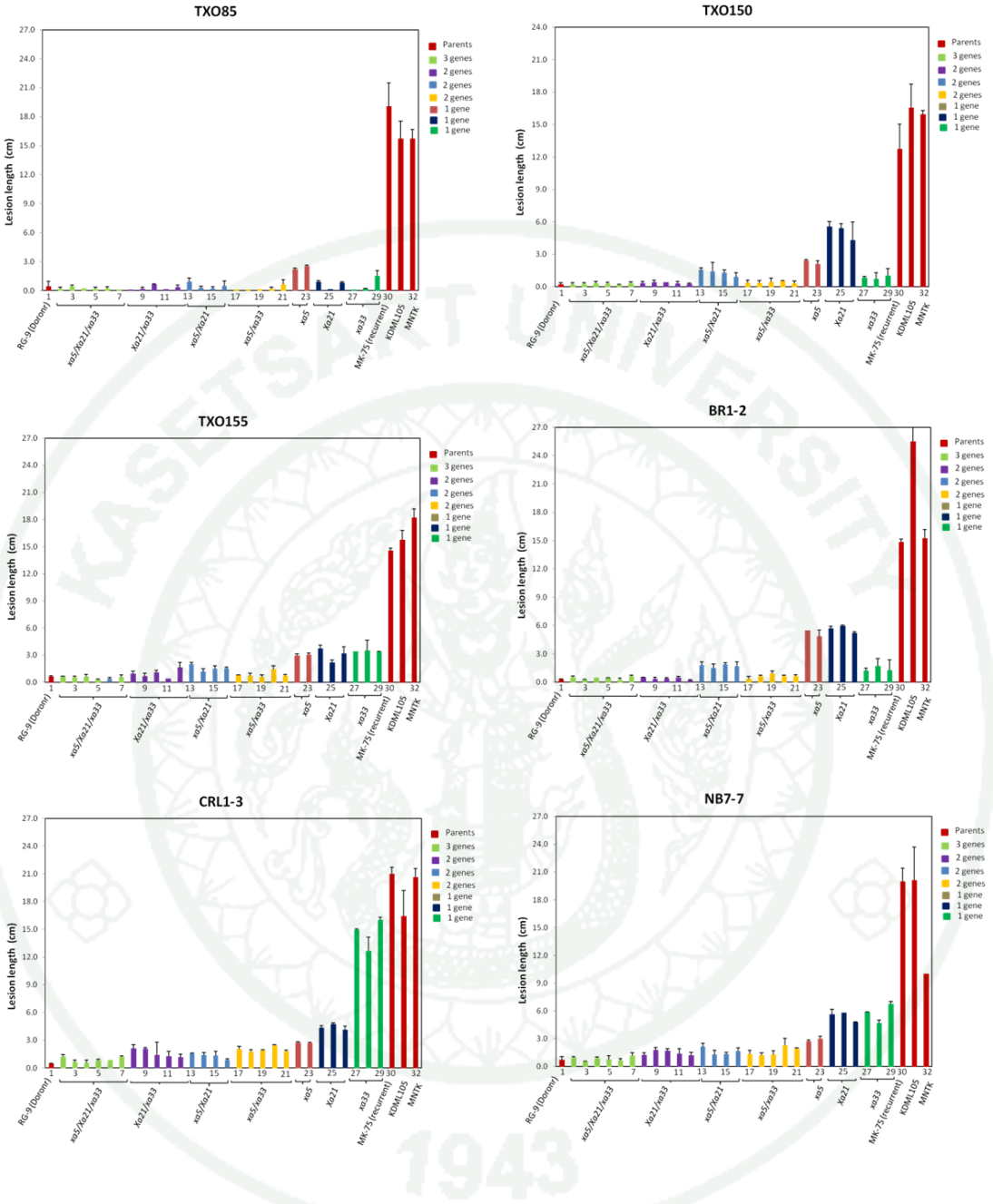

Figure 11 Lesion lengths of the two parents and MK-75ILs against 10 *Xoo* isolates at the tillering stage

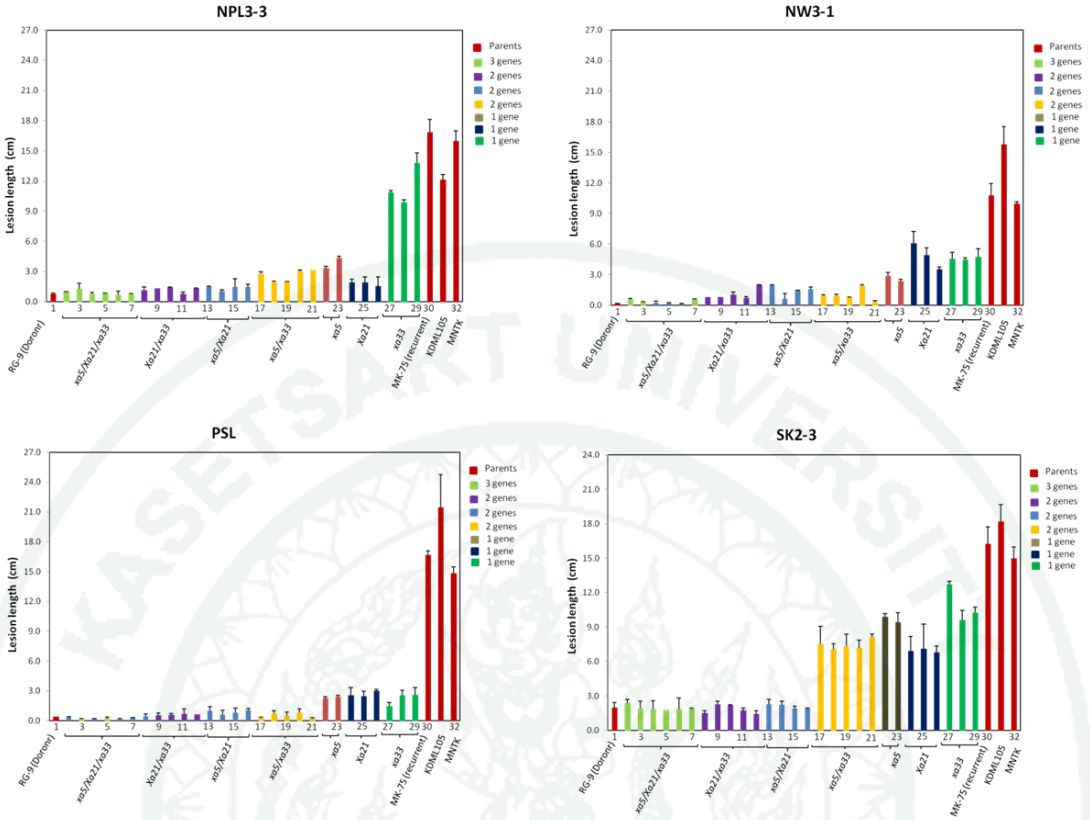

Figure 11 (Continued)

Table 15 Resistance reaction of the parents and MK-75ILs against 10 Thai isolates at the seedling stage

Gene combination	No. of lines	TXO85			TXO150			TXO155			BR1-2			CRL1-3		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
xa5.Xa21,xa33	6	0.3	0.6	0.4 [a]	0.2	0.7	0.5 [a]	0.2	0.3	0.2 [a]	0.2	0.9	0.5 [a]	0.6	0.9	0.8 [a]
xa5,Xa21	5	0.3	0.6	0.5 [a]	0.2	1.0	0.6 [a]	0.3	0.8	0.5 [a]	1.2	1.8	1.5 [a]	1.3	2.2	1.6 [a]
xa21,xa33	4	1.0	1.1	1.2 [a]	2.0	2.6	2.3 [c]	0.9	2.1	1.7 [c]	0.9	1.8	1.4 [a]	0.9	1.2	1.1 [a]
xa5,xa33	5	0.5	1.1	0.7 [a]	0.7	1.3	1.0 [a]	0.3	1.5	0.7 [a]	1.2	1.5	1.4 [a]	1.2	1.8	1.5 [a]
xa5	2	2.4	2.5	2.5 [c]	4.4	4.6	4.5 [d]	4.5	4.5	4.5 [d]	4.8	6.1	5.4 [c]	2.2	2.7	2.5 [c]
xa21	3	2.0	2.6	2.2 [c]	8.6	9.0	8.7 [b]	6.7	8.0	7.5 [e]	4.4	6.0	5.1 [c]	2.9	4.6	4.0 [c]
xa33	3	1.4	2.1	1.8 [c]	3.8	4.3	4.3 [d]	9.1	9.9	9.6 [f]	5.9	6.8	6.3 [c]	11.4	12.9	12.0 [d]
MK-75				13.2 [b]			14.6 [b]			17.3 [b]			15.0 [b]			15.9 [b]
RG-9				0.2 [a]			0.6 [a]			0.2 [a]			0.6 [a]			0.7 [a]
KDML105				21.6			18.4			26.7			24.8			24.0
MNTK				14.3			14.8			15.3			16.2			19.5
5% LSD				0.9			1.1			1.0			1.0			1.6
F test				**			**			**			**			**

Table 15 (Continued)

Gene combination	No. of lines	NB7-7			NPL3-3			NW3-1			PSL			SK2-3		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
xa5.Xa21,xa33	6	0.2	0.8	0.5 [a]	1.0	2.2	1.3 [a]	0.5	1.6	1.0 [a]	0.1	0.2	0.2 [a]	2.2	2.6	2.3 [a]
xa5,Xa21	5	0.7	1.6	1.0 [a]	1.0	1.5	1.3 [a]	0.6	2.3	1.3 [a]	0.2	0.3	0.2 [a]	0.9	2.4	1.8 [a]
xa21,xa33	4	0.7	1.3	1.2 [a]	1.3	2.1	1.8 [a]	1.9	2.7	2.3 [c]	0.6	1.1	0.9 [c]	1.2	3.3	2.2 [a]
xa5,xa33	5	0.6	2.7	1.3 [a]	4.0	6.1	6.2 [d]	1.1	1.4	1.2 [a]	0.2	1.1	0.5 [c]	7.3	9.0	8.5 [d]
xa5	2	1.8	2.5	2.2 [c]	6.3	7.0	6.6 [d]	4.1	4.4	4.2 [d]	2.0	2.1	2.1 [e]	9.7	10.1	9.9 [e]
xa21	3	4.6	5.1	4.8 [d]	3.2	3.4	3.3 [c]	3.9	5.4	4.6 [d]	1.5	1.8	1.6 [d]	6.1	8.0	7.1 [c]
xa33	3	5.7	6.2	5.9 [e]	14.5	17.8	15.9 [e]	4.8	5.9	5.2 [d]	0.8	1.7	1.3 [d]	10.0	14.1	11.9 [f]
MK-75				18.0 [b]			20.1 [b]			15.9 [b]			11.7 [b]			13.4 [b]
RG-9				0.4 [a]			0.8 [a]			0.6 [a]			0.1 [a]			1.1 [a]
KDML105				16.5			21.2			21.6			22.3			20.0
MNTK				15.8			18.2			12.4			10.6			13.7
5% LSD				0.9			1.2			1.0			0.3			1.3
F test				**			**			**			**			**

a = The variance of measurement was not significantly different from MK-75

b = The variance of measurement was not significantly different from RG-9

c, d, e, f= The variance of measurement was significantly different from MK-75 and RG-9

Table 16 Resistance reaction of the parents and MK-75ILs against 10 Thai isolates at the tillerling stage

Gene combination	No. of lines	TXO85			TXO150			TXO155			BR1-2			CRL1-3		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
xa5.Xa21,xa33	6	0.1	0.3	0.2 [a]	0.2	0.3	0.3 [a]	0.3	0.6	0.5 [a]	0.2	0.7	0.4 [a]	0.6	1.2	0.9 [a]
xa5,Xa21	5	0.1	0.7	0.3 [a]	0.3	0.4	0.3 [a]	0.4	1.6	0.9 [a]	0.2	0.5	0.4 [a]	1.2	2.1	1.6 [a]
xa21,xa33	4	0.1	0.9	0.4 [a]	0.9	1.6	1.3 [a]	1.2	2.0	1.6 [a]	1.5	1.8	1.7 [a]	0.9	1.6	1.3 [a]
xa5,xa33	5	0.1	0.2	0.1 [a]	0.2	0.5	0.4 [a]	0.6	1.5	0.9 [a]	0.4	0.9	0.7 [a]	1.8	2.5	2.0 [c]
xa5	2	2.2	2.6	2.4 [a]	2.1	2.5	2.3 [c]	3.0	3.1	3.0 [c]	4.9	5.5	5.2 [c]	2.7	2.7	2.7 [c]
xa21	3	0.1	0.9	0.6 [a]	4.3	5.6	5.1 [d]	2.1	3.7	3.0 [c]	5.2	6.0	5.6 [c]	4.1	4.8	4.4 [d]
xa33	3	0.1	1.5	0.6 [a]	0.7	1.0	0.9 [a]	3.4	3.5	3.4 [c]	1.2	1.5	1.3 [a]	12.7	16	14.5 [e]
MK-75				12.8 [b]			12.8 [b]			14.6 [b]			14.8 [b]			21.0 [b]
RG-9				0.2 [a]			0.2 [a]			0.7 [a]			0.3 [a]			0.5 [a]
KDML105				16.6			16.6			15.8			25.5			16.5
MNTK				16.0			16.0			18.2			15.3			20.7
5% LSD				1.2			1.2			0.8			1.0			1.4
F test				**			**			**			**			**

Table 16 (Continued)

Gene combination	No. of lines	NB7-7			NPL3-3			NW3-1			PSL			SK2-3		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
xa5.Xa21,xa33	6	0.5	1.2	0.8 [a]	0.7	1.3	0.9 [a]	0.2	0.6	0.2 [a]	0.2	0.4	0.2 [a]	1.8	2.4	1.9 [a]
xa5,Xa21	5	1.3	1.8	1.5 [a]	0.8	1.4	1.2 [a]	0.7	2.0	1.1 [a]	0.5	0.7	0.6 [a]	1.4	2.3	1.8 [a]
xa21,xa33	4	1.3	2.2	1.6 [a]	1.0	1.5	1.4 [a]	0.5	2.0	1.4 [c]	0.6	1.0	0.9 [a]	1.9	2.3	2.1 [a]
xa5,xa33	5	1.2	2.3	1.6 [a]	2.0	3.1	2.6 [c]	0.3	1.9	1.0 [a]	0.2	0.8	0.5 [a]	7.1	8.1	7.4 [c]
xa5	2	2.7	3.0	2.8 [c]	3.4	4.3	3.8 [d]	2.4	2.8	2.6 [d]	2.3	2.4	2.3 [c]	9.4	9.9	9.7 [d]
xa21	3	4.8	5.7	5.4 [d]	1.6	1.9	1.8 [c]	3.5	6.1	4.8 [e]	2.5	3.0	2.7 [c]	6.8	7.1	6.9 [c]
xa33	3	4.7	6.8	5.8 [d]	9.9	13.8	11.5 [e]	4.5	4.7	4.6 [e]	1.5	2.6	2.2 [c]	9.6	12.7	10.9 [d]
MK-75				20.0 [b]			16.9 [b]			10.8 [b]			16.7 [b]			16.3 [b]
RG-9				0.7 [a]			0.8 [a]			0.1 [a]			0.4 [a]			2.0 [a]
KDML105				20.1			12.2			15.8			21.5			18.2
MNTK				10.0			16.0			10.0			14.9			15.0
5% LSD				1.5			0.9			1.0			1.4			1.6
F test				**			**			**			**			**

a = The variance of measurement was not significantly different from MK-75

b = The variance of measurement was not significantly different from RG-9

c, d, e, = The variance of measurement was significantly different from MK-75 and RG-9

4. Evaluation of resistance in the parents and pyramiding lines with Myanmar isolates

Twenty eight BC_3F_3 pyramid lines fixed as homozygous at the target allele of BB resistant genes and parents were evaluated with five Myanmar isolates at the seedling stage. Myanmar *Xoo* isolate, Race I is the most virulent and widely distributed in Myanmar. The resistant reaction of the MK-75ILs obtained from DAR after inoculation with five isolates was shown in the Table 17. The MK-75ILs was found to be more resistance than the recipient MK-75 against 5 Myanmar *Xoo* isolates except for the MK-75IL+1 ($xa33^{RG}$) that was susceptible to all isolates. The MK-75IL+1 ($xa5^{RG}$) was susceptible to the Race I and Race III. The MK-75IL+1 ($Xa21^{RG}$) showed a moderately resistant reaction to all isolates. The MK-75IL+3 and MK-75IL+2 ($xa5^{RG}Xa21^{RG}$ and $Xa21^{RG}xa33^{RG}$) exhibited a high level of resistance to all isolates while the MK-75IL+2 ($xa5^{RG}xa33^{RG}$) showed susceptible reaction to the Race I and Race III and moderately resistant reaction to Race II, Race IV and Race V.

Table 17 Resistant reaction of the parents and MK-75ILs against five Myanmar isolates at the seedling stage

Sr.no	Line	Race I	Race II	Race III	Race IV	Race V
1	RGD12-79-11	MR	R	MR	R	R
2	RGD12-79-42	MR	R	MR	R	R
3	RGD12-95-38	MR	R	MR	R	R
4	RGD12-95-39	MR	R	MR	R	R
5	RGD12-95-45	MR	MR	MR	R	MR
6	RGD12-95-57	MR	R	MR	R	MR
7	RGD12-79-5	MS	MR	MR	R	MR
8	RGD12-95-69	MR	R	MR	R	R
9	RGD12-111-19	MS	R	MR	R	MR
10	RGD12-127-30	MR	MR	MR	R	MR
11	RGD12-142-49	MR	R	R	R	R
12	RGD12-79-24	MR	MR	MR	R	MR
13	RGD12-95-44	MS	R	MR	R	MR
14	RGD12-127-29	MS	MR	MR	R	MR
15	RGD12-154-34	MR	MR	MR	R	R
16	RGD12-95-66	S	MR	S	MR	MR
17	RGD12-104-50	S	MR	S	R	MR
18	RGD12-127-26	S	MR	S	R	MS
19	RDG12-142-8	S	MS	S	MR	MS
20	RDG12-154-44	S	MR	S	R	MR
21	RGD12-142-6	S	MR	S	MR	R
22	RGD12-142-38	S	MR	S	MR	MR
23	RGD12-79-41	MS	MR	MR	MS	MR
24	RGD12-104-8	MS	MS	R	MR	MR
25	RDG12-127-27	MR	MR	S	MS	S
26	RGD12-104-59	S	S	S	S	S
27	RGD12-111-8	S	S	S	S	S
28	RGD12-127-49	S	S	MR	S	S
29	MK-75	S	S	S	S	S
30	RG-9	MR	MR	MR	R	R
31	Manawthukha	S	S	S	S	S

5. Grain quality of Aromatic Manawthukha Introgression lines

Analysis of variance (ANOVA) for the chemical and physical grain quality traits evaluated at Kasetsart University were presented in Table 18. Aroma was evaluated with numerical score 0 to 3 (0 = non fragrance, 1 = mild fragrance, 2 = moderate fragrance and 3 = strong fragrance). Significant differences between the parents were observed for fragrance, AC and ASV. MK-75 had intermediate fragrance, intermediate AC (21.2%), low ASV (score 2), softer GC (123 mm). RG-9 had non fragrance, low AC and high ASV (score 6). All MK-75ILs had intermediate AC (18.6%-21.9%), intermediate fragrance (2) and low ASV (2) in which it was similar to those of the MK-75. Parental lines, MK-75 and RG-9 had similar GC values (123mm and 122 mm respectively). Most of the MK-75ILs had a little bit lower GC values (92-115 mm) than that of the MK-75. However, all of these MK-75ILs were defined as soft GC (the GC value greater than 60 mm) according to Cagampang *et al*., (1973).

6. Morphological feature of grains

Morphological feature of grains are presented in (Table 19). The donor parent (RG-9) has long slender grains as compared to recurrent parent, MK-75. Significant differences between the parents were observed for WGL, KGL, WL/WB and KL/KB. MK-75 had short GL and low ratio of KL/KB (2.78) whereas RG-9 possessed long GL and L/B ratio of 3.42. All MK-75ILs had intermediate KL (5.94-7.20 mm) in which it was similar to those of the MK-75.

Table 18 Chemical Characteristics of the parents and MK-75ILs

Sr.no	Entry	Aroma score	Amylose content	Alkali spreading value	Gel consistency
1	RGD12-79-11	2 [a]	19.8[a]	2 [a]	116
2	RGD12-79-42	2 [a]	20.8[a]	2 [a]	94
3	RGD12-95-38	2 [a]	19.9[a]	2 [a]	110
4	RGD12-95-39	2 [a]	20.4[a]	2 [a]	127
5	RGD12-95-45	2 [a]	19.4	2 [a]	77
6	RGD12-95-57	2 [a]	21.1[a]	2 [a]	118
7	RGD12-79-5	2 [a]	20.4[a]	2 [a]	96
8	RGD12-95-69	2 [a]	20.3 [a]	2 [a]	123
9	RGD12-111-19	2 [a]	20.4 [a]	2 [a]	85
10	RGD12-127-30	2 [a]	20.8 [a]	2 [a]	109
11	RGD12-142-49	2 [a]	21.0 [a]	2 [a]	104
12	RGD12-79-24	2 [a]	19.9 [a]	2 [a]	90
13	RGD12-95-44	2 [a]	20.1[a]	2 [a]	85
14	RGD12-127-29	2 [a]	20.1[a]	2 [a]	79
15	RGD12-154-34	2 [a]	19.3[c]	2 [a]	113
16	RGD12-95-66	2 [a]	20.5 [a]	2 [a]	96
17	RGD12-104-50	2 [a]	20.8 [a]	2 [a]	127
18	RGD12-127-26	2 [a]	21.2 [a]	2 [a]	121
19	RGD12-142-8	2 [a]	21.1 [a]	2 [a]	121
20	RGD12-154-44	2 [a]	21.5 [a]	2 [a]	109
21	RGD12-142-6	2 [a]	18.6[c]	2 [a]	109
22	RGD12-142-38	2 [a]	20.3 [a]	2 [a]	93
23	RGD12-79-41	2 [a]	21.9 [a]	2 [a]	92
24	RGD12-104-8	2 [a]	29.9 [a]	2 [a]	118
25	RGD12-127-27	2 [a]	20.3 [a]	2 [a]	123
26	RGD12-104-59	2 [a]	20.9 [a]	2 [a]	118
27	RGD12-111-8	2 [a]	20.3 [a]	2 [a]	82
28	RGD12-127-49	2 [a]	21.5 [a]	2 [a]	129
29	MK-75	2 [a]	21.2 [a]	2[a]	123
30	RG-9	0 [b]	14.0 [b]	6[b]	122
	F test		**	**	**
	Mean		20.3	2	107
	5%LSD		1.4	0.17	7

a = The variance of measurement was not significantly different from MK-75

b = The variance of measurement was not significantly different from RG-9

Table 19 Morphological features of grain of the two parents and MK-75ILs

Sr. no	Entry	Grain			Kernel		
		Seed length (mm)	Seed width (mm)	L/B	Seed length (mm)	Seed width (mm)	L/B
1	RGD12-79-11	9.51 [c]	2.46	3.86	7.08 [a]	2.14	3.31
2	RGD12-79-42	9.11[a]	2.68	3.40	6.30 [a]	2.19	2.88
3	RGD12-95-38	8.50 [a]	2.41	3.53	6.08 [a]	2.13	2.85
4	RGD12-95-39	7.77 [a]	2.52	3.08	5.99 [a]	2.19	2.74
5	RGD12-95-45	8.82[a]	2.69	3.27	6.35 [a]	2.25	2.82
6	RGD12-95-57	8.61[a]	2.58	3.34	5.98 [a]	2.22	2.70
7	RGD12-79-5	9.08 [a]	2.50	3.64	6.18 [a]	2.23	2.77
8	RGD12-95-69	8.83[a]	2.68	3.29	6.42 [a]	2.21	2.90
9	RGD12-111-19	8.18 [a]	2.54	3.22	6.24 [a]	2.21	2.82
10	RGD12-127-30	8.82[a]	2.41	3.66	6.11 [a]	2.16	2.83
11	RGD12-142-49	8.13 [a]	2.63	3.09	6.72 [a]	2.30	2.93
12	RGD12-79-24	9.15 [a]	2.49	3.67	6.36 [a]	2.24	2.85
13	RGD12-95-44	9.78 [b]	2.28	4.29	6.41 [a]	2.15	2.98
14	RGD12-127-29	8.68 [a]	2.50	3.47	6.13 [a]	2.22	2.77
15	RGD12-154-34	8.58 [a]	2.42	3.55	6.43 [a]	2.16	2.98
16	RGD12-95-66	8.72 [a]	2.67	3.28	6.83 [a]	2.36	2.89
17	RGD12-104-50	8.74 [a]	2.44	3.58	6.21 [a]	2.23	2.78
18	RGD12-127-26	8.27 [a]	2.63	3.15	6.21 [a]	2.18	2.85
19	RDG12-142-8	8.78 [a]	2.44	3.59	6.20 [a]	2.23	2.78
20	RDG12-154-44	8.43 [a]	2.58	3.27	6.30 [a]	2.26	2.79
21	RGD12-142-6	8.30 [a]	2.46	3.38	6.13 [a]	2.11	2.91
22	RGD12-142-38	8.55 [a]	2.48	3.44	6.32 [a]	2.26	2.80
23	RGD12-79-41	8.92 [a]	2.50	3.57	6.17 [a]	2.19	2.82
24	RGD12-104-8	8.84 [a]	2.55	3.47	5.94 [a]	2.21	2.69
25	RDG12-127-27	8.22 [a]	2.38	3.45	6.19 [a]	2.15	2.88
26	RGD12-104-59	9.37 [c]	2.81	3.33	6.60 [a]	2.26	2.91
27	RGD12-111-8	8.70 [a]	2.33	3.73	7.20 [a]	2.25	3.20
28	RGD12-127-49	8.51 [a]	2.48	3.43	6.47 [a]	2.18	2.96
29	MK-75	8.10[a]	2.46	3.46	6.52 [a]	2.32	2.78
30	RG-9	10.71[b]	2.64	4.05	7.47 [b]	2.18	3.42
	F test	**	ns		*	ns	
	Mean	8.8	2.52		6.4	2.12	
	5%LSD	1.12	0.24		0.75	0.382	

a = The variance of measurement was not significantly different from MK-75

b = The variance of measurement was not significantly different from RG-9

c = The variance of measurement was significantly different from MK-75 and RG-9

Discussion

Part I: Identification of genes conferring seedling resistance to bacterial blight from KDML105 introgression lines, for a non-aged-related broad spectrum resistance

One of the principal accomplishments of this study is the discovery of broad-spectrum BB resistance functioning at the seedling stage. In rainfed lowland ecosystems, rice plants are generally very susceptible to BB in the early phase but less susceptible in the late phase. In many plant-pathogen interactions, the expression of resistance depends on the developmental stage at which the plant is infected (Ou, 1985). The expression of *Xa21* is developmentally controlled. The resistance of the plant to *Xa21* increases progressively from the susceptible juvenile two-leaf stages through later stages, with 100% resistance at the adult leaf 9/10 stage (Century *et al*., 1999). In the present study, variations in lesion length (LL) at the seedling stage (30 days old) were observed in the KBILs after the challenges with 19 *Xoo* Thai isolates. Because *Xa21* is not fully expressed in the seedling stage, these observed variations in LL resulted from the segregation of other BB resistance genes present in the KBILs. The present study identified three genomic regions (*qBB1*, *qBB8.2* and *qBB11*) conferring a non-race-specific resistance at the seedling stage. The basis for this conclusion is that most of the KBILs carried these three genomic regions derived from the same BC family (104-4-1-19) and that IR1188 alleles at the three genomic regions reduced the LL against each of the Thai *Xoo* isolates. It is possible that these findings may result from epistatic interaction, may represent a pleiotropic effect of genes conferring resistance to a broad spectrum of *Xoo* isolates or may reflect linkages of race-specific genes in the coupling phase. To answer these questions about a single gene conferring broad-spectrum resistance or a cluster of resistance genes and about the way in which these genes function together (epistasis) in the KBILs, near-isogenic lines carrying small segments of the donor (IR1188) of these three regions need to be developed and challenged with the Thai *Xoo* isolates. However, the linkage of the resistant loci with the flanking molecular markers identified in this study should also be very useful for transferring these resistance genes in rice breeding programs.

The loci *qBB*1, *qBB8.2* and *qBB11* can be considered as new QTLs for broad-spectrum BB resistance. Locus *qBB1* was mapped to the long arm of chromosome 1, between the RM302 and RM212 markers. *Xa29(t)*, identified from the wild rice *Oryza officinalis*, is mapped between the RFLP markers C904 and R596 on chromosome 1 (Tan *et al*., 2004), but its location is distal to that of *qBB1*. Locus qBB8.2 was mapped to the long arm of chromosome 8, between the RM210 and RM149 markers. Its location is far from that of the broad-spectrum BB resistance gene *xa13* (Ogawa *et al*., 1987; Zhang *et al*., 1996; Sanchez *et al*., 1999). On chromosome 11, *Xa3*, *Xa4*, *Xa6*, *Xa9*, *Xa22* and *Xa26*, situated within a 230-kb region, are reported to belong to the BB resistance gene *Xa21* family (Song *et al*., 1997; Wang *et al*., 1998). Locus *qBB11*, flanked by markers RM287 and RM224, was found to overlap with the BB resistance gene *Xa21* family. Markers RM287 and RM224 are 676 kb and 130 kb distant, respectively, from *Xa21*. In this study, the *qBB1*1 locus, located in this region, showed resistance to BB at the seedling stage. No conclusive evidence can be drawn about the genetic relationship between *qBB11* and the reported BB genes in this study. It is possible that *qBB11* is a new gene or that it is the same locus as the previously reported BB resistance genes of the *Xa21* family. Further study needs to be conducted by developing an isogenic line that can be used to conduct an allelism test with the known resistance genes.

Xa21 is the most widely used gene in rice breeding programs because of its broad-spectrum resistance to most isolates of *Xoo*. Varieties IR1188 and IRBB21 are near-isogenic lines of IR24 for the bacterial blight resistance gene *Xa21* (Ronald *et al*., 1992; Ikeda *et al*., 1990). IR1188 carries *Xa21* and shows non-age-related broad-spectrum resistance to bacterial blight. IRBB21 is widely used for the improvement of bacterial blight resistance in Asia. It confers resistance to all Indian and Philippine races of *Xoo* tested (Ikeda *et al*., 1990; Khush *et al*., 1991). Singh *et al*. (2001) reported that the *Xa21* gene showed resistance against 17 *Xoo* isolates from Punjab. In China, *Xa21* has been introduced into the widely used restorer line Minghui 63, and the introgression lines showed a resistant reaction to Chinese isolates. *Xa21* is sufficient to confer resistance to all 7 Philippine races of *Xoo* and 27 isolates from China, Colombia, India, Indonesia, Korea, Malaysia, Nepal and Thailand (Wang *et*

al., 1996). Because IR1188 and IRBB21 are both near-isogenic lines of IR24 (Khush *et al*., 1991 Ronald *et al*., 1992; Chungwongse *et al*., 1993; Swamy *et al*., 2006), IRBB21 may show the non-age-related broad-spectrum resistance identified in IR1188. IRBB21 is broadly distributed in many countries in Asia because it is a donor of the *Xa21* gene. If such resistance is present, breeding lines derived from IRBB21 may also show this non-age-related broad-spectrum resistance. Currently, *Xa21* is the most important gene used against bacterial blight isolates in Myanmar. IR1188 was used as the donor to introgress the *Xa21* gene and non-age-related broad-spectrum resistance to the Myanmar rice cultivar Manawthukha and to other rice varieties in the countries of the Mekong region.

The development of non-age-related broad-spectrum-resistant KDML105 can be achieved by combining MAS with phenotypic screening. This process is a successful example of an integrated approach to plant breeding. In this study, *Xa21* was precisely selected by MAS, and additional, unidentified QTLs controlling broad-spectrum BB resistance at the seedling stage can be selected by phenotypic screening. In a rice breeding program, however, resistance genes expressed at all stages of rice growth are more desirable than resistance genes expressed at one particular stage. Accumulating major genes for resistance in an elite genotype solely through conventional breeding is laborious and time-consuming, especially if one or more of the genes are fully effective against all known isolates of the pathogen. It is very difficult to obtain plants with broad-spectrum resistance (BSR) based on phenotypic selection alone, as the action of one resistance gene may mask the action of another. Our results demonstrated the advantage of the combination of MAS with phenotypic screening to maximize genetic gain (non-age-related broad-spectrum resistance**)**. Resistance genes against bacterial blight, the green leafhopper and the brown planthopper can be identified based on recombinant inbred lines derived from the cross (Kinmaze × DV85) through MAS with phenotypic selection (Wang *et al*., 2005; Yasui and Yoshimura *et al*., 1999; Wang *et al*., 2004; Su *et al*., 2005). DNA markers associated with BSR genes could be used to increase selection efficiency through marker-assisted selection (MAS).

The selected KBI lines with non-age-related broad-spectrum resistance (carrying *Xa21* and the QTLs for seedling resistance) exhibited agronomic characters and physical and chemical grain quality (such as DF, PH, TSN, GY, aroma, AC, GT, GC, and SL and SS) similar to those of the original KDML105. Our current results (based on multi-location trials in a rainfed environment) showed clearly that the selected KBILs had agronomic characteristics similar to the original KDML105 and were well adapted to rainfed lowlands in Thailand. The analysis of aroma indicated that certain lines showed a mild aroma in certain locations. This outcome could be attributed to post-harvest handling (Wongpornchai *et al*., 2004; Yoshihashi *et al*., 2004, 2005) and temperature in the grain filling period (Itani, *et al*., 2004; Rohilla *et al*., 2000). However, in general, aroma was shown by all KBI lines. The chemical grain qualities of the KBILs were similar to those of the original KDML105. None of the physical grain qualities were found to differ from those of KDML105. Therefore, all of the selected KBILs were found to meet the KDML105 grain quality standards. From the breeders' perspective, KDML105 with *Xa21* will be subject to substantial losses if an epidemic occurs early in the growing season. Therefore, KBILs with *Xa21* and the QTLs for seedling resistance are suitable for recommendation for culture in rainfed lowlands in Thailand because of their wider spectrum of resistance, which includes different plant stages (both seedlings and adult plants). Certain KBILs, such as RGD9904-104-4-1-19-14, can be used as a new source of BB resistance in the breeding program because of the ease of QTL manipulation (co-location of resistant genes) through MAS (Wisser *et al*., 2005).

Part II: Marker assisted Introgression of multiple genes for bacterial blight resistance in Aromatic Manawthukha rice line, MK-75

Improvement for broad-spectrum BB resistance in the MK-75 can be achieved through marker assisted backcrossing (MAB). In this study, we successfully transferred three BB resistance genes including *xa5*, *Xa21* and *xa33* to susceptible elite line MK-75 through marker assisted backcrossing (MAB) within three years. Evaluation of BB resistance at seedling and maximum tillering stages against both Thai and Myanmar *Xoo* isolates indicated that MK-75 ILs had significantly improved

BB resistance to broad spectrum of *Xoo* compared with the original MK-75 which was very susceptible. This result is similar to previous reports on the successful utilization of MAB to transfer BB resistance genes into several elite rice varieties (Huang *et al*., 1997; Singh *et al*., 2001; Gopalakrishnan *et al*., 2008; Basavaraj *et al*., 2010; Bharam *et al*., 2010; Shanti *et al*., 2010; Rajpurohit *et al*., 2011; Deng *et al*., 2012; Suh *et al*., 2013). In the conventional breeding program, introgression of multiple genes with different gene actions (ex. dominant, recessive, growth-stage dependent) into an elite line is laborious and time-consuming, especially when one or more of the genes are fully effective against all known isolates of the pathogen (Singh *et al*., 2001). It was very difficult to obtain plants with all genes combined based on phenotypic selection alone as the action of one resistance gene may mask the action of another (Rao *et al*., 2002; Suh *et al*., 2013). In the present study, the feasibility of MAB clearly overcome those obstacles by the conventional breeding and demonstrated that MAB is generally an effective strategy for genes or QTL pyramiding.

The resistance conferred by most *Xa* genes has race specificity, with effectiveness only towards the *Xoo* race(s) that express a corresponding avr gene. Therefore, pyramiding *Xa* genes with different race specificity will lead to a broad spectrum resistance (Yoshimura *et al*., 1995; Singh *et al*., 2001; Deng *et al*., 2012). In this study, our results showed that the MK-75 ILs with more genes introgressed showed higher level of BB resistance to a broader spectrum of *Xoo* isolates (both Thai and Myanmar *Xoo* isolates) than those with lesser number of genes introgressed. For example, MK-75 ILs containing three *Xa* genes (*xa5+Xa21+xa33*) exhibited higher level of BB resistance (shorter LL) than the MK-75 ILs containing two *Xa* genes (*xa5+Xa21, xa5+xa33 and Xa21+xa33*). While MK-75 ILs containing two *Xa* genes exhibited higher level of BB resistance (shorter LL) than the MK-75 ILs containing single *Xa* gene (*xa5, Xa21 and xa33*) upon inoculation with ten Thai *Xoo* isolates at both seedling and maximum tillering stages. Although we observed that the MK-75 ILs with *xa5+xa33* genotype showed moderately susceptible reaction to two isolates, NPL3-3 and SK2-3 at seedling and maximum tillering stages, respectively but their LLs were shorter than that of MK-75 ILs carrying either *xa5* or *xa33* gene alone.

These results indicated that rice lines with higher number of *Xa* genes tend to display higher levels and/or wider spectra of resistance to BB than rice lines with single *Xa* gene. Similar result was reported by many authors. The combinations of *xa5*, *xa13* and *Xa21* provided a wider spectrum of resistance to the India isolates than the line carrying only one gene (Singh *et al*., 2001). Zhang *et al*. (2006) reported that the pyramid lines with both *Xa21* and *Xa7* genes were more resistant than other genotypes that have only one gene against China isolates. Suh *et al*. (2013) reported that pyramiding the resistance genes *Xa4*, *xa5* and *Xa21* provided a higher resistance to Korean isolates. All these evidences suggested that the gene interaction or quantitative complementation between the resistant genes might play the key role in increasing the level of resistance against broader spectrum of *Xoo* isolates (Yoshimura *et al*., 1995; Huang *et al*., 1997; Gopalakrishnan *et al*., 2008).

Our results showed that *xa5* was more effective against Thai *Xoo* isolates while *Xa21* was more effective against Myanmar *Xoo* isolates. At the seedling stage, the MK-75 ILs carrying the *xa5* conferred resistance to eight Thai *Xoo* isolates while the MK-75 ILs carrying the *Xa21* and *xa33* conferred moderate resistance to seven and six Thai *Xoo* isolates, respectively. When the MK-75 ILs were tested against five Myanmar *Xoo* isolates, the MK-75 ILs carrying the *Xa21* conferred moderate resistance to all *Xoo* isolates while the MK-75 ILs carrying the *xa5* were susceptible to Race I and II and the MK-75 ILs carrying the *xa33* were susceptible to all Myanmar *Xoo* isolates. This evidence suggested the genetic difference of avr genes of the *Xoo* races between Thailand and Myanmar. The wide distribution of the corresponding avr genes among *Xoo* races is suggested to be the reason for the broad host range of *Xa* genes. Geographical barriers, the similarity of rice varieties grown on a national basis, seed allocation and germplasm exchanged have been reported as major factors determining the genetic diversity of the *Xoo* population within a country or region (Adhikari *et al*., 1995; Kosawang *et al*., 2006; Chen *et al*., 2012). Therefore combining *xa5* and *Xa21* into a single genotype such as MK-75 will broaden the resistance spectra and may contribute to a longer life span of a new improved variety for the Mekong region in the future.

The epistatic effects between R genes revealed a complex genetic network (Li *et al*., 2006). The interactions between alleles at the rice R loci and alleles at the corresponding avirulence loci in *Xoo* lead to higher level of resistance. In this study, the MK-75 ILs with the *Xa21* and combinations such as *xa5+Xa21* and *Xa21+xa33* presented higher level of resistance than that without the *Xa21* within the combination (*xa5+xa33*) against the virulence *Xoo* isolate SK2-3. It was examined by Li *et al*. (2001) that dominant and recessive disease resistance genes against bacterial blight expressed different levels of resistance and that the residual effect of dominant genes is greater than the recessive genes. It suggested different mechanisms in expressing defense pathways of dominant and recessive genes and therefore the interaction of recessive and dominant genes in a pyramided manner may show more durable and higher level of resistance compared with single gene resistance. The *Xa21* encoded leucine-rich repeat (LRR) receptor kinase (Song *et al*., 1995; Sun *et al*., 2004) is inducibly expressed by *Xoo* (Yoshimura *et al*., 1998; Iyer and McCouch, 2004; Gu *et al*., 2005; Cao *et al*., 2007; Park *et al*., 2010). The broad host range of *Xa21* has been recently explained due to a PRR–PAMP interaction (Lee *et al*., 2009) and Ax21 (product of the *Xa21*) encodes a 198-amino acid protein that is highly conserved in all sequenced *Xanthomonas* species (Lee *et al*., 2009). The Ax21 functions as a quorum-sensing molecule to control genes involved in *Xoo* pathogenicity (Lee *et al*., 2006; Park *et al*., 2010; Han *et al*., 2011). Since the quorum sensing is a process in which bacterial molecules serve as signals to recognize bacterial population size, leading to changes in expression of a set of genes (Bassler and Losick, 2006). We hypothesized that the Ax21 may lead to better function of the *xa5* and *xa33*. However, more study need to be conducted to understand how *Xa21* interacts with other *Xa* gene remains.

Fragrance and amylose content are two of the most important determinants of rice quality. The selected triple, double and single resistance gene-introgressed lines of MK-75 exhibited similar physical and chemical grain quality (such as fragrance, AC, GT, GC, and seed length and width, kernel length and width) to the original MK-75. In case of fragrance, all MK-75 ILs are fragrant but small difference of score was observed. This outcome could be attributed to temperature in the grain filling period (Rohilla *et al*., 2000; Itani *et al*., 2004). The variation of score may be caused by

some minor genes or environmental factors. In case of amylose content, the MK-75 ILs had similar to the original MK-75, with intermediate amylose content. GT is a physical characteristic responsible for cooking time and the capacity of absorbing water as cooking. In this study, all improved lines carried the same alleles of *SSIIa* and similar ASV value was observed in MK-75 ILs and MK-75. Gel consistency helps in the determination of the texture of cooked rice. The two parental lines have high GC values (122 to 123 mm), indicating that their cooked rice is more tender and sticky. The GC value of MK-75 ILs ranged from 77mm to 129mm. Information from previous studies revealed that the major *Wx* gene plus several minor genes and or modifier governed the expression of GC (Lanceras *et al*., 2000; Tain *et al*., 2005). In the present study, the variation of GC might be controlled by two or more minor genes and their interaction made transgressive segregants in MK-75 ILs. In summary, fragrance was shown by all MK-75 ILs introgression lines and the chemical grain qualities of the MK-75 ILs were similar to those of the original MK-75. None of the physical grain qualities were found to differ from those of MK-75. Therefore, all of the selected MK-75 ILs were found to meet the Basamati grain quality standards. From the breeders' perspective, MK-75 with different gene combinations will be subjected to substantial losses if an epidemic occurs in the growing season. Therefore, the triple and double resistance gene-introgressed lines (*xa5Xa21xa33*, *xa5Xa21* and *Xa21xa33)* of MK-75 will have a wide range of benefits in rice breeding program. These lines are suitable for planting in rainfed lowlands in Thailand and Myanmar because of their wider spectrum of resistance, which includes different plant stages (both seedlings and adult plants).

CONCLUSION

The bacterial blight is one of the most destructive of rice in the world. The most effective approach is the use of the resistant varieties. Large-scale and long-term cultivation of varieties with single gene may enable the pathogen to overcome BB resistance. Therefore, identification of excellent genetic resources and resistance genes is need for an improvement of BB resistance in rice breeding program. Accumulating major genes for resistance in an elite genotype by only conventional breeding is laborious and time-consuming, especially when one or more of the genes are fully effective against all known isolates of the pathogen. It was very difficult to obtain plants with a broad spectrum resistance (BSR) based on a phenotypic selection alone as the action of one resistance gene may mask the action of another. Our results show that KBILs with *Xa21* and QTLs for seedling resistance were successfully developed through MAS over three generations. These KBILs are fragrant, have bacterial blight resistance and show broad adaptability to the target environments. The improved KDML exhibits BSR against a diverse range of bacterial blight isolates. The KBILs developed in this study will have an impact on the yield stability of KDML105 in the rainfed lowland areas of Thailand and will serve as breeding stock for the further improvement of bacterial blight resistance in Thai rice breeding programs.

The goal of our result is to develop rice variety combining high-yield, good-quality and multiple-resistance. With increasing number of cloned resistance genes and mapping of useful genes for rice genetic transformation, multiple gene pyramiding is a wise and efficient strategy in rice resistance breeding. Twenty eight aromatic Manawthukha introgression lines (MK-75 ILs) with bacterial blight resistance genes (triple, double and single resistance gene) were successfully developed using MAS in three generations. MK-75 ILs carrying three genes and two genes combination exhibited higher level of resistance to all tested Thai strains at both seedling and tillering stages and Myanmar strains at seedling stage. Thus the improvement of bacterial blight resistance in MK-75 with fragrance and intermediate AC quality may have a wide range of benefits in breeding program. Physical grain

quality of improved lines were similar with MK-75. The improved lines can be used as genetic resources for the improvement of bacterial blight resistance in rice breeding program.

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APPENDIX

Appendix Table 1 Lesion Length (cm) of the parents and MK-75ILs against 10 Thai isolates at the seedling stage

Sr.no	Line	TXO85	TXO150	TXO155	BR1-2	CRL1-3
1	RGD12-79-11	0.3	0.6	0.2	0.9	0.9
2	RGD12-79-42	0.4	0.3	0.3	0.5	0.8
3	RGD12-95-38	0.6	0.7	0.2	0.5	0.6
4	RGD12-95-39	0.6	0.5	0.2	0.4	0.7
5	RGD12-95-45	0.3	0.2	0.2	0.2	0.7
6	RGD12-95-57	0.6	0.4	0.2	0.7	0.8
7	RGD12-79-5	0.5	1.0	0.6	1.8	1.7
8	RGD12-95-69	0.5	0.5	0.3	1.2	2.2
9	RGD12-111-19	0.6	0.8	0.5	1.8	1.4
10	RGD12-127-30	0.4	0.3	0.8	1.4	1.3
11	RGD12-142-49	0.3	0.6	0.5	1.3	1.3
12	RGD12-79-24	1.0	2.0	2.1	1.3	1.2
13	RGD12-95-44	1.3	2.6	0.9	0.9	0.9
14	RGD12-127-29	1.3	2.6	1.9	1.5	1.2
15	RGD12-154-34	1.1	2.1	1.8	1.8	1.0
16	RGD12-95-66	0.7	1.3	0.5	1.5	1.2
17	RGD12-104-50	0.7	0.7	0.3	1.3	1.5
18	RGD12-127-26	0.5	0.8	0.5	1.4	1.6
19	RGD12-142-8	1.1	1.2	1.5	1.5	1.8
20	RGD12-154-44	0.5	1.2	0.7	1.2	1.6
21	RGD12-142-6	2.4	4.4	4.5	4.8	2.7
22	RGD12-142-38	2.5	4.6	4.5	6.1	2.2
23	RGD12-79-41	2.6	9.3	6.7	6.0	4.6
24	RGD12-104-8	2.0	8.6	8.0	4.8	4.5
25	RGD12-127-27	2.0	8.7	7.7	4.4	2.9
26	RGD12-104-59	1.9	3.8	9.1	6.1	11.6
27	RGD12-111-8	1.4	4.1	9.9	5.9	11.4
28	RGD12-127-49	2.1	5.2	9.8	6.8	12.9
29	MK-75	13.2	14.6	17.3	15.0	15.9
30	RG-9	0.2	0.6	0.2	0.6	0.7
31	KDML105	21.6	18.4	26.7	24.8	24.0
32	Manawthukha	14.3	14.8	15.3	16.2	19.5
	F test	**	**	**	**	**
	Mean	2.5	3.7	4.2	3.9	4.3
	5%LSD	0.9	1.1	1.0	1.0	1.6

Appendix Table 1 (continued)

Sr.no	Line	NB7-7	NPL3-3	NW3-1	PSL	SK2-3
1	RGD12-79-11	0.8	2.2	0.9	0.2	2.5
2	RGD12-79-42	0.6	1.2	1.0	0.2	2.3
3	RGD12-95-38	0.2	1.4	0.5	0.2	2.2
4	RGD12-95-39	0.3	1.0	1.1	0.2	2.6
5	RGD12-95-45	0.5	1.0	0.9	0.1	2.3
6	RGD12-95-57	0.8	1.1	1.6	0.2	2.2
7	RGD12-79-5	0.9	1.2	1.4	0.2	1.8
8	RGD12-95-69	0.7	1.5	1.1	0.2	2.1
9	RGD12-111-19	1.0	1.4	0.6	0.3	2.4
10	RGD12-127-30	1.0	1.0	0.8	0.2	1.6
11	RGD12-142-49	1.6	1.4	2.3	0.2	0.9
12	RGD12-79-24	1.3	1.8	1.9	1.0	1.8
13	RGD12-95-44	0.7	1.3	2.5	0.6	3.3
14	RGD12-127-29	1.3	2.1	2.7	1.1	2.4
15	RGD12-154-34	1.5	1.8	2.0	0.9	1.2
16	RGD12-95-66	1.2	4.0	1.3	0.2	7.3
17	RGD12-104-50	1.1	4.2	1.4	0.2	7.7
18	RGD12-127-26	0.6	6.0	1.1	0.4	8.5
19	RGD12-142-8	2.7	6.1	1.1	1.1	9.8
20	RGD12-154-44	0.8	5.8	1.2	0.4	8.6
21	RGD12-142-6	1.8	7.0	4.4	2.1	9.7
22	RGD12-142-38	2.5	6.3	4.1	2.0	10.1
23	RGD12-79-41	5.1	3.4	5.4	1.8	6.4
24	RGD12-104-8	4.8	3.4	4.5	1.5	8.0
25	RGD12-127-27	4.6	3.2	3.9	1.5	7.2
26	RGD12-104-59	5.7	17.8	4.8	0.8	14.1
27	RGD12-111-8	5.7	15.5	5.9	1.7	10.0
28	RGD12-127-49	6.2	14.5	4.9	1.3	11.4
29	MK-75	18.0	20.1	15.9	11.7	13.4
30	RG-9	0.4	0.8	0.6	0.1	1.1
31	KDML105	16.5	21.2	21.6	22.3	20.0
32	Manawthukha	15.8	18.2	12.4	10.6	13.7
	Ftest	**	**	**	**	**
	Mean	3.3	5.7	3.6	2.0	6.2
	5%LSD	0.9	1.2	1.0	0.3	1.1

Appendix Table 2 Lesion Length(cm) of the parents and MK-75ILs against 10 Thai isolates at the tillering stage

Sr.no	Line	TXO85	TXO150	TXO155	BR1-2	CRL1-3
1	RGD12-79-11	0.2	0.2	0.6	0.5	1.2
2	RGD12-79-42	0.5	0.3	0.5	0.3	0.6
3	RGD12-95-38	0.3	0.4	0.6	0.5	0.6
4	RGD12-95-39	0.2	0.3	0.3	0.4	0.8
5	RGD12-95-45	0.2	0.2	0.4	0.2	0.9
6	RGD12-95-57	0.1	0.3	0.6	0.7	1.2
7	RGD12-79-5	0.1	0.4	1.0	0.5	2.1
8	RGD12-95-69	0.2	0.4	0.7	0.3	2.1
9	RGD12-111-19	0.7	0.4	1.1	0.4	1.4
10	RGD12-127-30	0.1	0.3	0.4	0.5	1.3
11	RGD12-142-49	0.4	0.3	1.6	0.2	1.2
12	RGD12-79-24	0.9	1.6	2.0	1.8	1.6
13	RGD12-95-44	0.3	1.4	1.2	1.5	1.4
14	RGD12-127-29	0.2	1.3	1.5	1.9	1.4
15	RGD12-154-34	0.5	0.9	1.6	1.6	0.9
16	RGD12-95-66	0.1	0.4	0.8	0.4	2.1
17	RGD12-104-50	0.1	0.3	0.7	0.6	1.8
18	RGD12-127-26	0.1	0.5	0.6	0.9	1.9
19	RGD12-142-8	0.2	0.5	1.5	0.7	2.5
20	RGD12-154-44	0.7	0.3	0.7	0.7	1.8
21	RGD12-142-6	2.2	2.5	3.0	5.5	2.7
22	RGD12-142-38	2.6	2.1	3.1	4.9	2.7
23	RGD12-79-41	0.9	5.6	3.8	5.7	4.4
24	RGD12-104-8	0.1	5.4	2.2	6.0	4.8
25	RGD12-127-27	0.9	4.3	3.2	5.2	4.1
26	RGD12-104-59	0.1	0.8	3.4	1.2	15.0
27	RGD12-111-8	0.2	0.7	3.5	1.7	12.7
28	RGD12-127-49	1.5	1.0	3.4	1.3	16.0
29	MK-75	19.1	12.8	14.6	14.8	21.0
30	RG-9	0.5	0.2	0.7	0.3	0.5
31	KDML105	15.8	16.6	15.8	25.5	16.5
32	Manawthukha	15.8	16.0	18.2	15.3	20.7
	Ftest	**	**	**	**	**
	Mean	2.0	2.5	2.9	3.2	4.7
	5%LSD	1.2	1.2	0.8	1.0	1.4

Appendix Table 2 (continued)

Sr.no	Line	NB7-7	NPL3-3	NW3-1	PSL	SK2-3
1	RGD12-79-11	1.0	1.0	0.6	0.4	2.4
2	RGD12-79-42	0.5	1.3	0.3	0.2	1.9
3	RGD12-95-38	0.9	0.8	0.2	0.2	1.9
4	RGD12-95-39	0.8	0.8	0.2	0.3	1.8
5	RGD12-95-45	0.7	0.7	0.2	0.2	1.8
6	RGD12-95-57	1.2	0.8	0.6	0.3	1.9
7	RGD12-79-5	1.3	1.1	0.8	0.5	1.5
8	RGD12-95-69	1.8	1.3	0.8	0.5	2.3
9	RGD12-111-19	1.7	1.4	1.0	0.6	2.2
10	RGD12-127-30	1.4	0.8	0.7	0.7	1.7
11	RGD12-142-49	1.2	1.3	2.0	0.6	1.4
12	RGD12-79-24	2.2	1.5	2.0	1.0	2.3
13	RGD12-95-44	1.3	1.0	0.6	0.6	2.2
14	RGD12-127-29	1.3	1.5	1.4	0.8	1.9
15	RGD12-154-34	1.6	1.5	1.6	1.0	1.9
16	RGD12-95-66	1.3	2.8	0.9	0.3	7.5
17	RGD12-104-50	1.2	2.0	0.9	0.7	7.1
18	RGD12-127-26	1.3	2.0	0.8	0.5	7.3
19	RGD12-142-8	2.3	3.0	1.9	0.8	7.2
20	RGD12-154-44	1.9	3.1	0.3	0.2	8.1
21	RGD12-142-6	2.7	3.4	2.9	2.3	9.9
22	RGD12-142-38	3.0	4.3	2.4	2.4	9.4
23	RGD12-79-41	5.7	1.9	6.1	2.6	6.9
24	RGD12-104-8	5.8	1.9	4.9	2.5	7.1
25	RGD12-127-27	4.8	1.6	3.5	3.0	6.8
26	RGD12-104-59	5.9	10.9	4.6	1.5	12.7
27	RGD12-111-8	4.7	9.9	4.5	2.6	9.6
28	RGD12-127-49	6.8	13.8	4.7	2.6	10.3
29	MK-75	20.0	16.9	10.8	16.7	16.3
30	RG-9	0.7	0.8	0.1	0.4	2.0
31	KDML105	20.1	12.2	15.8	21.5	18.2
32	Manawthukha	10.0	16.0	10.0	14.9	15.0
	Ftest	**	**	**	**	**
	Mean	3.6	3.8	2.7	2.6	5.9
	5%LSD	1.5	0.9	1.0	1.4	1.6

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