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THESIS

QTL MAPPING FOR LEAF AND NECK BLAST RESISTANCE IN KHAO DAWK MALI105 AND JAO HOM NIN RECOMBINANT INBRED LINES

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Mapping of QTLs for resistance to leaf and neck blast disease of rice was the goal of this study. Khao Dawk Mali105 (KDML105) and Jao Hom Nin (JHN) were chosen to be the parents expecting to inherit their main good qualities to progenies i.e. good cooking quality for the former and blast resistance for the latter. The parents were primarily screened for broad resistance spectrum (BRS) of leaf and neck blast using 82 and 95 isolates collected from all over Thailand. BRS values for JHN confirmed its resistance quality as they were 0.99 and 1 for leaf and neck blast screenings, respectively. The values for KDML105 were only half and one-third of those recorded for JHN for corresponding screenings. For the assessment of phenotypic distribution for leaf and neck blast reaction, 587 recombinant inbred lines derived from the cross between KDML105 and JHN were inoculated with three selected blast isolates designated as THL191, THL318 and THL899. Chi-square (x^2) test had fitted a ratio of approximately 3: 1 reflecting the presence of 2 QTL for 4 out of 6 sets of data. Correlation coefficient between leaf and neck blast were low (0.28-0.56) suggesting that it might be a presence of two different pathosystems. Through Quantitative Trait Loci analysis, fourteen QTL were found on three chromosomes; 1, 11 and 12. The QTL on chromosome 11 were detected as major QTL against leaf and neck blast for all three isolates. High LOD values for both leaf and neck blast resistance might indicate the presence of major genes clustering on chromosome11. Three major genes *Pi 7(t), Pi 1* and *Pi lm*^{2} have been reportedly located on chromosome11 within the vicinity of the detected QTL in which the QTL peaks located close to RM224 marker and within the AC113249- RM139 interval. The QTL on chromosomes 1 and 12 showed race-specificity for leaf and neck blast. The peak of coincident QTL on chromosome1 was located very close to RM212 marker. The peak for QTL on chromosome12 was located between RM179 and RM309 markers. Four major genes being *Pi ta, Pi 4a(t), Pi 4b(t)* and *Pi 4(t)* were also reported as a cluster of blast resistance genes. These genes are located in the same marker interval as the detected QTL. Interactions were statistically significant and behaved additively for resistance. Clusters of QTL located on three chromosomes revealed the presence of broad spectrum resistance in JHN. Three QTL being detected by QTL analysis in comparison with 2 QTL obtained from the chi-square test on phenotypic reactions suggested that the RILs population used was reasonably large so that even a small QTL could be detected. The coincidence locations of leaf and neck blast resistance QTL on chromosomes 1 and 11 and associations of molecular markers with QTL will be a useful information for marker assisted selection.

Apichart Noenplab — Student's signature

Thesis Advisor's signature — 6 / 3 / 49

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QTL MAPPING FOR LEAF AND NECK BLAST RESISTANCE IN KHAO DAWK MALI105 AND JAO HOM NIN RECOMBINANT INBRED LINES

INTRODUCTION

Rice blast (*Pyricularia grisea* Sacc.) is one of the major rice diseases for rice growing areas all over the world. Panicle blast or neck blast could severely reduce yield (Ou, 1985) with low 1,000 grain weight, percentage of fully matured grains (Goto, 1965) and grain quality while increasing chalky kernels (Katsube and Koshimizu, 1970). The earlier the time of panicle infection, the greater the yield loss (Goto, 1965; Ou, 1985). Several reduction on grain yield caused by neck blast had been reported in Thailand thirteen years ago. Rice blast epidemics occurred in most provinces up north and some in the northeast causing the reduction on rice production of 650,000 tones leading to a loss of approximately 3,000 million baht in 1992 (Disthaporn, 1994). This is certainly a threat for farmers particularly those grown quality varieties such as Khao Dawk Mali 105 (KDML105) or RD6 which are highly susceptible to blast. Therefore, blast has become more and more significant for both national and international trades particularly when trading at the international level is rather tense nowadays. This blast problem is such a challenge for researchers with the fact that the breaking down of resistance do occur sometimes after the release of the variety. Breaking down of resistance to blast is known to be related to the diversity of the causal pathogen. A group of researchers in Thailand has reported up to 51 lineages for 653 isolates collected from two growing seasons in the north, northeast and central part of Thailand (Mekwatanakarn *et al*., 1997). The quantity of lineages found in Thailand was far greater than those reported anywhere else except China ofwhich 54 lineages have been characterized from 473 isolates (Shen *et al*., 1997). The same group of researchers in Thailand also found that the north and northeast regions have 15 lineages in common suggesting the potential use for shared information on resistant genes for both regions. In addition, they reported that three resistant genes; *Pi 1, Pi z-5* and *Pi ta*^{2} were incompatible with the common isolates found regularly indicating the possibility to use these genes in the breeding program. Results from three experiments carried out in Thailand by Na Lampang (2001) testing 16 NILs having 16 blast resistant genes against blast isolates collected from 3 rice eco-systems showed similar results to those of the previous findings mentioned above. In the first experiment, testing 39 selected blast isolates from the Chiangmai valley and Lampang, the upper north provinces, against 16 NILs revealed that *Pi ta* was the most effective resistant gene followed by *Pi k*^{p} and *Pi ta*^{2}. When inoculation was undertaken in the second experiment with 10 selected isolates from upland conditions in the upper north, 8 ideal resistant genes reported were *Pi ta, Pi ta*^{2}, *Pi 1(t)*^{ttp}, *Pi 4*^{a}*(t)*^{ttp}, *Pi k*^{m}, *Pi b, Pi k*^{p} and *Pi k*. Considering geographical difference, results from the third experiment using 37 selected isolates from the lowland areas in the upper and lower north provinces showed that *Pi 1* was incompatible to 35 isolates tested. Hence, the resistant gene(s) should be carefully chosen to be used depending upon the target areas otherwise the problem of breaking down of resistance will soon reappear.

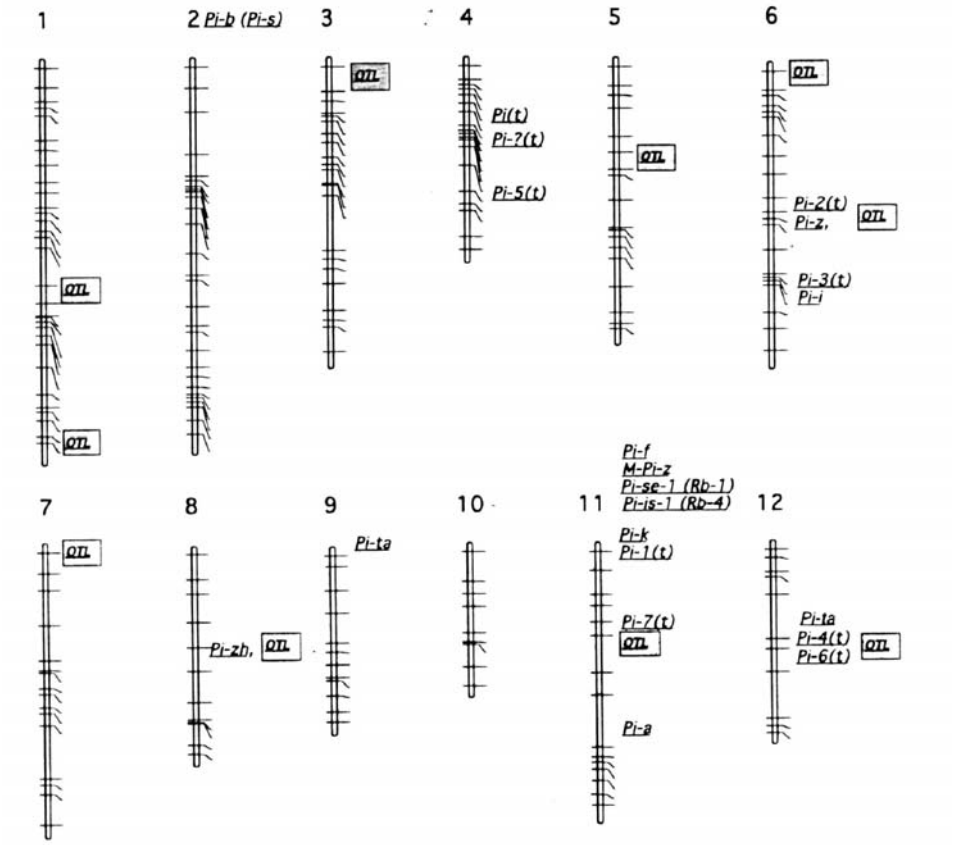


Figure 1 Map showing locations of known blast resistance genes along the 12 chromosomes of rice. Where no linkage information is available, genes are listed above the chromosomes onwhich they are located. (McCouch *et al*., 1994)

At least 30 blast resistance loci have been identified in *Oryza sativa* L. (Kinoshita, 1991). Of these, 20 are major genes and 10 are putative QTLs. Twelve of the major genes have been confirmed to be non-allelic. For example, Eight loci have been reported on chromosome 11 i.e., *Pi-f, M-Pi-z, Pi-se-1, Pi-is-1, Pi-k, Pi-1(t), Pi-7(t) and Pi-a*. Four loci have been reported on chromosome 6 being identified as *Pi-2(t), Pi-z, Pi-3(t)* and *Pi-i* (McCough *et al*., 1994)(Figure 1).

Molecular marker technology has been widely used nowadays. It has been applied for the identification and mapping of genes conferring both complete and partial resistance and has provided insight into the genetic basis of durable resistance (Wang *et al*., 1994). Many major genes for blast resistance have been identified using this method. Resistant rice varieties, especially when resistance is based on single major genes, may be rapidly overcome by compatible races of the pathogen (Kiyosawa, 1982). Gene pyramiding is one way to improve disease resistance in rice. Before this can happen, gene mapping and tagging should be carefully undertaken.

OBJECTIVE

To locate leaf blast (LB) and neck blast (NB) resistance QTLs in rice genome using the Recombinant Inbred Lines (RILs) derived from the cross between Khao Dawk Mali105 (KDML105) and Jao Hom Nin (JHN).

LITERATURE REVIEW

1. Blast : *Pyricularia grisea* Sacc.

Rice blast disease is considered to be the most important rice disease worldwidely. The causing agent of disease is the fungus called *Pyricularia grisea* Sacc. Saccardo was named as the type species of the genus in honour to his first report of *Pyricularia* on *Trichothecium griseum* grass in 1880. The other popular synonym of the fungus is *Pyricularia oryzae* named by Cavara who first found it on rice in Italy (Holliday, 1989). Apart from these two names for the genus *Pyricularia*, there were other names such as *Dactylaria grisea*, *D. oryzae* and *Pyricularia* but not as popular. Although *Pyricularia grisea* and *Pyricularia oryzae* have been originally found in two different hosts, these two species were morphologically indistinguishable and interfertile. In addition, as the fact that *Pyricularia grisea* was the first name given to this group of the fungi, it is recommended as the proper name for this group (Rossman *et al*., 1990)

Pyricularia oryzae has the teleomorph called *Magnaporthe grisea* (Hebert) Barr. The perfect stage of the fungus was first discovered and described as *Ceratophaeria grisea* by Hebert (1971) which had been changed later to the genus *Magnaporthe* by Barr (1977). As the teleomorph was rarely found in the nature but the asexual stage was known, Rossman *et al*. (1990) stated that it was acceptable to call this fungus as either *Pyricularia grisea* Sacc. or *Magnaporthe grisea* (Hebert) Barr.

2. Infection and Symptoms

Rice blast is destructiveness under favorable conditions (Ou, 1985). Rice is most susceptible to blast disease at seedling, tillering and heading stages (Anderson *et al*., 1947). Rice blast disease can cause damage to several parts of rice; leaf, node, internode, leaf sheath and panicles (Ou, 1985). The principle of rice blast pathosystem is derived into leaf and panicle blast pathosystems (Teng *et al*., 1991). Infection by *Magnaporthe grisea* is initiated when a conidium lands on a leaf surface. The hydrated conidium attaches firmly by releasing mucilage from the apex thereby preventing dislodgement by wind and rain (Hamer *et al*., 1988). One or more germ tubes may emerge from any of the three cells of the conidium, although germination from the middle cell is rare (Bourett and Howard, 1990). Apical growth ceases within a few hours when the germ-tube tip hooks to form an appressorium initial and begins to swell. During maturation, the appressorium becomes melanized, except at a well-defined pore between the appressorium and the substratum. A granular substance, believed to be an adhesive one, accumulates at the substratum interface (Howard and Ferrari, 1989). As the hydrostatic pressure increases within the appressorium, a narrow hypha emerges through the pore and penetrates directly into the plant (Howard, 1991).

The symptoms on leaves are elliptical. The centre of the lesion is usually gray or whitish and the margin is usually brown or reddish-brown. The shape and color

were related with environmental condition, age of spots and degree of susceptibility of the variety. The spots usually begin as small, water-soaked, whitish, grayish or bluish dots. Size of the lesion is 1-1.5 cm long, 0.3-0.5 cm broad and usually develop a brown margin. On the panicle, the symptoms are brown, called 'neck blast' or 'rotten neck' and the parts above are dead (Ou, 1985).

3. Mechanism of *P. grisea* Infection

Germination of the fungal germ tube is depended upon additional signal; infection signal and vegetative growth signal. Additional signal such as the contact of conidia to a solid surface would help the germination of germ tube (Lee and Dean, 1993). Sensing the infection signal such as hydrophobic leaf surface would cause the swelling of hyphal tip into appressorium which will penetrate into the leaf surface. After penetration, enzymes will take action and life cycle will be completed within 3-4 days.

4. Diversity of *P. Grisea* by DNA Fingerprinting

P. grisea, like those other fungi, can be classified into groups based on its visual appearance and morphology. However, since it is known to be extremely genetically diverse, a more accurate tool is needed for characterization of the fungus. Therefore, DNA fingerprinting method has become a valid approach to characterize genetic structure of the pathogen population (Lavy *et al*., 1991, 1993). It has been widely used in various countries such as the Philippines, Korea, China (Zeigler *et al.,* 1995) and Thailand (Mekwatanakarn *et al.,* 1997). All of these studies had effectively used the same tool i.e. a hybridization-based fingerprinting technique called RFLP using the dispersed repetitive probe MGR586 to characterize *P. grisea* population. As small number as 6 lineages were classified from more than 1,500 isolates in the Philippines (Nelson *et al*., 1994), 16 lineages from 62 isolates in Korea (Hen *et al*., 1993), 54 lineages from 473 isolates collected from 144 sites in China over 16 years (Shen *et al*., 1997) and 51 lineages from 653 isolates collected from all over Thailand (Mekwatanakarn, 1999). Apart from the use of the Amplified Fragment Length Polymorphism method (AFLP) was used to classify 97 isolates collected from four regions all over Thailand into 18 groups (Sirithunya, pers. Comm., Appendix Table 2).

5. Genetic of Blast Resistance

Resistant cultivar is one of the solution to prevent or reduce yield loss due to rice blast epidemics. Blast resistance was classified into two types according to gene expression induced by the attack of the pathogen. One type is called qualitative or complete resistance while the other is called quantitative or incomplete resistance (Ou, 1979).

Qualitative or complete resistance shows reaction indicating the absence of compatible type lesion being controlled by major genes(s) (Ahn, 1994) having race specificity (Marchetti, 1983) and expressing hypersensitivity to the pathogen. The

first study on blast resistance gene was reported by Sasaki (1923) who found a single dominant blast resistance gene in Japanese rice variety Tsurugi had initiated a light at the end of the tunnel. Forty-two years later, two resistant genes designated *Pi-1* and *Pi-6* were identified in the United States of America (Atkins and Johnson, 1965). Four dominant genes i.e., *Pi4, Pi13, Pi22* and *Pi25* were also identified (Hsieh *et al*., 1967). The total of 11 major genes designated *Pi-k, Pi-k^{5}, Pi-k^{h}, Pi-ta, Pi-z, Pi-z^{t}, Pi-a, Pi-b, Pi-f, Pi-i* and *Pi-lm* had been reported fourteen years later (Kiyosawa, 1981). The work on gene mapping had revealed that *Pi5(t)* and *Pi7(t)* mapped on chromosome4 and 11 were linked to marker RG778 and RG103, correspondingly (Wang *et al*., 1994). Based on these information, near isogenic lines (NILs) of rice with single resistant gene for each line were developed by backcrossing four donor cultivars to the recurrent parent CO39 (Mackill and Bonman, 1992).

Quantitative (Incomplete) resistance has been called field resistance or partial resistance, in general. It is characterized by lesions typically spindle-shaped, fewer in number, reduce in size, slower to develop and shorter-lived (Tabien *et al*., 2002). Partial resistance is more difficult to use than complete resistance due to its quantitative inheritance which usually polygenic and sensitive to environmental factors such as temperature, leaf wetness duration, nitrogen-fertilization, soil type and water stress (Ou, 1985; Roumen, 1994). It has also been stated that quantitative or partial resistance is usually controlled by polygenes that are minor genes (Bonman *et al*., 1992). Examples of partial resistance varieties had been reported as IRAT13, IAC24, IAC27 and Dourado Precose studied by Nottegham (1985). Quantitative resistance could be separated into two components. First, is the efficiency of quantitative resistance in eliminating an avirulent portion of any available inoculum. Second, is the ability to lower the infection efficiency of a virulent portion (Ahn and Koch, 1988).

6. Recombinant Inbred Line (RIL)

The basis for detection of resistant genes starts with the cross between resistant and susceptible parents. Segregation of alleles will appear at meiosis stage leading to equal frequency of alleles in the gametes. The progeny having independent segregation alleles from both parents is Recombinant Inbred Line (RIL). The segregation ratio for parents is 1: 1. Therefore, the percentage of parents and progenies have equal frequencies. Since crossing over will occur at meiosis stage, target loci could have been moved over to another chromosome. Thus, a genetic distance can be calculated from a recombinant frequency. RILs population had been used by several researchers recently. Wu and Tanksley, (1993) use RILs derived from Thong156 x Gumei2 population to locate blast resistant genes under field conditions in China. Sirithunya *et al*. (2002) detected QTLs associated with leaf and neck blast resistance on chromosome7 and 9 using RILs derived from KDML105 x CT9993-5-10-M. Tabien *et al.* (2000) also mapped blast resistant genes from RILs of Lemont and Teqing population.

RIL is generated by single seed descent(SSD). SSD is designed to maintain the total range of variation in a population by precluding loss of noncompetitive

plants by taking a single seed from each individual of the population, starting from F_2, to propagate the next generation by bulking. Selection is not practiced until F_5 or F_6, as individuals in the population are reasonably homozygous. It can be said that SSD is a modification of the bulk method of breeding .

Computer simulation studies revealed that at high heritabilities the pedigree method is more effective while at low heritabilities SSD is more effective. It has also been reported that SSD was more effective in situation inwhich competition effects are important.

Steps for preparation of RILs

First year : Screening the parents and make crosses between each pair of them. The parents may be varieties, single or multiple crosses.

Second year : Grow F_1 plants and parents for comparison. F_1 are hybrid or self fertilization. Harvest the F_1 plants in bulk for each cross.

Third year : Grow F_2 generation of each cross. Harvest the plants of each cross in bulk. Take single or equal number of seeds from each plant and composite them for raising the next generation.

Fourth to seventh year : In F_3, F_4, F_5 and F_6 generations, grow a single seed per line for each generation. Individual line is harvested in bulk.

The SSD method, as a modification of the bulk-population method, has features that overcome the problem of natural selection and inadequate sampling in the conventional bulk-population method. This method minimizes natural selection without eliminating it. Thus, if population size is limiting. It is expected that the SSD method will maintain more genetic variability.

The SSD method has the obvious advantage in that gene frequencies are stabilized. The other advantage is that the segregating generations can be advanced with the maximum possible seed, wherever facilities such as greenhouse and off-season nurseries are available. This can be extremely rapid in low nutrient, continuous-light environments. Depending on the crop plant, the breeding cycle can be reduced from about 8 years with mass selection to about 4 years with SSD. Thus, the SSD saves the time and labor and offers good possibilities in isolating superior genotypes. In crops such as lentils, where poor growth habit lacks of synchronized maturity make it difficult to practice the pedigree method, SSD should be preferred.

7. Rice Genome and Genome Size

Haploid rice genome consists of 12 chromosomes containing a complete set of genetic information for rice growth and development. Using the flow cytometry, the estimation rice nuclear DNA content of rice haploid genome is 4.3×10^5 which is

approximately six times smaller than maize genome and 40 times smaller than wheat genome making it attractive for genome structure study.

8. Linkage Map Construction

In genetic mapping construction, DNA marker is used to determine locations of targeted genes in the chromosome (Paterson *et al*., 1988; Lander and Botstein, 1989). DNA marker is a molecular or genetic marker which is a tool used to establish linkages between a marker and a gene or to enhance the establishment of genes controlling a targeted trait more precisely and rapidly than can be achieved by conventional breeding. Important agronomic traits such as plant height and heading (Li *et al*., 1995), grain yield and yield components (Lin *et al*., 1996), seedling vigor (Redora and Mackill, 1996) and root morphological character related to drought avoidance (Champoux *et al*., 1995) had been used for linkage mapping construction. Most of these traits are controlled by quantitative trait loci (Hallaner and Miranda, 1998) which simply involved with polygenes (Geldermann, 1975). Population ideal for mapping construction can be either segregated progenies of F_2 population (Mago *et al.,* 1998), back cross population (Paterson *et al*., 1988), double haploid population (Guiderdoni, 1989; Chen *et al*., 1997) or recombinant inbred population (Burr and Burr, 1989; Wang *et al*., 1994; Tabian *et al.,* 2002). Mapping distance can be calculated from recombination percentage as 1 percent recombinant is equivalent to 1 centimogan (cM) which is approximately 206 kb (Wu and Tanksley, 1993).

Once the test is carried out on used population, data on molecular marker and phenotype will be analyzed statistically. Primarily, scientists used single molecular marker analysis with polygene and the relationship between marker and phenotype was analyzed using linear regression (Thoday, 1961; Soller and Brody, 1976). The additive effect associated with the marker locus can be estimated by linear regression of marker and genotype while the relationship between a marker and quantitative trait locus was analyzed using one way ANOVA (Stuber *et al.,* 1992). However, this method can only detect a QTL near the marker. Thus, interval mapping using the likelihood approach (LOD score) (Lander and Botstein, 1989) and the use of a set of linkage markers with regard to effects on the quantitative trait loci were introduced to improve the results and therefore the program for data analysis was developed and commonly known as Mapmarker/QTL (Lincoln *et al*., 1992).

9. Mapping Populations

Different populations used in mapping study are differed in advantage and disadvantage. F_2 population can be achieved quickly but a quantitative estimation on the variation in the replicated progenies tested is not adequately accurate. Furthurmore, there is a limitation in availability of tissues for DNA extraction. Double haploid (DH) line derived from anther culture has the advantage that it can be homozygous in only one step as diploid plants from anther culture are obtained from haploid cells. Among populations used for mapping study , recombinant inbred lines (RILs) take longer time to reach than others as they need 6-7 generations in breeding program and undergo multiple rounds of meiosis before homozygosity is obtained.

These result in high recombination between closely linked loci which is approximately twice than that of DH (Haldane and Woddington, 1949). The other major advantage is that RILs are no longer segregated. Thus, they can be propagated easily. The use of DH and RILs was reported to be well suited to QTL analysis (Burr and Burr, 1991) and that the combination of a set of molecular markers and RILs make a perfect match for researchers to easily exchange the information on rice genes at the molecular level (Glenn, 1997).

10. Molecular Marker

In agriculture, molecular marker is the tool for generating genetic linkage maps and has provided a major contribution to the genetic knowledge of many cultivated plant species useful for crop improvement and increased breeding efficiency. In addition to being of basic importance to genetic and evolutionary studies, molecular marker is useful to localize monogenic and polygenic traits allowing the efficient introgression and selection of individuals with specific characteristics. Basically, any DNA sequence used to distinguish between individuals, lines, varieties or to localize agriculturally important genes and construct genetic linkage map can be considered as a molecular marker. Molecular marker is more specific and accurate than other markers i.e., morphological or biochemical markers. Other advantages are direct measurement on genetic materials, numerous markers in a single population and measurement not subjected to environmental or developmental effect. They could localize any positions on the chromosomes which can be detected and inherited to progenies. Molecular marker or DNA marker can be classified into 2 groups.

10.1 Hybridization-based fingerprinting technique.

This technique is represented by RFLP (Restriction Fragment Length Polymorphism), the use of cloned fragments of chromosomal DNA as genetic markers. In this technique, which depends on natural variation in DNA base sequence, DNA is digested with a restriction enzyme. Homologous restriction fragments of DNA which differ in size or length can be used as genetic markers to follow chromosome segments through genetic crosses. To use this technique, a set of chromosomal DNA fragments is prepared to use as probes. Such a set of probes is called a library. DNA isolated from the species of interest is digested with a restriction enzyme, and relatively small fragments(usually 2-5 kb) are used as DNA hybridization probes. Individual restriction fragments can be used as a probe, but a supply of the individual fragments in pure form is needed. Results will be shown through DNA hybridization as the detection of difference or variability of DNA fragments after being digested with restriction enzymes. As genetic compositions of different species are differed in DNA sequence, this leads to changes in recognition sites of enzymes. RFLP has several advantages such as numerous in each population, co-dominant, measured directly on DNA, not subject to effects of environment or development. Therefore, RFLP has been utilized in several aspects of plant breeding such as to distinguish between lines on plant germplasm, to use as a marker in

genome mapping, to study QTLs (Quantitative trait loci) and to use in back cross breeding.

However, RFLP has some disadvantages when it comes to analysis. These include a large size of restricted DNA fragments, high expense for the processes of the southern blot and hybridization and it's time consuming. Thus, PCR-based marker has been developed to solve these problems.

10.2 PCR-based fingerprinting technique

Polymerase chain reaction (PCR) is *in vitro* manipulation of specific DNA sequences by the simultaneously primer extension of complementary strands of DNA. The whole process starts from the denaturation of target DNA into a single strand, annealing of primers to target DNA and then the extension of the primers by DNA polymerase. DNA polymerase is an enzyme which synthesizes complementary strands of DNA. Given some specific conditions, this process of DNA synthesis can be mimicked *in vitro*. Once these 3 steps are repeated for many cycles, the end result is that a specific DNA sequence is amplified many times. The amplification can be calculated with

$$\text{Total amplification} = m \times 2^n$$

Where n is the cycle number and m is the copy of target sequence. At present, there is an availability of the programmable machine that performs automatic temperature cycling commonly called PCR machine. The products of PCR are DNA fragments. When DNA from two breeding lines are amplified by the same set of primers at locus specific condition, the molecular weight of PCR products might be different due to the physical change on the DNA sequence, e.g., deletion or addition of DNA sequence within the amplified region. This amplicon length polymorphism reflects the polymorphic DNA between individuals/breeding lines/varieties. The physical change of PCR products can be induced by the use of a restriction enzyme and the technique is called "Cleaved Amplified Polymorphic Sequences(CAPSs).

PCR products of progenies from parents of different genetic components are shown as co-dominant. Several advantages of PCR-based technique over RFLP are recorded, e.g., it is very rapid as the whole process can be finished within a day without the need for southern blotting, no radioisotope is involved, low cost and very little DNA is needed, the length of polymorphism is shorter than that of RFLP due to a specific detection on the amplified region. The maximum length of polymorphism is approximately 5 kb while the minimum length is around 1-2 kb. This leads to a high efficiency in the detection of difference of the PCR products. However, a major limitation of PCR is that prior DNA sequence information is required for primer synthesis. Details of three common PCR-based fingerprinting techniques are as followed.

10.2.1 Microsatellites or simple sequence repeats (SSRs)

Simple sequence repeats(SSRs) are tandemly repeated of neucleotide motifs, dinucleotide (AC)n, (AG); trinucleotides (TGT)n, (TTG)n; tetranucleotides (TATG); pentanucleotides and hexanucleotides. The DNA sequence flanking repeats are abundance in genome. The polymorphism among individuals is varied in number of repeat units. Satellite DNA have two to hundred repeats and found between gene and genome but can't be found in the gene. Microsatellites can be divided into three types; perfect, imperfect and compound. Perfect has only repeat sequences such as (AC)n, (AT)n. Imperfect has another base between the repeat sequences such as (AC)n CT (AC)n. Compound has different repeat sequences such as (AC)n (TG)n. Microsatellite marker is a simple technique, low cost, highly informative, co-dominant, highly abundance and is a specific marker. Microsatellite marker is based on PCR amplification of tandem repeats using unique DNA sequences flanking the repeats as oligonucleotide primers. Microsatellite marker has been developed in many crop species such as rice (Wu and Tanksley, 1993; Panaud *et al*., 1996; Akagi *et al*., 1996), Brassica (Kresovich *et al.,* 1995) and maize (Senior and Heun 1993). Microsatellite marker has been used for genotypic identification and varietal protection (Smith and Helentjaris, 1996; Rongwen *et al*., 1995; Olufowote *et al*., 1997), seed purity evaluation and germplasm conservation (Brown and Kresovich 1996; Hahn and Grifo, 1996; Bretting and Widrlechner, 1995; Powell *et al*., 1996; Olufowote *et al*., 1997), gene and quantitative trait locus (QTL) analysis (Blair and McCouch, 1997; Koh *et al.,* 1996, Xiao *et al*., 1996) pedigree analysis and marker-assisted breeding (Ayres *et al*., 1997; Yang *et al*., 1994). Microsatellite marker has been used to construct a map for basic genetic studies on rice and breeding applications. A map is constructed by using PCR amplification to generate DNA of population as banding patterns on gel revealing polymorphic bands of alleles. Relation between microsatellite marker and phenotypic reaction can be used for mapping onto the rice genome.

10.2.2 Amplified fragment length polymorphism (AFLP)

The amplified fragment length polymorphism(AFLP) method is the combination of restriction digestion and PCR amplification. Two restriction enzymes, such as *EcoRI* and *Tru91* are used to cut DNA template. The products obtained from cutting with such restriction enzymes are *EcoRI* adapter and *Tru91* adapter. The primer is designed in complementary with adapter and increased by 1-3 nucleotides for selected DNA template. PCR amplification is used to increase DNA fragments. AFLP can be used for DNA fingerprinting and marker assisted selection in breeding and gene mapping. Vos *et al*. (1995) was the first one who was interested in genome mapping using AFLP marker to construct high density genetic maps of either genomes or genome fragments. However, AFLP is costly and difficult to select the enzyme and primer.

10.2.3 Randomly amplified polymorphic DNAs (RAPDs)

RAPD is generated by PCR amplification of genomic DNA segment using a single, short primer under low annealing temperature (William et al., 1990). RAPD is a rapid method, need a small amount of DNA and no radioactive is involved but it is a dominant marker. Kristin *et al*. (1997) used RAPD for the selection of common bean in early generation. Liu *et al.* (2002) located *Pi9(t)* gene by three RAPD markers which tightly linked to *Pi2(t)* in 450 F_2 plants. These *Pi9(t)* and *Pi2(t)* genes are important blast resistant genes in rice. Zuuang *et al*. (1997) used RAPD to analyse neck blast resistant genes in Zhong156 and Gumei2 population and found resistant genes located between $K17_{1400}$ and $A7_{550}$. Through RAPD marker, Young and Kelly (1997) found 3 major genes for controlling anthracnose disease caused by *Colletotrichum lindemuthianum* in common bean (*Phaseolus vulgaris*) and Mayer *et al*. (1997) found resistant genes for controlling Fusarium wilt disease caused by *Fusarium oxysporum* in chickpea (*Cicer arietinum*)

MATERIALS AND METHODS

Materials

1. Plant Materials

Jao Hom Nin (JHN) and Khao Dawk Mali105 (KDML105) are two rice varieties used in this investigation. JHN is a commercial non-glutinous rice variety resistant to rice blast disease under natural condition and resistant to some selected blast isolates in preliminary screening (Appendix Table 1). It is grown in the central and northern part of Thailand. At present, it is considered to be highly resistant to a broad spectrum of blast isolates in Thailand. KDML105 is a popular commercial non-glutinous aromatic rice variety with low amylose content. However, it has a major disadvantage as a susceptibility to rice blast disease. KDML105 and JHN were cross-pollinated by Kasetsart University. F_1 to F_6 population were derived from this cross. After harvesting each hill of generation, F_3 was generated by means of single seed descent i.e. taking 4 hills from each line and sowing 1 seed from 1 hill. Thus, 1 line of F_2 would generate 4 lines of F_3. For F_4–F_6 generation, a single seed was taken from each harvested line, sown and harvested individually (Appendix, Figure1).

2. Blast Isolates

One hundred and two isolates were collected by Sirithunya (pers. comm.) around Thailand. These isolates were classified into 18 groups by AFLP markers (Appendix, Table 2). They were to be inoculated onto the parents by leaf and neck blast screening method for the assessment on correlation between leaf and neck blast severity.

Three diverse blast isolates from different groups of genetical compositions classified by AFLP as mentioned above were chosen on the basis of being aggressive to the parental set. They were designated THL191, THL318 and THL899. THL191 and THL318 were isolated from leaf blast lesions at Phitsanulok and Chiang Mai, respectively. The third isolate, THL899, was obtained from collar rot lesion at Surat Thani.These three isolates were used to screen RILs derived from KDML105 and JHN population.

Method

1. Preparation of Plant

1.1 Leaf blast screening

To identify resistant QTLs to blast, parental lines and 587 F_6 RILs were inoculated and evaluated. They were sown one line per row on moist soil in polyvinyl baskets (27.5 x 36.5 x 11.5 cm). Parental screening was under taken by growing the parents Population screening was carried out by planting tested lines along two sides of the baskets. Three rice cultivars designated as KDML105, KTH17 and RD23 were sown as susceptible checks while JHN was sown as a resistant check. Urea (46-0-0) was applied twice at 2 g per basket (approximately 184 kg N /ha) at one week interval. The first application was one week after sowing. Seedlings were kept outdoors in the nylon net to keep out from insect pests for 21 days prior to the commence of inoculation.

1.2 Neck blast screening

KDML105 and JHN were planted in the pot three times at one week interval for parental screening. Population screening (587 RILs) plants were sown in the field using dibbling method. Fertilizer was applied twice during growth stages, The first application was commenced using 15-15-15 at 187.5 kg/ha (28.12 kgN/ha) approximately 30-35 days after seeding. The second one was applied 55-60 days after seeding using (46-0-0) at 125 kg/ha (57.5kgN/ha).

2. Preparation of Inoculum

Each of the selected blast isolates was cultured on rice polished agar (Appendix, Table 3) and incubated at 26 $\pm$ 1 degree celcius under light condition for 14 days. After the fungal growth covered approximately ¾ of the agar surface, the culture was transferred to an ultraviolet-light chamber for 3 days to enhance sporulation. Sterilized distilled water was then poured onto the culture in each Petri-dish. Fungal conidia were scraped out from the surface using spatula. Inoculum was poured into a beaker and the spore count was carried out using a haemacytometer. The inoculum concentration was adjusted to $5x10^4$ conidia / ml. One percent of gelatin solution was added to the inoculum at 1 : 1 ratio for leaf blast screening and 1 : 100 ratio for neck blast screening to ensure a contact of conidia to the plant surface.

3. Inoculation and Scoring

3.1 Leaf blast

Inoculum of *P. grisea* was misty sprayed onto 21 day-old rice seedlings using the Air Brush Spray (BADGER 150-4 by BADGER AIR-BRUSH Co., IL 60131, USA). Inoculated seedlings were incubated in the plastic cover at 26 $\pm$ 1 degree celcius under 12-hour alternate light and dark conditions in the growth

room for 2 days afterwhich the cover was opened. The seedlings were maintained in the growth room for additional 5 days under the same conditions. High humidity condition was provided by the automatic overhead misty-sprayed springkler. The program was setup to spray for 5 seconds every hour. Leaf blast was recorded 7 days after inoculation on the basis of a 0 to 9 scale as described by Standard Evaluation System for Rice (Chaudhary,1996) as follow.

Lesion type score for leaf blast

0	No lesions observed
1	Small brown specks of pinpoint size or larger brown specks without sporulating center
3	Small, roundish to slightly elongated necrotic sporulating spots, about 1-2 mm in diameter with a distinct brown margin or yellow halo
5	Narrow or slightly elliptical lesions, 1-2 mm in breadth, more than 3 mm long with a brown margin
7	Broad spindle-shaped lesion with yellow, brown or purple margin
9	Rapidly coalescing small, whitish, grayish or bluish lesions without distinct margins

Note: Lesion types 0, 1, 3 are considered resistance and 5, 7, 9 are considered typical susceptible (Figure2).

Figure 2 The index value and corresponding levels of stress severity for leaf blast

3.2 Neck blast

Inoculum was injected into the leaf sheath at booting stage. The plants were maintained under the 60% light-allowance sarland net. High humidity was obtained from automatic misty-sprayed springkler. The program was setup to spray for 30 minutes 3 times a day at 11.00am, 2.00 pm and 3.30 pm, respectively. Neck blast scoring was recorded 3 times after inoculation at 7 days interval on the basis of a 0 to 9 scale as described by Standard Evaluation System for Rice (Chaudhary,1996) as follow:

Lesion type score for neck blast

0 = No visible lesion observed or lesions on only a few pedicels
1 = Lesions on several pedicels or secondary branches
3 = Lesions on a few primary branches or the middle part of panicle axis

5 = Lesion partially around the base (node) or the uppermost internode or the lower part of the panicle axis near the base
7 = Lesion completely around panicle base or uppermost internode or panicle axis near the base with more than 30 % of filled grains
9 = Lesion completely around panicle base or uppermost or the panicle axis near the base with less than 30 % of filled grains

Note: Lesion types 0, 1, 3 are considered resistance and 5, 7, 9 are considered typical susceptible (Figure3).

Figure 3 The index value and corresponding levels of stress severity for neck blast

3.3 Broad resistance spectrum

Broad - resistance spectrum (BRS) formula modified from Ahn (Sirithunya *et al*., 2002) was used to assess the broad-spectrum resistance.

BRS = **S/T**
S = Number of isolates giving a resistance reaction
T = Total number of isolates used for screening

The BRS was ranged from 0 to 1. The BRS equals to 0 or 1 indicated that the rice cultivar or line was considered as susceptible or resistant to all isolates, respectively

4. Genetic Analysis

Microsatellite markers or SSRs analysis described by Panaud *et al*. (1996) was utilized to detect polymorphism in this study. Sixteen SSRs markers developed by Cornell University were chosen for linkage map construction on 587 F_6 RILs population (Appendix, Table 4). The selection criteria was based on the information obtained from Theerayut (in press) who utilized 100 SSRs markers to analyse F_2 population and found that these 16 SSRs markers could detect QTLs effectively. The marker nomenclature followed the construction utilized by Cornell University and Japan's RGP. Six SSRs being detected on chromosome 1 were RM5, RM104, RM212, RM237, RM246 and RM319. Seven SSRs detected on chromosome 11 were RM21, RM139, RM144, RM206, RM224, RM254 and AC113249. Three SSRs detected on chromosome 12 were OSR32, RM179 and RM309

5. Linkage Map Construction

MAPMARKER/ QTL software (Lander *et al.,* 1987) was used for the linkage map construction from the F_6 RILs populations. The recombination frequency (rmax) of 0.30 and a LOD score of >2.5 were utilized to determine the final linkage map. The linkage group for corresponding chromosomes was assigned following the rice genetic map reported by Kurata *et al.* (1994) and Chen *et al.* (1997). The genetic distance (cM) was determined by recombination values using the Kosambi function**.**

6. QTL Analysis

QTLs were mapped by means of the interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures of NQTL, a softwaer for interval mapping (Tinker and Martha, 1995a, 1995b). The phenotypic data from each of the three isolates were analyzed separately. For the NQTL analysis, each data set was analyzed with 1000 permutations at a 5 cM walking speed and a type I error rate of 5%. For sCIM, four background markers with approximately even spacing were specified, with a maximum of three background markers per linkage group. STATGRAPHIC 2.1 software was used as a tool to reconfirm number, location and effect of the QTLs and to determine the phenotypic variance explained (PVE) or R^2 by QTLs. Two loci interactions of QTLs were determined using regression analysis and ANOVA. Chi square test was used to detect the distribution of this population.

RESEARCH LOCATION AND PERIOD OF STUDY

1. Location

1. Phitsanulok Rice Research Center
   Greenhouse – Neck blast screening on parental lines.

2. Rice Gene Discovery Unit, Kasetsart University, Kamphangsaen Campus
   Greenhouse – Leaf blast screening on parental lines and RILs population
   Experimental field – Neck blast screening on RILs population
   Laboratory – Molecular study and analysis

2. Period of study June 2001 – March 2005

RESULTS

1. Parental Screening

KDML105 and JHN were screened for leaf and neck blast resistance using 82-95 isolates for leaf and neck blast screenings, correspondingly. (Appendix Table 2) Data of broad resistance spectrum (BRS) showed that JHN was resistant to almost every isolates tested as the BRS of 0.99 and 1 were recorded for leaf and neck blast screenings, respectively. The BRS values of JHN obtained from leaf and neck blast screenings were 47 and 71% higher than those of KDML105, correspondingly (Figure 4).

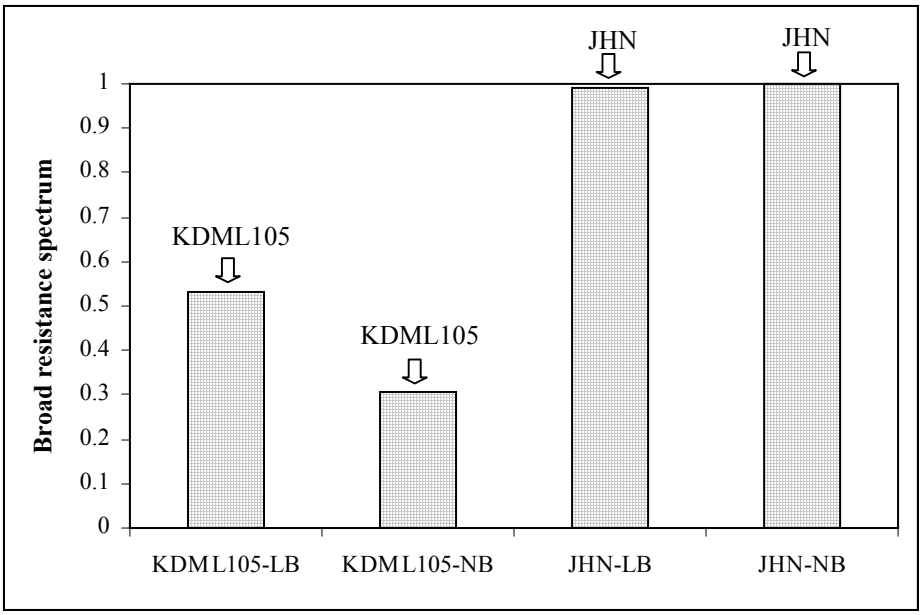


Figure 4 Broad resistance spectrum (BRS) of KDML105 and JHN as being screened for leaf and neck blast resistance using 82 - 95 isolates, respectively

For further clarification, the frequency of leaf and neck blast disease severity of JHN and KDML105 were investigated (Table1). Results showed that JHN gave the score of 0.0-1.0 (highly resistant) to 84 and 85 isolates for leaf and neck blast screenings, respectively. The scores for higher levels of disease severity for leaf blast screenings on JHN were limited to 1.1-2.0 and 2.1-3.0 with the frequency of 3 and 7 isolates, correspondingly. The higher scores for neck blast screenings on JHN were found as 1.1-2.0 with only 1 isolate. Different responses were recorded with KDML105 for leaf and neck blast screenings. KDML105 showed the reaction to leaf blast screenings as a skewed inverted parabola. The scores of 0.0-1.0 for 34 isolates were recorded. The number of isolates with higher levels of disease severity were reduced dramatically to 0 at the score of 3.1-4.0, afterwhich the number of isolates that caused KDML105 increasingly more susceptible to, was rising to 10-17 isolates. The maximum leaf blast scores of 8.1-9.0 were found on KDML105 with 10 isolates

tested. Data of neck blast screening showed that there were five recorded scores for KDML105 i.e. 0.0-1.0 (24 isolates) 4.1-5.0 (8 isolates), 5.1-6.0 (1 isolates), 6.1-7.0 (1 isolates) and 8.1-9.0 (48 isolates). Parents showed different reactions. Most of JHN reactions were resistant while those of KDML105 showed both resistant and susceptible but more towards susceptible. Consistency of leaf and neck blast screenings was seen as non-significant p-values from ANOVA of each screening set (Appendix Table 5-16).

Table 1 Number of isolates causing different blast scores for JHN and KDML105 screened for leaf and neck blast severity

Parental screening	0.0-1.0	1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0	5.1-6.0	6.1-7.0	7.1-8.0	8.1-9.0
Leaf blast JHN	84	7	0	0	1	0	0	0	0
Neck blast JHN	85	1	0	0	0	0	0	0	0
Leaf blast KDML105	34	9	6	0	1	16	0	17	10
Neck blast KDML105	24	0	0	0	8	1	1	0	48

2. Phenotypic Reaction of Leaf and Neck Blast Resistance

After inoculation, disease severity groups for leaf and neck blast were obtained and shown in Figure 5, Table 2 and Figure 6, Table 3, respectively. Phenotypic distribution of both leaf and neck blast severity revealed classes of resistant and susceptible reactions (Table 1, 2). There were some hypersensitivity and moderately susceptible reactions. However, if the data were to be classified into 2 groups; resistant (0, 1, 3) and susceptible (5, 7, 9), The chi-square (x^2) test showed that 4 out of 6 sets of data had fitted a ratio of approximately 3: 1, 2 set non fitted 1: 1 ratio for resistant: susceptible population. The ratio of 3: 1 was detected by leaf blast screening using THL191 (385: 147), THL318 (397: 124) and THL899 (444: 142) and neck blast screening using THL899 (268: 88). The ratio of 1: 1 was recorded from the inoculation of THL191 (247: 176) and THL318 (299: 220)for neck blast screening.

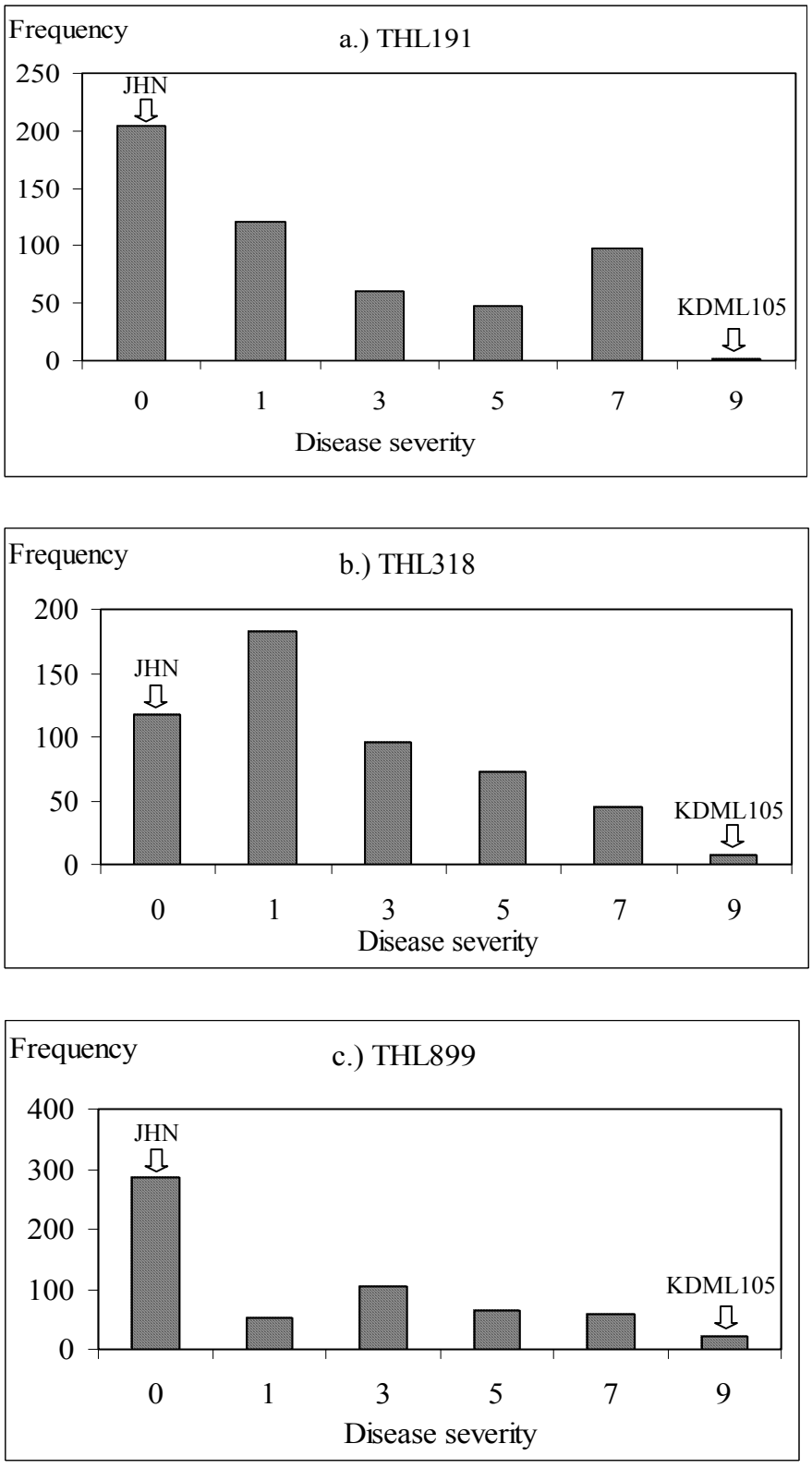
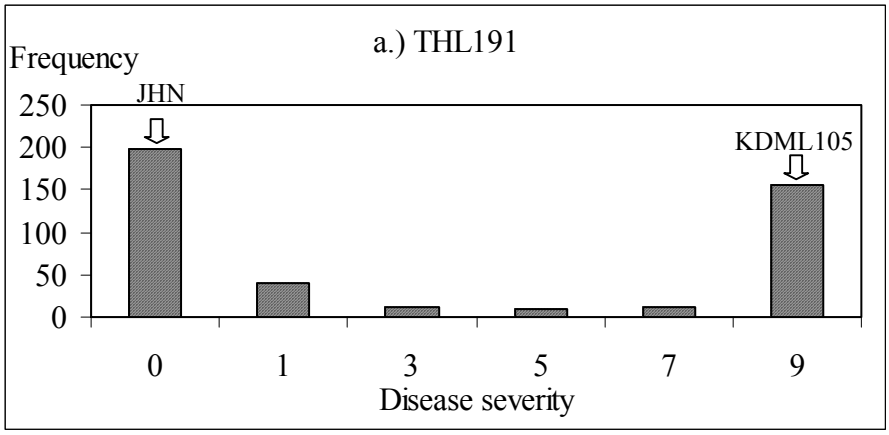
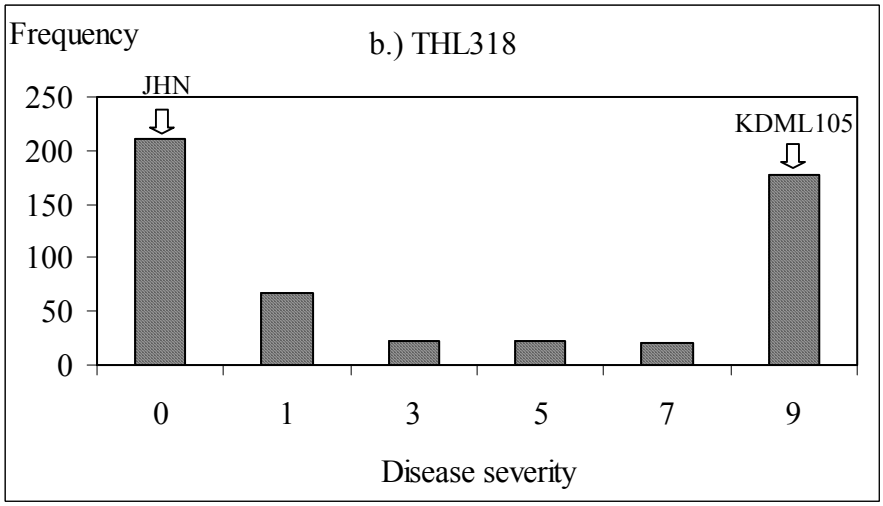


Figure 5 Distribution of disease severity of 587 RILs screened for leaf blast disease by 3 selected isolates.





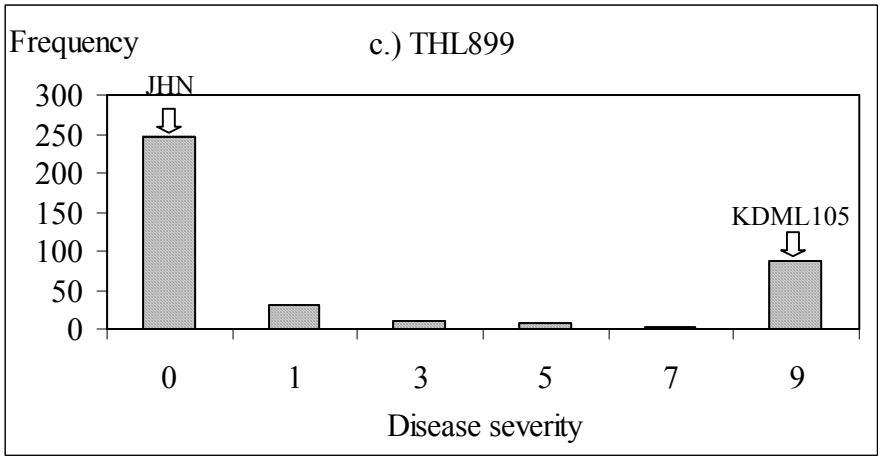


Figure 6 Distribution of disease severity of 587 RILs screened for neck blast disease by 3 selected isolates.

Table 2 Ratios between resistant and susceptible reactions and chi square of 587 RILs screened for leaf blast resistance by 3 selected isolates.

Isolate	Disease severity index						Total	Ratio	X^2
	0	1	3	5	7	9		R:S	
THL191	204	121	60	47	98	2	532	3 : 1	1.96^{ns}
THL318	118	183	96	72	45	7	521	3 : 1	0.38^{ns}
THL899	286	52	106	64	57	21	586	3 : 1	0.18^{ns}

Table 3 Ratios between resistant and susceptible reactions and chi square of 587 RILs screened for neck blast resistance by 3 selected isolates.

Isolate	Disease severity index						Total	Ratio	X^2
	0	1	3	5	7	9		R:S	
THL191	197	39	11	9	11	156	423	1 : 1	11.8**
THL318	210	67	22	22	20	178	519	1 : 1	12.02**
THL899	231	28	9	6	2	80	356	3 : 1	0.01^{ns}

3. Correlation Between Leaf and Neck Blast Severity

Regression analysis was carried out on disease severity obtained from three blast isolates to see the relationship between leaf and neck blast severity in the RILs population. Results showed a rather low correlation coefficient (r) in general. The highest r was obtained from THL899 being 0.56 followed by THL191 and THL318 being 0.32 and 0.28, respectively. Variance also followed the same trend as the highest variance being 0.48 was obtained from THL899 while those of THL191 and THL318 were 0.17 and 0.19, correspondingly (Table 4).

Table 4 Correlation between leaf and neck blast severity of 587 RILs population

Blast isolate	Correlation coefficient	Variances
THL191	0.32**	0.17
THL318	0.28**	0.19
THL899	0.56**	0.48

4. Linkage Map Construction and Quantitative Trait Loci (QTLs) Analysis

Data from sixteen SSRs markers were analyzed for the order of markers by MAPMARKER/QTL developed by Lander *et al*. (1987). Distances between markers were analyzed by Kosambi. The order of six markers on chromosome1 started from RM5, RM246, RM237, RM212, RM319 and RM104 with the distance between markers being 9.3, 7.6, 12.3, 1.5 and 22.7 cM, respectively. The total distance on chromosome1 was 51 cM while that on Chromosome11 was as long as 84.9 cM. There were seven markers being mapped on chromosome11 with the order as followed: RM21, RM206, RM254, AC113249, RM224, RM139 and RM144. Distances between these markers were 22.7, 20.7, 9.4, 13.3, 2.9 and 10.6 cM, correspondingly. The total distance on chromosome12 appeared to be the shortest

one being 32.8 cM with three markers designated OSR32, RM179 and RM309 being mapped onto this chromosome. Distances between markers were 12.7 and 27.4 cM, respectively (Figure 7).

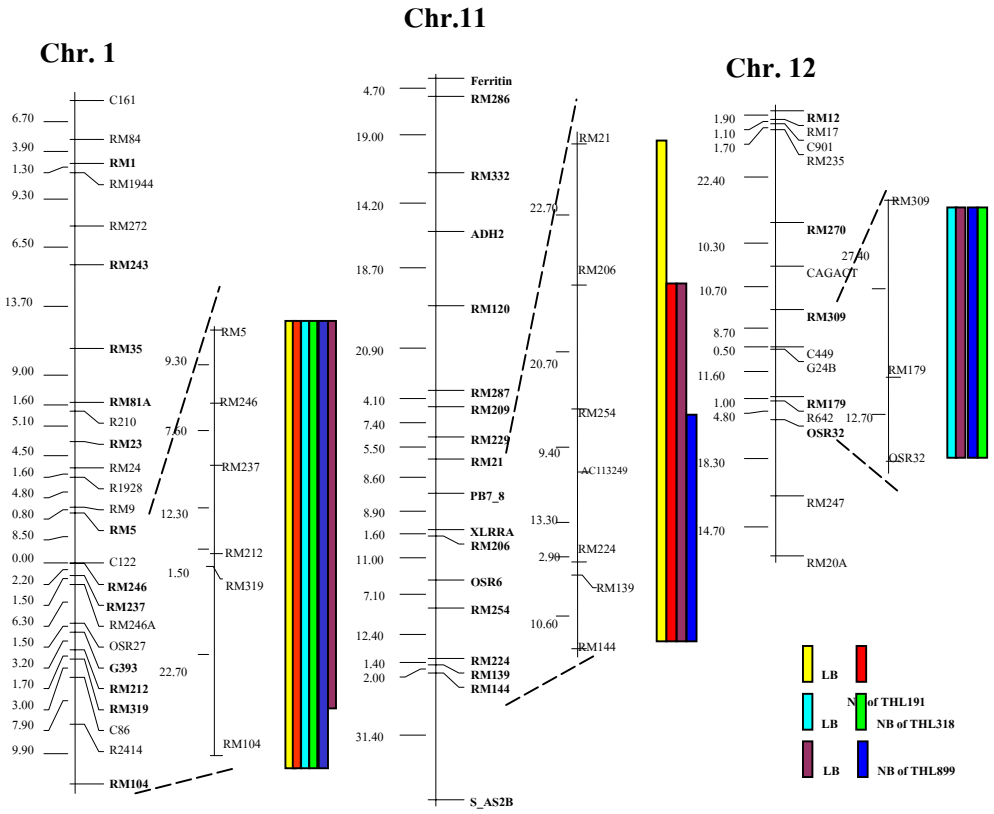


Figure 7 Linkage map from F_2 and F_6 587 RILs from KDML105 and JHN population with sixteen SSRs markers were used to locate QTLs on chromosome1, 11and 12.

Intervals and peaks of QTLs for leaf and neck blast resistance and linkage map of 587 RILs were shown in Table 5 and Figure 7 with details as followed.

Through the inoculation with THL191 and six markers designated RM5, RM246, RM237, RM212, RM319 and RM104 being mapped on chromosome1, one QTL for leaf blast resistance was detected with the peak between RM212 – RM104 markers having LOD being 13.15 while that for neck blast resistance have the peak at RM212 marker having LOD being 20.46. Results from the same isolate, THL191, also revealed the other QTL location on chromosome11 mapped with seven markers i.e. RM21, RM206, RM254, AC113249, RM224, RM139 and RM144, the peak for leaf blast was found between AC113249 – RM224 with LOD being 50.82 while that for neck blast was detected at RM224 with LOD being 40.19. The coefficient of

determination (R^2) for THL191 in identifying QTL for leaf blast and neck blast was 41.40 and 52.05 % respectively, with JHN as a sole contributor for all resistant alleles.

When the second isolate, THL318, was inoculated onto the same population, one QTL was also detected on chromosome1 between RM5 – RM104 markers having the peak at RM212 with LOD being 18.17 for leaf blast while that of neck blast having the peak between RM237 – RM319 with LOD being 25.10. Once again, JHN had contributed resistant alleles for this QTL. The other QTL was found on chromosome12. It was located between OSR32 – RM309 with the peak between RM179 – RM309 having LOD = 24.64 for leaf blast and 7.45 for neck blast. Interestingly, KDML105 was a sole contributor for resistant alleles of this QTL. The total coefficient of determination (R^2) for THL318 was 35.77% for leaf blast while 25.86% was calculated for neck blast .

Once the third isolate, THL899, was used, three QTLs were located on all three chromosomes i.e. 1, 11 and 12. The first one was detected on chromosome1 between RM5 – RM104 having the peak at RM212 with LOD being 12.18 for leaf blast while that for neck blast was detected between RM237 – RM104 having the peak at RM319 with LOD being 5.89. The second one was found on chromosome11 between RM206 – RM144 with the peak at RM224 having LOD being 42.63 for leaf blast. QTL for neck blast on the same chromosome was found between RM254 – RM144 with the peak around RM139 having LOD being 12.84. The third QTL location was detected on chromosome12 between OSR32 – RM309 having the peak between RM179 – RM309 with LOD being 7.72 for leaf blast while the peak for neck blast was recorded at RM179 with LOD being 10.25. For all these three QTLs, JHN was the sole contributing parent to resistant alleles. The total coefficient of determination (R^2) for leaf blast was 42.55 % while that of neck blast was 39.80 % .

Table 5 Intervals and peaks of QTLs on chromosome1, 11 and 12 located by 16 SSRs markers

Isolate	Plant	Chromosome	Contributor	Interval	Peak	LOD	Total R^2 (%)
THL191	Leaf	1	JHN	RM5-RM104	RM212-RM104	13.15	41.40
		11	JHN	RM21-RM144	AC113249-RM224	50.82	
	Neck	1	JHN	RM5-RM104	RM212	20.46	52.05
		11	JHN	RM206-RM144	RM224	40.19	
THL318	Leaf	1	JHN	RM5-RM104	RM212	18.17	35.77
		12	KDML105	OSR32-RM309	RM179-RM309	24.64	
	Neck	1	JHN	RM5-RM104	RM237-RM319	25.10	25.86
		12	KDML105	OSR32-RM309	RM179-RM309	7.45	
THL899	Leaf	1	JHN	RM5-RM104	RM212	12.18	42.55
		11	JHN	RM206-RM144	RM224	42.63	
		12	JHN	OSR32-RM309	RM179-RM309	7.72	
	Neck	1	JHN	RM237-RM104	RM319	5.89	39.8
		11	JHN	RM254-RM144	RM139	12.84	
		12	JHN	OSR32-RM309	RM179	10.25	

Significant correlation was found between blast isolates and markers used for both leaf and neck blast inoculation (Table 6). In general, coefficient of determination was improved by the additive effect of multiple markers in comparison with single markers. Details are as followed.

For leaf blast analysis, R^2 for the use of single markers was ranged from 0.85** - 32.87**%. The maximum R^2 of 32.87% was found following the inoculation with THL191 and the location of QTLs was detected by RM224 on chromosome11. Combining this detection with those on chromosome1 by marker RM212 had led to a total R^2 among leaf blast inoculation being 41.4%. JHN was a sole contributor to all resistant alleles in this case. The highest total R^2 being 42.55% was obtained through the inoculation with THL899 when QTLs were detected on chromosome1 by RM212 and on chromosome11 by RM224 and chromosome12 by OSR32 with resistant alleles being contributed by JHN. The use of THL318 provided the lowest total R^2 of 35.77% when QTLs were detected on chromosome1 by RM237 and RM212 having JHN as a donor for resistant alleles and chromosome12 by OSR32 and RM179 having KDML105 as donor for resistant alleles.

For neck blast inoculation, results revealed that R^2 for single markers were ranged from 5.01** - 34.58**%, higher than those of the leaf blast one. The highest R^2 was found with the use of THL191 and QTL was located on chromosome11 by RM224. This together with the detection on chromosome1 by RM212 had led to a highest total R^2 being 52.05% and JHN was responsible for all resistant alleles. The second highest total R^2 being 39.8 % was found through the inoculation with THL899 when QTLs were detected on chromosome1 by RM212, chromosome11 by RM139 and chromosome12 by OSR32 and that JHN was the sole donor to all resistant alleles. The inoculation with THL318 had the lowest total R^2 being 25.86% with the detection of QTL on chromosome1 by RM212 having JHN being the donor for resistant alleles while KDML105 was responsible for resistant alleles detected by RM179 on chromosome12.

Table 6 Correlation between single markers and 3 selected blast isolates on chromosome1, 11 and 12

Isolate	Chromosome1			Chromosome11		Chromosome12		Total R^2
	RM237	RM212	RM104	RM224	RM139	OSR32	RM179	(%)
Leaf								
THL191		-0.88,7.03**	-0.60,3.55**	-1.84,32.87**				41.40
THL318	-0.16,0.85*	-0.37,7.43**				0.25,1.26*	0.36,2.99**	35.77
THL899		-0.89,7.70**		-1.62,27.84**		-0.67,4.74**		42.55
Neck								
THL191		-1.54,16.57**		-2.05,34.58**				52.05
THL318		-1.89,19.90**					0.91,5.01**	25.86
THL899		-.74,9.02**			-1.3,22.12**	-1.08,17.51**		39.80

To classify the presence of QTLs, QTL likelihood map for leaf and neck blast resistance on chromosome1, 11 and 12 were shown in Figure 8-10. The regions of QTLs for blast resistance were located on chromosomal region flanked by marker loci detected by selected three isolates; THL191, THL318 and THL899. Details are as followed.

On chromosome1, a likelihood for the presence of QTLs for leaf and neck blast was found between RM5 and RM104 with the peaks between RM319 and RM212 using all three blast isolates i.e. THL191, THL318 and THL899 (Figure 8). The presence of QTLs on chromosome11 was found between RM206 and RM144 with the peaks at RM224 following the inoculation of THL191 and THL899 (Figure 9). On chromosome12, QTLs were shown between at RM309 – OSR32 with the peaks at RM179 using THL318 and THL899 (Figure 10).

In conclusion, inoculation with 3 selected isolates revealed QTLs for both leaf and neck blast on chromosome1 and 11 ofwhich JHN was responsible for all resistant alleles. However, the demonstration of QTLs for leaf and neck blast on chromosome12 when different isolates were used indicated that different donors were detected for the contribution of resistant alleles. QTLs for both leaf and neck blast on chromosome12 had resistant alleles from KDML105 when THL318 was used while those with resistant alleles from JHN were found with THL899 inoculated as leaf and neck blast screenings.

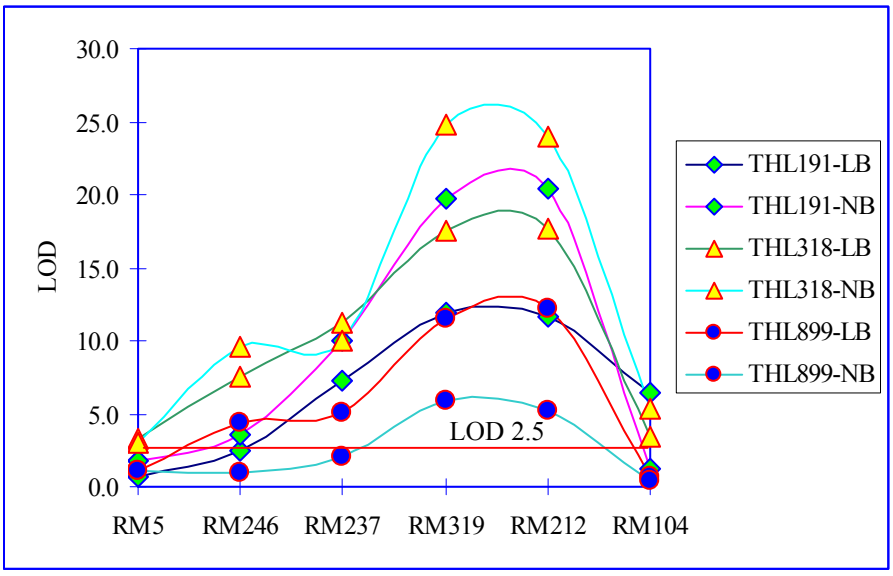


Figure 8 QTL likelihood map for leaf and neck blast resistance on chromosome1 constructed from LOD of 6 SSRs followed the inoculation of THL191, THL318 and THL899.

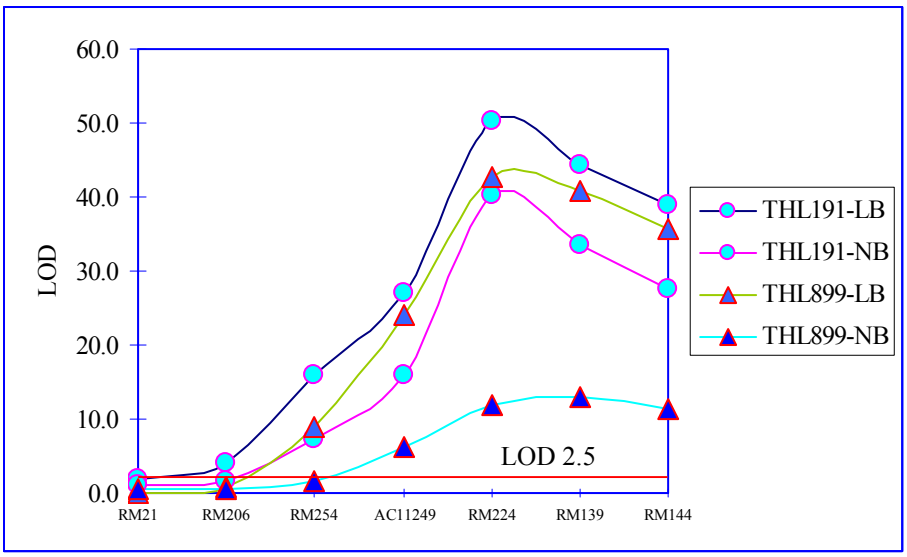


Figure 9 QTL likelihood map for leaf and neck blast resistance on chromosome11 constructed from LOD of 7 SSRs followed the inoculation of THL191 and THL899.

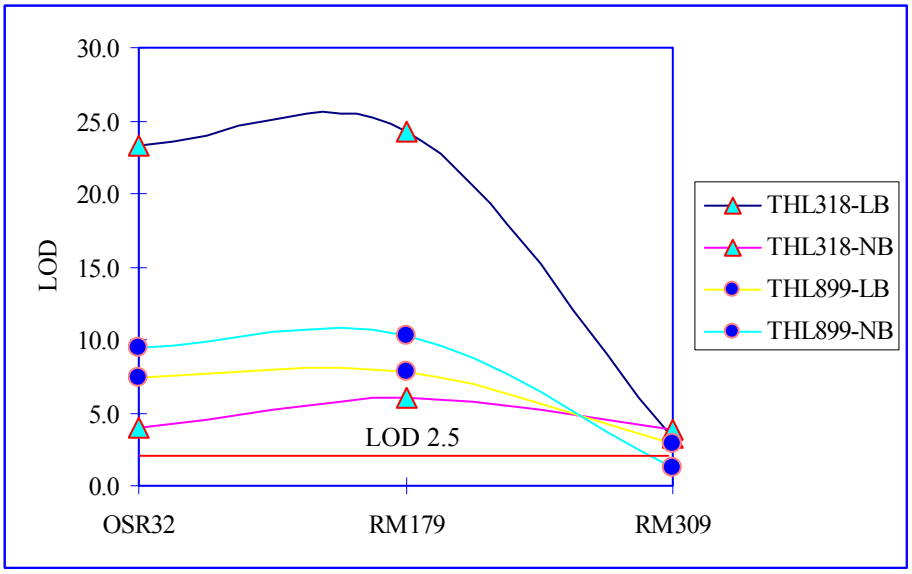


Figure 10 QTL likelihood map for leaf and neck blast resistance on chromosome12 constructed from LOD of 3 SSRs followed the inoculation of THL318 and THL899.

5. Main Effects and Interactions of Genes

5.1 Leaf blast screening

Results from phenotypic reaction and analysis have supported those of the genetical analysis. Using NQTL analysis, the main effect was detected on chromosome11 between RM206 and RM144 using THL191 and that JHN was the contributor for all resistant alleles. This was reflected as lower mean scores for disease severity ranging from 0.98 - 1.1 whenever JHN alleles were presented on chromosome11 in comparison with 3.99 as recorded for the homozygous alleles of KDML105 (Figure 11a). The minor effect was detected on chromosome1 and 12 following the inoculation of THL318. Results showed that resistant alleles on chromosome1 were obtained from JHN while those on chromosome12 were obtained from KDML105 as seen from low mean score of disease severity being 1.08 for heterozygous population with JHN alleles on chromosome1 and KDML105 on chromosome12 (Figure 11b). There were additive interactions between three QTLs on chromosome1, 11 and 12 followed the inoculation with THL899. Whenever JHN alleles were presented, mean scores of disease severity were low ranging from 0.38-3.62. The lowest mean score of 0.38 was recorded when JHN was the sole contributor to all resistant alleles on chromosome1, 11 and 12 suggesting a high degree of broad spectrum resistance of JHN (Figure 11c).

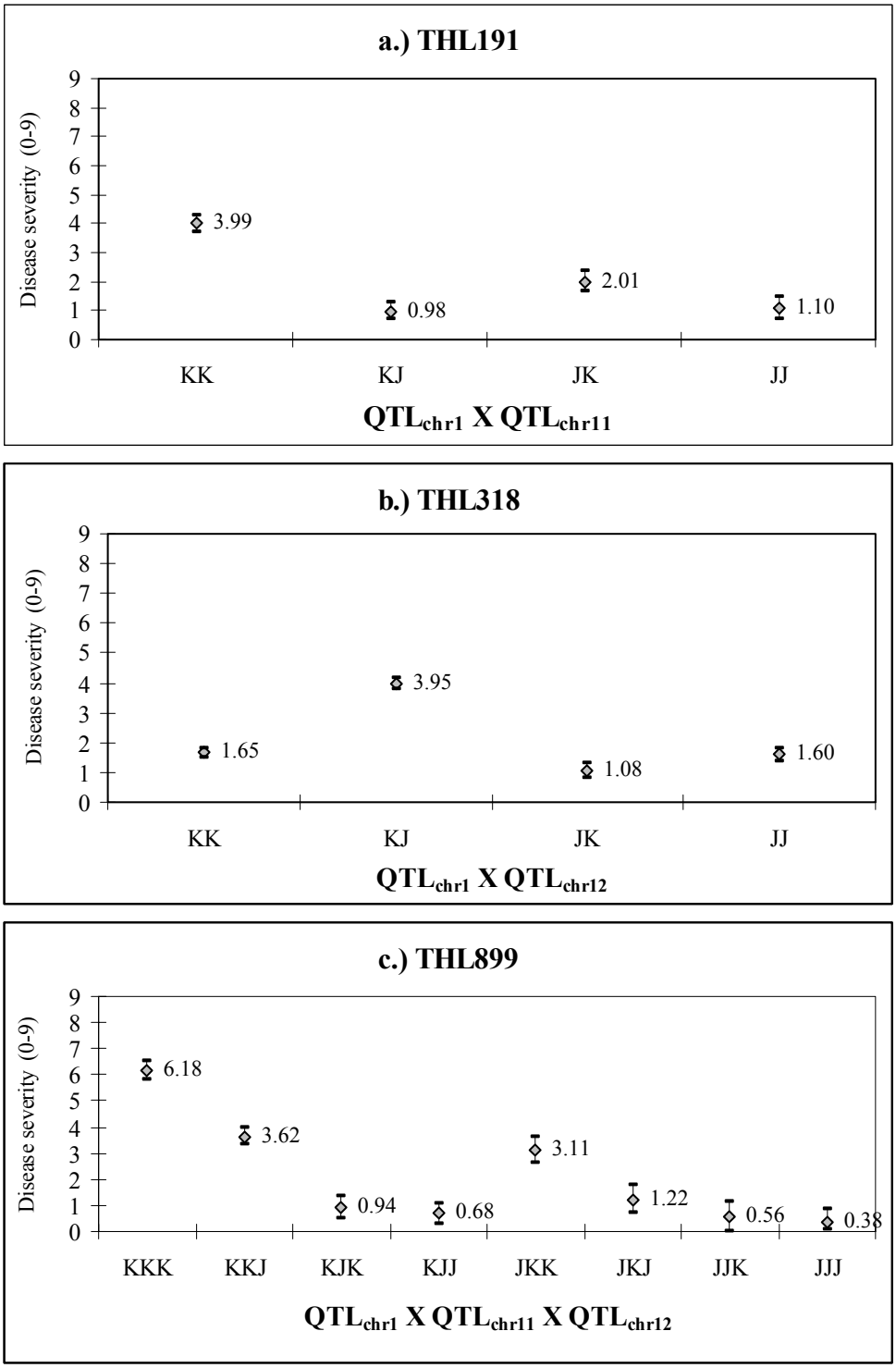


Figure 11 Interactions between QTLs of leaf blast resistance on chromosome1, 11 and 12 obtained from the inoculation with THL191, THL318 and THL899 having 95.0 % LSD interval

5.2 Neck blast screening

Similar results to those of leaf blast screening using THL191, neck blast resistant alleles on chromosome1 and 11 were contributed by JHN. Thus, homozygous population with all resistant alleles on chromosome1 and 11 had as low mean score of disease severity as 0.5. The presence of alleles from JHN had helped in reducing mean score of disease severity in heterozygous population by 73-88 % in comparison with that of homozygous population with alleles contributed by KDML105 (Figure 12a). When THL318 was used, population either homozygous with resistant alleles on chromosome1 and 12 contributed by JHN, homozygous with all resistant alleles on the same two chromosomes inherited by KDML105 or heterozygous with resistant alleles on chromosome1 and 12 contributed by JHN and KDML105, respectively, had a low range of mean score for disease severity between 1.17 - 3.69 while the mean score for the population without resistant alleles from any of the parents was as high as 6.5 (Figure 12b). Additive interactions between three QTLs on chromosome1, 11 and 12 were also observed followed the inoculation for neck blast screening using THL899. Since JHN was the sole donor for resistant alleles on chromosome1, 11 and 12, population either homozygous with the presence of JHN alleles or heterozygous with one or two alleles from JHN showed a low range of mean score for disease severity being 0.05-1.21 while that of the homozygous population without detected resistant alleles was as high as 5.95 (Figure 12c).

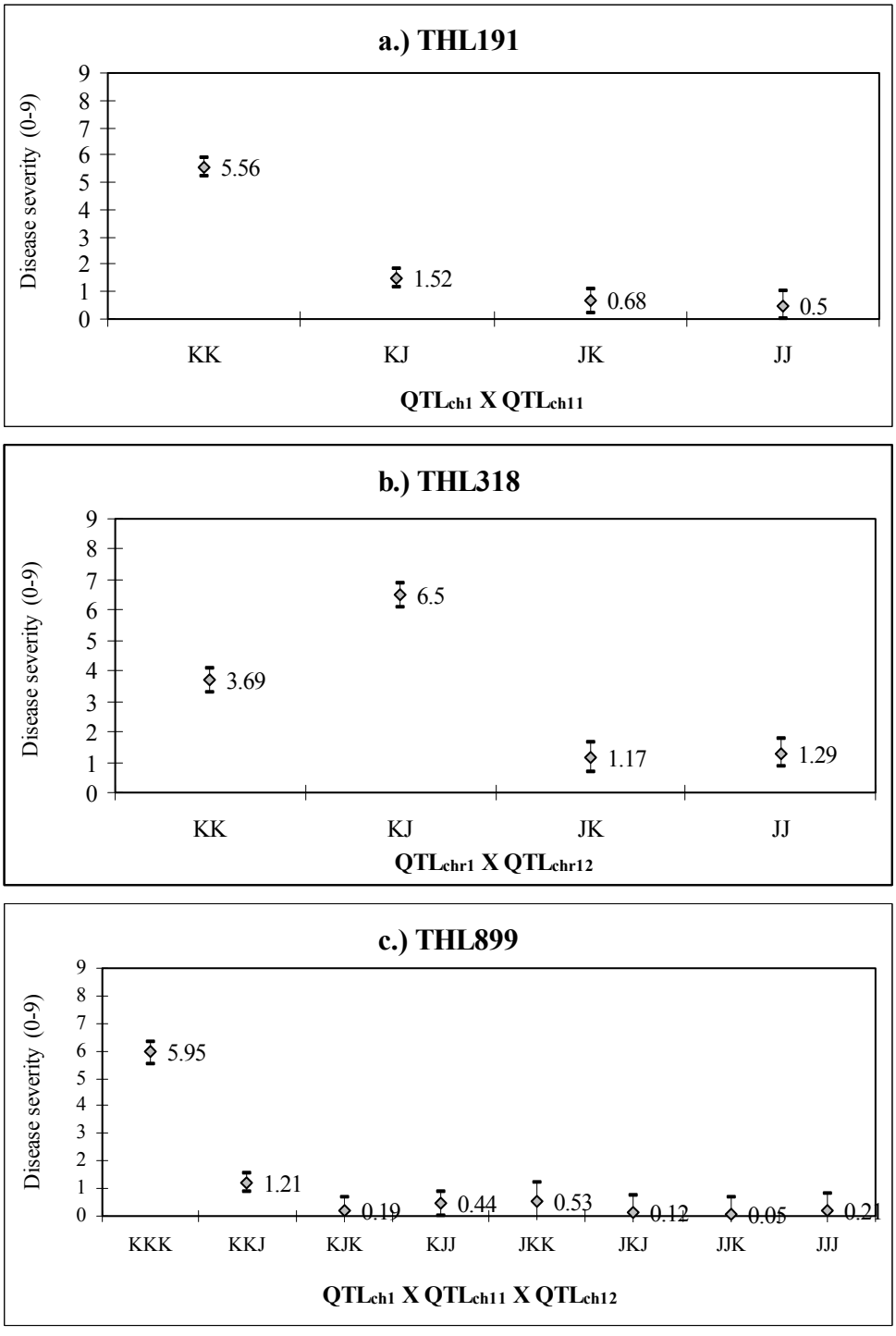


Figure 12 Interactions between QTLs for neck blast resistance on chromosome1, 11 and 12 obtained from the inoculation with THL191, THL318 and THL899 having 95.0 % LSD interval

Phenotypic reactions obtained from F_6 RILs population showed that when THL191 was used, There were 189 lines resistant to both leaf and neck blast inoculations. The high number of lines indicated inheritance of resistant alleles from the resistant parent, JHN. The figure of 190 lines was recorded as susceptible lines in response to both methods of inoculation with THL191 suggesting that these lines had susceptible alleles from the susceptible parent, KDML105. Apart from what has been mentioned, there were 2 groups of lines showing different reactions to leaf and neck blast screenings. The first group was resistant to leaf blast but susceptible to neck blast screenings. On the contrary, the second one showed reverse reactions for corresponding screenings. However, both of them represented lines having 16.25 and 11.28 % phenotypic recombination, respectively. These resulted in the total of 27.5% phenotypic recombination.

Results were slightly different when THL318 was inoculated onto the population, 231 lines were resistant to both leaf and neck blast inoculations. Once again, this suggested that resistant alleles were obtained from JHN. The difference was that there were 110 lines resistant to leaf blast but susceptible to neck blast inoculation recombination percentage being 24.07%. The high number of lines suggested that KDML105 was a contributor for their resistant alleles. The finding that THL318 could detect resistant alleles from both parents. The calculated phenotypic recombination percentage being 9.40% was obtained from the lines susceptible to leaf blast but resistant to neck blast infection. These resulted in the total of 34.47% phenotypic recombinant. The last group of lines being susceptible to both screenings suggested that susceptible alleles were contributed by KDML105.

Inoculation with THL899 followed the same trend as that of THL191. There were 269 lines being resistant to both leaf and neck blast screenings. The high number of lines suggested that resistant alleles were contributed by JHN. In contrast, 113 lines being susceptible to both screenings indicated that KDML105 was responsible for susceptible alleles. Recombination was shown in 2 groups of lines being resistant to leaf blast but susceptible to neck blast and *vice versa*. These 2 groups contained lines with 14.39 and 13.25% recombination leading to a total of 27.6% which was similar to that obtained from THL191 (Table 7).

Table 7 Phenotypic reaction of RILs screened for leaf and neck blast by three isolates

Isolates	LB	NB	No. of lines	The Recombination (%)	Total Recombination (%)
THL191	R	R	189	-	27.5
	R	S	85	16.25	
	S	R	59	11.28	
	S	S	190	-	
THL318	R	R	231	-	33.47
	R	S	110	24.07	
	S	R	43	9.40	
	S	S	73	-	
THL899	R	R	269	-	27.6
	R	S	76	14.39	
	S	R	70	13.25	
	S	S	113	-	

Results were further clarified by combining those obtained from phenotypic reactions and QTL analysis based on peaks of QTLs showing the presence of major QTL on chromosome11 and the peaks showing the absence of QTL on chromosome1. These peaks were detected by RM224 and RM212, correspondingly. Combined results are presented in Table 8.

Phenotypic reactions in response to leaf and neck blast screenings based on resistant alleles on chromosome 11 were classified into 4 groups; R,R: R,S: S,R and S,S. Lines showing R,S: S,R responses suggested a presence of recombination. Thus, recombinant lines detected by THL191 were 17 and 11, respectively.

Likewise, phenotypic reactions in response to leaf and neck blast screenings based on susceptible alleles detected on chromosome 1 and 11 by THL191 were also classified into 4 groups; R,R: R,S: S,R and S,S. Recombinant lines with R,S and S,R responses were 19 and 19 following THL191 inoculations, correspondingly.

Table 8 Number of lines showing response to leaf and neck blast screenings following THL191 inoculations based on resistant alleles only chromosome 11 and susceptible alleles on chromosome 1 and 11

Phenotypic response		THL191	THL191
LB	NB	Resistant alleles only chr.11	Susceptible alleles on chr. 1, 11
R	R	53	28
R	S	17	19
S	R	11	19
S	S	33	88
Total no. of lines		114	154

Results showing recombinant lines with different response to leaf and neck blast screenings using the same isolate suggested that there might be different genes for leaf and neck blast resistance situated within the same region in the chromosome.

DISCUSSION

1. Parental screening

JHN was chosen to be studied as one of the parents being expected to confer blast resistance to progenies while inheritance of good cooking quality was expected from the other parent, KDML105. Under natural conditions, JHN has long been resistant to both leaf and neck blast. Results from the details on leaf and neck blast scorings for parental lines extracted from 587 RILs population screening set together with results from parental screening have supported prior observation that JHN was broadly resistant to a wide range of blast isolates. This was seen as high values of BRS for JHN being 0.99 and 1 for leaf and neck blast screenings, respectively. BRS values for KDML105 was incomparable with those of JHN, as the value was only half and as small as one fifth in case of leaf and neck blast screenings, correspondingly. These can be explained by frequency of scores on RILs population tested against blast isolates when JHN had scores towards resistance (0.0-3.0) while KDML105 provided some resistant scores but more towards susceptible scores (4.1-9.0). Thus, progenies with alleles contributed by JHN would have a high potential to be more durable than those with alleles from KDML105 leading to a further investigation.

2. Phenotypic reaction of leaf and neck blast resistance

The use of 3 isolates for leaf and neck blast screenings resulted in 6 sets of data for blast reaction. Data from four out of six sets had fitted a ratio of 3: 1 meaning the presence of at least 2 QTLs while a ratio of 1: 1 meant the possibility of 1 QTL being detected.

For leaf blast screening, THL191 and THL318 caused the same phenotypic reaction ratio for R: S on the RILs population. Similarly, these two isolates also gave the same ratio for neck blast screening. The same trend for each screening following the inoculation of THL191 and THL318 indicated that these two isolates had triggered corresponding resistant genes/QTLs possibly at the same region on the chromosome. The ratio of 3:1 for leaf blast screening compared to 1: 1 fot the neck blast one suggested that some genes responsible for leaf blast resistance were ineffective at the reproductive stage.

The RILs population responded to THL899 infection slightly different from those of THL191 and THL318. The R: S ratio for both leaf and neck blast screening using THL899 were equivalent to 3: 1. In comparison to the response given by THL191 and THL318, it seemed that THL899 also triggered the activation process of genes/QTLs at the same region on the chromosome as occurred with the first two isolates following the inoculation at vegetative stage. The only difference was that these effective genes triggered by THL899 during vegetative stage remained effective at the reproductive stage too. Therefore, THL899 appeared to be ideal for both leaf and neck blast screenings.

3. Correlation between leaf and neck blast severity

An attempt was made to investigate the relationship between leaf and neck blast severity. Apparently, correlation coefficient (r) obtained from the inoculation of selected blast isolates was rather low, ranging from 0.28 – 0.56. The low correlation coefficient between leaf and neck blast severity followed the inoculation with THL191 and THL318 being 0.32 and 0.28 meant a weak relationship between leaf and neck blast pathosystems using these two isolates. This was possibly due to the inability to trigger some resistant genes at the reproductive stage indicated in the previous section. The higher correlation coefficient of 0.56 obtained from the inoculation with THL899 showed a stronger relationship between the two pathosystems and may be explained as the possible ability of THL899 to induce the action of some genes at both vegetative and reproductive stages. Some parts of the results were similar to the previous finding of Zuuang *et al*. (1997) who reported the difference in blast reaction at seedling and reproductive stages. Teng *et al*. (1991) mentioned that leaf and neck blast are two different pathosystems due to time discontinuity and the relationship between the two is yet to be defined. However, results in this section had shown and suggested the possible explanation for the relationship between the two pathosystems.

Apart from the reason on the inability to induce the action of resistant genes at reproductive stage causing low correlation coefficient between leaf and neck blast mentioned above, the other reason could be partly due to the fact that leaf and neck blast screenings were undertaken in different seasons as the workload had made it rather difficult to be handled in the same season.

4. Linkage map construction and QTL analysis

The linkage map on chromosome1, 11 and 12 obtained from this study revealed the same order for microsatellite markers as those reported by previous findings using the population derived from Azucena and IR64 (Wu and Tanksley, 1993; Chen *et al*., 1997; McCouch *et al*., 1997; Temnyhk *et al*., 2001).

In this study, QTLs with JHN as a sole contributor for resistant alleles were found on chromosome1 with high LOD for both leaf and neck blast resistance. However, the LOD for BRS score was higher for neck blast resistance. The peak of QTLs on chromosome1 was found near RM212 marker which was close to RZ19 – RG331 flanking marker reported by Prashanth *et al*. (2002) who also found QTL on this chromosome. On chromosome11, LOD values for both leaf and neck blast resistance were rather high ranging from 22 – 40 and so did the BRS scores (23 - 32). These indicated the strong presence of polygenes ofwhich the alleles were contributed by JHN. One of the possible major genes could be *Pi-7(t)* since it was reported to be linked to RG103 and Npb186 flanking marker (Wang *et al.,* 1994) which was mapped close to RM224 marker used in this study. The other possible qualitative gene on chromosome11 was *Pi 1* linking to the flanking marker RZ536 (Hittalmani *et al.,* 2000) which was mapped at only 6 cM far from RM224 (Chen *et al.,* 1997). Inukai *et al*. (1996) have reported that *Pi-7(t)* was allelic or closely linked to *Pi 1* on

chromosome11. Tabien *et al*. (2000) reported that *Pi-lm*^{2} was linked to RZ 536 which was close to RM224. Therefore, *Pi lm*^{2} could be the third major gene detected on chromosome11 in this study. Apart from major genes, QTL on chromosome11 could also contribute to a broad spectrum resistance as Prashanth *et al*. (2002) had reported the presence of QTL between RG103 – RZ536 flanking marker linking to RM224. Unlike those of chromosome1 and 11, QTLs on chromosome12 had resistant alleles contributed by both KDML105 and JHN. The presence of a resistant gene *Pi ta* linking to RZ397 at the distance of 3.3 cM on chromosome12 was reported by Hittalmani *et al*. (*2000*). Thus, *Pi ta* could be one possible resistant gene found in this study since RZ397 was 12.1 cM far from RM179 being used. Since Kiyosawa (1984) had found alleles between *Pi ta* and *Pi 4a(t)* and Inukai *et al*. (1994) reported that *Pi 4a(t)* was allelic to *Pi 4b(t),* therefore, *Pi 4a(t)* and *Pi 4b(t)* could be the other two possible major genes on chromosome12. In Addition to this, Mew *et al*. (1994) reported that there could be a cluster of QTls on chromosome12 with *Pi 4(t)* being closely linked to RG869 and RZ397. Thus, *Pi 4(t)* was highly likely to be the fourth major gene detected on chromosome12. Apart from four possible major genes mention above, QTLs also contributed to resistant alleles on this chromosome which supported previous findings of Sirithunya *et al. (*2001) that the detection of QTLs for blast resistance from DHL derived from IR64 and Azucena screened with 16 blast isolates collected from all over Thailand had located QTLs on chromosome1, 2, 6, 8, 11 and 12.

There was one interesting point involving the detection of QTLs followed the inoculation with THL899. Although R: S ratio from phenotypic reaction for both leaf and neck blast screenings using THL899 were equivalent to 3: 1 meaning 2 QTLs were present, data from the linkage map construction showed that 3 QTLs were detected by this isolate. This result may be due to the fact that the population used in this study was reasonably large, therefore, even a small QTL could be detected.

The homozygous and heterozygous lines with identified sources of alleles having different phenotypic reaction for leaf and neck blast screenings indicated the occurrence of crossing over.

Considering information obtained from combining the data between phenotypic reactions and QTL analysis based on peaks of QTLs showing the presence of major QTL on chromosome11 and the peaks showing the absence of QTL on chromosome1, Results showing recombinant lines with different response to leaf and neck blast screenings using the same isolate suggested that there might be different genes for leaf and neck blast resistance situated within the same region in the chromosome.

CONCLUSION

KDML105 and JHN, as the parents, were primarily screened for broad resistance spectrum (BRS) using 97 isolates collected from all over Thailand. BRS scores for JHN confirmed its resistance quality as they were 0.99 and 1.00 for leaf and neck blast screenings, respectively. These values were approximately 2-3 times higher than those of KDML105 for corresponding screenings. Five hundred and eighty-seven RILs population derived from KDML105 x JHN were inoculated with three selected blast isolates designated THL191, THL318 and THL899 aiming for phenotypic distribution of leaf and neck blast reactions. Chi-square (x^2) test revealed that 4 out of 6 sets of data had fitted a ratio of 3: 1 for resistant: susceptible population *i.e.* THL191 THL318 and THL899 for leaf blast screening and THL899 for neck blast screening. This suggested the presence of 2 QTLs apart from this, there were two sets of data, THL191 and THL318 for neck blast screening, fitted a ratio of 1: 1 for resistance: susceptible population meaning 1 QTL was highly likely to be presented. Investigation on the correlation between leaf and neck blast severity was carried out and resulted in a rather low correlation coefficient being 0.28-0.56 indicating the presence of two different pathosystems. The higher correlation coefficient for the relationship between leaf and neck blast severity being 0.58 for THL899 together with a phenotypic reaction, R: S ratio, being 3: 1 for both leaf and neck blast screenings using this isolate suggested that it was ideal to use for both screenings since it seemed to have an ability to trigger the action of genes/QTLs at both vegetative and reproductive stages. To locate QTLs on the chromosomes, linkage map construction using 16 SSRs markers and QTL analysis revealed the presence of QTLs on three chromosomes; 1, 11 and 12. The peak of QTLs on chromosome1 was close to RM212 marker. On chromosome11, high LOD values for both leaf and neck blast resistance (22-40) and the broad resistance spectrum scores (23-32) with JHN being a sole contributor for all resistant alleles indicated the presence of polygenes on this chromosome. The detection of QTLs on chromosome11 found the peaks with high LOD values (38-40) being close to RM224 and between AC113249 – RM224. Furthermore, a contribution to the broad spectrum resistance on chromosome11 could also be from QTL. While resistant alleles on both chromosome1 and 11 were contributed by JHN, those on chromosome12 were contributed by both JHN and KDML105. However, the peak for the detection of QTLs on chromosome12 was found between RM179 – RM309 with the highest LOD value being 25 and resistant alleles donated by KDML105. One extra QTL being detected from the linkage map construction and QTL analysis suggest that the size of population used was reasonably large so that even a small QTL could be detected. When phenotypic reactions were combined with QTL analysis based on peaks of QTLs showing the presence of major QTL on chromosome11 and the peaks showing the absence of QTL on chromosome1, Results showing recombinant lines with different response to leaf and neck blast screenings using the same isolate suggested that there might be different genes for leaf and neck blast resistance situated within the same region in the chromosome.

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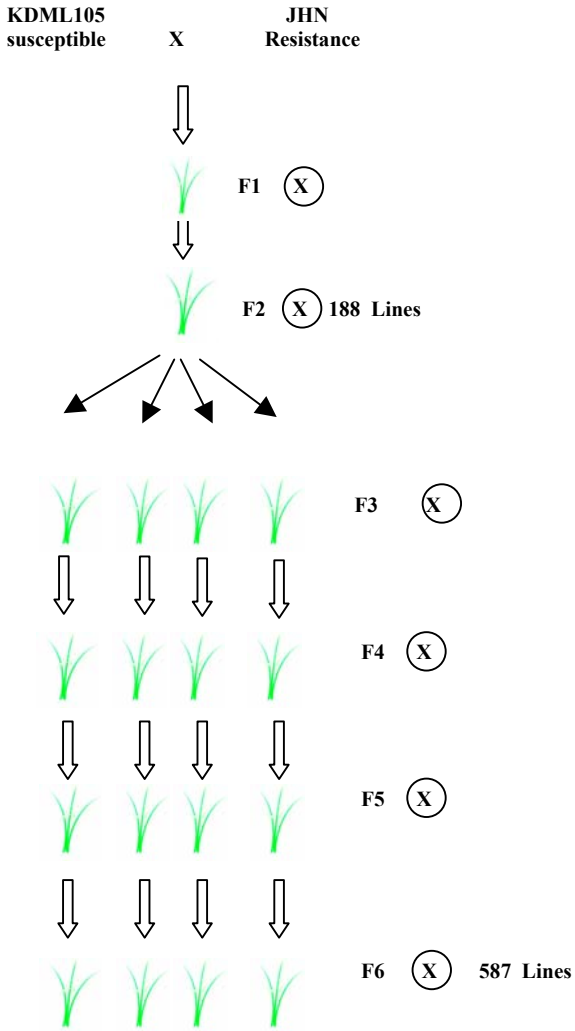
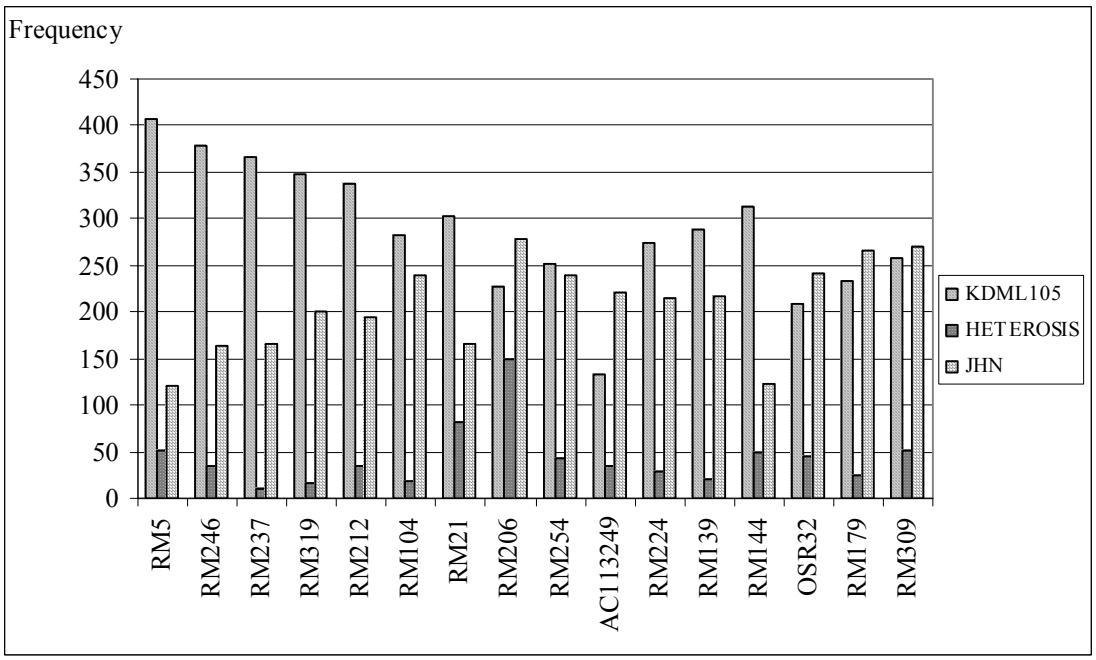
Appendix



Appendix Figure 1 Breeding diagram for the production of Recombinant Inbred Lines (RILs) from KDML105 X JHN population



Appendix Figure 2 Distribution of alleles on 587 RILs derived from KDML105 and JHN population detected by 16 SSRs markers

Appendix Table 1 Preliminary screening for leaf and neck blast resistance on differential parental set

No	Lines/Varities	THL191		THL861		THL329		THL862		THL149		THL318	Field
		LB	NB	LB	NB	LB	NB	LB	NB	LB	NB	NB	LB
1.	KDML105	5	2.8	5	9	5	9	5	8	3	7.4	-	9
2.	JHN	1	0	1	0	1	0	0	0	1	0.2	0	0
3.	Azucena	5	0	5	0.6	5	0	5	0	3	1.2	0	3
4.	IR64	1	0	5	0	5	1.8	1	0	0	0	0	5
5.	IR62266-42-6-2	5	0	1	0	1	0	5	0.2	0	4	0	5
6.	CT9993-5-10-1-M	1	0	1	0	1	0	1	0	1	1.8	0	0
7.	FR13A	-	0	-	0.2	-	1	-	0	-	9	7	9
8.	CT6241-17-1-5-1	1	0	1	0.2	5	0	1	0	1	2.2	0	0
9.	IR58821-23-1-3-1	5	7.8	5	9	5	6.3	5	3.2	5	2	0	9
10.	IR52561-UBN-1-1-2	5	9	5	9	9	0	5	8.6	5	9	9	9
11.	IR57514-PMI-5-B-1-2	1	0	1	0	5	2	1	0	1	9	9	0
12.	Abhaya	7	7.8	5	8.6	9	0	5	8.2	3	0	0	9
13.	SPR1	1	0	1	1	5	0	1	0	1	0	0	7
14.	PSL2	1	0	1	0.2	5	2	1	0	1	0	-	0
15.	HY71	5	-	1	9	5	0	3	2.8	1	-	0	0
16.	KTH17	5	9	5	1.8	-	5.4	5	-	5	7.8	9	9
17.	CO39	5	7	5	9	5	0	7	3	5	0.6	7	9

Appendix Table 2 Details of 102 blast isolates used for BRS screening on a differential parental set

NO	RFLP group	Isolate Code	Host cultivar	Plant Part	Location
1	1	THL 32	Carreon	leaf	Chiang Mai
2	1	THL 61	Salumpikit	leaf	Chiang Mai
3	1	THL136	KTH17	leaf	Chiang Mai
4	1	THL 1091	Weed	leaf	Chai Nat
5	2	THL 138	SPTUR-84027-TLG-B3-25	leaf	Chiang Mai
6	2	THL 149	KTH17	leaf	Chiang Mai
7	3	THL 104	RD 8	neck	Phrae
8	3	THL 126	RD 6	neck	Sakon Nakhon
9	3	THL 159	RD 6	leaf	Chiang Rai
10	**3**	**THL 191**	**Sinna Sivapu**	**leaf**	**Phitsanulok**
11	3	THL 215	KDML 105	neck	Nakhon Pathom
12	3	THL349	Aromatic Cultivar	leaf	Nakhon Ratchasima
13	3	THL 676	KDML 105	panicle	Karnchanaburi
14	3	THL 699	KDML 105	seed	Chanthaburi
15	3	THL715	Unknow	neak	Phayao
16	3	THL 791	KDML 105	neck	Lampang
17	3	THL 794	Khao nieaw sanpathong	neck	Chiang Mai
18	3	THL 829	Kaw koe deaw	neck	Sa kaeo
19	3	THL 839	KDML 105	neck	Buri Ram
20	3	THL 889	KDML 105	neck	Krabi
21	3	THL924	Exp. No.6	neck	Phuket
22	3	THL 961	KDML 105	seed	Ubon Ratchathani
23	3	THL966	Khao Ma-Eng	neck	Surin
24	3	THL 970	Yai kai (weed)	neck	Khon Kaen
25	3	THL1013	Unknow	neck	Sa Kaeo
26	3	THL 1089	KDML 105	leaf	Chanthaburi
27	3	THL1109	Khao mai tak	panicie	Krabi
28	3	THL1118	KDML105	neck	Ubon Ratchathani
29	3	THL 1127	Khaw lab nok	neck	Phuket
30	4	THL 105	Seomjinbyeo	leaf	Chiang Mai
31	4	THL 760	KDML 105	neck	Mae Hong Son
32	4	THL 797	RD15	neck	Mae Hong Son
33	4	THL 831	Khao jaeng(Khao nieaw dum)	seed	Mae Hong Son
34	5	THL 652	RD6	neck	Chiang Mai
35	5	THL 923	Exp.No. 6	neck	Phuket
36	5	THL 1103	Khaw jumpa	neck	Krabi
37	5	THL 1126	Khaw leb nok	neck	Phuket
38	6	THL 364	KDML 105	leaf	Nakhon Ratchasima
39	6	THL 802	RD6	neck	Lamphun
40	6	THL 812	Wild rice	seed	Ubon Ratchathani
41	6	THL 821	KDML 105	seed	Nan
42	6	THL 1020	Khao nieaw sanpathong	neck	Phayao
43	6	THL 1135	RD 6	neck	Lamphun
44	7	THL 55	IR 64	leaf	Phitsanulok
45	7	THL 84	CNT92001-PSL-18-6-1	leaf	Phitsanulok
46	7	THL 274	KTH17	leaf	Chai Nat
47	7	THL949	Unknow	neck	Suphan Buri
48	7	THL 951	Weed ; yah ta hang	leaf	Surat Thani
49	7	THL1001	Unknown	neck	Bangkok
50	7	THL 1008	RD	neck	Trat
51	7	THL 1010	Unknown	neck	Sa Kaeo

Appendix Table 2 (Cont'd) Details of 102 blast isolates used for BRS screening On a differential parental set

NO	RFLP group	Isolate Code	Host cultivar	Plant Part	Location
52	8	THL 127	SPR2	leaf	Pathum Thani
53	8	THL 360	KTH17	leaf	Phitsanulok
54	8	THL 903	Khao kaw ta hang (KTH)	leaf	Ratchaburi
55	9	THL 828	RD7 (radiated)	neck	Chiang Mai
56	9	THL 861	KDML 105	neck	Chiang Mai
57	10	THL 102	RD10	neck	Phrae
58	11	THL 68	Paikantao	leaf	Phitsanulok
59	11	THL 112	SPR2	leaf	Pathum Thani
60	11	THL 129	SPR2	leaf	Pathum Thani
61	11	THL 717	RD 23	neck	Pathum Thani
62	12	THL 139	KTH17	leaf	Chiang Mai
63	12	THL 908	Khao luk pla	panicle	Yala
64	13	THL 122	RD6	leaf	Chiang Rai
65	13	THL 959	Khaw nang phaya 132	seed	Phatthalung
66	13	THL 975	Khao! Very serious!	seed	Khon Kaen
67	14	THL 755	Khao hom khlong luang	neck	Mae Hong Son
68	14	THL 1140	KDML 105	seed	Mae Hong Son
69	not	TH3	Barley (*Hordeum vulgare*)	seed	Khon Kaen
70	not	THL 16	Azucena	leaf	Ubon Ratchathani
71	not	THL 59	NP125	leaf	Chiang Mai
72	not	THL116			
73	not	THL 258	H.Y.71	leaf	Chai Nat
74	not	THL 346	HY 71	leaf	Phitsanulok
75	not	THL 841	Unknown	neck	Phrae
76	**not**	**THL 899**	**PTT88114-10-1-1**	**collar**	**Surat Thani**
77	not	THL1111	Unknown	neck	Nakhon Si Thammarat
78	Not	THL1160	Unkhow	leaf	
79	UN	TH16	Barley (*Hordeum vulgare*)	seed	Khon Kaen
80	UN	TH21	Barley (*Hordeum vulgare*)	seed	Khon Kaen
81	UN	TH25	Barley (*Hordeum vulgare*)	seed	Khon Kaen
82	UN	THL48	KTH17	leaf	Phitsanulok
83	UN	THL80	Barley (*Hordeum vulgare*)	seed	Khon Kaen
84	UN	THL 82	SPR2	leaf	Pathum Thani
85	UN	THL 110	Cauvepy	leaf	Chiang Mai
86	UN	THL219	SPR2	leaf	Pathum Thani
87	**UN**	**THL 318**	**Salumpikit**	**leaf**	**Chiang Mai**
88	UN	THL 329	RD10	neck	Phrae
89	UN	THL 340	KTH17	leaf	Phitsanulok
90	UN	THL 472	CV Khao Prataan	leaf	Phichit
91	UN	THL 483	Khao Pruwang	collar	Tak
92	UN	THL498	Barley (*Hordeum vulgare*) Morex	leaf	Chiang Mai
93	UN	THL557	SPR90	neck	Tak
94	UN	THL800	Leuang pra thew	neck	Nakhon Ratchasima
95	UN	THL 843	RD6	neck	Nan
96	UN	THL925	Exp. No.6	neck	Phuket
97	UN	THL1000	wild rice,	seed	Uthai Thani
98	UN	THL1058	barley (*Hordeum vulgare*) cultivar BYT- II	leaf	Chiang Mai
99	UN	THL1107	khao no.4	seed	Narathiwat
100	UN	THL 1123	wild rice	seed	Loei
101		BT14	JHN	leaf	Ubon Rachathani
102		HY71	HY71	leaf	Phitsanulok

Appendix Table 3 Formula for blast culture medium

Component	Percentage to distilled water
Yeast extract	0.2
Polished rice	2
Agar	2

Appendix Table 4 Details of 16 SSRs markers

No.	Marker name	Accession number	Repeat type and length	Size range	Forward Primer	Reverse primer
1.	RM5	AF344007	(GA)14	108-130	tgcaacttctagctgctcga	gcatccgatcttgatggg
2.	RM21	AF344021	(GA)18	132-170	acagtattccgtaggcacgg	gctccatgagggtggtagag
3.	RM104	D24755	(GA)9	222-238	ggaagaggagagaaagatgtgtgtcg	tcaacagacacaccgccaccgc
4.	RM139	D48278	(CT)5	396-410	gagagggaggaagggaggcggc	ctgccatggcagagaaggggcc
5.	RM144	X67711	(ATT)11	214-255	tgccctggcgcaaatttgatcc	gctagaggagatcagatggtagtgcatg
6.	RM179	D47661	(TG)7	188-190	ccccattagtccactccaccacc	ccaatcagcctcatgcctcccc
7.	RM206	AF344027	(CT)21	128-202	cccatgcgtttaactattct	cgttccatcgatccgtatgg
8.	RM212	AF344033	(CT)24	112-134	ccactttcagctactaccag	cacccatttgtctctcattatg
9.	RM224	AF344045	(AAG)8(AG)13	124-158	atcgatcgatcttcacgagg	tgctataaaaggcattcggg
10.	RM237	AF344057	(CT)18	126-136	caaatcccgactgctgtcc	tgggaagagagcactacagc
11.	RM246	AF344066	(CT)20	97-118	gagctccatcagccattcag	ctgagtgctgctgcgact
12.	RM254	AF344073	(TC)6ATT(CT)11	183-193	agccccgaataaatccacct	ctggaggagcatttggtagc
13.	RM309	AF344134	(GT)13	165-169	gtagatcacgcacctttctgg	agaaggcctccggtgaag
14.	RM319	AF344144	(GT)10	132-134	atcaaggtacctagaccaccac	tcctggtgcagctatgtctg
15.	OSR32	X07515	(CTT)7	271-273	gagatggccccctccgtgatgg	Tgccctcaatcggccacacctc
16.	AC113249		(GGA)10	229	catacctagcccgaaccaa	aagtggaggacgcgaatg

Appendix Table 5 ANOVA of leaf blast data from JHN as check from 587 RILs population screening set inoculated with THL191

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	.0952381	2	.047619	.27	.7644
Within groups	3.14286	18	.174603		
Total (Corr.)	3.2381	20			

Appendix Table 6 ANOVA of neck blast data from JHN as check from 587 RILs population screening set inoculated with THL191

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	.166667	2	.0833333	.38	.6897
Within groups	2.83333	13	.217949		
Total (Corr.)	3.0	15			

Appendix Table 7 ANOVA of leaf blast data from KDML105 as check from 587 RILs population screening set inoculated with THL191

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	4.82581	2	2.41291	2.48	.1157
Within groups	15.5952	16	.974702		
Total (Corr.)	20.4211	18			

Appendix Table 8 ANOVA of neck blast data from KDML105 as check from 587 RILs population screening set inoculated with THL191

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	.466667	2	.233333	. 7	.9372
Within groups	46.5333	13	3.57949		
Total (Corr.)	47.0	15			

Appendix Table 9 ANOVA of leaf blast data from JHN as check from 587 RILs population screening set inoculated with THL318

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	.0952381	2	.047619	.19	.8306
Within groups	4.57143	18	.253968		
Total (Corr.)	4.66667	20			

Appendix Table 10 ANOVA of neck blast data from JHN as check from 587 RILs population screening set inoculated with THL318

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	.104167	2	.0520833	.20	.8187
Within groups	3.33333	13	.25641		
Total (Corr.)	3.4375	15			

Appendix Table 11 ANOVA of leaf blast data from KDML105 as check from 587 RILs population screening set inoculated with THL318

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	16.5255	2	8.26275	1.22	.3238
Within groups	94.5333	14	6.75238		
Total (Corr.)	111.059	16			

Appendix Table 12 ANOVA of neck blast data from KDML105 as check from 587 RILs population screening set inoculated with THL318

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	9.79248	2	4.89624	3.46	. 563
Within groups	22.6286	16	1.41429		
Total (Corr.)	32.4211	18			

Appendix Table 13 ANOVA of leaf blast data from JHN as check from 587 RILs population screening set inoculated with THL899

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	.0952381	2	.047619	.18	.8397
Within groups	4.85714	18	.269841		
Total (Corr.)	4.95238	20			

Appendix Table 14 ANOVA of neck blast data from JHN as check from 587 RILs population screening set inoculated with THL899

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	.0666667	2	.0333333	.15	.8641
Within groups	2.93333	13	.225641		
Total (Corr.)	3.0	15			

Appendix Table 15 ANOVA of leaf blast data from KDML105 as check from 587 RILs population screening set inoculated with THL899

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	1.96667	2	.983333	1.06	.3738
Within groups	12.0333	13	.925641		
Total (Corr.)	14.0	15			

Appendix Table 16 ANOVA of neck blast data from KDML105 as check from 587 RILs population screening set inoculated with THL899

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	13.0406	2	6.5203	2.78	. 918
Within groups	37.4857	16	2.34286		
Total (Corr.)	50.5263	18			

Protocols

1. DNA Isolation for DNA Trap

1. Grind tissue ~ 100 mg or use 100~200 µl of sample.
2. Add Extraction Buffer 1 ml, mix it well by vortexing and incubate at 65 °c for 10 min.
3. Add 100 µl of Neutralizer and mix by vortexing then place back on ice for another 5 min. Centrifuge the tube at 14,000 rpm for 5 min. at room temperature.
4. Transfer top liquid into new 1.5 ml tube and add 500 µl of Trapping buffer and mix gently and centrifuge at 14,000 rpm for 3-5 sec.
5. Pour liquid out and wash with 500 µl of Washing Buffer I, mix thoroughtly and centrifuge at 14,000 rpm for 3-5 sec. Repeat this step with 500 µl of washing buffer II.
6. Pour off the liquid and dry the pellet before adding 100 µl or Elution buffer and mix thoroughtly. Then place tube in 65 °c incubate for 5 min.
7. Centrifuge the tube at 14,000 rpm for 3-5 sec. Then, transfer the liquid part (DNA) into new tube.

2. Polymerase Chain Reaction (PCR) Amplification

1. set up a 100 µl in a 0.05 ml microfuge tube, mix and overlay with 1 drop of mineral oil.

	1X
Template DNA	2
DNTP	2
Buffer	1
$MgCl_2$	0.8
Primer Forward	0.25
Reward	0.25
Taq DNA	0.2
dH_2O	3.5
Total	10

2. Perform 35 cycles of PCR using the following temperature profile :

Denaturation	94°c	30	Seconds
Annealing	55°c	30	Seconds
Extension	72°c	2	Minutes

3. cycling should conclude with a final extension at 72°c for 5 minutes. Reaction are stopped by chilling to 4°c

3. Acrylamide Gel Preparation

1. Wipe a chamber once with 95% EtOH.
2. Wipe a chamber with clear view solution, let it dry.
3. Wipe a gel plate with 95% EtOH for 3 times.
4. Wipe a gel plate with 700 µl bind silane solution, let it dry then clean with

95% EtOH for 3 times again. (bind silane solution : 3 µl bine silane + 1 ml 0.5% acetic acid in 95% EtOH)

5. Set the chamber and gel plate carefully.
6. Prepare acrylamide gel : 50 ml of 4.5 acrylamide gel + 70 µl TEMED + 350 µl of 10% A.S.P. shake and use immediately.
7. Pour gel into the gel-set carefully, push the comb into the top of the filled Gel-set.
8. Let gel set for 30 min or 1 hour.
9. Pre-run by add 1XTBE buffer (1.5 1 for 1 gel-set) than remove the comb and clean up the wells.
10. Pre-running is done by run 100 V and set temperature at 50 ^{o}c
11. About 30 min, when the temperature is reaching to 49 ^{o}c, heat DNA samples that will be loaded onto gel.
12. Heat DNA samples at 94-95 ^{o}c for 3 min., then put immediately on ice, they are ready for loading onto the gel.
13. Stop pre-running, clean the wells and load DNA samples onto the gel.
14. Normally we run at 60 V for about 15-18 cm length.

4. <u>Silver staining</u>

1. Fix with 10% acetic acid, shake for 20 min.
2. Wash 3 times with dH_2O, 2 min. each.
3. Stain with silver staining solution : shake for 30 min. (Silver staining solution : 1 g/l Silver nitrate ($AgNO_3$) + 1.5 ml/l formaldehyde)
4. Quick wash for 10 s with dH_2O.
5. Stain with developer solution : Until DNA bands are appeared. Developer : 30 g/l Sodium carbonate anhydrous + 400 µl of Sodium thiosulfate + 1.5 ml/l formadehyde (keep in the fridge then use it when it is cold)
6. Stop by adding 10% acetic acid, shake.
7. Wash with dH_2O for 5 min. then let it dry.