UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

Identification of Non-Host Resistance Genes in Wheat to *Puccinia striiformis* f. sp. *hordei*

MASTER THESIS ON

RECURSOS GENÉTICOS
E MELHORAMENTO DE ESPÉCIES AGRÍCOLAS E FLORESTAIS

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ABSTRACT

Yellow rust, caused by *Puccinia striiformis* West., is an important foliar disease of wheat and barley throughout the world, and the development of resistant cultivars is the most economical and environmentally friendly method of control. Breeding for resistance to yellow rust has, for decades, been based on the use of race-specific resistance genes, which have shown to be short-lived. Non-host resistance has been studied as a possible source of durable resistance.

Two major genes, as well as an undetermined number of minor genes, for non-host resistance to the barley attacking form of yellow rust, *P. striiformis* f. sp. *hordei*, have been previously detected in the wheat cultivar 'Lemhi'. The present study aimed at quantifying and mapping those genes using QTL (quantitative trait loci) mapping procedures. For that purpose, an F_2 population of 114 individuals resulting from the cross of resistant 'Lemhi' with 'Chinese 166', a wheat cultivar susceptible to barley yellow rust, was used as the mapping population. QTL effects and significance were estimated by means of interval mapping and MQM mapping procedures.

A map for the F_2 population was constructed which included 116 DNA markers (14 SSRs and 102 AFLPs). Two major QTLs have been mapped to chromosome arms 1DS (*Psh1*) and 2BL (*Psh2*), with significant LOD values. These two QTLs account for 76.7% of the phenotypic variance for resistance to barley yellow rust. Two other QTLs, with a minor effect, were mapped to chromosome arms 5AL (*Psh3*) and 6AL (*Psh4*), explaining 5.1% and 10.9% of the phenotypic variation, respectively. The QTL on 5A was derived from the susceptible variety, 'Chinese 166'. In all cases the resistance towards *P. striiformis* f.sp. *hordei* was associated with a visual chlorosis/necrosis response typical of race-specific, host resistance.

RESUMO

A ferrugem amarela, cujo agente causal é *Puccinia striiformis* Westend, é uma doença particularmente importante nas produções de trigo e cevada em todo o mundo, principalmente em regiões de clima fresco e húmido (EVERSMEYER & KRAMER, 2000). Infecções severas deste patogénio podem causar drásticas reduções na altura da planta, no número de grãos por espiga, e no peso e qualidade dos grãos (MA & SINGH, 1996b). A espécie *P. striiformis* encontra-se dividida em *formae speciales*, em função do género vegetal que ataca. Por exemplo, o trigo é considerado hospedeiro para *P. striiformis* f. sp. *tritici*, a ferrugem amarela do trigo, mas não para a f. sp. *hordei*, a forma da ferrugem amarela que ataca a cevada. No entanto, a divisão de *P. striiformis* em *formae speciales*, e em particular a separação em f. sp. *tritici* e f. sp. *hordei*, tem sido fortemente questionada, uma vez que existem vários exemplos de *formae speciales* com capacidade de atacar genótipos de espécies que estão supostamente fora do seu leque de hospedeiros (hospedeiros 'inapropriados') (JOHNSON & LOVELL, 1994; CHEN *et al.*, 1995).

O desenvolvimento de cultivares resistentes à ferrugem amarela é actualmente considerado o melhor método de controlo da doença, tanto a nível económico como ambiental. No entanto, o melhoramento para a resistência a esta doença tem assentado, ao longo das últimas décadas, no uso de genes de resistência específica de planta hospedeira, que, na maioria dos casos, têm demonstrado baixa durabilidade (WELLINGS & MCINTOSH, 1990; BAYLES *et al.*, 2000; SING & HUERTA-ESPINO, 2001). O uso generalizado de cultivares portadoras deste tipo de resistência resulta geralmente numa elevada pressão de selecção sobre o patogénio e na sua consequente evolução para novas formas de virulência (BROWN, 1995). Formas de resistência alternativas à resistência específica têm sido estudadas como possíveis fontes de resistência durável.

A resistência de planta não hospedeira é considerada por vários autores, a forma mais eficaz de obter durabilidade (HEATH, 1991; CRUTE & PINK, 1996). Na sua generalidade, este tipo de resistência envolve um controlo genético complexo e uma multiplicidade de factores de defesa que impedem o microrganismo de formar uma interacção básica (compatível) com a planta (HEATH, 1991). No entanto, interacções não-hospedeiro entre espécies vegetais filogeneticamente

próximas (como é o caso do trigo e da cevada) e *formae speciales* do mesmo patogénio (*P. striiformis* f. sp. *hordei* e *P. striiformis* f. sp. *tritici*) parecem envolver mecanismos de resistência semelhantes aos envolvidos na resistência específica de planta hospedeira, que geralmente estão associados ao retardamento do desenvolvimento do patogénio na fase pós-haustorial e à morte das células invadidas (reacção de hipersensibilidade) (NIKS, 1988; GARROOD, 2001). As estratégias de exploração da resistência de planta não hospedeira, assim como a sua durabilidade efectiva, irão, neste sentido, depender de a resistência ser controlada por mecanismos de defesa específicos ou não-específicos (HEATH, 2001). Torna-se, portanto, indispensável a existência de informação detalhada sobre os genes que controlam os mecanismos de resistência de planta não hospedeira, por forma a determinar a viabilidade do uso deste tipo de resistência como fonte de resistência durável.

O progresso nos sistemas de marcadores moleculares de DNA e nos programas informáticos de análise genética tornou possível o mapeamento de genes e a identificação de QTLs (*Quantitative Trait loci*, *loci* para características quantitativas) com relativa precisão, o que permitiu uma revisão dos métodos de análise genética e das estratégias de melhoramento. A análise de QTLs, i.e., a dissecção genética de características quantitativas, atenta na determinação do número de *loci* envolvidos na resistência, assim como na localização no genoma da planta e contribuição para o fenótipo de cada um desses *loci*, através da associação entre a variação de marcadores genéticos numa população segregante e a variação fenotípica para a resistência apresentada por essa mesma população (MOHAN *et al.*, 1997).

A tecnologia de microsatélites ou SSRs (S*imple Sequence Repeats*, repetições de sequências simples), que consistem em repetições em tandem de motivos básicos de 2 a 6 bases (TAUTZ, 1989), emergiu na última década como o sistema de escolha no mapeamento molecular em plantas, e em particular no trigo. Tal ocorre devido ao elevado número de SSRs existente nos genomas das plantas, e porque nesta tecnologia se reúnem as principais vantagens dos diferentes sistemas de marcadores moleculares: são específicos do cromossoma, altamente informativos, co-dominantes, com uma boa cobertura do genoma e com elevado potencial de automatização (MORGANTE & OLIVIERI, 1993; RÖDER *et al.*, 1995; POWELL *et al.*, 1996a; KORZUN *et al.*, 1997). Têm como principal inconveniente o elevado custo de identificação e produção (POWELL *et al.*, 1996a). Vários mapas de ligação foram já desenvolvidos para o trigo baseados neste tipo de

marcadores moleculares (DEVOS *et al.*, 1995; PLASCHKE *et al.*, 1995; RÖDER *et al.*, 1995, 1998a, b; BRYAN *et al.*, 1997; STEPHENSON *et al.*, 1998; PESTSOVA *et al.*, 2000; VARSHNEY *et al.*, 2000; SOURDILLE *et al.*, 2001; GUPTA *et al.*, 2002), e têm sido amplamente usados na localização de genes e QTLs responsáveis por resistências a doenças, incluindo a resistência à ferrugem amarela (e.g. CHAGUÉ *et al.*, 1999; PENG *et al.*, 1999, 2000a, b; BOUKHATEM *et al.*, 2002; SUN *et al.*, 2002).

Com base num cruzamento entre as cultivares de trigo 'Lemhi' (resistente à ferrugem amarela da cevada) e 'Chinese 166' (susceptível à doença), JOHNSON & LOVELL (1994) identificaram dois genes *major*, independentes e dominantes, responsáveis pela resistência de planta não hospedeira à ferrugem amarela da cevada na cv. 'Lemhi'. Foi igualmente detectada a existência de um número indeterminado de genes *minor*, alguns dos quais com possível origem na cv. 'Chinese 166'. Pretendeu-se com o presente trabalho: 1) desenvolver um mapa genético para uma população F_2, constituída por 114 indivíduos, derivada do cruzamento 'Lemhi' x 'Chinese 166' usando marcadores do tipo SSR; 2) adicionar estes marcadores a um mapa de AFLPs previamente construído para a mesma população; e 3) localizar os genes responsáveis pela resistência do trigo à ferrugem amarela da cevada em segregação na população F_2 'Lemhi' x 'Chinese 166'.

Cento e dezoito indivíduos da população F_2 'Lemhi' x 'Chinese 166', assim como as plantas progenitoras desta população, foram previamente testados para resistência/susceptibilidade ao referido patogénio. 'Lemhi' apresentou um fenótipo totalmente resistente, enquanto 'Chinese 166' se apresentou moderadamente susceptível, o que confirmou a presença de gene(s) *minor* nesta cultivar. Os 118 indivíduos da F_2 analisados fenotipicamente segregaram 115 resistentes : 3 susceptíveis, sugerindo que a resistência de 'Lemhi' à ferrugem amarela é efectivamente controlada por dois genes *major*.

Foram testados 88 pares de *primers* de SSRs para a presença de polimorfismos entre 'Lemhi' e 'Chinese 166'. Desta análise resultou um total de 41 SSRs polimórficos, que foram analisados em 114 indivíduos da população F_2. Com base nestes SSRs e em 172 AFLPs (*Amplified Fragment Length Polymorphisms*, polimorfismos do comprimento dos fragmentos amplificados) anteriormente desenvolvidos para a mesma população, e recorrendo ao programa

informático de análise genética JoinMap® versão 3.0 (VAN OOIJEN & VOORRIPS, 2001), foi construído um mapa molecular com 18 mapas de ligação, integrando 116 marcadores de DNA (14 SSRs e 102 AFLPs), e abrangendo 680 cM, com uma densidade média de 1 marcador por cada 6 cM. Os restantes 97 marcadores moleculares não foram integrados no mapa, provavelmente por, dada a extensão do genoma do trigo, não haver marcadores suficientes para criar ligação entre eles. Oito dos 18 grupos de ligação foram ancorados a seis cromossomas (1D, 2B, 3A, 5A, 6A e 6B) pela presença de SSRs. Uma vez que os restantes grupos de ligação não foram associados a nenhum QTL (ver parágrafo seguinte), não foram desenvolvidos esforços no sentido de identificar SSRs específicos para esses grupos de ligação.

A identificação de QTLs foi efectuada usando o programa informático de análise de QTLs MapQTL™ versão 4.0 (VAN OOIJEN *et al.*, 2002). Os efeitos dos QTLs e a sua significância para a variação fenotípica total da resistência à ferrugem amarela da cevada foram estimados pelos métodos *Interval Mapping* e *MQM Mapping*. Através do método *Interval Mapping* foram identificados dois QTLs *major*, localizados nos cromossomas 1DS (*Psh1*) e 2BL (*Psh2*), com origem na cv. 'Lemhi'. Por forma a detectar possíveis QTLs *minor* mascarados por estes QTLs *major*, foi aplicado o método *MQM Mapping*. Neste método, recorre-se ao uso dos marcadores que flanqueiam os QTLs detectados por *Interval Mapping* como co-factores para eliminar o efeito daqueles e detectar QTLs *minor*. Após análise por *MQM Mapping*, foram localizados dois QTLs *minor* nos cromossomas 5AL (*Psh3*) e 6AL (*Psh4*), sendo que o QTL presente no cromossoma 5A deriva da variedade susceptível 'Chinese 166'. Os quatro QTLs detectados explicam, no seu conjunto, 92,7% da variação fenotípica total da resistência à doença, o que indica que, provavelmente, todos os *loci* que contribuem para a resistência de planta não hospedeira foram identificados.

Neste estudo, verificou-se que a resistência à ferrugem amarela da cevada estava associada a uma resposta fenotípica de clorose/necrose, típica de resistência específica de planta hospedeira. Para além disso, os genes *Psh1* e *Psh2*, genes de resistência de planta não hospedeira à ferrugem amarela da cevada, foram identificados em regiões do genoma do trigo onde se pensa (no caso do *Psh1*) e onde se sabe (no caso de *Psh2*) existirem genes de resistência de planta hospedeira (genes *Yr*) à ferrugem amarela do trigo.

Tendo em atenção estes factos, pode considerar-se a possibilidade de uma ligação entre genes *Psh* e genes *Yr*, que, a confirmar-se, pode levar a supor que se trata de genes que evoluíram de um mesmo gene de resistência ancestral, possuindo portanto estrutura e modo de acção semelhantes. Se tal se vier a verificar, então a durabilidade de ambos seria, também ela, semelhante. Patologistas e melhoradores teriam que repensar seriamente a validade da busca de genes de resistência de planta não hospedeira como fonte de resistência durável. A clonagem destes genes é, neste sentido, essencial para que estudos bioquímicos e de funcionamento dos genes possam ser posteriormente desenvolvidos, e para que seja determinada a viabilidade do uso dos genes *Psh* como genes de resistência com efeito duradouro.

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INTRODUCTION

All domesticated crops are hosts to a large variety of pests and pathogens, fungi being a class of pathogens with great impact on crop losses (AGRIOS, 1997). Protection against attack by pathogenic microorganisms is therefore one of the major aims in crop production. For some decades, control of plant diseases has depended heavily on the extensive use of chemicals and cultural techniques such as soil disinfection. The use of these compounds has, however, had extremely high costs, both economic and environmental. In the light of these events, much of the modern research in crop production aims at finding other environmentally friendly means of disease control. The use of resistant plants is, therefore, one of the most attractive approaches towards control and suppression of plant disease. RUBIALES (1996) estimated that the cost of breeding a new resistant cultivar was only 10% of the benefit of using it, as their use requires no additional inputs by the farmer, eliminating additional production costs and potential disruptive problems to the environment.

Knowledge about the genetics and biochemistry of plant disease resistance has accumulated since the turn of the century, when BIFFEN (1905) first recognized that resistance to yellow rust in wheat was controlled by genes with a Mendelian inheritance. After the demonstration that disease resistance could be conferred by single genes (FLOR, 1955), several breeding programs were started with the expectation that the resulting control of plant disease would be permanent.

However, the rapid evolution within the pathogen for pathotypes with matching virulence to previously resistant cultivars led to the "Boom and Bust" cycle of events, in which an extensively cultivated resistant cultivar rapidly succumbed to the pathogen (BROWN, 1995). This forced breeders into repetitive cycles of cultivar replacement, demanding the continual identification and introgression of new resistance specificities (BROWN, 1995).

Durability is therefore a key consideration in disease resistance breeding. Non-host and race-non-specific resistances are possibly effective and durable alternatives to race-specific resistance. Despite the fact that non-host resistance is the rule and susceptibility the exception, very little is known about the genetics of non-host resistance because, by definition (NIKS, 1987a), all plants of a non-host species are completely resistant to all isolates of the pathogen, making genetic studies almost impossible.

In attempts to uncover the mechanisms, genetics and pathogen specificity of non-host resistance to inappropriate pathogens, several crop-pathogen systems involving non-host resistance interactions have been studied. These include the work with legume and cereal rust fungi (HEATH, 1977, 1997, 2000a, b, 2001, 2002; ELMHIRST & HEATH, 1987, 1989; ECKENWALDER & HEATH, 2001; MELLERSH & HEATH, 2003), barley-rust interactions (NIKS, 1983a, b, 1987a, b, 1988; RUBIALES & NIKS, 1996; NIKS & RUBIALES, 2002), dicotyledonous-*Phytophthora* interactions (KAMOUN *et al.*, 1998, 1999; VLEESHOUWERS *et al.*, 2000; KAMOUN, 2001), and cereal-powdery mildew interactions (KUNOH, 1977; TOSA & SHISHIYAMA, 1984; TOSA *et al.*, 1987, 1988; 1990; TOSA, 1989a, b, 1992, 1996; MATSUOKA *et al.*, 1994; MATSUMARA & TOSA, 1995; SHIRAISHI *et al.*, 1995).

Cereals are the basis of human and animal food supplies worldwide, and wheat and barley play a major role in the total world cereal production. These cereals are, however, seriously attacked by cereal rusts (*Puccinia graminis*, *Puccinia striiformis* and *Puccinia recondita*), leading to considerable yield losses. For this reason, breeding for resistance to rusts has always been a concern to cereal breeders. In 1986, NEWTON *et al.* reported that, contrary to most wheat cultivars, the cultivar 'Chinese 166' could be infected by *P. striiformis* f. sp. *hordei*, the barley-attacking form of yellow rust, surpassing the barriers of non-host resistance. Another cultivar, 'Lemhi', thought at the time to be susceptible to all isolates of *P. striiformis* collected in wheat on the UK, was found to be fully resistant to barley yellow rust isolates (JOHNSON & LOVELL, 1994). In an effort to investigate the genetics of resistance in 'Lemhi' to *P. striiformis* f. sp. *hordei*,

JOHNSON & LOVELL (1994) crossed the two cultivars. They concluded that resistance in 'Lemhi' was controlled by two independent dominant genes of major effect, plus an undetermined number of genes of smaller effect. To determine the nature of non-host resistance in wheat to the barley-attacking form of yellow rust is of major interest for the future use of this resistance and its potential as a durable source of resistance. For this reason, the genes responsible for this resistance need to be identified and located in the wheat genome, and then cloned for further genetic and biochemical studies.

In the last two decades, the progress in DNA molecular marker systems and in statistical genetic analysis software has made it possible to identify and map major genes and QTLs (quantitative trait loci) with relative accuracy, which had opened the areas of genetic analysis approaches and breeding strategies to review. Microsatellite or SSR (simple sequence repeat) technology has emerged in the last decade as the system of choice in plant molecular marker mapping. Plant genomes contain large numbers of SSRs (consisting of tandemly repeated basic motifs) that are highly informative, stably inherited, co-dominant, have good genome coverage and have potential for automation (MORGANTE & OLIVIERI, 1993; RÖDER *et al.*, 1995; POWELL *et al.*, 1996a; KORZUN *et al.*, 1997). Several molecular linkage maps have been developed for wheat based on this type of molecular markers (DEVOS *et al.*, 1995; PLASCHKE *et al.*, 1995; RÖDER *et al.*, 1995, 1998a, b; BRYAN *et al.*, 1997; STEPHENSON *et al.*, 1998; PESTSOVA *et al.*, 2000; VARSHNEY *et al.*, 2000; SOURDILLE *et al.*, 2001; GUPTA *et al.*, 2002), and are being extensively used to locate genes and QTLs responsible not only for disease resistance, but for numerous other agronomic characteristics.

Using a cross between wheat cultivars 'Lemhi' and 'Chinese 166', JOHNSON & LOVELL (1994) identified two independent dominant genes originating from 'Lemhi' that confered non-host resistance to a barley-attacking isolate of yellow rust *(P. striiformis* f. sp. *hordei*). An undetermined number of genes of small effect, some possibly coming from 'Chinese 166', were also detected. The intended aims of this work were to develop a genetic map using chromosome specific SSR markers in an F_2 population derived from the cross 'Chinese 166' x 'Lemhi', to add these markers to an AFLP map previously developed for the same population, and to locate the major and minor genes for barley yellow rust resistance segregating in the 'Lemhi' x 'Chinese 166' F_2 population.

1. LITERATURE REVIEW

1.1. THE RUST-CEREAL RELATIONSHIP

1.1.1. The Host

Wheat, *Triticum* sp., is the 2nd most important cultivated cereal in the world, after rice, and its current world production is around 600 million tons (RAJARAM, 2001). In Portugal, wheat had an estimated production of 352,207 tons in 1999/2000 (GPPAA, 2001), being the most important cereal in the country. The two most cultivated species of the genus are *Triticum aestivum* L., common or bread wheat, and *Triticum turgidum* L., durum or macaroni wheat.

Common wheat is an allohexaploid with three genomes (AABBDD, 2n=6x=42), each containing seven pairs of chromosomes, showing homology across genomes, and with a total genome size of *ca*. 1.7 x 10^{10} base pairs (LAURIE & DEVOS, 2002). Hexaploid wheat originated from the natural hybridisation of three different diploid progenitors from the *Triticum* and *Aegilops* genera. The first step in its development is believed to have involved the hybridisation between *Triticum urartu* Thum. Ex Gandil. (AA) and an unknown species (BB) related to *Aegilops speltoides* Tausch (SS). The resulting tetraploid wheat (*T. turgidum*, AABB) then hybridised with *Aegilops tauschii* Coss. (DD) to produce hexaploid *T. aestivum* (AABBDD) (after KIHARA, 1944 and MCFADDEN & SEARS, 1946). Each of the tetraploid species of the *Triticum* and *Aegilops* genera, wild or cultivated, have contributed, and still are contributing, to the genetic variation of the present hexaploid cultivated wheats (MCINTOSH *et al.*, 1995).

Barley, one of the first plant species to be domesticated, is nowadays the fourth most important cereal crop in the world, with an estimated production, in 1999/2000, of 127.55 million tons worldwide (BROWN JR. *et al.*, 2001). Barley has persisted as an important cereal worldwide because of its unique characteristics for feed and food grain and for brewing (BROWN JR. *et al.*, 2001). In Portugal, this cereal occupies 7th place in importance inside the cereals group, with an estimated production of 33,119 tons, 2% of the total cereal production, for the period 1999/2000 (GPPAA, 2001). Cultivated barley, *Hordeum vulgare* L., is a diploid species (2n=2x=14), with a genome size of *ca.* 5.4 x10^9 base pairs (LAURIE & DEVOS, 2002).

Both wheat and barley are cultivated in cool, temperate climates, but barley has a wider geographical range and is cultivated in regions climatically unfavourable for producing wheat

and other major cereals (BROWN JR. *et al.*, 2001). Phylogenetic studies among grasses (reviewed in LAURIE & DEVOS, 2002) place *Hordeum*, *Aegilops* and *Triticum* species in the *Pooideae* taxa, showing a common ancestor and a very close evolutionary relationship.

1.1.2. The Pathogen

1.1.2.1. *Cereal rusts*

Cereal rusts are among the most devastating diseases of cereals. The causal agent is a group of obligate biotrophic fungi from the genus *Puccinia*, order *Uredinales*, and can usually attack wheat, barley, triticale, rye and other related grasses (AGRIOS, 1997). These fungi have a debilitating effect on young plants, and on adult plants they reduce foliage, root growth and yield, by reducing the rate of photosynthesis and increasing the rate of respiration (AGRIOS, 1997). The quantity of grain produced by rusted plants is usually greatly reduced and its quality is extremely poor, since it is devoid of starch, consisting mainly of cellulosic materials (AGRIOS, 1997). The geographical distribution and adaptability of the rusts is highly dependent on environment conditions, mostly temperature and humidity (EVERSMEYER & KRAMER, 2000).

There are three main cereal rust species, *Puccinia graminis* Pers. (black or stem rust), *Puccinia recondita* Roberge ex Desmaz (brown or leaf rust) and *Puccinia striiformis* Westend (yellow or stripe rust). Black rust is considered to be the most aggressive of the rusts, and is believed to be the most important rust disease of wheat worldwide, leading to reductions in tillering, and losses in grain weight and quality of up to 30% (EVERSMEYER & KRAMER, 2000). Brown rust is a cool temperature species (EVERSMEYER & KRAMER, 2000). It is spread throughout Europe and is regarded as an important disease in many European countries (PARK & FELSENSTEIN, 1998). These authors refer losses in wheat production of up to 25% caused by this disease, usually resulting from the reduction of the number of grains per spike and grain quality.

P. striiformis, the causal agent of yellow rust, is adapted to cooler and more humid climates than the other two rusts (EVERSMEYER & KRAMER, 2000). It is thought to have originated from Transcaucasia, where wild grasses are its primary host (LINE, 2002), and was later adapted to attack wheat and barley, as it spread from its centre of origin (WAHL *et al.*, 1984). The susceptibility of *Triticum dicoccoides* Korn and of diverse *Aegilops* species in Israel supports the theory that wild grasses were ancestral hosts of the fungus, which explains the numerous

resistance genes effective against wheat yellow rust that are present in them (WAHL *et al.*, 1984). Nowadays, yellow rust is a disease particularly important in wheat and barley production worldwide, but it can also attack rye and other grasses belonging to *Triticum*, *Aegilops*, *Hordeum* and other genera (WAHL *et al.*, 1984; reviewed in LINE, 2002). Severe infections from this pathogen can cause reduction of the number of grains per spike, grain quality and weight, and plant height (MA & SINGH, 1996b). In Europe, it has been a common disease of wheat for centuries, especially in the north-western countries (STEELE *et al.*, 2001). In the USA, it became important in the late 1950s and its distribution in 2000 was the most widespread in recorded history in that country (CHEN *et al.*, 2002). In Australia, yellow rust was only detected in 1979 and became established as an endemic disease, leading to substantial crop losses (WELLINGS & MCINTOSH, 1990). Yellow rust is also a serious disease in all barley producing regions in the world. It occurs within Western Europe, Middle East, South Asia, East Africa and across the American continent (SANDOVAL-ISLAS *et al.*, 1998). In 1975, barley cultivars in Colombia were severely infected by this fungus (DUBBIN & STUBBS, 1986) and some years later was responsible for yield losses on much of the cultivated barley in South America and Mexico (MARSHALL & SUTTON, 1995), causing losses of 30 to 70% (CHEN *et al.*, 1995). In the USA, this form of yellow rust was found for the first time in 1991 and the highest yield loss, 72%, occurred the next year (MARSHALL & SUTTON, 1995). There are no known references to the effect of this pathogen in portuguese cereal productions.

1.1.2.2. *Yellow rust disease symptoms*

Yellow rust on susceptible adult plants appears as golden-yellow small pustules in long, narrow stripes on leaves, usually between veins, and on leaf sheaths, glumes and awns. Once infection occurs on a leaf, the pathogen can continue to grow parallel to the leaf axis to produce long stripes of pustules (LINE, 2002; Figure 1.1). On seedlings, infection is not confined by leaf veins, and may grow to produce pustules that completely cover the leaf (LINE, 2002).

Figure 1.1 - Disease symptoms caused by *Puccinia striiformis* in wheat (courtesy of L.A. BOYD).

1.1.2.3. *Yellow rust life cycle*

Rust fungi form a group of obligate parasites with a complex life cycle that usually involves sexual and asexual stages with five different types of spores, spermatia (pycniospores), aeciospores, urediospores, teliospores, and basidiospores (Figure 1.2), and usually requires two different hosts (AGRIOS, 1997). In the case of *P. striiformis*, no known sexual stage or alternate hosts are known. It is not known why a sexual stage has not been found, maybe the teliospores germinate immediately after maturation in the late summer, and readily produce basidiospores at a time when leaves of an alternate host are not available (LINE, 2002). As far as is known, the telial stage has no function in the infection process (LINE, 2002). The fungus survives by repeated asexual uredial cycles (GARROOD, 2001). Even though it is generally considered that in rusts with no sexual stage the races are more stable (AGRIOS, 1997), *P. striiformis* populations are highly variable, mutation being the only mechanism known by which it can generate variability (STUBBS, 1988; LINE, 2002).

Under natural conditions (cool winter and warm spring), yellow rust infections can occur throughout autumn and winter, but mild winters and cool springs can favour the establishment and development of the rust in the spring (LINE, 2002). NEWTON & JOHNSON (1936) report minimum, optimal, and maximum spore germination temperatures to be 0 ºC, 10-12 ºC, and 13-16 ºC, respectively. Spore germination can originate from overwintering mycelium, since it can survive negative temperatures (LINE, 2002).

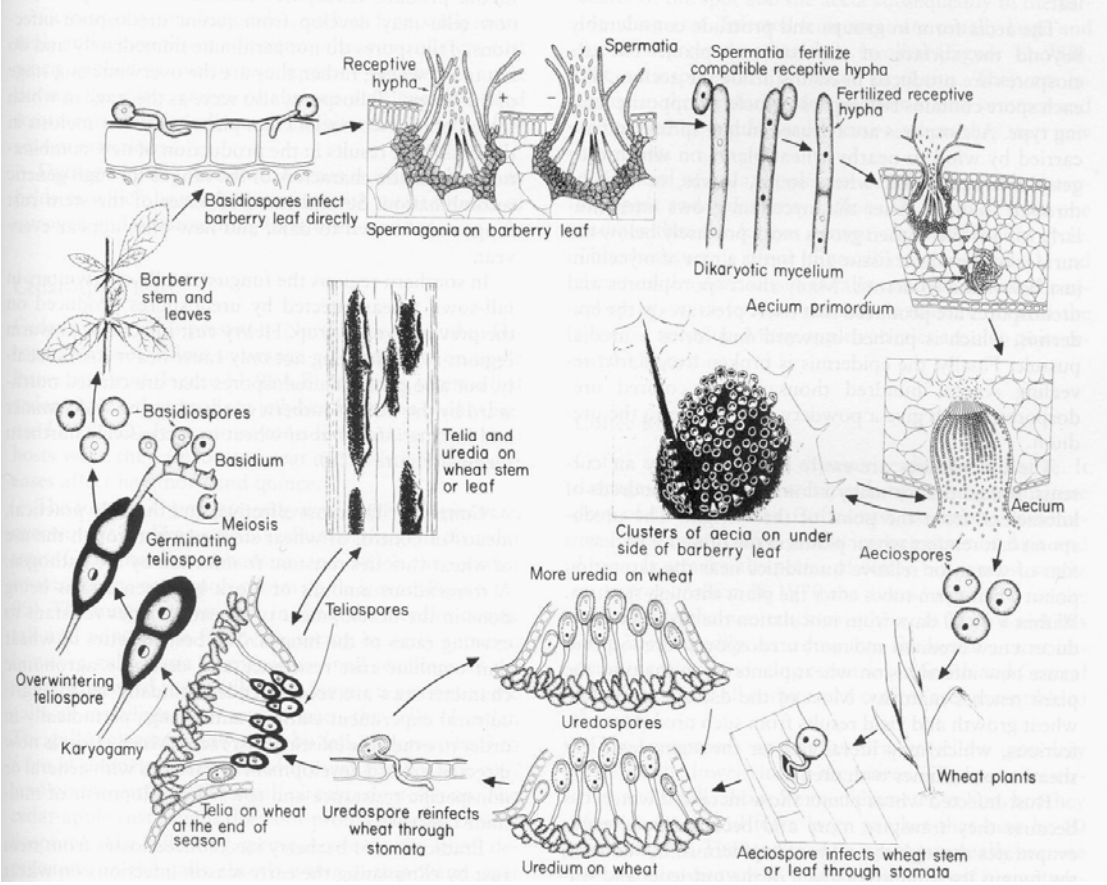

Figure 1.2 - Sexual and asexual cycle of rusts in wheat (From: AGRIOS, 1997).

1.1.2.4. *Yellow rust mode of infection*

The first step of rust infection is germination of the urediospore and growth of a germ tube towards a stomata, the result of a highly specialised response to topographic features of the leaf surface, like the size of stomatal lips (MENDGEN & HAHN, 2002). This step is followed by the formation of an appressorium above the stomatal opening and growth of an appressorial infection peg into the stomatal cavity, although the apressoria observed for yellow rust are small and often difficult to detect with any degree of certainty (RUBIALES, 1996; NIKS & RUBIALES, 2002). A substomatal vesicle is formed inside the cavity and an infection hypha grows intercellularly between mesophyll cells (BROERS & LÓPEZ-ATILANO, 1996; HEATH, 1997). The end of the infection hypha, when in contact with a mesophyll cell, swells to give rise to a haustorial mother cell, which produces an infection peg that breaches the mesophyll cell wall. Until this step, the host has not been in direct physical contact with the pathogen, since the development of the

infection structures happens in the intercellular spaces. When the infection peg develops a haustorium within the mesophyll cell, a parasitic relationship is finally established (RUBIALES, 1996). A diagrammatic representation of the fungal dikaryotic stage is shown in Figure 1.3.

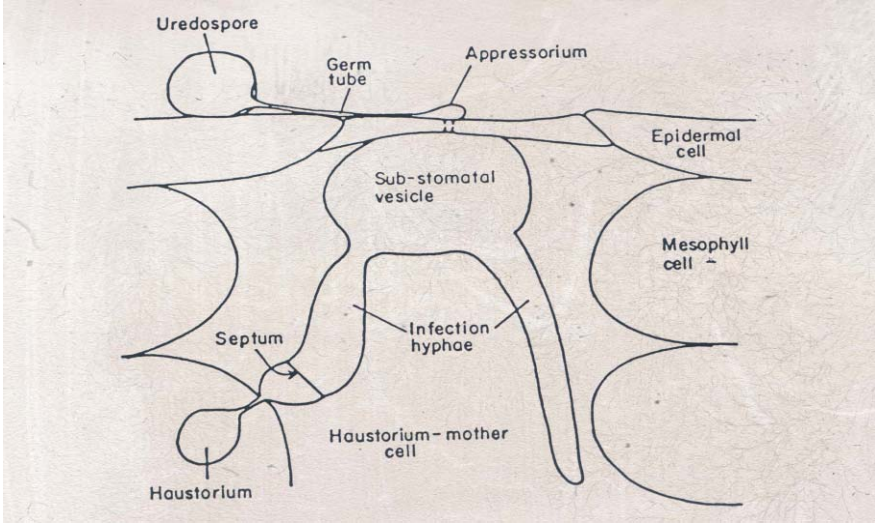

Figure 1.3 - Asexual infection of *Puccinia striiformis* on a cereal leaf (Courtesy of L.A. BOYD).

1.1.2.5. *Differentiation of* formae speciales

Rust fungi are very specialised parasites and each rust species attacks only certain genera or species of plant (AGRIOS, 1997). Rusts that are morphologically identical but attack different host species are regarded as *formae speciales* (f. sp.) or special forms. Within each special form, there are pathogenic (physiological) races, which can attack only certain genotypes within the species (AGRIOS, 1997).

P. striiformis was first divided into *formae speciales* by Eriksson in 1894, based on the host species (*P. striiformis* f. sp. *tritici* on wheat, *P. striiformis* f. sp. *hordei* on barley, *P. striiformis* f. sp. *secalis* on rye, *P. striiformis* f. sp. *elymi* on *Elymus* spp., and *P. striiformis* f. sp. *agropyri* on *Agropyron* spp.) (LINE, 2002). Other *formae speciales* have since been reported (reviewed in LINE, 2002). Such specificity is believed to be the outcome of coevolution (CRUTE, 1998), and several theories on the evolution of *formae speciales* and respective hosts have been developed. GREEN (1971) hypothesized that pathogens evolution progressed from broadly pathogenic, nonagressive forms to highly specialized, aggressive forms. HEATH (1991) developed a model of

speciation of parasites to their hosts assuming that different *formae speciales* of a given parasite have a common genetic background and thus share a number of genes that, being polymorphic for certain pathogenicity factors, may contain different and overlapping specificities towards related plant species. TOSA (1992), on the other hand, presented a model suggesting that evolution of *formae speciales* would be a process of pathogens losing avirulence genes in order not to be recognized by the corresponding resistance genes of the different plant species.

Some *formae speciales* have been reported to be able to attack certain genotypes of plant species that are supposedly out of their host range, as is the case with *P. striiformis* f. sp. *hordei*, reported to attack wheat (STUBBS, 1985; JOHNSON & LOVELL, 1994; CHEN *et al.*, 1995) and *P. striiformis* f. sp. *tritici*, reported to attack barley (CHEN *et al.*, 1995). The division of *P. striiformis* into *formae speciales*, especially the separation of f. sp. *tritici* and f. sp. *hordei*, has been questioned because of overlapping host ranges (NEWTON & JOHNSON, 1936). However, failed attempts to produce somatic hybrids between isolates of these two *formae speciales* (NEWTON *et al.*, 1986), and studies based on virulence and DNA polymorphisms (CHEN *et al.*, 1995), clearly demonstrated that *P. striiformis* f. sp. *tritici* and *P. striiformis* f.sp. *hordei* are distinct, however closely related, forms of the pathogen. These results support the idea that the level of subspeciation between *formae speciales* can be extremely low, and that genetic exchange could possibly occur (NIKS, 1987a).

1.1.3. Plant-Pathogen Interaction

The type of interaction between a plant and a microorganism is dictated by the ability of that microorganism to infect that plant species. For a microorganism to develop and cause disease in a plant, it must be able to overcome, negate or avoid the plant's defense (HEATH, 1991). This type of interaction is very specific and, when it happens, a compatible interaction is established (HEATH, 1991). The plant is said to be a susceptible host to that organism, and the organism is a pathogen to that plant. In a compatible interaction between a rust and its host, the infection hyphae often grow in the mesophyll intercellular spaces without any obvious response by the plant (HEATH, 1997), freely produce haustoria within living plant cells, and produce sporulating rust pustules on green leaf tissue (GARROOD, 2001).

When the host has the ability to resist the pathogen, a host, race-specific incompatible interaction is established, blocking disease development. The plant is said to be resistant to that pathogen,

and the pathogen is avirulent to that plant. In resistant host cultivars, plant defense reactions are usually associated with hypersensitive response, which is translated into localized induced plant cell death at the site of infection (JABS & SLUSARENKO, 2000). This is an active defense mechanism, generally considered to be of great importance in resistance to rusts which, as obligate biotrophs, require living cells to develop (JABS & SLUSARENKO, 2000). However, the hypersensitive response is not just a physical way of restricting the growth of obligate biotrophic fungi. A cascade of other defense responses usually occurs, such as the accumulation in the necrotic cells of various antimicrobial compounds (DE WIT, 1992; BOLLER & KEEN, 2000). This hypersensitive reaction usually occurs after haustorium formation, such resistance being referred to as post-haustorial, hypersensitive resistance (NIKS, 1987a; NIKS & RUBIALES, 2002).

The hypersensitive response is not the only defensive reaction triggered by resistant host plants. RUBIALES & NIKS (1995) report the association of *Lr34*, a wheat gene conferring resistance to wheat brown (leaf) rust, with reduced haustorium formation, and not with hypersensitivity. In a study aiming at revealing information about the mechanisms of resistance to *P. striiformis* in wheat, BROERS & LÓPEZ-ATILANO (1996) report that, on resistant cultivars where germ tubes were able to find stomata, but were not triggered to form appressoria and penetrate, the formation of appressoria was reduced by more than 50%, when compared to susceptible cultivars. The mechanisms behind this type of resistance are not clear.

When all the genotypes of a plant species are resistant to all isolates of a potential pathogen, a non-host interaction is established, and the organism is said to be non-pathogenic (NIKS, 1987a). Non-host interactions are thought to be due to a basic incompatibility between the plant species and the organism, at least in the cases where the host species is taxonomically distant from the non-host species under study (HEATH, 1991). In this type of interaction, avoidance is a common defense mechanism (NIKS, 1987a, b). Morphological features that inhibit spore germination, e.g. abundance of leaf hairs and waxy leaf surface (HEATH, 1977; RUBIALES & NIKS, 1996; NIKS & RUBIALES, 2002), germination inhibitors (JOHNSON *et al.*, 1982) and the topography of the leaf surface (GARROOD, 2001) have been associated with the inability of the pathogen to develop on non-host species. However, in cases where non-host species are morphologically and topographically similar to host species, many specialised pathogens are able to find and recognise stomata of non-host species, suggesting that stomata recognition is not a very specialised process (NIKS & RUBIALES, 2002). In cases where the stomata are found and recognised, haustorium

formation often fails at very high rates, in a type of resistance named pre-haustorial resistance (HEATH, 1981; NIKS, 1987a).

Complexed and largely unresolved perception systems exist for pathogen elicitors, possibly on the plant cell surface, that activate multiple intracellular defense signalling pathways and active defense responses to the inappropriate pathogen (HEATH, 2000b). The same rust fungus that on a susceptible host species grows an infection hypha between mesophyll cells without triggering any response in the plant, elicits a variety of defensive responses (e.g. deposition of silica, callose, saponins or phenolic materials on and in the plant cell wall) when it forms an infection hypha within non-host species (OSBOURN, 1996; HEATH, 1997). However, some examples of pathogens successfully penetrating cell walls of non-host plant species have been reported (HEATH, 1977; JOHNSON *et al.*, 1982; ELMHIRST & HEATH, 1987; NIKS & DEKENS, 1991; LEBEDA & REININK, 1994; KAMOUN *et al.*, 1998; VLEESHOUWERS *et al.*, 2000; GARROOD, 2001). In these cases, the invaded cells react rapidly with a hypersensitive reaction. Whether these induced responses are the same as those that protect resistant genotypes in host species, is not known.

The interaction of non-host species with yellow rust has not been widely studied. NIKS (1987a) considers that histological observations on the infection process can be of some help in distinguishing between host and non-host interactions. In cases where stomata recognition occurs, host resistant interactions are characterised as giving a post-haustorial, hypersensitive reaction, and non-host interactions as having pre-haustorial abortion of the infection hyphae, not associated with hypersensitive reaction and plant cell necrosis (NIKS, 1987a; NIKS & RUBIALES, 2002). However, in a microscopic study of the interaction between wheat and barley with *P. striiformis* f. sp. *hordei* and *P. striiformis* f. sp. *tritici*, GARROOD (2001) detected similar levels of primary haustoria in both non-host and host resistant interactions, indicating a post-haustorial, hypersensitive resistance in the non-host interaction, similar to that in the host, race-specific resistant interaction.

Given that an incompatible interaction between a *forma specialis* of a pathogen and a plant species that is a host to another *forma specialis* of the same pathogen usually results in a hypersensitive reaction (similar to host incompatible interactions), TOSA (1992) considers that plant species taxonomically related to host species of a *forma specialis* should not be considered as typical non-hosts. For example, wheat should be considered a host not only of *Erysiphe graminis* (DC.) Speer f. sp. *tritici* but also of *E. graminis* f. sp. *agropyri*, the wheatgrass attacking

form of powdery mildew. In this conformity, TOSA (1992) argues that Heath's model of basic incompatibility (Heath, 1991) can only be applied to non-host interactions if his concept of non-host is adopted. Following Heath's model, plant genera *X* and *Y* are non-hosts to f. sp. *Y* and f. sp. *X*, respectively, and the *formae speciales* are only able to establish basic compatibility with the appropriate hosts. The *forma specialis*-genus specificity is determined by resistance genes in the plant, and is not dependent on avirulence genes in the pathogen. In contrast, Tosa's model (Tosa, 1992) assumes that plant genera *X* and *Y* are hosts of both f. sp. *X* and f. sp. *Y*, and that basic compatibility can be established with both plant genera. The *forma specialis*-genus specificity is determined by avirulence genes and their corresponding resistance genes. According to this model, the genes involved in race-cultivar specificity evolved in the host population after the *formae speciales* association was established. This theory would probably explain the fact that inappropriate *formae speciales* carry avirulence genes for plant species other than the appropriate hosts, as detected by MATSUMARA & TOSA (1995).

1.2. GENETICS OF DISEASE RESISTANCE

Knowledge about the genetic basis of plant disease resistance has developed greatly since BIFFEN (1905) first recognised that resistance was controlled by genes showing Mendelian inheritance. During the last century, various pathosystems have been studied, and the characterisation of the plant-pathogen interactions has allowed the formulation of some of the key concepts in plant pathology and plant disease resistance. Different types of resistance, expressed at different stages of the plant's development, and having a different genetic basis, have been characterised.

1.2.1. Gene-for-Gene, Race-Specific Resistance

The genetic basis of race-specific resistance was first described by FLOR (1955), who demonstrated that the resistance of flax to the fungus *Melampsora lini* (DC.) Desm (flax rust) was a consequence of the interaction of single, major genes present in both plant and pathogen. The genetic analysis of resistance in numerous host species to specific avirulences in corresponding pathogens (see STASKAWICZ *et al.*, 1995 and CRUTE & PINK, 1996 for references), that followed Flor's discovery, has led to the general acceptance of the "gene-for-gene" theory as the genetic model for this type of single gene resistance, also referred to as race-specific, vertical, monogenic, major-gene or qualitative resistance. This type of resistance is usually expressed at all stages of a plant's development, and the plant is usually resistant to a subset of strains or races of the pathogen (BÖRNER *et al.*, 2001).

In gene-for-gene interactions, the activation of inducible defenses is triggered by a specific recognition of the attacking pathogen. Perception involves receptors in the plant with high degrees of specificity for pathogen ligands (STASKAWICZ *et al.*, 1995; HAMMOND-KOSACK & JONES, 1997), also called race-specific elicitors (DE WIT, 1992). The specific recognition of the pathogen elicitor (avirulence product) by the plant, which leads to resistance, requires the presence of matching avirulence (*Avr*) and resistance (*R*) genes in the pathogen and host, respectively. Elicitor recognition activates a cascade of host genes that leads to the hypersensitive response and inhibition of pathogen growth (BOLLER & KEEN, 2000). When mutation occurs in the pathogen avirulence gene, such that the product is no longer recognised by the resistance gene receptor in the plant, the pathogen isolate is now virulent on that plant genotype, and a compatible interaction is established (HEATH, 1991).

1.2.2. Quantitative Resistance

Quantitative resistance, also known as multigene, polygenic or minor gene resistance is governed by an array of genes, where each single gene contributes in an additive way to the overall level of resistance. In some cases, a significant proportion of the total variance in the expression of quantitative resistance may be attributable to one locus or a few loci (JOHNSON & LOVELL, 1994; MICHELMORE, 1995). This type of resistance is usually non-specific, as it shows no specificity to a single pathogen race, but confers resistance to all isolates of the pathogen.

While monogenic resistance usually gives phenotypes with a qualitative effect like no sporulation, necrotic spots or complete lack of symptoms, polygenic resistance is characterised by quantitative differences in the level of resistance (LINDHOUT, 2002). Minor resistance genes don't usually provide the same level of resistance as major genes do, but they are effective against all isolates of the pathogen.

1.2.3. Seedling and Adult Plant Resistances

Breeding efforts for resistance to rusts have identified resistances that are expressed at seedling growth stages (one or two leaf growth stage) and that are effective throughout the life of the plant (seedling resistance), and resistances that are effective at adult plant growth stages only (adult plant resistance, APR) (SINGH *et al.*, 2001a).

Seedling resistance is usually race-specific and can be recognized by its characteristic low infection type at all plant growth stages (ZHANG & KNOTT, 1993; MA & SINGH, 1996b). Genetic studies have indicated that seedling resistances are usually controlled by single genes fitting the gene-for-gene concept and usually result in hypersensitive defense responses (GARROOD, 2001). APR can be either race-specific or race-non-specific (MA and SINGH, 1996b) and is expressed after the seedling stage (3^{rd} to 4^{th} stage; GARROD, 2001). Such resistance is controlled mostly by temperature-sensitive, minor, or additive genes (QAYOUM & LINE, 1985; MILUS & LINE, 1986; SCHULTZ & LINE, 1992), but some single, major genes have also been identified (KERBER & DICK, 1990; ZHANG & KNOTT, 1993; MA & SINGH, 1996a; BÖRNER *et al.*, 2000; SINGH *et al.*, 2001a).

When considering rust infections, adult plant resistance is usually associated with slow rusting and partial resistance. Slow rusting is defined as a reduced rate of development of the pathogen, but resulting in a susceptible (non-hypersensitive) infection type, longer latent periods and reduced rates of spore production (PARLEVLIET, 1988). Cultivars carrying slow rusting resistance show high infection type at seedling growth stage (SINGH *et al.*, 2001b), and the level of protection in adult plants is highly dependent on the number of slow rusting genes carried by the cultivar (SINGH & RAJARAM, 1994; BARIANA *et al.*, 2001; SINGH *et al.*, 2001b). Even though partial resistance and slow rusting resistance are generally considered to be polygenic, major genes have been reported to control these types of resistance (RUBIALES & NIKS, 1995). However, the level of protection seen in a cultivar carrying single genes is usually not sufficient for commercial purposes, confirming that a few genes, with additive effect, would be necessary in order to retard disease progress to a rate that would make the final disease level acceptable (SINGH *et al.*, 2001b). Cultivars carrying the *Lr34* complex (*Lr34* plus three to four additional slow rusting genes) or the *Yr18* complex (*Yr18* plus three to four additional slow rusting genes) show lower brown and yellow rust infection levels, respectively, when compared to cultivars carrying *Lr34* or *Yr18*, alone (SINGH & RAJARAM, 1994; SINGH *et al.*, 2001b). Histological observations demonstrated that slow rusting of brown and yellow rust, due to *Lr34* and *Yr18*, is based on reduced rates of haustorium formation in the early stages of infection and is associated with no or relatively little plant cell necrosis (RUBIALES & NIKS, 1995; SINGH & HUERTA-ESPINO, 1997).

1.2.4. Non-Host Resistance

Resistance shown by all genotypes of a plant species to all pathotypes of a pathogen is known as non-host resistance (NIKS, 1987a), and is the most common form of disease resistance exhibited by plants, given that anyone pathogen is only able to attack a small range of plant species (HEATH, 2000b). Non-host resistance is usually considered to involve complex genetic control and a multiplicity of factors that prevent the microorganism from forming a basic, compatible interaction with the plant (HEATH, 1991). However, non-host resistance of species closely related to the host species seems to involve resistance mechanisms that may be similar to those involved in race-specific, host resistance.

Non-host resistance to obligate parasitic fungi has been described to involve a pre-haustorial reaction (NIKS, 1988), but cases of non-host plants allowing haustorial formation have been reported (HEATH, 1977; ELMHIRST & HEATH, 1987; JOHNSON *et al.*, 1982; NIKS & DEKENS, 1991; JOHNSON & LOVELL, 1994; LEBEDA & REININK, 1994; KAMOUN *et al.*, 1998; VLEESHOUWERS *et al.*, 2000; GARROOD, 2001), all of them resulting in a hypersensitive response. Hypersensitivity is usually associated with resistance gene/avirulence gene specific interactions, following the major, gene-for-gene model of host resistance. Several major genes are known to control resistance in non-host species (TOSA, 1989b; JOHNSON & LOVELL, 1994; WOOD *et al.*, 1994; MATSUMARA & TOSA, 1995; JEUKEN & LINDHOUT, 2002), possibly following a mechanism similar to that of host resistance. JOHNSON & LOVELL (1994) reported two major genes and an undetermined number of minor genes to be responsible for the non-host, hypersensitive reaction of wheat to *P. striiformis* f. sp. h*ordei*. Some authors have suggested that non-host resistance to inappropriate *formae speciales* may rest on a few major resistance genes that follow a gene-for-gene system with avirulence genes in the pathogen, with extremely high allele frequencies of these resistance genes, rather than a complex of genes that play a part in a general defense mechanism (NIKS, 1988; HEATH, 1991; TOSA, 1996). Recent studies on non-host resistance evoke several (and overlapping) layers of specific and non-specific defense responses (KAMOUN *et al.*, 1998; KAMOUN *et al.*, 1999; ARNOLD *et al.*, 2001; PEART *et al.*, 2002). The mode of action of the genes responsible for these reactions is still unknown and there is much interest in finding the factors, both in the plant and in the pathogen, responsible for the non-host relationships.

In cases of non-host interactions involving closely related plant species and *formae speciales* of the same pathogen species, the genetics and biology of resistance often resembles that of race-specific host resistance, usually associated with post-haustorial retardation of the pathogen and death of the invaded cells (NIKS, 1988; HEATH, 2000b). Non-host resistant genes in marigold to the parasite *Stiga asiatica* (L.) Kuntze (GOWDA *et al.*, 1999) and in *Arabidopsis* sp. to the *Brassica oleracea* pathogen *Albugo candida* (Pers.) Kuntze (HOLUB, 2002) were found to belong to the NBS-LRR (nucleotide-binding site-leucine-rich repeats) domains class of resistant genes, common to some genes that control host resistance (see Section 1.3.3). This structural similarity between host and non-host resistance genes could be evidence for identical biology and functionality against non-pathogens of closely related plant species.

In barley, the *Rpg1* gene conferred resistance to wheat attacking isolates of black rust (*P. graminis* f.sp. *tritici*) in North America for more than 60 years (STEFFENSON, 1992). This gene has recently been cloned, and encodes for a receptor kinase-like protein with two tandem kinase domains (BRUEGGEMAN *et al.*, 2002). This represents a novel structure from the host resistance genes currently isolated, showing greatest similarity to the tomato *Pto* gene, and therefore may represent a new class of plant resistance genes. This new class of resistance genes may also represent a more durable form of resistance, although even in this case the resistance was eventually overcome by the pathogen (STEFFENSON, 1992).

Comparative studies among several species belonging to the *Triticeae* have shown a high level of gene homology (i.e, common genetic ground) between resistance genes of related species (VAN DEYNZE *et al.*, 1995b, c; DEVOS & GALE, 1997; KELLER & FEUILLET, 2000; LAURIE & DEVOS, 2002). The isolation and transfer of homologous resistance genes between related plant species, e.g. wheat and barley, could allow the determination of whether the non-host status of individual plant species to *formae speciales* of a pathogen is caused by particular homologues of known host resistance genes (HAMMOND-KOSACK & JONES, 1997).

1.3. BREEDING FOR DISEASE RESISTANCE

1.3.1. Current Status of Yellow Rust Resistance in Wheat and Barley

The types of resistance which are easy to identify, the hypersensitive, monogenic and race-specific resistances, have been the most widely used in cereal breeding programs for rust resistance. The problem with this type of resistance is that major genes are rapidly overcome by the development of virulent races of the pathogen. Obligate biotrophic pathogens with great genetic diversity, high reproduction rates, several generations per growing season, and fast dispersal by air, as is the case with *P. striiformis*, have a very high potential to respond to the new selection pressure caused by introducing new major resistance genes (KELLER *et al.*, 2000). MCINTOSH (1988) considers that «breeding wheat for resistance to rusts is relatively easy; the problems come with the genetic plasticity of the pathogens».

The race structure in yellow rust populations is indeed complex, and constantly shifting, due to a high level of mutation (WELLINGS & MCINTOSH, 1990; STEELE *et al.*, 2001). CHEN & LINE (1992) report that, in 1987, 39 races of *P. striiformis*, based on 13 North American wheat differential cultivars, were identified in that territory. WELLINGS & MCINTOSH (1990) reported an additional 15 new races of *P. striiformis* f. sp. *tritici* in Australasia in the period between 1979 and 1988, all of them with close phenotypic similarity to pre-existing races, but showing virulence to cultivars previously resistant. The population of the barley yellow rust pathogen of the USA, initially thought to constitute exclusively race 24, turned out to be constituted by 14 different races (CHEN *et al.*, 1995).

In wheat, more than 50 yellow rust (*Yr*) resistance genes, showing different types of resistance, have been identified (MCINTOSH *et al.*, 1998) and some of them, mainly the ones showing monogenic host resistance, have already been overcome. WELLINGS & MCINTOSH (1990) reported virulence to genes *Yr1*, *Yr6*, *Yr7* and *YrA*, all of which are present in commercial Australasian wheat cultivars, due to the stepwise mutation to virulence of race 104E137A. Virulence for *Yr17*, a resistance gene introduced into northern European wheat cultivars in the mid 1970s, was detected in 1995 in the UK and Denmark, and later in other countries (BAYLES *et al.*, 2000). Virulences for *Yr27*, *Yr3* and *Yr9*, genes that confer resistance in the most important cultivars in northwestern Pakistan (cultivar 'Inquilab') and India (cultivar 'PBW343'), are known

to occur outside the region, namely Mexico, which makes those cultivars highly vulnerable (SINGH & HUERTA-ESPINO, 2001).

VANDERPLANK (1982) stated that foreign species could be a source of resistance genes with high durability, since pathogens are less likely to be able to overcome them. Some of the *Yr* genes present in commercial wheat cultivars were in fact introduced from foreign species, like *Yr9* from *Secale cereale* L. (MACER, 1975), *Yr15* from *T. dicoccoides* (GERECHTER-AMITAI *et al.*, 1989), *Yr17* from *A. ventricosa* (BARIANA & MCINTOSH, 1994), and *Yr26* introduced from *T. turgidum* (MA *et al.*, 2001). However, *Yr9* and *Yr17* have already been overcome (BAYLES *et al.*, 2000; HOVMØLLER, 2001).

In barley, little information on genetic resistance to yellow rust is available and only a few hypersensitive, major resistance genes (*Yr1*, *Yr2*, *Yr3*, *Yr4* and *Yr5)* have been detected (SANDOVAL-ISLAS *et al.*, 1998; TOOJINDA *et al.*, 1998; HAYES *et al.*, 2001). ROELFS & HUERTA-ESPINO (1994) detected a small number of cultivars showing effective hypersensitive resistance at the seedling stage among the American barley collection. MARSHALL & SUTTON (1995), on the other hand, refer to adult plant resistance present in two Chinese barley cultivars, 'Tambar 500' and 'Kold', and preliminary results obtained by SANDOVAL-ISLAS *et al.* (1998) with ICARDA (International Center for Agricultural Research in the Dry Areas)/CIMMYT (*Centro Internacional para Mejoramiento del Mais y Trigo*, International Maize and Wheat Improvement Center) barley advanced lines indicate that quantitative resistance of oligogenic or polygenic nature may be present in some of the lines, demonstrating that, as in other pathosystems, alternative types of resistance exist in the barley-yellow rust pathosystem. Quantitative resistance present in barley ICARDA/CIMMYT lines is seen to be more likely to be durable than the hypersensitivity resistance detected in seedlings. Varieties with quantitative resistance to yellow rust released in the late 1970s and early 1980s, such as 'IBTA-80' in Bolivia, 'Teran' in Ecuador and 'UNA-80' in Peru, were still resistant in 1998, which demonstrates the durable nature of quantitative resistance (SANDOVAL-ISLAS *et al.*, 1998). Novel, non-host sources of resistance to barley yellow rust, such as that in the wheat variety 'Lemhi', may be of great use in barley as an effective source of resistance against this pathogen.

1.3.2. Breeding for Durability

After the demonstration that single genes could be responsible for disease resistance, several breeding programs were developed with the expectation that the resulting control of plant disease would be permanent. However, the rapid evolution within the pathogen population of matching pathotypes virulent on previously resistant cultivars has forced breeders into a repetitive cycle of cultivar replacement, dependent on the continual introgression of new resistance specificities. Indeed, these 'Boom and Bust' cycles, in which new resistant cultivars become increasingly planted and then rapidly succumb to the pathogen, is a major problem resulting from agricultural practices that rely on monoculture crops and is one of the major worries of plant breeders (BROWN, 1995). As the popularity of the cultivar increases, and it occupies an increasing proportion of the crop area, selection pressure against the matching avirulence allele in the pathogen population also increases. Because new resistance specificities in new cultivars have generally been deployed singly, in theory a single mutation at the corresponding avirulence locus could result in a new virulent pathotype. Thus, for many crop diseases, the efforts of plant breeders simply guide the evolution of virulence in the pathogen.

In the light of these events, durable resistance (a resistance that remains effective in a cultivar during its widespread cultivation and prolonged commercial use; JOHNSON, 1981) has turned into a key consideration in disease resistance breeding. Several strategies aiming at reducing the selection pressure for matching virulence genes have been proposed and intensively studied.

Gene 'pyramiding', i.e., combining several resistances in a single variety, is a technique that aims at reducing the pathogen's effective rate of mutation to virulence (BROWN, 1995; PINK & PUDDEPHAT, 1999). Several gene combinations have demonstrated durability in controlling rusts in wheat (KOLMER *et al.*, 1991; SINGH & RAJARAM, 1994), but it has also been reported that high levels of resistance gene expression result in a reduction in fitness or even lethality in cultivars (STAHL *et al.*, 1999; TAO *et al.*, 2000).

The use of multilines and cultivar mixtures is also a strategy to reduce the selection pressure on the pathogen (BROWN, 1995; PINK & PUDDEPHAT, 1999; JONES, 2001). Some successful examples have been reported (GARRETT & MUNDT, 2000; ZHU *et al.*, 2000), but a problem inherent in the use of cultivar mixtures is that, besides disease resistance, varieties also differ in other agronomic characteristics that reduce profitability of the crop (PINK & PUDDEPHAT, 1999; JONES, 2001).

Another approach is to use resistance genes that do not exhibit gene-for-gene relationships. Partial resistance has sometimes been favoured as a potentially durable source of resistance (QI *et al.*, 1998). Complete resistance can be achieved by the additive effect of many genes, each of a small effect, but together expressing economically effective resistance. The pathogen is therefore at a disadvantage, as it would have to accumulate genes to match each of the resistance genes, rather than just one. The selection pressure would be low for each of the genes of small effect. Adult plant and partial resistances have proven their effectiveness in disease control (MCINTOSH, 1992; SINGH, 1992; MA & SINGH, 1996a, b; QI *et al.*, 1998).

Non-host resistance is possibly another effective way of achieving durable resistance (CRUTE & PINK, 1996; HEATH, 2000b) and some of these non-host interactions have already been identified and used in breeding programs, as is the case of resistance in barley to wheat black rust based on the gene *Rpg1*, which remained effective for over 40 years (BROWN, 1995). HEATH (2001) considers that detailed information on non-host resistance mechanisms is required in order to assess the durability of this type of resistance. The strategies for exploiting non-host resistance will strongly depend on whether the resistance is controlled by parasite-specific or parasite non-specific defense mechanisms (HEATH, 2001). The fact that some of the non-host interactions follow the major-gene model can make it easier for plant breeders to transfer resistance genes from a non-host species to the host species of interest (NIKS, 1988). Studies on non-host resistance imply that either interspecific crosses are necessary, or a susceptible variety has to exist in the non-host species (occasional or inappropriate host) in order to allow crosses between normal resistant and rare susceptible genotypes (NIKS, 1988). The problem rests on the absence of variation in plant resistance and in the sexual incompatibility between host and non-host plant species (KAMOUN, 2001).

There is no such thing as a formula to obtain durable resistance, as present evidence suggests that different types of resistance may all be durable. It is therefore important that we learn more about composition, genomic organization, allelic diversity and biochemical function of resistance genes and their role in disease resistance pathways, in order for us to define the best breeding program to follow in each particular case.

1.3.3. Plant Resistance Genes – The Molecular Evidence

One approach towards understanding the molecular mechanisms underlying resistance in plants to pathogens is the identification, isolation and functional analysis of genes that are involved in this resistance. A number of genes conferring race-specific resistance to a diversity of pathogens have been isolated from several different plant species (e.g. JOHAL & BRIGGS, 1992; MARTIN *et al.*, 1993; BENT *et al.*, 1994; PARNISKE *et al.*, 1997). In spite of the great diversity in pathogenic mechanisms of disease-causing organisms, resistance genes were found to encode proteins with certain common motifs. Five classes of resistance proteins encoded by these genes are now recognised (ELLIS *et al.*, 2000). The largest group of resistance genes carry leucine-rich repeats (LRRs) and nucleotide-binding site (NBSs) domains and are very abundant in plant genomes (MICHELMORE, 2000). The LRR domains are believed to mediate protein-protein interactions or to be involved in the recognition of specific pathogen elicitors and activation of protein kinase, phosphorylation reactions that initiate a signal cascade, resulting in the activation of defense response genes (DANGL, 1995; BENT, 1996). These highly conserved motifs in resistance proteins suggest that plants have evolved similar mechanisms to combat different pathogens and that plants use only a limited number of recognition/signal transduction systems to combat microbial attack (STASKAWICZ *et al.*, 1995; BAKER *et al.*, 1997; HAMMOND-KOSACK & JONES, 1997; ELLIS *et al.*, 2000; RICHTER & RONALD, 2000; YOUNG, 2000; HULBERT *et al.*, 2001).

Plant resistance genes are members of substantial, linked, multigene families, that are commonly clustered in the genome and are well conserved between plant families (CRUTE & PINK, 1995; HAMMOND-KOSACK & JONES, 1997; HULBERT *et al.*, 2001). Evolutionary and comparative studies between grasses (CHAO *et al.*, 1989; MOORE *et al.*, 1995; PATERSON *et al.*, 1995; VAN DEYNZE *et al.*, 1995b, c; DEVOS & GALE, 1997; KELLER & FEUILLET, 2000; LAURIE & DEVOS, 2002) reveal a common ancestor and a remarkable genetic proximity among *Triticeae* members. Several genes for important agronomic traits, including vernalization, flowering time and plant height (reviewed in DEVOS & GALE, 1997) have been reported to be common between cereals. When considering disease resistance genes, comparative analysis might be more complicated, since these genes evolve more rapidly than the rest of the genome (KELLER & FEUILLET, 2000; LAURIE & DEVOS, 2002). However, even though orthology and colinearity might not be as well conserved for resistance genes as for other functional genes, resistance genes against rusts and powdery mildew have been reported to be very well conserved on homoeologous group 1

chromosomes of oat, wheat and barley (VAN DEYNZE *et al.*, 1995b). Thus, resistance genes in different species seem to be closely related and possibly originate from the same ancestral gene (KELLER *et al.*, 2000).

In fact, many major genes that confer race-specific resistance in crop cereals have been successfully transferred from wild grasses or other cereal crop species. Major resistance genes to yellow rust have been transferred to wheat from rye (MACER, 1975), *T. dicoccoides* (GERECHTER-AMITAI *et al.*, 1989), *A. ventricosa* (BARIANA & MCINTOSH, 1994) and *T. turgidum* (MA *et al.*, 2001). HEATH (1991) suggests that the successful transference of specific major resistance genes from host to non-host species is limited to closely related species. The limits to the function of resistance genes moved between taxa could be related to the specificity of signal transduction pathways in distantly related plant species (CRUTE, 1998).

Gene clusters are thought to result from the occurrence of duplication and recombination events in resistance genes (RICHTER & RONALD, 2000). In the presence of distinct pathogens, and in an attempt to maintain a balance between creating new specificities and conserving old ones, some of these genes probably evolve into related but distinct resistance functions, leading to the generation of alternative recognition capabilities of the encoded proteins (MARTIN *et al.*, 1993; YOUNG, 2000; LAURIE & DEVOS, 2002). These complex loci may provide several selective advantages, as such arrangements allow multiple specificities to be assembled and retained in a single haplotype (i.e. set of genes in a complex locus), thus preserving the potential for variation and the evolution of novel specificities (CRUTE & PINK, 1996). BOUKHATEM *et al.* (2002) found genes for resistance to yellow rust to cluster in a region of wheat chromosome 2B where other resistance genes to yellow, brown and black rusts were already reported. ASHFIELD *et al.* (1998) demonstrated that *Rpg1*, a gene for soybean resistance to *Pseudomonas syringae*, maps to a cluster of previously identified resistance genes, including those effective against fungal, viral and nematode pathogens.

Clustering and rapid evolution of resistance genes suggests that a gene conferring resistance to one pathogen could evolve to recognize a different pathogen (RICHTER & RONALD, 2000). Further cloning and sequencing of resistance genes conferring resistance to different pathogens is needed in order to clearly demonstrate this evolutionary process.

1.4. GENETIC ANALYSIS AS A STRATEGY TO BREED FOR DISEASE RESISTANCE

The most economic and environmentally friendly strategy for disease control is genetic resistance. Plant breeding programs usually create new cultivars with resistance to diseases through the use of major genes, either singly or pyramided. These programs are based on crosses, backcrosses and selection, which are time-consuming processes and inadequate for coping with the rapid evolution of the pathogen populations. Furthermore, the lack of durable sources of resistance and the difficulties involved in resistance gene introgression from exotic species make classical breeding for resistance a difficult task.

In the last two decades, the progress in DNA molecular technology has resulted in a review of genetic analysis approaches and breeding strategies. The rapid generation of molecular markers has allowed approaches such as gene discovery and cloning, genetic engineering and marker-assisted selection techniques, among others (reviewed in DE LA VEGA, 1997; KOEBNER *et al.*, 2001).

The association of genes of interest with specific molecular markers makes marker-assisted selection a feasible technique in breeding for disease resistance. It eliminates the need for disease screening tests and helps reduce linkage drag (MICHELMORE, 1995), as well as allows the indirect selection for resistance in early segregating generations at the seedling stage, which saves time and resources in breeding programs. Furthermore, genes become easy targets, and map-based cloning, a technique of extreme importance in studies of gene function, is facilitated (MOHAN *et al.*, 1997). In a short review on the current status of QTL analysis in plants, KEARSEY & FARQUHAR (1998) conclude that the present QTL analysis technology is powerful enough for marker-assisted selection. QTLs with strong effects on the phenotype can be identified with enough accuracy for introgression by backcrossing. In fact, TOOJINDA *et al.* (1998) report the successful transfer of yellow rust resistance QTLs between barley accessions by applying marker-assisted selection techniques and the consequent development of barley germplasm for potential commercial production. For map-based cloning, greater mapping precision is still required (KEARSEY & FARQUHAR, 1998). Up to now, only five QTLs have been cloned based on map-based cloning techniques, two in tomato (FRARY *et al.*, 2000; FRIDMAN *et al.*, 2000), two in rice (YANO *et al.*, 2000; TAKAHASHI *et al.*, 2001) and one in *Arabidopsis* (JOHANSON *et al.*, 2000). None of them reports to disease resistance.

1.4.1. QTL Mapping

While some forms of disease resistance are genetically simple and phenotypically easy to identify, a large number of agronomic characters, including disease resistance, show a continuous range of values, indicating that they are controlled by several genes, each having a different effect on the phenotype. These genetic loci are named Quantitative Trait Loci (QTL) and, when considering quantitative resistance characters in particular, they are also termed Quantitative Resistance Loci (QRL; YOUNG, 1996). The genetics of quantitative resistance is hard to study, as it is influenced by the environment and the environment x genotype interaction, and each gene usually has a small effect on the phenotype. Since the individual effect of each locus on the trait is hard to observe, these traits cannot be studied in the same classical Mendelian way as single gene characters (HACKETT, 2002).

Until the late 1980s, quantitative traits where studied based on statistical analysis of the means, variance and covariance of relatives. This gave an indication of the number, but not the location, of the genes underlying them (KEARSEY & FARQUHAR, 1998). Since then, the development of molecular markers and genetic mapping techniques has had a large impact on quantitative genetics, as they allow the resolution of polygenic quantitative traits into discrete Mendelian factors (QI *et al.*, 1998). QTL analysis, i.e., the genetic dissection of a quantitative trait, aims at determining the number of loci involved, as well as the location and the contribution to the phenotype of each locus, by associating the variation in marker alleles segregating in a population with variation in the quantitative character. This is a highly effective tool for studying and manipulating genetically complex disease resistance (MOHAN *et al.*, 1997). Two steps are essential in QTL analysis: 1) the mapping of the markers; and 2) the association of those markers with the trait (KEARSEY & FAQUHAR, 1998).

1.4.1.1. *Molecular marker systems*

The development of molecular techniques that allows the detection of polymorphism at the DNA level has greatly improved mapping strategies. When compared to morphological and enzymatic markers, the type of markers classically used in mapping procedures, DNA molecular markers are much more numerous, independent of the environmental conditions, do not disturb the physiology of the organism, are morphologically neutral and detectable in all stages of plant development (JONES *et al.*, 1997b; MOHAN *et al.*, 1997). For mapping purposes, markers must

have some basic characteristics. They should be: 1) highly polymorphic, so that individuals are likely to carry different alleles at each locus; 2) abundant, so that good genome coverage is obtainable; 3) co-dominant, so all possible genotypes at a marker locus can be identified; 4) highly reproducible across laboratories; 5) economical and efficient, for large populations screening; and 6) user-friendly (FALCONER & MACKAY, 1996; MOHAN *et al.*, 1997).

From the numerous molecular marker techniques now available (KOEBNER *et al.*, 2001), RFLPs (Restriction Fragment Length Polymorphisms; BOTSTEIN *et al.*, 1980), RAPDs (Random Amplification of Polymorphic DNA; WILLIAMS *et al.*, 1990), Microsatellites or SSRs (Simple Sequence Repeats; TAUTZ, 1989) and AFLPs (Amplified Fragment Length Polymorphisms; VOS *et al.*, 1995) have been the ones most extensively used in genome mapping and gene tagging. Table 1.1 assembles some of the characteristics inherent to these marker systems (RAFALSKI & TINGEY, 1993; POWELL *et al.*, 1996b; JONES *et al.*, 1997a; MOHAN *et al.*, 1997).

RFLPs were the first molecular markers to be developed and used in genetic analysis, initially in humans (BOTSTEIN *et al.*, 1980), and later applied to plants (WEBER & HELENTJARIS, 1989). Even though these markers were extensively used for mapping approaches in various plant species, they didn't fulfil the initial expectations as universal genotyping assays, since they require large amounts of DNA, are expensive and time consuming. DEVOS & GALE (1993b) consider the RFLP technology too slow and too expensive to be used for routine screening of the mapping populations. Furthermore, the clustering of RFLP markers at certain chromosomal regions, mainly in the centromeric region, has been reported (DEVOS *et al.*, 1992; DEVOS *et al.*, 1993; MESSMER *et al.*, 1999), which reduces their potential for good genome coverage.

The advent of the polymerase chain reaction (PCR; MULLIS *et al.*, 1986) led to the development of a new class of molecular markers that includes RAPDs, SSRs and AFLPs. RAPD technology became popular because of its simplicity and ease of use, and because it provides a quick and efficient screen for DNA polymorphisms at a very large number of loci (RAFALSKI & TANGEY, 1993). However, this technique is highly dependent on laboratory conditions, and lack of reproducibility turned out to be a major impediment for its application in joint research projects (JONES *et al.*, 1997a).

Table 1.1 - Properties of four different systems for generating DNA molecular markers (compiled from: RAFALSKI & TINGEY, 1993; POWELL *et al.*, 1996b; JONES *et al.*, 1997a; MOHAN *et al.*, 1997).

MARKER	PRINCIPLE	ADVANTAGES	DISADVANTAGES
RFLP	Endonuclease restriction and Southern blotting. Polymorphism in the restriction fragment size detected by hybridisation with probe	Co-dominant Reproducible Tolerant to DNA contamination	Low EMR[a] Labour intensive and time consuming Difficult automation Large amount of DNA required (2-10 µg) Detection usually dependent on radioisotopes Low level of polymorphism in some species
RAPD	Genomic DNA template is primed by an arbitrary oligonucleotide primer	Simple and easy to use Low development costs No radioactivity required Small amount of DNA required (10-25 ng) High abundance High EMR Primers available commercially	Dominant Poorly reproducible Highly dependent on laboratory conditions Susceptible to contamination
AFLP	PCR amplification of restriction fragments generated by specific restriction enzymes and adapters of few nucleotide bases.	High EMR Very low amount of DNA required Reproducible No sequence information required Tolerant to DNA contamination	Expensive to generate Dominant Low polymorphism
SSR	PCR amplification of repeat units with different number of repeats, using primers flanking each sequence.	Co-dominant Highly reproducible Small amount of DNA required (30-100 ng) Highly polymorphic Genome- and chromosome-specific	Sequence information required High development costs Low EMR

[a] EMR: effective multiplex ratio, measures the number of fragments or loci generated per experiment.

In the last decade, SSR technology has emerged as the system of choice in plant molecular mapping. Plant genomes contain large numbers of SSRs, with tandemly repeated basic motifs of <6bp (MORGANTE & OLIVIERI, 1993). Polymorphisms in the number of repeats are relatively abundant between plants, highly informative, stably inherited, co-dominant, have good genome coverage and have potential for automation (MORGANTE & OLIVIERI, 1993; RÖDER *et al.*, 1995; POWELL *et al.*, 1996a; KORZUN *et al.*, 1997). They are, contrary to RAPDs, highly reproducible and the detection of polymorphic loci is rapid and accurate (MOHAN *et al.*, 1997). Furthermore, since polymorphism is detected by PCR, a large number of plants can rapidly be analysed at an early stage of development (KORZUN *et al.*, 1997), an advantage over RFLP technology. SSRs have hence been preferred over RFLPs and RAPDs, and their utility for molecular mapping has been largely demonstrated (see GUPTA & VARSHNEY, 2000 for a vast list of examples).

One major problem with SSRs is its high development cost, which has made it difficult to implement the technology in a wider way (BRYAN *et al.*, 1997). The detection and development of locus-specific SSRs require the construction and screening of a DNA library with specific probes, followed by sequencing of positive clones and subsequent PCR primer synthesis and testing (MA *et al.*, 1996; PLASCHKE *et al.*, 1996; BRYAN *et al.*, 1997; RÖDER *et al.*, 1998b). This technique is expensive and time consuming, and it has a low recovery rate of useful SSRs, so new methods for SSR generation are being developed in order to reduce the costs inherent to this technology (CONNELL *et al.*, 1998; HAYDEN & SHARP, 2001). Recently, a new source of large numbers of SSRs, the expressed sequence tags (ESTs), has become available for a number of species, at low cost (SCOTT, 2001). These markers derive from expressed gene sequences, which provide perfect markers for those genes (HOLTON, 2001).

Another drawback of SSRs is the amount of information that can be extracted from each experiment, since each primer pair usually only detects one locus (low effective multiplex ratio, EMR), and the preparation, running and scoring of the gel is by far the most time consuming part of the process (DONINI *et al.*, 1998). Multiplexing either the PCR reaction or the sample loading can be effective in augmenting the EMR, but that is only possible when the fragment sizes differ considerably and where non-specific amplification does not mask the true sign (DONINI *et al.*, 1998).

AFLP technology has overcome the problems of poor reproducibility of amplified bands associated with RAPDs (JONES *et al.*, 1997a) and, even though its level of polymorphism is lower

than that of RFLPs and SSRs, AFLP technology discloses a huge number of bands and polymorphisms per experiment, which elevates its EMR to levels higher than those exhibited by any other DNA marker detection system (POWELL *et al.*, 1996b). No prior sequence knowledge is required and the technique is relatively inexpensive when compared to other systems (JONES *et al.*, 1997a). The high reproducibility, rapid generation and high frequency of identifiable AFLP polymorphisms make this an attractive technique for identifying polymorphisms and for determining linkages (MOHAN *et al.*, 1997). However, the dominant way of inheritance results in some lack of informativeness, clustering, linkage gaps and failure in detecting linkage in repulsion phase (SAAL & WRICKE, 2002). VUYLSTEKE *et al.* (1999) propose AFLP bands in F_2 populations to be scored as co-dominant markers on the basis of signal intensity, but SAAL & WRICKE (2002) consider this procedure to be difficult to apply in routine usage, as amplification conditions may influence signal intensity. Automated scanning systems for AFLP co-dominant screening have been developed (BUNTJER, 2000), which avoid the inaccuracies introduced by eye observation and increase screening throughput.

1.4.1.2. *Molecular mapping*

JONES *et al.* (1997b) define mapping as «putting markers in order, indicating the relative genetic distances between them, and assigning them to their linkage groups on the basis of the recombination value». When constructing molecular marker maps, crosses are made between parent lines that differ in the character of interest to give heterozygous F_1 progeny, and this progeny is used to produce a segregating population, like F_2s, RILs (recombinant inbred lines), DHs (double haploids) or BC (backcross) populations.

If two markers are linked, they do not segregate independently and they will map together in the same linkage group, where each linkage group should correspond to a chromosome or chromosome region. The recombination value or recombination frequency between marker pairs is calculated from the mean number of recombination events that originate between them and is then converted into map distances (expressed in centimorgans, cM), using Haldane's or Kosambi's genetic mapping functions (HALDANE, 1919; KOSAMBI, 1943), on the assumption that the probability of recombination is proportional to the distance between the loci.

Various computer statistical software packages for map construction have been developed and improved over the last ten years in an attempt to enhance the accuracy and speed of analysis of

the large amounts of data (i.e., the large number of molecular markers) that is required for the construction of a genetic map. MapMaker (LANDER *et al.*, 1987) and JoinMap (STAM, 1993) are such examples. JoinMap 3.0 (VAN OOIJEN & VOORRIPS, 2001) was the statistical package used in the present study. Loci are placed together in linkage groups based upon a test of independence of segregation in a contingency table, which is translated into a Log-likelihood (LOD) score. The higher the LOD value for a population, the more confident the map. JoinMap then estimates the pairwise recombination frequencies and LOD scores from the original data in the locus genotype file for pairs of markers in each linkage group. Recombination estimates are obtained by maximum likelihood. A maximum recombination frequency (REC) threshold has to be defined to give results at the desired significance level. Furthermore, it allows individual genotype frequencies to be calculated, which reveals the genotype frequencies for each individual and detects individuals that have many missing data. JoinMap 3.0 also allows the identification of similar loci and similar individuals. Excluding one of the identical loci results in faster calculations, while one can be certain that identical loci map in the same position. For individuals, this command is intended to reveal identical individuals, which should be rare and thus indicate possible errors (VAN OOIJEN & VORRRIPS, 2001).

The mapping procedure is a sequential process of building a map by adding loci one by one, starting with closely linked loci. After choosing the first pair of loci and calculating their map distance (two mapping functions are available in JoinMap 3.0, Kosambi's and Haldane's), JoinMap determines which locus is to be added next and, by comparing the goodness of fit (expressed as χ^2 value) of the resulting map for each tested position, finds the best fitting position of the locus on the map. Since the addition of a new locus may influence the optimal map order, a 'ripple' is performed after each locus is added. If the difference in goodness of fit, before and after adding a new locus (the 'jump'), is high, i.e., when the jump value exceeds the imposed threshold, the locus is removed. After the first round of mapping, loci that were put aside in this round may fit well after a number of other loci have been placed on the map, JoinMap therefore makes two more attempts to place the loci previously excluded.

1.4.1.3. *Wheat linkage maps*

A good coverage of the wheat genome is harder to achieve than for other crops, given its low degree of DNA polymorphism (LIU & TSUNEWAKI, 1991), resulting from its relatively recent origin (DEVOS & GALE, 1993b), and because of its hexaploid structure and huge genome size (1.7 x 10^{10} bp; LAURIE & DEVOS, 2002).

RFLP genetic maps of bread wheat have been developed (CHAO *et al.*, 1989; LIU & TSUNEWAKI, 1991; ANDERSON *et al.*, 1992; DEVOS *et al.*, 1992; DEVOS & GALE, 1993a; DEVOS *et al.*, 1993; XIE *et al.*, 1993; NELSON *et al.*, 1995a, b; VAN DEYNZE *et al.*, 1995a; MARINO *et al.*, 1996; MESSMER *et al.*, 1999). However, the low level of polymorphic RFLP loci in wheat (<10%, RÖDER *et al.*, 1998b) has limited the use of RFLP maps in gene identification and marker-assisted selection in this crop.

The need for a more polymorphic genetic marker system in wheat has led to the application of SSR technology. Their robustness and informativeness have positioned them as the markers of choice for genetic mapping in wheat (RÖDER *et al.*, 1995). In fact, numerous SSR loci are being developed and used either to create new linkage maps or to incorporate into previously established ones (DEVOS *et al.*, 1995; PLASCHKE *et al.*, 1995; RÖDER *et al.*, 1995, 1998a, b; BRYAN *et al.*, 1997; STEPHENSON *et al.*, 1998; PESTSOVA *et al.*, 2000; VARSHNEY *et al.*, 2000; SOURDILLE *et al.*, 2001; GUPTA *et al.*, 2002).

While the creation of high resolution wheat linkage maps based exclusively on SSRs is still ongoing, these markers provide good reference ('anchor') points for specific regions of the genome when integrated into maps constructed with less informative markers, like AFLPs (POWELL *et al.*, 1996a; BUERSTMAYR *et al.*, 2002). The AFLP technology, with its capacity for rapid generation of a large number of markers and wide genome coverage, can create dense maps with reduced effort and fill the gaps left by SSRs (SAAL & WRICKE, 2002). Various integrated maps of AFLP and SSR markers have been successfully used in resistance gene and resistance QTL mapping in wheat (e.g. ANDERSON *et al.*, 2001; WENG & LAZAR, 2002; ZHOU *et al.*, 2002).

1.4.1.4. *Techniques of QTL analysis*

QTLs are mapped by estimation of the chance that a QTL is present at a certain position on the genome (in association with a marker) over the chance that a QTL is absent (LANDER & BOTSTEIN, 1989). The precision of the mapping is dependent on the marker density of the genetic map, on the accuracy of the phenotypic analysis of the population for the trait in question, on the size of the population under analysis and on the explained variation of the QTL (VAN OOIJEN, 1992).

Classical approaches for QTL mapping, namely Single Marker Analysis, involve the estimation of linkage between a QTL and a single marker (LUO & KEARSEY, 1989). This method has a few drawbacks, as the underestimation of the phenotypic effects of the QTL, the incapacity of distinguishing between linkage distance and small phenotypic effect and the necessity for large segregating populations (LANDER & BOTSTEIN, 1989; MARTÍNEZ & CURNOW, 1992). These problems have been overcome by the development of Interval Mapping, either by regression using least-squares estimates (KEARSEY & HYNE, 1994; http://web.bham.ac.uk/g.g.seaton/) or by maximum likelihood estimation (LANDER & BOTSTEIN, 1989), estimation methods based on the location of a given QTL between two flanking markers. Marker Regression fits a model to all the marker means on a given chromosome simultaneously, and obtains significance tests by weighted least-squares or by simulation (KEARSEY & HYNE, 1994; http://web.bham.ac.uk/g.g.seaton/). In the maximum likelihood estimation method (LANDER & BOTSTEIN, 1989), intervals between two adjacent markers are scanned for the presence of a segregating QTL at any point in that interval. The logarithm of the ratio of the likelihoods (LOD) of there being one versus no QTL at that particular point is calculated. When this LOD value is higher than a given pre-determined threshold, a QTL is considered to be present at that location. The subject of the significance thresholds above which a QTL can be inferred has been widely discussed (REBAÏ *et al.*, 1994; DOERGE & REBAÏ, 1996; VAN OOIJEN, 1999; PIEPHO, 2001).

The interval mapping method has a major problem: the mapping of a given QTL can be seriously biased by the presence of multiple QTLs located on the same chromosome (ZENG, 1993, 1994) or located elsewhere in the genome (JANSEN, 1993). ZENG (1994) suggests an alternative method, Composite Interval Mapping (CIM), in which the test statistic on a marker interval is made to be unaffected by QTLs located outside the defined interval. JANSEN (1993, 1994) and JANSEN & STAM (1994) suggest a similar method, Multiple QTL Model (MQM) Mapping, which allows the

detection of QTLs of small effect that are not detected by interval mapping given that they are masked by QTLs of major effect. In this method, genetic background 'noise' is removed by using markers as co-factors. In the case where a QTL explains a large proportion of the total variance, the use of markers linked to that QTL as co-factors in subsequent MQM mapping enhances the power in the search for other segregating QTLs (JANSEN, 1993). JANSEN (1994) demonstrates the power of MQM mapping in controlling the type I (detection of non-existing QTLs, false-positives) and type II (non-detection of existing QTLs, false-negatives) errors. These errors are frequently reported to occur with interval mapping analysis (HALEY & KNOTT, 1992; MARTINEZ & CURNOW, 1992; JANSEN, 1993). JANSEN *et al.* (1995) report the usefulness of this technique in dissecting the genotype-by-environment interaction in the mapping of multiple QTLs.

More recently, a method termed Multiple Interval Mapping (MIM) has been proposed (KAO et *al.*, 1999), where several QTLs involved in complex models of gene action, including epistasis, can be analysed simultaneously.

Numerous software packages making use of these and other mapping strategies are currently available, e.g. MapQTL (VAN OOIJEN *et al.*, 2002), QTL Cartographer (BASTEN *et al.*, s/d), QTL Café (http://web.bham.ac.uk/g.g.seaton), among others.

1.4.2. Mapping of Yellow Rust Resistance Genes

The location of some major genes and quantitative resistance loci for yellow rust resistance in wheat has already been identified by molecular mapping (Table 1.2). SINGH *et al.* (2001b) report slow rusting QTLs that confer resistance to both brown (leaf) and yellow rusts. To the moment, no genes for non-host resistance to yellow rust have been mapped.

Table 1.2 - List of yellow rust resistance genes and quantitative resistance loci located by DNA molecular mapping.

GENE	CHROMOSOME	MOLECULAR MARKERS	REFERENCE
Yr5	2BL	RAPD SSR AFLP/SSR RGAP[a]	ZHONG *et al.* (2002) SUN *et al.* (2002) P. SMITH (pers. commun.) YAN *et al.* (2003)
Yr7	2BL	SSR /AFLP	BARIANA *et al.* (2001)
Yr10	1BS	STS[b] SSR	BARIANA *et al.* (2002) SMITH *et al.* (2002) WANG *et al.* (2002)
Yr15	1BL	RAPD / RFLP RAPD / SSR AFLP / RAPD / SSR	SUN *et al.* (1997) CHAGUE *et al.* (1999) PENG *et al.* (2000a)
Yr17	2A	RAPD / RFLP	ROBERT *et al.* (1999)
Yr26	1BS	SSR	MA *et al.* (2001)
Yr28	4DS	RFLP	SINGH *et al.* (2000)
Yr29	1BL	AFLP	WILLIAM *et al.* (2003)
YrH52	1BS	SSR / RFLP AFLP / RAPD / SSR SSR	PENG *et al.* (1999) PENG *et al.* (2000a) PENG *et al.* (2000b)
Yrns-B1	3BS	SSR	BÖRNER *et al.* (2000)
QTLs	1BL, 3BS, 4B, 6A, and 6B	various	SINGH *et al.* (2001b)
QTLs	1BL, 3BS, 7B or 7D, and 7DS	various	SINGH *et al.* (2001b)
QTLs	2BS, 4AS, 6BS, and 7AL	various	BÖRNER *et al.* (2002)
QTLs	2B, 3D, 5A, 6D, and 7D	RFLP / SSR	BOUKHATEM *et al.* (2002)
QTLs	2A and 2B	RFLP / SSR	BOUKHATEM *et al.* (2002)
QTLs	2B, 4A, and 7D	AFLP/SSR	R. PRINS (pers. commun)

[a] Resistance Gene-Analog Polymorphism
[b] Sequence Tagged Sites

2. MATERIALS AND METHODS

2.1. PLANT AND PATHOGEN MATERIAL

An F_2 population of 118 individuals obtained from reciprocal crosses between cultivars 'Lemhi' and 'Chinese 166' of bread wheat was used for this study. The pathogen used to inoculate the population was the isolate BWR 80-1 of the barley-attacking form of yellow rust, *P. striiformis* f.sp. *hordei*. 'Lemhi' and 'Chinese 166' plants used as progenitors to this population had first been tested for resistance/susceptibility to isolate BWR 80-1 of *P. striiformis* f.sp. *hordei*.

2.2. PLANT INOCULATION AND DISEASE PHENOTYPING

Plant inoculation followed the procedure described by SHEN (2000).

Reactions to infection (infection types, IT) were previously scored using a scale ; to 4 as described in Table 2.1. 'Berac' was used as the barley susceptible control (IT 4) and 'Lemhi' and 'Chinese 166' as, respectively, resistant (IT ;) and susceptible (IT 1^{cn}/3) wheat controls (Figure 2.1).

Table 2.1 - Infection type (IT) scale used for scoring the disease reactions 14-16 days after inoculation.

IT	Disease Reaction
0	no visible symptoms
;	small necrotic flecks
$n^{;}$	necrotic regions greater than 1mm in diameter
0^{n}	necrotic regions greater than 2mm in diameter
0^{nn}	spreading necrotic regions greater than 4mm in diameter
1	small, sporulating uredia surrounded by necrotic and/or chlorotic tissue
2	moderately sized, sporulating uredia surrounded by necrotic and/or chlorotic tissue
3	moderately sized, sporulating uredia surrounded only by chlorotic tissue
4	large, sporulating uredia surrounded by green tissue
n	necrotic tissue
c	chlorotic tissue

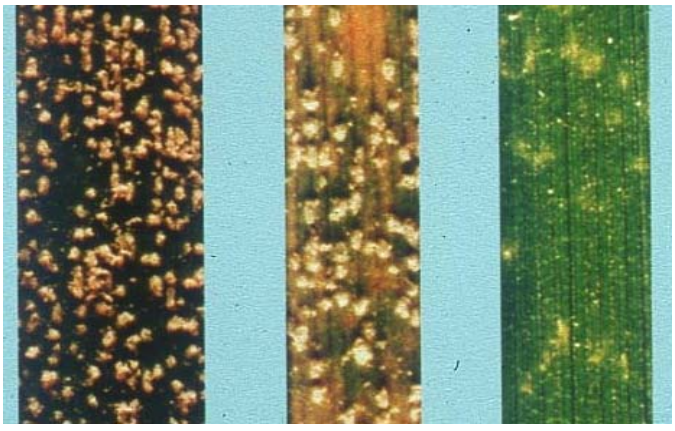

Figure 2.1 - Barley yellow rust infection. 1) Barley cultivar 'Berac' (susceptible control); 2) Wheat cultivar 'Chinese 166' (susceptible parent); and 3) Wheat cultivar 'Lemhi' (resistant parent) (Courtesy of L.A. BOYD).

Infection types were directly converted into a numerical scale ranging from 1 (resistant) to 6 (susceptible) as described in Table 2.2. Later, the F_3 population that resulted from selfing of the F_2 plants was also tested for resistance to BWR 80-1. The IT of the F_3 families allowed the IT of the F_2 plants to be converted into a 1 (susceptible) to 9 (resistant) scale based on segregation patterns of the F_3 families (Table 2.2). For example, if an F_3 family resulting from the selfing of an individual with a score of 2 in the 1-6 scale did not segregate for the characteristic (i.e., all the individuals showed the same level of resistance as the F_2 individual), a score of 8 in the 1-9 scale was attributed to that F_2 individual. If, on the other hand, the selfing of that F_2 individual resulted in an F_3 family segregating for IT 0^{nn} (less resistant) and IT 4 (susceptible), a score of 7 was attributed to the F_2 individual, since the occurrence of F_3 individuals showing those ITs meant a less resistant phenotype in the F_2 individual not easily observed by eye in the F_2 population. The same procedure was followed for the F_2 individuals with scores 3 and 4 in the 1-6 scale.

Table 2.2 - Numerical disease ratings assigned to each barley yellow rust disease phenotype.

Infection Type (IT)	**Disease score based on F_2 (scale 1-6)**	**Phenotypic segregation in F_3**	**Disease score based on F_2 and F_3 (scale 1-9)**
;	1	No sporulation	9
n;	2	Not segregating for IT 4 and IT 0^{nn}	8
		Segregating for IT 4 and IT 0^{nn}	7
0^{n}	3	Not segregating for IT 4 and IT 0^{nn}	6
		Segregating for IT 4 and IT 0^{nn}	5
0^{nn}	4	Not segregating for IT 4 and IT 0^{nn}	4
		Segregating for IT 4 and IT 0^{nn}	3
3-4	6	Susceptible	1

2.3. DNA EXTRACTION

Genomic DNA from the F_2 population used in this study was kindly provided by Dr. L.A. Boyd, John Innes Centre, Norwich UK. The DNA extraction procedure is described by SHEN (2000). From the 118 individuals used for phenotypic analysis, 4 DNA samples were lost, leaving 114 individuals for posterior DNA analysis. In order to confirm the quality and concentration of the DNA previously determined by spectrophotometry, a 1% agarose gel was run for the samples, and stained with ethidium bromide.

2.4. MOLECULAR MARKERS

2.4.1. AFLPs

The AFLP technique was developed by SHEN (2000, after VOS *et al*., 1995), which tested 23 primer combinations (Appendix I). From these primer combinations, 172 bands ranging in size from approximately 60 to 450 base pairs, and showing a distinct and scorable polymorphism in the population, were scored for the population.

2.4.2. SSRs

Eighty-eight SSR primer pairs covering the entire wheat genome were screened for polymorphism between 'Lemhi' and 'Chinese 166' and those showing scorable polymorphisms were screened in the population (Appendix I). Any markers giving a poor PCR product or a dubious scoring in the population were excluded. Several sources of SSR primers were used. Primers designated *Xgwm* originated from the Institute of Plant Genetics and Crop Research, Germany; primers designated *Xpsp* originated from John Innes Centre, UK; and the ones designated *Xbarc* originated from the US Wheat and Barley Scab Initiative, USA.

PCR reaction

Genomic DNA used as template for SSR PCR was diluted to a concentration of approximately 100 ng/μL. PCR was carried out in a ThermFast® 96 wells plate (Advanced Biotechnologies) and sealed with Microseal™ film. PCR mix contained 1 μL of genomic DNA, 2.0 μL 10x PCR Buffer (Roche), 0.1 μL Taq Polymerase (5U/μL, Roche), 1.04 μL dNTPs (2.5 mM each) and 2.0 μL of primer pairs (2 μM each), giving a total reaction volume of 20 μL with ultrapure water. A drop of paraffin oil was placed on the top of each PCR reaction mix, in order to avoid evaporation. Different PCR programmes (Appendix II) were used depending on the primer pair.

Acrylamide gel preparation, denaturation of samples and electrophoresis

The big and small plates used for vertical electrophoresis were cleaned with warm water and Alconox (Aldrich), rinsed with ddH_2O and dried with 100% ethanol. Repelcote V.S. (Pharmacia Biotech) was applied to the big plate and Bind Silane (Pharmacia Biotech) (30 μL) to the small

plate. Plates were allowed to dry for a few minutes. The big plate was then wiped with 100% ethanol and the small plate rinsed with ddH2O and dried with 100% ethanol, to remove excessive Repelcote and Silane, respectively. Two spacers (0.4 mm, Sigma) were aligned and sandwiched between the plates, to keep them separated.

Polyacrylamide (6%) denaturing gels were prepared by adding 30 µL of TEMED (BDH) and 300 µL of 10% ammonium persulphate (BDH) to 60 mL of gel mix (150 mL Acrylamide/bis solution 19:1 40% (Sigma), 100 mL 10x TBE, 480 g Urea, made up to 1 L with distilled water, filtered and kept at 4 ºC). Gels were immediately poured and a dry, clean comb (0.4 mm thick, Sigma) was inserted into the top of the gel, straight edge down. Gels were left to polymerise for at least one hour. After polymerisation, the comb was taken out and the well cleaned with distilled water. The comb was carefully replaced, this time with the teeth inserted a few millimetres into the gel. Gels were pre-run in 1x TBE buffer for 30 mins to remove existing ions in the gel and to allow the gel to reach a temperature of 45 to 50 ºC. Immediately prior to loading, samples were prepared by adding 5 µL of formamide dye (98% formamide, 10 mM EDTA pH 8.0, 0.25% bromophenol-blue and 0.25% xylene cyanol) to 5 µL of each PCR reaction mix and denatured for 5 min at 94 ºC in the PCR machine. Only 3 to 3.5 µL of each denatured sample was loaded, in order to avoid excessive background. 1 Kb DNA ladder (Biolabs) was used to determine the size of the bands. The gel was then run at 80 W for 75 to 90 mins, depending on the size of the SSR PCR product expected.

Silver staining (TIXIER *et al.*, 1997) was used to visualise the PCR products. After dismantling the plates, gels were fixed in 2 L of 10% glacial acetic acid for 30 mins, and rinsed 3 times with water. Gels were left shaking in the last water wash until all greasiness had gone and then placed in 2 L of silver stain (2 L distilled water, 12 mL 1.010N silver nitrate solution, 3 mL formaldehyde 40%) for 30 mins. Immediately prior to developing the gel, developing solution was prepared by adding 300 µL sodium thiosulphate solution (0.100 1N) and 3 mL formaldehyde (40% sol.) to the pre-chilled sodium carbonate solution (60 g anhidrous sodium carbonate, 2 L distilled water). After staining, gels were rinsed in distilled water for 10 secs and placed in the developing solution. When gels development was finished (when bands near the bottom started to show), the reaction was stopped by adding 10% glacial acetic acid and agitating until all bubbling ceased. Gels were rinsed off in water and left to dry at room temperature. The duplication of the

gels was obtained using DUPC/RA-1, Kodak duplicating films. The film was developed in a Fugifilm processor.

2.4.3. Data Analysis

Polymorphic AFLP bands were scored as dominant markers and polymorphic SSR were scored either as co-dominant or dominant markers. The code system used to classify the segregation type and the progeny genotype was the one indicated by VAN OOIJEN AND VOORRIPS (2001) and is described in Table 2.3. JoinMap® version 3.0 for MS-Windows® (VAN OOIJEN & VOORRIPS, 2001) was used to analyse SSR and AFLP segregation data.

Table 2.3 - Segregation type codes for an F_2 population (after VAN OOIJEN & VOORRIPS, 2001) and progeny genotype classification.

Code	Marker type	Ratio	Classification into genotype classes
(a,h,b)	Co-dominant		
a		1	Progeny homozygous as parent 'Chinese 166'
h		2	Progeny heterozygous as the F_1
b		1	Progeny homozygous as parent 'Lemhi'
(a,c)	Dominant		
a		1	Progeny homozygous as parent 'Chinese 166'
c		3	Progeny heterozygous as the F_1 or homozygous as parent 'Lemhi' (dominant b-allele, **h** and **b** included in class **c**)
(b,d)	Dominant		
b		1	Progeny homozygous as parent 'Lemhi'
d		3	Progeny heterozygous as the F_1 or homozygous as parent 'Chinese 166' (dominant a-allele, **a** and **h** included in class **d**)
u	-	-	Progeny genotype unknown

2.5. MAP CONSTRUCTION

Prior to the map construction, the genotype frequencies for each locus were calculated, in order to study segregation distortion. The segregation was tested against the normal expectation ratios using the Qui-square (χ^2) test. JoinMap® version 3.0 for MS-Windows® (VAN OOIJEN & VOORRIPS, 2001) was used to create a linkage map of the F_2 population from the cross 'Lemhi' x 'Chinese 166' (114 F_2 individuals). Markers with more than 50 missing values, or showing a χ^2 probability of >0.5% (p=0.005; i.e., χ^2>11) were removed from further analysis. For the population in study, the specified LOD thresholds were 2.0 to 10.0, at a rate of increment of 0.5, and the maximum recombination frequency (REC) of 0.45. The 'jump' value was set to 5.0. Linkage groups were determined using a minimum LOD score of 3.0, with most of the linkage groups holding between a range of LODs from 3.0 to 7.0. The recombination values were converted into genetic distances using the Kosambi function (KOSAMBI, 1943). The linkage maps used for QTL analysis were the ones obtained from the first cycle of mapping in JoinMap (i.e. the map generated under the most stringent mapping conditions).

2.6. QTL ANALYSIS

The QTL mapping package MapQTL™ version 4.0 for MS-Windows® (VAN OOIJEN *et al.*, 2002) was used to locate QTLs for resistance to *P. striiformis* f. sp. *hordei* in the 'Lemhi' x 'Chinese 166' F_2 mapping population. The F_2/F_3 resistance phenotypes fitted approximately to a normal distribution. Transformation of the phenotypic scores did not improve the normality of the distribution.

Both interval mapping (LANDER & BOTSTEIN, 1989) and MQM mapping (JANSEN, 1993, 1994; JANSEN & STAM, 1994) were used. For interval mapping, an imposed significance value of 5% gave a LOD significance threshold of 4.2, as calculated by VAN OOIJEN (1999). LOD values between 2.7 and 4.2 were considered as "suggestive QTLs" (LANDER & KRUGLYAK, 1995). MQM mapping was applied, where the co-factors were chosen based on the selection mode (the *automated cofactor selection* package) and the percentage of explanation (%expl) values of the markers flanking the QTLs detected by interval mapping.

3. RESULTS

3.1. YELLOW RUST DISEASE

'Chinese 166', 'Lemhi', 118 F_2 individuals derived from the cross 'Lemhi' x 'Chinese 166' and the F_3 families derived from each F_2 were tested for resistance to the *Puccinia striiformis* f. sp. *hordei* isolate BWR 80-1. The disease severity of barley yellow rust, first classified as infection type (IT, Figure 3.1), was later translated into two different numerical scales: 1-6 (based on F_2 phenotypes only) and 1-9 (based on F_2/F_3 segregation phenotypes). The F_2 segregation patterns for both scales are given in Figure 3.2. Appendix III lists the phenotypic scores for all the individuals of the F_2 population.

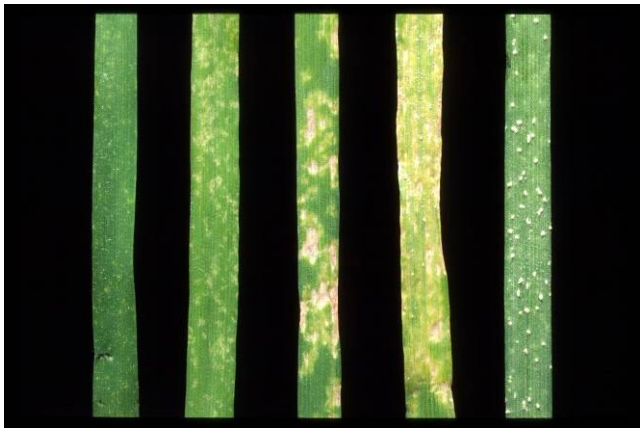

Figure 3.1 - Disease infection types seen on F_2 progeny from the wheat cross 'Lemhi' x 'Chinese 166' following inoculation with the *Puccinia striiformis* f.sp. *hordei* isolate BWR80/1. Infection types shown are (;) small necrotic flecks, ($n^;$) necrotic regions greater than 1 mm in diameter, (0^n) necrotic regions greater than 2 mm in diameter, (0^{nn}) spreading necrotic regions, greater than 4 mm in diameter and (4) large, sporulating uredia surrounded by green tissue (courtesy of L.A. BOYD).

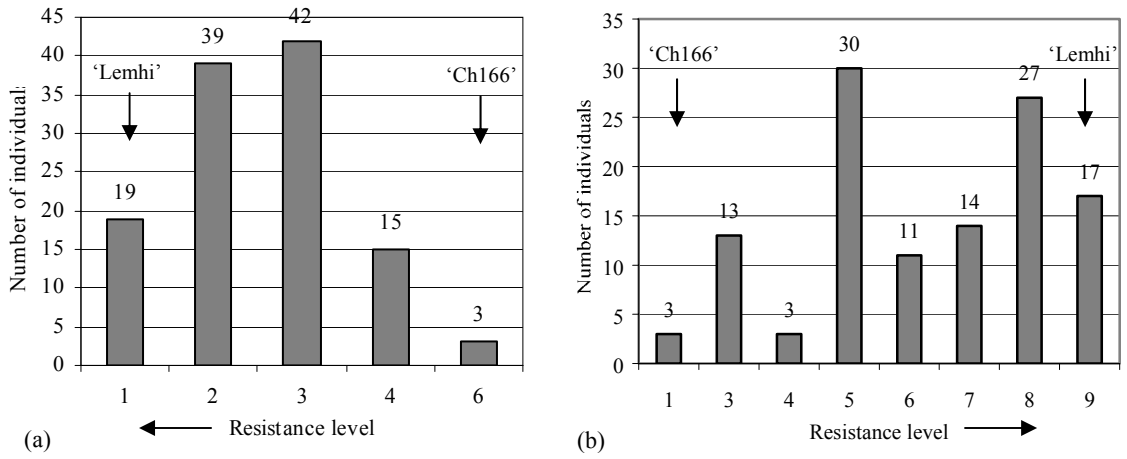

Figure 3.2 - F_2 and F_2/F_3 segregation patterns for resistance to barley yellow rust. (a) Scale 1-6, based on F_2 phenotypes; (b) Scale 1-9, based on F_2/F_3 phenotypes. Parental resistance levels are marked by arrows.

Whereas 'Lemhi' had a fully resistant phenotype (IT 0/;), 'Chinese 166' was moderately susceptible (IT 1^{cn}/3). The 118 F_2 individuals segregated 115 resistant (IT ;/0^{nn}) : 3 susceptible (IT 3/4), suggesting that the barley yellow rust resistance present in 'Lemhi' could be determined by two unlinked, dominant genes. A χ^2 test showed a slight deviation from the expected 15:1 ratio ($\chi^2_{15:1}$ = 2.77, $p<0.1$, df=1), due to fewer susceptible individuals than would be expected, suggesting the presence of extra gene(s), possibly with minor effect, contributing to the barley yellow rust resistance phenotype.

3.2. DNA ANALYSIS

Genomic DNA had previously been isolated from 'Lemhi', 'Chinese 166' and the 118 F_2 individuals from the cross 'Lemhi' x 'Chinese 166'. The quality and concentration of DNA was checked by running 4 μL of each DNA sample on a 1% agarose gel. Four F_2 individuals were lost from the population due to problems with mislabelling of the samples by a previous student who had used this population for AFLP mapping.

3.3. MOLECULAR MARKERS

From the AFLP analysis, 23 primer pair combinations (Appendix I) generated a total of 172 bands that, showing a distinct polymorphism between the parents, were scored in the population. Of these, 90 loci (52.3%) were dominant in 'Chinese 166' and 82 (47.7%) in 'Lemhi' (SHEN, 2000). Out of the 88 SSR primer pairs tested for polymorphism between 'Lemhi' and 'Chinese 166', 26 showed no polymorphism, 10 showed dominant inheritance and 5 produced more than one scorable and polymorphic locus. Of those, 4 produced 2 polymorphic loci (*Xbarc204*, *Xgwm174*, *Xpsp3003* and *Xpsp3131*) and 1 produced 3 loci (*Xgwm311*). Five SSRs showed a complex and unscorable band pattern and 3 were difficult to score (Appendix I). The rest of the SSRs (39) amplified only one polymorphic locus with co-dominant inheritance, even though some amplified extra non-polymorphic or unscorble bands. Figure 3.3 represents examples of band patterns obtained using different SSR primer pairs.

From the 44 SSR primer pairs analysed in the population, only 37 gave unambiguous scorable bands, resulting in 41 segregating loci used for mapping. The rest of the SSRs analysed in the population were not scored, either because there were too many unscorable individuals or because the band pattern was too complex to be genetically analysed with confidence. A total of 213 segregating loci (172 AFLPs and 41 SSRs) were scored and used for the current mapping analysis (Appendix IV).

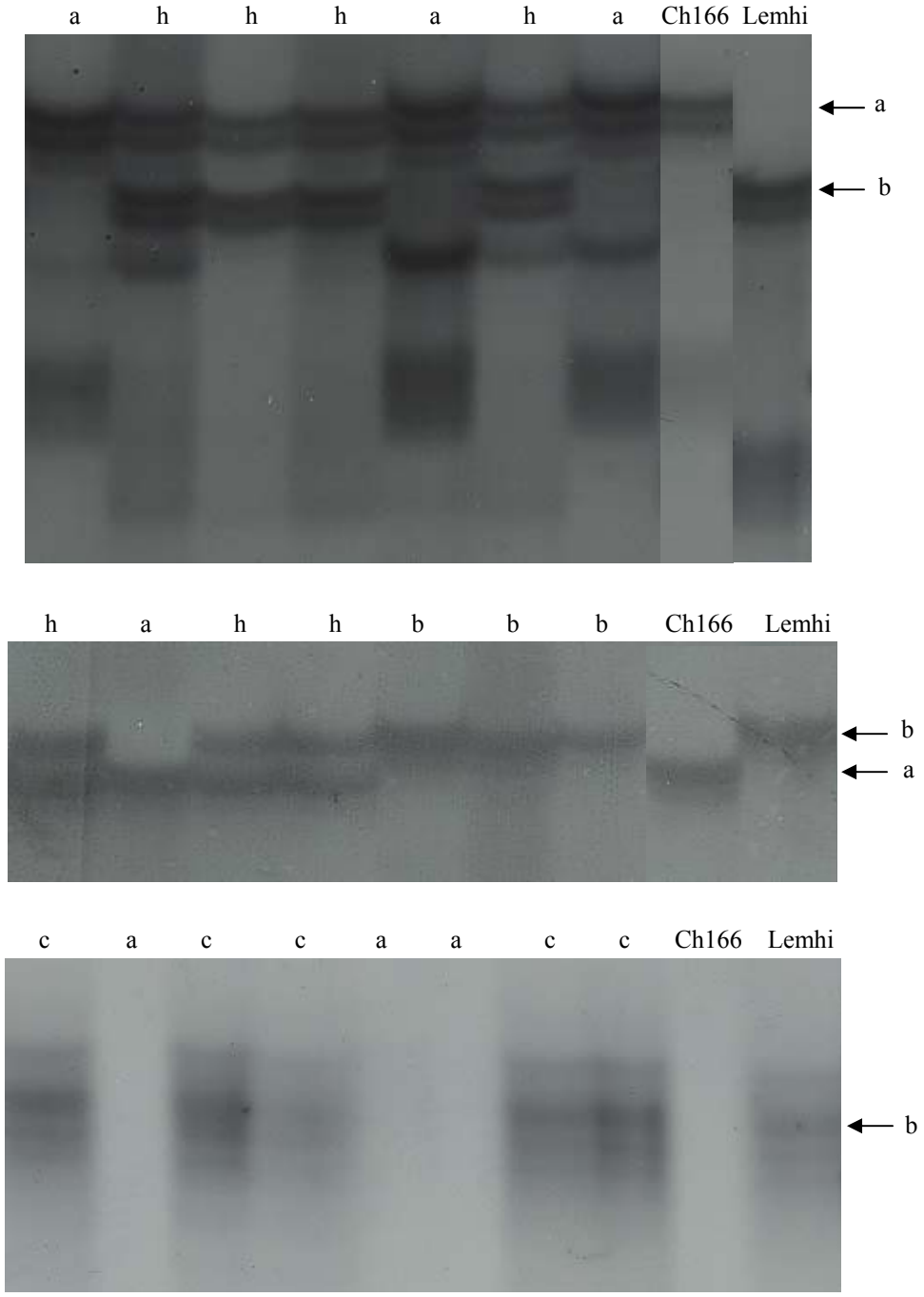

Figure 3.3 - Examples of band patterns resulting from different SSR primer pairs. *Top*: primer pair *Xpsp3000*, complex band pattern, only the bands marked by arrows were scored as a reliable polymorphism. *Centre*: primer pair *Xpsp3007*, simple co-dominant marker. *Bottom*: primer pair *Xgwm106*; dominant marker, the band is present on parent 'Lemhi' only. Ch166 = parent 'Chinese 166'; Lemhi = parent 'Lemhi'; a = like 'Chinese 166'; b = like 'Lemhi'; h = heterozygous; c = like 'Lemhi' or heterozygous.

3.4. MAP CONSTRUCTION

After testing the goodness-of-fit of the 213 segregating loci, 11 loci were excluded from further analysis, either because of a high χ^2 value (5 loci), too many missing data points (5 loci) or similarity between two markers (1 locus) (Appendix V). From the 202 undistorted marker loci, 22 linkage groups were created, but only 18 generated linkage maps (Figure 3.4), consisting of 3 to 13 loci per map. Eighty-six markers remained ungrouped. The 18 linkage maps resulted in a map with a total of 116 markers spanning 680 cM, with an average marker density of one marker every 6 cM. Eight of the 18 linkage maps were assigned to 6 wheat chromosomes (1D, 2B, 3A, 5A, 6A and 6B) by the presence of SSR markers (BRYAN *et al.*, 1997; RÖDER *et al.*, 1998b; STEPHENSON *et al.*, 1998; http://www.scabusa.org/index.html). For the other linkage groups, there were no SSR markers allowing us to assign them to any chromosome. As these linkage maps were not found to be associated with a QTL for barley yellow rust resistance, no further effort was made to identify SSR markers that would assign them to a chromosome (see section 3.5).

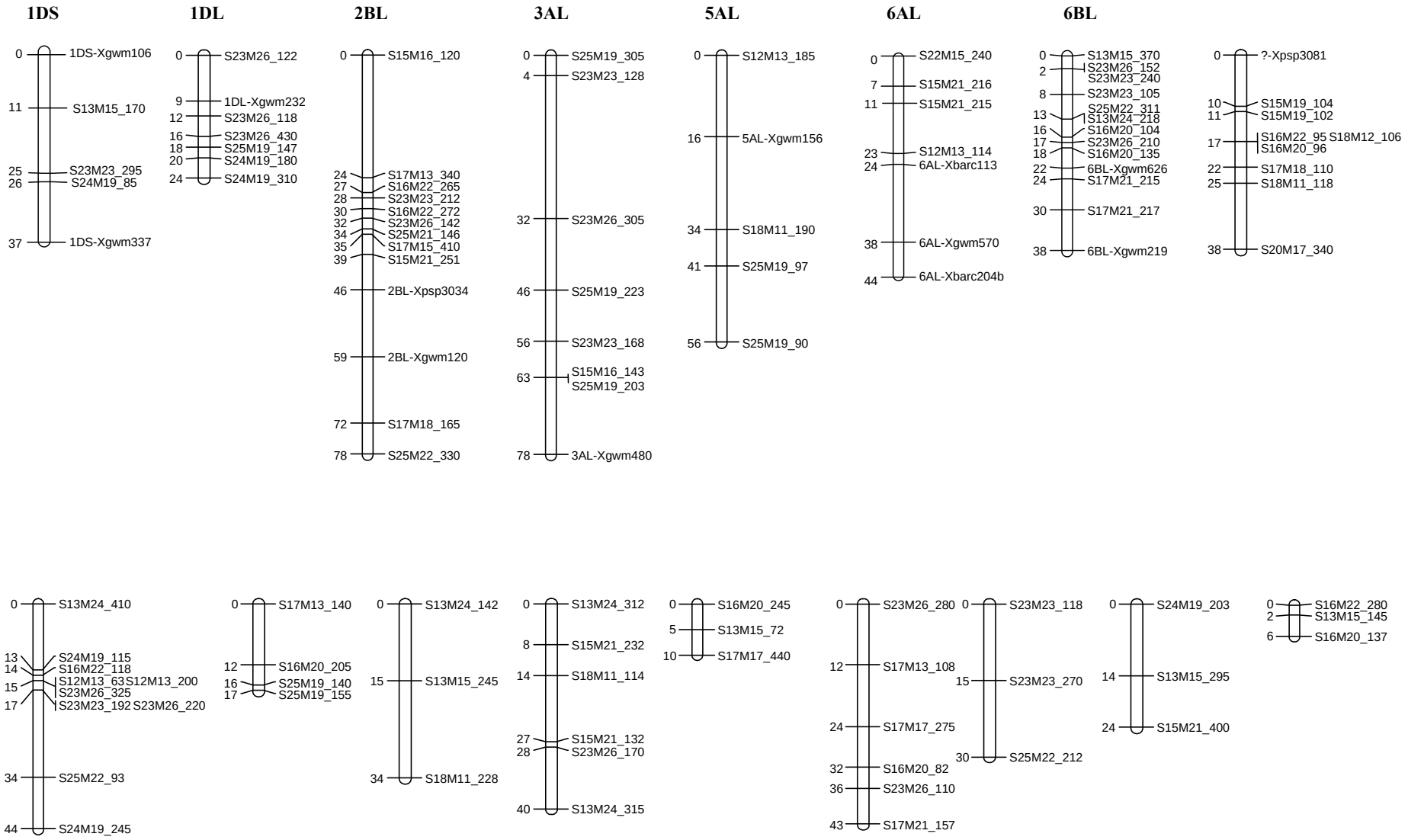

Figure 3.4 - Molecular linkage map (integrating AFLP and SSR markers) for hexaploid wheat 'Lemhi' x 'Chinese 166' F_2 population. For each linkage map, markers (*right*) and genetic distances, in cM (*left*) are given. Reference is made (*top*) to the chromosome arm to which the linkage maps were assigned by the presence of SSRs. Linkage maps with no top reference could not be assigned to a specific chromosome.

3.5. QTL ANALYSIS

As the F_2 segregation pattern using the 1-9 disease scale didn't show a perfect normal distribution (Figure 3.2; the parametric tests - interval mapping and MQM mapping - require normally distributed data), normalization of the data was attempted by several methods (angular transformation, $\log_{10}$, $\log_e$, reciprocal and square root). None of the methods used for data transformation resulted in a clearer approximation to a normal distribution, so parametric analysis was done on the non-transformed data. In order to detect any incorrect results obtained from using parametric analysis on non-normalised data, a non-parametric test (Kruskal-Wallis test) was also used, as this can be used for QTL detection on non-normalised data. However, no QTLs were detected using the Kruskal-Wallis test that were not detected using interval or MQM mapping, or vice-versa.

Two major QTLs for barley yellow rust resistance were detected, one on the long arm of chromosome 2B (2BL) and the other on the short arm of the chromosome 1D (1DS), with both non-parametric (Kruskal-Wallis test) and parametric (interval mapping) analysis. On chromosome arm 1DS, non-parametric analysis resulted in high K* values in two distinct positions: marker *Xgwm337* (K*=16.458, p<0.0001) and marker *Xgwm106* (K*=14.078, p<0.0001). In interval mapping analysis, there were also two peak LOD values: between markers *S24M19_85* and *Xgwm337*, with a LOD of 4.17, and between markers *S13M15_170* and *Xgwm106*, with a LOD of 4.27 (Figure 3.5; Table 3.1). With non-parametric analysis, the QTL present on chromosome arm 2BL was best associated with markers *Xpsp3034* (K*=25.344, p<0.0001) and *Xgwm120* (K*=24.934, p<0.0001). When analysed by interval mapping, the QTL fitted the interval between these two markers, giving a LOD score of 7.28 (Figure 3.6; Table 3.1).

After interval mapping analysis, MQM mapping was applied, in order to detect any possible QTLs of minor effect. The two markers with the highest LOD values associated with each of the QTLs detected by interval mapping (*S13M15_170* and *Xgwm106* for chromosome 1D, and *Xpsp3034* and *Xgwm120* for chromosome 2B) were used as co-factors. MQM mapping confirmed the QTL on 2BL, by sharpening the peak and enhancing the LOD value (Figure 3.6; Table 3.2). On 1DS, the high LOD values between markers *S24M19_85* and *Xgwm337* were eliminated, when using markers *S13M15_170* and *Xgwm106* as co-factors, leading to the conclusion that a false QTL was being detected in that map position (Figure 3.5). The QTL

between these co-factors was enhanced, now having a LOD value of 7.1 (Table 3.2). Both QTLs originated from the non-host resistant parent 'Lemhi'. The QTL on chromosome 1D accounted for most of the phenotypic variance (43.5%; Table 3.2) and was designated *Psh1*. The QTL on chromosome 2B accounted for 33.2% of the phenotypic variance (Table 3.2) and was designated *Psh2*. Besides the confirmation of the two major QTLs, two putative minor QTLs were identified by MQM mapping analysis, one on the long arm of chromosome 5A (5AL) and one on the long arm of chromosome 6A (6AL) (Figure3.7 and Figure 3.8; Table 3.2). The QTL on 5AL (designated *Psh3*) contributed 5.1% of the phenotypic variance, but had a LOD value of 2.1, below the LOD threshold. The QTL on chromosome 6A (*Psh4*) contributed 10.9% of the variance, with a LOD value of 4.2. The four QTLs account for 92.7% of the total variance.

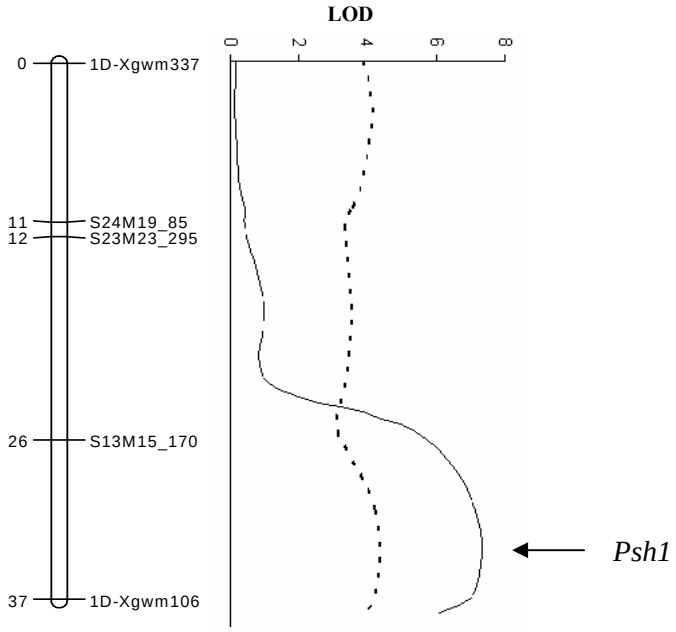

Figure 3.5 - Diagram representing linkage group 1DS. *Left*: Map with loci and distances (cM). *Right*: QTL detection by interval mapping ( ) and MQM mapping ( ), using as co-factors markers *S13M15_170* and *Xgwm106* for chromosome 1D, and *Xpsp3034* and *Xgwm120* for chromosome 2B.

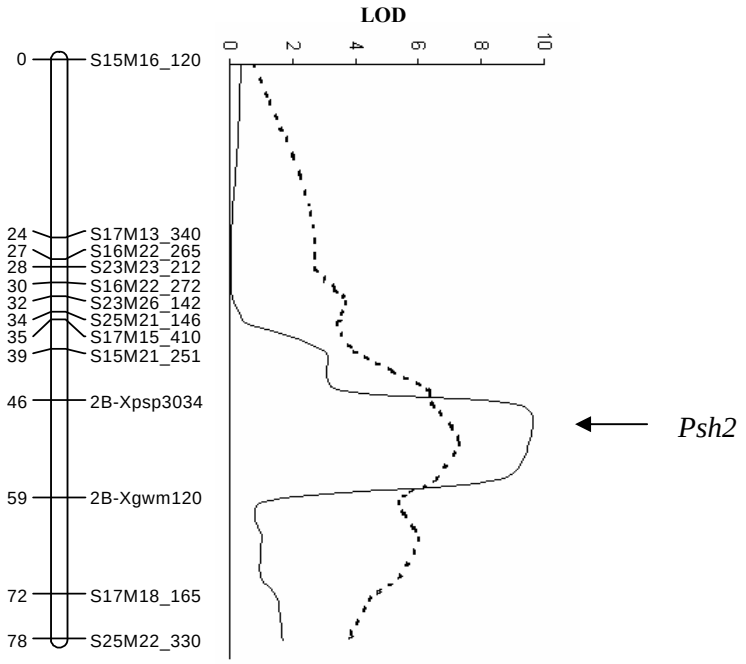

Figure 3.6 - Diagram representing linkage group 2BL. *Left*: Map with loci and distances (cM). *Right*: QTL detection by interval mapping ( ) and MQM mapping ( ), using as co-factors markers *S13M15_170* and *Xgwm106* for chromosome 1D, and *Xpsp3034* and *Xgwm120* for chromosome 2B.

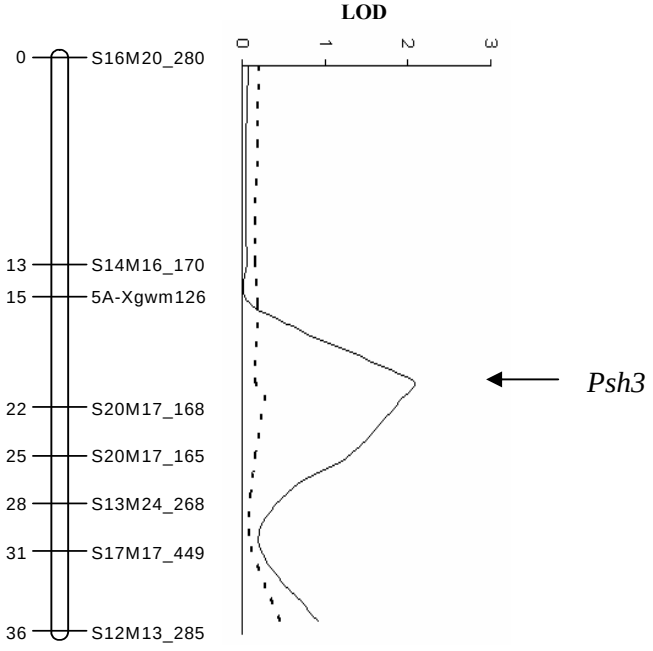

Figure 3.7 - Diagram representing linkage group 5AL. *Left*: Map with loci and distances (cM). *Right*: QTL detection by interval mapping (·····) and MQM mapping (——), using as co-factors markers *S13M15_170* and *Xgwm106* for chromosome 1D, and *Xpsp3034* and *Xgwm120* for chromosome 2B.

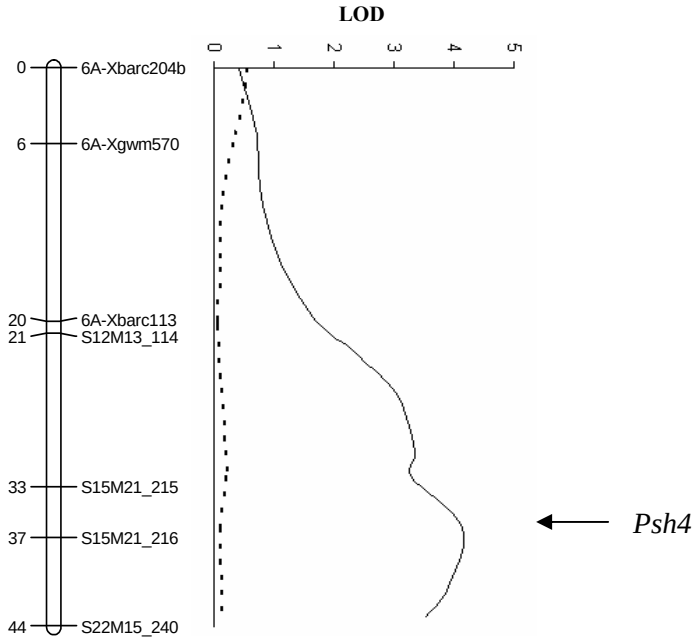

Figure 3.8 - Diagram representing linkage group 6AL. *Left*: Map with loci and distances (cM). *Right*: QTL detection by interval mapping (⋯⋯) and MQM mapping (──), using as co-factors markers *S13M15_170* and *Xgwm106* for chromosome 1D, and *Xpsp3034* and *Xgwm120* for chromosome 2B.

Table 3.1 - Details on QTLs detected by interval mapping, on linkage groups 1DS and 2BL.

LG	map	flanking markers	lod	mu_A	mu_H	mu_B	%expl
1DS	5.0	*Xgwm337 - S24M19_85*	4.2	5.10	6.29	7.49	18.2
1DS	35.6	*S13M15_170 - Xgwm106*	4.3	4.81	6.26	7.70	23.9
2BL	50.8	*Xpsp3034 – Xgwm120*	7.3	4.68	6.39	8.11	31.2

LG: linkage group (chromosome)
map: the current position on the map (distance in cM)
lod: the LOD score
mu_A: the estimated mean of the distribution of the quantitative trait associated with genotype a (parent 'Chinese 166')
mu_H: idem for genotype h (heterozigous)
mu_B: idem for genotype b (parent 'Lemhi')
%expl: the percentage of the variance explained for by the QTL

Table 3.2 - Details on QTLs detected by MQM mapping, when markers *Xpsp3034* and *Xgwm120*, from linkage group 2BL, and markers *S13M15_170* and *Xgwm106*, from linkage group 1DS, are used as co-factors.

LG	map	flanking markers	lod	mu_A	mu_H	mu_B	%expl
1DS	35.6	*S13M15_170 - Xgwm106*	7.1	3.85	6.76	7.80	43.5
2BL	50.8	*Xpsp3034 – Xgwm120*	9.5	4.54	6.15	7.86	33.2
5AL	20.5	*Xgwm126 – S20M17_168*	2.1	5.96	7.34	6.58	5.1
6AL	37.0	*S15M21_216*	4.2	5.76	6.92	5.20	10.9

LG: linkage group (chromosome)
map: the current position on the map (distance in cM)
lod: the LOD score
mu_A: the estimated mean of the distribution of the quantitative trait associated with genotype a (parent 'Chinese 166')
mu_H: idem for genotype h (heterozigous)
mu_B: idem for genotype b (parent 'Lemhi')
%expl: the percentage of the variance explained for by the QTL

As can be seen in Table 3.1 and Table 3.2, QTLs on chromosomes 1D and 2B both originate from 'Lemhi' (mu_B>mu_A), and show dominance, although the dominance is not complete. The minor QTLs on 5A and 6A seem to show heterosis (mu_H>mu_A or mu_B), with both the parents contributing alleles for resistance to barley yellow rust, and the heterozygotes showing greater resistance. In the case of the QTL on 6A, a larger effect appears to be contributed by the allele from the barley yellow rust susceptible parent, 'Chinese 166'.

In order to confirm the relative importances of *Psh1* and *Psh2* to the phenotype, we made the comparison between disease phenotypic scores and the presence of *Psh1* and *Psh2*, based on the DNA markers. The closer the marker from the gene, the less recombination is probable to occur between them, so the presence/absence of the gene in a genotype can be inferred from the presence/absence of a linked marker. The phenotypic scores of the individuals belonging to each infection type class (IT 1 to IT 9) and the respective genotypic data relative to the markers closer to each gene (*Xgwm106* for *Psh1*, and *Xgwm120* and *Xpsp3034* for *Psh2*) are condensed in Table 3.3.

Table 3.3 - Number of individuals belonging to each phenotypic class, and the respective genotypic data relative to the markers closer to each gene (*Xgwm106* for *Psh1*, and *Xgwm120* and *Xpsp3034* for *Psh2*).

	Homoz. 'Ch166' (a)	**Heteroz. (h)**	**Homoz. 'Lemhi' (b)**	**Heteroz. or homoz. as 'Lemhi' (c)**	**Heteroz. or homoz. as 'Ch166' (d)**	**Unscored (u)**
IT9 (18 individuals)						
1D – *Xgwm106* (d,b)	-	-	11	-	7	0
2B – *Xgwm120* (a,h,b)	1	7	9	-	-	1
2B – *Xpsp3034* (a,h,b)	0	4	9	-	-	5
IT8 (26 individuals)						
1D – *Xgwm106* (d,b)	-	-	7	-	18	1
2B – *Xgwm120* (a,h,b)	4	14	8	-	-	0
2B – *Xpsp3034* (a,h,b)	2	13	6	-	-	5
IT7 (13 individuals)						
1D – *Xgwm106* (d,b)	-	-	0	-	13	0
2B – *Xgwm120* (a,h,b)	1	11	1	-	-	0
2B – *Xpsp3034* (a,h,b)	1	9	0	-	-	3
IT6 (11 individuals)						
1D – *Xgwm106* (d,b)	-	-	4	-	7	0
2B – *Xgwm120* (a,h,b)	4	6	0	-	-	1
2B – *Xpsp3034* (a,h,b)	5	2	2	-	-	2
IT5 (28 individuals)						
1D – *Xgwm106* (d,b)	-	-	4	-	24	0
2B – *Xgwm120* (a,h,b)	11	16	1	-	-	0
2B – *Xpsp3034* (a,h,b)	10	14	1	-	-	3
IT4 (3 individuals)						
1D – *Xgwm106* (d,b)	-	-	0	-	3	0
2B – *Xgwm120* (a,h,b)	1	2	0	-	-	0
2B – *Xpsp3034* (a,h,b)	0	1	0	-	-	2
IT3 (13 individuals)						
1D – *Xgwm106* (d,b)	-	-	1	-	12	0
2B – *Xgwm120* (a,h,b)	2	10	0	-	-	1
2B – *Xpsp3034* (a,h,b)	2	7	0	-	-	4
IT1 (2 individuals)						
1D – *Xgwm106* (d,b)	-	-	0	-	2	0
2B – *Xgwm120* (a,h,b)	2	0	0	-	-	0
2B – *Xpsp3034* (a,h,b)	2	0	0	-	-	0

One major, seedling expressed, race-specific gene (*Yr5*) and one QTL (QYR1) for resistance to wheat yellow rust have already been mapped to the same chromosome arm as *Psh2*, at very near locations (Figure 3.9). The *Yr5* gene has been mapped 10.9 cM from marker *Xgwm120* (L.A. BOYD, unpublished), and 10.5-13.3 cM from *Xgwm501* (SUN *et al.*, 2002). A QTL for adult plant resistance to yellow rust has also been located in this region, less than 10 cM from marker *Xgwm120* in the wheat variety 'Camp Remy' (BOUKHATEM *et al.*, 2002). *Psh2* lay approximately 7.9 cM from the marker *Xgwm120*, but without a common flanking marker, we are unable to determine the orientation of *Psh2* in relation to these host yellow rust resistance genes.

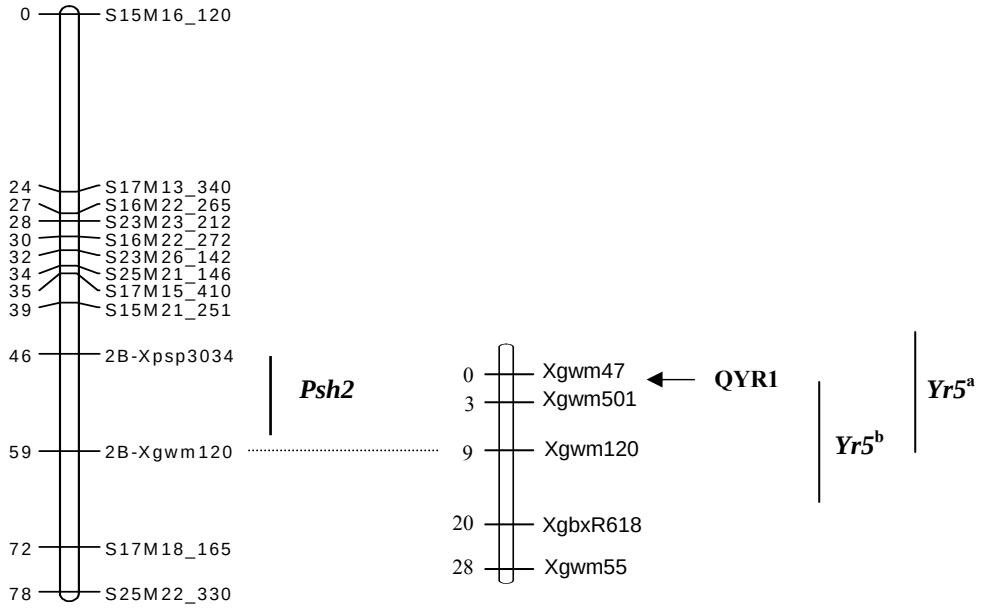

Figure 3.9 - Comparative locations of *Psh2*, QYR1 and *Yr5* on chromosome 2BL. On the left is the map obtained in this study, and on the right is the map obtained by BOUKHATEM *et al.* (2002), where the centromere is placed at the bottom of the map. QYR1 has been mapped in a position between markers *Xgwm47* and *Xgwm501* (BOUKHATEM *et al.*, 2002). *Yr5* was reported to be placed 10.5 to 13.3 cM from *Xgwm501* (*Yr5*[a]; SUN *et al.*, 2002), but no map was presented, and 10.9 cM from *Xgwm120* (*Yr5*[b]; L.A. BOYD, unpublished).

4. DISCUSSION

4.1. HOST VERSUS NON-HOST RESISTANCE

The molecular basis of non-host resistance remains one of the less known areas in plant-microbe interactions. The mechanisms involved in non-host resistance appear to be different when the distance between the host and non-host plant species is greater, than when the two plant species are more closely related. When considering a non-host plant species distantly related to the host species (e.g. monocotyledonous versus dicotyledonous), non-host resistance seems to involve avoidance mechanisms (resulting from topographical, morphological or biochemical differences between both species' leaf surface) or general active defense mechanisms (e.g. callose apposition, saponins production), that prevent a basic compatibility from being established between the pathogen and the non-host species (JOHNSON *et al.*, 1982; HEATH, 1991).

A study on the interaction of *Erysiphe cichoracearum* DC. (cucurbits powdery mildew) and *E. graminis* f. sp. *hordei* (barley powdery mildew) with Gramineae and with Cucurbitaceae species revealed that the surface environments of monocotyledonous and dicotyledonous had characteristics which selectively favoured fungal development by the pathogen adapted to each group (JOHNSON *et al.*, 1982). The same study revealed that, when comparing the reaction within Gramineae plant species (with similar leaf surface characteristics) to *E. graminis* f. sp. *hordei*, the fungus developed further on non-host species closely related to barley that are also hosts to other *formae speciales* of *E. graminis* (wheat, oats and rye), than on more distantly related plant species (several grasses, non-hosts to any form of *E. graminis*), revealing a negative correlation between pathogen development and taxonomic distance between non-hosts and appropriate host. Few cases of cell penetration were reported in wheat, oats and rye, and haustoria and infection hyphae were only produced in wheat, resulting in a hypersensitive reaction. GREEN (1971), working with the black rust-cereals system, and TOSA *et al.* (1990), working with the powdery mildew-cereals system, have found f. sp. *hordei* of both pathogens to be able to infect a wider host range than other *formae speciales* (namely f. sp. *tritici*), suggesting that f. sp. *hordei* are closer to the ancestral forms of the pathogens and are, therefore, less specific in their host range. This may suggest that the same applies to *P. striiformis* f. sp. *hordei*, and that resistance to f. sp. *hordei* and f. sp. *tritici* are controlled by the same resistance genes. If the same genes are found to control both host and non-host resistance in wheat to these *formae speciales*, then Tosa's theory that host range is determined by avirulence genes and not resistance genes (TOSA, 1992) would gain favour. If that was the case, then wheat should be considered a host to *P. striiformis* f. sp.

hordei, and its resistance genes would not be more durable than resistance genes to f. sp. *tritici*. However, non-host, hypersensitive-type resistance in barley to *E. graminis* f. sp. *tritici* was found to be independent of the major genes controlling resistance to the appropriate f. sp. *hordei* (TOSA & SHISHIYAMA, 1984), leading to the conclusion that, even though they act in a similar way, host and non-host hypersensitive resistance are probably controlled by different genes.

The study developed by JOHNSON *et al.* (1982) emphasizes that resistance of distantly related non-host species rests on external defenses leading to reduced development of the pathogen, while resistance in non-host species closely related to the host is the result of active responses within non-host cells (pre- or post-haustorial abortion and hypersensitive response), similar to those of host resistance. In the present work, we have found two major QTLs for non-host resistance to barley yellow rust in the wheat cultivar 'Lemhi', plus two minor QTLs, one in 'Lemhi' and one in 'Chinese 166'. Non-host resistance does not seem to be due to the absence of a basic compatibility, but to the presence of a genetic system similar to, if not the same as, that controlling host, race-specific resistance. Several other studies confirm the similarity of host and non-host resistance between related plant species and their pathogen *formae speciales*, involving resistance-gene-specific recognition events. TOSA (1996) refers to four major genes in wheat, following the gene-for-gene theory, responsible for non-host resistance (resulting in necrotic reaction) to *E. graminis* f. sp. *agropyri* (wheatgrass mildew), and JEUKEN & LINDHOUT (2002) identified, in an accession of the wild lettuce (*Lactuca saligna* L.), one major, race-specific QTL, plus three minor QTLs, against the cultivated lettuce (*Lactuca sativa* L.) pathogen downy mildew (*Bremia lactuca* Regel). NIKS (1987a) assumes that, given the similarity between *formae speciales* of related plant species, resistance to an inappropriate *formae speciales* or a non-pathogen will, in many instances, not be essentially different from resistance to an appropriate *formae speciales* and that, if several accessions of a population all give a post-haustorial hypersensitive response, there is a chance that the resistance is based on one or a few major genes as for host resistance.

Non-host resistant genes in marigold to the parasite *S. asiatica* (GOWDA *et al.*, 1999) and in *Arabidopsis* to *A. candida* (HOLUB, 2002) were found to belong to the NBS-LRR class of resistant genes, common to some genes that control host resistance. Furthermore, a gene from *Arabidopsis* (*RPM1*) was found to recognize *Pseudomonas syringae* pathovars carrying two completely unrelated avirulence genes (BISGROVE *et al.*, 1994; GRANT *et al.*, 1995), which

challenges the concept that each resistance gene is a specific receptor for a particular pathogen elicitor, and agrees with Tosa's theory (TOSA, 1992) that avirulence genes, and not resistance genes, are responsible for determining host range specificity.

If non-host resistance to *formae speciales* of a given pathogen is based on gene-for-gene interactions resulting in hypersensitive response, as seems to be the case with the 'Lemhi''s resistance to barley yellow rust under study, then the use of non-host resistance should not be considered to be more durable and effective against pathogens than host hypersensitive resistance, since the genetic bases of both types of resistance would probably be the same. But, it can also be the case that major and minor *Psh* genes function in a different way. While the major QTLs may represent a resistance triggering system similar to the gene-for-gene interaction common in host resistance, the minor QTLs may encode for products that trigger a general defense reaction of the non-host towards the non-pathogen.

NIKS (1988) considers non-host resistance with pre-haustorial abortion to be based on a mechanism different to that of host resistance, while non-host resistance with post-haustorial abortion (and hypersensitivity) is based on a mechanism similar to that of major gene host resistance. ANKER & NIKS (2001) report the pre-haustorial nature of resistance in *T. monococcum* to *P. triticina*. These authors consider that it may indicate a class of resistance genes different of that of genes for hypersensitive response. A gene for host resistance to yellow rust (*Yr18*) is responsible for moderate levels of durable resistance in wheat (MCINTOSH, 1992; SINGH, 1992). Cultivars carrying this gene show a phenotype of slow rusting, adult plant resistance, with no cell necrosis (MA & SINGH, 1996b). The phenotypic difference between this type of resistance and the one resulting from race-specific, gene-for-gene resistance leads to the conclusion that a different resistance basis controls these two types of resistance. Since host race-specific genes are known to be short-lived, the use of non-host resistance based on this type of reaction would probably have the same end. Only the use of non-host, non-race-specific resistance, similar to that resulting from *Yr18*, would broaden the pool of possibly durable forms of resistance.

4.2. MOLECULAR MARKER ANALYSIS

From the 88 SSR primer sets tested for polymorphism between 'Chinese 166' and 'Lemhi', 65 (74%) identified polymorphism. This level of polymorphism is considerably higher than those reported by several authors for other wheat mapping populations, ranging from 25% to 45% (BÖRNER *et al.*, 2000; MA *et al.*, 2001; GUPTA *et al.*, 2002; WANG *et al.*, 2002). This result is probably due to the phylogenetic distance between the two cultivars, since 'Chinese 166' is a winter wheat originating from a Chinese landrace population, while 'Lemhi' is a commercial cultivar originating from the USA. SHEN (2000) and GARROOD (2001) also reported a high level of polymorphism between 'Chinese 166' and 'Lemhi' when working with AFLP markers.

Most wheat SSR markers are genome-specific and amplify only one, chromosome-specific locus. However, some primer pairs amplify more than one locus, resulting in rates of approximately 1.5 loci per primer pair (BRYAN *et al.*, 1997; RÖDER *et al.*, 1998b). We report various primer pairs amplifying more than one locus, but only 5 of them showed more than one scorable and polymorphic band. From the 37 primer pairs used for mapping, 41 polymorphic loci were produced, giving a rate of 1.08 loci per primer pair. Five primer pairs (5.5% of the polymorphic SSRs) resulted in null alleles, i.e., no amplification for one of the parents. The missing amplifications are probably due to sequence alterations, such as point mutations, deletion or inversion, within the priming site (DEVOS *et al.*, 1995). Even though this level of null alleles is lower than that reported by PLASCHKE *et al.* (1995) and PRASAD *et al.* (2000) (up to 25%) for wheat, its use for mapping purposes can turn into a problem, since failed PCR reactions are difficult to distinguish from a null allele genotype (DONINI *et al.*, 1998). In order to reduce the possibility of miss-scoring, we only used these markers in cases where a low number of co-dominant markers was available for the respective chromosomes.

4.3. MAP CONSTRUCTION

The map of the 'Lemhi' x 'Chinese 166' population was created by integration of the SSR markers into an AFLP map previously constructed for the same population (SHEN, 2000). The AFLP technique has the advantage of producing a high number of markers and therefore giving good genome coverage, but a map based exclusively on AFLPs gives limited information when the aim is detecting QTLs, since the linkage groups cannot be associated with specific chromosomes. Therefore, SSR markers were used to assign linkage groups to chromosomes (BRYAN *et al.*, 1997; RÖDER *et al.*, 1998b; STEPHENSON *et al.*, 1998; http://www.scabusa.org/index.html).

From the 202 markers available, only 116 were incorporated in the map, leaving 97 markers unmapped. The LOD threshold used in this study was not highly stringent (3.0), so the reason for such a high number of ungrouped loci must be that insufficient numbers of markers were available given the size of the wheat genome, to incorporate all the loci within linkage groups. However, the map gave sufficient coverage to identify the two major QTLs/genes known to be present in 'Lemhi', and, in addition, two minor QTLs that contributed to the non-host resistance to barley yellow rust.

4.4. QTL ANALYSIS

In the present study, both parametric (interval mapping and MQM mapping) and non-parametric (Kruskal-Wallis rank sum test) QTL analysis was applied to the data. Non-parametric tests are used when quantitative data does not fit a good normal distribution, since these tests make no assumptions about the probability of distribution of the quantitative traits (VAN OOIJEN *et al.*, 2002). The Kruskal-Wallis test is performed on each locus separately and no use is made of the linkage map other than for sorting the loci. A segregating QTL closely linked to the tested marker results in large differences in average rank of the marker genotype classes (high K* value) (VAN OOIJEN *et al.*, 2002). In order to obtain an overall significance level of 0.005, as suggested by VAN OOIJEN & MALIEPAARD (1996), the significance level (P-value) used in this study was 0.005.

In interval mapping (LANDER & BOTSTEIN, 1989), a "QTL likelihood map" is calculated, which means that for each position on the genome the likelihood of the presence of a segregating QTL is determined by comparison to the likelihood that no QTL is segregating. This likelihood is translated into a LOD score, which is the 10-base logarithm of the quotient of the two respective likelihoods. When the LOD score exceeds the predefined significance threshold, a segregating QTL is detected (VAN OOIJEN *et al.*, 2002). The position with the largest LOD score on the linkage group is the estimated position of the QTL on that map. In order to reduce the probability of errors resulting from false positives in QTL analysis, the LOD significance threshold (i.e., the LOD score used as a threshold for accepting a given QTL) used in this study was calculated after the method proposed by VAN OOIJEN (1999). This method allows the calculation of the LOD significance threshold (for major QTLs), through the type of population under study, the size of the genome (or independent chromosome) of the species and the intended significance level. The significance level here imposed was 5% (which means that the detection of genome-wide false-positives is reduced to a rate of only 5%) and the chromosome size was considered to be approximately 100 cM (for a total of 21 chromosomes), resulting in a LOD significance threshold of 4.2. For results that are not significant, but point to a certain level of association between markers and traits (i.e., in cases where the LOD score is lower but not distant to the LOD significance threshold), to which LANDER & KRUGLYAK (1995) propose the term "suggestive linkage", VAN OOIJEN (1999) indicates a fixed LOD threshold of 2.7 when considering an F_2 population, a chromosome-wide significance level of 5% and a standard chromosome length of

100 cM, which fitted present work's conditions. However, some authors assume a LOD of 2.0 (BÖRNER *et al.*, 2002), or even 1.9 (MINGEOT *et al.*, 2002) as the minimum significance threshold for considering a 'suggestive QTL'.

The MQM mapping, developed by JANSEN (1993, 1994) and JANSEN & STAM (1994), is a method that allows the detection of QTLs not detected by interval mapping, given their small effect on the overall variance. In this method, after detecting putative QTLs by interval mapping, markers flanking the detected QTLs are selected as co-factors to take over the role of the nearby QTLs. In the case where a QTL explains a large proportion of the total variance, the use of linked markers as co-factors in subsequent MQM mapping enhances the power of the search for other segregating QTLs (VAN OOIJEN *et al.*, 2002).

A genetic analysis of F_2/F_4 families from the cross 'Chinese 166' x 'Lemhi' had previously identified two dominant genes of major effect in 'Lemhi' conferring resistance to *P. striiformis* f. sp. *hordei* isolate BWR80/1 (JOHNSON & LOVELL, 1994). We have now located these two loci to chromosomes 1D and 2B (Figure 3.5 and Figure 3.6). We propose the gene designations of *Psh1* and *Psh2*, respectively. Both genes were located with highly significant LOD values (Table 3.1). LOD values obtained by interval mapping relative to chromosome arm 1DS gave us some doubts about the correct location of *Psh1*, as two LOD peaks were detected. But, when applying co-factors for MQM mapping analysis selected by automated selection (markers *S13M15_170* and *Xgwm106*), the second peak disappeared (Figure 3.5). These results led us to conclude that this second peak was a "ghost" QTL.

From interval mapping analysis, *Psh2* appeared as the QTL with the strongest effect on the phenotype, explaining 31.2% of the phenotypic variation, against 23.9% explained by *Psh1* (Table 3.1). But with the MQM analysis, the percentages of phenotypic explanation were inverted. *Psh2* now explained 33.2%, and *Psh1* 43.5% (Table 3.2). This alteration should be the result of taking off the effect of the "ghost" QTL present on chromosome 1DS, that was probably reducing the importance of the real QTL on this chromosome. However, in order to confirm the relative importances of *Psh1* and *Psh2* to the phenotype, we analysed the phenotypic scores of the population. By comparing the genotypes of all the individuals belonging to each of the phenotypic classes, we could see that individuals showing higher levels of resistance had more alleles corresponding to *Psh1* than to *Psh2*, while individuals with moderate resistance levels had more alleles corresponding to *Psh2* than to *Psh1* (Table 3.3). So, the presence of *Psh1* had a

stronger effect on the level of resistance than *Psh2*. Furthermore, when running MQM mapping using as co-factors only the markers flanking *Psh2*, only the minor QTL on 6AL was detected (with a LOD value of 2.3, only), whereas when using the markers flanking *Psh1* as co-factors, two minor QTLs (the same minor QTLs as detected when using both *Psh1* and *Psh2* markers as co-factors) could be detected (Appendix VI). Since the use of markers as co-factors intends to eliminate the effect of the strongest QTLs in order to detect any minor QTLs, we can consider that this analysis confirms the stronger effect of *Psh1* over *Psh2*.

In addition to the two major genes, MQM mapping allowed the detection of two minor QTLs, located on chromosomes 5AL and 6AL, showing LOD values of 2.1 and 4.2, respectively (Table 3.2). Even though the maximum LOD value obtained for the QTL on chromosome 5AL (2.1) was below the proposed threshold (2.7), we have considered it as a "suggestive QTL". Both the parents appear to be contributing alleles for resistance to barley yellow rust at these QTL loci, with the heterozygotes showing greater mean resistance. In the case of the QTL on 6A, a larger effect appears to be contributed by the allele from the barley yellow rust susceptible parent, 'Chinese 166'. This is in agreement with the phenotypic observations, where 'Chinese 166' showed some level of resistance, giving infection types that range from IT 1^{cn} to 3.

The term polygenic is usually associated with the image of many minor genes, each of approximately equal and small effect on the phenotype (YOUNG, 1996), but many studies of QTL mapping indicate this is generally not the case. There are, in fact, few examples of more than 10 QTLs involved in quantitative resistance for any given trait/character (e.g. KELLER *et al.*, 1999; BÖRNER *et al.*, 2002), it being much more common to find only three to five QTLs, where one or two have a predominant effect (e.g. CHEN *et al.*, 1994; QI *et al.*, 2000; BOUKHATEM *et al.*, 2002; BUERSTMAYR *et al.*, 2002). YOUNG (1996) considers this observation of few QTLs as an artefact of small population sizes, inadequate choice of the cut-off for declaring a QTL, or inadequate disease scoring methods, but, on the other hand, the detection of numerous QTLs could be due to a cut-off that was too lenient (p value too high). VAN OOIJEN (1992) considers that at least 200 individuals are necessary for QTL mapping purposes, unless one is only interested in genes with a very large effect. YOUNG (1999) corroborates this idea and states that with a population of 100 to 200 progeny individuals, only a fraction of the true QTLs are usually discovered. But this author also considers that large populations can quickly become small if there are too many missing data, as QTL mapping requires accurate phenotypic scoring methods, which can be

difficult to optimize and to keep in large populations. The main purpose of this study was to locate the two major genes for non-host, barley yellow rust resistance previously reported by JOHNSON & LOVELL (1994), therefore the F_2 population of 114 individuals was adequate for this objective. Precise phenotypic evaluation is a pre-requisite for QTL mapping. Following the consideration that phenotypic data based on only one generation is a weakness in QTL mapping (YOUNG, 1999), the F_3 population, that resulted from selfing of the F_2 plants, was also tested for resistance, which allowed us to determine with more accuracy the phenotype of the corresponding F_2 individuals.

For both *Psh1* and *Psh2*, the interval between flanking markers is of 11 cM and 13 cM, respectively. If we consider that, in wheat, 10 cM equates 6 Mb (ASÍNS, 2002), this situation can be considered adequate for marker-assisted selection, but is not ideal for the map-based cloning of the genes. Finer mapping of the genes based on these and other markers should be conducted to find the co-segregated or closely linked markers of the gene (<0.5 cM; PENG *et al.*, 2000). This can be done with the use of high-resolution crosses, congenic strains, near-isogenic lines, and progeny testing, or by linkage disequilibrium (LD) mapping in experimental crosses (ASÍNS, 2002; GLAZIER *et al.*, 2002). Initial low-resolution linkage studies, like the one resulting from the present study, establish the map location to a resolution that is sufficiently precise to justify further study (GLAZIER *et al.*, 2002). Subsequent high resolution studies would allow the reduction of the size of the candidate interval, in order to functional studies being taken.

4.5. COMPARISON TO OTHER KNOWN RESISTANCE GENES AND QTLS

Both major *Psh* genes are known to originate from 'Lemhi'. In a study involving host and non-host resistances in cultivars 'Lemhi' and 'Chinese 166', GARROOD (2001) reported the presence of one major host resistance gene in 'Lemhi'. This host resistance showed a possible association or linkage with the non-host resistance in 'Lemhi'. A possible linkage of one of the *Psh* genes with the host resistance in 'Lemhi' was therefore considered to exist. Only two host *Yr* resistance genes are known to be present in 'Lemhi': *Yr21*, located on chromosome 1B (CHEN & LINE, 1992), and *Yr25*, located on chromosome 1D (CALONNEC & JOHNSON, 1998). In this study, *Psh1* has been mapped to the short arm of chromosome 1D. *Yr25* has not yet been mapped and is therefore possible that *Yr25* is associated / linked to *Psh1*.

Psh2 is located on chromosome 2B, between markers *Xpsp3034* and *Xgwm120*. From reference maps (RÖDER *et al.*, 1998b; John Innes Centre Database), *Xgwm120* and *Xpsp3034* lie approximately 16 cM and 20-25 cM from the centromere, respectively. *Yr5* lies 10 cM from the marker *Xgwm501* (SUN *et al.*, 2002), and 10.9 cM from *Xgwm120* (L.A. BOYD, unpublished). *Yr5* is closely linked to *Yr7* and *Sr9* (MCINTOSH *et al.*, 1998). BOUKHATEM *et al.* (2002) located a QTL for wheat yellow rust resistance between markers *Xgwm47* and *Xgwm501*. We therefore have two QTLs and a group of three race-specific resistance genes mapping very close to each other. A QTL for adult plant yellow rust resistance has been located on chromosome 5AL, close to marker *Xgwm126*, in the ITMI (Opata 85 x synthetic wheat) population (BOUKHATEM *et al.*, 2002), in a position near *Psh4*. This situation of several QTLs/major genes being mapped to the same location has been described for the yellow rust-wheat system (BOUKHATEM *et al.*, 2002) as well as for other resistance systems (resistance to rice blast: YU *et al.*, 1991; resistance to brown rust: FARIS *et al.*, 1999; resistance to powdery mildew: KELLER *et al.*, 1999; resistance to yellow rust in barley: CASTRO *et al.*, 2002).

Resistance genes determining responses to the same and/or different pathogens are known to cluster in specific regions of plants' genomes (MICHELMORE, 1995). Genetic and molecular analysis have placed several resistance genes for wheat yellow, brown and black rusts on chromosome 2B: *Lr13*, *Lr16*, *Lr23*, *Sr9*, *Sr10*, *Sr16*, *Sr19*, *Sr20*, *Sr28*, *Sr36*, *Sr39*, *Sr40*, *Yr5 Yr7* (considered to be allelic with *Yr5*), *Yr27*, *YrCv*, *YrLuc*, *YrSlk*, *YrSte*, *YrSu92* and *YrV23* (reviewed in MCINTOSH *et al.*, 1998 and Cereal Disease Laboratory databases, http://www.cdl.umn.edu/).

QTLs for resistance to rusts have also been mapped to 2B (BÖRNER *et al.*, 2002; BOUKHATEM *et al.*, 2002; R. PRINS, personal communication). The high number of resistance genes located on this chromosome indicates that it carries important factors for resistance to rusts, and particularly yellow rust. If a close linkage between *Psh* genes and *Yr* genes is confirmed, it would suggest that *Psh* genes, as major, non-host resistance genes, could have evolved from the same ancestral resistance gene as the host *Yr* genes, and therefore have a similar structure and mode of action.

5. CONCLUSIONS

From the present study, we have reached the following conclusions:

a) The genes for non-host resistance to *P. striiformis* f. sp. *hordei* present in the population under study are expressed in the form of post-haustorial, hypersensitive response, in every way similar to host resistance reaction.

b) The molecular marker systems (SSR and AFLP technologies) used in this study, as well as the mapping and QTL analysis software programmes (JoinMap and MapQTL), proved effective for the intended purpose.

c) Two major, non-host resistance QTLs to the barley-attacking form of yellow rust in the cultivar 'Lemhi', one on chromosome 1D (*Psh1*) and one on chromosome 2B (*Psh2*), plus two minor QTLs, on chromosomes 5A and 6A (*Psh3* and *Psh4*, respectively), one of them originating from 'Chinese 166', were identified and located.

d) The four QTLs account for 92.7% of the total phenotypic variance, which indicates that probably all of the loci contributing to non-host resistance in this cross have been identified.

e) *Psh* genes have been mapped to segments of the wheat genome where other wheat yellow rust resistance genes (*Yr* genes) and QTLs had previously been mapped, suggesting an association between host and non-host yellow rust resistance genes.

For both *Psh1* and *Psh2*, the interval between flanking markers is still too wide for map-based cloning purposes. Subsequent high map resolution, based on a higher number of markers, would be necessary in order to reduce the size of the candidate interval. Since the location of the genes has now been determined, this task is facilitated, through the use of markers specific for those chromosomes.

The cloning of both major and minor *Psh* genes, as well as the *Yr* genes present in 'Lemhi', would allow us to determine the similarity of their structure and function. Furthermore, if a close linkage between major *Psh* genes and *Yr* genes is confirmed, it would suggest that *Psh* genes, as major, non-host resistance genes, could have evolved from the same ancestral R gene as the host *Yr* genes, and therefore have a similar structure and mode of action. If that is to be the case, then their durability would be similarly perishable. Pathologists, breeders and scientists would therefore have to seriously rethink the value of pursuing non-host resistance genes as a source of durable resistance for our crop species.

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APPENDIXES

APPENDIX I – Lists of molecular markers (AFLPs and SSRs) scored and used for mapping.

1) List of AFLP primer combinations run in the population (SHEN, 2000), scored and used for mapping.

S12M13
S13M15; S13M24
S14M16
S15M16, S15M19, S15M21
S16M20, S16M22
S17M13, S17M17, S17M18, S17M21, S17M22
S18M11, S18M12
S20M17
S22M15
S23M23; S23M26
S24M19
S25M19, S25M21, S25M22

***SseI* primers (S--)**

S12	5' – GAC TGC GTA CAT GCA GG AC – 3'
S13	5' – GAC TGC GTA CAT GCA GG AG – 3'
S14	5' – GAC TGC GTA CAT GCA GG AT – 3'
S15	5' – GAC TGC GTA CAT GCA GG CA – 3'
S16	5' – GAC TGC GTA CAT GCA GG CC – 3'
S17	5' – GAC TGC GTA CAT GCA GG CG – 3'
S18	5' – GAC TGC GTA CAT GCA GG CT – 3'
S20	5' – GAC TGC GTA CAT GCA GG GC – 3'
S22	5' – GAC TGC GTA CAT GCA GG GT – 3'
S23	5' – GAC TGC GTA CAT GCA GG TA – 3'
S24	5' – GAC TGC GTA CAT GCA GG TC – 3'
S25	5' – GAC TGC GTA CAT GCA GG TG – 3'

***MseI* primers (M--)**

M11	5' – GTA GAG TCC TGA GTA A AA – 3'
M12	5' – GTA GAG TCC TGA GTA A AC – 3'
M13	5' – GTA GAG TCC TGA GTA A AG – 3'
M15	5' – GTA GAG TCC TGA GTA A CA – 3'
M16	5' – GTA GAG TCC TGA GTA A CC – 3'
M17	5' – GTA GAG TCC TGA GTA A CG – 3'
M18	5' – GTA GAG TCC TGA GTA A CT – 3'
M19	5' – GTA GAG TCC TGA GTA A GA – 3'
M20	5' – GTA GAG TCC TGA GTA A GC – 3'
M21	5' – GTA GAG TCC TGA GTA A GG – 3'
M22	5' – GTA GAG TCC TGA GTA A GT – 3'
M23	5' – GTA GAG TCC TGA GTA A TA – 3'
M24	5' – GTA GAG TCC TGA GTA A TC – 3'
M26	5' – GTA GAG TCC TGA GTA A TT – 3'

2) List of SSRs screened for polymorphism between parents and run in the population, scored and used for mapping.

CHROM.	PRIMER	ANNEALING TEMP. (T°)	POLYMORPHISM	SCREENED IN POPULATION	SCORED	USE FOR MAPPING
1A	Xpsp2999	55	0	-	-	-
	Xpsp3003a	63	dominant	✓	✓	✓
	Xpsp3027	61	✓	✓	✓	✓
1B	Xpsp3000	55	complex	✓	✓	✓
	Xgwm18	50	0	-	-	-
1D	Xpsp3137	61	0	-	-	-
	Xpsp3139	55	0	-	-	-
	Xgwm106	60	dominant	✓	✓	✓
	Xgwm232	55	✓	✓	✓	✓
	Xgwm337	55	✓	✓	✓	✓
	Xgwm458	60	0	-	-	-
	Xgwm642	60	dominant	-	-	-
	Xbarc66	55	0	-	-	-
	Xbarc152	50	✓	✓	difficult	-
	Xbarc169	50	✓	✓	✓	✓
2A	Xpsp3039	55	✓	✓	✓	✓
	Xpsp3153	61	0	-	-	-
	Xgwm311a,b,c	60	✓	✓	✓	✓
2B	Xpsp3034	55	✓	✓	✓	✓
	Xpsp3131a	61	✓	✓	✓	✓
	Xgwm120	60	✓	✓	✓	✓
	Xgwm257	60	0	-	-	-
2D	Xgwm261	55	✓	✓	✓	✓
	Xgwm320	55	0	-	-	-
	Xgwm539	60	✓	✓	✓	✓
3A	Xpsp3047	61	✓	✓	✓	✓
	Xgwm5	50	complex	-	-	-
	Xgwm32	55	0	-	-	-
	Xgwm162	60	dominant	-	-	-
	Xgwm369	60	✓	✓	✓	✓
	Xgwm480	60	✓	✓	difficult	✓
	Xgwm674	60	✓	-	-	-
	Xbarc45	52	0	-	-	-
	Xbarc54	60	✓	✓	✓	✓
3B	Xpsp3144	61	✓	✓	difficult	-
	Xgwm299	55	dominant	-	-	-
	Xgwm389	60	✓	✓	✓	✓
3D	Xpsp3019	61	0	-	-	-
	Xgwm161	60	✓	✓	✓	✓
4A	Xpsp3119	63	difficult	-	-	-
	Xgwm160	60	0	-	-	-
	Xgwm397	55	difficult	-	-	-
	Xgwm601	60	0	-	-	-
	Xgwm637	60	0	-	-	-
4B	Xpsp3030	63	difficult	-	-	-
	Xgwm6	55	complex	✓	difficult	-
	Xgwm368	60	0	-	-	-
4D	Xpsp3007	50	✓	✓	✓	✓
	Xpsp3103	61	complex	✓	difficult	-
	Xpsp3112	63	✓	-	-	-

5A	Xgwm126	60	✓	✓	✓	✓
	Xgwm156	60	✓	✓	✓	✓
	Xgwm304	55	✓	-	-	-
5B	Xgwm67	60	dominant	✓	difficult	-
	Xgwm159	60	✓	✓	✓	✓
	Xgwm213	60	0	-	-	-
5D	Xgwm174a,b	55	✓	✓	✓	✓
	Xgwm182	60	0	-	-	-
	Xgwm192	60	✓	✓	✓	✓
6A	Xpsp3071	61	0	-	-	-
	Xgwm169	60	dominant	-	-	-
	Xgwm459	55	✓	✓	✓	✓
	Xgwm494	60	✓	-	-	-
	Xgwm570	60	✓	✓	✓	✓
	Xbarc3	52	dominant	-	-	-
	Xbarc37	55	complex	-	-	-
	Xbarc113	50	✓	✓	✓	✓
	Xbarc204b	52	✓	✓	✓	✓
6B	Xpsp3131b	61	✓	✓	difficult	-
	Xgwm88	60	✓	-	-	-
	Xgwm132	60	✓	✓	✓	✓
	Xgwm219	60	✓	✓	✓	✓
	Xgwm626	50	✓	✓	✓	✓
	Xbarc48	55	0	-	-	-
	Xbarc134	52	✓	✓	✓	✓
6D	Xgwm325	60	✓	✓	difficult	-
	Xgwm469	60	0	-	-	-
	Xbarc204a	52	✓	✓	✓	✓
7A	Xpsp3094	61	✓	no PCR product	-	-
	Xpsp3114	63	0	-	-	-
	Xgwm60	60	0	-	-	-
	Xgwm233	50	0	-	-	-
	Xgwm332	60	dominant	✓	difficult	-
7B	Xpsp3033	61	dominant	-	-	-
	Xpsp3081	61	complex	✓	✓	✓
	Xgwm537	60	✓	✓	✓	✓
7D	Xpsp3003b	63	complex	✓	difficult	-
	Xpsp3123	63	0	-	-	-
	Xgwm37	60	✓	-	-	-
	Xgwm111	55	✓	✓	✓	✓
	Xgwm295	60	0	-	-	-

APPENDIX II – PCR programmes used for SSR analysis.

GWM Tº (*Xgwm* primers):

Step 1 – 94 ºC	3 min.
Step 2 – 94 ºC	1 min.
Step 3 – T ºC	1 min. (T ºC = annealing temp. specific of each primer; see Appendix I)
Step 4 – 72 ºC	1 min.
Step 5 – 30x to step 2	
Step 6 – 72 ºC	10 min.
Step 7 – 10 ºC	for ever
Step 8 – end	

RAMP Tº (*Xpsp* and *Xbarc* primers):

Step 1 – 94 ºC	5 min.
Step 2 – 94 ºC	1 min.
Step 3 – 0.5 ºC/sec to Tº	
Step 4 – T ºC	1 min. (T ºC = annealing temp. specific of each primer; see Appendix I)
Step 5 – 0.5 ºC/sec to 72 ºC	
Step 6 – 72 ºC	1 min.
Step 7 – 0.5 ºC/sec to 94 ºC	
Step 8 – 35x to step 2	
Step 9 – 72 ºC	5 min.
Step 10 – 10 ºC	for ever
Step 11 – end	

APPENDIX III – Phenotypic scores (ITs) for each individual of the F_2 population for the IT scale (;-4) and the numerical scales (1-6 and 1-9).

nr	; - 4	1-6	1-9
8131	n;	2	7
8132	4	6	1
8133	;	1	9
8134	0n	3	5
8135	0n	3	5
8136	n;	2	8
8137	0nn	4	3
8138	0nn	4	4
8139	0nn	4	4
81310	0n	3	5
8221	0n	3	6
8222	n;	2	8
8223	0n	3	6
8224	0n	3	5
8225	;	1	9
8226	0n	3	5
8227	n;	2	8
8228	;	1	9
8229	0n	3	5
82210	0n	3	5
8311	0n	3	5
8312	0nn	4	3
8313	n;	2	8
8314	4	6	1
8315	n;	2	7
8316	0n	3	6
8317	;	1	9
8318	0n	3	5
8259	n;	2	8
82510	n;	2	8
8521	0nn	4	3
8522	;	1	9
8523	0nn	4	3
8524	n;	2	8
8525	4	4	1
8526	0n	3	5
8527	0n	3	6
8528	n;	2	7
8621	0n	3	5

(cont.)

8622	;	1	9
8623	0n	3	5
8624	0n	3	5
8625	0n	3	5
8626	n;	2	8
8627	n;	2	8
8628	;	1	8
8629	;	1	9
86210	n;	2	8
8811	n;	2	8
8812	;	1	9
8813	n;	2	8
8814	0n	3	6
8815	;	1	9
8816	n;	2	8
8817	n;	2	8
8818	0n	3	5
8819	n;	2	8
88110	0nn	4	3
9141	0nn	4	3
9142	n;	2	7
9143	n;	2	7
9144	0n	3	5
9145	0nn	4	3
9146	0nn	4	3
9147	4	3	3
9148	0n	3	5
9149	n;	2	7
91410	;	1	9
9231	0n	3	5
9232	0n	3	5
9233	0nn	4	3
9234	0n	3	5
9235	0nn	4	3
9236	;	1	9
9237	0n	3	6
9238	n;	2	7
9239	0n	3	5
92310	n;	2	7
9451	n;	2	8

(cont.)

9452	0n	3	5
9453	n;	2	7
9454	;	1	9
9455	n;	2	8
9456	n;	2	7
9457	0n	3	6
9458	n;	2	8
9459	0n	3	5
94510	n;	2	8
9511	0n	3	5
9512	n;	2	7
9513	0n	3	6
9514	0n	3	5
9515	;	1	9
9516	;	1	8
9517	;	1	9
9518	0n	3	5
9519	0n	3	5
95110	n;	2	8
9651	n;	2	8
9652	n;	2	7
9653	n;	2	8
9654	n;	2	8
9655	n;	2	7
9656	0nn	4	4
9657	0n	3	5
9658	;	1	9
9659	0n	3	5
96510	0n	3	5
9731	n;	2	8
9732	0n	3	6
9733	0n	3	6
9734	;	1	9
9735	0nn	4	3
9736	0nn	4	3
9737	n;	2	8
9738	0n	3	6
9739	n;	2	7
97310	;	1	9

APPENDIX IV – Genotypic scoring of each individual (ordered as in Appendix III) for each locus.

S12M13_345 (b,d)
bbbbd ddddd ddddb dddb- ddddb dbddd ddbdb dbddd bbddd bdddb ddbdb dbddd dddbd dbdbd dbdbd dddbd ddddd bdbdb bdddd ddbbb dbbbd ddddd ddbb

S12M13_285 (a,c)
aaccc caccc cccac cacc- accaa ccccc ccacc cccac acccc ccccc ccaaa caccc caacc cccca aaaac cccac cccac ccccc caccc cccca ccccc caaaa aaca

S12M13_280 (b,d)
ddbdd ddddb ddddd dddd- ddddd bddbb dddbd ddbdd ddddd ddddd dbbbd ddbbb bdddb dbddd dbddd bdbdd dbddd dddbd bddbd dbddd bdbdb dddbd dddd

S12M13_200 (a,c)
caacc ccccc acacc cccc- acccc caccc cccca aaccc accca accac acccc ccacc acccc ccccc ccaca ccccc cacaa cccaa aaacc ccacc ccacc cccaa accc

S12M13_185 (b,d)
dbdbd dbddd dddbd dbbd- bdbdd bdbdd ddbdd ddbbd ddddd dddbd ddddd ddddd ddddd ddddd ddbdd bddbd ddbbd bdbbd ddddd bdddd bbdbb bddbd dddd

S12M13_114 (b,d)
dddbd ddddd ddddd dddd- dddbd dbddd ddddd ddddb bdddd ddddd dddbd ddddd ddbdd dddbd dbddd dbddd dbddd ddbdd ddddd bdddb bddbb ddddd dbdd

S12M13_111 (a,c)
ccccc caccc ccccc ccca- ccccc ccccc ccccc caccc ccccc ccaac ccaaa ccccc ccaca caccc cacac cccca ccccc ccccc ccccc ccaca ccccc ccccc cccc

S12M13_63 (a,c)
caacc ccccc acacc cccc- acccc caccc cccca aaccc accca accac acccc ccacc a-ccc ccccc ccaca ccccc cacaa cccaa aaacc ccacc ccacc cccaa accc

S13M15_398 (b,d)
ddddd dbdd- -dddb bbddd bdddd dd-dd d-ddd ddddd dbbdb dddbd dbbdd ddddd ddbd- ddddd bdddd ddddd
-dddd dbddd -bddd dd-db ddd-d ddd-d dddb

S13M15_370 (b,d)
ddddd bddb- -dbdb dddbd bdddd dd-dd b-bdd ddbdb ddddb ddbbb dbddb bdddd dddbb bdddd bbdbb bbbdd ddbdd bdddd -dddd dd-db ddd-b ddd-b dddd

S13M15_295 (b,d)
bddbd dbdd- -bddd dddbd bdbbd dd-dd d-ddd ddddd ddddd bbdbd ddbdd bdddd dbdbb bddbd dddbd ddddd
-dddd ddddb -dddd dd-dd ddd-d ddd-d dbdb

S13M15_245 (b,d)
bdbdb ddbb- -dddd ddbdd dddbd dd-bd b-ddb ddbdd ddddb bdddd bbbbd dbbdd ddddb ddddd dbdbb dbbdd ddbdd bdddb -bddb db-dd bdd-b bbdbd dbdb

S13M15_170 (b,d)
dddbd bddd- -dbdb dbbdb ddbbd db-bd d-dbb ddbbb bdbdb bbbdb dbbdd dbbdd ddddd ddddd dbdbd bbdbd dbbdd ddddd -dddd dd-dd bdd-b bbbdd dbdd

S13M15_145 (b,d)
ddddd dddd- -bbdd bbdbd bbddd bd-dd d-bdb dbddd dbddd bddbd ddddd dbdbd dbddd dbbbd ddddd ddbdd dbddd ddddd -dddd bd-dd ddd-d ddddd dddd

S13M15_72 (b,d)
ddddd ddbd- -bddd dbddd ddbdd dd-dd b-bdd ddddd bdddd ddddb ddddd ddddb bdddb ddddd ddddb ddbdd bdddd ddddd -dddd dd-dd bdd-d bdddd ddbb

S13M24_410 (b,d)
dddbd -bbdd ddd-d dd-d- db-dd dddbb bddbd dddbd -ddbd dbddb dbddd bddbb d-bdb bdddb bbdbd bbdbb ddddd bdddd ddddd bdbbd dbdbd dbd-d ddbd

S13M24_315 (a,c)
ccccc -caac cac-a cc-cc accaa ccccc acaac cccaa -c-ca caccc cccca aaccc a-cca cccca cccaa acccc aacac aaccc ccccc caacc caccc acc-a aaac

S13M24_312 (b,d)
dbbdd -dddd ddb-d dd-bb dbddd bbddd dbddd bdbdd -bbbd ddbbb ddbbd ddddb d-bdb dbdbd dbbbd ddbdd ddddb ddddb ddbbd bdbdb bddbb dbd-d ddbd

S13M24_275 (b,d)
ddddd -dddd ddd-b dd-dd ddddd ddddd ddddd ddddd -bddd bdddd ddddb ddddd d-ddd ddddd dbdbd dddbd ddddd bdddd dbddd dddbb dbbdd dbd-d ddbd

S13M24_268 (b,d)
dbddd -dbbb dbd-d dd-db dbbdd dddbb bdddd bdbbd -bdbd dbddd ddddd d-ddb d-ddd ddbbd dbdbd ddddd dddbd ddbbd bdddd bd-db bdddd bdddb dddd

S13M24_218 (a,c)
caccc -cacc ccc-c ccccc cccaa cccac cccca acccc -cccc ccccc cccac caacc c-acc ccccc ccccc cccca cccac cacac ccccc ccccc acacc ccccc caaa

S13M24_150 (a,c)
-cccc -cccc acc-c ccccc ccccc ccccc acccc ccccc --ccc ccaca ccaca ccaca c-ccc cccac ccccc ccccc cccca cacaa cccac cccca acccc cacac cccc

S13M24_142 (a,c)
caccc -ccca acc-c caacc cccca acccc caccc aaccc -acac ccaca cccca cccaa c-ccc aaaac c-ccc accac cccac ccacc acacc accac ccccc ccacc acac

S13M24_112 (b,d)
ddddd -dddb ddddd ddddd bddbd bddbb bdddd dddbd -dddd ddbdd ddbdd dbdbd d-ddb ddbbd ddddb ddbbd ddddd ddddb ddbdd ddddb ddddb dbddd dbbd

S14M16_360 (b,d)
----d -bbd- -b--- bd--- -ddd- --b-- -dd-- --d-- -d-d- d---- --ddd ddddd dd--b dbd-d db-db ddd-d dbddd --ddd d--d- -d-dd -d--- d--dd -d--

S14M16_350 (a,c)
----c -ccc- -c--- ca--- -cca- --c-- -ca-- --c-- -a-c- c---- --acc cccac cc--c acc-c cc-ac caa-a accac --cca c--c- -a-ca -a--- c--cc -a--

S14M16_198 (b,d)
----d -dbd- -d--- db--- -bdb- --d-- -bd-- --b-- -b-b- d---- --dbd bbdbd db--d ddd-d dd-dd ddddd dbddb --dbd d--d- -b-dd -b--- d--bd -b--

S14M16_197 (a,c)
----c -cca- -a--- ca--- -ccc- --c-- -ca-- --c-- -c-a- a---- --ccc cccaa ac--c aaa-a aa-ac aacaa acacc --ccc c--a- -c-aa -c--- c--ca -c--

S14M16_180 (b,d)
----b -dbd- -b--- dd--- -ddd- --b-- -bd-- --d-- -d-d- d---- --bbb ddddb dd--b ddd-d bd-dd bdddb ddddb --ddd d--d- -b-dd -d--- d--bd -d--

S14M16_170 (b,d)
----d -dbb- -b--- bd--- -bdd- --d-- -dd-- --d-- -d-d- d---- --ddd ddddb dd--d ddb-d dd-dd dbbdd ddddd --bdd d--d-
-d-dd -b--- d--dd -d--

S14M16_105 (b,d)
----d -ddd- -d--- dd--- -bdd- --d-- -dd-- --d-- -d-b- d---- --ddd -dddd dd--b bdd-b bb-dd ddddb ddddd --ddd d--d-
-d-bd -b--- d--dd -d--

S15M16_143 (b,d)
ddddb -dbdd ddddd ddddd dbddb ddbdd dbddd ddbdb bbddd bd-dd dd-dd dbbdd bd-db ddddd dd-bd bdddd ddddd
bdddd -dddd ddddd ddddd dd-dd dd-d

S15M16_120 (a,c)
caccc accca cca-c ccccc aaccc cccaa ccccc cccca ccc-a -acac cc-cc ccaaa ccaca cacca ac-cc ccccc ccacc aa---
-cacc acccc ccccc -cccc caac

S15M16_110 (a,c)
cacca cccac ccaac caccc cccca ccacc ccacc ccccc acccc caccc ccccc ccccc ccacc ccccc cacac caaca caaac ccccc -caca
ccccc acccc ccccc cccc

S15M19_226 (a,c)
cccca ccccc ccccc ccaaa -cccc cc-ca acccc cccac cca-c ccccc ccacc cacca ccccc cc-cc cccaa aacaa -accc ccccc cccca
ccaca ccccc acccc ccac

S15M19_208 (b,d)
ddddd ddddd dddbb ddddb ddddd dd-dd dbddd ddddd ddb-b dddbd dbbbd ddddb ddbdd ddddb bdddd ddddb
-ddbd dbbdd dbddd bddbb dbdbd ddddd dddd

S15M19_205 (a,c)
caccc cccca ccacc caccc aacac cc-aa ccccc cccca ccc-c caccc ccccc ccaaa cccca ccccc accac ccccc -accc acacc ccacc
acccc ccccc accac cacc

S15M19_115 (a,c)
ccccc ccaac ccccc -cccc ccccc cc-cc ccc-a accca ccc-c ccccc ccccc cccca ccccc ccacc caacc ccccc -ccac caccc ccccc
ccccc acccc ccccc ccac

S15M19_104 (b,d)
ddddd ddbdd ddddd dbddd ddddd bd-bd ddddd ddddd ddb-d dbddd dbddd ddddd ddbdd bdbdb ddddd ddddb
-dddd ddddd ddddd bbbdd dbbdd ddddd ddbd

S15M19_102 (b,d)
ddddd ddbdd ddddd dbddd ddddd bd-bd ddddd ddddd ddb-d dbddd ddddd ddddd ddbdd bdbdb ddddd ddddb
-dddd ddddd ddddd bbbdd dbbdd ddddd ddbd

S15M21_400 (b,d)
bddb- dbd-b dbbbd -bdbd bdbdd bd-dd dbdbd ddddd b-ddd bbdbb ddbdd --ddd --db- dddbd dddbd dd-dd -dddd
ddddb ddddd dddd- dddbd d-ddd dbdb

S15M21_380 (b,d)
bdbd- d-d-b ddddd ddddd bdddd bddbb dddbd ddbdd d-ddd dddbb dbbbd bdbbb b-dbb d-ddd d-ddd bd-dd dbddb
ddbbd bbdbb ddbdd bdbdb dddbd dddb

S15M21_251 (b,d)
ddbb- d-d-d bbddb dddbd bdddd dbddb dbdbd bdddd d-ddb ddddd bdddd ddddd d-bd- b-ddd dddbd ddddd ddbdd
ddddd bbddb dbbdb ddddd ddbdd bddb

S15M21_250 (a,c)
cacc- ccacc ccaca ccccc cccca caacc cacca ccccc c-acc acccc ccccc caccc c-cc- ccacc ccccc caccc acaa- acccc ccccc
accca ccccc aaccc ccac

S15M21_235 (b,d)
dddd- ddd-b ddbbd bdddd dbddd bdddd ddbbb dbddb b-dbd bddbd ddddb ddbbd d-ddb bbdbd dbddd ddddd ddbdd dbdbd ddbdd ddddd bdbdb ddbbd dbdd

S15M21_232 (a,c)
cccc- c-a-c ccccc caccc accaa cccaa accac cccac c-ccc ccccc cccca caccc c-ccc ccccc cacca acccc acccc caccc cccca ccccc cacac accca cacc

S15M21_216 (a,c)
cacc- ccc-c caccc aacac ccccc ccccc cccca ccccc c-cac ccacc ccccc accac a-c-c ca-cc ccccc cccca ccccc caccc cccca ccacc
cccc cccac cccc

S15M21_215 (b,d)
dddb- d---d ddddd b-ddd bdbbd dbbdd d-ddd ddddd bdddd ddddd ddbbd d---- --bd- ---b- --d-- ----- ----- dd---
-dd-d d-d-- b-dbd -dd-- d-dd

S15M21_132 (a,c)
ccccc aca-c cacca ccccc ccccc ccccc acaac cccac ccccc ccccc cccca aaccc a-ccc cccca cccca acacc acccc caccc ccccc ccccc caccc accca caac

S15M21_120 (a,c)
cacc- ccc-a ccaac cccca accac ccccc ccacc ccaca ccccc cacca ccccc cccca ccaca ccaac ccccc ccacc caccc ccacc ccaac ccccc ccaca caccc cccc

S15M21_118 (a,c)
cccc- ca--c ccccc acccc ccccc cc-aa ccccc caccc ccccc -cccc ccaaa c---- ---c- ---c- --a-- ----- ----- -c--- -ac-- c-c--
c-cca -cc-- ---c

S16M20_315 (b,d)
d-ddd dbddd dbdbd bbddd bddbb dbddd bdddd -dddd ddd-b ddddb ddddd d-ddd bdbb- dbbdd ddddd ddddd ddbdd db-db bb-db bdbdb dddbb dddbd dbbb

S16M20_305 (a,c)
cccac acccc ccccc caaac acaca ccccc cccca -cacc aaccc caccc cacac cccca cccaa ccccc ccaaa ccccc caccc ccccc cacac ca-cc caccc ccacc acac

S16M20_280 (a,c)
acacc ccccc ccccc cccac accac acccc ccccc -cccc acacc ccacc acaaa ccccc cacc- cacca acaaa cccac ccacc cacaa cacac caccc ccccc ccaac caca
S16M20_245 (a,c)
aaccc accca ccaca ccccc aacca aaacc cccaa -ccac ccaac caaac caccc acccc caccc ccccc acaac aacca cccaa caaaa acaac caacc ccaac ccccc cccc

S16M20_210 (a,c)
aacac cccac accca c-ccc caacc accaa caccc -ccaa caccc c-cca ccccc ccccc cccca cccca aaccc aaccc ccccc acacc accaa ccccc accac ccacc acaa

S16M20_205 (b,d)
dbdbd ddddd dddbb ddddd ddddd bddbb ddddd -dbdb ddddd dbddd ddddd ddddd bddd- bbdbd ddbdd bdbdd dbddb ddddd dddbb bbddd bddbb dbddd ddbd

S16M20_137 (a,c)
ccaca accca cccac ccccc ccccc ccccc cacac -cccc acaac ccccc caacc ccccc acacc ccccc accaa ccccc ccaca caccc acacc cccca caccc ccccc accc

S16M20_135 (b,d)
ddddd bddbd bddd- ddddd ddddd ddddd ddbdd -dbdd ddddd bdddb d-ddb ddddd ddddd ddddd bddbb dbbdd ddbdd bdddd bdddd ddddb ddddd ddddb dddd

S16M20_104 (b,d)
-dbdd bddbd bdddd ddddd ddddd d-ddd ddbd- -d--d ddddd b-bdb dbddb bdddd d-ddd ddddd bddbb db-dd ddbdd bdddd bdddd ddddb ddddb dd-db dddd

S16M20_96 (b,d)
ddddd ddbdd ddddd bbddd ddddd bddbb ddddd -dddd ddbbd dbbdd ddddd ddddd ddbdd bdbdb ddbdd ddddd ddddd ddddd ddddd bbbdd dbbdd ddddb dddd

S16M20_82 (b,d)
ddddd bdddd ddbbd dbdbd dbddb bddbb ddddd -ddbd bbdbd bdddb ddddb bbbdd dbddd bdddd bdbdb ddddd ddddb bddbd bdddd dbbdd dddbd bdddb bddd

S16M22_400 (a,c)
cca-c -acac aaaa- caaca aaaca a-caa cccc- ccc-a aca-- acaaa caccc caaa- acaaa aacca caccc -accc -ccca cacaa caaac acaca acaca aa-ac acaa

S16M22_350 (a,c)
ccccc -cccc ccaac accac aaacc a-caa aca-- ccc-c cccac acccc aacac accca caaac acccc aaaac aa-ac --cac caccc cccac ac-cc cccca ca-cc ccac

S16M22_280 (a,c)
caaca -caca cccac ccccc ccccc ccccc cacac acccc acaac ccacc caaac ccccc acacc ccccc aacaa -cccc -caca ccccc acacc cccca caccc ccccc accc

S16M22_272 (b,d)
ddbbb -dddd bbddb dddbd ddddd bbbdd dbd-d bdddd ddddb bdddd bdddd ddddd ddddd bbd-d dddbd dddbd
-dbdd ddddd bbddb ddddb ddddd ddbdd bd-b

S16M22_265 (a,c)
caccc -ccca ccaac acccc cacac ccccc ccccc cccca acccc cacaa c-cac ccaaa ccccc cccca a-ccc -cacc -accc aaacc cccac aaccc ccaca a-cac cacc

S16M22_155 (b,d)
dddbd ddddb dddb- dbddd dddbd --ddd -ddbb bdbdd -dddb d--bb d-dbd ddddd dddbd dbd-b ddddd dddd- -dddd ddddd bbdd- ddbdd ddbdb bbbbd -bbd

S16M22_154 (a,c)
caccc cac-c acacc accac accca cccaa ccacc ccccc ccaac acccc ccacc ccccc ccccc ccccc caacc accac -accc cc-cc cccac caccc accac cccca ccca

S16M22_145 (a,c)
ccacc -cccc caacc ccacc caaac acccc accca cccca cccac accca cccca caccc cccac caccc cccca caacc ccccc ccccc cccaa caacc ccaca caaac aaaa

S16M22_118 (a,c)
caacc -cccc acacc ccccc acccc caccc cccca aaccc accca accac acccc ccacc acccc ccccc ccaca ccccc cacaa cccaa aaacc cc-cc ccacc cccaa accc

S16M22_95 (b,d)
ddddd -dbdd ddddd bbddd ddddd bddb- ddddd ddddd ddbbd dbbdd ddddd ddddd ddbdd bdbdb dbbdd ddddd ddddd ddddd ddddd bbbdd dbbdd ddddb dd-d

S16M22_90 (b,d)
----d -dbd- -d--- bb--- -ddd- --d-- -dd-- --d-- ---b- d---- --ddd d-d-d dd--d bdb-b db-dd ddddd bdddd --ddd d--d-
-b-d- -b--- d--db -d--

S16M22_85 (b,d)
----d -bdd- -d--- dd--- -bdd- --d-- -bd-- --d-- -d-d- d---- --ddb ddbb- bd--d bbb-d -d-dd -bddd -dddd --bdb d--d-
-d-bd -d--- d--dd -d--

S17M13_340 (b,d)
d-bbb -dddd bb-db dddbd bdddd bb-dd dbdbd ddddd dddb- bd--d bdddd ddddd dd-bd bdddd db--d d-dbd dd-dd
--ddd ---d- dd--b ddd-d dd-dd bd--

S17M13_198 (a,c)
c-aca -ccca ac-ac cccaa cacaa cccac cccca -ccaa cccc- cc--c aaaac ccacc aacac ccccc cc--a ccccc cccaa --aac ---a- cc--
c acc-c cc-cc ca--

S17M13_140 (a,c)
c-acc -acca ac-cc c-cac ccc-c ca-cc ac-ca --cac acc-- ac--a aa-c- ccaac ccc-c ccccc ca--c ccccc ccccc --ccc ---c-
-c--a ca--c c---a c---

S17M13_108 (b,d)
d-ddd -dddd dd-dd dbdbd ddddd bd-dd bdddd -dddd dddb- bdd-b ddddb dbdbd dbddd bdddd -d-bb dddbd ddddb --d-
d ---d- dd--d ddd-d dd-bb dd--

S17M15_410 (b,d)
----b -ddd- -b--- dd--- -ddd- --b-- -bd-- --d-- -d-d- b---- --ddd dbddd d---d bbd-d dd-bd bddbd ddbdd --ddd b--d-
-d-db -d--- d---d -d--

S17M15_135 (a,c)
caacc ccccc ccc-c ccccc accca caccc c-cca acccc ccccc ccccc ac-cc cccca a-ccc cccca aaccc ccccc c-ccc ccccc ccccc
ccccc ccccc acc-c cc-c

S17M17_449 (a,c)
ccacc ccccc acacc cccac ccccc -cacc ccacc ccccc aca-c -cacc ccaca ccccc acc-c cacca aca-a -cc-a -ca-c cccca caacc
ccccc ccccc ccccc cccc

S17M17_440 (b,d)
ddddd ddbbd dbddd dbddd bdbdd ddddd bdbdd ddddd bddbd -dddb ddddd ddbdb bdd-b ddddd ddd-b -db-d -dd-d
ddddd ddddd ddddd bdddd bdddd ddbb

S17M17_275 (a,c)
ccaca ccaac ccccc acccc accac ccacc cccac ccccc ccacc ccacc aacac cccca acc-a cacac ccccc aacc- -acac ccccc cacac
accac ccccc ccccc cacc

S17M17_111 (b,d)
ddddb ddddd ddddd ddddd bdddd dbbdd bddbd ddddd ddddd ddbbd bbdbb ddbdd ddd-d ddbdd ddddb ddddd
-ddbb ddddd ddddd bddbb ddddd ddddb dddd

S17M18_345 (a,c)
c-ccc acca- ca-ac a-a-a aacaa aaccc a-aca ccccc accaa a-c-a c-acc cccca -ca-a aacc- aaccc acccc ccaaa --aac ---ac -c-ac
acc-c -c-cc cc--

S17M18_223 (a,c)
c-ccc cacc- cc-aa c-acc cccca ccaac c-ccc ccccc cccaa ccc-a caccc ccccc ccc-a caccc cc-cc ccacc aa-cc --acc ---cc -c-
cc ccc-c -c-ac cc--

S17M18_222 (b,d)
d-ddd bbdd- dd-dd d-bdb ddddd -bddd b-d-d bbddd ddbdd ddd-d dbddd ddddd ddb-d dbddd dd-dd ddddd dd-bd
--ddd ---bb -d-bd ddd-d -b-dd dd--

S17M18_212 (a,c)
c-ccc caaac cca-a ccccc cccca caacc cacca ccccc ccacc acccc ccccc caccc ccccc ccacc cccca ccccc acacc ccccc
-cccc accca ccacc aaccc cca-

S17M18_165 (a,c)
a-ccc cccaa aca-c cccca cccac acccc ccccc caaca ccccc accca ccccc caccc caccc ccaca acccc ccccc caccc caacc
--cca cc-cc ccaca aaccc cac-

S17M18_118 (a,c)
c-aaa accca acc-c cccaa cacac cccca cccca cccca cccca acccc caaac ccacc aacac caccc cccac ccccc cccaa ccacc
-cccc ccacc acccc cccac caa-

S17M18_110 (b,d)
d-ddd dbbdd ddd-d bbddd ddddd bddbd ddddd ddddd ddbbd dbbbd ddddd ddddd ddbdd bdbdb ddbdd ddddd ddddd
ddddd -ddbd bbddd dbddd ddddb dddb

S17M21_292 (a,c)
-c-c- ccaca accca ca-cc ccccc acc-c cccca caccc c-caa cccca -accc cccc- c-cca cccaa caccc ccacc cacc- acccc acccc
acccc cacac cc-cc cccc

S17M21_217 (a,c)
caccc ccacc cccac acacc cccca acccc cacca accca acccc ccccc cccac caacc acacc ccccc ccccc cccca cccac accac ccccc
ccacc acaac cc-cc cacc

S17M21_215 (b,d)
ddddd bddbd bdddd ddbdd ddddb ddddd ddbdd ddbdd ddddd bdbdb dbddb bdddd ddddd ddddd bddbb dbbdd ddbdd
ddddd bdddd ddddb ddbdb dd-db ddbd

S17M21_202 (a,c)
cccac cccac caccc ac-cc ccacc cacca accca caaac ccacc ccccc -accc acccc ccaac acccc caacc cacca ccacc accac accac
acccc ccccc acacc acca

S17M21_157 (b,d)
---db -dddd ddbdd db-bd dbddb bdd-b dddd- bddbd b-dbd bddbb -dddb bbbdd d-ddd bdddd bdbdb bdddd dddd-
dddbd bdbdd dbddd -d--- b--db bdbd

S17M21_140 (a,c)
caccc c-ccc ccccc acacc ccccc -cccc ccaac acccc ccccc acaac acc-c ccaca -ccca c-ccc acccc cccca aaccc aaacc
-ccac ccaac ccccc aacac ccac

S17M21_102 (b,d)
---d- dbd-d dddbd bd-db dddbd ddd-b dbbd- bdddd d--dd dd-dd -dbbd dddbd d-bdd ddbdb ddddb ddddd dddd- bdddd
bbbdd -dddb -d-d- d--bd dddd

S17M21_98 (b,d)
---c- ccc-c caaa- cc-cc caccc c-c-a cccc- acaca c-aaa aacca --cca ccccc c-cca ccc-c ccccc acccc cacc- cccac cccaa -
cccc -c-a- a--cc accc

S17M22_110 (b,d)
dbddd --bdb bdbbd ddbdd dddb- ddd-d ddddd dbddd dbbdb ddddd bbddd ddddd dddbb d-ddd bdd-b bbbdd bd-dd d-
ddd --dbd bddbd dddbd dd-bd dddd

S17M22_85 (b,d)
dddbd --ddd dddbd ddddd ddddd dddbd bddbd ddddd ddddd dbddb -bddd ddddd ddddb d-ddd ddd-b d-bbb bd-dd
ddddd -ddbd dddbb ddddd dd-bd dddd

S18M11_245 (b,d)
dbdbd ddddd ddbdd ddddd ddddd dbddb ddbdb ddddd dddbd dbdbd ddddd dbdbd bdddd bdddd dddbd ddddd ddddb
-dbdb d-ddd bb-bd bdddd bdddd bbd-

S18M11_230 (b,d)
dddbd dbddd ddddb bbddd ddddb dbdbd ddddb ddbbb ddddd dbddd dbddd ddddb ddddd bbbdb bddbd ddddb dbddd
ddddd d-ddb bb-db bddbd ddddd ddb-

S18M11_228 (b,d)
dddd- ddd-d dbddd ddddd ddddd dd-dd b-ddb ddbdb ddddb bdddb dbbbd dbbdd d-ddb -dddd dddbb ddddd ddbdd ddddb d-ddb dd-dd bdddb bbddd bdd-

S18M11_190 (b,d)
bdddd bdddd dbdbd bdbdd bdbdd bbbdd dbbdd dddbd ddddd ddddd bdddd ddddd ddddd dbdbd dbbbd bdddd ddbdb bddbd d-ddd dd-dd bdddb dbdbd ddd-

S18M11_168 (a,c)
ccacc cacac acccc ccccc ccacc cccca ccaac cacca cacaa ccccc accca ccacc acccc cccac cccac cacca caccc ccccc c-aac ca-ac ccacc ccaac aca-

S18M11_118 (b,d)
ddddd ddbdd ddddd bbddd ddbdd bddbb bdddd ddbdd ddbbd dbbdd ddddd ddddd ddbdb bdbdb dbbdd ddddb ddddd ddddd d-ddd bb-dd bbbdb ddddb ddd-

S18M11_114 (a,c)
ccccc caaac caccc caccc acccc ccccc accac cccac ccccc ccccc cccca caccc ccccc acccc cacca acccc acccc caccc c-cca cc-cc cacac accca cac-

S18M12_362 (b,d)
ddddb ddddd dbd-d ddddd bdbdd ddddd bdddd ddbdd ddddb ddddd ddd-d dddd- ddbdd dbddb ddddb dd-bd bdddd bdddd bddbd bdddd ddbdd ddd-d bdbd

S18M12_265 (a,c)
acaca ccccc ccc-c cccca aaccc acccc acacc caacc ccccc ccccc ccc-a cccaa acaac caccc cccaa ca-ac caacc caacc acaaa accac cc-ca ccc-c ccac

S18M12_240 (b,d)
bddbd ddddd ddb-d ddddd dddbd ddddd ddbdd ddddd dbdbd dbdbd ddd-d dbdbd bdddd bdddb dddbd dd-bd ddddd ddbdb dbddd bbdbd bdddd bdd-d dbdd

S18M12_215 (b,d)
dbddd ddddd ddb-d dddbd bbddd dbddd dbddd bdbdd ddbdb ddbbd dd--b ddbdb bdbbd bbbdd dddbd -b-dd bdddd ddbdb ddd-b bddbd ddbbb bdd-d dddd

S18M12_132 (b,d)
ddbdd ddddb ddd-d ddddd ddddd bddbb dddbd ddbdd ddddd dddbd dbb-d bdbbb bbddb dbddd dbddd bd-dd dbddb dddbd bddbb dbddd bdbdb ddd-d dddb

S18M12_106 (a,c)
ccaaa aacca acc-c cccac cacac ccccc cccca cccca cccca acccc caa-c ccacc aacac caccc cccac cc-cc cccaa acacc accac cccac acccc ccc-c caca

S20M17_340 (b,d)
bbddd d-bdd ddddd bbddd bdddb bdddd dddbb dd-dd ddbbd dbbdd ddddd dbddd ddbdd bdbdb ddddb bdddd dbddd ddd-d ddddd dbddd dbddd dbddb dddb

S20M17_332 (a,c)
caccc c-cac ccacc ccaaa cccac ccccc ccccc cc-ac ccccc caccc ccccc cacca ccacc ccaca ccacc acccc caccc caa-c cc-cc acacc ccc-c acacc cacc

S20M17_168 (a,c)
acccc c-ccc accac caacc accac ccccc ccacc cc-cc acccc cccca ccaaa caccc cacac cccca aacac ccccc cccac ccc-c caacc ccccc acacc ccaaa cacc

S20M17_165 (b,d)
dbdbd d-bbd dbddb bdddd dbbdb bdbbd bdddb dd-bb ddddd bbdbd bdddd ddddb bdddd ddbdd ddddb dbbdd ddbdd bdb-d bdddd ddddd ddddd dbddd bddb

S20M17_95 (a,c)
caccc c-ccc ccccc ccacc caaca accac ccccc cc-cc ccccc cccaa ccccc caacc ccacc acccc ccccc cccca cccac acc-c c-acc ccccc ccccc aacac cccc

S20M17_77 (a,c)
caccc c-ccc ccccc ccacc caaca accac ccccc cc-cc ccccc cccaa ccccc caacc ccacc acccc ccccc cccca cccac acc-c c-acc ccccc ccccc aacac cccc

S22M15_320 (a,c)
caaca accaa aaccc c-ccc aacac acacc cacca cacac caccc ccacc ccacc ccccc acccc acc-c cccac ccaac aacac caacc -accc aaacc cccca ccacc aaca

S22M15_240 (b,d)
-ddbd dbbdd ddddd d-ddd dddbd dbbdd dbddd ddddb bdddd ddddd bdbdd ddbdd ddbdd bdbbd ddddd ddddd bdddd dd-dd -dddd dbddb bddbd ddbdb dbdd

S23M23_295 (b,d)
d-db- bdddd ddddb db-dd ddbbd dbddd dbdbd ddddb ddd-d bdbbb dbbdd dbbdd ddddd bdddd ddd-d -bd-d -bdbd ddddd ddbdd ddbdd bdddb d--dd dbdd

S23M23_270 (a,c)
caca- cacac ccacc ccacc caacc cacac ccccc ccccc aac-a accac ccccc caacc acccc acaca ccccc -ac-- -acc- cccac ccaac acacc ccc-c c-cac acac

S23M23_258 (a,c)
cccca ccccc ccacc caccc caacc caacc acccc ccacc acacc aaccc accac ccccc ccccc cccca ccc-a -ca-c -ccca cccac acccc acaca aaccc c-aac cccc

S23M23_240 (a,c)
caccc ccccc cccac cccc- cacca cccaa ccccc acccc acccc ccccc acccc caacc ccacc ccccc ccccc -cc-a -ccac cacac ccccc acccc ccacc a-cac ccaa

S23M23_212 (b,d)
ddbbb ddddd bbddb dddbd ddddd bbbdd dbdbd bdddd dddbb bdddd -dddd ddddd ddddd bbddd ddddd -ddbd -dbdd ddddd dbddb ddddb ddddd d-bdd bddb

S23M23_192 (a,c)
caaca cc-cc acacc ccccc acccc caccc cccca aaccc a-cca accac acccc cc-cc acccc ccccc ccaaa -cc-c --c-a -ccaa aaacc cca-c cca-c c-caa ac-c

S23M23_180 (b,d)
d-ddd d--dd dddbd bdddb ddddd dddbb dbbdd bdddd ddddd ddddd ddbbd dd--d ddbdd d-bdb ddd-- -dd-d --d-b dbddd bdbdd d-d-b dddbd d-dd- dddd

S23M23_168 (a,c)
ccccc cccca acccc ccccc ccccc cccac cccaa ccccc cccaa aacca acaaa cccaa cccac acaaa ccacc -ccac -ca-a -cccc ccccc acc-c acc-a c-caa ac-a

S23M23_128 (a,c)
ccacc cc-aa ccccc ccaac a-ccc ccccc ccccc cccca c-cca accaa ccacc ac--a -cccc -cacc -ccc- --c-c --c-a cacac ccccc acc-c aaaca c-cc- acca

S23M23_124 (b,d)
ddddd bd-dd bdbdd bdddd d-ddd bddbb dbdbd bdddd d-ddd ddddd ddddd -d--d dbddb -ddbd -dd-- --d-b d-d-d ddddb ddddd db--b ddddd b-bb- dddd

S23M23_118 (b,d)
ddbdd ddbdd ddddb bdddd ddddd ddbdd bdddb bdddb dbbdd dbddb bdbbb bdddd dbbdb ddddd dddbb -ddbd -dbbd bdddd ddddd dddbb dbddd d-bdb dddd

S23M23_105 (b,d)
ddddb bddbd bdddb ddddd ddddd ddddd ddbdd ddbdd ddddd ddbdb dbddb -dddd dddbb ddddd bdddb -bbdd -dbdd bdbdd ddddd ddddb ddddb d-ddb bddd

S23M26_430 (a,c)
caccc cccca cccac caccc acccc ccccc cccca caccc aaacc ccccc ccccc cccca accca caaac ccccc cccca -caac cccca cccac cccac ccacc ccacc cccc

S23M26_325 (a,c)
caacc ccccc acacc ccccc acccc caccc cccca aaccc accca accac acccc ccacc acccc ccccc ccaca ccccc aacaa ccca- aaacc ccacc ccacc cccaa accc

S23M26_305 (a,c)
ccccc cccca acccc cccac acccc caccc cacac cccac cccaa accac accaa cccaa cc-ac ccacc ccccc cccac caaca caaa- ccccc acaac aaaaa ccaac acac

S23M26_280 (a,c)
ccaca ccaca acccc acccc accac ccccc cccac caccc ccacc ccacc acaac ccaca ccaac ccaaa ccccc caccc caccc cccaa cacac cacac caccc cccac aacc

S23M26_220 (b,d)
dddbd bbddb dddbd dddbd dbddd dddbb bddbd ddddb dddbd dbddb dbddd bdddd ddddb bdddb bbddd dddbb -dddd ddbdd ddddd dddbd dbddd d-ddd dddd

S23M26_210 (b,d)
ddddd bddbd bdddd ddddd ddddd ddddd ddbdd ddbdd ddddd bdbdb dbddb ddddd ddddd ddddd bddbb dbbdd ddbdd bdddd bdddd ddddb ddddb ddddb dddd

S23M26_170 (b,d)
dbbdd ddddd ddbdd ddddd dbddd bbddd ddbbd bdbdd bbdbd dbdbb ddddd ddddd ddddd dbddb ddddd ddbdd -bddb ddddd ddbdd ddbbb bdddb d-ddd dddd

S23M26_160 (a,c)
caaca aacca aacac ccaaa aacaa ccacc cccca cccca cacca acccc caaac cccc- aaca- caccc cccac cc-cc cacaa acaac acaac cccac acccc cacac caca

S23M26_152 (a,c)
caccc ccccc cccac ccccc cacca cccaa ccccc acccc acccc ccccc acccc caac- cc-cc ccccc ccccc cccca cccac cacac ccccc acccc ccacc accac ccaa

S23M26_142 (b,d)
ddbbb ddddd bbddb dddbd ddddd bbddd dbdbd bdddd ddddb ddddd bdddd ddddd dd-dd bbddd dddbd dddbd ddbdd ddddd bbddb ddddb ddddd ddbdd bddb

S23M26_122 (b,d)
ddddd bdbbd bbbdd dddbb dddbd ddddd bbdbd dddbb ddddd ddddb ddddb dbdbd ddadd ddddd dbddb ddddd ddddd ddddd bbddd ddddd bdddb ddddb dbdd

S23M26_118 (a,c)
c--cc cccca cccaa -accc acccc ccccc cccca caccc aaccc caccc ccccc ccccc ac-ca caaac ccccc cccca acaac cccc- cccaa cccac cc-cc c--cc cccc

S23M26_110 (b,d)
bdddd bdddd dbbbd dbdbb dbddb bbdbb ddddd bddbd bbdbd bdddb ddddb bbbdd db-dd bdddd bdbdb bdddd bdddb bd-bd bdddd dbbdd dddbd b-ddb bddd

S24M19_310 (a,c)
caccc accca cccac caccc acccc ccccc cccca caccc aaaaa acccc ccccc cccca accca caaac cca-c -cc-a cc-ac ccc-a cccac cc-ac ccacc cccac cccc

S24M19_250 (b,d)
dddbd dbdbb dddbd dbdbd ddddd ddddd d--dd ddddd ddbdd ddddd ddbdd ddddd ddbdd ddbbd ddddb -ddbd -dbbd dbddd ddddd ddddb bddbd ddd-d dddd

S24M19_245 (b,d)
bdddd dbbdd dddbd bddbd ddbdd bdddd bddbd ddddd dddbd dbbdd dbddd dbdbd bbddd bbddd bdddd -dbbb ddddd ddddd ddddb ddddb dbddd bdddd dddd

S24M19_203 (b,d)
bddbd dbdbd dbddd d-dbd ddbbd ddddd dbddd ddddd dbdbd bbdbd dbbdd bdddd dbdbb bddbb ddddb ddddd ddddd ddddb ddddd ddddb ddbbd dddbb dbbd

S24M19_180 (a,c)
c-ccc accca cccac caccc acccc ccccc cccca caccc aaaac ccccc ccccc ccccc accca caaac ccccc cccca ccaac cccca cc-ac cccac cca-c ccccc cc-c

S24M19_115 (b,d)
dddbd dbddd dddbd ddddd dbddd dddbd bddbd ddddd dddbd dbddb dbddd -dddd ddddb bdddb bbddd dddbb ddddd ddddd ddddd dddbd dbddd ddddd dddd

S24M19_108 (b,d)
dddbd dddbd bbdbd ddbbd ddddd ddbdd ddddd dbdbd bbddd bbdbd dbbdd ddbbb ddddd bdddb ddddd ddddd ddbdd ddddd ddddd ddddd ddbbd ddddd dddd

S24M19_85 (a,c)
aaacc cacac cacac cccac acccc accaa acacc aaccc ccccc ccccc ccccc acccc caaac ccccc acacc ccccc acccc cccca cccac acccc ccccc cccaa cccc

S25M19_305 (a,c)
ccacc cccaa ccccc ccaac acccc ccccc ccccc ccc-a cc-c- cccaa ccacc cccca ccccc acccc ccccc ccccc cacca cacac ccc-c acccc aaaca cccac acca

S25M19_280 (b,d)
ddddd bdddd ddbdd b-ddd ddd-d dddbd dbdbd ddddd dddb- ddbdd ddddd ddbdd dbddb dddbd ddddd ddddb ddddd ddddb ddd-d dddd- dddbd bdddd dddd

S25M19_223 (a,c)
dbddd bdbbd ddddb bbddd bbddb ddbdd ddddd dbbdb dbbd- ddbdd ddddd ddbdd bdddb ddddd bdbbd bdddd bdddd bdddd ddbbd ddddd ddddd ddddd dddd

S25M19_203 (a,c)
ccccc cccca acccc ccccc ccccc cccaa cccaa ccccc ccaa- cacca acaaa cccaa cccac aaaaa ccacc cccac caaaa ccccc cccac accac accca ccccc aca-

S25M19_155 (a,c)
cccca ccccc ccacc ccccc ccc-a ccacc accca caccc ccca- ccaca caaca ccaac ccccc ccccc caccc ccccc ccccc acccc ccacc ccaaa caacc ccccc cacc

S25M19_147 (a,c)
caccc cccca cccac caccc acccc ccccc cccca caccc aaac- ccccc ccccc cccca accca caaac ccccc cccaa ccaac cccca cccac cccac ccacc ccccc cccc

S25M19_140 (a,c)
cccca ccccc cca-c cc-cc ccca- cca-c accca cac-c ccca- ccac- -aaca ccaac ccccc ccccc caccc ccccc ccccc acccc ccacc ccaaa ca-cc acccc cacc

S25M19_97 (b,d)
ddddd bdddd dbd-d bdbdd bdbdd bbbdd ddddd dddbd dddd- ddddd bdddd ddddd ddddd dbdbd dbbdd bdddd ddbbb bdddd ddddd ddbdd bdddb ddddd dddd

S25M19_90 (a,c)
ccccc ccac- acccc ccccc cacca cccaa ccccc acacc cacc- ccacc ccaaa aaaac aaaaa aaacc ccccc caccc acccc ccccc ccccc aacaa caacc accaa -ccc

S25M21_146 (a,c)
caccc cccca ccaac acccc cacac ccccc ccccc cccca acccc cacca cccac acaaa ccccc ccaca ccccc ccacc caccc caacc ccccc caccc ccaaa cccac ca-c

S25M22_379 (b,d)
ddddd bbddd ddbbb ddddd dbddd bbddb -ddbd dddd- ddbbd ddddb dbdbd --ddb ddbdb bdddb bbddd -ddbb ddddd ddddd ddddd dddb- dbddd ddddd dddd

S25M22_330 (b,d)
aaccc cccaa acacc c-ccc cccac acccc ccccc caac- ccccc accaa ccccc aaccc caccc ccaca acccc ccccc caccc ccacc cccca ccccc caaaa aaccc cacc

S25M22_311 (b,d)
ddddd bddbd ddddd ddddd ddddd ddddd ddbdd ddbd- ddddd bdbdb dbddb bdddd ddddd dbddd bddbb dbbdd ddbdd bdddd ddddd ddbdb ddddb ddddb dddd

S25M22_212 (b,d)
ddddb ddbdb ddddb dddd- bdddb dbbdd bdddb dddb- dd-dd dbddb bddbb --ddd ddddb ddddd bbbdb ddbbd ddbbb ddddd ddddd dbddb dbbbd ddddb dddd

S25M22_203 (b,d)
bbbdd ddbbb bdddd ddddd ddddd ddbdd dbddd bbdb- ddddd bdbdd ddddd ddddd ddddd bdddd ddddd dddbd bdddd bdddd ddddb bdddb ddddd bbddd ddbd

S25M22_200 (a,c)
cacc- ccacc c-aca --ccc cc--a ca-cc c--cc cc-c- cca-c -cccc cc--- a-ccc caccc ccacc c-cca caccc ccccc ac--- -cccc acccc ccccc -ac-c c-ac

S25M22_93 (b,d)
b-dbd dbbdd dddbd bdbbd dbddb dddbb bddbd dddd- dddbd dbd-b dbdbd bbddd dbddd bbddd bdddd dddbb ddddd --ddd b--dd dd-db bbb-b ddddd dd--

1A-Xpsp3027 (a,h,b)
ahaba bahbh bbhbh hhhha aahab hhbbh ahahh bahhh bbhbh hbhbh hbbha bhbhh ahaah hahbb bhhaa hahah hahhb ahahh aa-aa ahbah hbhaa -hbhh hhhh

1A-Xpsp3003 (b,d)
dd-bd bddbd bddbd ddddd dd-db ddbbb ddddd bdddd b-dbd dbddd ddbdd b-bdd dbddb dddbb bdddd ddddd bdddb ddddd ddbdd ddbdd dbddd ddddd dddb

1B-Xpsp3000 (a,h,b)
ahaa- hahhh ahahh ahhah abh-h -aahb hahhh abhbh hbhha hhbhh bhhbh -h-hh habhh bhhbh hhbhh haaha hbhhh bhbhh hhbba ahaah hhbhh hhbah ab--

1D-Xgwm337 (a,h,b)
haahh baha- hahhb hbbaa ahbah hb-ah ababh aahhb hhbbh ahah- -bbhh abbhh -aaah bhhhh aaabh bbhba abhbh hbaaa bhhah ahhhh bhhhb hhbaa hb--

1D-Xgwm232 (a,h,b)
hhhhh bhbha bbbah aahbh ahhch hhhhh bbhba hahhb ahhhh hhbhb hhbhh hbhbh aahha haaah hbhhb bhhha bhhah hhhha bhbah hhhah bhahb hhahb ----

1D-Xgwm106 (b,d)
ddddd bdddd ddddb dbbbd ddddd dbddd dbbdd ddddb ddddb bbbdb d-bdd ddddd ddddd bdddd dbddb dbdbd dbddd dbdbd ddddd ddddd bdddb ddbdd dbdd

1D-Xbarc169 (b,d)
bddbb ddddd ddddb dbddd ddbdd dbddb dddbd ddddb ddddd bdbdb dbbdd -dbd- ddddd bdbdd ddddd bbdbd dbd-d dbddd dbddd ddddd bdddb ddbdd dbbd

2A-Xpsp3039 (a,h,b)
ahahh hhhhh bhhbh hbab- -abhb hhbah hhhah bhhhb hhabb abhh- hhhhh hbhah hhhah aabhh babbh hhahb bhbhb ahhhh hahbh hahaa hbhba ahhbh ahbh

2B-Xgwm120 (a,h,b)
babhh hhhaa hb-ah hbbha hhhah hbhhb hhhbh hhaha hbhbh habba bhhhh a-hha hhahh -haha hhhah hhhhh hhaah hhaah hbahh hhbhh hbaha aabhh bahb

2B-Xpsp3034 (a,h,b)
habhb hh-ha bbaa- hhbha ha-ah -bhhb -bhbh b-h-- h---- h-bba bhhah hahha h-hhh hhaha hhh-h hhabh habhh
aa-hh bhahh habh- hha-a ahb-- ----

2B-Xpsp3131 (a,h,b)
aahhh hhhaa bbaah bbbha bhbah hbhhb -hhhh hh-ha bbbbh ha-ba bhhbh hahah hhahh bhhha hhbhh hhhbh hahhh haahb hahhh hhbhh hbaha aab-h baab

2D-Xgwm539 (b,d)
bdbbd bdddd ddddd ddddd ddbdd ddddd ddddd ddddd ddddd ddbdd dbbdd ddddd dbddd ddbdd bdddd dbddd dddbd ddddd dbbbd ddddd bbbdb dddbd dddb

2D-Xgwm261 (a,h,b)
aahah hahhh hhhba hbbah hbbba ahaba abahb hbbhb bhhhh abbhb hbhhb bbaah hbaah hhhab aahbh ahahh abhhh bbabb ahbha habbb bhbh- bhbhh ahbh

3A-Xbarc54 (a,h,b)
hhbhh hhahh hb-aa hhab- -aaba ahbhb hbbhb hhhbb hhhbh hbh-h -habb chbah hhbbb bbbh- abbbh hhaah ahahh hahhh hbhbb ahaah abahh hbhhh babh

3A-Xgwm369 (a,h,b)
hahha hhhhh bhhha bbaaa ah-ah bhhah ahhbh hbhbh ahhbh bhhhh ah-ab -hbbh haaab bba-b hbhha ahhhb ahhhh bhbbh hbhhb hhhaa -hhhb ahbbh hbbh

3A-Xgwm480 (a,h,b)
hbhab ahbha aha-- bhhbb bbbh- hhbhh --h-h -bbab bbhaa aa-ha aahah hhbha bhha- a---- --abh bah-- ---aa ahhhh bbaba hhaah ahhhh hhhah --bh

3A-Xpsp3047 (a,h,b)
bhhhh hhbha bhabh bbbbb bhahh bb-aa bhhha bbhba ahaba hhhhh abaab bhhbh haahb ahhhb hhhah ahahh aabba ahhhh abhbb a-aah b-bhb haahh habb

3B-Xgwm389 (a,h,b)
haaaa a-aah ahhbh hbhhh bhbhh hhhah haabb a-hhh abha- -h-ah hahaa h---- ----- hhhbh aahaa hhbhh hhaha h-hh-ahhhh hahha hahhh hhhbh ahhh

3D-Xgwm161 (b,d)
dbbdb bddbb b-ddd dbddb bbdbd bdbdd dbddb dbdbd dbddd ddbbd ddbdd ddddd bdddd bdddd dddbd ddbbd bbdbd bdddd ddddd bbbdd ddddb ddbdd bbdb

4D-Xpsp3007 (a,h,b)
hahh- -bh-a hahhb bbaa- hhhhb abhbh hahhb habbb hahhh hbhhb h-hha hahhb hahhh bbbhb bhabh hhaab hbaah hhhhh ahahb hhhhh baahh aaahh ahbb

5A-Xgwm156 (a,h,b)
hbhbh hbh-a abhbh -bbaa babhh bbbhh abbaa aabbh aaaa- ahhhh bbaha hhhhh aahaa ahaha hbbah bahhh aabbh hbbbh babab haaaa bbahb hhahh hhbh

5A-Xgwm126 (a,h,b)
abahh bhbbb ababh hh-ab abaah aha-b bhbhh bhbbb ahabb hbab- hhaha bhbh- -aaab ha-ba hbaha hhhaa ahabh aabb- baha- bhahb -h-h- b-aab h-ha

5B-Xgwm159 (b,d)
d-ddb dddbd d--bd b--dd b-bdb bdd-d db-db dddbd ddbbb d-ddd ddddb dbddd dddbd bbbbd bbddd dddbd dddbb bdbdd bdbdd d-ddd bbdbb dbbdb ddd-

5D-Xgwm192 (a,h,b)
baaah hhbah haabb hhhhb bbaab bh-hb ----- --bhh -bbhh -ahbh hbhb- -bhhh h-bhh hhahh hhahh hahaa hbahh
hh-hh hh-ha hbbah bbb-- -baba hb-b

5D-Xgwm174b (a,h,b)
bhhhh hhhbb hbhhh hhbah hbhhb habha bhabh haahh ahbhb hbbhh hhbab abhhb hbhhh hbhbh hbhah hbhha bhaha hbhbb ahhhh ahahh hb-bh hbhha hhha

6A/6D-Xbarc204a (a,h,b)
bhhab hhbbb bbhaa hhabb haahh ahbhb hbbhh bbbbb aahhh bhhbh h--hb bhhah hhhha hbhab hhahh a-hbh hhahb hbhhh -bhhb hhhaa hhahh bhbha hhhh

6A/6D-Xbarc204b (a,c)
caccc ccaca caaac cccac ccacc acccc accca ccacc accac cccca c--cc accca acccc caccc ccccc cccac ccccc acccc cccca ccacc cacc- cccaa cccc

6A-Xgwm459 (b,d)
dbddd ddddd ddbdd dddbd dbdbb dbddd dbdbd bdddd ddbbd ddddb ddbdb bdbd- bdbbd bbbdd ddd-d bbddd bdddd dbb-b ddbdb -ddbd ddbd- bddbd dbbd

6A-Xgwm570 (a,h,b)
aahbh hhaha -a-ah hb-ah hhabb hbhhb ahaha hhahb -bhah bhahh hhhbh ahhba hhbbh habhh hbhha bbhah bbhhh ahbhh bhbha bhahb -a-hb h-haa hbhh

6A-Xbarc113 (a,c)
aaccc ccaca caaac ccccc ccccc ccccc cccca ccacc cccac ccccc ccccc acccc accca caccc ccccc cccaa ccccc a-cac cccca ccccc ccacc cccac ccaa

6B-Xgwm219
hahhh haabh hhhah hahhh bhhha ahhhb hhbaa aabhh a-hhh hhhhh ahhab bhahh ahhhh -bhah ahhbb -bbha hhbhh aaaab -hbbh hhaa- ahaab hhhh- ----

6B-Xbarc134 (a,c)
aaaac caacc ccccc ccccc caacc caccc cc-aa cccaa accca accac cccac ccccc aaccc ccccc accca cccca ccccc ccccc ccccc cccac acaac ccccc cccc

6B-Xgwm132 (a,c)
caccc ccccc cccac cccca cacca cccca ccccc acccc -cccc ccccc acccc caacc ccacc ccc-- -ccca cccca ccccc ccccc ccccc caccc caaca ccacc ccca

6B-Xgwm626 (a,h,b)
hahhh bhhba bhhh- -ahhh hhhhh ahhab habaa ahbha ahhhh bhbhb abhab baahh ahahh hhhh- bhhbb hbbha hhbah aaaah bhhhh hhahb ahaab hhhhb habh

7B-Xgwm537 (a,h,b)
hbhbb habbh hbhhh hbbah -hhhh hbb-b -bb-h h---- -babb bhaah hhaaa --hhh haaba --hhh aaaah bhhhh bahhb hahbh hhhba abbba bb-aa hbbhh baba

7D-Xgwm111 (a,h,b)
hhhbh bhbha hhhbh aahhh ahhbb ahaha hhbha habah -hha- aahhh hhhah hbahh hahhh abh-h hahhh hhahh hhhhb abbaa aabhb ahhaa ahhh- hhhbh hbbb

Xpsp3081 (a,h,b)
hhaba aabhh hhhhh bbhah hhhah bhhba baabh hhhhh hhbbh abhbh haahh bbaab aabah babhb hhbah hhhhb hahaa hhhhh ahhaa bbaa- abbbh hahah haah

Xgwm311a (a,c)
cacac -cccc ac-cc ac-cc cccca aac-- accc- caccc ccacc accc- ccaac caccc cccca ccccc acaca cacca caa-- ----- ----- aaccc cacac aaccc cccc

Xgwm311b (a,h,b)
ahahh hhhhh bhhbh hb-bh bahhb hhbah bhba- bhhhb hhabh abhhh hhhhh hbhah hhhah aabhh babah hhahb bhb-- ----- ----- hahaa hbhba ahhhh ahbh

Xgwm311c (b,d)
bdddb dddbb dbddd dd-d- dd-dd ddddd ddbb- ddddd ddddd dbddd dbddd bdbdd dbddd dbddd dbddd dddbd
ddd-- ----- --dbb ddbbb ddddb dddbb dddb

Xgwm174a (a,h,b)
bhhhh hhhbb hbhhh hhbah hbhhb habha bhabh haahh ahbhb hbbhh hhbab abhhb hbhhh hbhbh hbhah hbhha bhaha hbhbb ahhhh ahahh hbhbh hbhha hhha

APPENDIX V - Loci used in JoinMap analysis.

a: number of individuals homozygous as parent 'Chinese 166'; **h**: number of heterozygous individuals; **b**: number of individuals homozygous as parent 'Lemhi'; **c**: number of individuals heterozygous or homozygous as parent 'Lemhi'; **d**: number of individuals heterozygous or homozygous as parent 'Chinese 166'; **u**: number of unscored individuals; χ^2: Qui-square value; **Df**: degree of significance.

Nr	Locus	a	h	b	c	d	u	χ^2	Df	Signif.	Classes
1	S12M13_345	0	0	36	0	77	1	2.8	1	*	[a+h+d:b]
2	S12M13_285	33	0	0	80	0	1	1.1	1	-	[a:h+b+c]
3	S12M13_280	0	0	28	0	85	1	0.0	1	-	[a+h+d:b]
4	S12M13_200	31	0	0	82	0	1	0.4	1	-	[a:h+b+c]
5	S12M13_185	0	0	29	0	84	1	0.0	1	-	[a+h+d:b]
6	S12M13_114	0	0	18	0	95	1	5.0	1	**	[a+h+d:b]
7	S12M13_111	16	0	0	97	0	1	7.1	1	***	[a:h+b+c]
8	S12M13_63	31	0	0	81	0	2	0.4	1	-	[a:h+b+c]
9	S13M15_398	0	0	17	0	87	10	4.2	1	**	[a+h+d:b]
10	S13M15_370	0	0	32	0	74	8	1.5	1	-	[a+h+d:b]
11	S13M15_295	0	0	22	0	83	9	0.9	1	-	[a+h+d:b]
12	S13M15_245	0	0	38	0	69	7	6.3	1	**	[a+h+d:b]
13	S13M15_170	0	0	40	0	67	7	8.8	1	****	[a+h+d:b]
14	S13M15_145	0	0	23	0	84	7	0.7	1	-	[a+h+d:b]
15	S13M15_72	0	0	18	0	89	7	3.8	1	*	[a+h+d:b]
16	S13M24_410	0	0	35	0	71	8	3.6	1	*	[a+h+d:b]
17	S13M24_315	36	0	0	71	0	7	4.3	1	**	[a:h+b+c]
18	S13M24_312	0	0	40	0	68	6	8.3	1	****	[a+h+d:b]
19	S13M24_275	0	0	15	0	93	6	7.1	1	***	[a+h+d:b]
20	S13M24_268	0	0	31	0	76	7	0.9	1	-	[a+h+d:b]
21	S13M24_218	20	0	0	90	0	4	2.7	1	*	[a:h+b+c]
22	S13M24_150	18	0	0	90	0	6	4.0	1	**	[a:h+b+c]
23	S13M24_142	32	0	0	77	0	5	1.1	1	-	[a:h+b+c]
24	S13M24_112	0	0	25	0	86	3	0.4	1	-	[a+h+d:b]
25	S14M16_360	0	0	10	0	48	56	1.9	1	-	[a+h+d:b]
26	S14M16_350	18	0	0	40	0	56	1.1	1	-	[a:h+b+c]
27	S14M16_198	0	0	20	0	39	55	2.5	1	-	[a+h+d:b]
28	S14M16_197	26	0	0	33	0	55	11.4	1	*****	[a:h+b+c]
29	S14M16_180	0	0	16	0	43	55	0.1	1	-	[a+h+d:b]
30	S14M16_170	0	0	11	0	48	55	1.3	1	-	[a+h+d:b]
31	S14M16_105	0	0	10	0	48	56	1.9	1	-	[a+h+d:b]
32	S15M16_143	0	0	18	0	88	8	3.6	1	*	[a+h+d:b]
33	S15M16_120	27	0	0	77	0	10	0.1	1	-	[a:h+b+c]
34	S15M16_110	23	0	0	90	0	1	1.3	1	-	[a:h+b+c]
35	S15M19_226	23	0	0	86	0	5	0.9	1	-	[a:h+b+c]
36	S15M19_208	0	0	24	0	87	3	0.7	1	-	[a+h+d:b]
37	S15M19_205	25	0	0	86	0	3	0.4	1	-	[a:h+b+c]
38	S15M19_115	13	0	0	96	0	5	9.9	1	****	[a:h+b+c]
39	S15M19_104	0	0	18	0	93	3	4.6	1	**	[a+h+d:b]
40	S15M19_102	0	0	17	0	94	3	5.5	1	**	[a+h+d:b]
41	S15M21_400	0	0	27	0	73	14	0.2	1	-	[a+h+d:b]
42	S15M21_380	0	0	36	0	70	8	4.5	1	**	[a+h+d:b]
43	S15M21_251	0	0	27	0	80	7	0.0	1	-	[a+h+d:b]
44	S15M21_250	23	0	0	86	0	5	0.9	1	-	[a:h+b+c]
45	S15M21_235	0	0	33	0	77	4	1.5	1	-	[a+h+d:b]
46	S15M21_232	23	0	0	86	0	5	0.9	1	-	[a:h+b+c]
47	S15M21_216	17	0	0	91	0	6	4.9	1	**	[a:h+b+c]
48	S15M21_215	0	0	14	0	56	44	0.9	1	-	[a+h+d:b]
49	S15M21_132	23	0	0	89	0	2	1.2	1	-	[a:h+b+c]
50	S15M21_120	25	0	0	87	0	2	0.4	1	-	[a:h+b+c]
51	S15M21_118	11	0	0	55	0	48	2.4	1	-	[a:h+b+c]
52	S16M20_315	0	0	32	0	75	7	1.4	1	-	[a+h+d:b]
53	S16M20_305	29	0	0	83	0	2	0.1	1	-	[a:h+b+c]
54	S16M20_280	32	0	0	80	0	2	0.8	1	-	[a:h+b+c]
55	S16M20_245	42	0	0	71	0	1	8.9	1	****	[a:h+b+c]
56	S16M20_210	33	0	0	78	0	3	1.3	1	-	[a:h+b+c]
57	S16M20_205	0	0	28	0	84	2	0.0	1	-	[a+h+d:b]

58	S16M20_137	25	0	0	88	0	1	0.5	1	-	[a:h+b+c]
59	S16M20_135	0	0	18	0	93	3	4.6	1	**	[a+h+d:b]
60	S16M20_104	0	0	21	0	83	10	1.3	1	-	[a+h+d:b]
61	S16M20_96	0	0	21	0	92	1	2.5	1	-	[a+h+d:b]
62	S16M20_82	0	0	35	0	78	1	2.1	1	-	[a+h+d:b]
63	S16M22_400	56	0	0	46	0	12	48.6	1	*******	[a:h+b+c]
64	S16M22_350	37	0	0	67	0	10	6.2	1	**	[a:h+b+c]
65	S16M22_280	29	0	0	82	0	3	0.1	1	-	[a:h+b+c]
66	S16M22_272	0	0	27	0	82	5	0.0	1	-	[a+h+d:b]
67	S16M22_265	31	0	0	77	0	6	0.8	1	-	[a:h+b+c]
68	S16M22_155	0	0	27	0	74	13	0.2	1	-	[a+h+d:b]
69	S16M22_154	26	0	0	85	0	3	0.1	1	-	[a:h+b+c]
70	S16M22_145	34	0	0	79	0	1	1.6	1	-	[a:h+b+c]
71	S16M22_118	30	0	0	82	0	2	0.2	1	-	[a:h+b+c]
72	S16M22_95	0	0	21	0	90	3	2.2	1	-	[a+h+d:b]
73	S16M22_90	0	0	12	0	43	59	0.3	1	-	[a+h+d:b]
74	S16M22_85	0	0	14	0	41	59	0.0	1	-	[a+h+d:b]
75	S17M13_340	0	0	21	0	69	24	0.1	1	-	[a+h+d:b]
76	S17M13_198	30	0	0	63	0	21	2.6	1	-	[a:h+b+c]
77	S17M13_140	20	0	0	59	0	35	0.0	1	-	[a:h+b+c]
78	S17M13_108	0	0	18	0	74	22	1.4	1	-	[a+h+d:b]
79	S17M15_410	0	0	14	0	43	57	0.0	1	-	[a+h+d:b]
80	S17M15_135	14	0	0	93	0	7	8.1	1	****	[a:h+b+c]
81	S17M17_449	22	0	0	83	0	9	0.9	1	-	[a:h+b+c]
82	S17M17_440	0	0	21	0	86	7	1.6	1	-	[a+h+d:b]
83	S17M17_275	28	0	0	83	0	3	0.0	1	-	[a:h+b+c]
84	S17M17_111	0	0	21	0	91	2	2.3	1	-	[a+h+d:b]
85	S17M18_345	38	0	0	52	0	24	14.2	1	******	[a:h+b+c]
86	S17M18_223	18	0	0	75	0	21	1.6	1	-	[a:h+b+c]
87	S17M18_222	0	0	17	0	74	23	1.9	1	-	[a+h+d:b]
88	S17M18_212	23	0	0	87	0	4	1.0	1	-	[a:h+b+c]
89	S17M18_165	27	0	0	81	0	6	0.0	1	-	[a:h+b+c]
90	S17M18_118	32	0	0	78	0	4	1.0	1	-	[a:h+b+c]
91	S17M18_110	0	0	22	0	89	3	1.6	1	-	[a+h+d:b]
92	S17M21_292	23	0	0	80	0	11	0.4	1	-	[a:h+b+c]
93	S17M21_217	26	0	0	87	0	1	0.2	1	-	[a:h+b+c]
94	S17M21_215	0	0	25	0	88	1	0.5	1	-	[a+h+d:b]
95	S17M21_202	32	0	0	80	0	2	0.8	1	-	[a:h+b+c]
96	S17M21_157	0	0	32	0	65	17	3.3	1	*	[a+h+d:b]
97	S17M21_140	27	0	0	81	0	6	0.0	1	-	[a:h+b+c]
98	S17M21_102	0	0	22	0	72	20	0.1	1	-	[a+h+d:b]
99	S17M21_98	24	0	0	68	0	22	8.0	1	****	[a+h+d:b]
100	S17M22_110	0	0	27	0	76	11	0.1	1	-	[a+h+d:b]
101	S17M22_85	0	0	18	0	87	9	3.5	1	*	[a+h+d:b]
102	S18M11_245	0	0	25	0	85	4	0.3	1	-	[a+h+d:b]
103	S18M11_230	0	0	30	0	81	3	0.2	1	-	[a+h+d:b]
104	S18M11_228	0	0	24	0	81	9	0.3	1	-	[a+h+d:b]
105	S18M11_190	0	0	29	0	82	3	0.1	1	-	[a+h+d:b]
106	S18M11_168	31	0	0	80	0	3	0.5	1	-	[a:h+b+c]
107	S18M11_118	0	0	28	0	83	3	0.0	1	-	[a+h+d:b]
108	S18M11_114	23	0	0	88	0	3	1.1	1	-	[a:h+b+c]
109	S18M12_362	0	0	20	0	89	5	2.6	1	-	[a+h+d:b]
110	S18M12_265	34	0	0	75	0	5	2.2	1	-	[a:h+b+c]
111	S18M12_240	0	0	25	0	85	4	0.3	1	-	[a+h+d:b]
112	S18M12_215	0	0	34	0	73	7	2.6	1	-	[a+h+d:b]
113	S18M12_132	0	0	31	0	79	4	0.6	1	-	[a+h+d:b]
114	S18M12_106	32	0	0	78	0	4	1.0	1	-	[a:h+b+c]
115	S20M17_340	0	0	27	0	84	3	0.0	1	-	[a+h+d:b]
116	S20M17_332	24	0	0	85	0	5	0.5	1	-	[a:h+b+c]
117	S20M17_168	29	0	0	82	0	3	0.1	1	-	[a:h+b+c]
118	S20M17_165	0	0	34	0	77	3	1.9	1	-	[a+h+d:b]
119	S20M17_95	20	0	0	90	0	4	2.7	1	*	[a:h+b+c]
120	S20M17_77	20	0	0	90	0	4	2.7	1	*	[a:h+b+c]
121	S22M15_320	39	0	0	72	0	3	6.1	1	**	[a:h+b+c]
122	S22M15_240	0	0	24	0	86	4	0.6	1	-	[a+h+d:b]
123	S23M23_295	0	0	27	0	77	10	0.1	1	-	[a+h+d:b]
124	S23M23_270	31	0	0	74	0	9	1.1	1	-	[a:h+b+c]
125	S23M23_258	28	0	0	81	0	5	0.0	1	-	[a:h+b+c]
126	S23M23_240	22	0	0	87	0	5	1.4	1	-	[a:h+b+c]
127	S23M23_212	0	0	26	0	84	4	0.1	1	-	[a+h+d:b]
128	S23M23_192	30	0	0	71	0	13	1.2	1	-	[a:h+b+c]
129	S23M23_180	0	0	19	0	78	17	1.5	1	-	[a+h+d:b]
130	S23M23_168	32	0	0	74	0	8	1.5	1	-	[a:h+b+c]

131	S23M23_128	25	0	0	71	0	18	0.1	1	-	[a:h+b+c]
132	S23M23_124	0	0	20	0	75	19	0.8	1	-	[a+h+d:b]
133	S23M23_118	0	0	32	0	79	3	0.9	1	-	[a+h+d:b]
134	S23M23_105	0	0	24	0	86	4	0.6	1	-	[a+h+d:b]
135	S23M26_430	24	0	0	89	0	1	0.8	1	-	[a:h+b+c]
136	S23M26_325	31	0	0	82	0	1	0.4	1	-	[a:h+b+c]
137	S23M26_305	38	0	0	74	0	2	4.8	1	**	[a:h+b+c]
138	S23M26_280	34	0	0	80	0	0	1.4	1	-	[a:h+b+c]
139	S23M26_220	0	0	27	0	85	2	0.1	1	-	[a+h+d:b]
140	S23M26_210	0	0	21	0	93	0	2.6	1	-	[a+h+d:b]
141	S23M26_170	0	0	27	0	85	2	0.1	1	-	[a+h+d:b]
142	S23M26_160	45	0	0	66	0	3	14.3	1	******	[a:h+b+c]
143	S23M26_152	21	0	0	91	0	2	2.3	1	-	[a:h+b+c]
144	S23M26_142	0	0	26	0	87	1	0.2	1	-	[a+h+d:b]
145	S23M26_122	1	0	26	0	87	0	0.3	1	-	[a+h+d:b]
146	S23M26_118	22	0	0	84	0	8	1.0	1	-	[a:h+b+c]
147	S23M26_110	0	0	42	0	69	3	9.8	1	****	[a+h+d:b]
148	S24M19_310	28	0	0	80	0	6	0.1	1	-	[a:h+b+c]
149	S24M19_250	0	0	20	0	89	5	2.6	1	-	[a+h+d:b]
150	S24M19_245	0	0	28	0	85	1	0.0	1	-	[a+h+d:b]
151	S24M19_203	0	0	32	0	81	1	0.7	1	-	[a+h+d:b]
152	S24M19_180	23	0	0	87	0	4	1.0	1	-	[a:h+b+c]
153	S24M19_115	0	0	20	0	93	1	3.2	1	*	[a+h+d:b]
154	S24M19_108	0	0	25	0	89	0	0.6	1	-	[a+h+d:b]
155	S24M19_85	28	0	0	86	0	0	0.0	1	-	[a:h+b+c]
156	S25M19_305	24	0	0	86	0	4	0.6	1	-	[a:h+b+c]
157	S25M19_280	0	0	16	0	93	5	6.2	1	**	[a+h+d:b]
158	S25M19_223	0	0	28	0	85	1	9.3	1	****	[a:h+b+c]
159	S25M19_203	35	0	0	77	0	2	2.3	1	-	[a:h+b+c]
160	S25M19_155	24	0	0	88	0	2	0.8	1	-	[a:h+b+c]
161	S25M19_147	24	0	0	89	0	1	0.8	1	-	[a:h+b+c]
162	S25M19_140	23	0	0	82	0	9	0.5	1	-	[a:h+b+c]
163	S25M19_97	0	0	23	0	89	2	1.2	1	-	[a+h+d:b]
164	S25M19_90	36	0	0	75	0	3	3.3	1	*	[a:h+b+c]
165	S25M21_146	28	0	0	85	0	1	0.0	1	-	[a:h+b+c]
166	S25M22_379	0	0	26	0	82	6	0.1	1	-	[a+h+d:b]
167	S25M22_330	29	0	0	83	0	2	9.7	1	****	[a+h+d:b]
168	S25M22_311	0	0	22	0	91	1	1.8	1	-	[a+h+d:b]
169	S25M22_212	0	0	32	0	77	5	1.1	1	-	[a+h+d:b]
170	S25M22_203	0	0	24	0	89	1	0.8	1	-	[a+h+d:b]
171	S25M22_200	16	0	0	73	0	25	2.3	1	-	[a:h+b+c]
172	S25M22_93	0	0	33	0	70	11	2.7	1	*	[a+h+d:b]
173	1A-Xpsp3027	31	55	26	0	0	2	0.5	2	-	[a:h:b]
174	1A-Xpsp3003	0	0	27	0	83	4	0.0	1	-	[a+h+d:b]
175	1B-Xpsp3000	24	63	20	0	0	7	3.7	2	-	[a:h:b]
176	1D-Xgwm337	32	47	28	0	0	7	1.9	2	-	[a:h:b]
177	1D-Xgwm232	21	64	24	1	0	4	3.5	2	-	[a:h:b]
178	1D-Xgwm106	0	0	27	0	86	1	0.1	1	-	[a+h+d:b]
179	1D-Xbarc169	0	0	29	0	82	3	0.1	1	-	[a+h+d:b]
180	2A-Xpsp3039	22	63	26	0	0	3	2.3	2	-	[a:h:b]
181	Xgwm311a	28	0	0	67	0	19	1.0	1	-	[a:h+b+c]
182	Xgwm311b	20	57	23	0	0	14	2.1	2	-	[a:h:b]
183	Xgwm311c	0	0	24	0	77	13	0.1	1	-	[a+h+d:b]
184	2B-Xgwm120	26	66	19	0	0	3	4.9	2	*	[a:h:b]
185	2B-Xpsp3034	22	50	18	0	0	24	1.5	2	-	[a:h:b]
186	2B-Xpsp3131	25	60	25	0	0	4	0.9	2	-	[a:h:b]
187	2D-Xgwm539	0	0	22	0	92	0	2.0	1	-	[a+h+d:b]
188	2D-Xgwm261	28	48	37	0	0	1	4.0	2	-	[a:h:b]
189	3A-Xbarc54	22	53	32	1	0	6	1.9	2	-	[a:h:b]
190	3A-Xgwm369	23	58	28	0	0	5	0.9	2	-	[a:h:b]
191	3A-Xgwm480	28	41	23	0	0	22	1.6	2	-	[a:h:b]
192	3A-Xpsp3047	30	48	33	0	0	3	2.2	2	-	[a:h:b]
193	3B-Xgwm389	29	59	10	0	0	16	11.4	2	****	[a:h:b]
194	3D-Xgwm161	0	0	39	0	74	1	5.5	1	**	[a+h+d:b]
195	4D-Xpsp3007	25	58	26	0	0	5	0.5	2	-	[a:h:b]
196	5A-Xgwm156	36	42	33	0	0	3	6.7	2	**	[a:h:b]
197	5A-Xgwm126	34	34	33	0	0	13	10.8	2	****	[a:h:b]
198	5B-Xgwm159	0	0	37	0	66	11	6.5	1	**	[a+h+d:b]
199	5D-Xgwm192	19	48	28	0	0	19	1.7	2	-	[a:h:b]
200	?-Xgwm174a	18	66	30	0	0	0	5.4	2	*	[a:h:b]
201	5D-Xgwm174b	18	65	30	0	0	1	5.1	2	*	[a:h:b]
202	6A/6D-Xbarc204a	19	60	31	0	0	4	3.5	2	-	[a:h:b]
203	6A/6D-Xbarc204b	26	0	0	85	0	3	0.1	1	-	[a:h+b+c]

204	6A-Xgwm459	0	0	37	0	72	5	4.7	1	**	[a+h+d:b]
205	6A-Xgwm570	25	56	26	0	0	7	0.3	2	-	[a:h:b]
206	6A-Xbarc113	23	0	0	90	0	1	1.3	1	-	[a:h+b+c]
207	6B-Xgwm219	28	59	17	0	0	10	4.2	2	-	[a:h:b]
208	6B-Xbarc134	27	0	0	86	0	1	0.1	1	-	[a:h+b+c]
209	6B-Xgwm132	19	0	0	91	0	4	3.5	1	*	[a:h+b+c]
210	6B-Xgwm626	28	60	23	0	0	3	1.2	2	-	[a:h:b]
211	7B-Xgwm537	24	44	32	0	0	14	2.7	2	-	[a:h:b]
212	?-Xpsp3081	32	55	26	0	0	1	0.7	2	-	[a:h:b]
213	7D-Xgwm111	28	63	19	0	0	4	3.8	2	-	[a:h:b]

APPENDIX VI – Details on QTLs detected by MQM mapping when using different markers as co-factors

Table VII-1 – Details on QTLs detected by MQM mapping, when markers *Xpsp3034* and *Xgwm120*, from linkage group 2BL, are used as co-factors.

LG	map	flanking markers	lod	mu_A	mu_H	mu_B	%expl
1DS	35.6	*S13M15_170 - Xgwm106*	7.1	3.85	6.76	7.80	43.5
2BL	50.8	*Xpsp3034 – Xgwm120*	7.7	4.94	6.17	8.39	32.1
5AL	20.5	*Xgwm126 – S20M17_168*	1.8	6.16	7.53	6.57	7.9
6AL	37.0	*S15M21_216*	2.3	5.90	7.16	5.86	8.9

LG: linkage group (chromosome)
map: the current position on the map (distance in cM)
lod: the LOD score
mu_A: the estimated mean of the distribution of the quantitative trait associated with genotype a (parent 'Chinese 166')
mu_H: idem for genotype h (heterozigous)
mu_B: idem for genotype b (parent 'Lemhi')
%expl: the percentage of the variance explained for by the QTL

Table VII-2 – Details on QTLs detected by MQM mapping, when markers *S13M15_170* and *Xgwm106*, from linkage group 1DS, are used as co-factors.

LG	map	flanking markers	lod	mu_A	mu_H	mu_B	%expl
1DS	35.6	*S13M15_170 - Xgwm106*	4.5	4.23	6.51	7.62	33.3
2BL	43.6	*S15M21_251 - Xpsp3034*	9.9	3.90	5.91	7.10	45.5
5AL	21.5	*S20M17_168*	2.9	5.75	7.33	6.19	12.0
6AL	25.8	*S12M13_114 - S15M21_215*	3.3	6.14	6.82	4.82	16.3

LG: linkage group (chromosome)
map: the current position on the map (distance in cM)
lod: the LOD score
mu_A: the estimated mean of the distribution of the quantitative trait associated with genotype a (parent 'Chinese 166')
mu_H: idem for genotype h (heterozigous)
mu_B: idem for genotype b (parent 'Lemhi')
%expl: the percentage of the variance explained for by the QTL