MOLECULAR CHARACTERIZATION OF QTL FOR SLOW LEAF RUSTING RESISTANCE, AND A GENE FOR POWDERY MILDEW RESISTANCE IN WHEAT

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PREFACE

Wheat is an important crop and is grown on an area over 200 million hectares from which almost 600 million tones of grain are harvested annually (FAO; http://www.fao.org/). Wheat leaf rust and powdery mildew are two wheat diseases that affect wheat yield stability. The combination of durable resistance genes and race-specific resistance genes is a promising way to control leaf rust and powdery mildew. This study was conducted to characterize quantitative trait loci (QTL) for slow leaf rusting resistance in wheat line CI 13227, and powdery mildew resistance gene Pm3a in wheat cultivar Suwon 92. QTLs associated with slow leaf rusting resistance, leaf rust infection rate, leaf rust infection duration, and latent period were identified and characterized. The Pm3a gene was mapped on chromosome 1A, and AFLP markers, SSR marker, and morphological marker closely linked to Pm3a were detected. The identified SSR marker and morphological marker can be directly used in marker-assisted selection.

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FORMAT OF THESIS

This thesis present in the Theoretical and Applied Genetics style and format allowing for independent chapters (Chapter II, Chapter III, and Chapter IV) to be suitable for submition to scientific journals. Three papers have been prepared from research data collected at Oklahoma State University to partially fulfill the requirements for the degree of Doctor of Philosophy. Each paper is complete in itself containing an abstract, introduction, materials and methods, results, discussion, and references section.

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Chapter I

Introduction and Literature Review

Wheat is a globally important food crop both in terms of acreage and production, and is grown on an area of over 200 million hectares and yields about 600 million tones annually (FAO; http://www.fao.org). Wheat production is crucial for the security of world food. As world population is constantly growing, demand on food supply is rapidly increasing. Therefore, continually increasing wheat yield is essential to meet the growing population in the world. However, wheat diseases such as leaf rust and powdery mildew continue to be major constraints to increasing wheat yield.

Wheat leaf rust

The pathogen.

Wheat leaf rust is a common and widely distributed wheat disease. It is caused by theobligate, biotrophic fungus *Puccinia triticina,* which belongs to family *Pucciniaceae* of the order *Uredinales* of the class *Basidiomycetes* (Agrios 1997). The complete life cycle of the leaf rust pathogen is complicated, including both sexual and asexual stages. The asexual stage occurs on wheat or related grasses and the sexual stage occurs on an alternate host, typically meadow rue (*Thalictrum spp*) (Agrios 1997; Knott 1989). The complete life cycle includes five types of spores: uredospores, teliospores, basidiospores, spermatia, and aeciospores. Pustules which form on the wheat leaf are called uredia, and contain urediospores that directly damage the wheat leaf. The uredia sporulate primarily on the upper wheat leaf surfaces. The urediospores develop within the uredia. When the spores mature, they rupture the epidermis of the leaf surface to form the rust symptom on wheat leaves. The matured urediospores are then blown by wind and land on other wheat

plants to initiate new infections. The spores can also be dispersed long distances by wind. Urediospores are adapted to long distance dispersal by wind.

In the central Great Plains of the United States, the leaf rust pathogen doesn't have the alternate host for the completion of its sexual stage during the off-seasons, and the urediospore is the only functional spore type of the pathogen in this area. Instead, the urediospores move and develop wheat leaf rust from northern Mexico and southern Texas northward across the Great Plains into Canada in the spring, and then move from those northern areas back to the south during the fall. This annual cycle of pathogen movement and disease development in Great Plains is known as the "Puccinia pathway" (Eversmeyer et al. 2000). Due to the lack of a sexual stage, mutation become s a major means of genetic variation of the pathogen.

The leaf rust pathogen is considered a cool temperature species, with the favorable temperature for rust development between15 °C to 24 °C. When winter wheat resumes growth in early spring, cool temperatures favors leaf rust infection to facilitate rust epidemics. Severity of rust epidemics may vary with wheat growing stage when primary infection occurs, host resistance level, and environmental conditions. Highly resistant cultivars can slow or prevent disease epidemics. However, growing cultivars with the same resistance gene over a large area also increases the risk of disease epidemics.

Methods to control leaf rust include cultural practices, application of fungicides and deployment of resistant cultivars. Cultural practices such as eliminating susceptible hosts at a critical stage can break the life cycle of the pathogen and reduce primary inoculum. In some areas winter wheat is infected soon after emergence, by spores from nearby infected spring wheat. Delaying planting, or grazing for dual-purpose wheat cultivars,

may slow or prevent this early infection. On the other hand, in areas where rust inoculum normally arrives late in growing season, planting early may allow a crop to mature before rust becomes serious (Knott 1989). This method is less effective in Great Plains because the primary inoculum is dispersed long distances from northern areas. Fungicides can effectively control the disease but it is not preferred due to high costs, potential danger of residues in food, and environmental pollution. Growing resistant cultivars is the most economic, effective, and environment-friendly method to control leaf rust. Therefore, breeding wheat cultivars with durable resistance to leaf rust takes the central stage in wheat breeding programs worldwide.

Pathogen-host interaction

Working with flax (*Linum usitatissimum*) rust (*Melampsora Lini*), Flor (1946) demonstrated the gene-for-gene relationship between the flax rust pathogen and flax. He concluded that for each gene conditioning resistance in the host, there is a corresponding locus in the pathogen with alternate alleles that conditioned pathogenicity in the pathogen. The gene-for-gene relationship has been thoroughly studied in the *T. aestivum-P. triticina* pathosystem, and has since served the basis for resistance breeding. According to the gene-for-gene concept, resistance will not occur if the pathogen acquires the corresponding virulence allele, hence lose the avirulence allele that elicits resistance. Hence the identification and deployment of diverse resistance genes is imperative to control plant disease. Another implication of the gene-for-gene concept is that pyramiding resistance genes into cultivars is a possible way to breed wheat cultivars with durable resistance. Based on the gene-for –gene concept, if the resistance genes are

combined together, the pathogen will not be virulent until it accumulates enough necessary changes at specific loci to overcome each gene.

Host resistance

Vanderplank (1963) first classified resistance as vertical or horizontal. Vertical resistance often demonstrates a typical gene-for-gene relationship (Flor 1946), while horizontal resistance reacts uniformly across all races of a pathogen (Vanderplank 1963). Usually, vertical resistance is synonymous with race-specific resistance and horizontal resistance is synonymous with race-nonspecific resistance.

Vertical (race-specific) resistance genes are known for their ease of identification, evaluation, and incorporation. Therefore genes for vertical leaf rust resistance have been extensively used in wheat breeding programs. However, the vertical resistance genes typically have a "boom-and –bust" cycle because the deployment of a single vertical resistance gene results in a shift of pathogen races and subsequent loss of resistance in a relatively short period. In contrast, horizontal (race-nonspecific) resistance is usually polygenic, and relatively difficult to identify, evaluate, and be applied in breeding programs; However, it is expected to be durable since it is relatively difficult for the pathogen population to overcome multiple resistance genes and develop a highly virulent and aggressive race.

Leaf rust resistance is also classified as seedli ng resistance or adult plant resistance (APR) based on the time at which the genes express. Seedling resistance can be effective from seedlings throughout the life of the plant. Most of the known leaf rust resistance genes are of this type. Among them, Lr26 from rye, Lr9 from *T. umbellulatum*, Lr28 from

Aegilops speltoides, Lr19, Lr24 and Lr29 from *Agropyron elongatum* are reported to have a high levels of resistance, and have been extensively used in breeding programs (Sawhney 1995 McIntosh 1995). APR is described as resistance that is ineffective in seedlings but effective in adult stages of plant growth. Hence Lr12 (Dyck et al. 1966), Lr13 (Dyck et al. 1966), Lr22a (Dyck and Kerber 1970), Lr22b (Dyck 1979), Lr34 (Dyck 1977), Lr35 (Kerber and Dyck 1990), Lr37 (McIntosh et al. 1995), Lr46 (Singh 1998), Lr48 (McIntosh et al. 1998), and Lr49 (McIntosh et al. 1998) have been identified as APR genes. But, Lr13 is also expressed in seedlings (Dyck 1966; McIntosh et al. 1995).

Leaf rust resistance also has been classified as slow or fast rusting, and is based on infection type and the ability to retard rust development. The term "slow rusting" was coined to characterize what has also been called as "partial", "incomplete", or "general" resistance in the wheat cultivar Knox (Caldwell 1970), in which a susceptible infection type was observed. Slow leaf rusting resistance is assumed to be conditioned by minor genes whose effects are too small to be detected individually (Caldwell 1968). Such resistance is more durable than resistance conditioned by single genes (Kolmer 1996). In general, slow rusting wheat has a reduced frequency of penetration, increased latent period, reduced number of uredinia per unit area of host surface, reduced pustule size, reduced pustule expansion, and reduced sporulation (Cadwell 1968; Bjarko et al. 1988a; Shaner et al. 1997). Among these parameters, latent period and pustule size were considered the most important components for identifying slow rusting genotypes (Kuhn et al. 1978; Singh et al. 1986&1993; Knott 1991&1989).

Among the known leaf rust resistance genes, Lr34 and Lr46 are slow-rusting genes. Lr34 is well characterized and widely used in breeding programs. Many wheat cultivars

showing slow leaf rusting or partial resistance were derived from the same source of Lr34. Kolmer (1996) provided a detailed review on the inheritance of Lr34. Some studies have showed that the combination of slow leaf rusting resistance genes with race-specific resistance genes is a promising strategy to breed cultivars for durable resistance. However, the expression of slow rusting genes is more vulnerable to environments than that of race-specific resistance genes. Also it is more difficult to incorporate slow rusting resistance genes into breeding lines largely due to the lack of simple and effective methods to evaluate the slow rusting trait in segregating progenies.

Breeding for durable leaf rust resistant cultivars

Pyramiding major leaf rust resistance genes in a wheat cultivar is an attr active way to enhance durable leaf rust resistance of a cultivar. Successful application of this strategy requires precise genetic analyses, critical pathogen surveys, and suitable pathogen races available to discriminate each gene in the segregating popul ation of a cross. Therefore, this strategy is time-consuming and labor-intensive. More important, surveys showed that multiple-pathogenicity was common in rust isolates, suggesting that gene pyramiding may provide only slightly more durable protection against the leaf rust pathogen (Sawhney 1995).

The combination of major resistance genes and durable resistance genes, such as slow leaf rusting genes, is probably an ideal strategy to breed durable resistant cultivars. Using this strategy, the major genes provide a high level of resistance, while the slow rusting resistance genes provide durable resistance. Durable leaf rust resistant cultivars identified so far usually carry the slow rusting resistance gene Lr34 and some other race-

specific resistance genes (Roelfs 1988), which strongly supports this strategy. However, this strategy may not be universally feasible in classic breeding programs due to the mask effects of major genes over durable genes. The inoculation procedures are still complicated and labor-intensive. Effective methods are not available to identify progenies that carry durable resistance genes based on phenotype. Molecular marker-assisted selection (MAS) circumvents this problem and facilitates breeding for durable rust resistant cultivars. Marker-assisted selection has several advantages over conventional breeding methods, primarily because no confounding non-genetic effects is involved. In general, marker-assisted selection has two immediate advantages in breeding for durable leaf rust resistant cultivars: shortening breeding timelines and pyramiding major and durable resistance genes into an elite breeding line without time-consuming and labor intensive inoculation.

Molecular characterization of leaf rust resistance genes or QTL

The identification of molecular markers linked to leaf rust resistance genes, or QTL, is a prerequisite for MAS. Molecular markers linked to some leaf rust resistance genes have been identified for Lr1 (Feuillet et al. 1995), Lr3 (Sacco et al. 1998), Lr9 (Autrique et al. 1995; Schachermayr et al. 1995), Lr10 (Schachermayr et al. 1997; Feuillet et al. 19978; Nelson et al 1.997), Lr13 (Seyfarth et al. 1998), Lr18 (Yamamori et al.1994), Lr19 (Autrique et al. 1995), Lr23 (Nelson et al. 1997), Lr24 (Autrique et al. 1995), Lr27 (Nelson et al. 1997), Lr28 (Naik et al. 1998), Lr29 (Procunier et al.1995), Lr31 (Nelson et al. 1997), Lr34 (Nelson et al.1997; Williams et al.1997), Lr35 (Seyfarth et al. 1998), and Lr47 (Helguera et al. 2001). Molecular markers linked to QTL for leaf rust resistance also

have been reported (Messmer et al. 2000;Faris et al.1999; Nelson et al.1995b). These studies provide valuable information for breeding durable leaf rust resistant cultivars by using MAS.

Wheat powdery mildew

Pathogen and control.

Powdery mildew, caused by *Blumeria graminis* (DC.)E.O.Speer f. sp. tritici Em. Marchal, is a common wheat leaf disease in wheat growing areas with a humid or semi - arid environment. High-yielding semi-dwarf cultivars and heavy use of irrigation and nitrogen fertilizer greatly favor powdery mildew infection and have allowed this disease to become a worldwide wheat disease (Bennett 1984). Powdery mildew is found in all wheat growing areas of the United States, but tends to be most severe in the east and southeast.

Blumeria graminis belongs to the family *Erysiphaceae* of the class *Ascomycetes* of the *Ascomycota phylum* (Agrios 1997). It reproduces by means of two spore types: asexual spores (conidia) and sexual spores (ascospores). The fungus overwinters as cleistothecia on plant debris. In warmer climates, the fungus also survives as conidia or mycelium on infected plants. Cleistothecia are closed, thick-walled, tiny, black, spherical structures that house sacs called asci. The oval sexual spores, ascospores, are produced within the asci. The conidia are usually barrel-shaped or oval, and produced on the mycelium. Both types of spores germinate when the relative humidity is between 85% to100%. The fungus grows superficially on the top of the leaf surface. Fine, thread-like infection pegs penetrate the epidermal cells of the leaves to form haustoria. Severe infection can result

in premature death of leaves, failure to produce wheat heads, lodging, light kernel weight, and reduced photosynthesis and plant vigor.

Conidia formation and host tissue penetration are carried out under light conditions, but most other stages of the life cycle occur in the dark. Cool and damp weather with temperatures between 15.6°C to 21°C favor disease development. Disease development is arrested at temperatures above 25°C. Other factors that may promote powdery mildew development are use of susceptible cultivars, dense planting, low air circulation, low light conditions, and high nitrogen fertilization.

Cultural practices such as crop rotation, use of fungicides, and growing resistant cultivars are major approaches to control powdery mildew. Planting cultivar mixture with different resistance genes is also an effective way to reduce losses caused by powdery mildew.

Race-specific powdery mildew resistance genes.

To date, 30 race-specific powdery mildew resistance genes have been identified and officially named in the Catalogue of Gene Symbols for Wheat (McIntosh et al. 1998). Most of these genes come from primitive and wild wheats or related genera such as *T. dicoccum* (Pm5, Pm16), *T. timopheevi* (Pm6), Secalis cereale (Pm8), *T. turgidum* (Pm4&Pm26), *T. speltoides* (Pm12), *H. villosa* (Pm21) and *T. longissimum* (Pm13) et al (McIntosh et al 1998). These genes typically follow the gene-for-gene concept and demonstrate "boom-and-bust" cycles. The wide application of these short-lived race-specific genes makes breeding for mildew resistant cultivars a never-ending task in many breeding programs. Hence, breeding for durable resistance has become a top priority

worldwide, especially in developing countries where frequent replacement of cultivars entails an enormous drain on scarce resources (Sawhney 1995).

Pyramiding race-specific powdery mildew resistance genes may provide relatively longer resistance. However, since multiple races of the powdery mildew fungus exist and new ones continue to be formed as a result of genetic recombination, this strategy does not appear to be a viable solution to the problem caused by *Blumeria graminis*. For example, the Swedish cultivar Timmo, which contains Pm2, Pm4b, and Pm6 and was highly resistant when first released in the United Kingdom in 1977, rapidly became susceptible so that by 1982 the resistance rating for Timmo had declined from an '8' to a '1' on a '0 to 9' scale (Bennett 1984).

Slow mildewing and durable resistance.

Durable resistance was defined as " resistance that has remained effective for many years while (the cultivar) was cultivated widely enough to have favored the selection of more virulent races of the pathogen if these had evolved" (Johnson and Law 1975). Slow mildewing is a race-nonspecific resistance, and is regarded as a major source of durable powdery mildew resistance. Slow mildewing was first identified in the wheat cultivar 'Knox' (Roberts & Caldwell 1970), and was characterized by a susceptible reaction in the seedling stage but only moderate infection in the adult-plant stage. Slow mildewing was associated with a longer latent period, lower mildew severity, lower infection rate, and smaller colony size (Shaner 1973). Segregation for mildew severity was found to occur in a continuous manner and transgressive segregation may be observed, which demonstrates the multigenic nature of slow mildewing resistance. For example, 'Diplomat', a German

wheat cultivar that was reported to have slow mildewing resistance, demonstrated durable resistance to powdery mildew. Monosomic analysis indicated that 14 chromosomes were involved in its resistance (Chae & Fischbeck 1979). Additive gene action was the most important genetic component of variance for slow mildewing resistance. Additive by additive and additive by dominance effects also were found (Heutea et al. 1987).

Durable resistance was also recognized in the United Kindom cultivar 'Maris Huntsman', which contains Pm2 and Pm6 genes and also confers race-nonspecific (quantitative) resistance. After Pm2, Pm6, and the quantitative resistance were separately overcome by the pathogen, 'Maris Huntsman' was still effective against powdery mildew in the field. Therefore, it appears that a unique combination of some or all of these genes is responsible for the durability of mildew resistance in this cultivar (Johnson 1979). This suggests that the combination of race-specific and race-nonspecific resistance is a promising strategy to breed durable powdery mildew resistant cultivars. However, the detection of quantitative resistance or slow mildewing resistance is a time-consuming process involving extensive quantitative measurements (Gustafson and Shaner 1982). The successful introgression of quantitative powdery mildew resistance genes into wheat cultivars by traditional breeding methods is still difficult. MAS is clearly an ideal alternative to selection of durable powdery mildew resistant cultivars.

Molecular characterization of powdery mildew resistance in wheat.

The prerequisite for breeding durable powdery mildew resistant cultivars by MAS is to develop molecular markers that are closely linked to the powdery mildew resistance gene or QTL. Several studies have been conducted to tag powdery mildew resistance

genes with molecular markers. These genes include Pm1 (Hu et al. 1997; Ma et al. 1994; Hartl et al.1995), Pm2 (Ma et al. 1994), Pm3 (Hartl et al. 1993; Ma et al. 1994), Pm4 (Hartl et al. 1999; Ma et al.1994), Pm5 (Keller et al. 1999), Pm6 (Tao et al.2000), Pm8 (Hsam et al. 2000), Pm12 (Jia et al. 1996), Pm13 (Donimi et al. 1995), Pm17 (Hsam et al. 2000), Pm18 (Hartl et al.1993), Pm21 (Qi et al. 1996), Pm24 (Huang et al. 2002), Pm25 (Shi et al. 1998), Pm26 (Rong et al. 2000), Pm27 (Jarve et al. 2000), and Pm30 (Liu et al. 2000). Molecular markers were also used to characterize QTL for powdery mildew resistance (Keller et al. 1999; Liu et al. 2001). The markers identified in these studies are valuable for breeding durable powdery mildew resistant cultivars by using MAS. However, most of the markers identified are RFLP (restriction fragment length polymorphisms) or RAPD (randomly amplified polymorphic DNA), which are not ready for direct application in MAS. Development of simple and cheap PCR-based markers is essential for MAS.

Molecular marker systems

Several types of molecular markers are available to detect DNA sequence polymorphism between individuals, including RFLP, RAPD, amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR). Extensive wheat genetic mapping has been conducted using segregating RFLP markers (Chao et al. 1989; Devos et al. 1992 Ma et al. 1994; Gale et al. 1990; Jia et al. 1996; Nelson et al. 1995; Liu et al. 1991), which provided the framework for subsequent genetic analyses.

RFLP markers are effective in detecting polymorphisms in wheat. The probes used to detect RFLPs are frequently derived from cDNA clones and therefore represent

expressed genes. RFLP markers can be used to study the relationships between chromosomes of different species. The disadvantages of RFLPs include (1) requirement of large amount of DNA, (2) high cost for analyses of large populations, and (3) time consuming and not suitable for automation, which limits its application in applied breeding programs.

AFLP is a PCR-based marker system developed by Keygene NV company (Vos et al. 1995). The AFLP technique involves the digestion of genomic DNA, followed by the ligation of adapters to genomic restriction fragments, and subsequent PCR amplification of a subset of these fragments. The sources of polymorphism are (1) mutations in the restriction sites, (2) mutations in the sequences adjacent to the restriction sites and complementary to selective primer extensions, and (3) insertions or deletions within the amplified fragments.

AFLP is a robust and reliable marker system. The advantages of AFLP include (1) high level of polymorphism; (2) no requirement of prior knowledge of DNA sequence, (3) requirement of a small amount of DNA template. The high multiplex ratio of AFLP markers makes them ideal markers for high-resolution mapping and gene tagging of loci that control commercially important traits. AFLP is finding increased use in wheat gene mapping and gene tagging and rapidly becoming the preferred molecular technique for many types of studies. However AFLP markers are random, and it is necessary to combine AFLP markers with other genome-specific markers such as SSR, or RFLP, to physically locate the target gene position.

Microsatellites, also known as SSR, provide a simple, tandem -repeated di- or tetra-nucleotide sequence motifs flanked by unique sequences. SSR is usually codominant.

The major advantages of SSRs are their ease of use, low cost of analysis, chromosome specificity and the ability to detect genetic differences even among closely related individuals. The first two advantages are critical to the widespread use of molecular markers in large scale breeding programs. The fourth one is of paramount importance in modern plant breeding programs in which crosses are often made between elite parental lines that are genetically similar. The third one makes it a promising strategy to create "skeletal' genetic maps with SSR markers, and 'fill' the gaps between the SSR markers with other high throughput makers, such as AFLP markers. The development of SSRs in plant research is accelerating, and SSR loci are now being incorporated into established genetic maps in wheat (Roder et al. 1998a; Roder et al. 1998b).

Marker development methods

Three methods are commonly used to find markers linked to traits of interest: (1) the construction of complete linkage maps with the entire mapping population, (2) identify markers between isogenic lines, or (3) use of a pooled strategy such as bulked segregant analysis (BSA). The first method involves screening a large number of individuals from a segregation population with an entire set of markers that are distributed evenly throughout the genome. Subsequently, statistical analysis is performed to identify the regions in the genome involved in the trait. This method is suitable for simultaneous identification of molecular markers linked to several QTL or traits.

Although near- isogenic lines are ideal materials for marker identification, development of near isogenic lines is time consuming. The BSA method provides a rapid and cost-effective procedure for identifying markers associated with traits of interest

(Michelmore et al. 1991). The essence of BSA is to pool individuals from two phenotypic extremes of a segregating population. DNA isolated from two pools (bulks) is then screened with DNA markers, and the polymorphisms detected between two bulks are supposed to be derived from regions of the genome that are common between the individuals that form each pool but different between pools. BSA was successfully applied to develop markers linked to agronomically important traits in wheat (Williams et al. 1997; Eastwood et al. 1994). However, the drawback of this method is that it can only detect molecular markers linked to one trait each time. Also, recombination between the marker and the gene of interest greatly compromises its power to detect target markers.

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Chapter II

Molecular Characterization of Slow Leaf Rusting Resistance in Wheat

Abstract

Slow leaf rusting resistance in wheat (*Triticum aestivum* L) is becoming more attractive due to its durability in comparison with race-specific resistance. CI 13227 was reported to provide a new source of slow leaf rusting resistance, and have the highest level of slow rusting resistance ever identified. The objective of this study was to characterize the slow leaf rusting resistance conferred by CI 13227 with molecular markers. A recombinant inbred line (RIL) population derived from CI 13227 x Suwon 92 was evaluated for final leaf rust severity, area under disease progress curve (AUDPC), leaf rust infection rate (AUDPC/day), and leaf rust infection duration. Four hundred fifty nine AFLP markers and 16 SSR markers were analyzed with the population. Two quantitative trait loci (QTLs), designated as QLR.osu-2B1 and QLR.osu-5BL/7BL, were consistently associated with leaf rust resistance (AUDPC and final severity) and leaf rust infection rate (AUDPC/day). The percentages of phenotypic variance explained by each QTL varied with environments and traits, ranging from 13.4% to 18.8% for AUDPC, 11.9% to 16.6% for final rust severity, and 12.9% to 16.1% for infection rate. A third QTL, designated as QLR.osu-2B2, was only detected in 1995 and explained 12.47%, 13.4%, and 13.5% of the phenotypic variance for AUDPC, final severity, and infection rate, respectively. A fourth QTL for leaf rust infection duration, designated as QLRID.osu-2D, was located on chromosome 2D and explained 26.4% and 21.47% of the phenotypic variance in 1994 and 1995, respectively.

Key words: wheat, slow leaf rusting resistance, AUDPC, final severity, infection rate, infection duration.

Introduction

Leaf rust, caused by *Puccinia triticina* (previously *P. recondita* Rob. Ex Desm. f. sp. tricici), is one of the major wheat diseases worldwide. Its relative importance depends on climate and the degree of resistance of the predominant cultivars. Yield losses due to leaf rust can be as high as 63% for susceptible cultivars (Sayre et al. 1996). Losses in grain yield are mostly due to reductions in kernel weight, kernel number per square meter, and grain fill rate (Sayre et al. 1996). Genetic resistance is the most effective and environment-friendly way to control leaf rust. However, the short-lived nature of race-specific leaf rust resistance genes, which are widely used in breeding programs, makes breeding for leaf rust resistant cultivars a never-ending task.

In recent years, there has been increasing interest in understanding and using slow leaf rusting resistance because of its prospects in breeding for durable resistance (Caldwell et al. 1970;Kuhn et al. 1978;Bjarko et al. 1988a; Bjarko et al. 1988b). Some slow leaf rusting resistant materials have been identified and characterized (Singh et al. 1998; Messmer et al. 2000; Shaner et al. 1980; Shaner 1980; Kuhn et al. 1978). Methods used to assess slow leaf rusting resistance have included measuring the disease severity either once at the peak of epidemic development or several times from beginning to end of the epidemic. The former is assumed to represent the cumulative result of all resistance factors operating during the epidemic, and the latter data are used to measure the infection rate and AUDPC (Paralevliet 1979). The AUDPC has been widely used to characterize foliar disease resistance (Jeger et al. 2001), and is thought to be a stable

measurement of slow rusting because it reflects both severity and rate of disease development (Wilcoxson et al. 1975). Infection rate and infection duration were also considered to be important factor of the disease epidemics (Paelevliet 1979).

Genetic studies on slow leaf rusting have generally produced very similar results and conclusions (Bjarko et al. 1988; Das et al. 1992; Broers 1989). Slow rusting capacity is under oligogenetic control with moderately high heritability (Bjarko et al. 1988; Das et al. 1992; Gavinlertvatana et al. 1978). These indications show that slow rusting resistance can be selected in a breeding program. It was also reported that two to five genes control longer latent period (Kuhn 1980; Shaner et al. 1997; Lee et al. 1985; Das et al. 1992 Vander Gagg et al. 1997). Gene action for slow leaf rusting is mainly additive or additive x additive interaction (Bjarko et al. 1988; Das et al. 1992).

Two genes associated with slow leaf rusting resistance were identified, Lr34 (Dyck 1977) and Lr46 (Singh et al.1998). The Lr34 gene has been widely used in wheat breeding programs because of its durable resistance to leaf rust, its association with the stripe leaf rust resistance gene, Yr18, and with its tolerance to barley yellow dwarf virus (McIntosh 1992; Singh 1993). The combination of Lr34 with other genes, such as Lr12 and/or Lr13, is the basis of many durable leaf rust resistant cultivars worldwide (Roelfs 1988). Several attempts were made to tag the Lr34 with molecular markers (Williams et al.1997; Nelson et al.1997; Messmer et al. 2000). Nelson et al. (1997) found two loci linked with leaf rust resistance. One was located on 7DS, the expected position of Lr34, and another one on 2BS. Both loci together explained 45% of the phenotypic variance. Williams et al. (1997) used bulked segregant analysis (BSA) to detect markers that distinguished pooled samples with and without the Lr34 gene, and found three RAPD

markers associated with leaf rust resistance. Two of the markers were located on 7BS and another one on 4DL. Faris et a.l (1999) also found that a region of 7BL contributed to leaf rust resistance under the condition of natural infection. Using a RIL population derived from Forno X Oberkulmer, Messmer et al (2000) determined the genetic basis of durable leaf rust resistance in the European cultivar, Forno, which has a high level of resistance against leaf rust and shows leaf tip necrosis. This characteristic is in agreement with that of Lr34, which is closely linked (or pleiotropic) with leaf tip necrosis caused by the major gene Ltn (Singh 1992). Messmer et al. (2000) detected 6 QTL for leaf rust resistance, and the major QTL located on 7BL explained 35% of the phenotypic variance. Singh et al. (1998) identified another gene (Lr46) involved in slow leaf rusting resistance in the wheat cultivar 'Pavon76', and assigned it on chromosome 1B.

Although slow rusting resistance is durable, the pathogen also evolves to overcome it in agroecosystems (McDonald et al. 2002). The nature of this evolution differs from the evolution against major gene resistance and was characterized as a process of "erosion" rather than as a process of breakdown (McDonald et al. 2002). Hence, it is wise to identify and deploy diverse slow leaf rusting resistance genes because there is no evidence to assume that isolates of *Puccinia triticina* with virulence to Lr34 or Lr46 will not eventually appear and quickly be selected in the pathogen population. CI 13227 was identified as a new source of slow leaf rusting and confers the highest level of resistance ever reported (Shaner et al. 1980 & 1997). Although pathogenic and genetic studies were conducted to investigate the slow leaf rusting resistance conferred by CI13227 (Shaner et al. 1980 & 1997), little is known about its molecular basis. The objectives of this study are to identify and locate QTL responsible for slow leaf -rusting resistance in wheat

cultivar CI 13227, and develop tools that can be used in MAS to facilitate breeding for durable leaf rust resistant cultivars.

Materials and methods

Plant materials

A population of 104 RILs developed by single seed descent (SSD) from the F2 generation of a cross between CI 13227 X Suwon 92 was used in this study. CI 13227 has a high level of slow rusting resistance to leaf rust and Suwon 92 is very susceptible to leaf rust. Nether of these parents are known to carry any race-specific resistance genes.

The 104 RILs, together with five replicated entries of the parental lines CI 13227 and Suwon 92, were evaluated at West Lafayette, IN, in 19 94 and 1995 using a randomized complete-block design with two replications. Leaf rust severity was determined for all RILs and parents according to the modified Cobb Scale (Peterson et al. 1948). Leaf rust severity was evaluated seven times in 1994 (from May 29 to June 19) and 1995 (from May 30 to June 25), respectively. Area under the disease progress curve (AUDPC) and infection rate (AUDPC/day), were calculated according to Shaner and Finney (1980). The final severity and infection duration, defined as the period over which the diseased tissue sporulates, also were analyzed.

Analysis of amplified fragment length polymorphism (AFLP)

The CTAB (Cetyltrimethyllammonium) method was used to isolate genomic DNA from two-week-old wheat seedlings (Saghai-Maroof et al. 1984). About 300 ng of wheat genomic DNA was double-digested with *Pst* I and *Mse* I restriction enzymes in 1X H

buffer at 37 ° C for 2 hours. After digestion, 8 µl of ligation mixture containing 4 pmol of P*st I* and 40 pmol of M*seI* adapters, 0.8 unit of DNA ligase and 1X ligase buffer was added to the digested DNA. The ligation was conducted at 20 °C overnight. The resulting 38 µl DNA mixture was diluted with 362 µl sterilized deioned water for subsequent PCR reactions.

For preamplification, 40 µl of PCR reaction included 10 µl ligated DNA, 75 ng of each of *Pst* I and *Mse* I pre-amplification primers, 0.75 unit Taq enzyme, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 1x PCR buffer. The following PCR profile was used: 25 cycles of 30 seconds at 94 ° C, 1 minute annealing at 56° C and 1 minute extension at 72 ° C. Five microliters of PCR product was used for quality check on a 1% agarose gel. The remaining PCR product was diluted 10-fold by adding 315 µl of sterilized deioned water.

Selective amplification was conducted using *Pst* I and *Mse* I primers with two to four additional selective nucleotides. *Pst* I primers were labeled with IR fluorescent dye. Each PCR reaction contained 3 µl diluted pre-amplified DNA, 0.4 µl *Pst* I primer, 0.4 µl *Mse* I primer, 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.2 unit of Taq DNA polymerase, 1 x PCR buffer and 3.8 µl water. The following PCR profile was used for selective PCR amplification: denaturing at 94°C for 2 minutes followed by 13 cycles of 30 seconds at 94 °C, 30 seconds annealing at 65 °C with a decreasing of 0.7 °C per cycle, 1 minute of extension at 72 °C, and then 23 cycles of 30 seconds denaturing at 94 °C, 30 seconds of annealing at 65 °C, and 1 minute of extension at 72 °C. Finally, an extension at 72 °C for 5 minutes was followed. PCR was conducted in a MJR PTC-100 thermal cycler. The PCR product was denatured at 95 °C for 5 min after adding 5 µl loading buffer and chilled quickly on the ice.

AFLP products were separated in a 6.5% denaturing polyacrylamide gel running in 1x TBE buffer on Li-Cor IR-4200 DNA sequencer. The electrophoresis condition was set at 1500V voltage, 40W power, 35 mA current with 50 °C constant temperature for 3 h. The gel was prepared as described in the user's manuals from the manufacturer. A 68 well comb was used for gel-well formation and 0.8 μl of each sample was loaded into separated wells using Hamilton 8-channel syringe (Hamilton, Reno, NV). The gel image file was stored in a computer and the segregation patterns were manually scored by visual inspection. The AFLP markers were designated according to the selective nucleotides of *Pst* I primers and *Mse* I primers, and their molecular weight. For example, XACA.CGT150 represents the AFLP marker generated by *Pst* I primer, pACA, and *Mse* I primer, mCGT, and the molecular weight of this marker is 150 bp.

Analysis of simple sequence repeat (SSR) markers

PCR. SSR analysis was conducted using the silver staining method. For each reaction, 20 ng of genomic DNA was used in a solution containing 200 μM of each dNTP, 1x PCR buffer, 2.5 pmol of each primer with 2.0 mM $MgCl_2$ and 2 units of Taq polymerase. The touch-town program was used for PCR amplification, in which the reaction mixture was denatured at 95 °C for 5 minutes followed by 5 cycles of 45 seconds at 95 °C, 5 minutes of annealing at 68 °C with a decrease of 2 °C in each of following cycles, and 1 minute of extension at 72 °C; For another 5 cycles the annealing temperature starts at 58 °C for 2 minutes with a decrease of 2°C for each of following cycles. Then, PCR went through additional 25 cycles of 45 seconds at 94 °C, 2 minutes

annealing at 50 °C, and 1 minute of extension at 72 °C with a final extension at 72 °C for 5 minutes.

Gel preparation and electrophoresis. The large and small glass plates were cleaned thoroughly with Rain-X and 75% ethanol, respectively. Then, the inner side of the small glass plate was wiped with 1.5ml of freshly prepared gel binding solution containing 5 µl of bind silane, 150 µl of acetic acid and 1.345 ml of 95% ethanol. After the solution dried, two 0.5 mm side spacers were placed between the glass plates and the gel plates were assembled with two clamps. Six hundred microliters of 20% ammonium persulfate solution and 60 µl ofTetramethyl ethylene-diamine (TEMD) were added to 100 ml of 5% polyacrylamide gel solution that contained 4.75 g of acrylamide, 0.25 g of Bis-acylamide, 45 g of urea, and 5 ml of 10X TBE buffer. The gel mix was injected between the glass plates by a syringe and a comb was inserted from the top to form wells for loading samples.

After the gel was polymerized, the electrophoresis apparatus was assembled and 2L of 0.5x TBE buffer was added into the upper and bottom buffer chambers. The gel was pre-run at 100 Walt for 40 minutes to achieve a gel surface temperature of approx. 40 °C. After the gel wells were flushed with buffer using a syringe, the denatured PCR product was loaded into gel wells. The gel was run at 100 w constant power for about 90 min.

Silver staining of gels. When electrophoresis was finished, the buffer solution was drained and glass plates were removed and carefully separated with a spatula. The gel usually stuck on the small plate. To fix the gel, the plate with gel was immersed in 1.5 L of fixation solution (10% w/w glacial acetic acid) for 30 min. After the gel was washed twice with 2 L of DD-water for 2 min, it was stained with 1.5 L of silver staining solution

containing 1.5 g of silver nitrate and 2.3 ml of 37% formaldehyde for 35 min, and then washed with DD-water for 5 to 10 sec. The gel was moved to 1.5 L of freshly prepared cold developing solution which contained 45 g of sodium carbonate, 2.3 ml of 37% formaldehyde, 300 µl of 10mg/ml sodium thiosulfate. After the gel was developed for 5 to 15 min, DNA bands showed up in the gel and then fixation solution was added to the development solution to fix the gel for 5 minutes. Then the gel was washed in distilled sterile water for 2 min. Gel was air-dried overnight and scanned in a scanner to collect data.

Bulked segregant analysis (BSA)

BSA based on phenotypic data was used for initial screening of informative AFLP primers. After 612 *Pst* I / *Mse* I primer pairs were screened (Table 2.1.), 85 primer pairs showed polymorphism between the two bulks and therefore they were used to genotype the population. A total of 459 AFLP markers were analyzed in the population. AFLP markers linked to QTL for leaf rust resistance were identified in the initial QTL analysis. To determine the tentative chromosome locations of these QTL, a revised BSA method was applied to screen informative SSR primers. A total of two pairs of bulks contrasting in presence and absence of an individual QTL for leaf rust resistance were constructed based on AFLP markers flanking the target QTL. In each pair of bulks, resistant bulks consisted of equal amount of DNA from each of five RILs that had AFLP alleles flanking a QTL for leaf rust resistance, and susceptible bulks consisted of equal amount of DNA from each of five RILs that had alternative AFLP alleles. The selected RILs in each pair of bulks showed excellent contrast in leaf rust resistance. DNA from two parents and the

four bulks were used to screen SSR primers. Totally, 146 SSR primers were screened, and 16 SSR primers that detected polymorphism between at least one pair of bulks were used to genotype the entire population. Data from both SSR and AFLP were combined for further QTL analysis.

Data analysis

One-way ANOVA was used to find AFLP markers that were significantly associated with slow rusting resistance ($p < 0.05$). Genetic linkage maps were constructed by using MapMaker 3.0 (Lander et al. 1987). A threshold log likelihood ratio (LOD) of 4.0 was used to group the significant markers into linkage groups. Centimorgan (cM) values were calculated by the Korsambi mapping functio n (Korsambi 1944). The interval function of Qgene (Nelson 1997) was used to detect QTL for slow rust resistance at a LOD threshold of 3.0. AUDPC, final severity, infection rate and infection duration were used for QTL mapping. The QTL were designated according to the guidelines for nomenclature of quantitative trait loci in wheat (McIntosh et al. 1998): Lr (leaf rust) and LRID (leaf rust infection duration) were used for trait designator, and "osu" (Oklahoma State University) was used for laboratory designator. The SAS program "PROC CORR" was used to calculate the correlation coefficients. "PROC GCHART" was used to produce phenotype distribution histograms.

Results

Phenotypic segregation

CI 13227 showed a high level of slow rusting resistance to leaf rust. It had significantly lower AUDPC, final rust severity (FS), infection rate (IR) and longer infection duration than those of Suwon 92 in both years (Table 2.2.).

In each year, the population of RILs of CI 13227 x Suwon 92 showed continuous distributions for AUDPC, FS, IR and ID. Across the two years, they ranged from 27.9 to 548.7 for AUDPC, 11.5% to 87.5% for FS, 1.54 to 35.32 for IR, and 16.5 days to 23.5 days for ID (Fig.2.1. to Fig.2.4.).

Significant correlations were detected between two years for AUDPC ($r=0.53$ $p<0.0001$), FS ($r=0.42$ $p<0.0001$), IR ($r=0.63$ $p<0.0001$) and ID ($r=0.83$ $p<0.0001$). Correlation coefficients were also high among AUDPC, FS, and IR, ranging from 0.93049 to 0.98875 (Table 2.3.). Infection duration (ID) was negatively correlated with AUDPC, FS, and IR with correlation coefficients ranging from –0.58 to –0.72 (Table 2.3.).

Marker analysis

A total of 612 pairs of AFLP (*Pst* I/*Mse* I) primers were screened using BSA. The primers used are listed in Table 2.1. Eighty-five primers detected polymorphism between both parents and bulks, and were used further to screen the 104 RILs. A total of 459 AFLP markers and 16 SSR markers were generated. Molecular markers closely linked to leaf rust resistance with a P value less than 0.05 were used for further linkage analysis. Table 2.4. lists molecular markers that were significantly linked to AUDPC, FS, and IR in both 1994 and 1995. Among them, 11 were significantly associated with all three traits. Linkage analysis showed that these markers belonged to two linkage groups

(Fig.2.5. A & B). This result suggested that the two linkage groups were important for leaf rust resistance and leaf rust infection rate. The determination coefficients of these markers ranged from 3.9% to 19.4% for AUDPC, 5.8% to 19% for FS, and 4.3% to 18.1% for IR in 1994, and 5.0% to 14.8% for AUDPC, 3.9% to 18.0% for FS, and 5.5% to 13.7% for IR in 1995. The allele substitution effects of these markers ranged from 25.4 to 58.3 for AUDPC, 3.9% to 8.5% for FS, and 1.4 to 2.7 for IR in 1994, and 21.7 to 35.4 for AUDPC, 3.4 % to 7.0% for FS, and 1.4 to 2.2 for IR in 1995. In all cases higher values of AUDPC, FS, and IR were detected for the parental allele of Suwon 92. This suggested that all positive alleles for resistance, i.e., smaller AUDPC, FS, and IR values, were contributed by CI 13227. Fig.2.6. to Fig.2.14. show the segregation of AFLP markers that were closely associated with leaf rust resistance in the RIL population derived from CI 13227 x Suwon 92.

All the molecular markers linked to infection duration (ID) were located on chromosome 2D and were grouped into one linkage group (Table 2.5.), which covers 45.3 cM (Fig. 2.5. C). Their determination coefficients ranged from 4% to 27.9% in 1994, and 5.7% to 29.2% in 1995, respectively. The allele substitution effects of these markers ranged from 0.6 days to 1.5 days in 1994; and 0.4 days to 0.9 days in 1995, respectively. Fig. 2.15. shows the segregation pattern of the AFLP marker that was most closely associated with leaf rust infection duration in the RIL population derived from CI 13227 x Suwon 92, XGCTG.CGCT118.

Interval analysis

Based on interval mapping, two to three QTL were detected for leaf rust resistance (AUDPC and FS) and leaf rust infection rate (IR) in each year (Table2.6.). Chromosome 2B is important for three slow leaf rusting traits. A QTL for AUDPC, FS, and IR, designated as QLR.osu-2B1, was identified in both 1994 and 1995 (Fig.2.17. and Fig.2.18.). QLR.osu-2B1 was located between AFLP markers XCATG.ATGC60 and XCAG.CGAT70. It explained 18.8%, 16.6%, and 15.6% of the phenotypic variance for AUDPC, FS and IR in 1994, and 13.4%, 15.2% and 13.6% of the phenotypic variance in 1995, respectively. An additional QTL on 2B, designated as QLR.osu-2B2, was identified in 1995. The likelihood plots of QLR.osu-2B2 peaked between XCAT.CGTA146 and XCAT.CGTA150, and explained about 13% of the phenotypic variance for AUDPC, FS, and IR (Table 2.6.).

Another QTL, designated as QLR.osu-5BL/7BL, was also identified in both 1994 and 1995. This QTL was putatively assigned on 5BL or 7BL according to the location of the SSR marker linked to it, XBarc32. XBarc32 was previously mapped on 5BL or 7BL. In 1994, QLR.osu-5BL/7BL peaked between AFLP marker XTGC.ACAG198 and XCAT.CTA155, and explained 17.2%, 19.4% and 19.1% of phenotypic variance for AUDPC, FS, and IR, respectively. In 1995 QLR.osu-5BL/7BL was located between XCGA.CAT85 and XCATG.ATGC125, and explained 14.8%, 11.9% and 12.9% of the phenotypic variance for AUDPC, FS and IR, respectively.

The QTL located on 2D, designated as QLRID.osu-2D, was associated with leaf rust infection duration (ID). QLRID.osu-2D was located in the interval between XTGC.CTA208 and XGCTG.CGCT118 with a LOD score of 6.73 and 5.3 for ID in 1994

and 1995, respectively (Fig. 2.20.). This QTL was quite stable, and explained 24.4% and 21.5% of the phenotypic variance in 1994 and 1995, respectively. However, the positions of the QTL were a little different in 1994 and 1995. It was 1.3 cM away from XGCTG.CGCT118 in 1994. In 1995 it was mapped on the exact location of XGCTG.CGCT118. The longer infection duration was inherited from CI 13227 allele. Further analysis showed that infection duration was positively associated with heading date ($r=0.69$ $p<0.0001$), i.e. later heading corresponded with longer the infection duration. The heading date of CI13227 was 7 days later than that of Suwon 92 in 1994 and 12 days later in 1995. Since the infection duration was negatively correlated with AUDPC, FS, and IR (Table 2.3.), the RILs with longer infection duration allele still had good resistance.

Discussion

CI 13227 was previously reported to confer the highest level of slow rusting resistance (Shaner et al. 1980). Understanding the genetic basis of the slow leaf rusting resistance conferred by CI13227 is imperative for its application in breeding for durable leaf rust resistant cultivars. Our results suggested that at least three major QTL contribute to slow leaf rusting resistance in CI 13227. This is in agreement with previous reports based on biometric analysis (Das et al. 1992). Two out of the three QTLs, QLR.osu-2B1 and QLR.osu-2B2, were located on chromosome 2B. Another one, QLR.osu-5BL/7BL, was tentatively mapped on 5BL or 7BL. Since the known slow leaf rusting resistance genes Lr34 and Lr46 were previously mapped on 7DS and 1B, respectively, the two QTLs on 2B detected in this study constitute new slow leaf rust resistance sources. Application of

these two QTLs in wheat breeding should diversify the slow leaf rusting sources and be helpful for breeding durable leaf rust resistant cultivars. It is interesting to note that Nelson et al. (1995) detected a QTL on chromosome 2BS in a synthetic wheat, and Messmer et al. (2000) found a QTL on 2BS explaining 8% of the phenotypic variance in one out of four environments.

Dyck et al. (1977) located slow leaf rusting resistance gene Lr34 on 7DS. However, Dyck (1994) also found evidence that Lr34 might be translocated onto another chromosome. Similarly, Williams et al. used BSA to identify molecular markers linked to the Lr34 gene, and found a RAPD marker on 7BL instead of the expected 7DS (1997). In a study investigating the inheritance of a durable leaf rust resistant cultivar, Forno, Messmer et al. expected to detect QTL resembling Lr34 on 7DS because of the occurrence of leaf tip necrosis, a trait closely linked to Lr34 (Singh et al. 1992). However, they did not detect significant QTL on 7DS, instead, found QTL on 7BL. They concluded that Forno might carry one or more homoeologous loci of Lr34 carrying different alleles. In this study, we consistently detected a QTL for leaf rusting resistance and leaf rust infection rate, QLr.osu-5BL/7BL, putatively located on 5BL or 7BL. At this time we can not pinpoint its location and determine its relationship with Lr34. However, due to the lack of leaf tip necrosis in CI 13227, QLr.osu-5BL/7BL is unlikely to be a homoeologous locus of Lr34. Further physical mapping is necessary to pinpoint its location.

Considering the high adaptability and the rapid distribution of virulent isolates of *Puccinia triticina* over long distances, the best strategy for breeding durable leaf rust resistant cultivars should be the combination of race-specific resistance gene(s) with race

non-specific resistance gene(s) or QTL. In fact, most of the identified durable leaf rust resistant cultivars carry Lr34, a slow leaf rusting resistance gene, and other race-specific gene(s). The South American cultivar 'Frontana', which has been regarded as one of the best sources of durable resistance to leaf rust, carries Lr34, Lr13, and LrT3 (Dyck et al. 1982). Chinese Spring, a popular wheat cultivar whose resistance to leaf rust has lasted for about a century in North America (Kolmer 1996), carries Lr34, Lr12 (Dyck 1991), and Lr31 (Singh et al.1984). However, this strategy is not practical in traditional breeding programs due to the time-consuming process involving complex inoculation tests and extensive measurements, but is feasible when linked markers are available. Molecular markers linked to race-specific leaf rust resistance genes and slow leaf rusting resistance gene, including Lr1 (Feuillet et al. 1995), Lr3 (Sacco et al. 1998), Lr9 (Autrique et al. 1995; Schachermayr et al.1994), Lr10 (Schachermayr et al. 1997; Feuillet et al. 19978; Nelson et al 1997), Lr13 (Seyfarth et al. 1998), Lr18 (Yamamori et al.1994), Lr19 (Autrique et al. 1995), Lr23 (Nelson et al. 1997), Lr24 (Autrique et al. 1995), Lr27 (Nelson et al. 1997), Lr28 (Naik et al. 1998), Lr29 (Procunier et al. 1995), Lr31 (Nelson et al. 1997), Lr34 (Nelson et al. 1997; Williams et al. 1997), Lr35 (Seyfarth et al. 1998), and Lr47 (Helguera et al.2001), has been identified. Three of these markers are STS markers that can be directly used in MAS (Naik et al 1998; Seyfarth et al 1998; Helguera et al. 2001), and the others have the potential to be converted into STS markers. In this study we identified two SSR markers closely linked to QTL for slow leaf rusting resistance and leaf rust infection rate, XBarc18 and XBarc167. They can be directly used in MAS. The further conversion of other markers flanking the QTL, such as

XCAG.CGAT70, XCATG.ATGC60, XCAT.CGTA150, and XTGC.ACAG198, into STS markers will greatly facilitate the introgression of the identified QTL into other cultivars.

It was hypothesized that quantitative resistance loci are simply variants of qualitative resistance loci that have been (partially) overcome by their respective pathogen (Yong 1996). Among the known major leaf rust resistance genes, Lr13, Lr16, Lr23, and Lr35 were mapped on chromosome 2B (McIntosh 1995). Lr13, Lr23 and Lr35 are adult plant resistance (APR) genes. Because of the limited information on the precise chromosomal position of these genes, it is not possible to orthologically compare them with the QTLs on 2B detected in this study at this time. One alternative way is to test the race-specificity of the QTL by inoculating the RILs with specific races. Previous study indicated that QTL showed distinctly different resistance effects against different races (Leonards-Schippers et al.1994).

Correlation and genetic analysis for slow-rusting components determining resistance to leaf rust were described to be either tightly linked or under pleiotropic genetic control (Singh et al. 1991; Das et al. 1993). The three QTL for AUDPC detected in this study were coincident with QTL for final severity and infection rate. Coincident QTL support the observed pattern of phenotypic correlations. The correlation coefficients among AUDPC, final severity, and infection rate ranged from 0.93 to 0.98. The highly significant associations among the three parameters maybe attributed to the fact that the calculations of AUDPC and infection rate were based on leaf rust severity. This result suggested that AUDPC, final severity, and infection rate reflect different aspects of the same process, slow leaf rusting. We find it's reasonable to select for slow rusting genotypes based on only final severity as suggested by Das et al. (1993).

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Table2.1. Primer combinations for pre-amplification and selective-amplification of AFLP analysis. "p" and "m" represent the pre-amplification primer sequences of *Pst*I and *Mse*I, respectively.

Pre-amplification primers:
Pst I:GACTGCGTACATGCAG
Mse I: GATGAGTCCTGAGTAA
Selective amplification primers:
Pst I: pACT, pACTG, pAGT, pCAT, pCATG, pCTC, pTGC, pAGC, pCGA, pAG, pACA, pAGG, pCAG, pCGT, pCTCG, pGCTG, pGTG
Mse I: mACGC, mAGC, mCAA, mCAC, mCACG, mCAG, mCAGT, mCAT, mCGAC, mCTA, mCTC, mCTG, mCTGA, mCTT, mGAC, mGCG, mTGC, mGCAG, mGTG mATGC, mACAG, mACGT, mAGAC, mAGCT, mAGGC, mAGTG, mCACG, mCGCT, mCGAT, mCGTA, mCTCG, mTGCG, mTCGA, mGGCT, mGCAT

Table 2.2. Area under disease progress curve (AUDPC), final severity (FS), infection rate (IR), and infection duration (ID) of the parental lines, CI13 227 and Suwon 92, and 104 RILs derived from CI 13227 x Suwon 92 in 1994 and 1995.

	Year	AUDPC	IR	FS (%)	ID(days)
CI 13227	1994	48.3	1.9	11.1	26
	1995	36.9	1.7	25.6	22.5
Suwon 92	1994	464.2	23.5	76	19.5
	1995	335.3	15.3	69	18
RIL means	1994	305.7	13.6	52.1	22.7
	1995	234.8	12.7	56.1	19.7
RIL ranges	1994	33.1-666.9	1.3-29.9	8-92.5	18-26
	1995	22.2-427.9	1.0-33.56	15-90	16-22.5

Table 2.3. Phenotypic correlations among area under disease development curve (AUDPC), final severity (FS), infection rate (IR), and infection duration (ID) obtained from the RIL population derived from CI 13227 X Suwon 92. Correlation coefficients were calculated based on data combined across 1994 and 1995.

	AUDPC	FS	IR
FS	0.9544		
IR	0.98875	0.93048	
ID	-0.64531	-0.57692	-0.72107

Table 2.4. One - way ANOVA for markers linked to area under disease progress curve (AUDPC), final severity (FS), and infection rate (IR) in a RIL population derived from CI 13227 x Suwon 92 in 1994 and 1995. All markers have a p value less than 0.05.

			AUDPC				FS				IR			
Marker	Chro*.	Year	R^2(%)	Cl. All.*	Su. All.*	ASE.*	R^2(%)	Cl. All.	Su. All.	ASE.	R^2(%)	Cll. Al.	Su. All.	ASE.
XCATG.ATGC60	2B	1994	19.4	244.2	358.3	57.1	16.2	44.4	59.6	7.6	16.5	11.1	16.2	2.5
		1995	13.3	201.6	269.3	33.9	14.8	50.4	63.2	6.4	13.7	12.2	16.5	2.2
XTGC.ACAG198	5BL/7BL	1994	19.1	230.7	347.2	58.3	18.7	41.7	58.6	8.5	18.1	10.3	15.8	2.7
		1995	8.7	205.5	259.9	27.2	7.8	51.5	60.9	4.7	7.7	12.6	15.8	1.6
XBARC18	2B	1994	17	251.2	356.2	52.5	13.5	45.7	59.3	6.8	14.8	11.4	16.1	2.4
		1995	11.9	203.3	267.2	32	13.6	50.4	62.6	6.1	11.9	12.3	16.4	2
XACA.CACG126	5BL/7BL	1994	16.3	350	249.7	50.1	19	44	60.2	8.1	13.7	11.3	15.8	2.2
		1995	13.4	269	200.7	34.2	10.4	51	61.9	5.5	11	12.3	16.2	1.9
XCATG.ATGC125	5BL/7BL	1994	15.3	254.9	356.6	50.9	18.9	44.6	61	8.2	14.6	11.5	16.2	2.4
		1995	13.7	202.7	271.3	34.3	10.2	51.5	62.2	5.4	11.9	12.4	16.4	2
XCAT.CTA155	5BL/7BL	1994	15.8	248.3	352.8	52.2	18.1	43.6	59.9	8.1	15.1	11.2	16.1	2.4
		1995	13.2	201.3	268.4	33.5	12	50.4	62	5.8	11.3	12.4	16.3	2
XCGA.CAT85	5BL/7BL	1994	13.3	254.5	346.9	46.2	15	45	59.5	7.3	12.3	11.5	15.8	2.1
		1995	14.4	201.2	271.5	35.1	12.8	51	63	6	12	12.3	16.4	2
XCAG.CGAT70	2B	1994	11.1	256.4	342.2	42.9	11.2	45.5	58.1	6.3	9.1	11.7	15.4	1.9
		1995	9.9	203.8	262.9	29.6	12.4	50.2	62.3	6	9.3	12.4	16	1.8
XBARC167	2B	1994	11	258.9	342.3	41.7	10.5	46.3	58.2	6	8.9	11.8	15.4	1.8
		1995	14.8	198.2	268.9	35.4	18	49.1	63.1	7	13.7	12.1	16.3	2.1
XAGC.TGC135	2B	1994	9	271.4	354.2	41.4	7.1	48.4	59	5.3	6	12.4	15.7	1.6
		1995	5	215	258.4	21.7	5.2	53	60.9	4	5.5	13	15.8	1.4
XCAT.CGTA150	2B	1994	5.9	274	336.6	31.3	5.8	48.2	57.3	4.5	4.9	12.5	15.2	1.4
		1995	11.5	205.5	269.9	32.2	12.2	51.2	63.1	5.9	12.3	12.4	16.6	2.1
XGCTG.CGAT7	2B	1994	4.3	273.5	328.8	27.6								
		1995	9.2	208	264.4	28.2								
XCAT.CGTA146	2B	1994	3.9	274.6	325.3	25.4								
		1995	9.1	204.8	261.8	28.5								
XBARC32	5BL/7BL	1994					8.1	45.5	56.3	5.4				
		1995					3.9	52.2	59	3.4				
XCATG.CGTA152	2B	1994					4.3	48.4	56.2	3.9				
		1995					7.1	52.4	61.7	4.6				

*Chro.: chromosome location.
*CI. All.: CI 13227 allele.
*Su. All.: Suwon 92 allele.
*ASE: allele substitution effect .

Table 2.5. One-way ANOVA for molecular markers linked to infection duration in a RIL population derived from CI 13227 x Suwon 92 in 1994 and 1995.

Marker	Chro*.	Year	R^2(%)	CI. All.*(days)	Su. All.*(days)	ASE* (days)
XGCTG.CGCT118	2D	1994	27.9	24.43	21.39	1.51
		1995	25.3	18.74	17.64	0.54
XGCTG.CGCT60	2D	1994	23.7	24.09	21.32	1.38
		1995	19.6	18.6	17.64	0.47
XBARC95	2D	1994	6.6	23.6	22.15	0.72
		1995	13.8	18.6	17.76	0.41
XCAT.CGTA237	2D	1994	5.5	23.38	22.04	0.67
		1995	6.8	18.43	17.83	0.29
XTGC.CTA208	2D	1994	4.0	23.18	22.04	0.57
		1995	8.0	18.37	17.74	0.31

*Chro.: chromosome location.

*CI. All.: CI 13227 allele.

*Su. All.: Suwon 92 allele

*ASE: allele sustitution effect

Table 2.6. Summary of QTLs for AUDPC, final severity and infection rate detected in RIL population derived from CI13227 x Suwon 92 in 1994 and 1995

		AUDPC					Final severity					Infection rate				
QTL	Chro. *	Year	Interval	Posi.*	R^2(%)	LOD	Interval	Posi.	R^2(%)	LOD	Interval	Posi.	R^2(%)	LOD
QLR.osu-2B1	2B	1994	XAGC.TGC135/XCATG.ATGC60	89	18.8	4.57	XCGAT.ATGC60/XCAG.CGAT70	90.9	16.6	3.97	XCGAT.ATGC60/XCAG.CGAT70	89.7	16	3.81
		1995	XCGAT.ATGC60/XCAG.CGAT70	91.2	13.4	3.16	XCGAT.ATGC60/XCAG.CGAT70	92.2	15.2	3.61	XCGAT.ATGC60/XCAG.CGAT70	90.6	13.6	3.21
QLR.osu-2B2	2B	1995	XCAT.CGTA150/XGTG.CAGT303	41.8	12.5	2.92	XCAT.CGTA146/XCAT.CGTA150	40.6	13.4	3.16	XCAT.CGTA150/XGTG.CAGT303	41.8	13.5	3.19
QLR.osu-5BL/7BL	5BL or 7BL	1994	XTGC.ACAG198/XCAT.CTA155	1.6	17.2	4.13	XTGC.ACAG198/XCAT.CTA155	2	19.4	4.72	XTGC.ACAG198/XCAT.CTA155	1.9	16.1	3.84
		1995	XCGA.CAT85/XCATG.ATGC125	5.3	14.8	3.52	XCGA.CAT85/XCATG.ATGC125	4.8	11.9	2.77	XCGA.CAT85/XCATG.ATGC125	5.3	12.9	3.04

* Chro.: chromosome location;
* posi.: position.

Table 2.7. Summary of QTL for leaf rust infection duration in RIL population derived from CI 13227 x Suwon 92 in 1994 and 1995.

QTL	Chrom.*	Year	Interval	Position(cM).	R2	LOD
QLRID.osu-2D	2D	1994	XGCTG.CGCT60/XGCTG.CGCT118	40.3	26.42	6.73
		1995	XGCTG.CGCT60/XGCTG.CGCT118	42	21.18	5.22

* chrom.: chromosome location

Legends

Fig. 2.1. Frequency distribution of leaf rust AUDPC in a RIL population derived from CI13227 x Suwon 92. Data were averaged over two years.

Fig. 2.2. Frequency distribution of leaf rust final severity in a RIL population derived from CI 13227 x Suwon 92. Data were averaged over two years.

Fig. 2.3. Frequency distribution of leaf rust infection rate (AUDPC/day) in a RIL population derived from CI 13227 x Suwon 92.

Fig. 2.4. Frequency distribution of leaf rust infection duration in a RIL population derived from CI 13227 x Suwon 92.

Fig. 2.5. Linkage groups associated with leaf rust resistance (A & B) and leaf rust infection rate (C). Recombination frequency (in brackets) and genetic distance were given on the left side. AFLP markers and SSR markers were shown on the right side. Name of AFLP markers consist of three parts: *Pst* I selective primer, *Mse* I selective primer, and molecular weight. For example, XGCTG.CGAT125 represents AFLP marker generated by *Pst* I primer pGCTG and *Mse* I primer mCGAT, and the molecular weight of this marker is 125 bp. Barc18, XBARC167, and XBARC32 and XBARC95 are SSR primers which were used as anchor markers.

Fig. 2.6. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XCAG.CGAT70 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XCAG.CGAT70 linked to QLR.osu-2B1.

Fig.2.7. An AFLP image generated from Li-cor DNA Analyzer to show the segregation

of AFLP marker XAGC.TGC135 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XAGC.TGC135 linked to QLR.osu-2B1.

Fig.2.8. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP markers XCAT.CGTA60 and XCAT.CGTA125 in the population of RILs derived from CI 13227 x Suwon 92. AFLP marker XCAT.CGTA60 as pointed by the lower arrow, and XCAT.CGTA125 as pointed by the upper arrow link to QLR.osu-2B1 and QLR.osu-5BL/7BL, respectively.

Fig.2.9. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP markers XCAT.CGTA146 and XCAT.CGTA150 in the population of RILs derived from CI 13227 x Suwon 92. Both AFLP marker XCAT.CGTA146 as pointed by the lower arrow, and XCAT.CGTA150 as pointed by the upper arrow link to QLR.osu-2B2.

Fig.2.10. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XGTG.CAGT303 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XGTG.CAGT303 linked to QLR.osu-2B2.

Fig.2.11.An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XACA.CACG126 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker linked to QLR.osu-5BL/7BL, XACA.CACG126.

Fig.2.12. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XCAT.CTA105 and XCAT.CTA155 in the population of RILs derived from CI 13227 x Suwon 92. AFLP marker XCAT.CTA105 as pointed by the

lower arrow links to QLR.osu-2B2 and AFLP marker XCAT.CTA155 as pointed by the upper arrow links to QLR.osu-5BL/7BL

Fig. 2.13. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XCGA.CAT85 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XCGA.CAT85linked to QLR.osu-5BL/7BL.

Fig. 2.14. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XTGC.ACAG198 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XTGC.ACAG198 linked to QLR.osu-5BL/7BL.

Fig. 2.15. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XGCTG.CGCT118 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker linked to QLRID.osu -2D.

Fig. 2.16. The likelihood plots of QLR.OSU-2B1 in 1994. QLR.OSU-2B1 was associated with AUDPC (left curve), final severity (middle curve) and infection rate (right curve).

Fig. 2.17. The Likelihood plots of QLR.OSU-2B1 and QLR.OSU-2B2 in 1995. Both QLR.OSU-2B1 and QLR.OSU-2B2 are associated AUDPC (the coincident curve on the right), final severity (left curve) and infection rate (the coincident line on the right). The arrows show the most likely positions of QLR.osu-2B1 (upper) and QLR.osu-2B2 (lower).

Fig. 2.18. The likelihood plots of QLR.OSU-5AL/7BL in 1994. QLR.OSU-5BL/7BL was

associated with AUDPC (middle curve), final severity (left curve) and infection rate (right curve). The arrow shows its most likely position.

Fig. 2.19. The Likelihood plots of QLR.OSU-5AL/7BL in 1995. QLR.OSU5BL/7BL was associated with AUDPC (left curve), final severity (right curve) and infection rate (middle curve). The arrow shows its most likely position.

Fig. 2.20. The likelihood plots of QTL for leaf rust infection duration, QLRID.osu-2D, in 1994 (left) and 1995 (right).

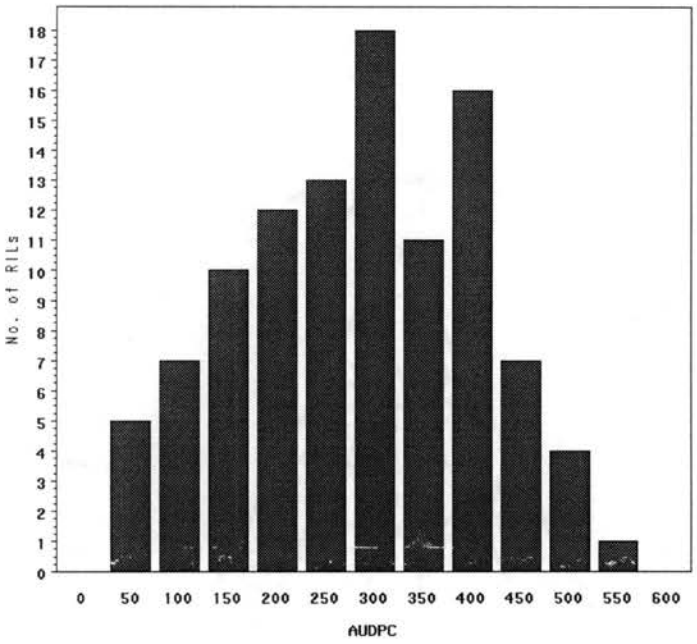

No. of RILs
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
0
50
100
150
200
250
300
350
400
450
500
550
600
AUDPC

Fig. 2.1.

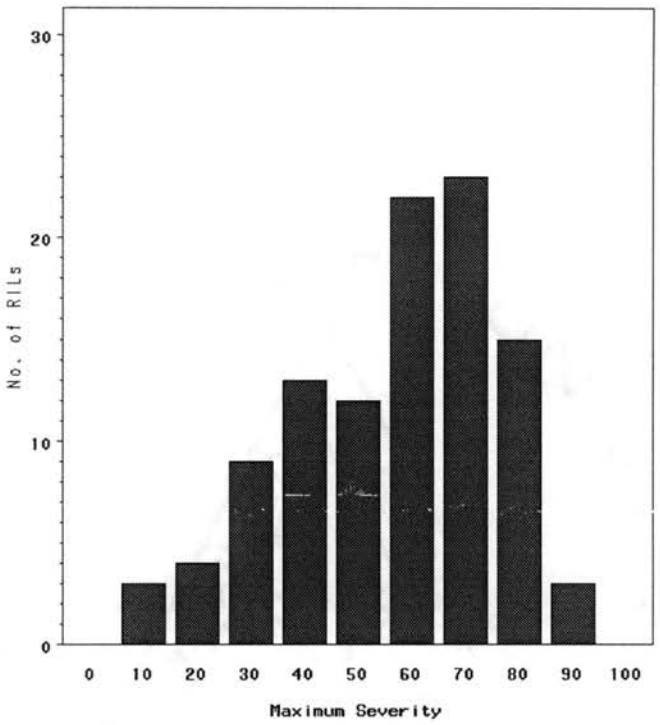

30
20
10
0
No. of RILs
0
10
20
30
40
50
60
70
80
90
100
Maximum Severity

Fig. 2.2.

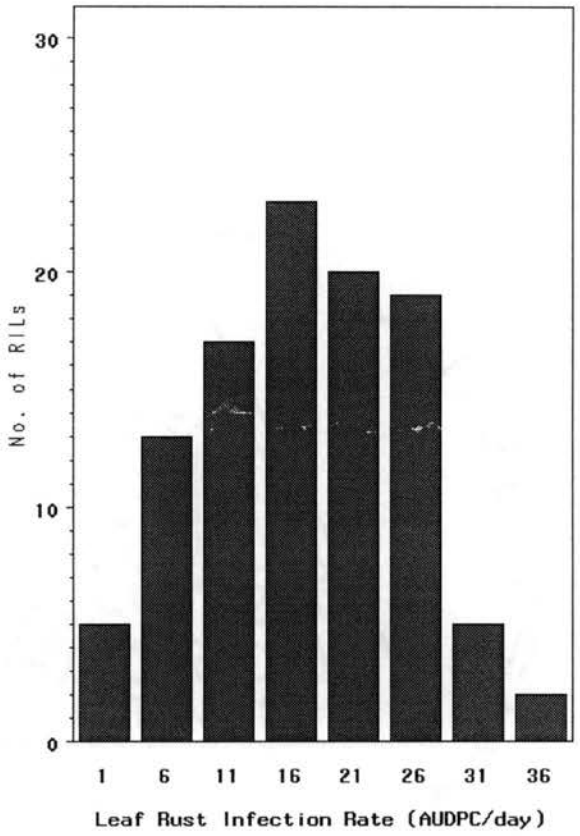
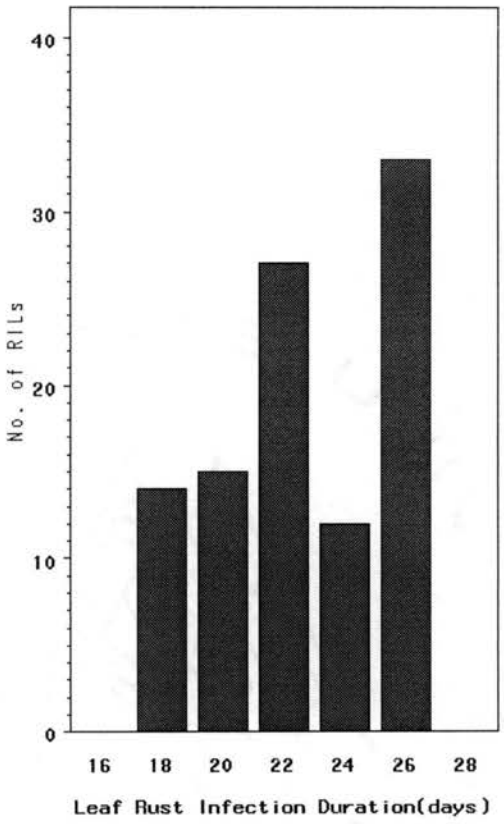

30
20
10
0
No. of RILs
1
6
11
16
21
26
31
36
Leaf Rust Infection Rate (AUDPC/day)

Fig. 2.3.


40
30
20
10
0
No. of RILs
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18
20
22
24
26
28
Leaf Rust Infection Duration(days)

Fig. 2.4.

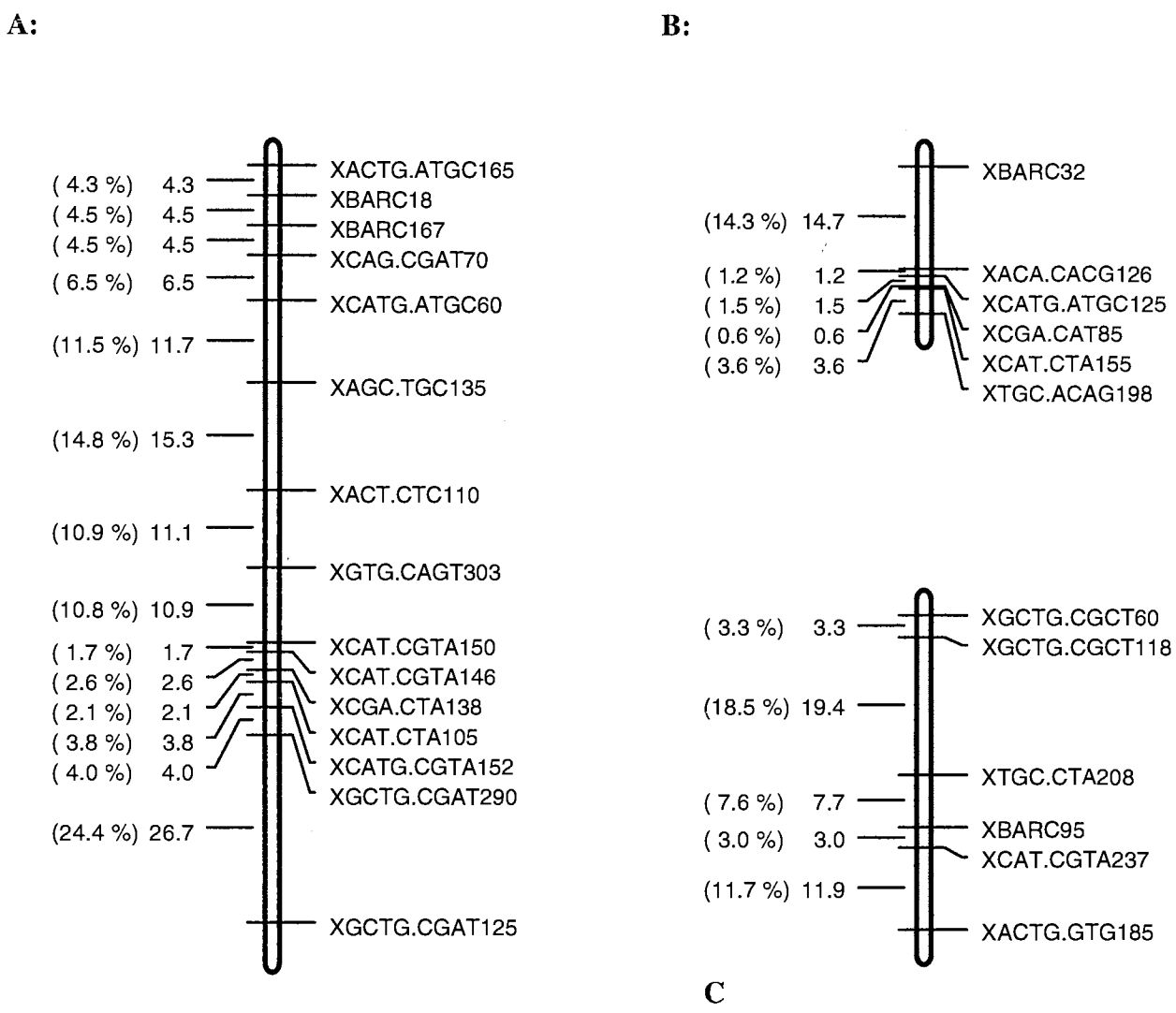
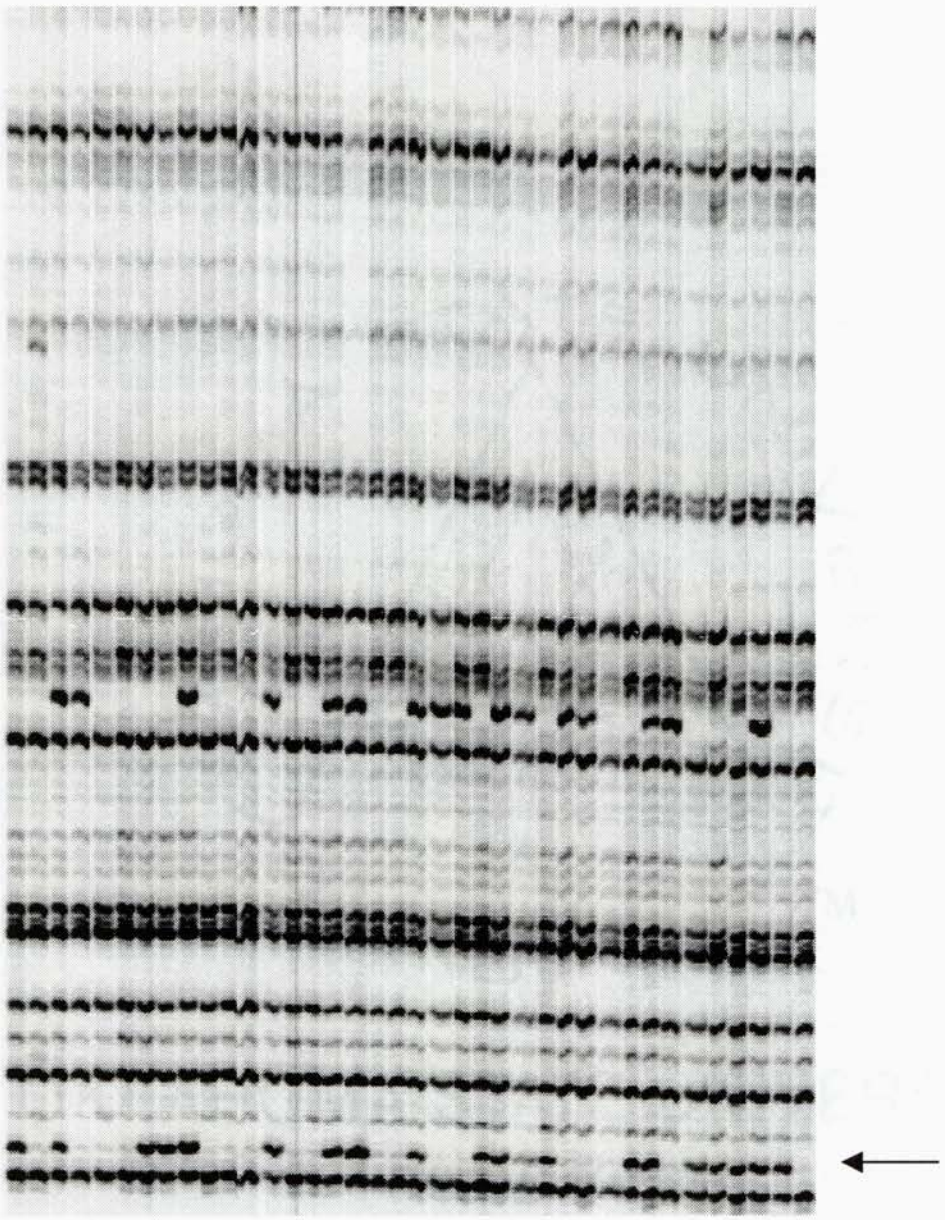

A:
B:
(4.3 %) 4.3
(4.5 %) 4.5
(4.5 %) 4.5
(6.5 %) 6.5
(11.5 %) 11.7
(14.8 %) 15.3
(10.9 %) 11.1
(10.8 %) 10.9
(1.7 %) 1.7
(2.6 %) 2.6
(2.1 %) 2.1
(3.8 %) 3.8
(4.0 %) 4.0
(24.4 %) 26.7
XACTG.ATGC165
XBARC18
XBARC167
XCAG.CGAT70
XCATG.ATGC60
XAGC.TGC135
XACT.CTC110
XGTG.CAGT303
XCAT.CGTA150
XCAT.CGTA146
XCGA.CTA138
XCAT.CTA105
XCATG.CGTA152
XGCTG.CGAT290
XGCTG.CGAT125
(14.3 %) 14.7
(1.2 %) 1.2
(1.5 %) 1.5
(0.6 %) 0.6
(3.6 %) 3.6
XBARC32
XACA.CACG126
XCATG.ATGC125
XCGA.CAT85
XCAT.CTA155
XTGC.ACAG198
(3.3 %) 3.3
(18.5 %) 19.4
(7.6 %) 7.7
(3.0 %) 3.0
(11.7 %) 11.9
XGCTG.CGCT60
XGCTG.CGCT118
XTGC.CTA208
XBARC95
XCAT.CGTA237
XACTG.GTG185
C

Fig. 2.5.



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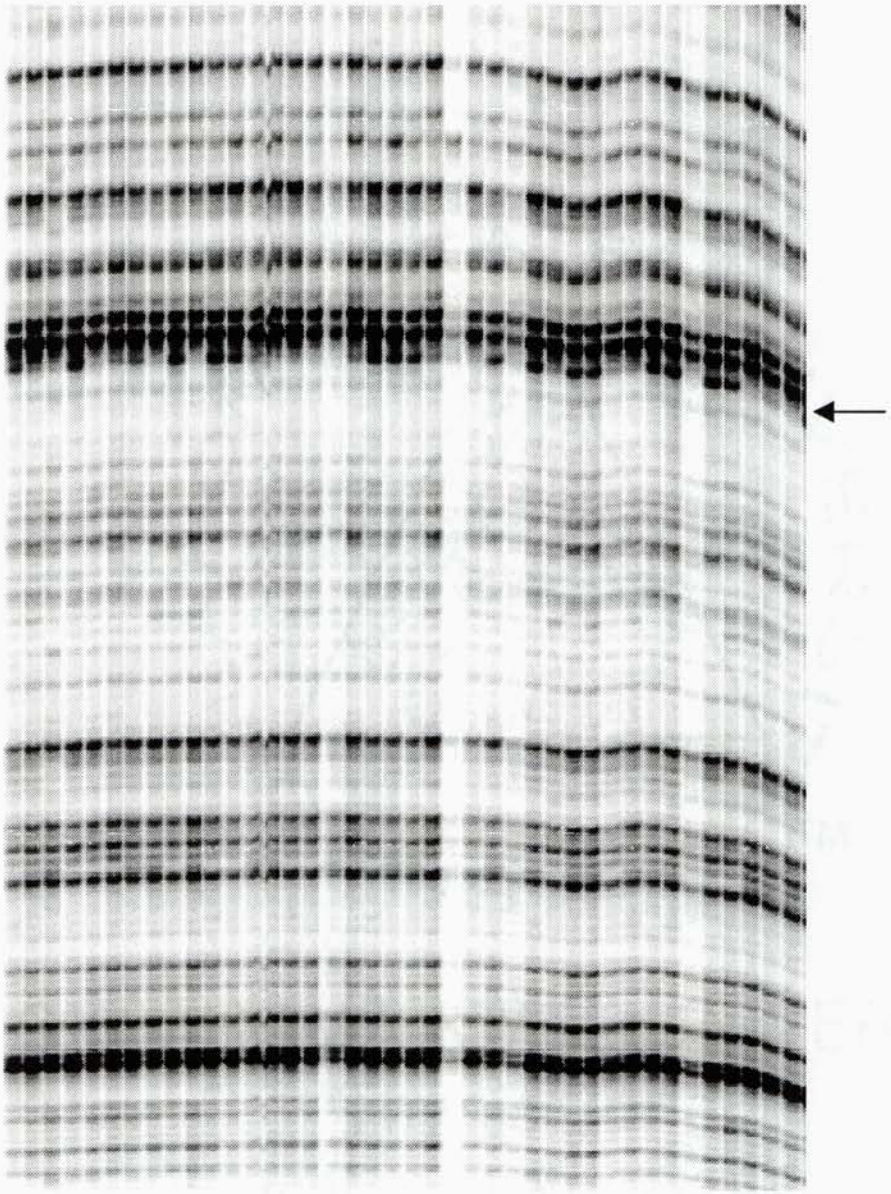

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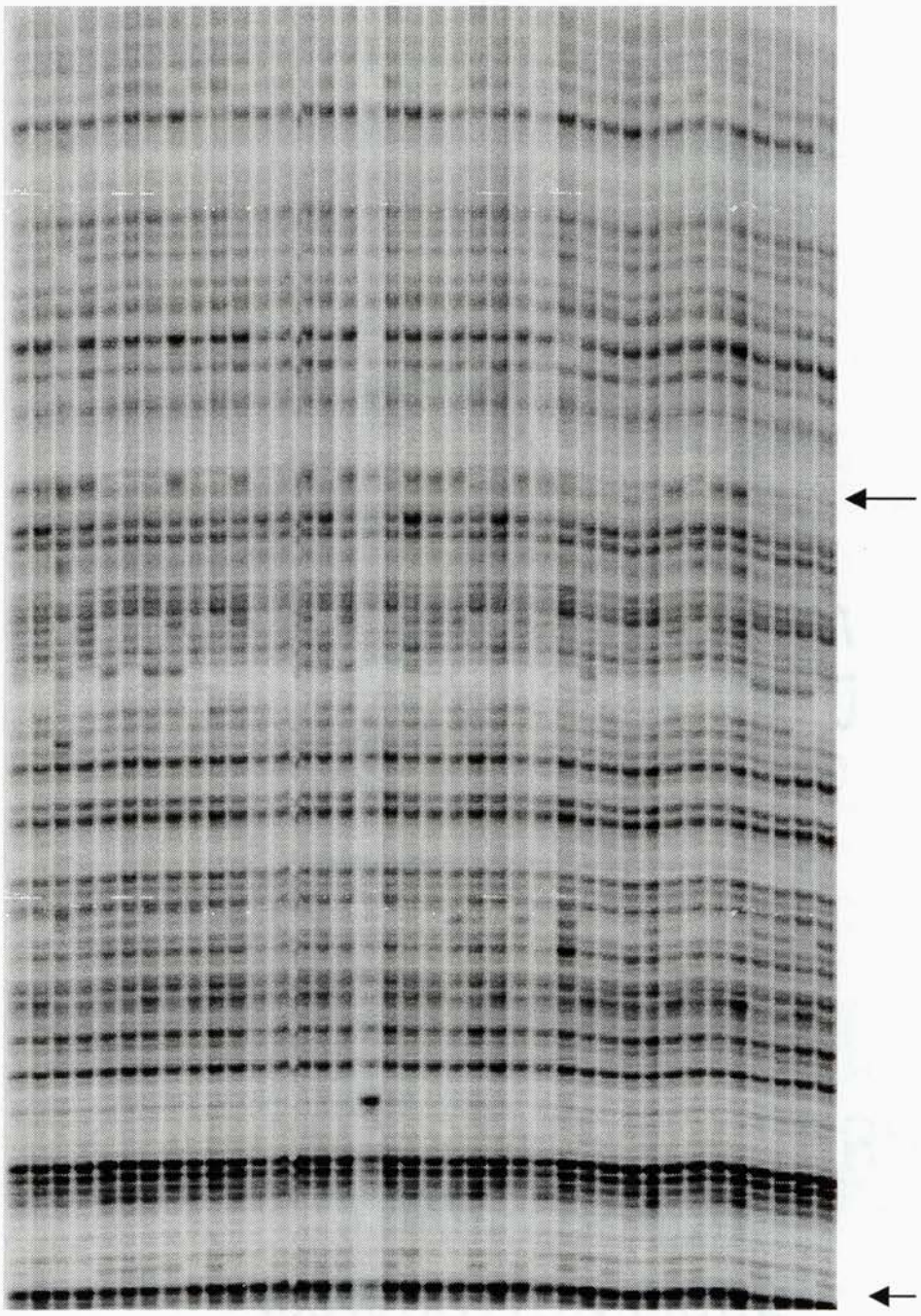

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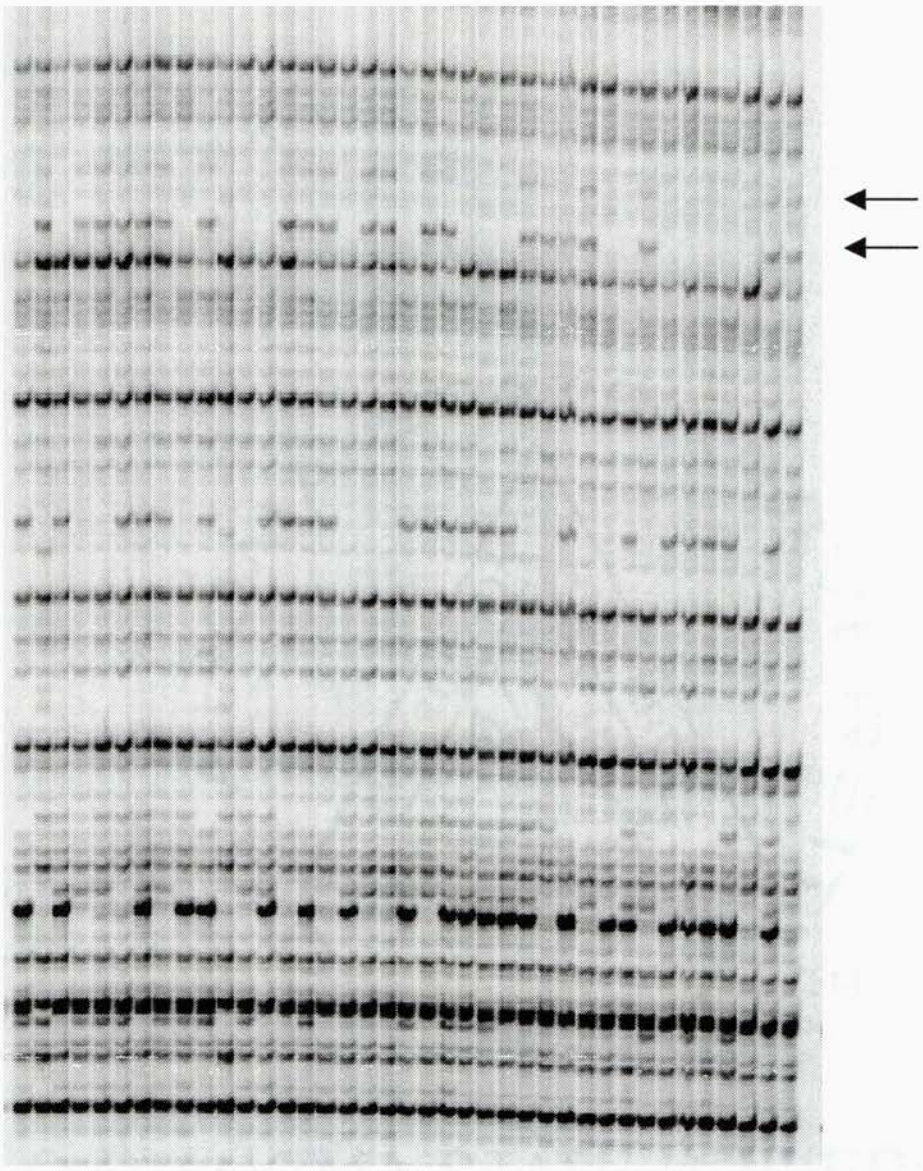

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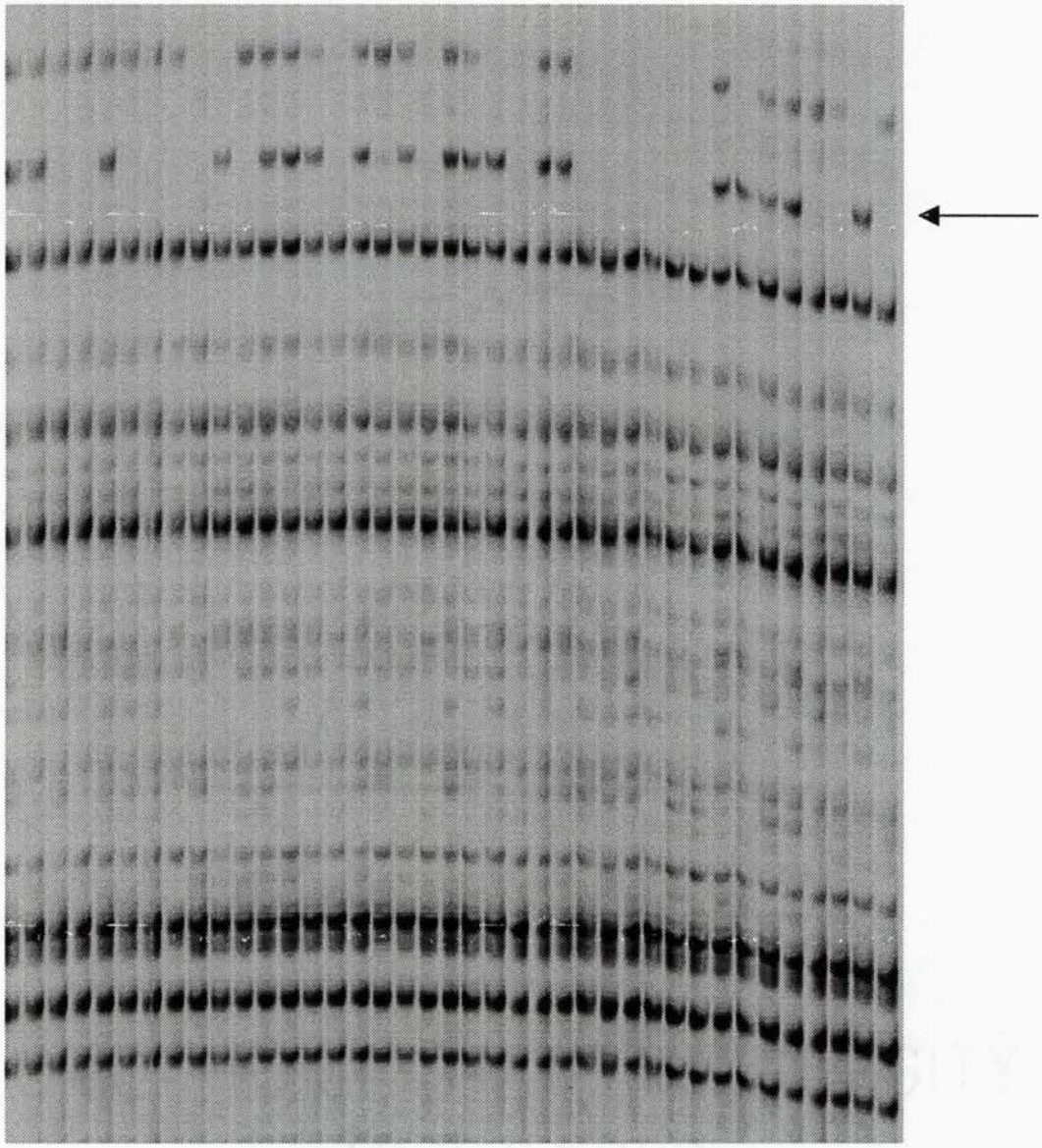

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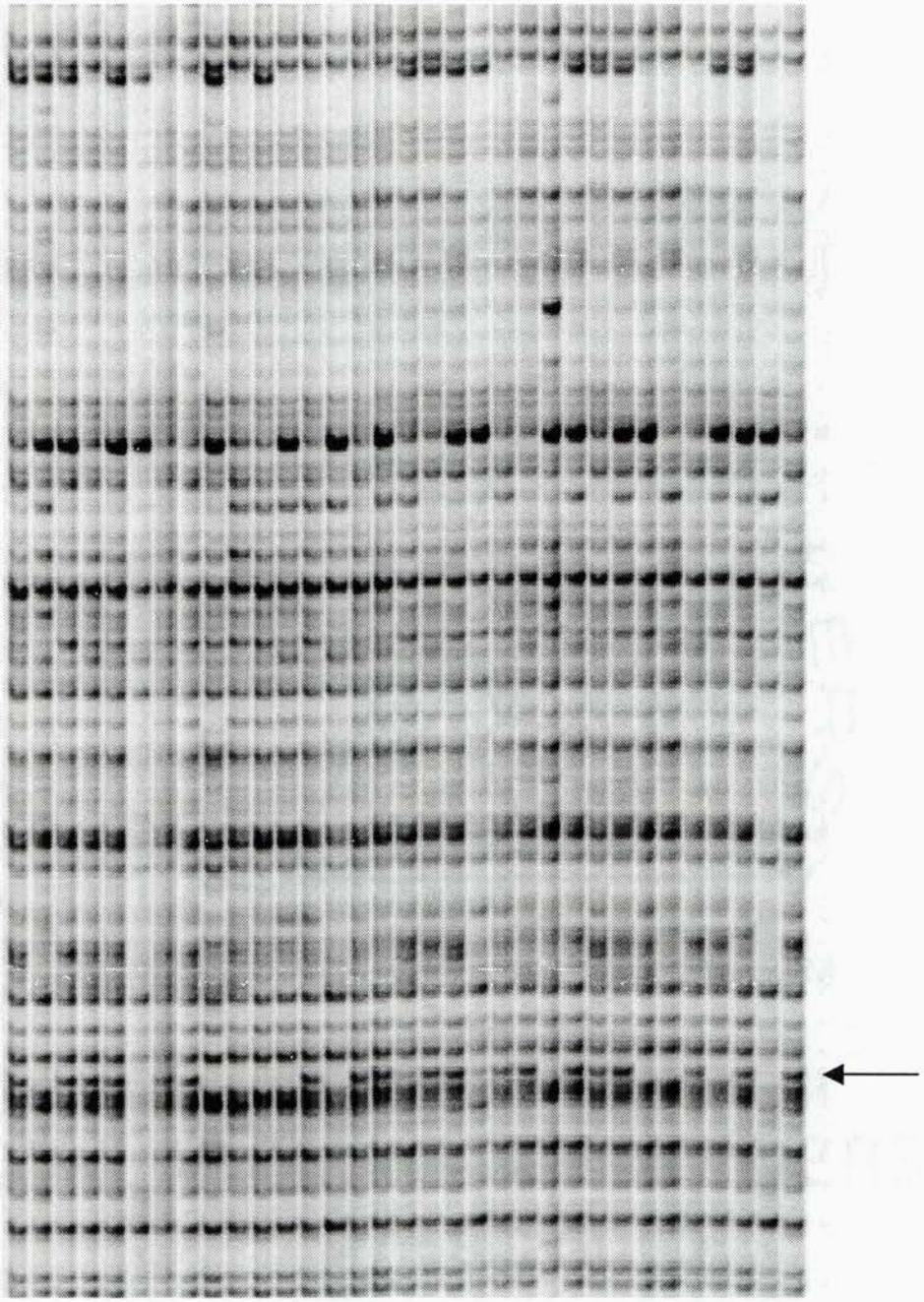

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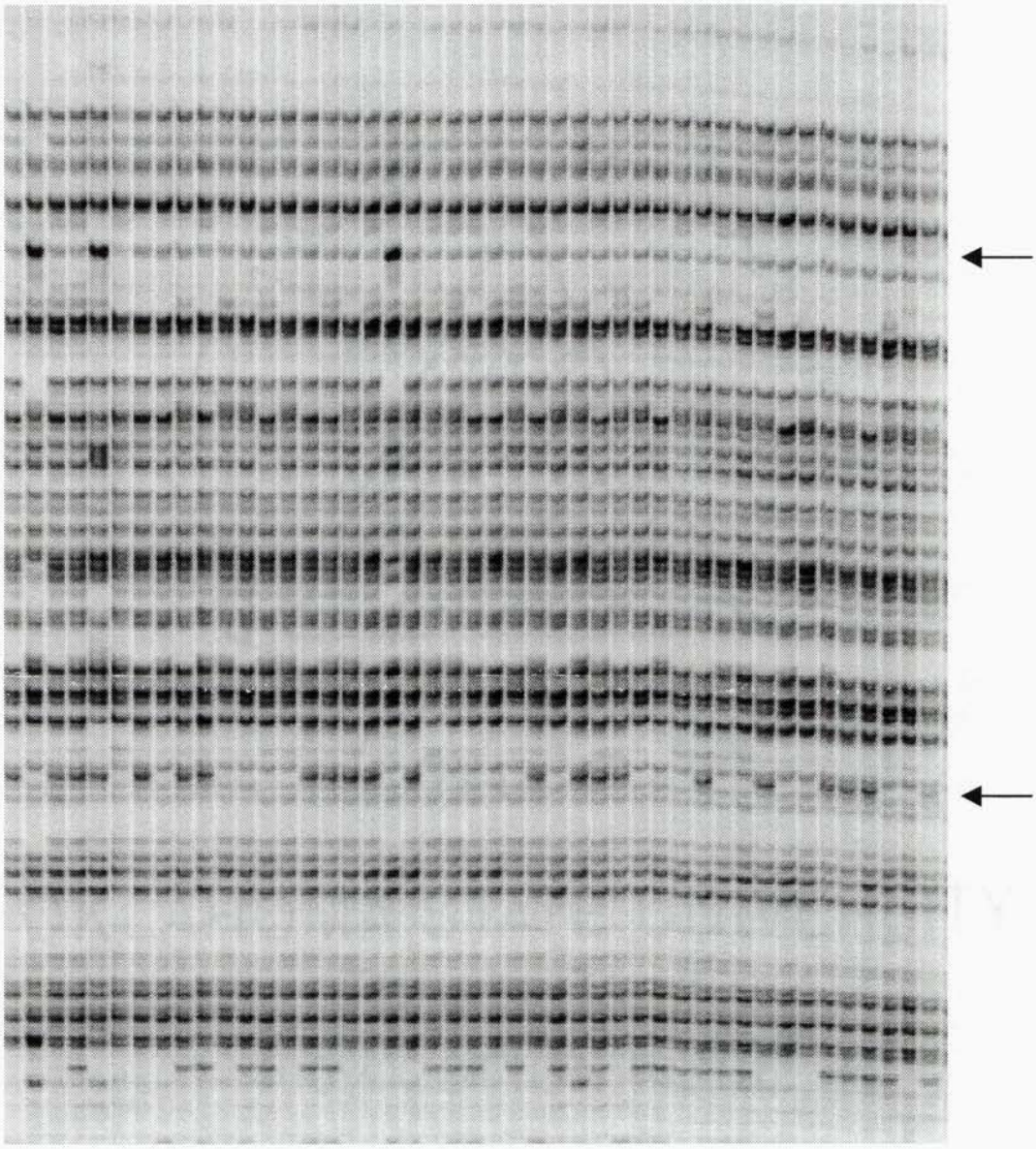

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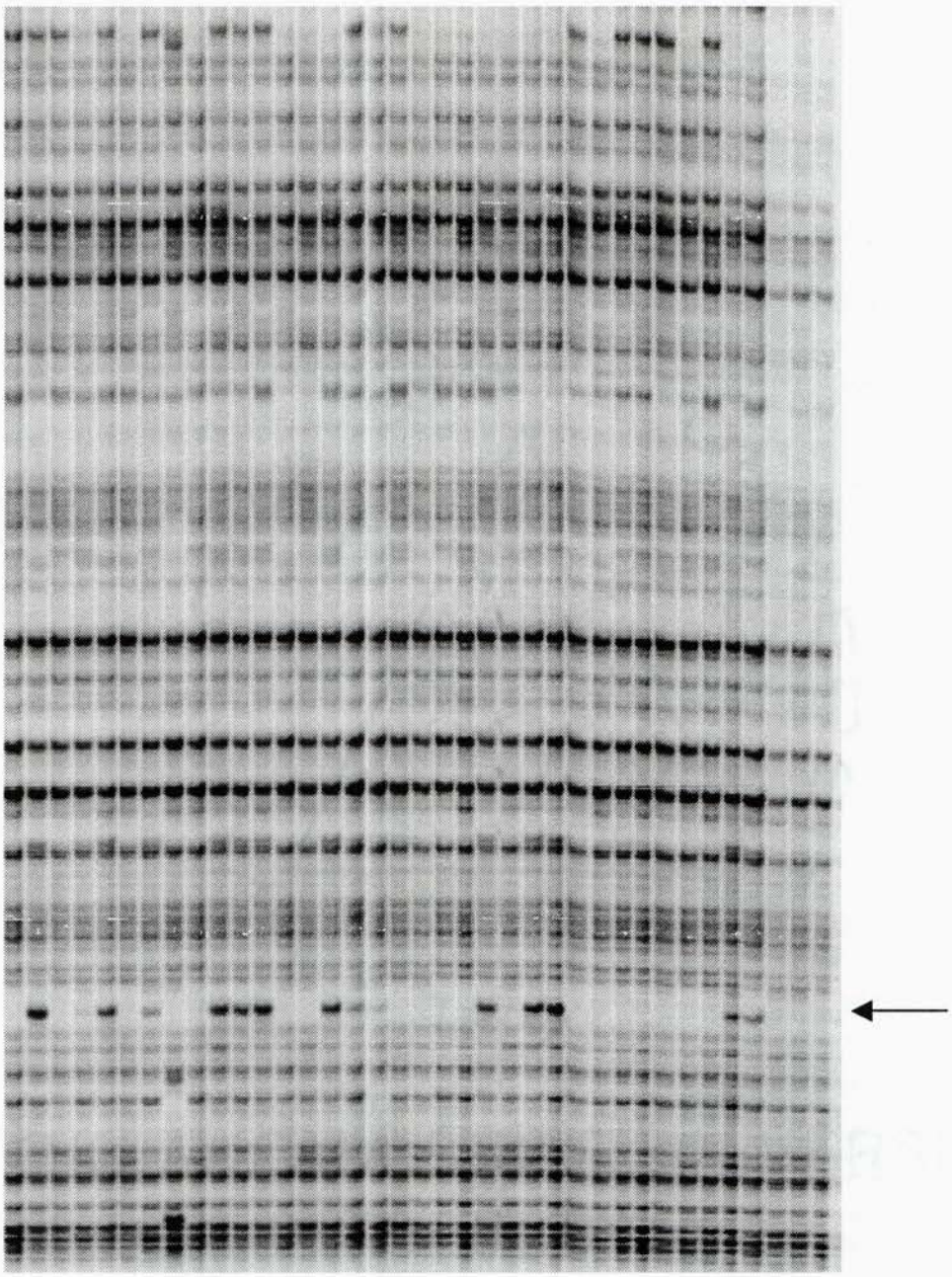

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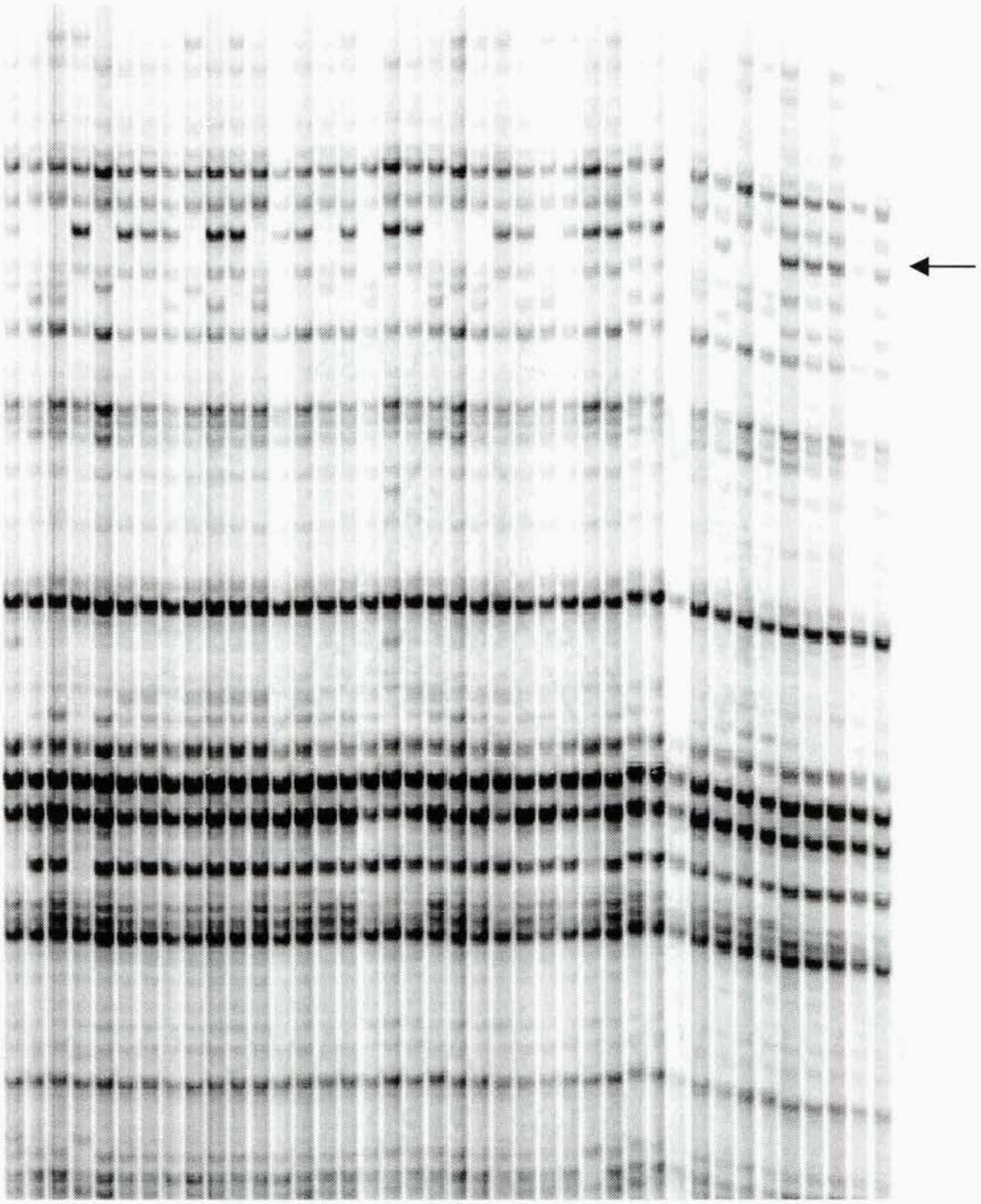

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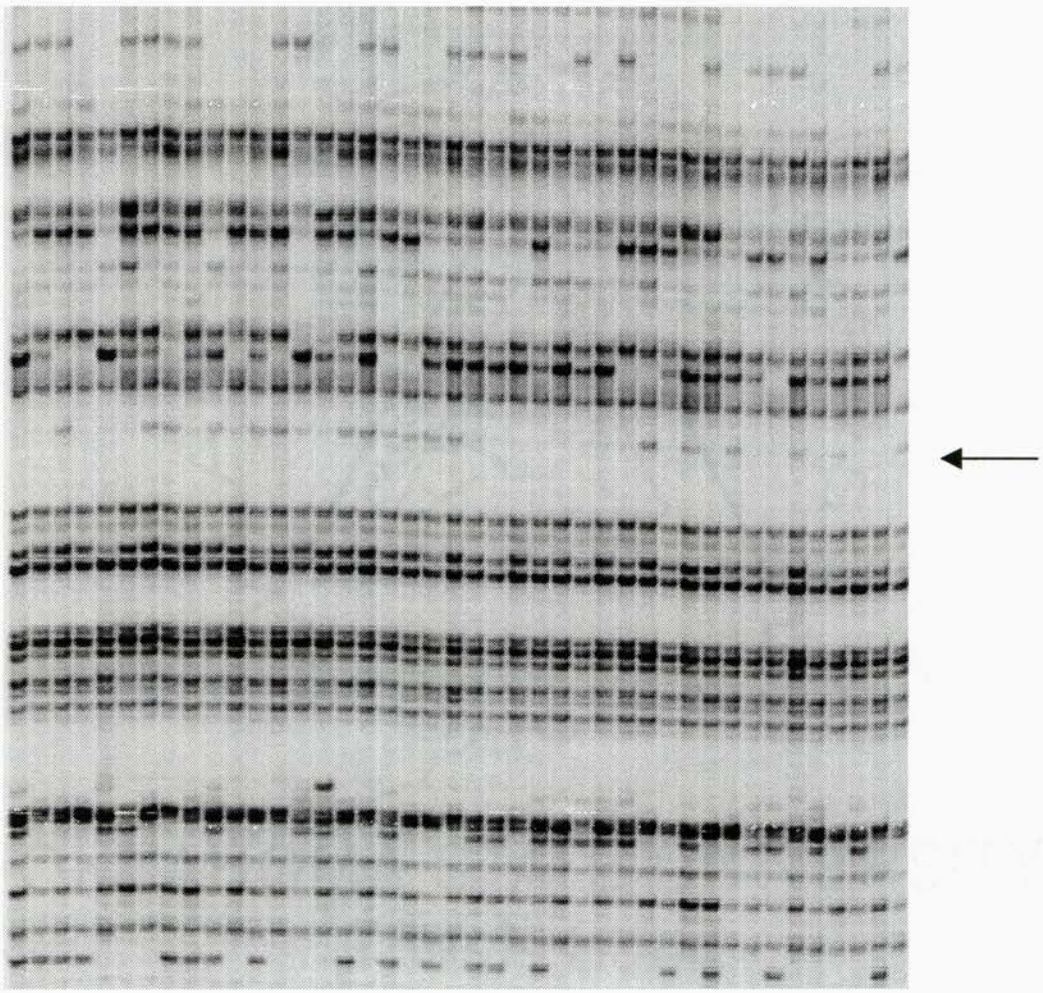

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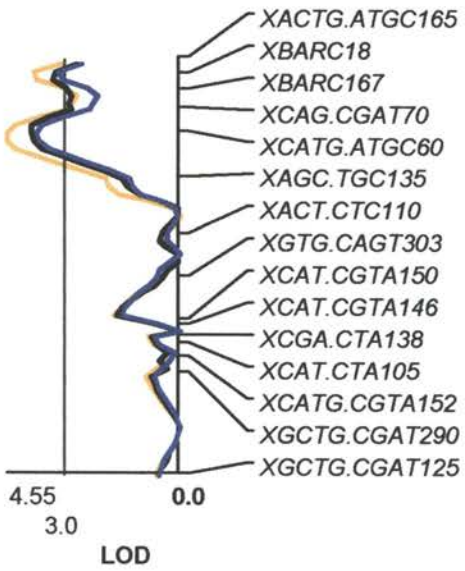
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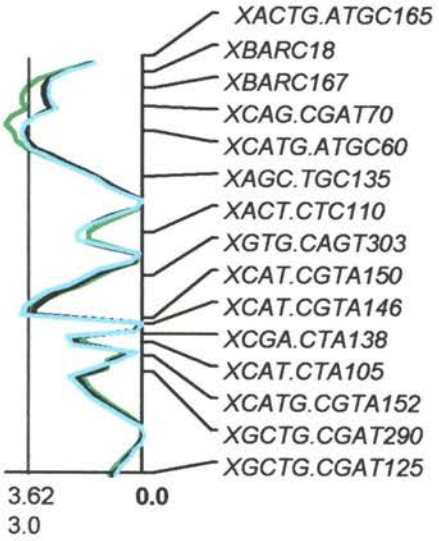
XBARC167
XCAG.CGAT70
XCATG.ATGC60
XAGC.TGC135
XACT.CTC110
XGTG.CAGT303
XCAT.CGTA150
XCAT.CGTA146
XCGA.CTA138
XCAT.CTA105
XCATG.CGTA152
XGCTG.CGAT290
XGCTG.CGAT125
4.55
3.0
0.0
LOD

Fig. 2.16.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XBARC167
XCAG.CGAT70
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XAGC.TGC135
XACT.CTC110
XGTG.CAGT303
XCAT.CGTA150
XCAT.CGTA146
XCGA.CTA138
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XGCTG.CGAT125
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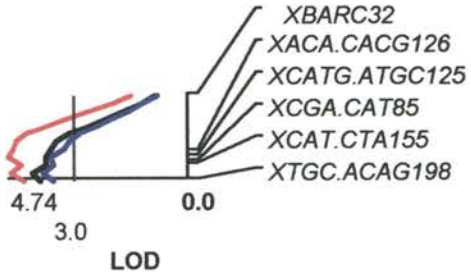
3.0
0.0

Fig. 2.17.


XBARC32
XACA.CACG126
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XCAT.CTA155
XTGC.ACAG198
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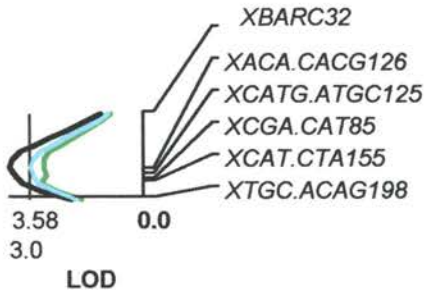
3.0
0.0
LOD

Fig. 2.18.


XBARC32
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XCAT.CTA155
XTGC.ACAG198
3.58
0.0
3.0
LOD

Fig. 2.19.

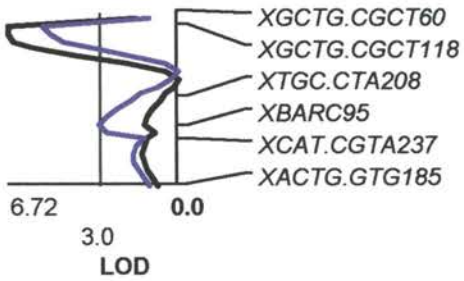
XGCTG.CGCT60
XGCTG.CGCT118
XTGC.CTA208
XBARC95
XCAT.CGTA237
XACTG.GTG185
6.72
0.0
3.0
LOD

Fig. 2.20.

Chapter III

Molecular Mapping of Quantitative Trait Loci for Latent Period of *Puccinia triticina* in Wheat

Abstract

Slow rusting is an important character for durable resistance to wheat leaf rust, while long latent period (LP) is a crucial component of slow rusting. Selection for longer LP is an effective approach to improve wheat cultivars with durable resistance to leaf rust. CI 13227, a US soft red winter wheat cultivar, was identified to have a long latent period. A recombinant inbred line (RIL) population derived from CI 13227 X Suwon 92 was phenotyped for latent period in two years, and AFLP and SSR markers were analyzed in the same population. The LP frequency distributions were continuous in the RILs, and LP of the RILs was significantly correlated between two years ($r=0.94$, $p<0.0001$). Three quantitative trait loci (QTL) for latent period were identified. A major QTL located on chromosome 2D, designated as QLRLP.osu-2D, explained 45.7% and 33.2% of the phenotypic variance in two years. QLRLP.osu-2D is the first QTL identified and mapped for latent period. Multiple regression analysis showed the three QTLs acted independently, and collectively explained 61.2% of the phenotypic variance in 1988 and 54.2% in 1989. Fourteen RILs with all three long latent period alleles at three AFLP loci flanking each QTL had an average LP of 12.7 days in two years, while thirteen RILs without any long latent period alleles at three AFLP loci flanking QTL had an average LP of 7.3 days in two years. Molecular markers closely linked to these QTLs have the potential to be applied in marker-assisted selection for long latent period.

Key words: wheat, slow rusting, latent period, QTL, AFLP, SSR and MAS

Introduction

Leaf rust, caused by *Puccinia triticina* (previously *Puccinia. recondite* Rob. Ex Desm), is one of the most common and widely distributed diseases in wheat worldwide. The yield losses caused by leaf rust mainly derived from shriveled kernels produced after pre-mature senescence. In many wheat-producing regions, breeding for leaf rust resistant cultivars is a daunting task due to the wide deployment of race-specific resistance genes. Race-specific genes usually are short-lived in nature because of the occurrence of pathogen races with matching virulence alleles after they are widely deployed. As a result, slow leaf rusting is becoming more and more attractive due to its durability (Kuhn et al. 1978; Rajaram et al. 1984). Slow leaf rusting resistance is quantitative and race-nonspecific. In slow rusting cultivars, susceptible reaction is still visible but the rate of disease progress is much slower than in susceptible cultivars (Bjarko et al. 1988; Caldwell et al. 1970, Shaner et al. 1980 & 1997). Slow rusting consists of several components, including longer latent period, reduced receptivity (number of uredinia per unite area of leaf), smaller uredinia size, and lower spore production (Ohm et al. 1976; Minus et al. 1980; Parlevlit 1975; Shaner et al.1978, 1980 & 1983).

Among these components, latent period is crucial in determining the apparent leaf rust resistance (Parlevliet 1997) and is negatively associated with area under disease progress curve (AUDPC), final rust severity, and uredinium size and receptivity (Das et al. 1993). By simulating epidemics with parameters that approximate the range found in cereal rust, cereal powdery mildew, and potato late blight, Zadoks et al. (1971) found that latent

period is more important to infection rate than any other component. Data collected with barley also suggested that the differences in partial resistance in the field are largely explained by differences in latent period (Parlevliet 1975). In another study, Neervoort et al. (1978) found that the correlation coefficients for total spore production verse partial resistance is the same as that for latent period verse partial resistance (r=0.85), and thus they concluded that latent period was as suitable for estimating partial resistance as all other parameters combined.

The genetic mechanisms of latent period are not well characterized. Clifford (1972) studied the course of infection and colonization of the leaf rust pathogen in two barley cultivars contrasting in resistance to leaf rust and found that the resistant cultivar Vada had two times as many infections arrested in the substomatal vesicle phase than that in the susceptible cultivar Midas. The pustules in Midas ruptured the epidermis 7 to 8 days after the inoculation, but those on Vada ruptured after 9 to 14 days. Vada also had significantly smaller and fewer pustules than Midas.

Heritability of latent period is moderately high, ranging from 0.46 to 0.90 (Das et al. 1993; Lee et al. 1985; Jacobs et al. 1989). Three to five genes were reported to control longer latent period (Vander Gaag et al. 1997; Das et al.1992). Shaner et al. (1997) found four loci with epistatic effects to control latent period and one of them showed a major effect. Transgressive segregation of latent period in the progenies was also observed (Lee et al.1985; Broers et al. 1989). Hence, selection for long latent period by pyramiding several quantitative trait loci (QTL) appears feasible.

Since phenotypic evaluation of latent period is labor-intensive and requires complicated measuring procedures, traditional breeding methods may be inappropriate to

select for long latent period. Molecular markers linked to QTL for latent period may simplify selection and enhance selection efficiency.

Molecular characterization of QTL for long latent period has not been documented. The objectives of this study were to identify the number and genome positions of QTL for long latent period of *Puccinia triticina* in wheat and understand its genetic basis, and develop molecular markers that can be used to enhance durable rust resistance in wheat cultivars through marker-assisted selection.

Materials and methods

Plant materials

A mapping population with 98 recombinant inbred lines (RILs) was developed by single seed descent from a cross between the US resistant wheat line CI 13227 and a Korean susceptible cultivar Suwon 92. CI 13227 showed a high level of slow leaf rusting resistance with a long latent period, ranging from 12.8 to 13.9 days (Shaner et al., 1980). Suwon92 was very susceptible to leaf rust with a short latent period of 7 to 8 days. Nether parent is known to carry any race-specific Lr genes. Shaner et al. (1997) provided detailed information on the population.

Greenhouse experiments were conducted at West Lafayette, IN, in the fall of 1988 and spring of 1989 using a randomized complete-block design with seven replications. Each replication contained one plant from each F_7 family and five plants from each parent. Plants with flag leaves fully emerged were inoculated with uredinospores of *P. triticina* culture 7434-1-1T. Latent period was measured by counting the number of days

from inoculation to the time of uredinia rupturing the epidermis. For each line, mean latent period (MLP) was calculated and used for analysis herein (Shaner et al. 1997).

Analysis of amplified fragmant length polymorphism (AFLP)

The CTAB (Cetyltrimethyllammonium) method was used to isolate genomic DNA from two-week-old wheat seedlings (Saghai-Maroof et al. 1984). About 300 ng wheat genomic DNA was double-digested with *Pst* I and *Mse* I restriction enzymes in 1X H buffer at 37 ° C for 2 hours. After digestion, 8 µl of ligation mixture containing 4 pmol of P*st I* and 40 pmol of M*seI* adapters, 0.8 unit of DNA ligase and 1X ligase buffer was added to the digested DNA. The ligation was conducted at 20 °C overnight. The resulting 38 µl DNA mixture was diluted with 362 µl sterilized deioned water for subsequent PCR reactions.

For preamplification, a 40 µl of PCR reaction included 10 µl ligated DNA, 75 ng of each of *Pst* I and *Mse* I pre-amplification primers, 0.75 unit Taq enzyme, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 1x PCR buffer. The following PCR profile was used: 25 cycles of 30 seconds at 94 ° C, 1 minute annealing at 56° C and 1 minute extension at 72 ° C. Five microliter of PCR product was used for quality check on a 1% agarose gel. The remaining PCR product was diluted 10-folds by adding 315 µl of sterilized deioned water.

Selective amplification was conducted using *Pst* I and *Mse* I primers with 2 to 4 additional selective nucleotides. *Pst* I primers were labeled with IR fluorescent dye. Each PCR reaction contained 3 µl diluted pre-amplified DNA, 0.4 µl *Pst* I primer, 0.4 µl *Mse* I primer, 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.2 unit of Taq DNA polymerase, 1 x PCR buffer

and 3.8 µl water. The following PCR profile was used for selective PCR amplification: denaturing at 94°C for 2 minutes followed by 13 cycles of 30 seconds at 94 °C, 30 seconds of annealing at 65 °C with a decreasing of 0.7 °C per cycle, 1 minute of extension at 72 °C, and then 23 cycles of 30 seconds of denature at 94 °C, 30 seconds of annealing at 65 °C, and 1 minute of extension at 72 °C. Finally, an extension at 72 °C for 5 minutes was followed. PCR was conducted in a MJR PTC-100 thermal cycler. The PCR product was denatured at 95 °C for 5 min after adding 5 µl loading buffer and chilled quickly on the ice.

AFLP products were separated in a 6.5% denaturing polyacrylamide gel running in 1x TBE buffer on Li-Cor IR-4200 DNA sequencer. The electrophoresis condition was set at 1500 V voltage, 40 W power, 35 mA current with 50 °C constant temperature for 3 h. Gel was prepared as described in the user's manuals from the manufacturer. A 68 well comb was used for gel well formation and 0.8 µl of each sample was loaded into separated wells using Hamilton 8-channel syringe (Hamilton, Reno, NV). The gel image file was stored in a computer and the segregation patterns were manually scored by visual inspection.

Analysis of simple sequence repeat (SSR) markers

PCR. SSR was conducted using silver staining method. For each reaction, 20 ng of genomic DNA was used in a solution containing 200 µM of each dNTP, 1x PCR buffer, 2.5 pmol of each primer with 2.0 mM $MgCl_2$ and 2 units of Taq polymerase. The touch-town program was used for PCR amplification, in which reaction mixture was denatured at 95 °C for 5 minutes followed by 5 cycles of 45 seconds at 95 °C, 5 minutes of

annealing at 68 °C with a decreasing of 2 °C in each of following cycle, and 1 minute of extension at 72 °C.; For another 5 cycles the annealing temperature starts at 58 °C for 2 minutes with a decrease of 2°C for each of following cycle. Then, PCR went through additional 25 cycles of 45 seconds at 94 °C, 2 minutes annealing at 50 °C, and 1 minute of extension at 72 °C with a final extension at 72 °C for 5 minutes.

Gel preparation and electrophoresis. The large and small glass plates were cleaned thoroughly with Rain-X and 75% ethanol, respectively. Then, the inner side of the small glass plate was wiped with 1.5 ml of freshly prepared gel binding solution containing 5 µl of bind silane, 150 µl of acetic acid and 1.345 ml of 95% ethanol. After the solution dried, two 0.5 mm side spacers were placed between the glass plates and the gel plates were assembled with two clamps. Six hundred microliter of 20% ammonium persulfate solution and 60 µl ofTetramethyl ethylene-diamine (TEMD) were added to 100 ml of 5% polyacrylamide gel solution that contains 4.75 g of acrylamide, 0.25 g of Bis-acylamide, 45 g of urea, and 5 ml of 10X TBE buffer. The gel mix was injected into between the glass plates by a syringe and a comb was inserted from the top to form wells for loading samples.

After the gel was polymerized, the electrophoresis apparatus was assembled and 2 L of 0.5x TBE buffer was added into the upper and bottom buffer chambers. The gel was pre-run at 100 Walt for 40 minutes to achieve a gel surface temperature of approx. 40 °C. After the gel wells were flushed with buffer using a syringe, the denatured PCR product was loaded into gel wells. The gel was run at 100 w for about 90 min.

Silver staining of gels. When the electrophoresis was finished, buffer was drained and glass plates were removed and carefully separated with a spatula. The gel usually

stuck on the small plate. To fix the gel, the plate with gel was immersed in 1.5 L of fixation solution (10% w/w glacial acetic acid) for 30 min. After the gel was washed twice with 2 L of DD-water for 2 min, it was stained with 1.5 L of silver staining solution containing 1.5 g of silver nitrate and 2.3 ml of 37% formaldehyde for 35 min, and then washed with DD-water for 5 to 10 sec. The gel was moved to 1.5 L of freshly prepared cold developing solution which contained 45 g of sodium carbonate, 2.3 ml of 37% formaldehyde, 300 µl of 10 mg/ml sodium thiosulfate. After the gel was developed for 5 to 15 min, DNA bands showed up in the gel and then fixation solution was added to the development solution to fix the gel for 5 minutes. Then the gel was washed in distilled sterile water for 2 min. Gel was air-dried overnight and scanned in a scanner to collect data.

Bulk segregant analysis (BSA)

BSA based on phenotypic data was used for initial screening of informative AFLP primers. After 612 *Pst* I / *Mse* I primer pairs were screened (Table 3.1.), 85 primer pairs showed polymorphism between two bulks and therefore they were used to genotype the population. A total of 459 AFLP markers were analyzed in the population. AFLP markers linked to three QTL for MLP were identified in initial QTL analysis. To determine the tentative chromosome locations of these QTL, a revised BSA method was applied to screen informative SSR primers. A total of three pairs of bulks contrasting in presence and absence of an individual QTL for MLP were constructed based on AFLP markers flanking the target QTL. In each pair of bulks, long MLP bulks consisted of equal amount of DNA from each of five RILs that had AFLP alleles flanking a QTL for

long MLP, and short MLP bulks consisted of equal amount of DNA from each of five RILs that had alternative AFLP alleles. The selected RILs in each pair of bulks showed excellent contrast in MLP. DNA from two parents and the six bulks were used to screen SSR primers. Totally, 146 SSR primers were screened, and 16 SSR primers that detected polymorphism between at least one pair of bulks were used to genotype the entire population. Data from both SSR and AFLP were combined for further QTL analysis.

Data analysis

One-way ANOVA was used to identify AFLP markers that were significantly associated with mean latent period (MLP) ($p < 0.05$). Genetic linkage maps were constructed by using MapMaker 3.0 (Lander et al. 1987). A threshold of log likelihood ratio (LOD) was set at 4.0 for the construction of linkage groups. Centimorgan (cM) values were calculated according to the Kosambi mapping function (Kosambi 1944). "Single marker analysis" and "Interval analysis" from Qgene (Nelson 1997) were used to characterize the effects of each individual marker and to detect QTL for MLP. The SAS "PROC GCHART" was used to generate the histograms of phenotypic frequency distribution, and the SAS PROC REG was used to perform multiple regression analysis. QTL was designated according to the guidelines for nomenclature of quantitative trait loci in wheat (McIntosh et al. 1998). "LRLP" (leaf rust latent period) and "osu" (Oklahoma State University) were used for trait designator and laboratory designator, respectively.

Results

Phenotype segregation

For both experiments in 1988 and 1989, a significant difference in mean LP was observed between CI 13227 and Suwon 92 (Table 3.1.) The average LP of the slow rusting parent CI 13227 was about 6 days longer than that of the susceptible parent Suwon 92. The RIL population showed continuous distribution for LP, varying from 7 to 15 days (Fig. 3.1. and Fig. 3. 2.) among lines. The population mean LP of RILs (9.2 days) was about one day shorter than the mid-parent (10.4 days). Transgressive segregation for mean LP was found in both years (Fig.3.1. and Fig. 3. 2.). The RIL LPs were highly correlated between years ($r=0.94$ $p<0.0001$).

Molecular markers linked to latent period

Molecular markers closely linked to mean LP were identified by one-way ANOVA based on two years' phenotypic data (Table 3.2.). The determination coefficients (R^2) ranged from 6.3% to 43.9% in 1988 and from 4.9% to 31.6% in 1989. The additive effects of individual markers ranged from 0.4 days to 1.3 days. Linkage analysis putatively assigned these markers to three chromosomes: 2D, 2B and 5BL/7BL (Fig. 3.5.). Three AFLP markers and one SSR marker on chromosome 2D showed high R^2 for mean LP, indicating that these AFLP and SSR markers may be tightly linked to a major QTL for long latent period. The segregation patterns of these markers in the population of RILs derived from CI 13227 x Suwon 92 were given in Fig. 3.3. to Fig. 3.6.

Interval analysis

Interval mapping indicated that a putative QTL for long LP was between AFLP markers XACTG.GTG185 and XCAT.CGTA237 on chromosome 2D. This QTL, designated as QLRLP.osu-2D, resides about 2.8 cM away from XACTG.GTG185 with LOD values of 12.5 and 8.7 in 1988 and 1989, respectively. This QTL had a major effect on LP and explained 45.7% and 33.2% of the phenotypic variance in 1988 and 1989, respectively (Table 3.3.).

The second QTL was detected on chromosome 2B and designated as QLRLP.osu-2B. This QTL appeared between AFLP XCAG.CGAT70 and XCATG.ATGC60 with a LOD value of 3.3 in 1988, 3.6 in 1989, and was 1.2 cM away from XCAG.CGAT70 and 5.3 cM away from XCATG.ATGC60. This QTL explained 15.0 and 16.2% of the phenotypic variance for mean LP in 1988 and 1989, respectively.

The third QTL detected in this study was designated as QLRLP.osu-5BL/7BL, based on the chromosome location of the linked SSR marker XBarc32. Since XBarc32 was previously located on 5BL or 7BL, location of this QTL cannot be assigned to a specific chromosome. This QTL resides between AFLP markers XACA.CACG126 and XCATG.ATGC125 with LOD values of 2.4 in 1988 and 3.6 in 1989. It explained 11.1 and 16.0% of the total phenotypic variance in 1988 and 1989, respectively.

Multiple regression analysis of the three QTL on LP

Three markers with the highest R^2 values of each QTL for LP in the three linkage groups were selected for multiple regression analysis (Table 3.4.). A multicollonearity

test did not show significant correlations among the three QTL (p=0.17-0.58). Thus, the three QTL were assumed to be independent QTL. The respective partial R^2s for QLRLP.osu-2D, QLRLP.osu-2B, and QLRLP.osu-5BL/7BL were 43.2%, 11.9%, and 6.1% in 1988, and 30.8%, 13.3%, and 10.0% in 1989. The three QTL collectively explained 61.2 and 54.2% of the total phenotypic variance in 1988 and 1989, respectively.

Discussion

Slow leaf rusting is also known as horizontal resistance, general resistance, or race-nonspecific resistance. This type of plant response is thought to retard the onset of disease epidemics. The major components of slow leaf rusting resistance include longer latent period, smaller uredinium size, lower receptivity and less spore production as compared to leaf rust susceptible cultivars. Among these components, latent period is regarded as a more stable parameter when plants are inoculated under controlled greenhouse conditions (Shaner et al. 1997). Selection for slow rusting based on one or more of these components, especially for long latent period, can effectively retard the disease development in selected lines (Knott 1989). In areas where the rust infection season is short, a longer latent period can substantially reduce or reduce spore accumulation, such that damage is minimizedin a rust epidemic (Knott 1989).

Two to five genes were estimated for long LP depending on genetic basis (Das et al. 1993; Lee et al. 1985; Broers et al. 1989; Kuhn et al.1980). Lee et al. (1985) reported that three recessive genes controlled the long latent period of CI 13227, the slow rusting

parent used in this study. Based on early-generation analysis of a cross between CI 13227 and Suwon 92, Shaner et al. (1997) estimated two to five genes responsible for long LP. They further analyzed LP segregation in a RIL population derived from the same cross and concluded that four loci with epistatic effects were involved in long LP. One of the loci exerted a major effect on long LP. In present study, three QTLs for long latent period were identified in the same RIL population. The QTL with greatest effect, QLRLP.osu-2D, explained up to 46% of the phenotypic variance for LP. We conclude that this is a major QTL for long LP.

As discussed previously, long latent period is a reliable component of slow rusting resistance (Shaner et al. 1997). However, measuring LP is time-consuming and labor-intensive in large breeding populations. Therefore, marker assisted selection (MAS) for long LP may simplify the selection process and improve selection efficiency. In this study, we identified molecular markers closely linked to QTL for LP. These markers provide a new tool for screening progenies with long latent period in breeding programs. With molecular markers, the genotype of each plant can be easily determined without time-consuming inoculation and disease measurement. Based on our results (Table 3.5.), the RILs with all longer latent period alleles at the loci of three markers closely linked to QTL (XACTG.GTG185, XCAG.CGAT70, and XACA.CACG126) have an average LP of 12.7 days for both years, which is similar to that of CI 13227. In contrast, the RILs without any longer latent period allele had an average LP of 7 days which is similar to that of Suwon 92. These results suggest that the three QTL detected in this study explained most of the genetic variation of LP and indicated that if resistant RILs carry all the three resistance QTL, their LP will be about 5 days longer than that of genotypes

without any long LP QTL. Therefore, selection for long LP based on these marker genotyping data is very effective. Direct application of AFLP in MAS still poses some practical problems due to its complicated procedure. More recently, AFLPs have been transferred into sequence tag site (STS) markers which can be directly used in breeding programs (Guo et al 2003). Therefore, these AFLPs closely linked to the QTL should be further transferred into STS markers for MAS. Another alternative is to use SSR markers linked to QLRLP.osu-2D and QLRLP.osu-2B as selectable markers for the corresponding long LP QTL. For example, XBARC95 is 12.1 cM away from QLRLP.osu-2D and explained 30.1 and 23.3% of the total variance of latent period in 1988 and 1989, respectively. Acceptable results should be obtained using this as a selectable marker. XBARC18 had similar R^2s as those of XCAG.CGAT70 (Table 3.2.). Therefore similar results (for QLRLP.osu-2B) can be achieved using the markers.

Although many race-specific leaf rust resistance genes have been identified from wheat and its relatives, sources of slow leaf-rusting resistance are still limited. Lr34 and Lr46 are two known slow rusting resistance genes available to breeding programs. Previous studies mapped these two genes on 7DS and 1B. In this study the major QTL for LP, QLRLP.osu-2D, was mapped on chromosome 2D, which appears to be different from Lr34 and Lr46. To our knowledge, this is the first QTL for latent period that has ever been identified and mapped. This QTL played a major role in determining the latent period of *P. triticina* in wheat. Pyramiding of this QTL with other slow rusting genes such as Lr34 and Lr46 may enhance rust resistance levels in wheat cultivars. In addition, further fine mapping of this QTL may facilitate the cloning of this QTL.

Two additional QTL, QLRLP.osu-2B and QLRLP.osu-5BL/7BL, were also identified in this study. These QTL was also associated with other parameters of leaf rust resistance, such as AUDPC, final rust severity, and rust infection rate (See chapter 2), while QLRLP.osu-2D was only associated with latent period. This suggested that different mechanisms determining latent period of *Puccinia. triticinia* in wheat exist. Further studies to understand these mechanisms are imperative for breeding durable leaf rust resistant cultivars.

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Table 3.1. Mean latent period (days) of parents and their RILs in 1988 and 1989's experiments.

	Suwon 92	CI 13227	RILs means	RILs ranges
1988	7.5	13.0	9.3	6.8-15.2
1989	7.2	13.7	9.1	7.0-14.3

Table 3.2. Determination coefficients (R^2) and allelic substitution effects (days) of AFLP, SSR markers linked to MLP across two years.

Marker	Chrom.*		R^2(%)	P	CI. All.*	Su. All.*	ASE*
XACTG.GTG185	2D	1988	43.9	<0.0001	10.7	8.1	1.3
		1989	31.6	<0.0001	10.2	8.1	1.1
XBARC95	2D	1988	30.1	<0.0001	10.2	8.3	1.0
		1989	23.3	<0.0001	10.0	8.3	0.8
XCAT.CGTA237	2D	1988	27.1	<0.0001	10.2	8.3	1.0
		1989	19.0	<0.0001	9.8	8.3	0.7
XTGC.CTA208	2D	1988	18.4	<0.0001	9.8	8.3	0.7
		1989	14.1	0.0003	9.5	8.3	0.6
XCAG.CGAT70	2B	1988	14.8	0.0002	9.8	8.5	0.6
		1989	15.8	0.0001	9.6	8.3	0.6
XTGC.ACAG198	5BL/7BL	1988	13.9	0.0006	10.0	8.7	0.7
		1989	18.9	0.0001	10.0	8.5	0.8
XBARC18	2B	1988	12.3	0.0009	9.8	8.5	0.6
		1989	13.4	0.0005	9.6	8.3	0.6
XACA.CACG126	5BL/7BL	1988	11.1	0.0014	9.8	8.5	0.6
		1989	15.3	0.0002	9.6	8.3	0.6
XCATG.ATGC60	2B	1988	10.6	0.0018	9.8	8.5	0.6
		1989	12.3	0.0007	9.6	8.3	0.6
XCATG.ATGC125	5BL/7BL	1988	9.8	0.0022	9.8	8.5	0.6
		1989	15.1	0.0001	9.6	8.3	0.6
XBARC167	2B	1988	9.8	0.0034	9.8	8.5	0.6
		1989	12.4	0.0009	9.6	8.3	0.6
XCGA.CAT85	5BL/7BL	1988	8.6	0.0049	9.6	8.5	0.5
		1989	12.9	0.0005	9.6	8.3	0.6
XCAT.CTA155	5BL/7BL	1988	8.4	0.0057	9.6	8.7	0.4

		1989	12.5	0.0006	9.6	8.3	0.6
XAT.CGTA146	2B	1988	8.1	0.0059	9.6	8.5	0.5
		1989	5.3	0.0268	9.3	8.5	0.4
XCATG.CGTA152	2B	1988	7.5	0.0101	9.6	8.5	0.5
		1989	5.6	0.0269	9.3	8.5	0.4
XCAT.CGTA150	2B	1988	7.4	0.0101	9.6	8.5	0.5
		1989	4.9	0.0363	9.3	8.5	0.4
XGCTG.CGAT290	2B	1988	6.3	0.0179	9.6	8.7	0.4
		1989	5.1	0.0331	9.3	8.5	0.4
XACTG.ATGC165	2B	1988	7.0	0.0181	9.6	8.5	0.5
		1989	5.1	0.0448	9.1	8.5	0.6

* CI. All.: mean of CI 13227 allele;

* Su. All.: mean of Suwon 92 allele;

* ASE: allele substitution effect.

Table 3.3. Determination coefficients (R^2), chromosome locations, marker interval and LOD values of QTLs for long latent period of *P. triticina* in CI13227.

QTL	Chro.*	Year	Interval	Posi.*(cM)	R^2 (%)	LOD
QLRLP.OSU-2D	2D	1988	XACTG.GTG185/XCAT.CGTA237	2.8	45.7	12.5
		1989	XACTG.GTG185/XCAT.CGTA237	2.8	33.2	8.2
QLRLP.OSU-2B	2B	1988	XCAG.CGAT70/XCATG.ATGC60	95.2	15	3.3
		1989	XCAG.CGAT70/XCATG.ATGC60	95.2	16.2	3.6
QLRLP.OSU-5BL/7BL	5BL/7BL	1988	XACA.CACG126/XCATG.ATGC125	6.5	11.1	2.4
		1989	XACA.CACG126/XCATG.ATGC125	6.5	16	3.6

* Chro.: chromosome location

* Posi.: position

Table 3.4. Multiple regression of three QTLs on MLP in RIL population derived from CI 13227 x Suwon 92

QTL	Markers	Chrom.*	1988		1989	
			Partial R^2(%)	Model R^2(%)	Partial R^2(%)	Model R^2(%)
QLRLP.osu-2D	XACTG.GTG185	2D	43.2	43.2	30.8	30.8
QLRLP.osu-2B	XCAG.CGAT70	2B	11.9	55.1	13.3	44.1
QLRLP.osu-5BL/7BL	XACA.CACG126	5BL/7BL	6.1	61.2	10	54.2

* Chrom.: Chromosome

Table 3.5. The mean LPs and their ranges for genotypes with different allelic combinations of three QTL in the RIL population derived from CI 13227 x Suwon 92.

Genotypes	Year	Mean LP (days)	Range (days)
Q1Q1Q2Q2Q3Q3	1988	12.7± 0.8	10.8-15.2
	1989	12.2± 0.7	10.6-14.3
Q1Q1Q2Q2q3q3	1988	10.1± 0.8	9.1-11.3
	1989	9.7±1.1	8.5-11.3
Q1Q1q2q2Q3Q3	1988	11.5±1.4	10.7-12.4
	1989	11.2 ± 1.4	9.9-11.8
Q1Q1q2q2q3q3	1988	9.5 ± 0.6	8.3-11.3
	1989	9.0 ± 0.6	8.0-11.1
q1q1Q2Q2Q3Q3	1988	8.7 ± 0.8	7.2-12.4
	1989	8.9 ± 1.0	7.4-13.2
q1q1Q2Q2q3q3	1988	8.7 ± 1.1	7.6-12.6
	1989	8.5 ± 0.8	7.5-10.7
q1q1q2q2Q3Q3	1988	8.0 ± 0.4	7.2-9.0
	1989	8.0 ± 0.4	7.2-8.7
q1q1q2q2q3q3	1988	7.3± 0.2	6.8-8.1
	1989	7.4 ± 0.3	6.8-8.8

*Q1Q1, Q2Q2, Q3Q3 represent CI 13227 allele at XACTG.GTG185, XCAG.CGAT70, XACA.CACG126 locus, respectively; q1q1, q2q2, q3q3 represent Suwon 92 allele at XACTG.GTG185, XCAG.CGAT70, XACA.CACG126 locus, respectively.

Legends:

Fig.3.1. Frequency distribution of MLPs of RILs derived from CI 13227 x Suwon 92 in 1988

Fig. 3.2. Frequency distribution of MLPs of RILs derived from CI 13227 x Suwon 92 in 1989

Fig. 3.3. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XACTG.GTG185 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XACTG.GTG185 flanking QLRLP.osu-2D.

Fig. 3.4. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XCAT.CGTA237 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker flanking QLRLP.osu-2D, XCAT.CGTA237.

Fig. 3.5. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XTGC.CTA208 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker flanking QLRLP.osu-2D, XTGC.CTA208.

Fig. 3.6. The segregation of SSR marker XBARC95 in the population of RILs derived from CI 13227 x Suwon 92. The gel was developed by silver staining method.

Fig. 3.7. The likelihood plots of QTL for MLP in the experiments conducted in1988 (red curves) and 1989 (black curves). Upper left: QLRLP.osu-2D; Lower left: QLRLP.osu-5BL/7BL; Right: QLRLP.osu-2B. The vertical lines represent the threshold LOD values of 3.0.

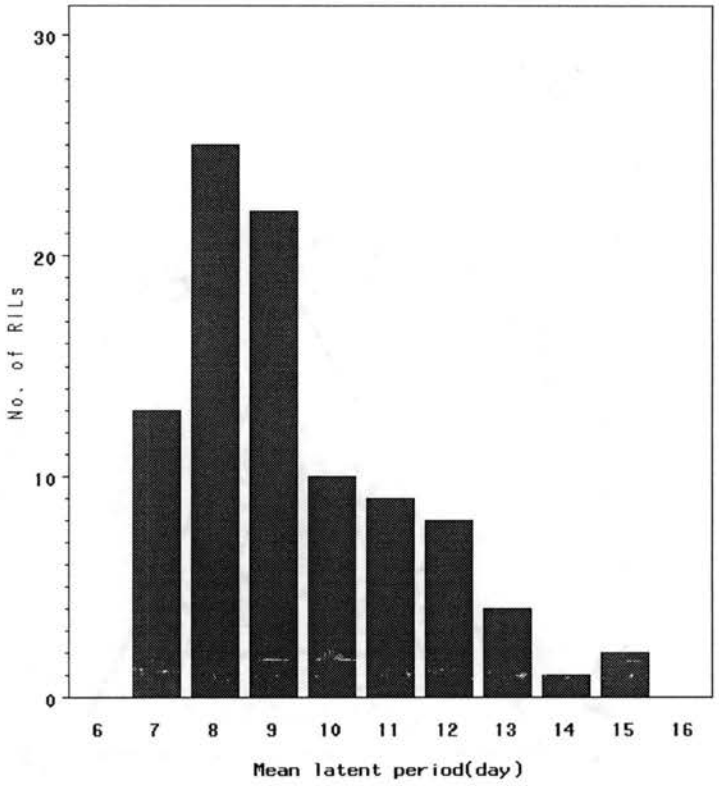
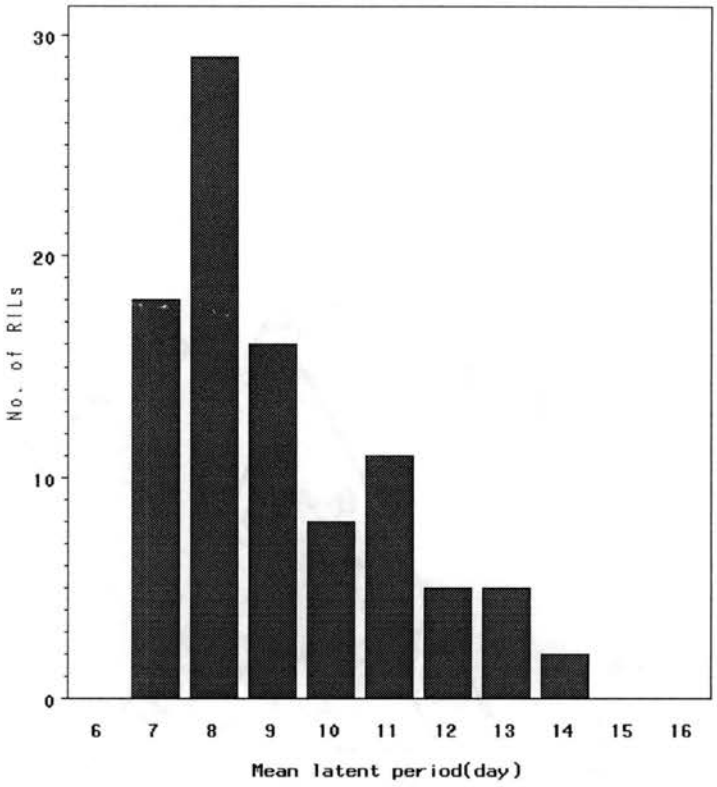

30
20
10
0
No. of RILs
6
7
8
9
10
11
12
13
14
15
16
Mean latent period(day)

Fig. 3.1.


30
20
10
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No. of RILs
6
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8
9
10
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12
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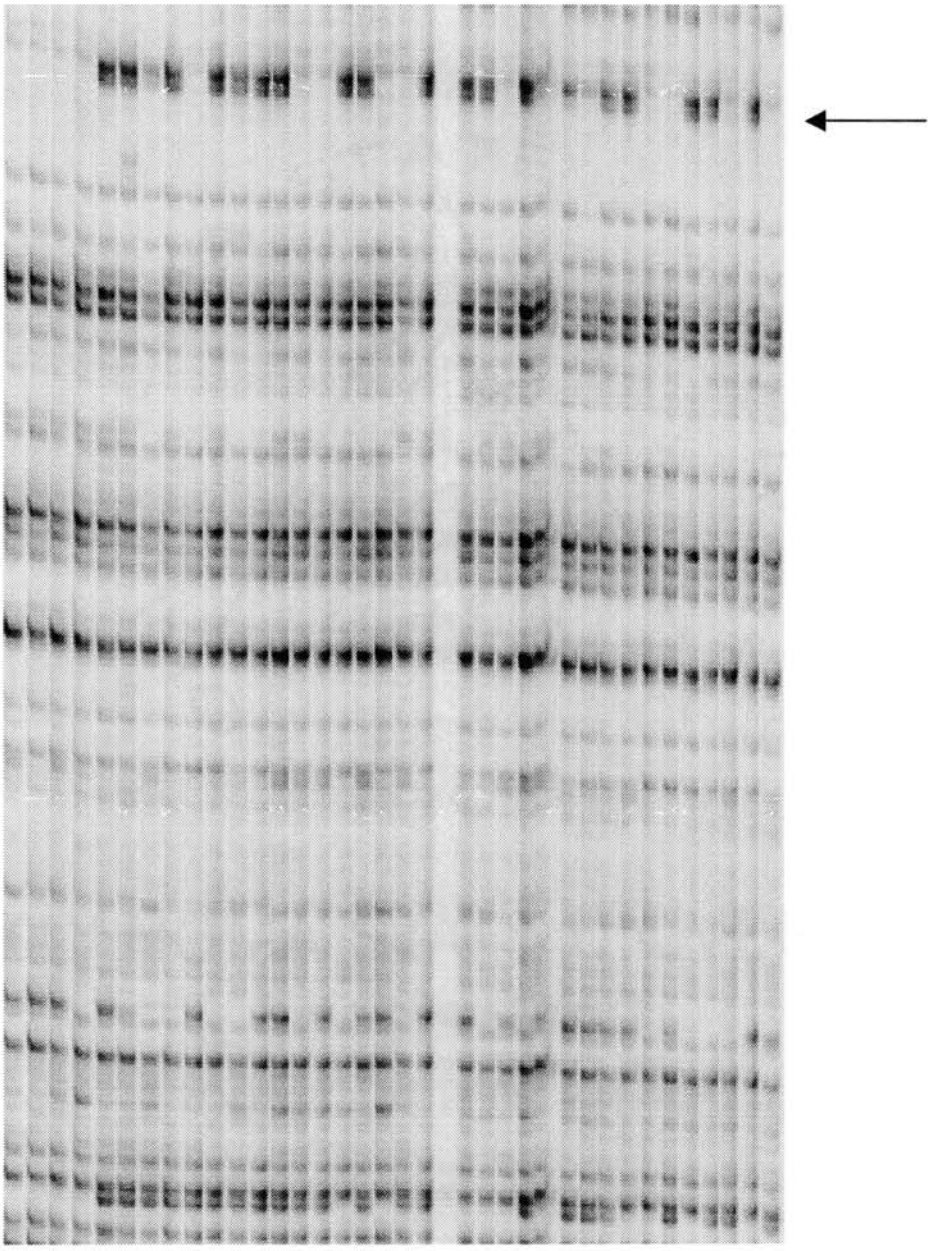
14
15
16
Mean latent period(day)

Fig. 3.2.



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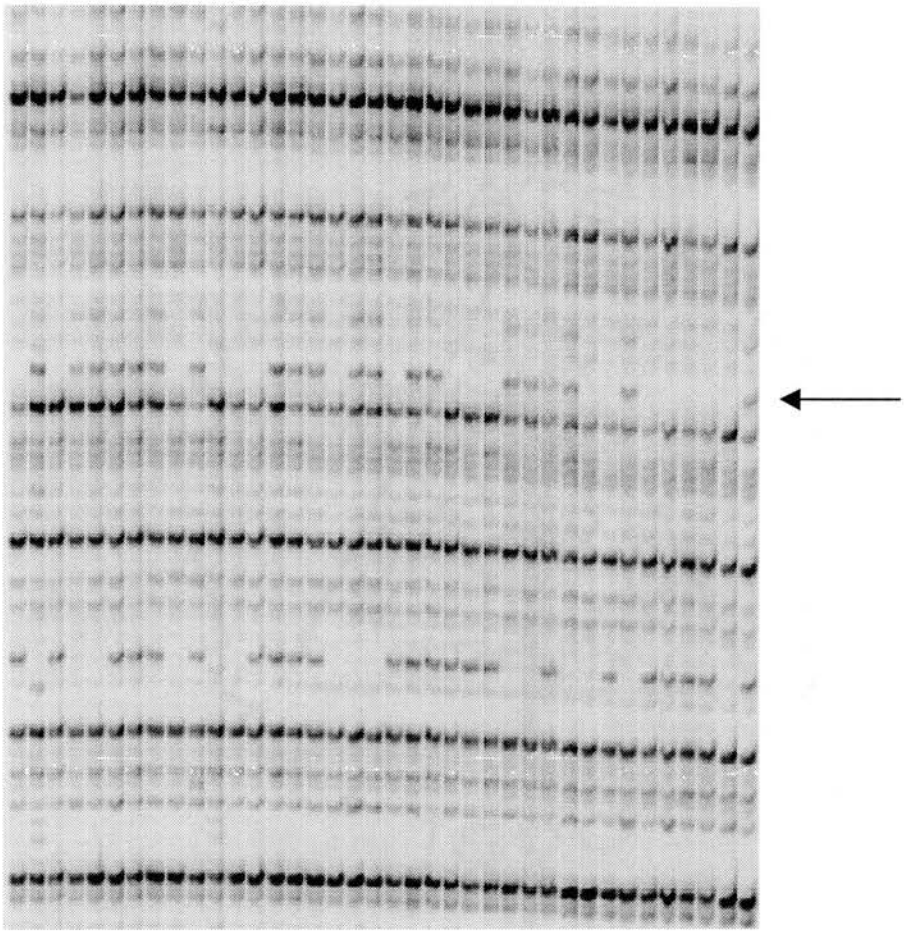
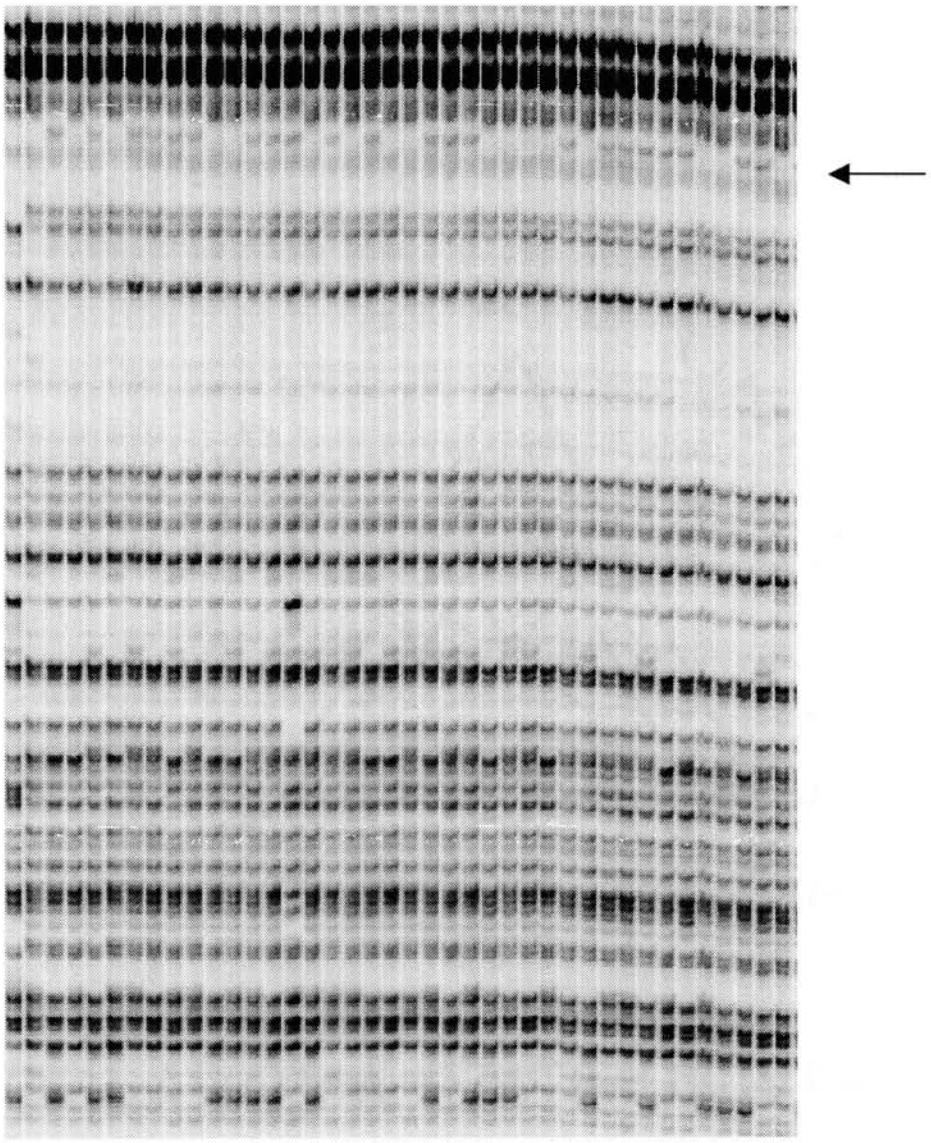


Fig. 3.4.



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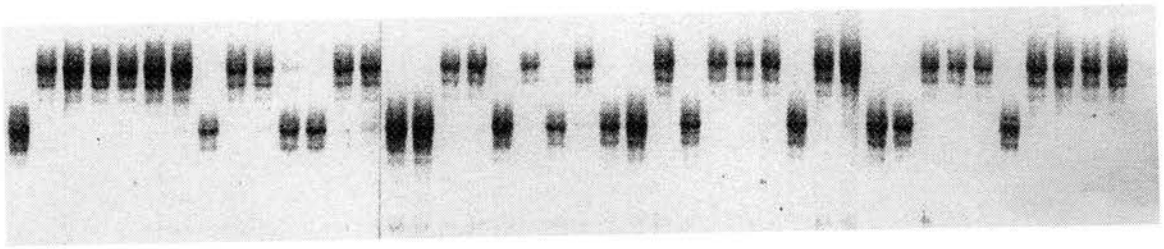
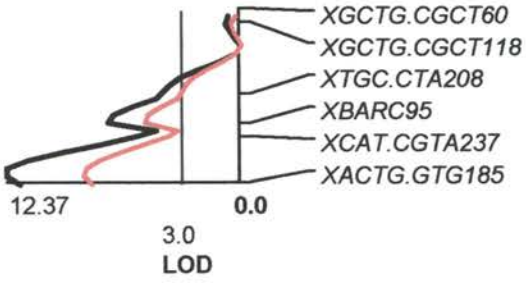


Fig. 3.6.


XGCTG.CGCT60
XGCTG.CGCT118
XTGC.CTA208
XBARC95
XCAT.CGTA237
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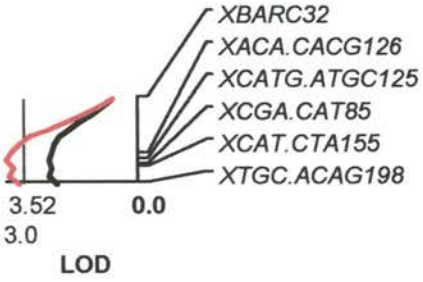
3.0
LOD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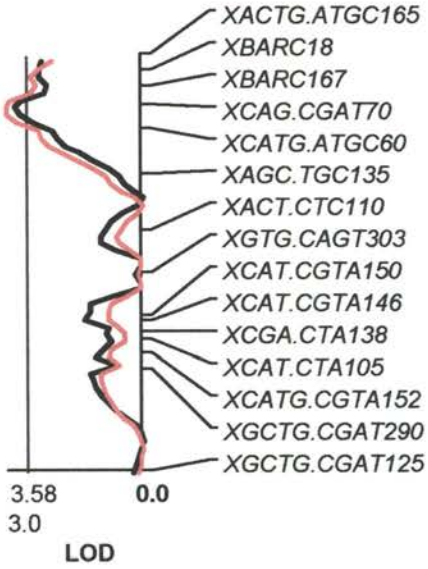
LOD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XBARC18
XBARC167
XCAG.CGAT70
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XAGC.TGC135
XACT.CTC110
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XCAT.CGTA150
XCAT.CGTA146
XCGA.CTA138
XCAT.CTA105
XCATG.CGTA152
XGCTG.CGAT290
XGCTG.CGAT125
3.58
0.0
3.0
LOD

Fig. 3.7.

Chapter IV

Characterization of a Powdery Mildew Resistance Gene, Pm3a, Using Molecular and Morphological Markers in Wheat

Abstract

Powdery mildew, caused by *Blumeria gramminis* (DC) E.O.Speer f.sp.*tritici*, is an important foliar disease of wheat worldwide. Pyramiding of race-specific genes into a single cultivar and combining race-specific resistance genes with durable resistance genes are the preferred strategies to improve the durability of powdery mildew resistance. The objectives of this study were to identify the chro mosome location of a powdery mildew resistance gene Pm3a, and to identify molecular markers tightly linked to Pm3a for use in marker-assisted selection (MAS). A recombinant inbred line (RIL) population was derived by single seed descend of the F2 from a cross between the U.S. susceptible wheat cultivar CI13227 and a Korean cultivar carrying the Pm3a gene. RILs were characterized for powdery mildew infection type in 1994 and 1995 and with AFLP, SSR, and morphological markers in 2002 and 2003. One SSR marker on chromosome 1A, one morphological marker (hairy glume), and seven AFLP markers closely linked to Pm3a were identified. They explained 50.3% to 72.0% of the phenotypic variance in 1994 and 39.4% to 64.8% in 1995. The SSR marker, XPSP2999, and hairy glume trait can be directly used in MAS for Pm3a in breeding programs.

Key words: Wheat, powdery mildew, BSA, Pm3, AFLP, SSR, and MAS

Introduction

Wheat powdery mildew, caused by the obligate biotrophic parasite fungus *Blumeria gramminis* (DC) E.O.Speer f.sp.*tritici*, is a destructive foliar disease of wheat in regions with cool or maritime climates. High-yielding semi-dwarf cultivars and heavy irrigation and nitrogen fertilizer application greatly favor powdery mildew infection (Bennett 1984). Hence breeding for powdery mildew resistant cultivars is a major objective in wheat breeding programs worldwide. Unfortunately, powdery mildew resistance genes typically have a "boom-and –bust" cycle, and the deployment of single resistance genes results in a shift of pathogen races and subsequent loss of resistance in a relatively short time period. Pyramiding several race-specific powdery mildew resistance genes and combining race-specific genes with durable, race-nonspecific genes into a single cultivar are the preferred strategies to improve durability of powdery mildew resistance. However, both strategies are time-consuming and labor-intensive and may be limited by the availability of corresponding races of the pathogen.

The advent of molecular mapping techniques makes it possible to simplify the gene pyramiding procedure. Molecular markers linked to several powdery mildew resistance genes have been identified. These genes include Pm1 (Hu et al. 1997; Ma et al. 1994; Hartl et al.1995), Pm2 (Ma et al. 1994), Pm3 (Hartl et al.1993; Ma et al. 1994), Pm4 (Hartl et al. 1999; Ma 1993), Pm5 (Keller et al. 1999), Pm6 (Tao et al.2000), Pm8 (Hsam et al. 2000), Pm12 (Jia et al. 1996), Pm13 (Donimi et al. 1995), Pm17 (Hsam et al. 2000),

Pm18 (Hartl et al.1993), Pm21 (Qi et al. 1996), Pm24 (Huang et al. 2000), Pm25 (Shi et al. 1998), Pm26 (Rong et al. 2000), Pm27 (Jarve et al. 2000), and Pm30 (Liu et al. 2000). Quantitative trait loci for powdery mildew resistance also have been characterized (Liu et al. 2000; Keller et al. 1999). These mapping efforts provide a solid foundation for marker-assisted breeding to improve powdery mildew resistance in wheat cultivars.

Pm3, formerly called MLs, Mla, or MLc, is a powdery mildew resistance gene originally identified in the wheat cultivars 'Indian', 'Sonora', 'Chul' and 'Asosan' (Briggle 1966). Its chromosome location was determined by cytological method to be 1AS (McIntosh et al. 1969). Several alleles have been proposed for this gene (Zeller et al.1998). One of these alleles, Pm3a, is widely distributed in North American cultivars (Leath et al. 1990). In Europe, Hartl et al. (1993) anticipated that Pm3a and Pm3b would play a major role in improving powdery mildew resistance of wheat cultivars because of the low frequency of virulence alleles in the mildew pathogen population. To facilitate the introgression of Pm3 alleles into commercial cultivars and further cloning of them, several studies were conducted to tag the Pm3 locus. Hartl et al. (1993) found that one RFLP marker, Xwhs179, was closely linked (3.3±1.9 cM) to Pm3 locus. In another study, this marker was assigned to 1B and 1D (Van Deynze et al 1995). Using NILs, Ma et al. (1994) identified a RFLP marker linked to Pm3b with a distance of 1.3 cM. Sourdille et al. (1999) found that Pm3g was 5.2 cM away from Xgli-A1, which is a locus encoding for storage protein. However, due to the complexity associated with RFLP marker, their application in marker-assisted selection (MAS) for Pm3 is impracticable. Our objective was to map the Pm3a allele and identify selectable markers tightly linked to Pm3a so that they can be indirectly used in breeding durable powdery mildew resistant cultivars.

Materials and methods

Materials and phenotyping

One hundred and four recombinant inbred lines (RILs) were developed from a cross between CI 13227 and Suwon 92 by single-seed descent. Suwon 92, a Korean cultivar introduced into the USA in early 1900s, is semidwarf with short apical awns (awnless), hairy glumes, and early maturity. Suwon 92 demonstrated resistance to powdery mildew under field conditions and was reported to carry the Pm3a gene (Briggle 1966). In contrast, CI13227, a breeding line from the USA, is tall and susceptible to powdery mildew with awns, hairless glumes, and late maturity. In addition, CI 13227 is resistant to leaf rust and carries slow rusting resistance genes (Shaner et al. 1997), while Suwon 92 is highly susceptible to leaf rust.

The 104 RILs, together with the two parents 'CI 13227' and 'Suwon 92', were grown and evaluated for powdery mildew resistance under natural infection at West Lafayette, IN in 1994 and 1995. Each experimental line was planted in one-meter rows with 3-row per plot. The experiments were arranged in a randomized block design with two replications for RILs and five replications for parents.

Infection type (IT) of powdery mildew was recorded on a 1 to 6 scale for each full spot. Increasing values for this scale represent highly resistant, resistant, moderate resistant, moderate susceptible, susceptible, and highly susceptible, respectively. Glume type was scored as a qualitative trait: 0 and 1 represent hairless and hairy glume, respectively.

Analysis of amplified fragment length polymorphism (AFLP)

The CTAB (Cetyltrimethyllammonium) method was used to isolate genomic DNA from two-week old wheat seedlings (Saghai-Maroof et al. 1984). About 300 ng wheat genomic DNA was double-digested with *Pst* I and *Mse* I restriction enzymes in 1X H buffer at 37 ° C for 2 hours. After digestion, 8 µl of ligation mixture containing 4 pmol of P*st I* and 40 pmol of M*seI* adapters, 0.8 unit of DNA ligase and 1X ligase buffer was added to the digested DNA. The ligation was conducted at 20 °C overnight. The resulting 38µl DNA mixture was diluted with 362 µl sterilized deioned water for subsequent PCR reactions.

For preamplification, 40 µl of PCR reaction included 10µl ligated DNA, 75ng of each of *Pst* I and *Mse* I pre-amplification primers, 0.75 unit Taq enzyme, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 1x PCR buffer. The following PCR profile was used: 25 cycles of 30 seconds at 94 ° C, 1 minute annealing at 56° C and 1 minute extension at 72 ° C. Five microliters of PCR product was used for quality check on a 1% agarose gel. The remaining PCR product was diluted 10-fold by adding 315 µl of sterilized deioned water. Selective amplification was conducted using *Pst* I and *Mse* I primers with two to four additional selective nucleotides. *Pst* I primers were labeled with IR fluorescent dye. Each PCR reaction contained 3 µl diluted pre-amplified DNA, 0.4µl *Pst* I primer, 0.4µl *Mse* I primer, 0.2mM dNTP, 2.5mM $MgCl_2$, 0.2 unit of Taq DNA polymerase, 1 x PCR buffer and 3.8 µl water. The following PCR profile was used for selective PCR amplification: denaturing at 94°C for 2 minutes followed by 13 cycles of 30 seconds at 94 °C, 30 seconds annealing at 65 °C with a decreasing of 0.7 °C per cycle, 1 minute of extension at 72 °C, and then 23 cycles of 30 seconds denaturing at 94 °C, 30 seconds of annealing at

65 °C, and 1 minute of extension at 72 °C. Finally, an extension at 72 °C for 5 minutes was followed. PCR was conducted in a MJR PTC-100 thermal cycler. The PCR product was denatured at 95 °C for 5 min after adding 5 µl loading buffer and chilled quickly on the ice.

Analysis of simple sequence repeat (SSR) markers.

To detect SSR markers linked to Pm3a gene, a revised bulk segregant analysis (BSA) method was applied. Two bulks contrasting in presence and absence of the Pm3a gene were constructed based on AFLP markers flanking the Pm3a gene. Resistant bulk consisted of equal amount of DNA from each of five RILs with Suwon 92 alleles at all seven AFLP loci, and susceptible bulk consisted of equal amount of DNA from each of five susceptible RILs with CI 13227 alleles at all seven AFLP loci. The selected RILs in the two bulks showed excellent contrast in resistance to powdery mildew in 1994 and 1995 field experiments. The two bulks, together with DNA from two parents, were used to screen SSR primers.

Tailed PCR method was used for SSR analysis. In brief, a 19-base oligo sequence (M13 tail) was conjugated to the 5'- end of one of the two SSR primers, forward primer or reverse primer. So each SSR reaction used 3 primers: two unlabeled SSR primers with one attached by M13 sequence tail,and one IR fluorescent dye labeled M13 primer w ith the sequence same as the tail sequence attached to one of the SSR primers. For each 10µl SSR reaction, 50ng of genomic DNA was added to a PCR mix containing 0.25 mM of each dNTP, 1x PCR buffer, 2.5mM $MgCl_2$ and 0.6 unit Taq polymerase, 0.5 pmol SSR primers, and 1 pmol M13 primer. PCR was performed using a touchdown program in which PCR cycles started at 95°C for 5 min and then 5 cycles of 95°C for 45 s, 68°C for 5

min, and 72°C for 1 min with a subsequently lowering the annealing temperature (68°C) by 2 °C per cycle followed by another 5 cycles of 95 °C for 45 s, 58 °C for 2 min, and 72 °C for 1 min with a subsequently lowering the annealing temperature (58°C) by 2°C per cycle; then an additional 25 cycles of 95°C for 45 s, 50°C for 2 min, and 72°C for 1 min. A 5 min final extension step at 72 °C is processed before soaking the PCR at 4 °C. After PCR amplification, 5 µl of formamide stopping buffer was added to the PCR before the samples were denatured at 95°C for 5 min. The denatured PCR products were kept on ice before electrophoresis.

AFLP and SSR were separated in a 6.5% denaturing polyacrylamide gel running on Li-Cor IR-4200 DNA sequencer (Li-Cor Inc, Lincoln NE). The electrophoresis condition was set at 1500 V of voltage, 40W of power, 35 mA of current with 50 °C constant temperature for 2.5 h in 1x TBE buffer. Gel was prepared as described in the user's manuals from manufacturer. A 68-well comb was used for lane formation and 0.8 µl of each sample was loaded into separated wells using Hamilton 8-channel syringe (Hamilton, Reno, NV). The segregation patterns were manually inspected on a computer screen.

Data analysis

One-way ANOVA was used to identify AFLP markers that were significantly associated with powdery mildew resistance ($p < 0.05$). Marker data from 101 RILs were analyzed. Genetic linkage maps were constructed by using MapMaker 3.0 (Lander et al. 1987). A threshold log likelihood ratio (LOD) of 4.0 was used to determine linkage groups. Centimorgan (cM) values were calculated based on the Kosambi mapping

function (Kosambi 1944). The "single marker analysis" and "interval analysis" were performed to characterize the effects of each marker and to detect the location of Pm3 using Qgene (Nelson 1997). The SAS "PROC GCHART" was used to produce phenotype distribution histograms.

Results

Powdery mildew resistance in RILs

Reactions to natural infections of powdery mildew for the RILs were consistent between 1994 and 1995. In each year, the two parents showed a significant contrast in powdery mildew resistance ($p<0.05$). Suwon 92 was highly resistant with a score of "1" in 1994 and "2" in 1995, while CI 13227 was highly susceptible with a score of "5" for both years. The 104 RILs showed significant and continuous variation in Pm IT (Fig. 4. 1.). Mean Pm IT of individual RILs ranged from 1 to 6 in 1994 and 2 to 5 in 1995 with an average of 3.3 and 3.5 in 1994 and 1995, respectively. Transgressive segregation of susceptible plants was observed in 1994, but not in 1995. An RIL with better resistance than Suwon 92 was not observed in either year.

Considering ITs from 1-3 as resistant and 4-6 as susceptible, the ratio of resistant to susceptible did not deviate from a 1:1ratio for both years ($X^2=0.346$, $p>0.5$ in 1994; $X^2=0.154$, $p>0.5$ in 1995), suggesting that a major gene segregates for powdery mildew resistance in this population. This result supports the previous report that Suwon 92 possessed one powdery mildew resistance gene, which was named as Pm3a (Briggle 1966). The correlation coefficient of Pm ITs among RILs between two years was highly

significant (r= 0.890 p<0.0001). The glume type data from the RILs were also consistent between 1994 and 1995.

Molecular marker analysis

A total of 612 pairs of AFLP (*Pst* I/*Mse* I) primers were screened using BSA. Eighty-five primers detected a polymorphism between both parents and bulks, and were used further to screen the 104 RILs. A total of 459 unambiguous polymorphic AFLP bands were generated. The polymorphic bands generated by each pair of primers ranged from 1 to 14. Their molecular weights ranged from 50 bp to 405 bp. Among them, more than 80% had a molecular weight of 100bp to 200bp. Seven AFLP markers significantly associated with Pm3a were used to construct a linkage map. Fig. 4.2. to Fig. 4.4. showed the segregation of AFLP markers that were highly associated with Pm3a in the RIL population.

A total of 158 SSR primers were used to screen two bulks and parents to identify SSR markers linked to Pm3a. The resistant bulk was constructed by pooling equal amounts of DNA from five RILs: A3, A8, A15, A22, and A31. All of them showed high resistance to powdery mildew in both years. Each possessed Suwon 92 alleles at seven AFLP marker loci. Following the same protocol, a susceptible bulk with CI13227 alleles at all seven AFLP marker loci were constructed with five highly susceptible RILs, A5, A13, A23, A20 and B1.

Among these SSR primers screened, PSP2999 generated a polymorphic band between the resistant bulks and between parents. The sizes of the polymorphic bands were 158 bp in the resistant bulk and Suwon 92, and 166 bp in the susceptible bulk and

CI13227. Although 18 SSR primers generated polymorphisms between parents, only primer XPSP2999 showed polymorphism between bulks; therefore, it was used to genotype the RIL population. Fig. 4.5. shows the segregation of XPSP2999 in the RIL population derived from CI 13227 x Suwon 92.

Seven AFLP, one SSR and one morphological marker were mapped in the same linkage group spanning 20.3 cM (Fig. 4.6.). Among these markers, SSR marker XPSP2999 was previously mapped on chromosome 1A (Devos et al. 1995). Hg, the gene for hairy glume, was assigned on the short arm of the same chromosome (McIntosh et al. 1969). Therefore, the Pm3a gene is tentatively mapped on chromosome 1AS. All nine markers showed highly significant associations with powdery mildew resistance in both 1994 and 1995 field tests. The coefficients of determination (R^2) of these markers ranged from 50.3% to 72.0% in 1994, and 37.3% to 64.8% in 1995 (Table 4.1.). The effects of single marker allele substitution as reflected by Pm IT values varied from 1.4 to 1.7 in 1994 and 0.8 to 1.1 in 1995. They accounted for 69.6% to 83.5% of the difference between two parents in 1994 and 53.3% to 66.7% in 1995.

Mapping Pm3a gene

Iinterval mapping indicated that the Pm3a gene was located between the AFLP marker XCGT.GCAG145 and the SSR marker XPSP2999 with a LOD score of 27.7 and 20.1 in 1994 and 1995, respectively. Four markers were mapped within this region covering 4.8 cM. In 1994, Pm3a gene was mapped between XCGT.GCAG145 and XCGT.ACAG145 with a genetic distance of 0.9 cM away from XCGT.ACAG145, and 0.8 cM away from XCGT.GCAG145. Pm3a explained 72.7% of the phenotypic variance

in 1994. In 1995, Pm3a was mapped between XCAG.ATGC193 and XPSP2999, which was only 0.3 cM from XCAG.ATGC193 and 0.9 cM from XPSP2999. It explained 62.1% of the phenotypic variance.

Discussion

In this study, we successfully detected AFLP, SSR and morphological markers closely linked to Pm3a. Pm3a gene was mapped in a region covering 4.8 cM. SSR marker XPSP2999, which was located in chromosome 1A (Devos et al.), was mapped onto a locus that was 4.0 cM and 0.3 cM away from Pm3a in 1994 and 1995, respectively. This supports the previous conclusion that Suwon92 confers Pm3a gene (Briggle 1966), which was located in chromosome 1A (McIntosh et al. 1969). The four markers (Fig 4.2. to Fig. 4.5.) mapped around Pm3a are particular useful for the further map-based cloning of Pm3a gene. Since the existence of interaction of year x powdery mildew infection type, further phenotyping the RILs in multiple environments and fine mapping are necessary for map-based cloning of Pm3a gene.

Methods for breeding durable powdery mildew resistant cultivars have included pyramiding major genes/QTL, using partial resistance genes and the combining major genes and partial resistance genes. Due to the high adaptability and the fast spread of virulent isolates over long distance, pyramiding race-specific major genes into a single cultivar may provides limited durability of powdery mildew resistance. On the other hand, partial resistance gene itself sometime can't provide satisfactory protection. Therefore, combination of race-specific genes and partial resistance genes is the best strategy for breeding durable powdery mildew resistant cultivars. However, the

combination of major resistance genes with partial resistance genes/QTL can be hardly achieved by traditional phenotypic selection because culture identification and maintenance is difficult and time-consuming. Molecular mapping may provide promising method to simplify the selection process. Some markers associated with race-specific resistance genes and partial resistance QTL have been identified. However, ma ny were RFLP markers which are difficult to be used for MAS duo to the complicated procedure for marker analysis. Therefore breeder-friendly markers are still limited for MAS.

AFLP marker XCAG.ATGC193, XCGT.ACAG145 and XCGT.GCAG145 are all closely linked to the Pm3a gene. They have the potential to be used in marker-assisted selection of Pm3a gene. For example, AFLP marker XCAG.ATGC193 consistently showed highly significant association with powdery mildew resistance across the two years. In both 1994 and 1995, 98% (47 out of 48) of RILs with CI 13227 allele at XCAG.ATGC193 locus were susceptible to powdery mildew (IP>3). Among the RILs with Suwon 92 allele, 92.45% and 83% were scored as resistant in 1994 and 1995, respectively. The further conversion of these AFLP markers into a breeder-friendly marker, such as STS marker, will greatly facilitate the application of them in maker-assisted selection for Pm3a in breeding programs.

Among the 9 markers, XPSP 2999 is a SSR marker which is considered as "breeder-friendly" marker. The distance between SSR marker XPSP2999 and Pm3a locus was about 4.0 cM and 0.8 cM in 1994 and 1995, respectively. The determination coefficients of this marker were 65.3% in 1994, and 56.0% in 1995. The single allele substitution effects of this locus on Pm IT value were 1.6 and 0.9 in 1994 and 1995, respectively. T-test showed that the difference in Pm IT values between these RILs with CI13227 allele

and those with Suwon 92 allele was highly significant ($p<0.0001$) in both years (Table 4.2.). In 1994 and 1995, 89.4% and 76.6% of the RILs with Suwon 92 allele at XPSP2999 locus were resistant to powdery mildew, respectively; 98.1% and 88.9% of the alleles with CI 13227 allele were susceptible to powdery mildew (Fig. 4.10. and Fig. 4.11.). These results suggest that satisfactory results can be obtained if XPSP2999 is used to select Pm3a gene in wheat breeding programs.

Hairy glume, a morphological marker that can be easily identified in field, was also closely associated with powdery mildew resistance. The distance between Hg gene and Pm3a was about 11.6 cM and 8.4 cM in 1994 and 1995, respectively. It explained 53.8% and 44.0% of the total variance in 1994 and 1995, respectively. In 1994, 41 out of 48 RILs with hairy glume were resistant (IT<=3), accounting for 85.4%, while 47 out of 56 RILs with hairless glume were susceptible (IP>3), accounting for 83.9% phenotypic variation (Fig. 4.8.). In 1995, similar results were observed: 85.4% of the RILs with hairy glume were resistant, while 76.8% RILs with hairless glume were susceptible (Fig. 4.9.).

T-test showed that the differences in mean IT score between the lines with hairy glume and those without hairy glume are highly significant (Table 4.2.). This suggests that acceptable results can be obtained based on the presence or absence of hairy glume. Further analysis showed that selection for Pm3a gene based on both hairy glume and SSR marker XPSP2999 may increase accuracy of MAS (Table 4.2.).

Wheat protein content and protein quality play a vital role in determining industrial quality. Both high and low molecular weight glutenin subunits (HMW-GS or LMW-GS, respectively) contribute to the dough mixing time, dough strength and loaf volume (Khelifi et al. 1992; Khatkar et al. 1996). A higher ratio of HMW-GS to LMW-GS was

reported in good bread-making cultivars whereas flour of poor bread-making wheat cultivars exhibited a low ratio of HMW-GS to LMW-GS (Gupta et al.1992). Hence the development of suitable methods to select for/against HMW-GS and LMW-GS gene (family) is of great importance. SSR primer PSP2999 amplified the genome-specific (CAG)(CAA)-microsatellite in the coding region of LMW-glutenin gene (Devos et al. 1995). The genetic distance between XPSP2999 and the gene encoding hairy glume, Hg, is 7.6 cM in this study. This provides a clue for further investigating the linkage relationship between morphological marker, hairy glume, and the LMW-GS gene family, and the possibility to use hairy glume to select against LMW-GS genes.

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Table 4.1. Determination coefficients (R^2) and allelic substitution effects of AFLP, SSR and morphological markers linked to Pm3a gene in 1994 and 1995. All of them have a P value less than 0.0001.

Marker	Year	R^2(%)	LOD	Cl. All.*	Su. All.*	ASE*
XCAG.ATGC193	1994	72	25.7	5	1.6	1.7
	1995	64.8	21.1	4.6	2.5	1.1
XCGT.ACAG145	1994	68.9	24.9	4.9	1.6	1.6
	1995	60.6	19.8	4.5	2.5	1
XCGT.GCAG145	1994	69.7	23.8	4.9	1.6	1.6
	1995	57.5	17.1	4.5	2.6	0.9
XPSP2999	1994	65.3	23	4.8	1.6	1.6
	1995	56	17.8	4.4	2.5	0.9
XCGA.CTA125	1994	62.9	21.5	4.8	1.7	1.6
	1995	56	17.8	4.4	2.5	0.9
XCAT.AGAC180	1994	57.1	18.2	4.7	1.7	1.5
	1995	44.2	12.6	4.3	2.6	0.8
Hairy glume	1994	53.8	16.8	4.7	1.8	1.4
	1995	43.6	12.4	4.3	2.6	0.8
XCAG.GTG65	1994	50.3	15.4	4.6	1.8	1.4
	1995	37.3	10.2	4.2	2.7	0.8
XCAG.AGAC96	1994	50.3	14.3	4.5	1.7	1.4
	1995	39.4	10.2	4.2	2.6	0.8

* Cl. All.: Cl 13227 allele;

* Su. All.: Suwon 92 allele;

* ASE: Allele substitution effect

Table 4.2. The effects of selection for powdery mildew infection type (IT) by morphological marker hairy glume (Hg), SSR marker XPSP2999, or both markers based on 1994 and 1995 field data. IT difference: infection type difference between the RILs with CI 13227allele and those with Suwon 92 allele

	Selection based on hairy glume			Selection based on SSR Marker PSP2999			Selection based on hairy glume and XPSP2999		
	IT difference	t value	P value	IT difference	t value	P value	IT difference	t value	P value
1994	2.9±0.5	10.6	<0.0001	3.2±0.5	13.7	<0.0001	3.5±0.4	18	<0.0001
1995	1.7±0.4	8.2	<0.0001	1.91±0.3	11.3	<0.0001	2.6±0.5	23.7	<0.0001

Legends:

Fig. 4.1. Powdery mildew IT frequency distributions of RILs derived from CI 13227 x Suwon 92 in 1994 (A) and 1995 (B).

Fig. 4.2. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XCAG.ATGC193 in the population of RILs derived from CI13227 x Suwon 92. The arrow indicts AFLP marker XCAG.ATGC193, which was closely linked to Pm3a gene.

Fig. 4.3. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XCGT.ACAG145 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XCGT.ACAG145, which was closely linked to Pm3a gene.

Fig. 4.4. An AFLP image generated from Li-cor DNA Analyzer to show the segregation of AFLP marker XCGT.GCAG145 in the population of RILs derived from CI 13227 x Suwon 92. The arrow indicts AFLP marker XCGT.GCAG145, which was closely linked to Pm3a gene.

Fig. 4.5. An SSR image generated from Li-cor DNA Analyzer to show the segregation of SSR marker PSP2999 in the population of RILs derived from CI 13227 x Suwon 92. The molecular weight of Suwon 92 allele and CI 13227 allele is 160 bp and 164 bp, respectively.

Fig. 4.6. The linkage map harboring the Pm3a gene. XPSP2999 is SSR marker which was previously mapped on chromosome 1A. Hg represents hairy glume gene which was previously mapped on chromosome 1AS.

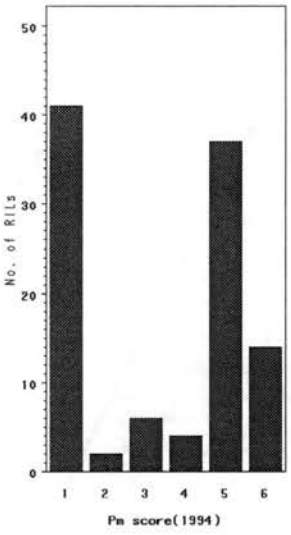
Fig. 4.7.The likelihood plots of Pm3 in 1994 (black) and 1995 (yellow). The peaks of the contours showed the positions of Pm3 gene.

Fig. 4.8. Powdery mildew IT frequency distributions of RILs with hairy glume (A) and hairless glume (B) in 1994 in the population derived from CI 13227 x Suwon 92 in 1994.

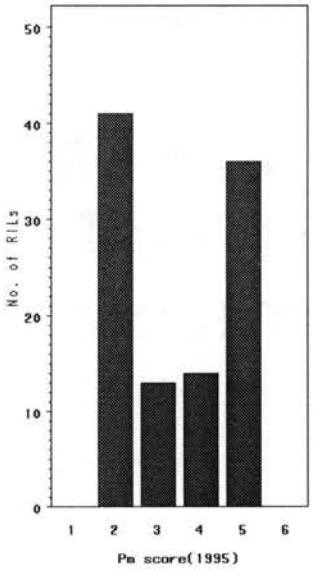
Fig. 4.9. Powdery mildew IT frequency distributions of RILs with hairy glume (A) and hairless glume (B) in 1994 in the population derived from CI 13227 x Suwon 92 in 1995.

Fig. 4.10. Powdery mildew IT frequency distributions of RILs with Suwon 92 allele (A) and CI 13227 allele (B) at XPSP2999 locus in the population derived from CI13229 X Suwon 92 in 1994.

Fig. 4.11. Powdery mildew IT frequency distributions of RILs with Suwon 92 allele (A) and CI 13227 allele (B) at XPSP2999 locus in the population derived from CI 13229 X Suwon 92 in 1995


No. of RILs
0
10
20
30
40
50
1
2
3
4
5
6
Pm score(1994)

A


No. of RILs
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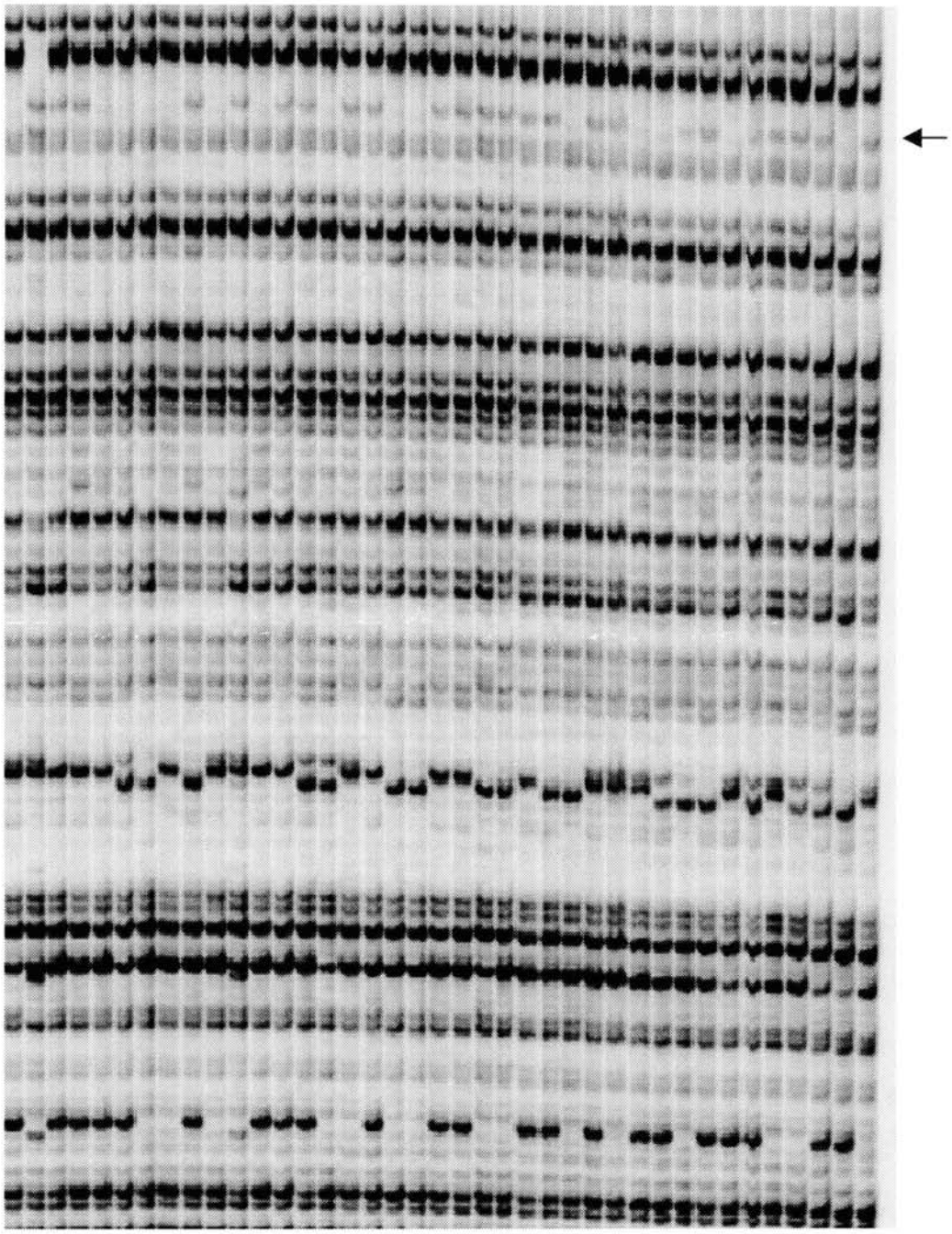
20
30
40
50
1
2
3
4
5
6
Pm score(1995)

B

Fig. 4.1.



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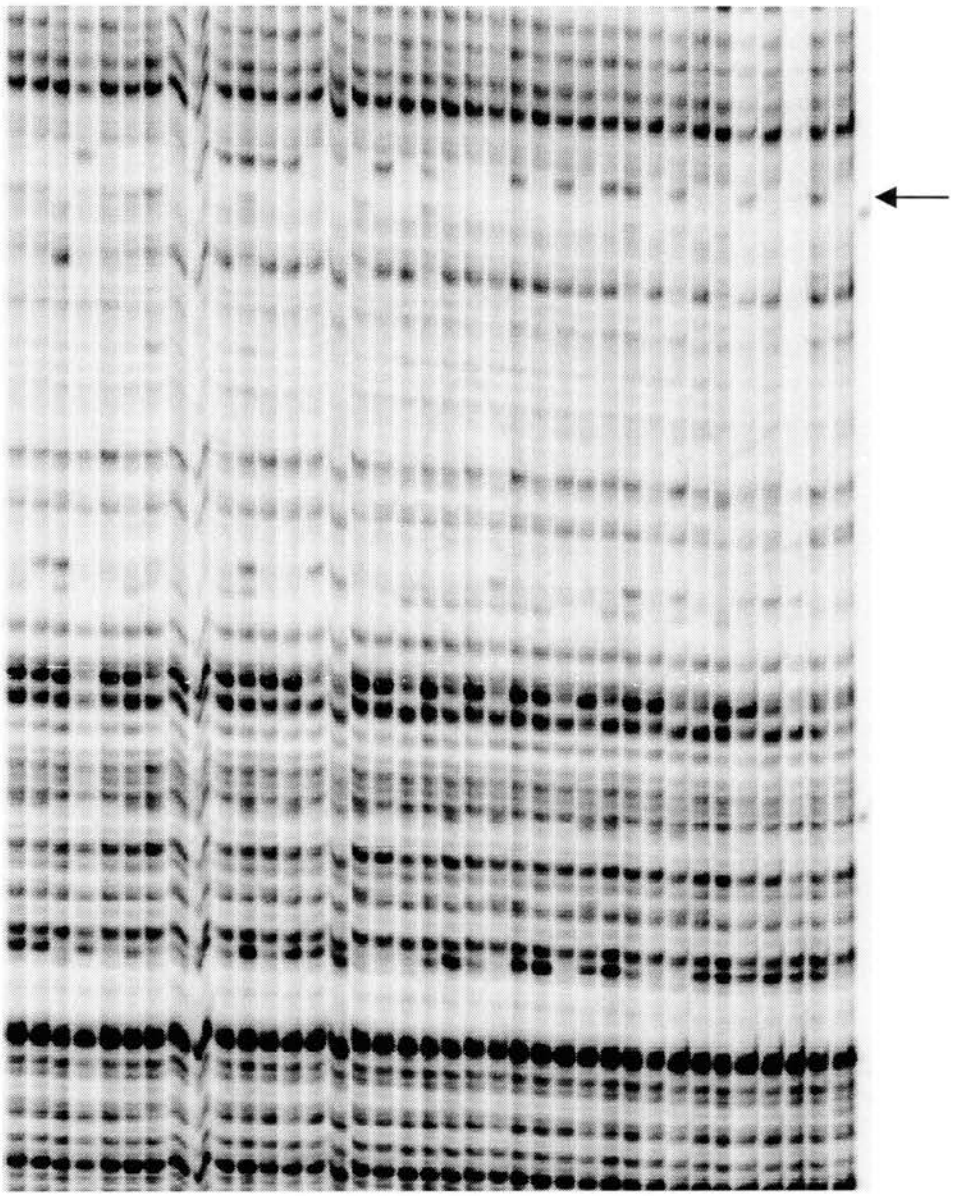

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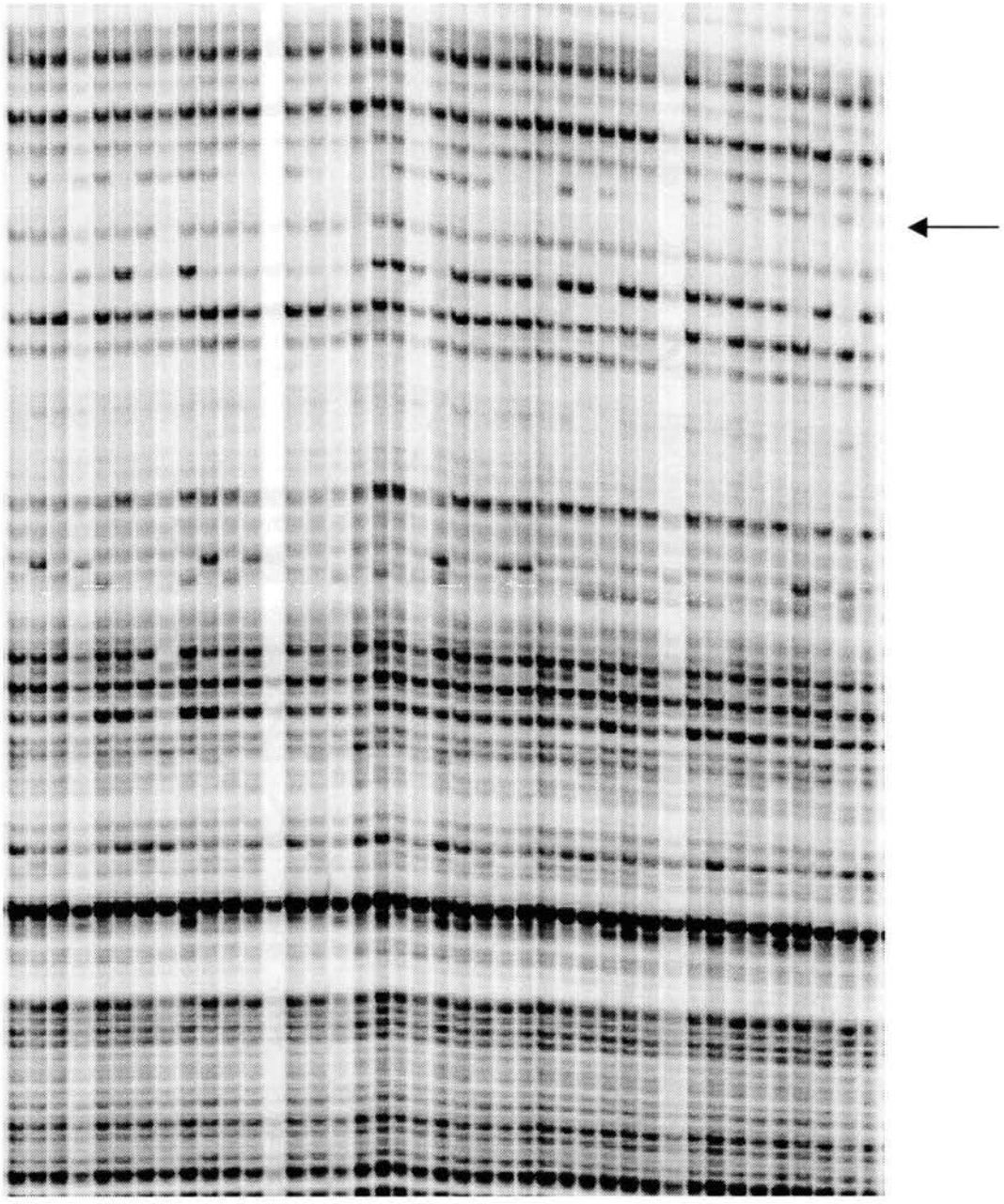

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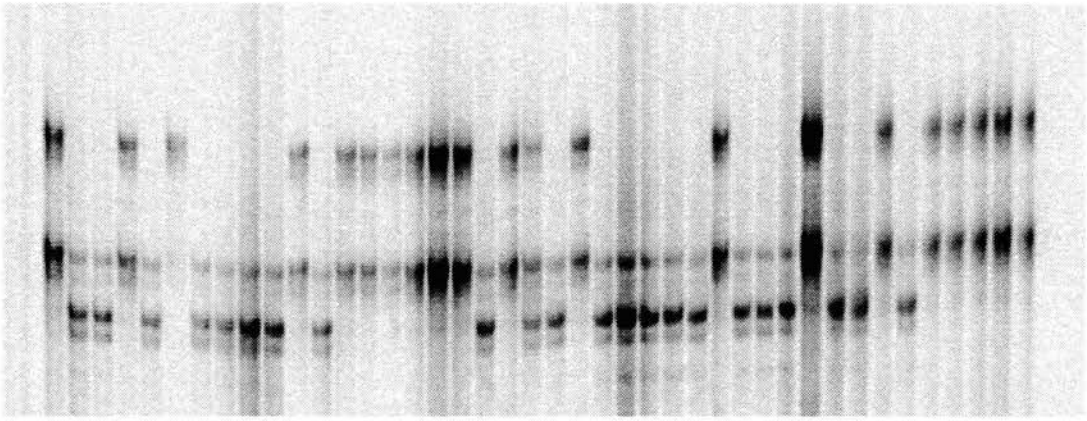

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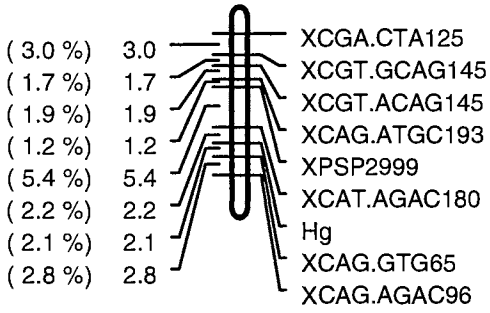

(3.0 %) 3.0
(1.7 %) 1.7
(1.9 %) 1.9
(1.2 %) 1.2
(5.4 %) 5.4
(2.2 %) 2.2
(2.1 %) 2.1
(2.8 %) 2.8
XCGA.CTA125
XCGT.GCAG145
XCGT.ACAG145
XCAG.ATGC193
XPSP2999
XCAT.AGAC180
Hg
XCAG.GTG65
XCAG.AGAC96

Fig. 4.6.

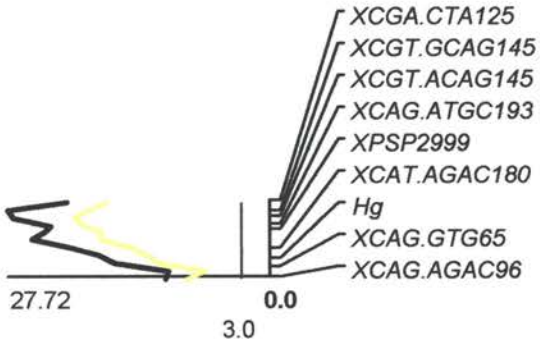
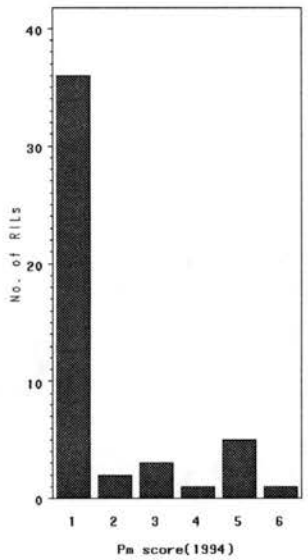

XCGA.CTA125
XCGT.GCAG145
XCGT.ACAG145
XCAG.ATGC193
XPSP2999
XCAT.AGAC180
Hg
XCAG.GTG65
XCAG.AGAC96
27.72
0.0
3.0

Fig. 4.7.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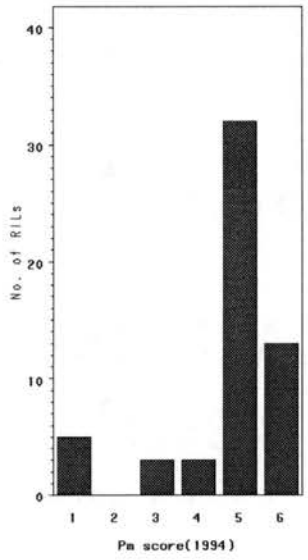
30
20
10
0
No. of RILs
1
2
3
4
5
6
Pm score(1994)

A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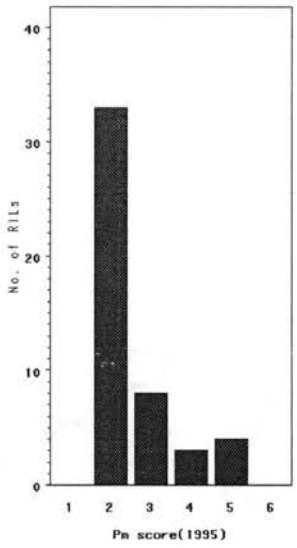
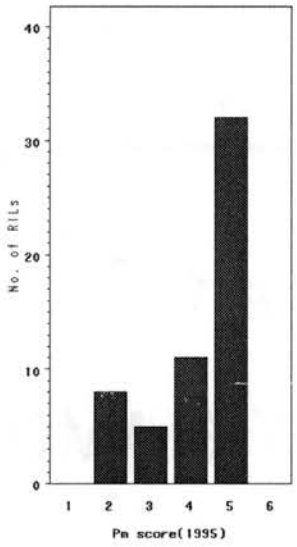
20
10
0
No. of RILs
1
2
3
4
5
6
Pm score(1994)

B

Fig. 4.8.


40
30
20
10
0
No. of RILs
1
2
3
4
5
6
Pm score(1995)

A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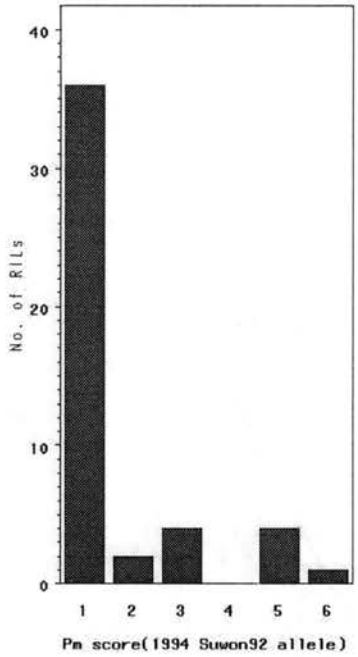
30
20
10
0
No. of RILs
1
2
3
4
5
6
Pm score(1995)

B

Fig. 4.9.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20
10
0
No. of RILs
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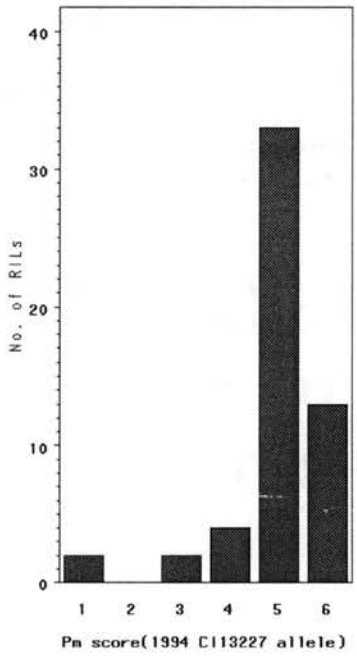
2
3
4
5
6
Pm score(1994 Suwon92 allele)

A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30
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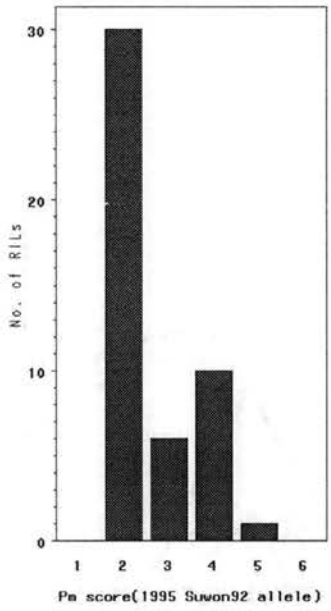
2
3
4
5
6
Pm score(1994 CI13227 allele)

B

Fig. 4.10.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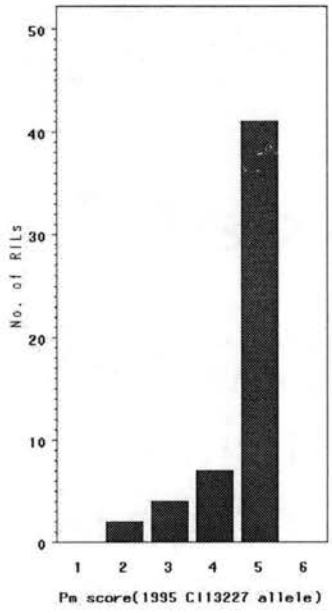
20
10
0
No. of RILs
1
2
3
4
5
6
Pm score(1995 Suwon92 allele)

A


50
40
30
20
10
0
No. of RILs
1
2
3
4
5
6
Pm score(1995 CI13227 allele)

B

Fig. 4.11.

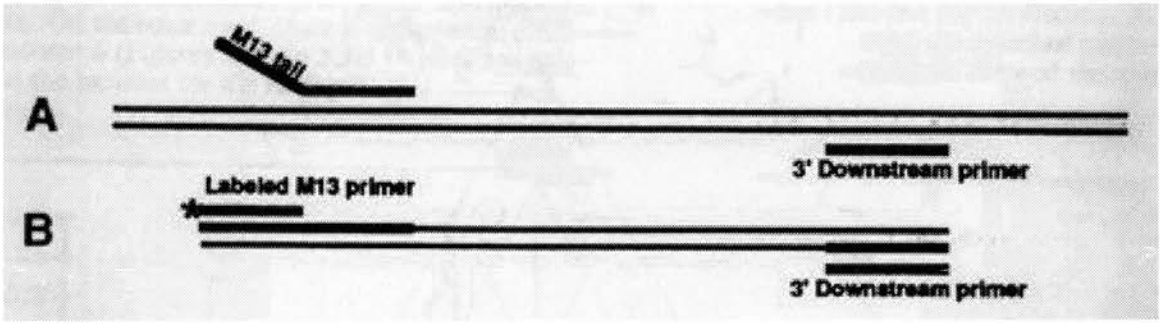
Appendix I. SSR Primer Sequences

Primer	Chrom.	An. Te.*		Primer Sequence
BARC018	2BS	52°C	Forward	CGC TTC CCA TAA CGC CGA TAG TAA
			Reverse	CGC CCG CAT CAT GAG CAA TTC TAT CC
BARC032	5BL/7BL	52°C	Forward	GCG TGA ATC CGG AAA CCC AAT CTG TG
			Reverse	TGG AGA ACC TTC GCA TTG TGT CAT TA
BARC095	2D	52°C	Forward	GGG GTG TGG TTG TTT GTA AGG
			Reverse	TGC GAA TTC TAT ATA CGA TCT TGA GC
BARC167	2BS	50°C	Forward	AAA GGC CCA TCA ACA TGC AAG TAC C
			Reverse	CGC AGT ATT CTT AGT CCC TCA T
PSP2999	1A	60°C	Forward	TCCCGCCATGAGTCAATC
			Reverse	TTGGGAGACACATTGGCC

* An. Te.: annealing temperature

Appendix II. M13 sequence and a diagram illustrating the principle of tailed M13 primer for SSR amplification

M13 tail sequence: GTAAAACGACGGCCAGT



This Diagram illustrates the principle of tailed M13 primer for SSR amplification. The upstream primer in the initial amplification (A) has an M13 tail that is incorporated into the initially amplified PCR products. This provide the priming site for the subsequent amplification by the fluorescence-labeled M13 primer which allows PCR detection by a laser scanner (B)

VITA

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