Phenotypic and genetic analysis of yellow rust resistance in the UK winter wheat cultivar Claire.

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A thesis submitted in fulfilment of the requirements of the University of East Anglia for the degree of Doctor of Philosophy

John Innes Centre in collaboration with Advanta-Nickerson Seeds Ltd, UK.

March 2010

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Abstract

Yellow rust resistance in the winter wheat cultivar Claire is considered durable, as Claire has been grown extensively within the UK for over 10 years and the resistance has remained effective. To characterise this potentially durable source of resistance a doubled haploid (DH) population was produced between Claire and the yellow rust susceptible cultivar Lemhi. Construction of a genetic linkage map, together with phenotypic data allowed the estimation of the number, chromosomal position and degree of effect of yellow rust resistant QTL(s). Claire was found to exhibit a quantitative, adult plant resistance phenotype (APR) controlled by two major QTLs, both located on chromosome 2DL (*QTLAPR.2Da* and *QTLAPR.2Db*) and two minor QTLs, located on 2BL (*QTLAPR.2B*) and 7BL (*QTLAPR.7B*). These QTLs act additively to achieve a resistance response that has remained effective since Claire's release in 1999. Marker loci associated with the two major QTLs and knowledge of Claire's pedigree suggests *QTLAPR.2Da* could be a APR QTL, known to confer complete immunity to yellow rust, originating from the winter wheat cultivar Alcedo and that *QTLAPR.2Db* is the durable yellow rust resistance gene *Yr16* derived from Cappelle-Desprez. However, DH lines containing *QTLAPR.2Da* exhibited either a complete or partial yellow rust resistance indicating a potential suppressor effect against the resistant phenotype conferred by this major QTL could be segregating within the population. Analysis of transcript levels of defence genes was used to examine the effect of the hypothesized suppressor on the expression of basal defence in DH lines containing *QTLAPR.2Da*. No correlation between the presence of the hypothesized suppressor and expression of basal defence genes was observed. The identification of four APR QTLs in Claire and a potential suppressor in Lemhi that affects *QTLAPR.2Da* will serve as a precursor to help elucidate the defence mechanisms of the yellow rust resistance in Claire.

Acknowledgements

Firstly, I would like to thank my supervisor Lesley Boyd for giving me the opportunity take on this PhD; I am grateful for her support and guidance throughout thick and thin. Also to all members of the Boyd lab, both past and present, who have made my time at JIC an enjoyable one. I would also like to say thank you to Ruth MacCormack, who has continually shown her support and given help wherever needed. Similarly, I am also thankful for the support of my committee members and for their useful suggestions in my research, particularly Simon Berry at Advanta-Nickerson Seeds Ltd. A big thank you goes to Eddie Arthur, who has endured many a coffee time talking about statistics, which, against the odds, he has made an enjoyable task.

However, within the JIC community, the biggest thanks go to my friend and colleague Graham McGrann for his continued support and belief. He has always been there with advice and guidance on project ideas and for this he will never know just how grateful I am. Most importantly he has kept my spirits high with countless cups of tea and motivational speeches when the going got especially tough.

I will be forever grateful for the support and love my family and friends have given me. Their love, humour, patience and understanding have been invaluable for the duration of my PhD, and always will be.

Finally, my thanks go to the JIC canteen for their delicious scones, which have brought a smile to my face on many mornings.

This thesis is dedicated to my parents, Beverly and Stephen Powell,
for their unconditional love and belief.

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Chapter one
General introduction

1.1 The host - wheat

The tribe *Triticeae* is economically the most important group of the family Gramineae, containing cereal crops grown around the world, as well as lawn and forage grasses (Kellogg, 1998). Wheat is the leading cereal grain consumed and traded in the world today. The Grain Market Report (February, 2010) of the International Grains Council (IGC) estimated global wheat production for 2009 at 675 million tonnes, the second largest harvest on record, with global consumption forecast at 643 million tonnes (http://www.igc.org.uk). The wheat area planted in the UK in 2010 has risen 11.4 % on last year to 1.94 million hectares (HGCA, UK wheat survey www.hgca.co.uk).

Wheat crops cultivated today can be traced back to their origin over 10,000 years ago, being descended from the einkorn and emmer wheat that grew wild in the Middle East, in a region known as the Fertile Crescent in the Transcaucasia corridor (Dvorak *et al*., 1998). The cultivated tetraploid wheat (*Triticum durum* AABB) derived from the wild emmer tetraploid *Triticum dicoccoides* (AABB), which in turn was derived from the natural hybridisation between diploid wild einkorn wheat (*Triticum monococcum*) and an ancestor of *Aegilops speltoides* (diploid BB; Figure 1.1). A chance hybridisation between cultivated emmer wheat (AABB) with the wild grass *Triticum tauschii* (diploid DD) gave rise to the progenitor of modern bread wheat, *Triticum aestivum* (hexaploid AABBDD) (Snape and Pánková, 2006).

Hexaploid wheat has the largest genome among agricultural crops estimated to be 16,000 Mb, with an average of 810 Mb per chromosome (Gupta *et al*., 1999), approximately eight times the size of the maize genome and forty times the size of the model cereal crop rice (Arumuganathan and Earle, 1991). More than 80% of the wheat genome consists of repetitive DNA sequences which have resulted from extensive duplication events (Roder *et al*., 1998). Even though *T. aestivum* is an allohexaploid with three genomes (designated A, B and D) each containing seven chromosomes, it behaves as a diploid. Pairing of chromosomes during meiosis occurs only between homologues of the same genome (Gill *et al*., 2004).

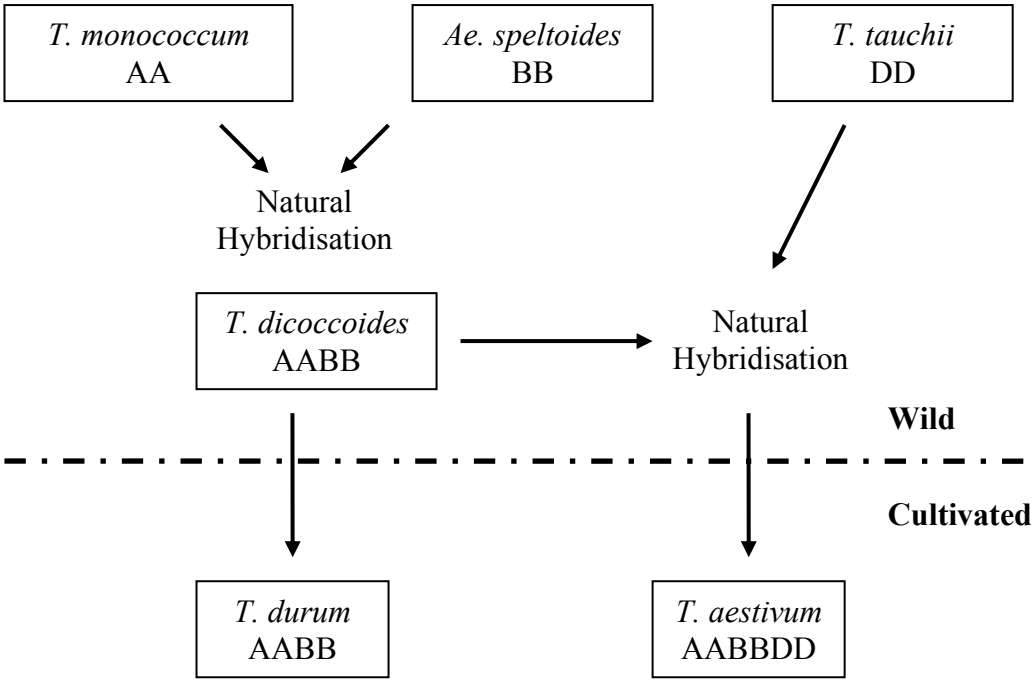


Figure 1.1 Evolution of polyploid wheat.

Eight cereals (wheat, rice, maize, barley, oats, rye, sorghum and millets) account for 66% of the worlds food supply (Dyson, 1999). An enormous improvement in the productivity of cereal crops has been achieved in recent decades. Plant breeding has lead to the introduction of hybrid maize and the Green Revolution varieties of wheat and rice averaged an increase in grain yield of 2.1% per year (Pingali *et al*., 1999). More recently, however, the average annual yield increase has declined by 0.5% as a result of limited genetic biodiversity and environmental factors. At the same time, there is a greater demand for cereal grains as a direct result of our rapidly growing global population, economic development and changing dietary habits. By the year 2030 the demand for food is expected to double, with the demand for wheat being 40% greater than the current level of 643 million tons per annum (House of Commons Environment, Food and Rural Affairs Committee, 2009, Pingali *et al*., 1999). The challenges faced in today's wheat market are as great now as they were three decades ago, before the Green Revolution.

1.2 The pathogen – rust fungi

Rust fungi are basidiomycete, biotrophic pathogens that are dependent on the host plant to complete their life cycle. The obligate biotrophic nature of rust fungi has resulted in these pathogens having evolved highly specialised relationships with their host plants, with host ranges being very specific. The three economically important rust fungi of wheat are stem (also know as black), leaf (also known as brown) and yellow (also known as stripe) rust caused by *Puccinia graminis*, *Puccinia triticina* and *Puccinia striiformis* respectively. Optimal temperature and light requirements for germination and infection differ for stem, leaf and yellow rust resulting in each rust fungus having distinct geographical distributions (Table 1.1). Environmental conditions appear to be more critical for the development of wheat yellow rust in comparison to stem and leaf rust, with this pathogen prevailing at lower temperatures (minimum 0 ˚C, optimum 11 ˚C and maximum 23 ˚C temperatures, Roelfs *et al*., 1992). Yellow rust is typically inhibited by temperatures over 23 ˚C although strains tolerant of high temperatures do exist (Milus *et al*., 2009). Yellow rust within plant tissue is able to survive the coldest UK winters and only if the host plant is killed by frost will the fungus die within the plant tissues (Roelfs, 1985). Typically, yellow rust is associated with higher elevations and northern latitudes since it is suited to cooler, wetter climates (Singh *et al*., 2002). The distribution of yellow rust is global (Saari and Prescott, 1985) being common in Northern Europe (Jagger, 2009, Melichar *et al*., 2008), the Middle East (Akat *et al*., 2007), China (Chen *et al*., 2009), Eastern and Southern Africa (Pretorius *et al*., 1997), Australia (Wellings *et al*., 2002) and America (Line, 2002).

Table 1.1 Optimal environmental conditions required for development of wheat rust (Roelfs *et al*., 1992).

Stage	Temperature ˚C			Light intensity		
	Stem	**Leaf**	**Yellow**	**Stem**	**Leaf**	**Yellow**
Germination	15-24	20	9-13	Low	Low	Low
Germ tube formation	20	15-20	10-15	Low	Low	Low
Appresorium development	16-27	15-20	Not formed	None	None	None
Stomatal penetration	29	20	9-13	High	No effect	Low
Hyphal growth	30	25	12-15	High	High	High
Sporulation	30	25	12-15	High	High	High

Yellow rust can be further subdivided into *formae speciales* depending on the specific host to which a particular form of the fungus is adapted and on which it is able to complete its asexual lifecycle. *P. striiformis* f.sp. *tritici* is capable of infecting wheat and triticale whilst *P. striiformis* f.sp. *hordei* only infects barley. Within each *formae speciales* of *P. striiformis* there are specific races that are able to propagate on particular host varieties (Anikster, 1985). Pathogen races are defined by the resistance genes of the host genotypes on which they are virulent, allowing the completion of their asexual life cycle. This plant-pathogen association is commonly described as the gene-for-gene hypothesis (Flor, 1955) (Section 1.4).

1.2.1 Rust life cycle

P. graminis and *P. triticina* have a heteroecious life cycle involving two different unrelated hosts, a microcyclic asexual stage on a gramineous host and a sexual stage which occurs on an alternative host. The sexual stage of the life cycle initiates late in the cereal growing season with mature telia producing teliospores. These teliospores remain dormant over winter, but following the onset of warm weather in the spring they develop into basidia which produce basidiospores. These basidiospores infect the alternative host plant forming after 3 – 4 days spermangonium that rupture the surface of the leaf revealing receptive hyphae and spermatia. Following fertilisation, aesciospores are produced that are wind dispersed to infect the primary cereal host.

The life cycle of *P. striiformis* was believed to be autoecious, having only a gramineous host upon which its reproduction was exclusively asexual (Figure 1.2). However, teliospores are produced by *P. striiformis* on cereals, indicating a possible sexual cycle (Roelfs *et al*., 1992). More recently evidence has been found that suggests that *Berberis* species may act as an alternative host for the wheat yellow rust pathogen (Jin *et al*., 2010).

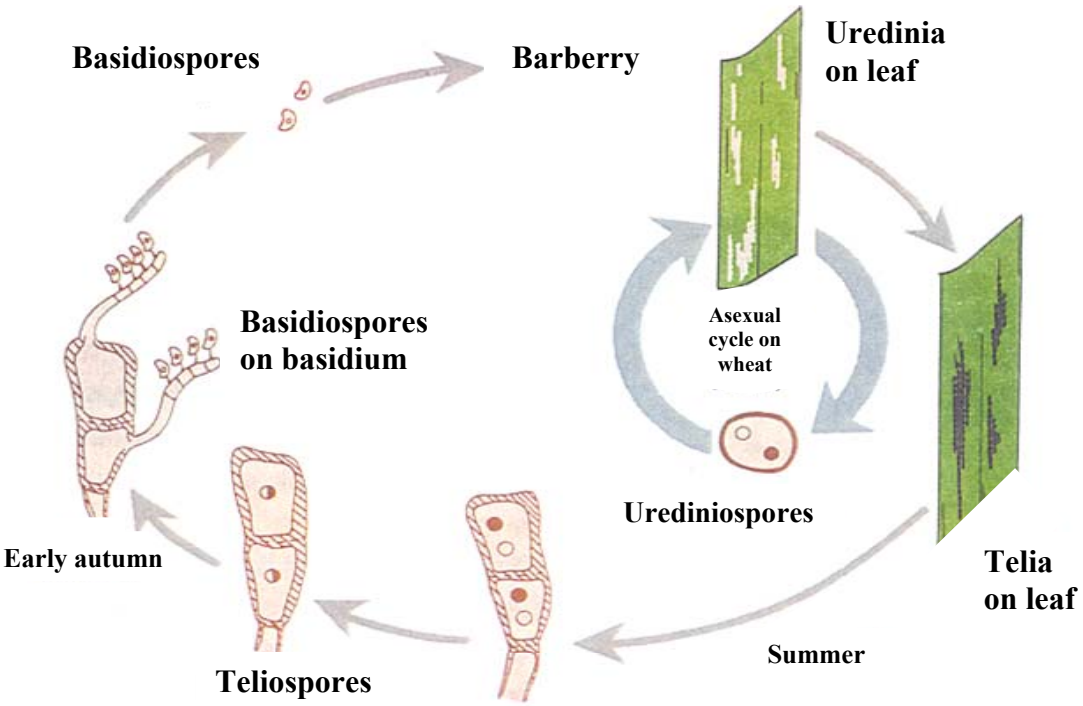


Figure 1.2 The autoecious life cycle of *Puccinia striiformis* (figure modified from Roelfs *et al.,* 1992).

1.2.2 Yellow rust infection

P. striiformis is spread by the dispersal of wind borne urediniospores released from pustules on the leaf surface. The fungus is able to survive the winter as dormant mycelium in early autumn sown crops. In the spring, particularly in cool moist weather, the fungus starts to grow and produces actively sporulating pustules. Urediniospores germinate within 12 hours (Moldenhauer *et al*., 2006, Mares and Cousen, 1977) forming germ tubes which grow perpendicular to the venation of the leaf surface (Hu and Rijkenberg, 1998). The germ tube enters the host plant through the stomatal opening, sometimes forming an appressorium above the stomata (Wang *et al*., 2007), although this is rare for *P. striiformis* (Moldenhauer *et al*., 2008, De Vallaveille-Pope *et al*., 1995). An infection peg develops from the appressorium base growing between the guard cells into the sub-stomatal cavity. Once the infection peg is inside the sub-substomatal cavity it swells to form a sub-stomatal vesical (SSV). From the SSV infection hyphae (IH) develop and grow into the mesophyll layer of the leaf. Upon contact with a mesophyll cell the tips of IH differentiate forming a

haustorial mother cell (HMC). A septum separates the HMC from the main body of the IH (Moldenhauer *et al*., 2006). A penetration peg grows from the HMC, breaching the mesophyll cell wall and forms a haustorium between the plasma membrane and cell wall (Heath, 1977). The haustoria are thought to function as feeding structures, allowing the uptake of nutrients from the living host plant cell (Hahn *et al*., 1997). Voegele *et al*. (2003) and Mendgen *et al*. (2000) reported that haustoria also act as a communication bridge, manipulating the host plant defence responses and metabolic processes.

Hyphae develop off the hyphal side of the HMC septum, growing within the intercellular space between the mesophyll cells. Further haustoria are produced when hyphae make contact with an adjacent mesophyll cell. The fungus can grow away from the initial site of infection by producing runner hyphae (RH) that extend for some distance before establishing further haustoria. Ten days post inoculation (dpi) vertical hyphae grow upwards, rupturing the leaf epidermis, followed by the emergence of pedicels bearing urediniospores. A large number of uredia pustules and urediniospores can be produced from a single infection site (Roelfs *et al*., 1992).

The complete cycle from infection to the production of new urediniospores can take as little as ten days under ideal conditions. Therefore, the disease cycle can be repeated many times in a single growing season. Parallel rows of yellow/orange coloured pustules on the leaf surface of adult plants gives the disease its characteristic names of yellow or stripe rust (Figure 1.3). Severe attacks can quickly give rise to necrotic, senescing tissue and under warm and dry conditions lead to desiccation as the pustules tear open the leaf epidermis. At the end of the growing season, telia bearing teliospores are sometimes produced from infected leaf tissues.

Figure 1.3 Wheat leaf showing symptoms of yellow rust infection (image from www.hcga.co.uk).

1.2.3 Origins, distribution and economic impact of yellow rust

Yellow rust infection has been documented in more than 60 countries around the world and on all continents excluding Antarctica. Viable urediniospores can be dispersed over 2000 miles (Staples, 2000) with long distance dispersal accomplished through human migration, enabling the pathogen to spread to new geographical areas. In 1915 yellow rust was documented in the USA (Boyd, 2005, Line, 2002), but epidemics were scarce until serious outbreaks were reported in Western USA in the 1960s (Line, 2002). Eastern Australia did not see the introduction of yellow rust until 1979 (O' Brien *et al*., 1980), believed to have been introduced from Europe on a farmers clothing (Wellings *et al*., 1988). Wind dispersal then carried the disease to

New Zealand a year later (Wellings and McIntosh, 1990). However, yellow rust was not detected in Western Australia until August 2002, again as a new introduction (Wellings *et al*., 2002). In Africa the disease was reported in Northern Zambia in 1958 (Angus, 1965), but took until 1996 to reach Southern Africa (Pretorius *et al*., 1997). This recent introduction into Southern Africa highlights that the global range of yellow rust is ever-increasing. New pathogen races with wider virulence and increased aggressiveness and adaptability are some of the underlying causes of these new introductions (Milus *et al*., 2009, Zhang *et al*., 2009).

Yellow rust is considered one of the most damaging global diseases of wheat, causing yield and quality losses as a consequence of shrivelled grains and decreased tillering (Roelfs *et al*., 1992). Most wheat producing areas have seen yield losses ranging from 10% to 70% (Chen, 2005), resulting in estimated losses of 20 million tons per annum (Kosina *et al.*, 2007). Early onset of disease, combined with good pathogen growing conditions throughout the cereal growing season can result in extreme epidemics (Dedryver *et al*., 2009). Seed produced from disease damaged crops often have low vigour and show poor emergence at germination. Increasing the yield potential of wheat is a target of all wheat breeders. Disease clearly remains a major constraint to wheat production, making resistance breeding a target of fundamental importance.

1.2.4 Control of yellow rust

A major objective of plant breeding, agronomists and the agricultural chemical industry is the control of disease epidemics (Martin *et al*., 2003). In many parts of the developed world plant diseases have been controlled through the use of agrochemicals as well as disease resistant varieties. In contrast to agrochemicals, breeding for disease resistance is an economical and environmentally safe measure to reduce crop losses.

1.3 Durable resistance

Resistance has been deliberately incorporated into new wheat cultivars through conventional breeding strategies since the discovery of the genetic control of

resistance to yellow rust in the cultivar Rivet (Biffen, 1905). Whilst this approach initially proved effective in controlling yellow rust outbreaks, it soon became apparent that over time the resistance in some cultivars broke down (Johnson, 1983), whereas other cultivars, such as Cappelle-Deprez (Johnson, 1983) and Camp Remy (Boukhatem *et al*., 2002) were grown extensively for many years and retained a level of resistance. The term 'durable resistance' was introduced by Dr. Roy Johnson and describes resistance that remains effective in a cultivar during its widespread cultivation, over a long period of time, in an environment favourable to the disease (Johnson, 1981, Johnson and Law, 1973). Breeding strategies strive to accumulate sources of durable resistance within modern wheat cultivars to control yellow rust.

1.4 Seedling resistance

Seedling resistance genes are expressed from an early growth stage of the plant's development (Dyck and Kerber, 1985), conferring a completely resistant phenotype which is easy to select for in breeding programs. This type of resistance is however normally race-specific, the resistance gene conforming to the gene-for-gene hypothesis (Flor, 1971, 1956, 1955). The gene-for-gene hypothesis refers to the specific plant-pathogen interactions between the host resistance gene (R-gene) and a corresponding avirulence (*avr*) gene in the pathogen, resulting in an incompatible interaction (McDowell and Woffenden, 2003, Dangl and Jones, 2001). Usually a hypersensitive defence response is triggered by this incompatible interaction. A compatible interaction results if either the R-gene or the corresponding *avr* gene is absent (Hammond-Kosack and Jones, 1997).

In the last decade several R-genes have been cloned and sequenced from many plant species (Dangl and Jones, 2001). These R-genes can be divided into five classes based on the domains within the predicted amino acid sequence (Martin *et al*., 2003, Aarts *et al*., 1998 Table 1.2). Research into the molecular mechanisms underlying disease resistance has been accelerated by the identification of numerous plant R-genes (Martin *et al*., 2003, Cohn *et al*., 2001, Martin, 1999). R-gene class one is characterised by a serine/threonine kinase region and a myristylation motif at the N-terminus (Martin *et al*., 1999). The presence of a nucleotide binding site (NBS), a stretch of leucine rich repeats (LRR) and an N-terminal putative leucine zipper (LZ)

or coiled–coil (CC) sequence, classifies class two R-genes (Martin *et al*., 2003). R-genes of class three are similar to that of class two, except the CC sequence contains a protein region of similarity to the Toll and Interleukin 1 Receptor (IL-1R) known as the TIR region. These three classes of R-genes are thought to be localised intracellularly as they lack a transmembrane domain. In R-gene classes four and five the lack of the NBS region is replaced by a transmembrane domain which locates the LRR region extracellularly. Class four R-genes are distinguished by a small cytoplasmic tail, whilst class five R-genes have a serine/threonine kinase region (Dixon *et al*., 1996, Jones *et al*., 1994).

Table 1.2 Major classes of seedling R-genes cloned from plant species.[1]

R-gene class	Species	R protein	Pathogen
1	Arabidopsis	*PBS1*	*Pseudomonas. syringae*
	Tomato	*Pto*	*P. syringae*
2	Arabidopsis	*RPS2, RPM1, RPS5*	*P. syringae*
	Arabidopsis	*RPP8, RPP13*	*Peronospora parasitica*
	Barley	*Mla1, Mla6*	*Blumeria graminis*
	Lettuce	*Dm3*	*Bremia lactuae*
	Maize	*Rp1-D*	*Puccinia sorghi*
	Pepper	*Bs2*	*Xanthomonas campestris*
	Potato	*R1*	*Phytophthora infestans*
	Potato	*Rx1, Rx2*	Potato virus X
	Rice	*Pi-ta*	*Magnaporthe grisea*
	Tobacco	*N*	Mosaic virus
	Tomato	*Sw-5*	Tomato spotted wilt virus
	Tomato	*Mi*	*Macrosiphum euphorbiae*
	Wheat	*Lr21, Lr10*	*P. triticina*
	Wheat	*Pm3b*	*Erysiphe graminis*
3	Arabidopsis	*RPP1, RPP4, RPP5*	*P. syringae*
	Flax	*M, L, P*	*Melampsora lini*
4	Tomato	*Cf-2, Cf-4, Cf-5, Cf-9*	*Cladosporium fulvum*
5	Rice	*Xa21*	*X. oryzae*

[1] Table compiled from Hammond-Kosack and Kanyuka, 2007, Martin *et al*., 2003, Hammond-Kosack and Jones, 1997.

The majority of the seedling R-genes that have been cloned fall into class two. In many plants species, NBS-LRR genes have been shown to cluster within the genomes, usually in the highly recombinogenic subtelomeric ends (Bai *et al*., 2002,

Meyers *et al*., 1999). The clustered arrangement of these genes may be a critical attribute, allowing the generation of novel resistance specificities via recombination or gene conversion (Hulbert *et al*., 2001). However, recombination immediately around R-genes is usually suppressed, conserving genomic regions of functional importance (Meyers *et al*., 1999). The LRR motif has been implicated in protein-protein interactions, being a major determinant of recognition specificity of the pathogen *avr* proteins (Dodds *et al*., 2001, Ellis *et al*., 1999, Meyers *et al*., 1999, Dixon *et al*., 1996). However the majority of R-proteins do not interact directly with *avr* proteins (Tornero *et al*., 2002, Axtell *et al*., 2001, Kajava, 1998). The NBS region has been proposed to control plant cell death, NB-ARC domains being activated following LRR dependent recognition of the pathogen (Van der Biezen and Jones, 1998).

1.4.1 Defence response

An array of defence mechanisms accompanies plant recognition of a pathogen (Kombrink and Somssich, 1995). Changes in protein phosphorylation are one of the first responses of the host plant (Yang *et al*., 1997). In addition to phosphorylation rapid ion fluxes, the export of Cl^- and K^+ and influx of Ca^{2+} and H^+ are seen (Dangl *et al*., 1996, Levine *et al*., 1996, Atkinson *et al*., 1990). This flux of charged ions causes the host plant cell to acidify and with the accumulation of reactive oxygen species (ROS) within the cell leads to the production of hydrogen peroxide (H_2O_2) resulting in an oxidative burst (Hammond-Kosack and Jones, 1997, Wojtaszek, 1997). Plant cells are known to be directly killed by the accumulation of H_2O_2 (Peng and Kuc, 1992).

ROS and H_2O_2 play a variety of roles within the cell leading to cell death (Lamb and Dixon, 1997). They can function directly, having antimicrobial activity (Brisson *et al*., 1994, Bradley *et al*., 1992), have signalling roles as messengers that trigger fluxes of ions and the production of secondary metabolites (Levine *et al*., 1996), as well as being implemented in defence gene induction (Mellersh *et al*., 2002). Cross linking of proteins within the cell wall occurs in the presence of ROS (Brisson *et al*., 1994, Bradley *et al*., 1992) providing reinforcement (Bolwell, 1996). All these roles could contribute to the inhibition of hyphal establishment and haustorial

development, as well as reducing the amount of nutrients made available to the pathogen from the host plant cell.

Other major biochemical defence responses associated with resistance include the synthesis of phytoalexins (Pedras *et al*., 2000). Phytoalexins are antimicrobial molecules that accumulate rapidly around sites of incompatible interactions (Maleck and Lawton, 1998, Hammond-Kosack and Jones, 1996). Many novel plant proteins are also induced as part of the plant defence response, being referred to as pathogenesis related (PR) proteins (van Loon and van Stien, 1999). Many have antifungal properties, including the enzymes chitinase and β-1, 3 glucanase which can directly degrade the invading pathogen cell walls (Melchers *et al*., 1994).

1.4.2 Seedling yellow rust resistance genes

To date 49 resistance genes have been identified in wheat and designated a permanent *Yr* number (Tables 1.3 and 1.5), the majority seedling expressed and being race-specific. The large number of seedling resistance genes identified goes some way to explain the common breakdown of resistance in wheat cultivars (Table 1.4). Therefore, when cultivars containing the same seedling resistance genes are deployed over large areas resistance breakdown can lead to large scale epidemics (Rosewarne *et al*., 2006).

Table 1.3 Classified seedling yellow rust resistance genes

Yr gene	Chromosome	Source	Reference
1	2AL	Chinese 166	McIntosh and Arts, 1996
2	7B	Heines VII	Labrum, 1980
3a	1B	Cappelle-Deprez	Lupton and Macer, 1962
3b	1B	Hybrid 46	Lupton and Macer, 1962
3c	1B	Minister	Lupton and Macer, 1962
4a	6B	Cappelle-Deprez	Lupton and Macer, 1962
4b	3B	Hybrid 46	McIntosh *et al*., 1995
5	2BL	*Triticum spelta*	Law, 1976
6	7BS	Heines Kolbern	El-Bedewy and Röbbelen, 1982
7	2BL	*Triticum turgidum*	McIntosh *et al*., 1981
8	2D	*Triticum comosa*	Riley *et al*., 1968
9	1BL	*Secalis cereale*	Mettin *et al*., 1978
10	1BS	Moro	Metzger and Silbaugh, 1970
15	1BS	*T. dicoccoides*	McIntosh *et al*., 1996
17	2AS	*Triticum ventricosa*	Bariana and McIntosh, 1993
19	5B	Compair	Chen *et al*., 1995
20	6D	Fielder	Chen *et al*., 1995
21	1B	Lemhi	Chen *et al*., 1995
22	4D	Lee	Chen *et al*., 1995
23	6D	Lee	Chen *et al*., 1995
24	1BS	*T. turgidum*	McIntosh and Lagudah, 2000
25	1D	TP1295	Calonnec and Johnson, 1998
26	1BS	*Haynaldia villosa*	Ma *et al*., 2001
27	2BS	Selkirk	Singh *et al*., 2003
28	4DS	*T. tauschii*	Singh *et al*., 1998
31	2BS	Lalbahadur	Singh *et al*., 2003
32	2AL	Carsten V	Eriksen *et al*., 2004
33	7DL	Batavia	McIntosh *et al*., 2003
34	5AL	WAWHT2046	Bariana *et al*., 2001
35	6BS	*T. dicoccoides*	Marais *et al*., 2005a
37	2DL	*Aegilops kotschyi*	Marais *et al*., 2005b
38	6A	*Aegilops sharonenses*	Marais *et al*., 2006
40	5DS	*Aegilops geniculata*	Kuraparthy *et al*. 2007
41	2BS	Chuannong	Luo *et al*., 2008
42	6AL	*Aegilops neglecta*	Marais *et al*., 2009
43	2BL	IDO377	Cheng and Chen, 2010
44	2BL	Zak	Cheng and Chen, 2010
45	3DL	PI181434	Li *et al*., 2009

The race-specific resistance genes *Yr6*, *Yr9* and *Yr17*, deployed in the cultivar Brigadier illustrate the most recent example of yellow rust resistance breakdown Table 1.4). At the time of cultivar release virulence to *Yr6* and *Yr9* were already present within the UK *P. striiformis* population, as both R-genes had been deployed extensively in UK cultivars. Virulent isolates for *Yr17* were found in 1996 in the UK (Bayles *et al*., 2000). Even pyramiding several race-specific resistance genes is only partially more effective (Parlevliet, 1995). The resistance is still vulnerable to breakdown, as the pathogen continues to mutate to virulence against multiple resistance genes (McDonald and Linde, 2002a, b).

Table 1.4 Breakdown of yellow rust resistance in major cultivars grown within the UK since 1955.

Cultivar	Year of cultivar release	Year of resistance breakdown	*Yr* genes in cultivar
Brigadier	1993	1996	*Yr6, Yr9, Yr17*
Hornet	1987	1988	*Yr2, Yr6, Yr9*
Stetson	1983	1983	*Yr1, Yr9*
Clement	1975	1975	*Yr2, Yr9*
Talent	1971	1972	*Yr7*
Maris Ranger	1968	1969	*Yr3a, Yr4a, Yr6*
Rothwell Purdix	1964	1966	*Yr1, Yr2, Yr6*
Heines VII	Unknown	1955	*Yr2*

1.5 Adult plant resistance (APR)

The term APR describes resistance that is expressed at post seedling growth stages (Bariana *et al*., 2001). Race-specific seedling resistance genes do not provide long term protection against disease, therefore in recent years breeding efforts have focussed on non-specific APR (Chen, 2005, Line, 2002). Cultivars that contain only APR show a susceptible phenotype at seedling growth stages. The cultivars Cappelle-Deprez (Johnson, 1983) and Camp Remy (Boukhatem *et al*., 2002) comply with Dr. Roy Johnson's definition of 'durable resistance' having been found to possess APR genes for yellow rust. In wheat currently eleven APR genes for yellow rust have been formally classified (Table 1.5) although many more have been reported.

Table 1.5 Classified yellow rust APR genes

Yr gene	Chromosome	Source cultivar	Reference
11	-	Joss Cambier	Priestley, 1978
12	-	Mega	Priestley, 1978
13	-	Maris Huntsman	Priestley, 1978
14	-	Hobbit	Priestley, 1978
16	2D	Cappelle-Deprez	Worland and Law, 1986
18[1]	7DS	Frontana	Suenega *et al*., 2003
29	1BL	Lalbahadur	Bariana *et al*., 2001
30	3BS	Opata35	Singh *et al*., 2001
36[2]	6BS	*Triticum dicoccoides*	Uauy *et al*., 2005
39[2]	7BL	Alpowa	Lin and Chen, 2007
46[2]	2DS	Compair	Chen and Zhao, 2007

[1]Low temperature induced seedling resistance, [2]High temperature APR (HTAP).

Genetic analysis of cultivars with APR in general finds the resistance to be conferred by the additive effects of several minor genes (Singh *et al*., 2005, Navabi *et al*., 2004). Individually APR genes express a partially resistant phenotype (Singh *et al*., 2005), while stacking multiple APR genes can achieve a complete resistant phenotype (Singh *et al*., 2005). Some cultivars exhibit a slow rusting phenotype (Rosewarne *et al*., 2006), the pathogen developing at a slower rate than if the resistance gene was absent. Slow rusting resistance genes have proven to be durable, e.g. the linked APR genes effective against yellow rust (*Yr18*) and brown rust (*Lr34*) and have remained durable for more than 50 years (Spielmeyer *et al*., 2008, Lagudah *et al*., 2006, Singh *et al*., 2003 Sawhney, 1998). The complex quantitative inheritance of APR makes genetic analysis a challenging task. A statistical quantitative trait loci (QTL) analysis is often used to predict the number and chromosomal location of genes contributing to APR and the proportion of the phenotypic variance accounted for by each gene. QTL analysis is discussed further in Section 6.1.

Yellow rust APR genes are often more sensitive to environmental variables compared to seedling resistance genes. Expression of yellow rust resistance at higher temperatures have been documented, however relatively few reports have been published characterising high temperature APR (HTAP) in wheat (Carter *et al*., 2009, Lin and Chen, 2009, 2007, Santra *et al*., 2008, Uauy *et al*., 2005, Chen and

Line, 1995). This type of resistance proves most effective during day temperatures of 25 - 30˚C and night temperatures that average 10˚C (Qayoum and Line, 1985 and 1984). Mild winters, followed by cool, wet springs and dry summers typical of California and the Pacific Northwest USA provide yellow rust with ideal conditions (Carter *et al*., 2009). In the USA many wheat cultivars including Louise (Carter *et al*., 2009), Express (Lin and Chen, 2009), Stephens (Santra *et al*., 2008) and Alpowa (Lin and Chen, 2007) have been reported to contain HTAP resistance. The cooler climate within the UK does not provide suitable environmental conditions for these types of resistance to be effective (Johnson, 1983).

1.6 DNA marker systems

The development of comprehensive genetic maps was not possible until the introduction of DNA based molecular marker technologies. DNA markers are relatively simple tools that are completely independent of plant environmental conditions and can be detected at any stage of development. DNA markers can be either dominant or co-dominant (Table 1.6). Dominant markers detect only one allele per locus, while co-dominant markers generally have the potential to reveal all alleles of a locus (Gupta *et al*., 1999).

Restriction Fragment Length Polymorphisms (RFLPs) was the first DNA marker technology to be developed (Botstein *et al*., 1980). This hybridisation based technique uses the variation in the length of DNA fragments following digestion of genomic DNA with one or more restriction enzymes. The DNA fragments are separated by size electrophoretically and detected following hybridisation of a fluorescent or radio labelled genomic or complementary DNA probe. Size polymorphisms result from differences in restriction enzyme site locations between genotypes. The amount of DNA required is large (5-10 μg), but a single Southern blot can be re-probed many times making the technology very efficient (Rafalski and Tingey, 1993). Genetic maps have been developed in wheat that utilise RFLPs (Gale *et al*., 1995, Devos *et al*., 1993).

Table 1.6 Summary of the relative attributes of DNA marker technologies.

	Co-dominant			Dominant	
Marker	**RFLP**	**SSR**	**SNP**	**AFLP**	**RAPD**
Principle	Endonuclease restriction Southern blotting	PCR of simple sequence repeats	Direct sequencing, electronic SNPs	PCR amplification of genomic restricted fragments	DNA amplification with random primers
Type of Polymorphism	Single base changes, insertions, deletions	Changes in length of repeats	Single base changes	Single base changes, insertions, deletions	Single base changes, insertions, deletions
Level of polymorphism	Medium	High	Very high	Very high	High
Amount of DNA required	High	Low	Low	Medium	Low
PCR based	No	Yes	Yes	Yes	Yes
Ease of automation	Low	High	High	Low	Medium
Reliability	High	High	High	Medium	Low
Sequence information	No	Yes	Yes	No	No
Procedure time	High	Low	Low	Medium	Low
Cost per analysis	Medium	Low	Low	Medium	Low
Development cost	Medium	High	High	Low	Low

Even though the RFLP assay has been the choice for many species to measure genetic diversity and construct genetic linkage maps, this technique is time consuming and laborious. Polymerase chain reaction (PCR) technology has become a widespread research tool and has led to the development of several novel genetic assays based on selective amplification of DNA. The discovery that the PCR along with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers. Random Amplification of

Polymorphic DNA (RAPD) uses genomic DNA primed by an arbitrary oligonucleotide primer, resulting in the amplification of several discrete DNA products between regions homologous to the primer sequences (Rafalski and Tingey, 1993). Polymorphisms arise when a genotype has lost or gained a primer recognition site or the amplified sequence contains a deletion or insertion. RAPD offer a fast, cheap and easy to perform marker technology, but they lack reproducibility, especially in wheat (Jones *et al*., 1997). RAPD is sensitive to reaction conditions, DNA quality and PCR temperature profiles, limiting their application in wheat, along with a lack of polymorphism in the wheat genome (Mohan *et al*., 1997, Koebner, 1995).

Simple sequence repeats (SSR) also known as microsatellites are stretches of DNA consisting of tandem repeating mono-, di-, tri-, tetra-, or penta-nucleotide units that are arranged throughout the genome (Powell *et al*., 1996). Polymorphism is revealed by PCR amplification using two unique short primers that match the SSR flanking regions and hence define the SSR locus. The co-dominant nature of SSRs allows homozygote and heterozygote loci to be distinguished. Chapter 4 discusses the use of SSR marker technologies in further detail.

The DNA fingerprinting technique Amplified Fragment Length Polymorphism (AFLP) was developed in 1993 (Vos *et al*., 1995). Restriction fragments for amplification are generated by two restriction enzymes, a rare cutter and a frequent cutter. The procedure results in predominant amplification of those restriction fragments which have a rare cutter sequence on one end and a frequent cutter sequence on the other end. Adapters are ligated to the ends of the DNA fragments to generate template DNA for amplification. The sequence of the adapter and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments (Vos *et al*., 1995). Selective nucleotides are included at the 3' ends of the PCR primers resulting in amplification only if the DNA sequence contains the complementary nucleotide sequence, this way large numbers of polymorphic fragments are generated per PCR (Gupta *et al*., 1999). The level of polymorphism detected by AFLP in wheat is high in comparison to other available marker systems (Mohan *et al*., 1997). A single AFLP primer combination can detect

up to eight times more polymorphisms than a polymorphic RFLP marker (Gupta *et al*., 1999).

Several conserved motifs, such as the P-loop (phosphate binding) and kinase-2, have been identified within the NBS domain of R-genes (Figure 1.4). The identification of conserved domains has enabled marker systems to be developed that allow specific targeting of R-genes (van der Linden *et al*., 2004). NBS-specific primers have been developed from alignments of the conserved motifs. Slightly different primer positions and nucleotide compositions can result in different NBS profiles. The advantage of NBS profiling compared to other marker technologies is its gene targeting nature, with the possibility of producing markers linked to R-genes and R-gene clusters (van der Linden *et al*., 2004)

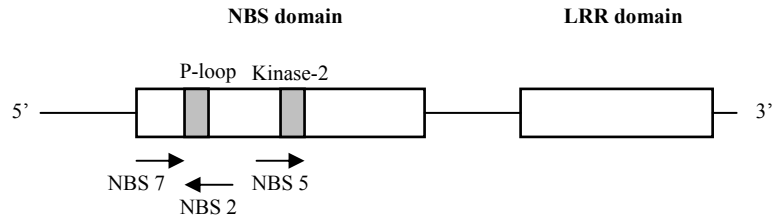


Figure 1.4 NBS domain of disease resistance genes with relative position of NBS 2, NBS 5 and NBS 7 primer.

Single Nucleotide Polymorphisms (SNP) represent sites where the DNA sequence differs by a single base (Gupta *et al*., 2001), such polymorphisms being considered to be of high abundance within the wheat genome (Koebner and Summers, 2003). Numerous approaches for SNP discovery have been described, the main ones being based on comparison of locus-specific sequences generated from different genotypes. The simplest way to detect SNP's, when targeting a defined region containing a candidate gene, is to perform direct sequencing of genomic PCR products obtained in different individuals. Depending on the frequency of polymorphisms present in the germplasm under investigation, it may be beneficial to pre-screen amplicons for the presence of polymorphisms. Several methods may be employed, including denaturing high pressure chromatography, Single Strand Conformation Polymorphism (SSCP) or one of several chemical or enzymatic cleavage methods.

Recently the development of the molecular technique Diversity Array Technology (DArT; Figure 1.5) has been applied to rice (Jaccoud *et al*., 2001), barley (Wenzl *et al*., 2004) and wheat (Akbari *et al*., 2006, Semagn *et al*., 2006c). Essentially DArT is a microarray hybridisation technology for AFLP. Genomic representations of the wheat genotypes to be screened are prepared by restriction enzyme digestion, followed by adapter ligation and selective primer amplification (Figure 1.5 a). PCR amplicons are purified and hybridised to the DArT array (Jaccoud *et al*., 2001). The DArT microarray chip contains a diversity of AFLP fragments from genetically distinct wheat genotypes allowing polymorphic clones (DArT markers) to be distinguished based on differential hybridisation intensities. Two samples can be screened in the same analysis by labelling each with a different fluorescent dye (Figure 1.5 b).

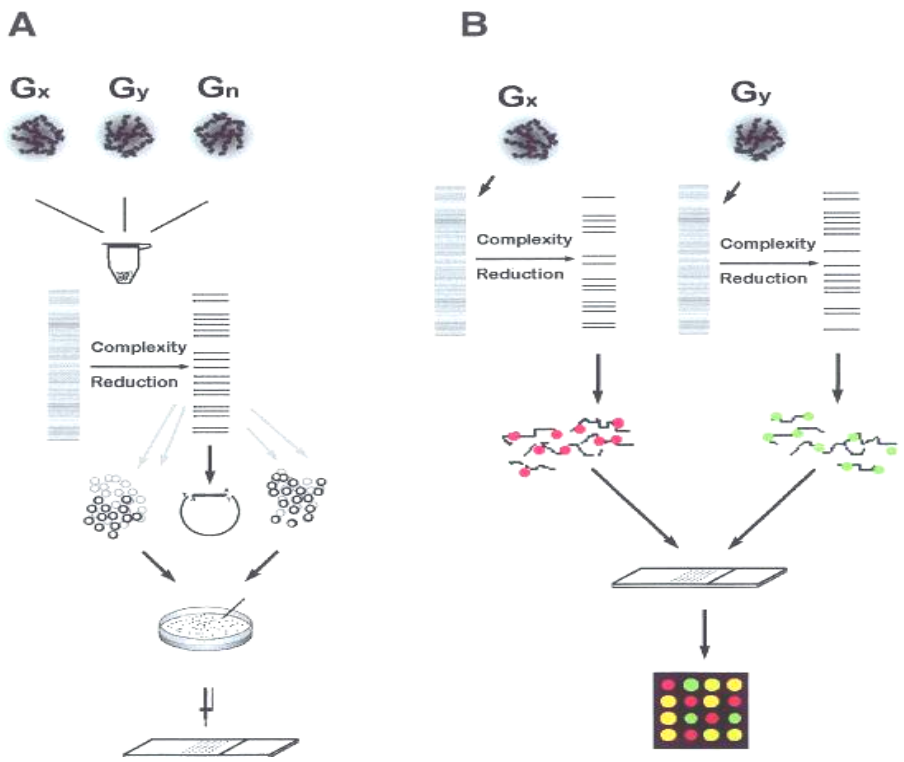


Figure 1.5 (**a**) Schematic representation of the development of the DArT array. (**b**) Methodology of the DArT array when screening for polymorphism between genotypes. Individual plant genotypes are labelled with a green or red fluorescent dye (Jacoud *et al*., 2001).

The advantages and disadvantages of the marker technologies used in this study are discussed in detail in Section 4.1.

1.6.1 Comparative genomics within the grass family *Poaceae*

Over the past 20 years plant comparative genomics has shown that the organisation of genes within genomes has remained more conserved over evolutionary periods than previously thought (Gales and Devos, 1998). One of the virtues of comparative genomics lies in the transfer of structural and functional information from one genome to another (Vandepoele *et al*., 2002). The similarities between two genomes can be investigated either at the macro or microsyntenic level. Macrosyntenic studies focus on genomes as a whole, analysing large regions by comparing the order of genes and genetic markers. Comparative genomic mapping has demonstrated that plants have retained different levels of conservation in their genomes during evolution depending on their phylogenetic separation (Paterson *et al*., 2000). These conserved regions (syntenic or orthologous) have co-linear gene contents when compared either genetically or physically. Microsyntenic studies identify smaller regions of colinearity between genomes, allowing the order and orientation of coding sequences, as well as the non-coding DNA sections to be investigated.

The grass family *Poaceae* contains over 10,000 species including the major cereals rice, wheat, maize, barley, rye and millet (Kellogg, 2001). Members of this grass family diverged evolutionary around 60 million years ago resulting in a variety of genome sizes, differing as much as 40-fold amongst the family (Gale and Devos, 1998). Despite this diversity a remarkable degree of genome conservation has been detected by comparative genetic mapping studies in the *Poaceae* family (Gale and Devos, 1998, Moore *et al*., 1995). Early investigations demonstrated the extent of synteny among the cereal genomes, exploring the relationship between rice and maize (Ahn and Tanksley, 1993), the alignment of the wheat genome with rice (Kurata *et al*., 1994) and the ability to combine the maps (Moore *et al*., 1995). The synteny within the *Poaceae* family was used to produce a circularised consensus map, comprising the major members of the family *Poaceae* (Devos and Gale, 1997). The alignment in Figure 1.6 is based upon rice (located in the centre) with the other grass genomes arranged relative to rice so that any radius passes through

homoeologous (orthologous) genes. Any particular radius taken on the consensus grass map can be used to predict the position of orthologous genes in other cereals (Gale and Devos, 1998).

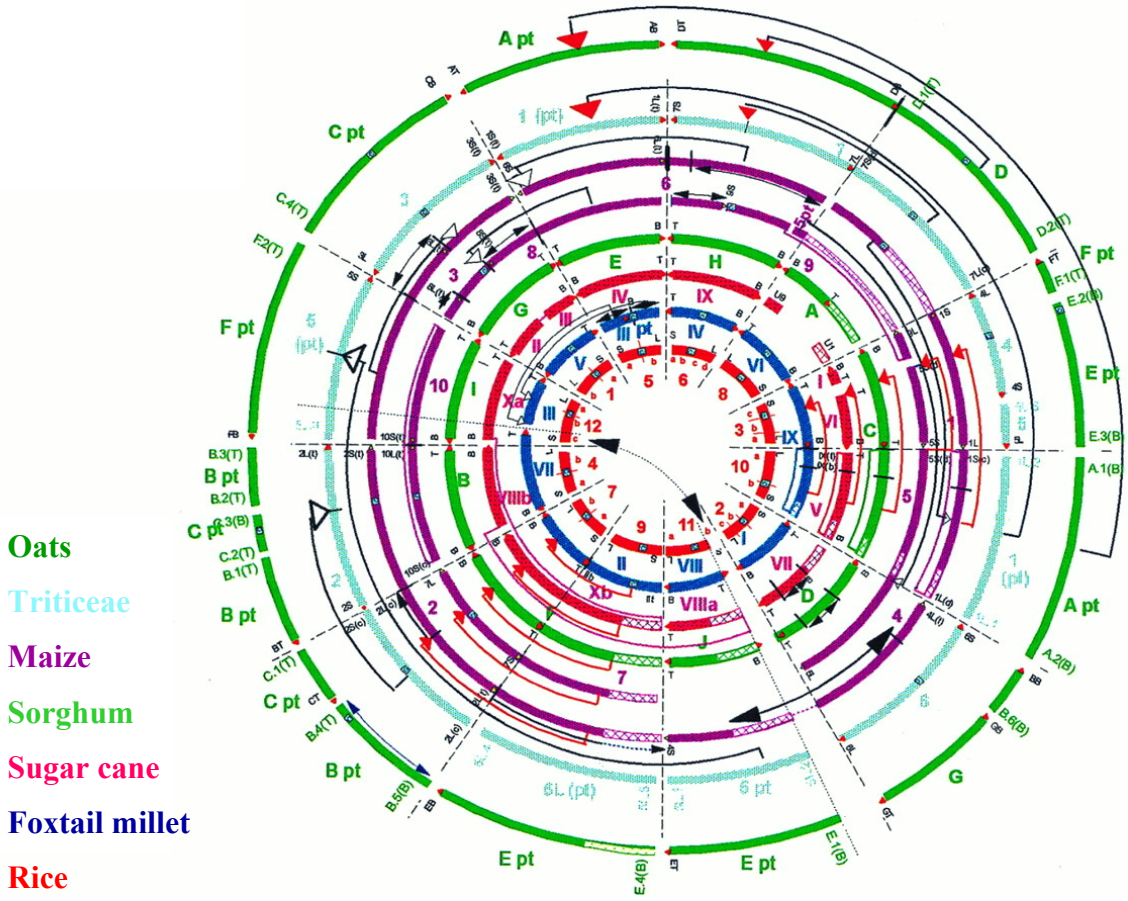


Figure 1.6 Consensus grass comparative map of six major grass crop species (Gale and Devos, 1998). Oats, triticeae, maize, sorghum, sugar cane, foxtail millet grass species are arranged relative to the rice genome which is located centrally.

1.6.2 Marker Assisted Selection

Conventional cereal breeding is time consuming. It can typically take between 8-10 years to breed a new variety and even then the commercial release cannot be guaranteed. Molecular marker technology provides tools that can make the breeding procedure more efficient, improving selection strategies. Constant improvement of marker detection systems and the identification of markers linked to useful traits can aid the introgression of more favourable genes into new cultivars. There is now the potential to intentionally pyramid yellow rust resistance genes by marker-assisted

selection (MAS) (Paterson *et al*., 1991). MAS has made a significant contribution in efforts to efficiently screen for resistance early in the breeding process. Breeders are able to select indirectly for the genetic determinant of a trait of interest (linked genetic markers to an allele) without the need for phenotyping. DNA markers must meet certain essential criteria to be useful in a MAS breeding programme. Marker loci must be tightly linked to the gene of interest, be diagnostic in a wide range of valuable germplasm and usable in a high throughput, reproducible and cost effective screening procedure (Gupta *et al*., 1999). The current yellow rust resistance genes tagged with a closely linked genetic marker are shown in Table 1.7.

Table 1.7 Yellow rust resistance genes for which linked molecular markers are reported.

***Yr* gene**	**Chromosomal location**	**Resistance**	**Linked molecular marker locus**	**Reference**
*Yr5/Yr7**	2BL	Seedling	*Xwgp17, Xwgp18*	Yan *et al*., 2003
Yr9	1B	Seedling	*Xwgp4, Xwgp7, Xwgp8, Xwgp9*	Shi *et al*., 2001
Yr10	1BS	Seedling	*Xpsp3000, S26M47-150*	Wang *et al*., 2002
Yr15	1BS	Seedling	*Xgwm33, UBC199*$_{700}$	Smith *et al*., 2002 Chagué *et al*., 1999
Yr17	2AS	Seedling	*SC-Y15*	Robert *et al*., 1999
Yr18	7DS	Adult	*Xgwm295*	Lagudah *et al*., 2009
Yr24	1BS	Seedling	*Xgwm11*	Zakari *et al*., 2003
Yr26	1BS	Seedling	*Xgwm11, Xgwm18*	Ma *et al*., 2001
Yr28	4DS	Seedling	*Xbcd265, Xmwg634*	Singh *et al*., 2000
Yr32	2AL	Seedling	*Xwmc198, M62/P19-156, M59/P37-375*	Eriksen *et al*., 2004
Yr34	5AL	Seedling	*Xgwm6*	Bariana *et al*., 2006
Yr36	6BS	Adult	*Xucw129, Xucw148*	Fu *et al*., 2009
YrH52	1BS	Seedling	*Xgwm413, Xgwm273a*	Peng *et al*., 1999

*allelic (Zhang *et al*., 2009).

Pyramiding of resistance genes is now possible where conventional breeding methods failed if suitable races of the pathogen were not available to test for the presence of each resistance gene (Kumar *et al*., 2008). Genetic markers will now aid the development of wheat cultivars carrying durable yellow rust resistance (Song *et al*., 2005).

1.7 Yellow rust resistance with UK wheat breeding

The French yellow rust resistant wheat cultivar Cappelle-Desprez conforms to Johnson's description of durable resistance. Cappelle-Desprez (*Yr16*$^{+}$) occupied more than 80% of the total UK wheat area for more than 10 years and remained resistant to yellow rust during this time (Parry, 1990). The German yellow rust resistant winter wheat cultivar Alcedo remained resistant over the 15 years it was grown, at its peak occupying 47% of German wheat acreage in 1981 (Meinel, 1997). It is believed that Cappelle-Desprez and the German cultivars Carstens V and Alcedo are the progenitors of many modern European cultivars and the source of some of the known seedling and APR genes that confer yellow rust resistance today (Angus, 2001; Figure 1.7). Carstens V carries both seedling and adult plant expressed yellow rust resistance genes, although in the UK all the seedling yellow rust resistance genes have been overcome. Three genes for seedling resistance identified using UK *P. striiformis* isolates were characterised in Carsten V (Calonnec *et al*., 2002), one was designated *Yr32* (Eriksen *et al*., 2004) and another an allele of *Yr25* (Calonnec *et al*., 2002). The yellow rust APR in Carstens V is considered a potential source of durable resistance, which may still be conferring effective field resistance in many modern cultivars (Lewis, 2006).

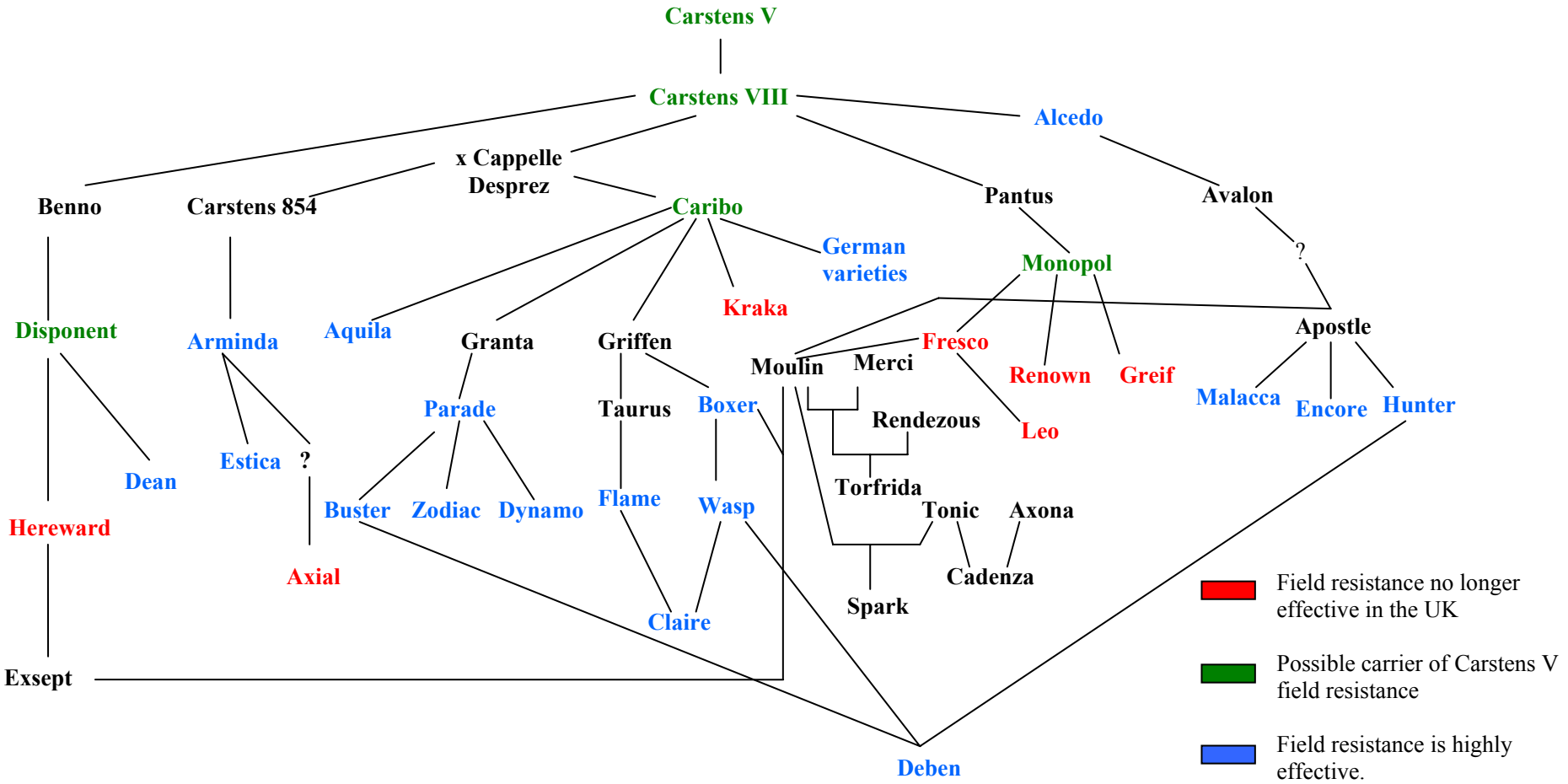


Figure 1.7 Pedigree illustrating the relationship between UK winter wheat cultivars.

1.8 Research summary

This thesis aims to determine the genetic basis of the yellow rust seedling and APR in the UK winter wheat cultivar Claire. Using a doubled haploid (DH) population from a cross between Claire and the susceptible spring wheat cultivar Lemhi (USA) the phenotypic traits percentage infection (PI) and infection type (IT) will be assessed together with height and latent period. A genetic linkage map will be developed using DArT, SSR, NBS, AFLP and EST-derived markers. Phenotypic data sets, in combination with the genetic map will be used in QTL analysis to determine the number and location of seedling and APR genes segregating within the population.

Chapter 2
Materials and Methods

2.1 Plant Material

A DH population consisting of 77 lines from a cross between the yellow rust resistant cultivar Claire and the susceptible cultivar Lemhi was supplied by Dr. L. A. Boyd at the John Innes Centre.

2.2 DNA extraction

All seed material was surface sterilised by agitation in 70% ethanol for 10 seconds followed by a 10 minute wash in 20% commercial bleach and rinsing twice with sterile dd.H_2O. The seed was cold shocked at 4°C for 24 hours and germinated for 3 days at 20°C. A 0.5cm piece of coleoptile was harvested for DNA extraction. The 0.5cm of coleoptile was incubated at 65°C for one hour in 400 μl of extraction buffer (200 mM Tris.HCL pH 7.5, 250 mM NaCl, 25 mM EDTA. 0.5 % SDS) which had been pre-heated to 60°C. After incubation, 135 μl of 5 M CH_3COOK was added and the samples vortexed. The extractions were chilled on ice for 10 minutes and centrifuged at 4°C at 13,000 *g* for 10 minutes. The supernatant was transferred to a clean eppendorf, 0.8 volumes (approximately 400 μl) of isopropanol added and the DNA precipitated at -20°C for 30 minutes. Following centrifugation at 13,000 *g* for 15 minutes the pellet was washed twice with 70% ethanol. The DNA pellet was then air dried and re-suspended in 100 μl TE-buffer. DNAeasy kit (Qiagen, Hilden, Germany) was also used for DNA extraction from freshly germinated leaf material following the manufacturer's instructions.

2.3 Marker analysis

2.3.1 DArT marker screen

DArT marker assays were performed by Triticarte Pty Ltd (http://www.triticarte.com.au/; Yarralumla, ACT, Australia). Genomic DNA (20-100 ng) of both parents and each DH line from the Claire x Lemhi cross was placed into a 96 well Thermo-fast® plate (ABgene) and the plate sealed with Microseal™ 'A' film (MJ Research, 590 Lincoln Street, Waltham, MA 02451, USA) before sending to Triticarte Pty Ltd for analysis. Data from Triticarte Pty Ltd was received

electronically and added to the marker data file in Chapter 4 for the construction of the genetic linkage map.

2.3.2 EST primers screened at CASE partner Advanta-Nickerson Seed (Pty) Ltd

Twenty two EST-derived primer pairs were screened in the DH population at CASE partner Advanta-Nickerson Seed using a 'rapid' throughput method. This method used fluorescently labelled EST-derived primers, with the PCR product being analysed on a DNA sequencer (3700 Data Analyser, Applied Biosystems). The method uses a sequencer with capillaries, which are filled with a separation matrix (POP-6™, Applied Biosystems) on which the PCR products are run. See Chapter 5 (Section 5.3) for development of and conditions used to analyse these primer pairs. Several PCR products can be amplified in one sample and run in the same capillary. An oligonucleotide labelled with a different fluorescent dye (6FAM™, VIC™, NED™, PET™, and HEX™, Applied Biosystems) is attached to each forward primer resulting in PCR products that fluoresce at different wavelengths.

2.3.3 SSR markers

SSR primers were used to amplify genomic DNA. Primers were selected from the GrainGenes database (www.graingenes.org) using the criteria described in Chapter 4 (Section 4.3.2 and 4.3.3). See Appendix Table 1 for primer sequences. SSR marker PCR reactions were carried out in a 96 well Thermo-fast® plate (ABgene, ABgene House, Blenheim Road, Epsom, KT19 9AP, UK). The plate was then sealed with Microseal™ 'A' film (MJ Research). All SSR primer pairs had an annealing temperature of 60°C, unless otherwise stated. Amplifications were performed using a PTC-225 DNA engine™ PCR machine and PCR products were stored at -20°C. DNA amplifications were carried out in 15 μl reaction volumes, each containing 3 μl (10 *ng*) of template DNA, 0.07 μl Hot Star Taq DNA polymerase, 1.5 μl of 0.05 pmol of SSR-derived primer pair, 1 μl of 2 mM each of dATP, dCTP, dGTP and dTTP, 1.5 μl 10 x PCR buffer and 8 μl dd.H_2O. PCR conditions were as follows: an initial denaturing step at 94°C for 3 minutes, followed by 35 cycles at 94°C for 1 minute, 60°C for 1 minute, 72°C for 2 minutes, with a final extension step at 72°C

for 10 minutes. A 0.8 μl aliquot of each PCR product was denatured in order to separate the DNA strands for 3 minutes at 94°C and cooled on ice before loading onto a 6.5% polyacrylamide gel.

2.3.4 NBS Profiling

The restriction enzymes *Mse*I and *Rsa*I were used in combination with NBS2 and NBS2cer (cereal specific) primers (Chapter 4 Section 4.3.4) (van der Linden *et al*., 2004). Genomic DNA is digested with a restriction enzyme, adapters ligated to each end of the cut DNA and an NBS-specific primer is used to amplify towards the adapter-specific primer (Figure 2.1).

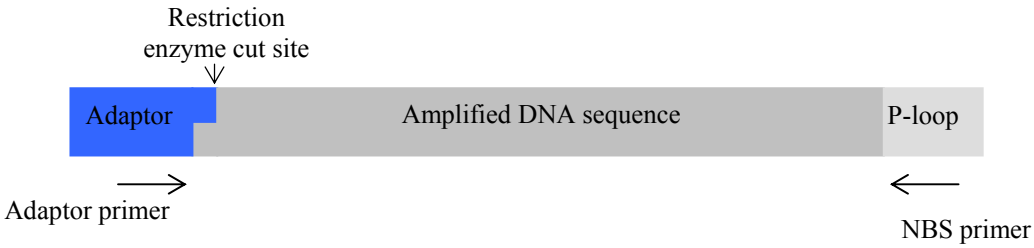


Figure 2.1 NBS-profiling procedure.

2.3.4.1 Digestion/Ligation

DNA digestion and ligation were carried out in 30 μl reaction volumes, each containing 2 μl of template DNA, 0.5 μl T_4 ligase (1u μl-1), 0.5 μl *Mse*I or *Rsa*I restriction enzyme (10u μl^{-1}), 3 μl of 10 mM ATP, 1.5 μl annealed adapter (0.25 μl *Mse*I upper adapter, 0.25 μl *Mse*I lower adapter and 1 μl dd.H_2O), 6 μl RL buffer (10 mM Tris. HCL pH 7.5, 10 mM MgAc, 50 M KAc, 5 mM DTT and 50 ng μl-1 Bovine Serum Albumen (BSA) and 16.5 μl dd.H_20. Digest conditions were as follows: 37ºC for 3 hours, 65ºC for 15 minutes, to then be held at 4ºC. All samples were diluted with 30μl dd.H_2O before the amplification step (Section 2.3.4.2). See Appendix Table 2 for primer sequences.

2.3.4.2 Amplification

DNA amplification was carried out in two steps (first and second PCR). The first PCR reaction contained 5 μl diluted digested/ligated DNA template together with

12.92 μl dd.H_2O, 2.5 μl 10 x PCR buffer, 2.5 μl of 2.5mM dNTP, 2.0 μl of 10 μM NBS primer and 0.08 μl (5U μl^{-1}) Hot Star Taq DNA polymerase. All samples were diluted with 50 μl dd.H_2O. The second PCR reaction contained 5 μl diluted first round PCR together with 6.92 μl dd.H_2O, 2 μl 10 x PCR buffer, 2 μl of 2.5 mM dNTP, 2 μl of 10 μM NBS primer, 2 μl of 10 μM pigtail primer and 0.08 μl (5U μl^{-1}) Hot Star Taq DNA polymerase. See Appendix 1 Table 2 for primer sequences. The PCR conditions used for the first and second round PCR had an initial denaturation step at 95°C for 15 minutes, followed by a 1 minute 40 seconds annealing step where the annealing temperature was determined by the primer pair (60ºC) and an extension step at 72°C for 2 minutes. Then 29 cycles were performed as follows, 95°C for 1 minute, 60ºC for 1 minute 40 seconds and 72°C for 20 minutes.

2.3.5 AFLP

2.3.5.1 Restriction/Ligation

The restriction/ligation reaction was carried out together in a 30 μl reaction of 200 *ng* of template DNA, 1 x RL buffer (10 mM Tris. HCL pH7.5, 10 mM MgAc, 50 M KAc, 5 mM DTT and 50 *ng* μl-1 BSA), *Pst*I adapter (5 pM), *Mse*I adapter (50 pM), 1 mM ATP, 5 U *Pst*I and *Mse*I and 0.5 U of T4 DNA ligase (Invitrogen Ltd, 3 Fountain Drive, Inchinnan Business Park, Paisley, UK). The reactions were incubated at 37°C for 3 hours followed by 15 minutes at 65°C.

2.3.5.2 Non selective PCR

Template DNA was pre-amplified using the non-selective primers M00 and P00 (Appendix Table 2 for sequences), which have sequences which are complimentary to the *Mse*I and *Pst*I adapters, respectively. The reaction consisted of 2.5 μl of the restriction/ligation product and 22.5 μl of the non-selective amplification reaction mix (1 X PCR buffer (Roche Diagnostics, Charles Avenue, Burgess Hill, West Sussex, RH15 9RY, UK), 0.2 mM of each dNTP, 0.5 U Taq DNA polymerase (Roche), 30 *ng* of M00 primer and 30 *ng* of P00 primer. PCR conditions were as follows: 95°C for 30 seconds, 56°C for 1 minute and 72°C for 2 minutes, for 30 cycles. The PCR product was diluted 1:50 with dd.H2O prior to selective PCR amplification.

2.3.5.3 Selective PCR

To 3 µl of the diluted non selective PCR 17 μl of selective PCR reaction mixture was added (1 X PCR buffer (Roche), 0.4 mM of each dNTP, 0.4 U Taq DNA polymerase (Roche), 30 *ng* S + 2 or P + 2 primer and 30 *ng* of M + 3 primer (Appendix 1 Table 2 for sequences). The PCR conditions were: denaturing at 95°C for 30 seconds, annealing at 65°C for 30 seconds with 0.7°C touchdown with each subsequent cycle and extension at 72°C for 1 minute for 12 cycles, followed by 95°C for 30 seconds, 56°C for 30 seconds and 72°C for 1 minute for a further 22 cycles.

2.4 Polyacrylamide gel preparation

Products of PCR amplifications were visualised by separation on a 6% denaturing polyacrylamide gel. A pair of glass plates, S2 sequencing system (Whatman Biometra, Gottingen, Germany), was used to support this matrix.

2.4.1 Preparation of the gel

The large and small glass plates were cleaned with Alconox (Sigma Aldrich, Poole, BH12 4QH, UK), rinsed with dd.H_2O and wiped twice with 100% ethanol. Repelcote, 5 ml, (VWR International Ltd, Hunter Boulevard, Magna Park, Lutterworth, Leicestershire, LE17 4XN, UK) was then applied to the large plate and bind silane, 60 μl, (GE Healthcare UK Ltd, Pollards Wood, Nightingales Lane, Chalfont, St Giles, Buckinghamshire, HP8 4SP, UK) applied to the smaller plate. These were allowed to dry for at least 30 minutes before being gently wiped with 100% ethanol to remove the excess bind silane and repelcote. The premixed Sequagel 6 gel matrix (National Diagnostics, Unit 4 Fleet Business Park, Itlings Lane, Hessle, Yorkshire, HU13 9LX, UK) made up of 45 ml Sequagel 6, 11 ml Sequagel complete and 257 μl 10% APS (Amersham Biosciences, Little Chalford, Buckinghamshire, HP7 9NA, UK), was poured between the two glass plates which had been securely clamped and separated by 0.4 mm spacers. The gel was left for one hour to polymerise.

Once polymerised, the plate is loaded onto the BRL sequencing system gel rig

(Gibco) and 500 ml 1 X TBE solution placed in the bottom and top reservoirs. The gel was run for 30 minutes at 80W/40mA, until the temperature of the glass plate reaches 45°C, before the samples are loaded.

2.4.2 Loading and electrophoresis of the gel

Prior to loading the gel 5 μl of the PCR products (generated from Sections 2.3.3, 2.3.4 and 2.3.5) were mixed with 5 μl of loading buffer (19.6 ml formamide, 0.4 ml 0.5M EDTA, 20 mg bromophenol blue dye, 20 mg xylene cyenol dye) and denatured by heating to 95°C for 5 minutes. After denaturation 6 μl of each sample was loaded onto the 6 % polyacrylamide gel together with 3 μl of a 1 kb ladder (Invitrogen) at the ends of each gel. The gel was run at 80W/40mA for 2 hours or until the dark blue front of the formamide dye had run to the bottom.

2.4.3 Silver nitrate staining

The two glass plates were separated to leave the 6 % polyacrylamide gel attached to the smaller of the two plates. With gentle shaking, the gel was washed in 2 L of 10% acetic acid fixer solution (1800 ml d.H_2O and 200 ml 100% acetic acid), followed by two washes with d.H_2O and a 30 minute soak in dH_2O with gentle shaking. The gel was then gently shaken for 30 minutes in silver nitrate staining solution (12 ml 1.01 N silver nitrate, 3 ml formaldehyde 1985 ml d.H_2O), followed by a rinse with d.H_2O. To develop the gel it was placed in 2 L of developer solution (60 g sodium carbonate, 3ml formaldehyde solution (40% solution), 300 μl of sodium thiosulphate (0.1001 N) made up to 2 L with d.H_2O) until the stained PCR bands appear. Once the PCR bands at the bottom of the gel could be seen (i.e. 70 bp marker on the 1kb ladder) the fixer solution was added to stop the staining reaction, followed by a final wash step in d.H_2O for 20 minutes. After drying overnight the gel was exposed to autoradiograph duplicating film (Kodak) which was developed using a Fuji x-ray processor RGII and the image scanned (ScanMaker 9800 XL, Microtek Ltd, Intec 4, Wade Road, Basingstoke, Hants, RG24 8NE).

2.5 Linkage Map Construction

Joinmap version 3.0 for Windows (van Ooijen and Vooripps, 2001) was used for the genetic linkage map construction of the AFLP, DArT, EST-derived, NBS and SSR markers screened in the DH population (77 lines) from the cross Claire x Lemhi. A chi-squared goodness-of-fit analysis (Snedecor and Cochran, 1989) was carried out on the segregation ratios of each marker to determine whether any marker loci deviated significantly from the expected 1:1 ratio. The genetic map was constructed in Joinmap by using the weighted least squares procedure as described by Stam (1993). One thousand permutations were used (as recommended by Van Ooijen, 2004) to establish genome wide levels of significance (LOD value at the 95% threshold) in order to determine linkage groups for map construction. Recombination fractions (RF) of less than 0.4 between marker loci were used (a RF of below 0.5 denotes linkage). RF values were converted to map distances in centimorgans (cM) using the Kosambi mapping function (Kosambi, 1944).

2.6 Growth conditions

2.6.1 Screening for seedling resistance

The phenotypic seedling data was supplied by Dr. L. A. Boyd (Section 3.3.1).

2.6.2 Screening for Adult Plant Resistance – glasshouse trial

Seeds of all 77 Claire x Lemhi DH lines and the parents Claire and Lemhi were surface sterilised (Section 2.2) and placed in Petri dishes (Sterlin, Aberbargoed, Caephily, CF81 9FW, UK) upon dampened Whatman filter paper. The seeds were vernalised by covering the Petri dishes with tin foil and incubating at 4°C for eight weeks. Vernalised seed was sown into a mix of peat and sand in the glasshouse. Each DH line was transplanted into 1 L pots containing cereal mix after three to four weeks. The DH lines were sprayed every four weeks with the fungicide Fortress (Dow AgroSciences, Latchmore Court, Brand Street, Hertfordshire, SG5 1NH) in order to prevent powdery mildew infection. Glasshouse conditions were maintained at a day length of 16 hours, with the temperature during the day reaching a maximum of 18°C and a minimum of 14°C at night. The adult plant screen for yellow rust

resistance was carried out over the winter of 2008. Each DH line and parents (Claire and Lemhi) had three replicates. As the first four flag leaves emerged they were indentified with corresponding coloured tags in order to facilitate scoring of individual flag leaves post inoculation. Plants were inoculated once they had reached the heading stage of development, growth stage 50 (Zadoks, 1974). Yellow rust isolates are stored as urediniospores over liquid nitrogen. Mixtures of isolates collected on different dates containing the same virulence profile were selected (Table 3.3). Urediniospores were transferred to glass vials and heat shocked at 42°C in a water bath for five minutes to break spore dormancy. An equal volume (ratio of 1:1) of un-perfumed talcum powder (Boots chemist, simple range) was added to the glass vial. Incubator cabinets were sprayed with H_2O and a trough filled with H_2O was placed at the bottom of each cabinet to help create a humid microclimate to facilitate pathogen germination and infection. Plants were placed inside the incubator cabinets and sprayed to run off with H_2O containing 5 drops Tween 20 (Sigma Aldrich) per 2.5 L^{-1} H_2O. This solution acts as a surfactant lowering the surface tension of the H_2O to aid spore adhesion. The urediniospore and talcum powder mixture was air blown using a puffer device onto the adult wheat plants until a covering of talc could be seen. Cabinets were transferred to a humidity chamber (100% humidity) at 10°C in darkness for 24 hours to aid urediniospore germination. After 24 hours plants were transferred back into the glasshouse under the growth conditions described previously.

2.6.3 Screening for Adult Plant Resistance - field trial

To assess the yellow rust resistance of each Claire x Lemhi DH line field trials were carried out at the John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH, UK over two consecutive years (scored in May 2006 and May 2007). Each field trial had a single replicate (a single plant of each DH line and parents) within each of two blocks. The parents Claire and Lemhi, together with the 77 DH lines were randomised within the replicates. A mixture of isolates containing different virulence profiles were selected (Table 3.3). Urediniospores were prepared as described in Section 2.6.4. Avocet S was inoculated with the isolate mixture and seven days post inoculation were sown in two opposite corners of each block to aid the spread of the disease.

2.7 Phenotypic data

2.7.1 Phenotypic scoring of field data

Plants in the field trials were closely monitored for the development of yellow rust infection. Upon detection of yellow rust pustules individual plants were scored for percentage of the leaf area showing sporulating colonies using the modified Cobb scale (Ma and Singh, 1996, Stubbs *et al.*, 1986). Example symptoms and associated percentage infection scores are shown in Figure 2.2.

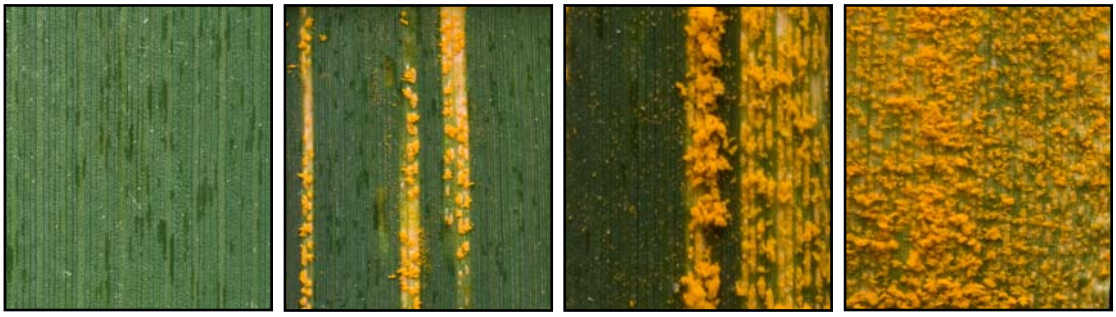


Figure 2.2 Percentage infection phenotypes observed. The numbers below indicate the percentage of leaf area covered with sporulating uredinia.

The defence response of the host plant was also recorded by scoring infection type which ranged from small necrotic flecks (;), resistant necrotic areas with no sporulation (R), moderately resistant, necrotic areas with small sporulating uredinia (MR), moderately susceptible, chlorotic areas with sporulation (MS) and susceptible, abundant sporulation surrounded by green tissue (S) (Figure 2.3). The infection types were converted to numerical values to give an infection type nominal (IT) (; = 0.1, R = 0.2, R/MR=0.3, MR = 0.4, MR/MS=0.5, MS = 0.6, MS/S=0.7, S = 0.8 adapted from McNeal *et al*., 1971). The field trial conducted in 2006 had two score dates approximately 14 days apart, to identify the natural progression of disease. In 2007 three score dates were recorded.

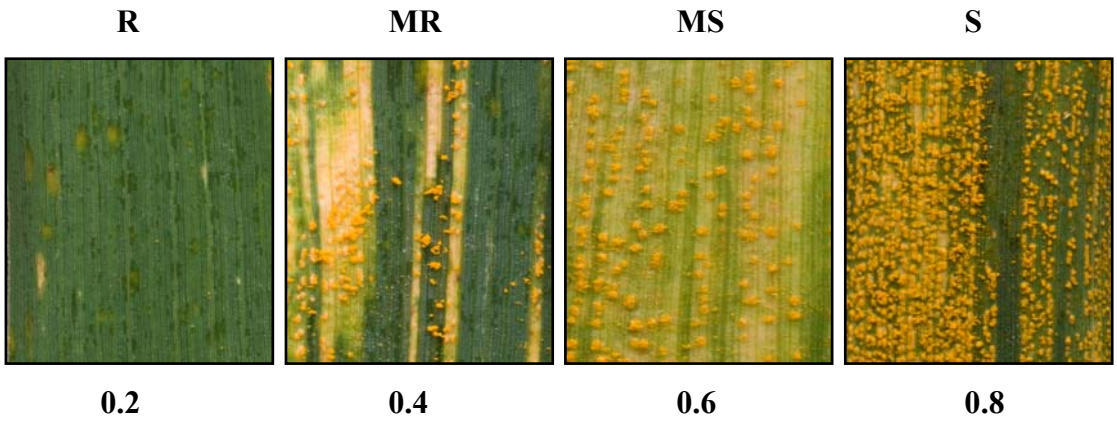


Figure 2.3 Infection type phenotypes observed. R = resistant, necrotic areas with no pustules, MR = moderately resistant, necrotic areas with some pustules, MS = moderately susceptible, chlorotic areas with sporulation, S = susceptible, abundant sporulation surrounded by green tissue. Numbers below are the associated infection type nominals.

2.7.2 Phenotypic scoring of glasshouse data

Seven days following inoculation plants in the glasshouse were monitored for yellow rust development. After the initial signs of yellow rust infection were detected, each DH line was scored for latent period. Latent period was scored as the number of days post-inoculation (dpi) at which 10% of the inoculated leaf surface showed sporulating uredinia (Boyd and Minchin, 2001). The phenotypic PI and IT scores for the whole plant were recorded 17 dpi. The first four flag leaves of each DH line were also scored separately for PI. The scoring system for PI and IT was as described for field trial data (Section 2.7.1, Figures 2.2 and 2.3). Plant height of the three replicates of each DH line was also recorded.

2.8 Statistical analysis

GenStat® for Windows, 9th Edition (GenStat Release 9 Committee, 2007) was used to perform all statistical analysis on phenotypic data sets. Analysis of variance using Generalised Linear Modelling was performed on PI and IT. PI data sets underwent LOGIT^{+} transformation to achieve independence of mean and variance and IT underwent an adjusted log transformation (see Chapter 3). The correlation co-

efficient (R^2) between PI and IT, for each line and at each score date, was calculated. Chi-squared analysis and broad sense heritability was also determined (see Section 3.3.3).

2.9 QTL analysis

Quantitative trait loci (QTL) analysis was carried out using MapQTL version 5 (van Ooijen, 2004). A Kruskall and Wallace test was initially used to identify possible markers associated with the yellow rust resistance found in Claire, at a minimum significance of $p = 0.05$. Interval mapping (Lander and Bostein, 1989) was then used to locate regions within the genome associated with the resistance phenotype. The automatic cofactor selection tool in MapQTL was used to confirm the location of significant QTL ($p = 0.001$) by using a backward elimination procedure. Identified cofactors were then used in MQM mapping (Jansen and Stam, 1994). Permutation analysis (Churchill and Doerge, 1994) was performed with each phenotypic data set to minimise any chance associations between QTL and markers. One thousand permutations were used (as recommended by Van Ooijen, 2004) to establish genome wide levels of significance (LOD value at the 95% threshold) and to verify the presence of a QTL in a given genomic region.

Chapter 3

Genetic analysis of adult plant and seedling yellow rust resistance

3.1 Introduction

To study the genetics of yellow rust resistance it is essential to have a reliable and reproducible method of recording infection levels. Disease responses can be assessed either qualitatively, quantitatively or a combination of both. Most commonly in adult plant tests estimation of disease severity is achieved by using the modified Cobb scale. The scale was first developed by N. A. Cobb and later modified by Peterson *et al*., (1948). The modified Cobb scale has been extensively used to measure yellow rust resistance in wheat (Jagger, 2009, Chhuneja *et al*., 2008, Melichar *et al*., 2008). Infected plants are scored for the percentage leaf area covered with sporulating uredinia, percentage infection (PI), reflecting the extent to which the pathogen has been able to infect the plant. The scoring system can be somewhat subjective, particularly when the upper limit of the scale is reached, becoming increasingly difficult to differentiate between and score highly susceptible individuals. By scoring PI over consecutive dates, the rate of pathogen development can be calculated as the area under the disease progression curve (AUDPC). This is a useful quantitative summary of disease severity over time, allowing comparisons across years and locations, integrating all aspects of disease progression in relation to host growth and development (Jeger *et al*., 2001). Dedryver *et al*. (2009) used AUDPC to identify the APR genes associated with yellow rust resistance in the winter wheat cultivar Renan.

Together with PI it is useful to score the infection types (IT), a qualitative score that reflects the defence response shown by the plant. McIntosh *et al*. (1995) adapted the IT scoring system for analysis of adult plant phenotypes, proposing the categories resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Intermediate categories have subsequently been added to reflect the whole range of possible infection phenotypes. Data sets obtained using the IT score require conversion to an arbitrary numerical scale to allow statistical analysis and subsequent QTL identification. The McNeal numerical scale, 0-9 (McNeal *et al*., 1971) originally devised to score yellow rust on seedlings has also been adapted to score APR. Additional yellow rust scoring systems are shown in Table 3.1.

Table 3.1 The major infection type classes for three scoring systems developed for yellow rust infection of wheat (McIntosh *et al*., 1995).

Infection Type				
Gassner and Straib (1932)	**McNeal *et al*., (1971)**	**PBI Cobb**	**Host response**	**Symptom**
I	0	0	Immune	No visible symptoms
00	1	;	Very resistant	Necrotic flecks
0	2	;n	Resistant	No sporulation with some necrotic areas
I	3-4	1	Resistant	Limited sporulation with chlorotic and necrotic regions
II	5-6	2	Resistant/ moderately resistant	Moderate sporulation with chlorotic and necrotic regions
III	7-8	3	Resistant/ moderately susceptible	Sporulation with chlorosis
IV	9	4	Susceptible	Abundant sporulation without chlorosis

PI and IT scores can be combined to give a coefficient of infection (CI) score, thereby weighting the modified Cobb scale rating by the disease response phenotype. CI has been reported to be more closely related to crop loss than either IT or PI (McIntosh *et al*., 1995) and has therefore been used to identify numerous genes associated with resistance to yellow rust. The problem with the CI is that the two variables it is calculated from are not independent (McIntosh *et al*., 1995), therefore correlation between PI and IT is required in order to accurately predict the associated QTL. Recently CI was used to identify two APR genes, *QYrtm.pau*-2A and *QYrtb.pau*-5A, in the wild wheat species *T. monococcum* and *T. boeoticum,* respectively (Chhuneja *et al*., 2008). Yellow rust QTL have been identified that are associated with both PI and IT (Suenaga *et al*., 2003), suggesting that the resistance QTL affects both pathogen growth and plant response. However, resistance QTL have also been found that affect one phenotype, but not the other (Melichar *et al*.,

2008) suggesting that different QTL may inhibit pathogen infection by different mechanisms.

Roelfs (1985) proposed a further three criteria that could be used to assess yellow rust infection, namely the number of uredinia expressed as the receptivity of the host or ability of the pathogen to infect, length of the latent period and the subsequent duration of sporulation. The latent period, measured as the time between inoculation and the first appearance of yellow rust pustules on the leaf surface can be a useful phenotype of yellow rust infection. Genotypes which have similar final PI and IT may display very different latent period lengths. Environmental factors strongly influence latent period, so field trials are not always suitable for this type of score. Trials done under controlled environmental conditions, however, are ideal for evaluating latent period. A long latent period provides a certain degree of resistance in the field by delaying the rust epidemic, whilst a short latent period results in more rapid onset of disease, leading to faster disease spread in the field.

The phenotypic distribution of yellow rust PI and IT within a population enables the genetic basis of resistance to be determined. The presence of multiple, strong resistance genes segregating within a population results in a distribution that is skewed towards resistance (Carter *et al*., 2009). However, when a single major resistance gene is present within a population a bimodal distribution, with peaks for resistant and susceptible classes is observed (Carter *et al*., 2009, Borner *et al*., 2000). Frequently, in yellow rust field resistance numerous genes, with partial effects are found to contribute to the resistance phenotype, resulting in a near normal distribution (Lu *et al*., 2009, Lillemo *et al*., 2008, Lin *et al*., 2007). By grouping the individuals within a population into resistant and susceptible classes the segregation ratio can be compared to expected gene ratios in order to predict the number of genes that are accountable for the resistance.

3.2 Aims of the study

Claire (Nickerson Seeds UK) is a winter wheat cultivar with good field yellow rust resistance, having a NIAB rating of nine on the Home Grown Cereals Authority (HGCA) 2009/2010 Recommended List (http://www.hgca.com). This rating has

remained unchanged since its release in 1999. In the UK Claire was the market leader until it was superseded in 2004 by the cultivar Einstein which had superior eyespot (*Oculimacula yallundae* and *Oculimacula acuformis*) and powdery mildew (*Blumeria graminis*) resistance. The wheat cultivar Carstens V carries both seedling and APR genes and is thought to be in the pedigree of Claire (Figure 1.7). In the UK the Carstens V seedling resistance has now been overcome. A wide cross, between two distantly related cultivars (Claire and Lemhi) was chosen for the DH mapping population with the aim of obtaining a higher frequency of DNA polymorphism for genetic map construction (Chapter 4). Lemhi, an American spring wheat, is susceptible to all UK *P. striiformis* f.sp. *tritici* isolates. In order to identify the APR genes in Claire phenotypic data on 77 DH lines of the Claire x Lemhi mapping population was obtained. This chapter analyses two years of field data, a subsequent controlled environment data set conducted under glasshouse conditions and seedling data sets. The phenotypic data collected, together with the genetic linkage map of the Claire x Lemhi DH population (Chapter 4) was subsequently used for the identification and localisation of the APR genes present in Claire (Chapter 6).

3.3 Materials and Methods

3.3.1 Seedling data

Previous multiple isolate race seedling tests suggested that Claire carried the seedling resistance genes *Yr2*, *Yr3*, *Yr4*, *Yr25*, *YrHVII* and another unknown seedling resistance gene (Lewis, 2006). To confirm the yellow rust seedling resistance genes the 77 DH individuals from the Claire x Lemhi cross were inoculated with two yellow rust isolates, WYR 76-10 and WYR 68-2 (Table 3.2). Claire was previously found to be resistant and Lemhi susceptible to these two isolates (Lewis, 2006).

The scoring system used in this study was based upon the PBI scale (Table 3.1), initially devised by Gassner and Straib (1932). This scale takes into account the seedlings response to infection, as well as uredia levels, classifying infection type (IT) on a scale of 0 to 4.

Table 3.2 The virulence profile of the *P. striiformis* isolates used to identify seedling resistance gene(s) within Claire.

Isolate	Virulent on yellow rust resistance genes
WYR 76-10	2, 3, 4, 6, 8, 17
WYR 68-2	1, 2, 3, 6, 7

3.3.2 Adult plant data

The DH lines from the Claire x Lemhi cross were planted in the field and glasshouse (as described in Section 2.6). In order to identify the APR genes associated with Claire the DH lines were inoculated with isolates of *P. striiformis* virulent on seedlings of Claire and Lemhi (Table 3.3). Yellow rust resistance was evaluated measuring the extent of uredinia formation as the percentage of the leaf surface infected (PI) and the defence response exhibited by the host plant (IT). The PI and IT phenotypes were scored in the first and second year of field trials and under glasshouse conditions. Also under glasshouse conditions each of the first four flag leaves, for each DH plant, were scored for PI. The height of each DH line was also measured in the glasshouse experiment in order to map the semi dwarfing gene present in Claire, thereby providing a control for the mapping.

Table 3.3 The virulence profile of the *P. striiformis* isolates used to identify adult plant resistance gene(s) within Claire.

Year	Source	Isolate	Virulent on yellow rust resistance genes
1 and 2	Field	WYR 96/502	*Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr9* and *Yr17*
		WYR 95/12	*Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr9*, *Yr13* and *Yr17*
3	Glasshouse	WYR 96/502	*Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr9* and *Yr17*

3.3.3 Statistical analysis

IT scores obtained from the two years of field trials and the glasshouse adult plant trial were converted to a numerical IT nominal to allow for statistical analysis (Table 3.4).

Table 3.4 Scale used to convert qualitative infection type (IT) scores into numerical IT nominals.

IT score	IT nominal
;[1]	0.1
R[2]	0.2
R/MR[3]	0.3
MR[4]	0.4
MR/MS[5]	0.5
MS[6]	0.6
MS/S[7]	0.7
S[8]	0.8

[1]necrotic flecks with no visible uredia, [2]necrotic tissue with or without small non-sporulating dry pustules, [3]small pustules surrounded by necrotic tissue, [4]pustules surrounded by necrotic tissue, [5]pustules surrounded by necrotic and chlorotic tissue, [6]pustules surrounded by chlorosis, [7]pustules surrounded by chlorotic and green tissue, [8]pustules surrounded by green tissue with no necrosis or chlorosis.

In order to achieve near normal distributions each phenotypic data set for PI and IT were transformed. The PI data sets were transformed using $LOGIT^{+}$, which is specifically tailored to deal with percentage data where the upper and lower limits of the individual data sets are incorporated into the formula (Arraiano *et al.,* 2006). $LOGIT^{+}$ transformation was used by Lewis (2006) and Jagger (2009) to transform PI data.

$$LOGIT^{+}=Log_n\,[(\%\ score + (minimum\ \% + 0.25))/((maximum\ \% + 0.25) - \%\ score))]$$

Log_n = natural logarithm
% score = individual data point score
Minimum = lowest percent recorded for score date
Maximum = highest percent recorded for score date

Adjusted log transformation was used to achieve near normality for the IT nominal scores.

$$\text{Log transformation} = \text{In. (IT nominal +1)}$$

IT nominal = individual IT score after conversion to arbitrary numerical scale

Genstat 9th edition (Lawes Agricultural Trust (Rothamsted Experimental Station)) was used to conduct ANOVA on the transformed data sets using General Linear Modelling (GLM). Outputs obtained from Genstat were then used to calculate narrow sense heritability values.

$$\text{Heritability}^2 = \sigma_g^2 / \sigma_p^2$$

Where: $\sigma_g^2 = \sigma_l^2 - \sigma_e^2/r$ and $\sigma_p^2 = \sigma_g^2 + \sigma_e^2$

σ_g^2 = genetic variance

σ_p^2 = phenotypic variance

σ_l^2 = variance in DH lines

σ_e^2 = error variance

r = number reps

Chi-squared analysis was carried out to test the distribution of observed phenotypic frequencies against those expected for given gene number segregation models. Chi-squared analysis was used to predict the number of genes responsible for seedling yellow rust resistance in Claire.

$$\chi^2 = \sum(O - E)^2/E$$

O = observed number of lines in each gene group

E = expected number of lines in each gene group

3.4 Results

3.4.1 Distribution of phenotypic adult plant data

The PI field data from year one and year two together with one year of glasshouse whole adult plant data (Figure 3.1) display a positively skewed, continuous distribution.

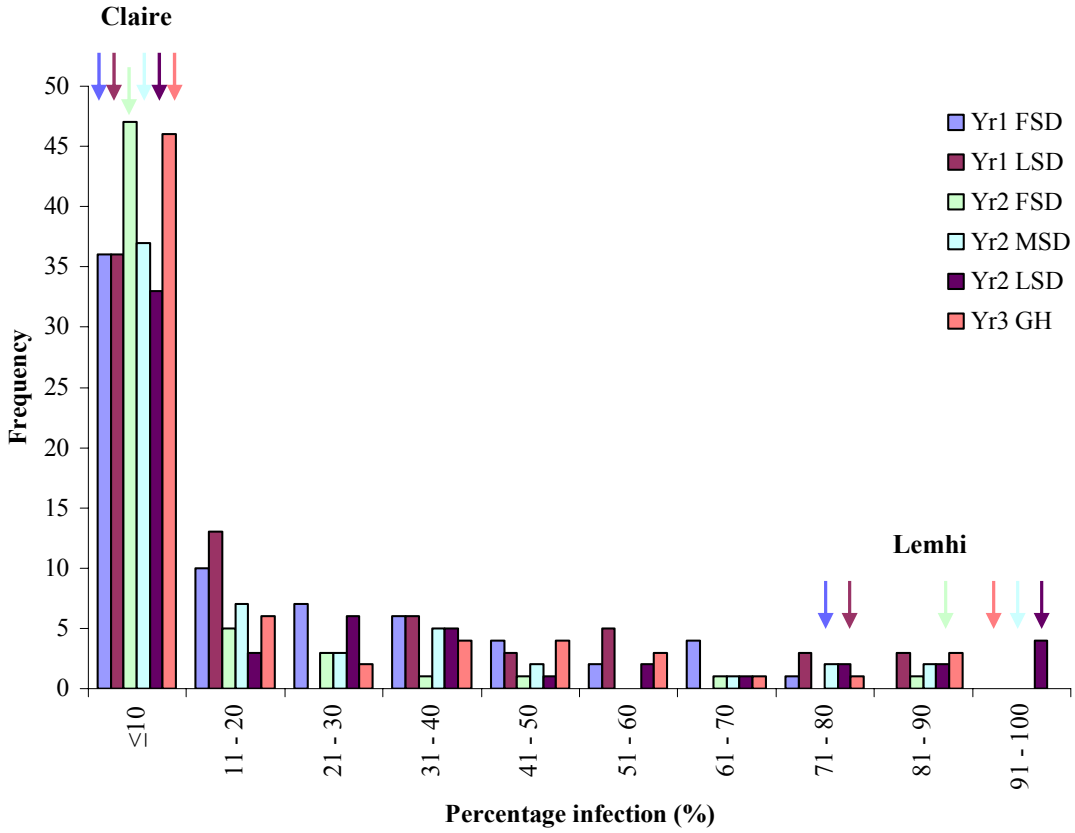


Figure 3.1 Frequency distribution of percentage infection (PI) at consecutive score dates in the Claire x Lemhi DH population recorded over two years in the field and the glasshouse. FSD = First score date, MSD = middle score date, LSD = last score date, GH = glasshouse. Parental scores are shown with an arrow.

Under glasshouse conditions and in field trials the DH lines exhibited a range of PI values from 0% to 96%. No yellow rust pustules formed on Claire under field or glasshouse conditions (Figure 3.2). Lemhi had a consistently high PI in all data sets (80 – 100%). DH 12, DH 40 and DH 60 had a higher PI score than displayed by Lemhi in year one, replicate two, suggesting that Lemhi may contribute a small effect towards the field yellow rust resistance segregating in the Claire x Lemhi

cross. In year one, replicate one, Lemhi died, therefore no scores were obtained. In year two PI values for Lemhi were 80 – 85% at the first score date, rising to 100% by the intermediate score date in both replications, suggesting a stronger disease pressure in this year. In the glasshouse experiment the PI value for Lemhi remained constant at 90% over all three replicates.

Figure 3.2 Phenotypes observed under glasshouse conditions of the parents of the DH population, Claire (two flag leaf segments on the left) and Lemhi (two flag leaf segments on the right). Claire shows a typical PI and IT phenotype rating of 0 R (0% coverage of uredinia, resistant) and Lemhi 90 S (90% coverage of uredinia, susceptible).

The frequency distribution of IT scores from field data in year one and year two and of glasshouse data show normal, continuous distributions (Figure 3.3). The resistant parent Claire had a stable IT nominal of 0.1 throughout all score dates and replicates, under both field and glasshouse conditions. Lemhi had a constant score for IT of 0.8, at all score dates, in all experiments. None of the DH lines had higher IT scores than Lemhi.

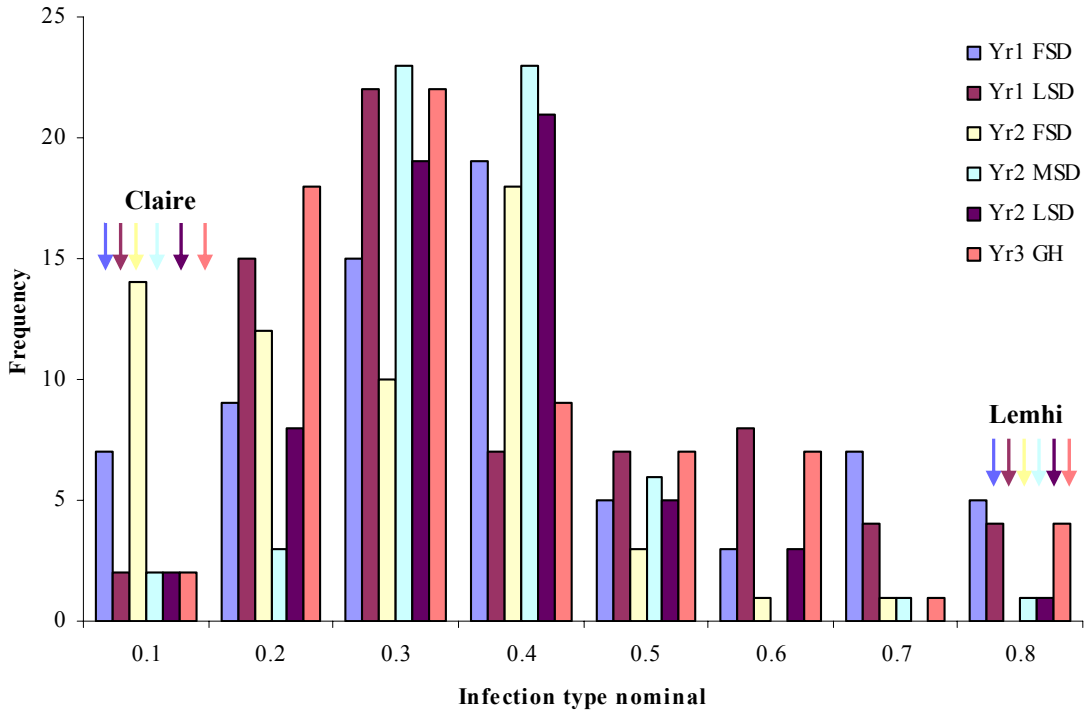


Figure 3.3 Frequency distribution of infection type (IT) at consecutive score dates in the Claire x Lemhi DH population recorded over two years in the field and the glasshouse. FSD = first score date, MSD = middle score date, LSD = last score date, GH = glasshouse. Parental scores are shown with an arrow.

The frequency distribution of the plant height data collected from the glasshouse shows a normal, continuous distribution (Figure 3.4). Transgressive segregation was observed within the population, as some DH lines were taller than the tall parent Lemhi and others smaller than the semi-dwarf parent Claire. DH line 17 (average 124.5 cm over three replicates) was the only individual that was consistently taller over all three replicates than Lemhi (average 106.7 cm). A number of DH lines (DH 12, DH 13, DH 24, DH 25, DH 36, DH 40, DH 42, DH 48, DH 66 and DH 75) were shorter than the semi-dwarf parent Claire (average 71.9 cm).

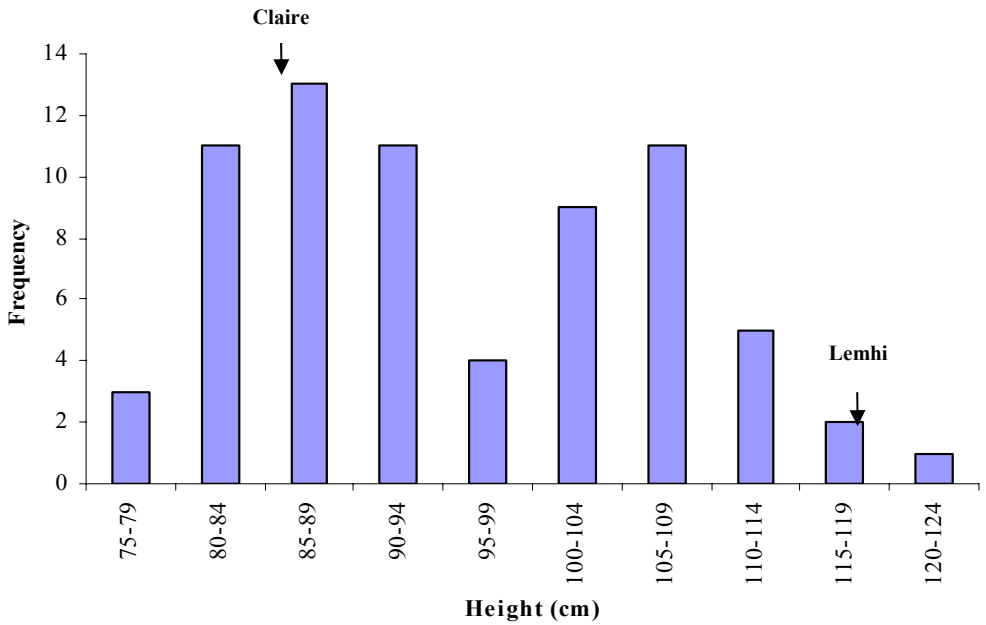


Figure 3.4 Frequency distribution of the height (centimetres) of each Claire x Lemhi DH line in the glasshouse.

The latent period scores and the final PI scores collected from the glasshouse adult plant tests were highly correlated (Figure 3.5). The longer the latent period the lower the final PI. No DH lines showed evidence of sporulation before the susceptible parent Lemhi. Lemhi showed early signs of sporulation on the leaf surface eight to nine dpi, whereas the resistant parent Claire showed no sporulation.

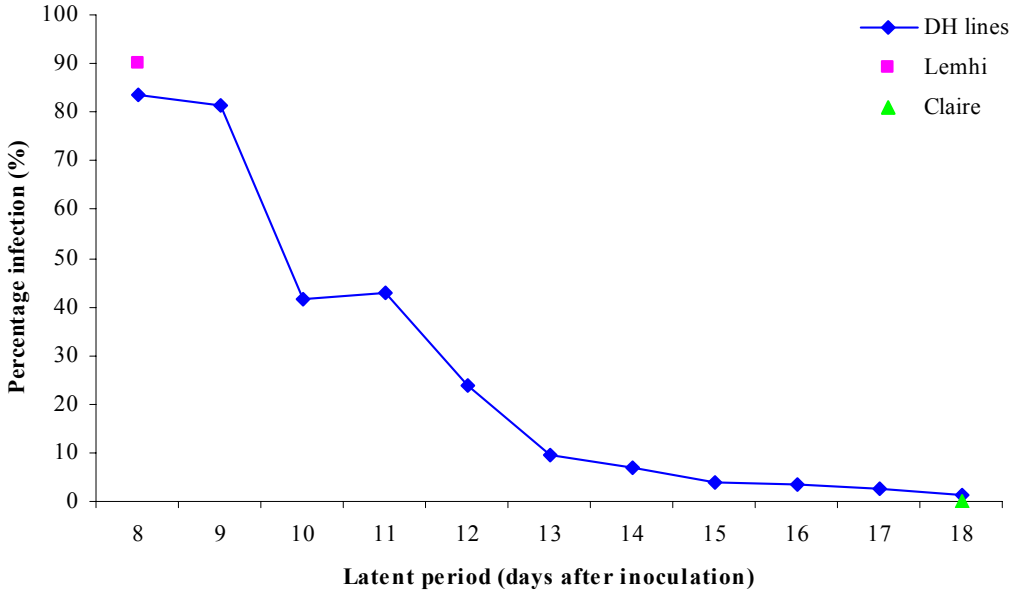


Figure 3.5 Correlation between the latent period and final levels of percentage infection (%) under glasshouse conditions.

3.4.2 Genetic analysis of adult plant data

Correlations between the PI and IT scores for the DH lines recorded over two years in the field and the glasshouse showed high correlation coefficient (R^2) values, ranging from 0.75 to 0.90 (Table 3.5). The highest correlation was seen in the glasshouse experiment, with an R^2 of 0.9. The correlations between the two phenotypic scores increased at later score dates. As the PI and IT scores are correlated the CI could be calculated and used in the QTL analysis, however, the PI and IT scores were used independently to identify the QTL associated with each phenotype (Chapter 6).

Table 3.5 Correlation coefficient (R^2) between PI and IT at each score date in two years of field trials and the glasshouse data for the Claire x Lemhi DH population

Year	Source	Score date	R^2
2006	Field	First	0.84
	Field	Last	0.88
2007	Field	First	0.75
	Field	Middle	0.82
	Field	Last	0.85
2008	Glasshouse	Last	0.9

3.4.2.1 Percentage infection

ANOVA, using the general linear regression (GLR) model showed significant differences in PI ($p<0.001$) between the DH lines across both years of field and glasshouse data (Table 3.6). A replicate effect was exhibited in both years of field data, for the first score dates ($p<0.001$). However, variability between the replicates decreased once the disease had become established. The glasshouse data displayed a very significant replicate effect ($p<0.001$), probably reflecting variation in inoculum density. The heritability value decreased from 0.80 to 0.78 over the score dates in the first year field trial. With the second year field data the heritability value increased for the middle score date, but was found to decrease by the last score date.

Table 3.6 ANOVA of the percentage infection (PI) scores from the Claire x Lemhi DH population for each score date, over two years of field data and one year of glasshouse data.

Data	Score	Source	d.f[1]	m.s[2]	v.r[3]	F.pr.[4]	H[2(5)]
Year 1 Field	First	Block	1	9.52	10.96	0.001	0.80
		DH line	71	8.53	9.82	<0.001	
		Residual	68	0.87			
		Total	140	4.81			
	Last	Block	1	8.1	7.00	0.010	0.78
		DH line	70	10.16	8.78	<0.001	
		Residual	67	1.16			
		Total	138	5.77			
Year 2 Field	First	Block	1	15.69	17.45	<0.001	0.78
		DH line	60	9.17	10.19	<0.001	
		Residual	60	0.89			
		Total	121	5.12			
	Middle	Block	1	4.18	4.25	0.044	0.85
		DH line	60	15.10	13.33	<0.001	
		Residual	60	1.13			
		Total	121	8.09			
	Last	Block	1	17.09	8.71	0.005	0.79
		DH line	60	18.79	9.58	<0.001	
		Residual	60	1.96			
		Total	121	10.43			
Year 3 Glasshouse		Block	2	21.20	17.44	<0.001	0.64
		DH line	71	12.48	10.27	<0.001	
		Residual	132	1.22			
		Total	205	5.31			

[1]degrees of freedom, [2]mean sum of squares, [3]variance ratio, [4]significance probability of variance ratio, [5]narrow sense of heritability.

An ANOVA was carried out to compare PI across score dates (Table 3.7). Significant differences PI ($p<0.001$) were seen between the DH lines in both years of field trials and no significant differences were seen between PI values of each DH line over score dates, and no interaction between DH lines and PI scores in year 1. However, in year 2 the PI values of each DH line did differ significantly over score dates, suggesting that the disease did not progress at the same rate in all DH lines. Consequently, the correlation between PI values over the two score dates in year 1 was higher than over the three score dates in year 2 (Table 3.8).

Table 3.7 ANOVA of the percentage infection (PI) scores from the Claire x Lemhi DH population for the two score dates in year 1 and three score dates in year 2, field trial data sets.

Score	Source	d.f[1]	m.s[2]	v.r[3]	F.pr.[4]
First Year Field Trial	DH lines over score dates	72	18.97	19.15	<.001
	PI score over score dates	1	0.76	0.77	0.382
	Interaction between DH lines and score date	72	0.69	0.70	0.953
	Residual	138	0.99		
	Total	283			
Second Year Field Trial	DH lines over score dates	61	43.06	28.90	<.001
	PI score over score dates	2	103.53	69.49	<.001
	Interaction between DH lines and score date	122	1.75	1.17	0.162
	Residual	185	1.49		
	Total	371			

[1]degrees of freedom, [2]mean sum of squares, [3]variance ratio, [4]significance probability of variance ratio.

Table 3.8 Correlation coefficient (R^2) of percentage infection (PI) between first and last score dates from two consecutive years of field trials.

Score	R^2
Year 1	0.89
Year 2	0.71

3.4.2.2 Infection type

Significant differences in IT values (p<.001) were seen between the DH lines with all data sets (Table 3.9). No significant repetition effect was seen in any data set, unlike with the PI scores, indicating that IT is less affected by disease pressure, establishment of disease and environmental variation across field trials than PI. The heritability of IT showed a similar pattern to that of PI. The first year of field data showed a decrease in heritability by the final score date, while in the second year the middle score date produced the highest heritability value. The glasshouse data set again produced the lowest heritability value for IT (the same as for PI scores), reflecting the higher environmental pressure found under glasshouse conditions, being conducive to disease and creating higher disease pressure.

Table 3.9 ANOVA of infection types (IT) of the Claire x Lemhi DH population for each score date over two years of field data and one year of controlled environment data.

Data	Score	Source	d.f[1]	m.s[2]	v.r[3]	F.pr.[4]	H²[5]
Year 1 Field	First	Block	1	0.001	0.34	0.56	0.80
		DH line	71	0.037	8.99	<0.001	
		Residual	68	0.004			
		Total	140	0.02			
	Last	Block	1	0.004	0.01	0.926	0.75
		DH line	70	0.034	6.89	<0.001	
		Residual	67	0.005			
		Total	138	0.019			
Year 2 Field	First	Block	1	0.039	5.27	0.025	0.68
		DH line	60	0.042	5.66	<0.001	
		Residual	60	0.007			
		Total	121	0.025			
	Middle	Block	1	0.016	7.65	0.008	0.76
		DH line	60	0.016	7.75	<0.001	
		Residual	60	0.002			
		Total	121	0.009			
	Last	Block	1	0.005	1.89	0.174	0.74
		DH line	60	0.017	7.02	<0.001	
		Residual	60	0.002			
		Total	121	0.009			
Year 3 Glasshouse		Block	1	0.013	2.03	0.135	0.60
		DH line	72	0.05	7.60	<0.001	
		Residual	133	0.006			
		Total	207	0.02			

[1]degrees of freedom, [2]mean sum of squares, [3]variance ratio, [4]significance probability of variance ratio, [5]narrow sense heritability.

Comparing DH, over score dates within each year of field trials, significant differences in IT ($p = <0.001$) were observed amongst the DH lines (Table 3.10). A similar pattern was observed for IT as for PI. No significant difference was found between the two score dates within year 1, but a significant difference was found between the three score dates in year 2 ($p<0.001$), with no significant interaction between DH lines and score dates in either year. The IT scores again showed significant correlation coefficient (R^2) values between the first and last score dates in both years (Table 3.11).

Table 3.10 ANOVA of the infection type (IT) scores from the Claire x Lemhi DH population for the two score dates in year 1 and three score dates in year 2, field data sets.

Score	Source	d.f[1]	m.s[2]	v.r[3]	F.pr.[4]
First Year Field Trial	DH lines over score dates	72	0.07	16.31	<.001
	IT score over score dates	1	0.003	0.70	0.40
	Interaction between DH lines and score date	71	0.004	0.98	0.54
	Residual	138	0.004		
	Total	283			
Second Year Field Trial	DH lines over score dates	61	0.07	16.66	<.001
	IT score over score dates	2	0.30	71.55	<.001
	Interaction between DH lines and score date	122	0.30	0.006	1.49
	Residual	181	0.004		
	Total	367			

[1]degrees of freedom, [2]mean sum of squares, [3]variance ratio, [4]significance probability of variance ratio.

Table 3.11 Correlation coefficient (R^2) of infection type (IT) between first and last score dates from two consecutive years of field trials.

Score	R^2
Year 1	0.81
Year 2	0.74

3.4.2.3 Genetic analysis of individual flag leaves

Flag leaf PI scores showed significant differences ($p<0.001$) across the DH lines for all four flag leaves (Table 3.12). Unlike with the whole plant glasshouse scores, no replication effect was seen. Heritability values varied between the four flag leaves, with the third flag leaf giving the highest heritability (0.65). A positively skewed distribution was displayed for PI (Figure 3.6).

Table 3.12 ANOVA of the percentage infection (PI) scores from the Claire x Lemhi DH population for the first to fourth flag leaf in the glasshouse plant test.

Score	Source	d.f[1]	m.s[2]	v.r[3]	F.pr.[4]	H[2(5)]
First Flag Leaf	Block	2	3.31	1.87	0.159	0.53
	DH line	71	8.94	5.03	<0.001	
	Residual	108	1.77			
	Total	181	4.60			
Second Flag Leaf	Block	2	0.04	0.02	0.982	0.49
	DH line	71	10.22	4.47	<0.001	
	Residual	109	2.29			
	Total	182	5.35			
Third Flag Leaf	Block	2	0.84	0.49	0.617	0.65
	DH line	71	12.56	7.27	<0.001	
	Residual	107	1.73			
	Total	180	5.99			
Fourth Flag Leaf	Block	2	0.53	0.21	0.815	0.53
	DH line	70	12.31	4.73	<0.001	
	Residual	100	2.61			
	Total	172	6.53			

[1]degrees of freedom, [2]mean sum of squares, [3]variance ratio, [4]significance probability of variance ratio [5]narrow sense heritability.

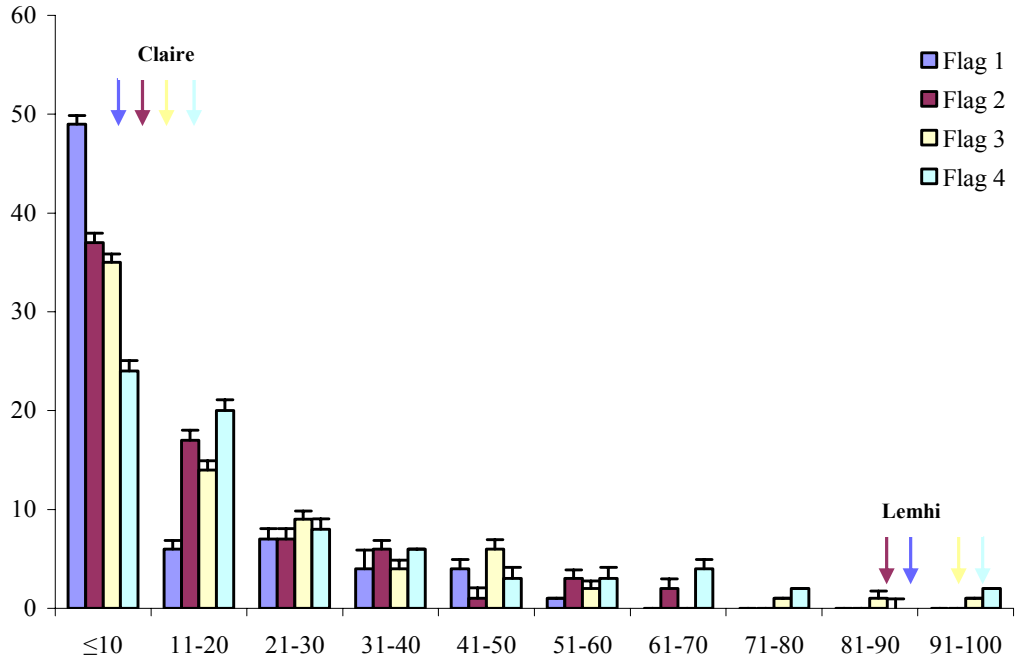


Figure 3.6 Percentage infection (PI) of flag leaves 1 to 4 of the Claire x Lemhi DH population in the glasshouse, adult plant test. Values represent the predicted means. Error bars represent standard error.

Flag leaf PI scores showed no significant differences (p = 0.099) within the four flag leaves for each DH line (Table 3.13). Since this was near the significance threshold least significant differences (LSD) analysis was performed by pairwise comparison of all four flag leaves. No significant differences were found within the four flag leaves (p>0.28).

Table 3.13 ANOVA of the percentage infection (PI) scores across flag leaves from the Claire x Lemhi DH population.

Score	Source	d.f[1]	m.s[2]	v.r[3]	F.pr.[4]
Flag leaf Glasshouse	Block	2	1.05	0.55	0.578
	DH line	71	39.0	20.39	<0.001
	Flag Leaf	3	4.02	2.10	0.099
	Residual	641	1.91		
	Total	717	5.6		

[1]degrees of freedom, [2]mean sum of squares, [3]variance ratio, [4]significance probability of variance ratio.

3.4.2.4 Genetic analysis of seedling data

Chi-square gene estimations (Table 3.14) were used to determine the number of seedling resistance genes segregating in the Claire x Lemhi DH population. Seedling infection scores were not available for DH 12, DH 19, DH 23, DH 28, DH 45, DH 50, DH 59, DH 61, DH 63, DH 63, DH 69, DH 74 and DH 78.

From the chi-squared analysis of the DH segregation ratios the most likely explanation is that there is one gene associated with seedling resistance in Claire that is effective against isolate WYR76-10 and two genes effective against WYR68-2.

Table 3.14 Segregation ratios observed in the Claire x Lemhi DH population when inoculated with *P. striiformis* f.sp. *tritici* isolates WYR 76-10 and WYR 68-2.

Isolate	Phenotype[1]	Observed	Expected 1 gene model	χ^2 - 1 gene model		Expected 2 gene model	χ^2- 2 gene model	
				Ratio	Pr.		Ratio	Pr.
WYR76-10	R	36	32.5	1:1	<0.39	48.75	1:3	<0.003
	S	29	32.5			16.25		
WYR68-2	R	42	32.5	1:1	<0.02	48.75	1:3	<0.05
	S	23	32.5			16.25		

[1] R = resistant, S = susceptible.

3.5 Discussion

The resistant parent Claire exhibits complete, adult plant yellow rust resistance under field and glasshouse conditions. On early assessment of the field data (first score) the phenotype of Lemhi displayed 75-100 S (denoting susceptible with 75-100% of the leaf area infected) while Claire showed no signs of infection (0% R). At the final assessment (last score date) of field and glasshouse data Lemhi consistently scored 100% leaf area infected and Claire 0; (fleck phenotype). Individual DH lines, while exhibiting both parental phenotypes covered the full range of yellow rust infection phenotypes. The DH population did not exhibit transgressive segregation, all genes contributing to yellow rust resistance being derived from Claire.

A positively skewed, continuous distribution was observed for the PI score. This indicates the presence of major resistance genes, as well as the possibility of several minor genes involved in the yellow rust APR in Claire. The IT phenotype showed a near normal, continuous distribution, indicating the presence of a number of minor resistance genes. However, the PI and IT data sets were found to be highly correlated, although the distributions were different. Carter *et al*. (2009) also found PI and IT to be highly correlated although the distributions were different.

Yellow rust APR expressing high heritability has been found in the Italian wheat cultivars Libellula and Strampelli (Lu *et al*., 2009), in Alcedo (Jagger, 2009), Saar (Lillemo *et al*., 2008), Guardian (Melichar *et al*., 2008) and Alpowa (Lin *et al*., 2007). Previous work on a Claire x Lemhi $F_{2:3}$ population found Claire to have a low

heritability of $H^2 = 0.04$ (Lewis, 2006). The DH Claire x Lemhi population exhibited high levels of heritability for both phenotypic traits scored over two field trials, however lower heritability values were observed under glasshouse condition and for individual flag leaves. The low heritability found by Lewis (2006) was credited to the small number of F_3 progeny examined for each $F_{2:3}$ family. By using a DH population the residual variance is reduced (no within family variance) which consequentially increases the estimates of heritability. Having a highly heritable genetic component makes the transferability of APR genes into modern wheat cultivars a more reliable process than for a genetic component of low heritability. The high heritability of the yellow rust APR in Claire should therefore be easier to transfer in a breeding program than initially thought from the $F_{2:3}$ study (Lewis, 2006).

The heritability of PI and IT phenotypes was found to be similar across the years and score dates. Jagger (2009), Melichar *et al*. (2008) and Ramburan *et al*. (2004) all identified higher heritability's for IT than PI. Yellow rust naturally progresses throughout the growing season, resulting in variation in PI scores as the disease progresses at a rate dependent on environmental conditions (Mallard *et al*., 2005). Since the heritability of PI over the two years of field trials was high from the first score date it suggests a rapid onset of disease and a resistance that expresses early in the plants development. A number of environment factors can influence disease severity and progression, including temperature and humidity. Low relative PI heritability values, compared to IT may suggest that the environmental conditions affect PI scores more than IT (Jagger, 2009, Melichar *et al*., 2008). In the first year field trial the heritability of PI and IT differed only marginally between the first and final score dates indicating a strong influence of resistance genes throughout the growing season. In the second year field trial the heritability of IT increased throughout the growing season, while PI heritability had decreased by the final score date. The lowest heritability value, for both phenotypic scores, was obtained from the glasshouse experiment, indicating a greater environmental effect on PI and IT than in the field. This could be attributed to the controlled environment conditions being more favourable to the development of the disease, resulting in a higher disease pressure under glasshouse conditions. Under such high disease pressure the yellow rust APR genes may prove less effective.

The association between yellow rust PI and IT values were always highly significant ($R^2 = 0.75$ to $R^2 = 0.9$, $p<0.001$). Lewis (2006) also found a high level of correlation between PI and IT in the $F_{2:3}$ populations. This high level of correlation between the two phenotypes suggests that the same genes are influencing both phenotypes (Mallard *et al*., 2005). Any APR QTL identified using the PI or IT data set in Chapter 6 are therefore likely to represent the same genetic component. The lowest correlation between PI and IT values was found in the second year field trial at the first score date. Jagger (2009) found that the highest and lowest correlations corresponded to the score dates that had the lowest and highest environmental effect, respectively. Lewis (2006) observed differences between the AUDPC profile of the glasshouse and field trials, indicating that the APR genes from Claire have environmental sensitivities. The frequency distribution was discontinuous within the glasshouse, whilst in the field a continuous distribution was observed (Lewis, 2006). This suggests that under glasshouse conditions the yellow rust resistance in the Claire x Lemhi F_2 population was less effective, resulting in more disease development. Under conditions of high disease pressure the APR genes in Claire may therefore prove less effective.

In the glasshouse trial variation was observed for the four flag leaves across the different DH lines. However, no variation was observed within the four flag leaves of each DH line. Developmental factors can influence resistance, with older flag leaves being more resistant. Once a yellow rust epidemic has been initiated, infection on the lower leaves of the crop is the main source of inoculum for newly emerging leaves. The disease severity on lower leaves would therefore have the potential to provide indications of epidemic development on upper leaves (Young *et al*., 2003). The high disease pressure achieved under glasshouse conditions could explain why no variation was found within flag leaves of each DH line.

Variation between experimental replicates was greater for PI than IT values, indicating a greater effect of environmental variables on the PI phenotype. The significant repetition effect seen in the glasshouse indicates that the controlled environment was probably not uniform. In the field a replicate effect was seen for the first score date in the first year. This is not unexpected as the disease is progressing naturally through the field at different rates, these differences levelling

out by the final score date. To remove variation due to replicate effects and missing data the predicted means were used for QTL analyses (Chapter 6).

A variation in latent period was clearly evident between the DH lines. This variation in yellow rust development has been reported in several studies (Dehghani *et al*., 2004, 2002 and Ghannadha *et al*., 1995) and is considered to be a very important component of quantitative resistance. Zadoks (1974) and Teng *et al*. (1977) showed that a small change in latent period can have a strong impact on the development of rust epidemic. A high level of correlation between the latent period and PI was clearly evident in the glasshouse experiment. The longer the latent period the less severe the yellow rust infection resulting in a lower PI. Having a longer latent period is a desirable characteristic that breeders strive for resulting in a slower development of epidemics throughout the field.

Environmental conditions can alter disease pressure, affecting pathogen infection and development (De Vallaveille-Pope, 1995). In the glasshouse extreme environmental conditions found in the field are removed, and optimal conditions for yellow rust infection provided. At least three hours of continuous moisture on the plant surface is required for spore germination and infection (Rapilly, 1979). Light rain creates the optimal conditions for yellow rust infection to occur in the field (Geagea *et al*., 2000, Fitt *et al*., 1989). Yellow rust spores are known to be dispersed by wind (Rapilly, 1979) and are released from the host tissue by the impact of water drops (Park, 1990, Rapilly, 1979). Differences in the phenotypic data sets obtained in the first and second year field trials could be due to the weather conditions in each year. Humidity levels are important for yellow rust infection, the higher the humidity the greater the adhesion of spores to the leaf surface, increasing the disease by increasing infection frequency (Beest *et al*., 2008). Temperature affects several aspects of yellow rust infection, from spore germination and infection, the latent period, sporulation, spore survival and host resistance. Stubbs (1985) reported that night temperature has a crucial role in disease establishment, demonstrating that dew formation and cool temperatures resulted in increased infection. Hot weather limits the development of the disease, inhibiting sporulation or causing the fungus to become dormant, compromising the survival of the fungus (Line, 2002). Temperatures higher than 23˚C slow down yellow rust epidemics (Hogg *et al*.,

1969), periods of hot weather halting the spread of disease, although temperatures exceeding 25˚C are rare in England (Beest *et al*., 2008). Meteorological data from the Weather Link at John Innes Centre suggests that humid and wet conditions in the second year field trial could have been the reason for the rapid onset of disease and high levels of infection observed.

Lewis (2006) suggested that the UK cultivar Claire carries the seedling resistance genes *Yr2*, *Yr3*, *Yr4*, *Yr25* and *YrHVIII* and another unknown seedling resistance gene. The presence of *Yr3* and *Yr4* in Claire is not unexpected as they are common seedling resistance genes found in many European wheat cultivars (Stubbs, 1985). However, these two resistance genes have been overcome by most UK *P. striiformis* races and are therefore not likely to contribute to the resistance phenotype observed in Claire. Chi-squared analysis of the seedling data revealed that there is probably one gene associated with resistance towards isolate WYR 76-10 and two genes associated with resistance towards isolate WYR 68-2 present in Claire.

The PI and IT nominal scores obtained for the Claire x Lemhi DH population under glasshouse conditions and in two consecutive years of field trials will be used for the identification of the QTLs associated with yellow rust resistance found in the cultivar Claire (Chapter 6).

Chapter 4

Construction of a genetic marker linkage map for the Claire x Lemhi doubled haploid population

4.1 Introduction

Until the introduction of DNA-based molecular markers the development of comprehensive genetic linkage maps was not possible. Advances in DNA marker technologies have provided virtually unlimited DNA markers. Genetic mapping locates the position of and distance between genetic loci (genes and markers), providing a one dimensional graphical representation that reflects the linear structure of the chromosomes. Relative position and distance are determined on the basis of how often the loci are inherited together, referred to as linkage, or become separated by genetic recombination (Jansen *et al*., 2001). Molecular marker loci are considered fixed points on the chromosome with the distances between loci being measured in centimorgans (cM). These map units are calculated from observed recombination frequencies, however there is no direct relationship between the cM units of genetic distance and physical, kilobase pair (Kb) distances (Semagn *et al*., 2006b). Construction of a detailed genetic map, with a high level of genome coverage is the first step towards locating genes or QTL associated with economically important traits. Genetic mapping allows the identification of markers for MAS, enables comparative mapping between related species, provides a framework for anchoring physical maps and forms the basis for map-based gene cloning (Semagn *et al*., 2006a, b). At present there are several computer packages available for linkage map construction, the most extensively used are JoinMap (Stam, 1993), MAPMAKER (www.broad.mit.edu/ftp/distribution/software/mapmaker3/) (Lauder *et al*., 1987), MapDisto (http://mapdisto.free.fr/) (Lorieux, 2007) and Map Manager (www.mapmanager.org) (Manly *et al*., 2001). JoinMap is a commercial program, the other computer packages being freely available from the internet.

In this study five marker technologies were used to construct a genetic map of the Claire x Lemhi DH population. The marker technologies used were AFLP, DArT, NBS profiling, SSRs and expressed sequence tag (EST) derived markers (Chapter 5). SSR markers remain a standard for map construction as they are highly polymorphic, require only a small amount of test DNA, can be easily automated enabling high throughput screening and are highly reproducible, thereby allowing exchange between laboratories and transferability between populations (Gupta *et al*., 1999). The identification and development of SSR markers is however time consuming and

expensive, but once developed produces a simple, high-throughput and cost effective marker system (Song *et al*., 2005, Kota *et al.,* 2001, Roder *et al*., 1995) (Table 4.1). SSR loci are fairly evenly distributed throughout the wheat genome. Physical mapping of SSRs on group 2 chromosomes confirmed that they do not cluster to specific regions (Roder *et al*., 1998a, b), making them highly suitable for the construction of molecular maps.

AFLP is a highly sensitive technology, having the capability of detecting multiple polymorphisms in different regions of the genome simultaneously. AFLP can generate a vast number of marker loci which is useful for saturating genetic maps (Semagn *et al*., 2006a, Vos *et al*., 1995). No prior sequence knowledge is required for marker development and the stringent reaction conditions used for primer annealing results in robust and reliable markers. AFLP marker distribution is generally genome wide, although AFLP marker loci detected with some restriction enzymes have been found to cluster within the genome (Vuylsteke *et al*., 1999). NBS profiling is a modification of AFLP which identifies polymorphisms within regions of the plant genome containing the conserved NBS motifs common to many cloned *R*-genes. Amplification is from the conserved NBS motif towards the restriction enzyme sites identifying polymorphism associated with presumed resistance genes, and resistance gene analogues (RGAs).

ESTs provide a valuable resource for the development of gene-targeted molecular markers as they are derived from gene mRNA transcripts (Kota *et al*., 2001). The usefulness and development of EST-derived markers is discussed in Chapter 5.

DArT is a microarray hybridisation-based technique that enables the simultaneous typing of several hundred polymorphic loci allowing comprehensive genome profiling without the need for sequence information (Wenzl *et al*., 2004). DArT analysis is high-throughput and robust, having the capability of producing high marker density within a short period of time at low cost (reported to be 10-fold lower per data point than the cost of SSRs; Xia *et al*., 2005).

Table 4.1 Comparison of the marker technologies used to construct a genetic map of the Claire x Lemhi DH population.

	SSR	AFLP	NBS-AFLP	EST-derived	DArT
DNA amount used	10-20*ng*	0.2-1.0μg	0.2-1.0 μg	10-20*ng*	20-100*ng*
DNA quality required	Moderate	Moderate	Moderate	Moderate	High
Number of loci/assayed	High	High	High	Low	High
Inheritance	Co-dominant	Dominant	Dominant	Co-dominant	Dominant
Ease of use	Easy	Easy	Easy	Easy	Easy
Amenability to automation	High	Low	Moderate	High	High
Reproducibility	High	Moderate	Moderate	Moderate	High
Development costs	High	Low	Moderate	Low	High
Cost per analysis	Low	Moderate	Moderate	Low	Low

4.2 Aims of current study

This chapter describes the development of a genetic linkage map using DArT, SSR, AFLP, NBS and EST-derived markers of a DH mapping population from a cross between wheat cultivars Claire x Lemhi. Linkage analysis was used to calculate the pairwise recombination between marker loci to establish linkage groups, estimate map distance and determine marker order using the statistical program JoinMap. Establishing a detailed genetic map of the Claire x Lemhi DH population is the first step towards locating the QTL associated with the yellow rust resistance found in Claire.

4.3 Results

4.3.1 Diversity array technology ® (DArT) marker screen

DArT marker assays were performed by Triticarte Pty Ltd (http://www.triticarte.com.au/; Yarralumla, ACT, Australia) and provided widespread genome coverage producing linkage groups of all 21 wheat chromosomes. A total of 501 DArT markers were found to be polymorphic between Claire and Lemhi, 147 of these had an unknown genetic location while 352 had a known location (Table 4.2). The polymorphic markers were screened against the

whole DH population of 77 lines and the data used in the construction of the genetic linkage map.

Table 4.2 Total number of polymorphic DArT markers with known chromosomal location.

	Chromosome Number						
Genome	**1**	**2**	**3**	**4**	**5**	**6**	**7**
A	15	17	22	27	5	22	32
B	19	23	25	6	30	21	42
D	20	10	2	5	5	1	5

4.3.2 Transfer of SSR and AFLP markers from the Claire x Lemhi F_2 population to the DH mapping population

Claire and Lemhi were previously screened with a total of 287 publicly available SSR primers (Lewis, 2006) obtained from the Graingenes database (www.graingenes.org) (Carollo *et al*., 2005, Matthews *et al*., 2003). SSR primer sets were obtained from the BARC (Song *et al*., 2005), the CFD (Guyomarc'h *et al*., 2002), the D genome specific GDM (Pestova *et al*., 2000), the GWM (Roder *et al*., 1998), the PSP (Bryan *et al*., 1997) and the WMC marker collections (Edwards *et al*., 1996). Eighty two of these SSRs showed either presence/absence or size polymorphism, 37 of which were easily scored and previously mapped in the Claire x Lemhi F_2 population. These SSRs were transferred to the Claire x Lemhi DH population (Table 4.3). BARC106 did not produce a clear polymorphic banding pattern that could be scored on the 77 Claire x Lemhi DH lines so was excluded from the mapping data to avoid inaccurate association of markers. Three of the SSR markers CFD21, GWM044 and GWM570 amplified two polymorphic loci, these were given an additional lower case letter ($_a$ or $_b$) for identification.

Table 4.3 Polymorphic SSR markers screened on the Claire x Lemhi F_2 population and transferred to the Claire x Lemhi DH population. nbs = no band size data available, null = absent band, CS = band size in Chinese Spring and C= band size in Courtot. Opata 85 and W7984 are the parents of the International Triticeae Mapping Initiative (ITMI) spring wheat reference population developed by van Deynze *et al.* (1995) (www.scri.sari.ac.uk/ITMI).

SSR marker[1]	Published chromosomal location	Expected fragment size (bp)				SSR marker	Published chromosomal location	Expected fragment size (bp)			
		Observed		Expected				Observed		Expected	
		Claire	Lemhi	Opata 85	W7984			Claire	Lemhi	Opata 85	W7984
BARC24	6BL	200	190	nbs	nbs	GWM304	5AS	196	200	202	208
BARC108	7AL	198	200	nbs	nbs	GWM333	7BC	156	158	154	166
BARC148	1AS	190	205	nbs	nbs	GWM357	1AS/L	123	125	123	120
BARC171	6AS	220	200	nbs	nbs	GWM368	4BS	244	248	259	271
BARC 212	2AS	214	230	nbs	nbs	GWM383	3DL	193	189	188	199
CFD21a	1AS	240	235	nbs	nbs	GWM369	3AS	196	156	184	null
CFD21b	1DS	270	268	268(C)	272(CS)	GWM413	1BS	85	95	91	95
GWM044a	7ASa	185	175	nbs	nbs	GWM458	1DL	114	110	115	119
GWM044b	7DSb	185	175	178	176	GWM469	6DS	173	167	172	170
GWM098	6DL	159	158	nbs	nbs	GWM540	5BS	130	135	133	117
GWM132	6BS	100	95	118	116	GWM570a	6AL	146	157	149	143
GWM153	1BL	240	245	183	195	GWM570b	6BL	210	null	nbs	nbs
GWM160	4AL	180	176	184	196	GWM601	4AS	164	166		
GWM161	3DS	160	175	154	145	PSP3047	3AL	186	181	184(CS)	184(CS)
GWM174	5DL	190	210	233	204	WMC25	2DS	160	155	166(CS)	166(CS)
GWM179	5A	190	184	181	null	WMC47	4BL	143	149	141(CS)	141(CS)
GWM190	5DS	211	216	201	253	WMC222	1DS	142	145	188(CS)	188(CS)
GWM205	5AS	153	165	158	152	WMC407	2AS	128	132	135(CS)	135(CS)
GWM296	2DS	165	174	182	null	WMC457	4D	166	168	159(CS)	159(CS)
GWM297	7BC	150	145	150	168						

[1]Appendix Table 1 for primer sequences.

A previous investigation using a Claire x Lemhi $F_{2:3}$ population identified a significant QTL for yellow rust field resistance on a linkage group that could not be assigned a chromosomal location (Lewis, 2006). This linkage group spanned 17 cM in length and was comprised of two AFLP loci, S15/M32-155 (Figure 4.1) and S15/M45-190. These AFLP primer pairs were also screened against the Claire x Lemhi DH mapping population, all polymorphic bands produced by these two AFLP primer pairs being mapped in the DH population (Table 4.4).

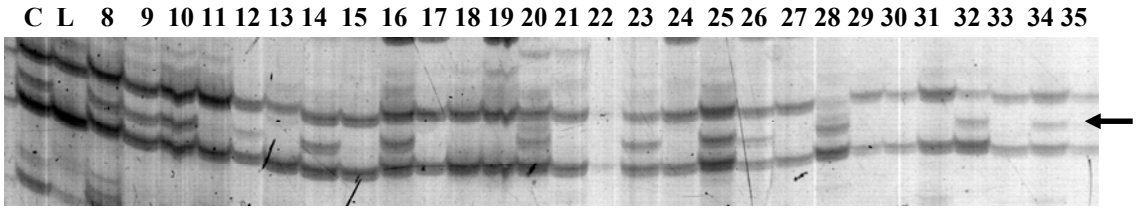


Figure 4.1 Polyacrylamide gel showing the presence/absence polymorphism S15M32-155 in the parents Claire (C) and Lemhi (L) and 28 DH lines (DH 8-35).

Table 4.4 Selective nucleotides of the AFLP primers and the size of the polymorphic bands produced.

Primers	Selective nucleotides[1]		Fragment sizes (bp)
	*Sse*I	*Mse*I	
S15/M32	CA	AAC	155[2], 300
S15/M45	CA	ATG	165, 175, 190[2], 230, 350, 360

[1]Selective nucleotides of the primers for the *Sse*I and *Mse*I restriction sites, [2]polymorphism associated with QTL for field yellow rust resistance in the Claire x Lemhi $F_{2:3}$ populations.

4.3.3 Additional SSR marker selection

Preliminary linkage mapping of the DArT, SSR and AFLP data found that the unknown linkage group, consisting of the two AFLP markers (S15M32-155 and S15M45-190) was located on the long arm of chromosome 2D. Preliminary QTL analysis using this linkage map together with two years of field data and one year of adult plant glasshouse data (Chapter 3) found QTL effects to be located on 2BL and 2DL. An additional QTL associated with seedling resistance was also identified on

chromosome 3B. Further SSR primers were selected to saturate 2BL, 2DL and 3B to allow for fine mapping of the QTL associated with seedling and APR (Chapter 6). These were selected from the Graingenes database (www.graingenes.org) and by comparative mapping using the wheat consensus map (Somers *et al*., 2004), the wheat composite map and the ITMI map (Song *et al*., 2005) (Table 4.5). A total of 39 SSR were chosen based upon chromosome location in order to saturate 2BL, 2DL and 3B. Of these, 20 SSRs were polymorphic between Claire and Lemhi and screened against the DH mapping population. SSRs BARC219, BARC59, WMC179, WMC181 (Figure 4.2), WMC817, WMC441, GWM311 and GWM16 each amplified two polymorphic loci, while WMC78, GWM382, GWM301 each amplified three polymorphic loci. These SSR loci were given a lower case letter ($_a$ or $_b$) and ($_a$, $_b$, or $_c$), respectively for identification.

Table 4.5 Additional SSR markers screened against Claire and Lemhi. X = no polymorphism, nbs = no band size data available, null = absent band, CS = band size in Chinese Spring. Opata85 and W7984 are the parents of the International Triticeae Mapping Initiative (IMTI) spring wheat reference population developed by van Deynze *et al*. 1995.

SSR marker[1]	Published chromosome location	Expected fragment Size (bp)				SSR marker	Published chromosome location	Expected fragment Size (bp)			
		Observed		Expected				Observed		Expected	
		Claire	Lemhi	Opata 85	W7984			Claire	Lemhi	Opata 85	W7984
BARC59a	2D	200	190	nbs	nbs	GWM320	2DL	X	X	nbs	226
BARC59b	-	140	145	nbs	nbs	GWM349	2DL	X	X	243	225(CS)
BARC68	3B	203	205	nbs	nbs	GWM382a	2AL	X	220	nbs	184
BARC77	3B	null	275	nbs	nbs	GWM382b	2DL	X	115	nbs	108
BARC159	2D	298	290	nbs	nbs	GWM382c	-	X	85	nbs	86
BARC219a	2D	215	220	220(CS)	220(CS)	GWM441	2D	X	X	nbs	nbs
BARC219b	7A	130	132	nbs	nbs	GWM526	2BL	X	X	148	138
BARC228	2D	X	X	nbs	nbs	GWM539	2DL	143	null	143	157
GWM12	2D	X	X	nbs	nbs	GWM608a	2DL	X	X	166	181
GWM16a	2B	220	225	224	225	GWM608b	-	X	X	151	144
GWM16b	5D	200	null	206	204	PSP519	3B	X	X	nbs	nbs
GWM16c	7B	X	X	181	176	PSP2151	2A/3B	X	X	nbs	nbs
GWM120	2B	158	165	162	174	PSP3001	3B	X	X	207(CS)	207(CS)
GWM157	2DL	X	X	106	110	PSP3039	2AL	X	X	152(CS)	152(CS)
GWM189	2BL	X	X	nbs	nbs	WMC41	3B	X	X	163(CS)	163(CS)
GWM311	2DL	160	155	157	143	WMC54	-	X	X	142(CS)	142(CS)
GWM312	2AL	152	155	216	219	WMC78a	3B	240	230	241	nbs
GWM301a	2DL	340	null	210(CS)	171	WMC78b	-	160	null	nbs	nbs
GWM301b	-	160	null	nbs	nbs	WMC78c	-	155	null	nbs	nbs
GWM301c	-	142	null	nbs	nbs	WMC154	2B	140	145	147(CS)	147(CS)

[1]Appendix Table 1 for primer sequences.

Table 4.5 continued. Additional SSR markers screened against Claire and Lemhi. X = no polymorphism, nbs = no band size data available, null = absent band, CS = band size in Chinese Spring. Opata85 and W7984 are the parents of the International Triticeae Mapping Initiative (IMTI) spring wheat reference population developed by van Deynze *et al*. 1995.

SSR marker[1]	Published chromosome location	Expected fragment Size (bp)				SSR marker	Published chromosome location	Expected fragment Size (bp)			
		Observed		Expected				Observed		Expected	
		Claire	Lemhi	Opata 85	W7984			Claire	Lemhi	Opata 85	W7984
WMC167	2DL	190	null	185(CS)	185(CS)	WMC322	3B	X	X	95(CS)	95(CS)
WMC175	2DL	260	255	253(CS)	253(CS)	WMC441a	2D	270	null	nbs	nbs
WMC179a	3B	195	200	201(CS)	201(CS)	WMC441b	2B	163	158	158(CS)	158(CS)
WMC179b	-	160	155	nbs	nbs	WMC445	2D	X	X	229(CS)	229(CS)
WMC181a	2AL	260	255	260(CS)	260(CS)	WMC754	3B	X	X	174(CS)	174(CS)
WMC181b	2DL	155	null	nbs	nbs	WMC817a	2D	210	205	199(CS)	199(CS)
WMC243	2DL	X	X	nbs	nbs	WMC817b	2A	80	75	nbs	nbs

[1]Appendix Table 1 for primer sequences.

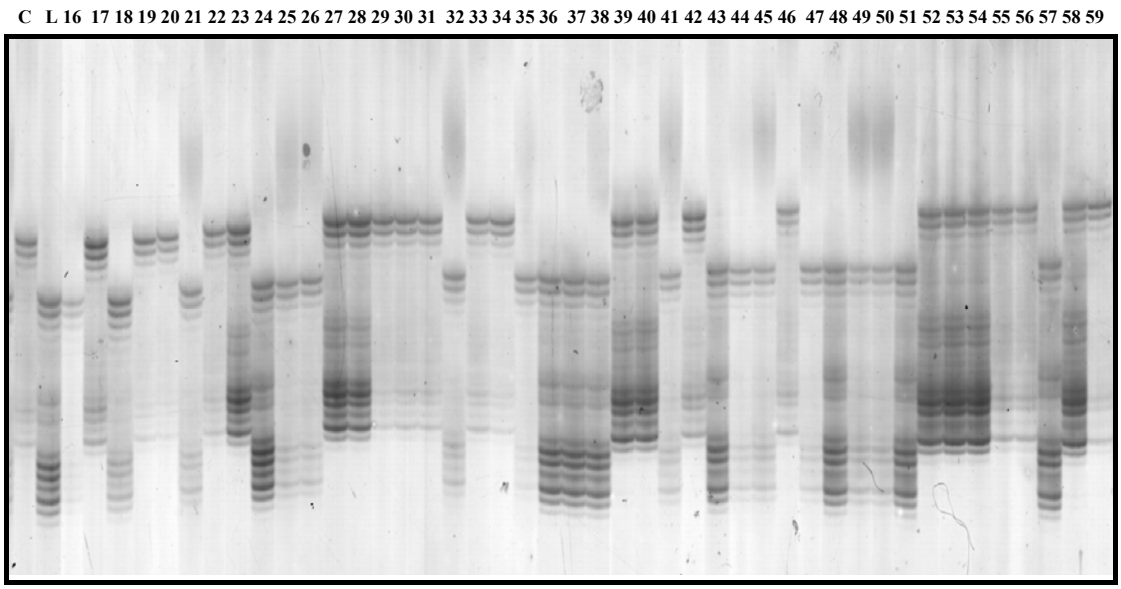


Figure 4.2 Polyacrylamide gel of SSR marker WMC181 showing size polymorphism between the parents Claire (C) and Lemhi (L) and 43 DH lines (DH16-59) for marker loci *Xwmc181a*.

4.3.4 NBS markers

NBS2 and NBS2cer (cereal specific) primers were used in combination with the restriction enzymes, *Mse*I and *Rsa*I. Nine polymorphisms were identified and screened against the mapping population (Table 4.6). The polymorphisms are due either to the absence/presence of the restriction enzyme sites or variation in the NBS motifs between Claire and Lemhi. The polymorphisms identified by NBS2/*Mse*I are shown in Figure 4.3.

Table 4.6 NBS profiling polymorphisms found between Claire and Lemhi

NBS primer	Restriction enzyme	Fragment size (bp)
NBS 2	*Mse*I	288, 298, 310, 396
NBS 2cer	*Mse*I	396, 506
NBS 2cer	*Rsa*I	205, 215, 220

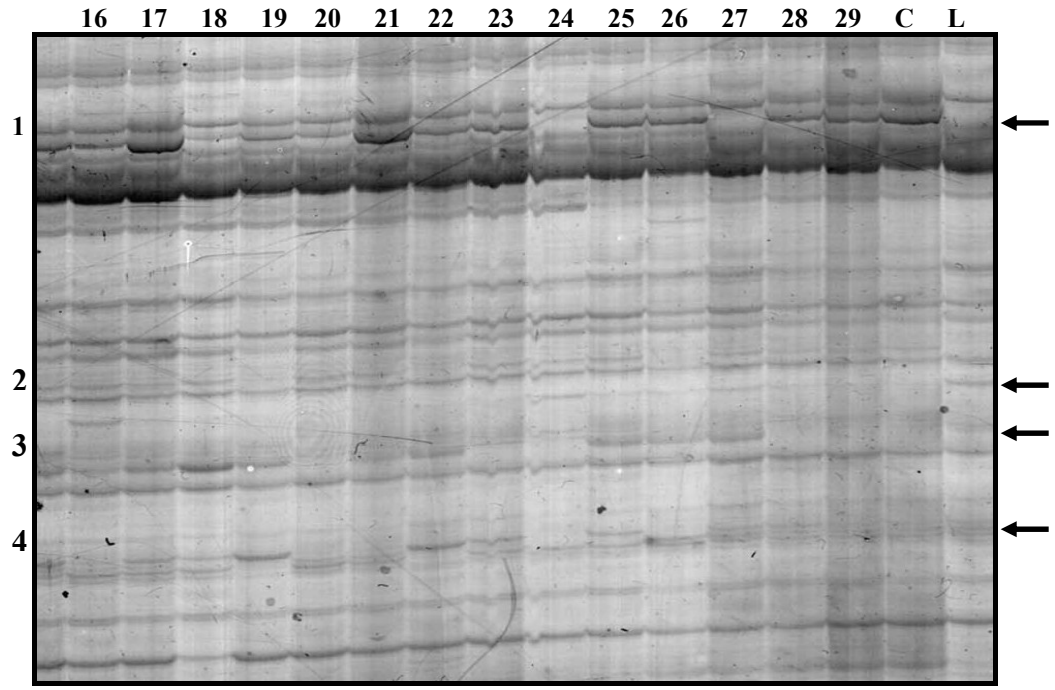


Figure 4.3 Polyacrylamide gel showing NBS profile produced by NBS2/*Mse*I in parents Claire (C) and Lemhi (L) and 20 DH lines (DH16-35). Four polymorphisms detected, 1 to 4 of 396, 310, 298 and 288bp, respectively.

4.3.5 EST-derived markers developed for chromosome 2DL

The 22 2DL EST-derived markers developed in Chapter 5 were screened against Claire and Lemhi. Eleven of the EST-derived primer pairs were found to be polymorphic (EST 4, 6, 7, 8, 9, 10, 11, 12, 16, 18 and 22) and were subsequently screened against the entire mapping population (Chapter 5). One EST-derived marker (EST 18) amplified three polymorphic loci, these were given a lower case letter ($_{a}$, $_{b}$ and $_{c}$) for identification.

4.3.6 Linkage Map construction

Prior to map construction pre-mapping analysis of the SSR, AFLP, DArT, EST and NBS marker data set was performed to determine the quality of the data. Any marker that had over 10% missing data was excluded from the analysis in order to prevent inaccurate association of markers. Most DH lines had a very low proportion of

missing marker data with 76 DH individuals containing less than 6.2% missing data points, the exception being DH60, with 86.8% of the marker data missing. This individual was subsequently excluded from further analysis. For each segregating marker a chi-squared goodness-of-fit analysis (Snedecor and Cochran, 1989) was conducted to test for deviation from the expected segregation ratio of a DH mapping population. A high proportion of the markers (550 markers, 90.4%) conformed to the expected 1:1 ratio for a DH population. Those markers that exhibited segregation distortion ($p<0.01$) were removed from the linkage analysis. These 58 (9.6%) skewed markers could result in false associations of markers to otherwise unlinked regions (Knox and Ellis, 2002). Of these 58, markers exhibiting segregation distortion in favour of Lemhi alleles were more frequent (67.2%) than those in favour of Claire alleles (32.8%). Chi-squared analysis identified 50 DArT (10%), four SSRs (5.3%), two AFLPs (33.3%), and two NBS-derived (22.2%) markers with segregation distortion, all of which were removed from further analysis. Each DH line had a low proportion of missing field data, the exception being DH78 which had no phenotypic field or glasshouse data, this DH line was therefore also excluded from any further analysis. Five of the DH lines (DH1, DH2, DH3, DH5 and DH7) were found to be self's of the resistant parent Claire. These lines were used as controls for genetic mapping to assess the quality of the marker data, however they were removed prior to linkage map construction.

Joinmap version 3.0 for windows (van Ooijen and Vooripps, 2001) was used to create a linkage map of the Claire x Lemhi DH population. A total of 580 markers displaying 608 polymorphism loci (500 DArT loci, 75 SSRs loci, 6 AFLP loci, 18 EST-derived loci and 9 NBS loci) were finally used in the Claire x Lemhi mapping analysis. The genetic map produced consisted of 491 marker loci (80.3% of loci) consisting of 424 DArT (94.2%), 52 SSRs (73.2%), 10 EST-derived (55.6%), 3 AFLP (75%) and 2 NBS markers (28.6%). These yielded 49 linkage groups with a total map length of 1560cM and represented all 21 chromosomes of wheat. The linkage groups selected for map construction had a minimum LOD of 3.0 with recombination fractions of less than 0.4 between marker loci. Map length was determined by the Kosambi mapping function (Kosambi, 1944) converting recombination fractions into cM distances. Several linkage groups that carried diagnostic SSR or DArT markers for the same chromosome could not be mapped

together on one linkage group, despite lowering the LOD values below the selected threshold.

A high proportion (43.9%) of the DArT markers tended to cluster to the same locus thereby reducing the amount of unique information on the genetic map. Of the clustered DArT markers 184 (43.4% of mapped DArT loci) were removed, leaving 240 DArT markers in the map. Subsequent removal of these clustered loci did not substantially alter the genetic map. The translocation linkage group 5BL/7BL had the highest level of clustered DArT markers, i.e. 36 loci clustered. Removal resulted in a 1cM increase in linkage group length however, loci order was not affected. Position and distance were not altered in linkage groups containing less than five clustered DArT loci, those containing more than five clustered loci resulted in increased length in linkage group of 1-3cM. The final map (Figure 4.4) had an average of 14.5 loci per chromosome, ranging from two on linkage group (6D) to 28 on the 5B/7B translocation chromosome. The longest linkage group was on 3B (83 cM), whilst the shortest was on an unknown linkage group (2 cM). The average linkage group length was 31.8 cM. Marker density ranged from 1 cM marker^{-1} on unknown linkage group 1, to 13 cM marker^{-1} on 5D, with an average density of 5.3 cM marker^{-1}. Map distances between two consecutive marker loci varied from 0 to 32 cM, 80.3% of loci having less than a 10 cM distance between them. The largest distance between linked loci was observed on chromosome 5A (32 cM).

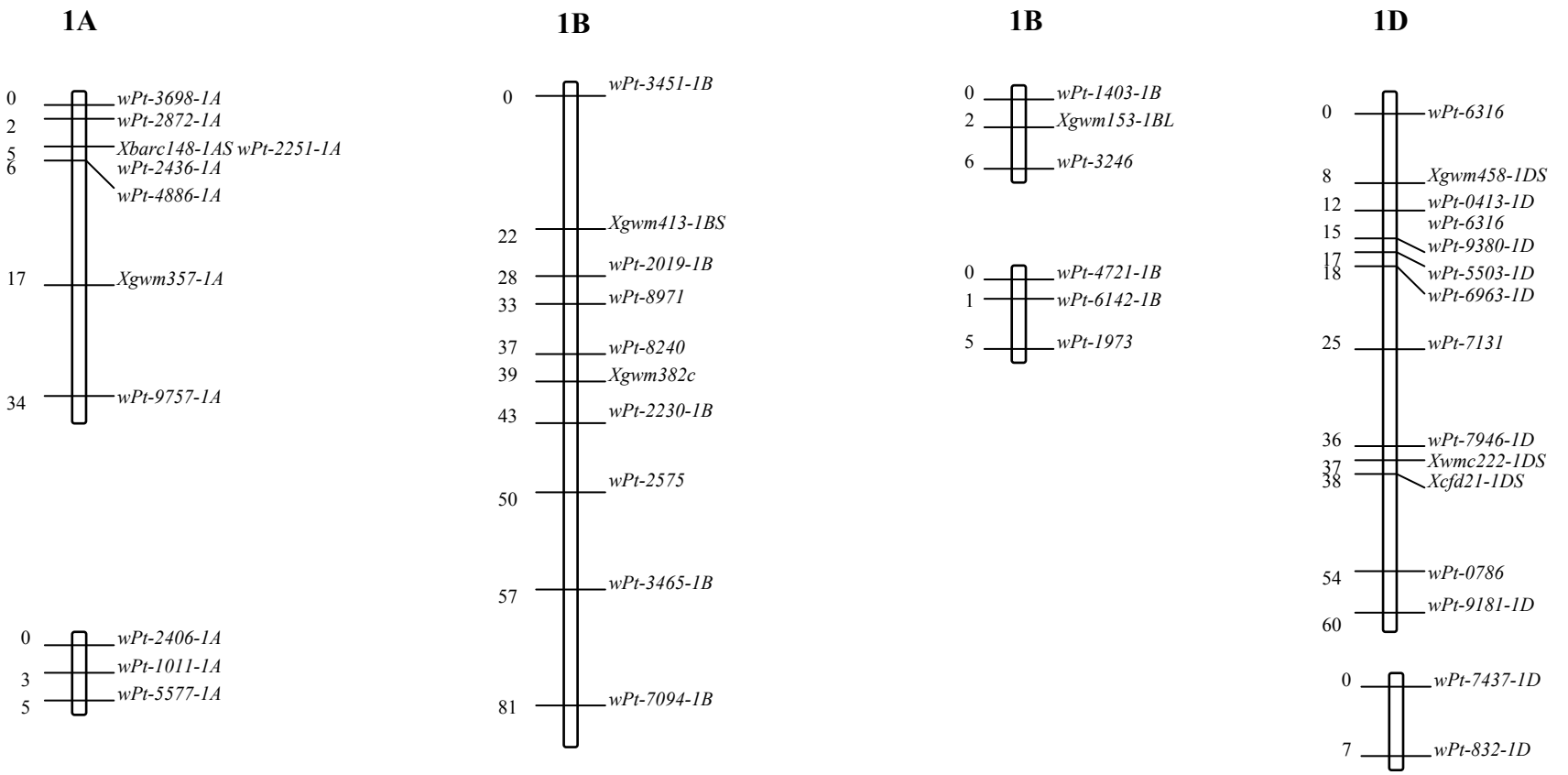


Figure 4.4 Genetic marker map of the Claire x Lemhi cross. Linkage groups cover all 21 chromosomes of wheat being constructed using AFLP, NBS, DArT, SSR and EST-derived markers by Joinmap v 3.0 (van Ooijen and Voorrips, 2001). The short arm of each chromosome is located towards the top and the long arm towards the bottom of the page, the marker loci are ordered in centimorgans (cM) distances from the top.

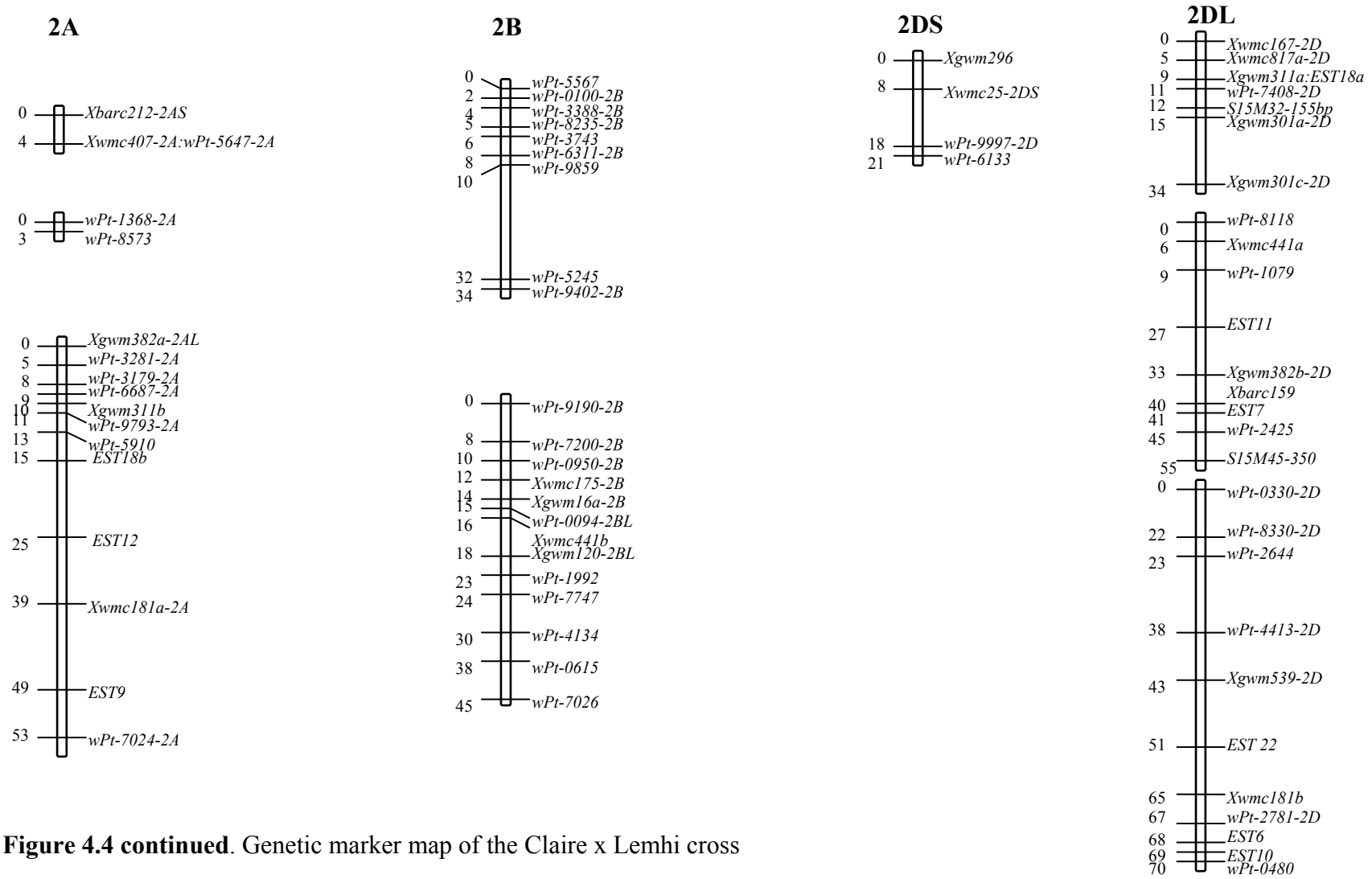


Figure 4.4 continued. Genetic marker map of the Claire x Lemhi cross

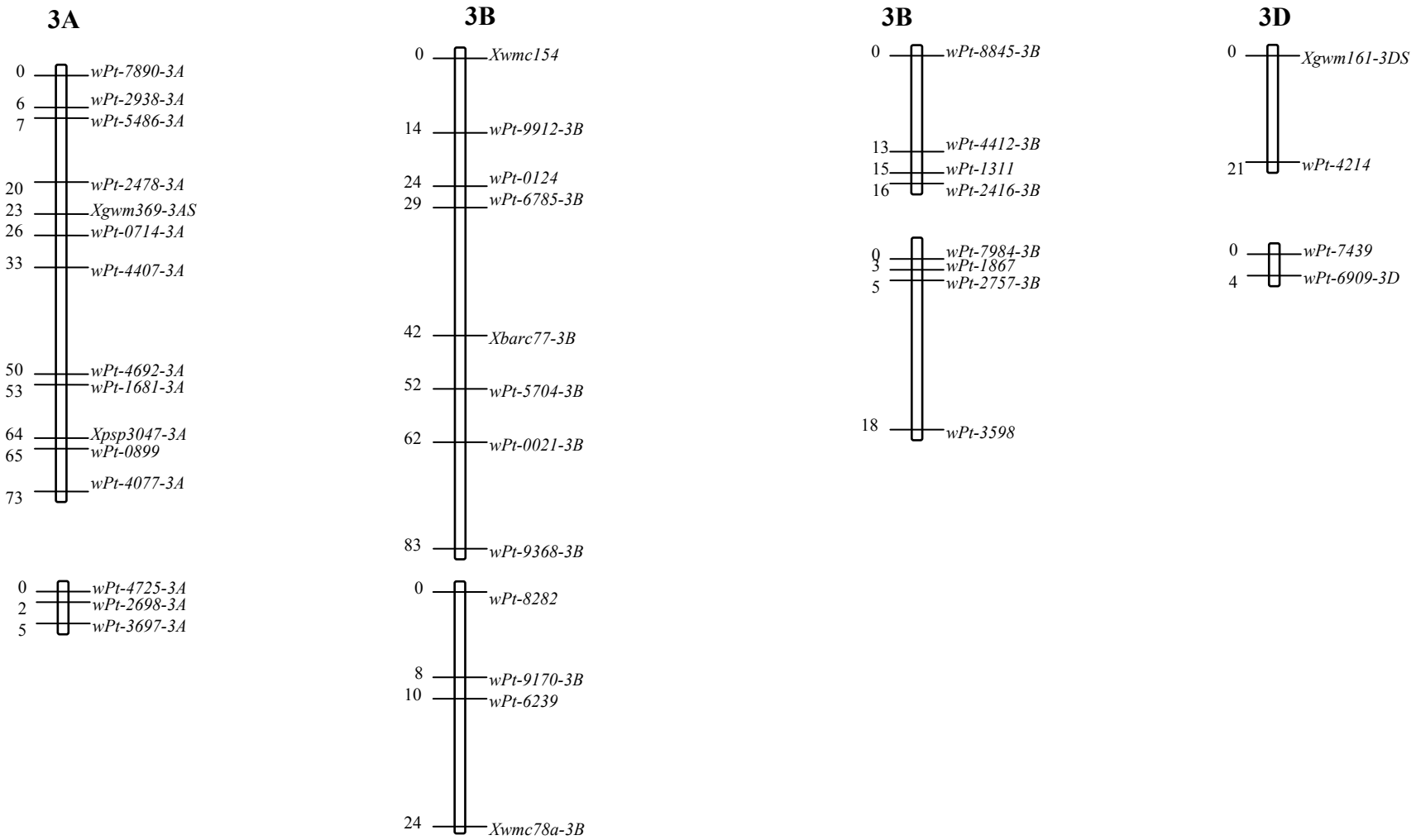


Figure 4.4 continued. Genetic marker map of the Claire x Lemhi cross

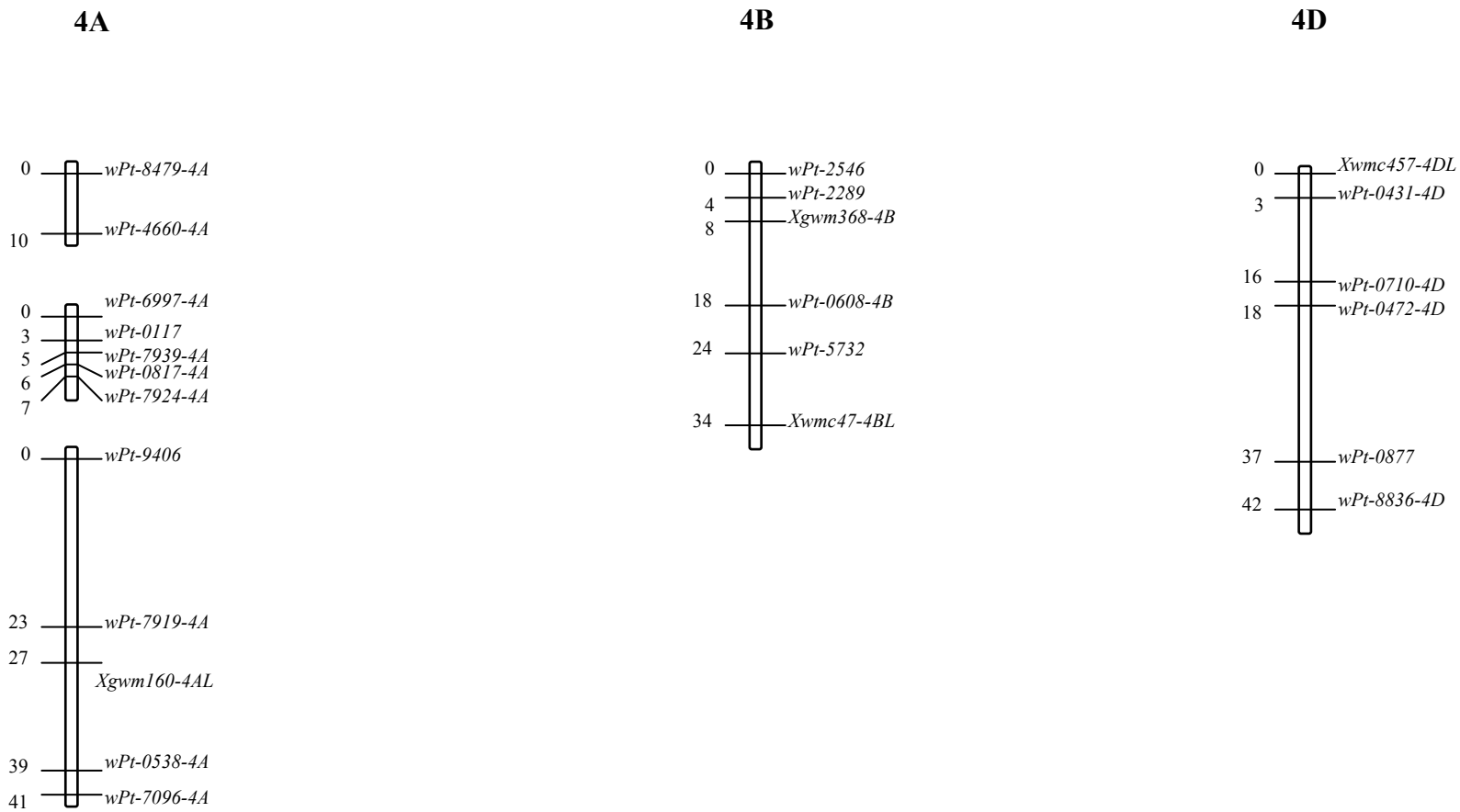


Figure 4.4 continued. Genetic marker map of the Claire x Lemhi cross

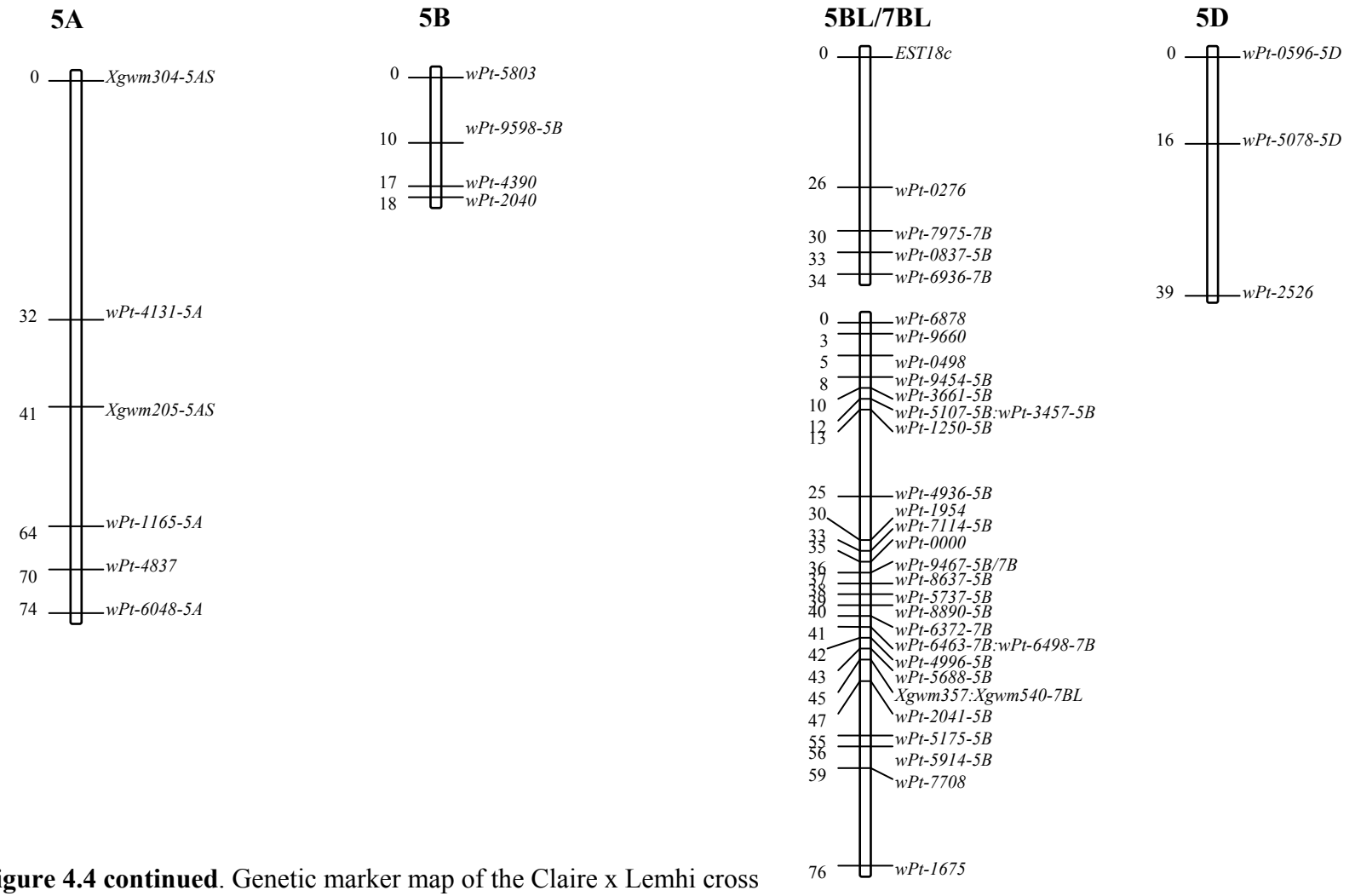


Figure 4.4 continued. Genetic marker map of the Claire x Lemhi cross

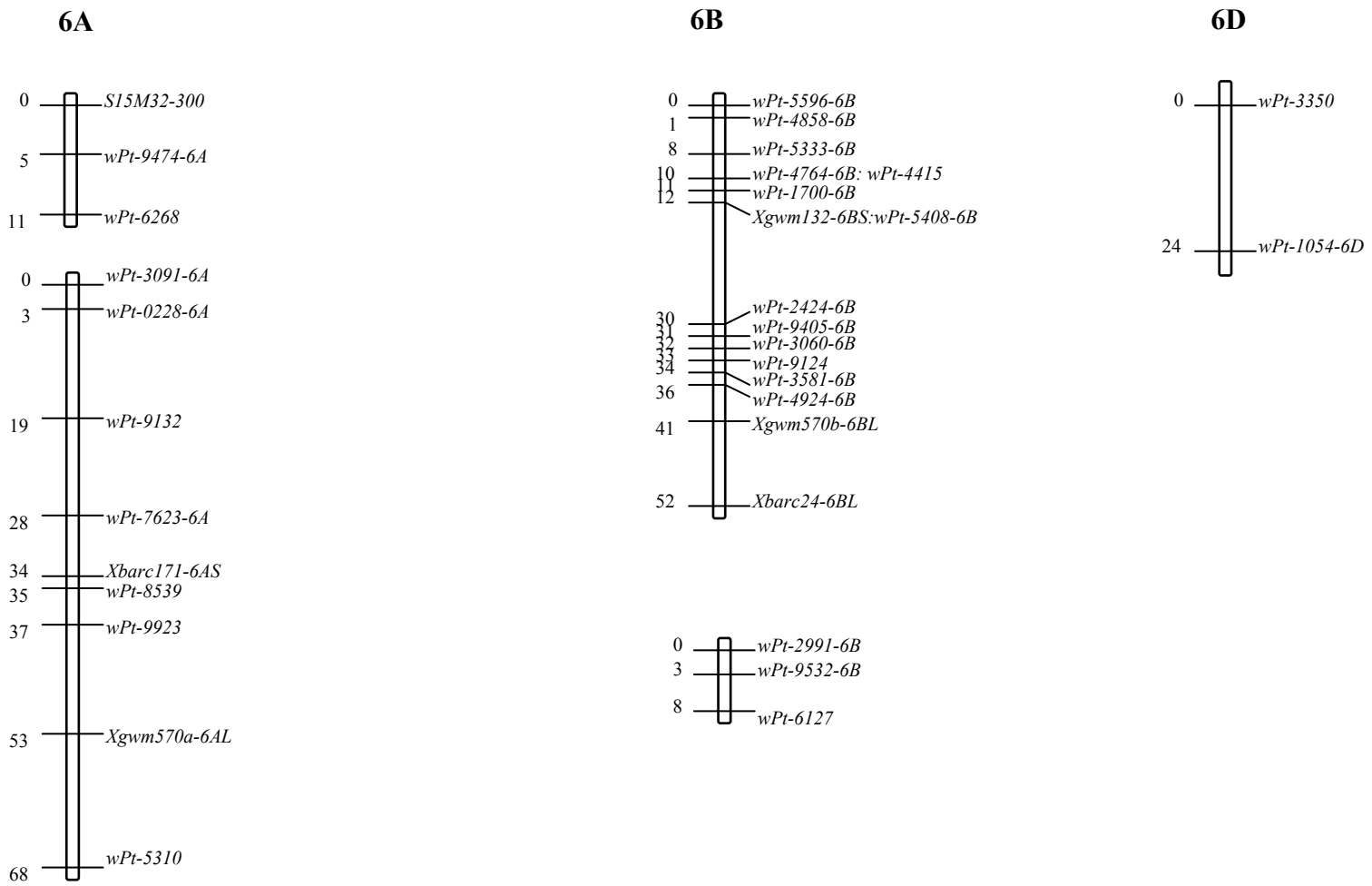


Figure 4.4 continued. Genetic marker map of the Claire x Lemhi cross

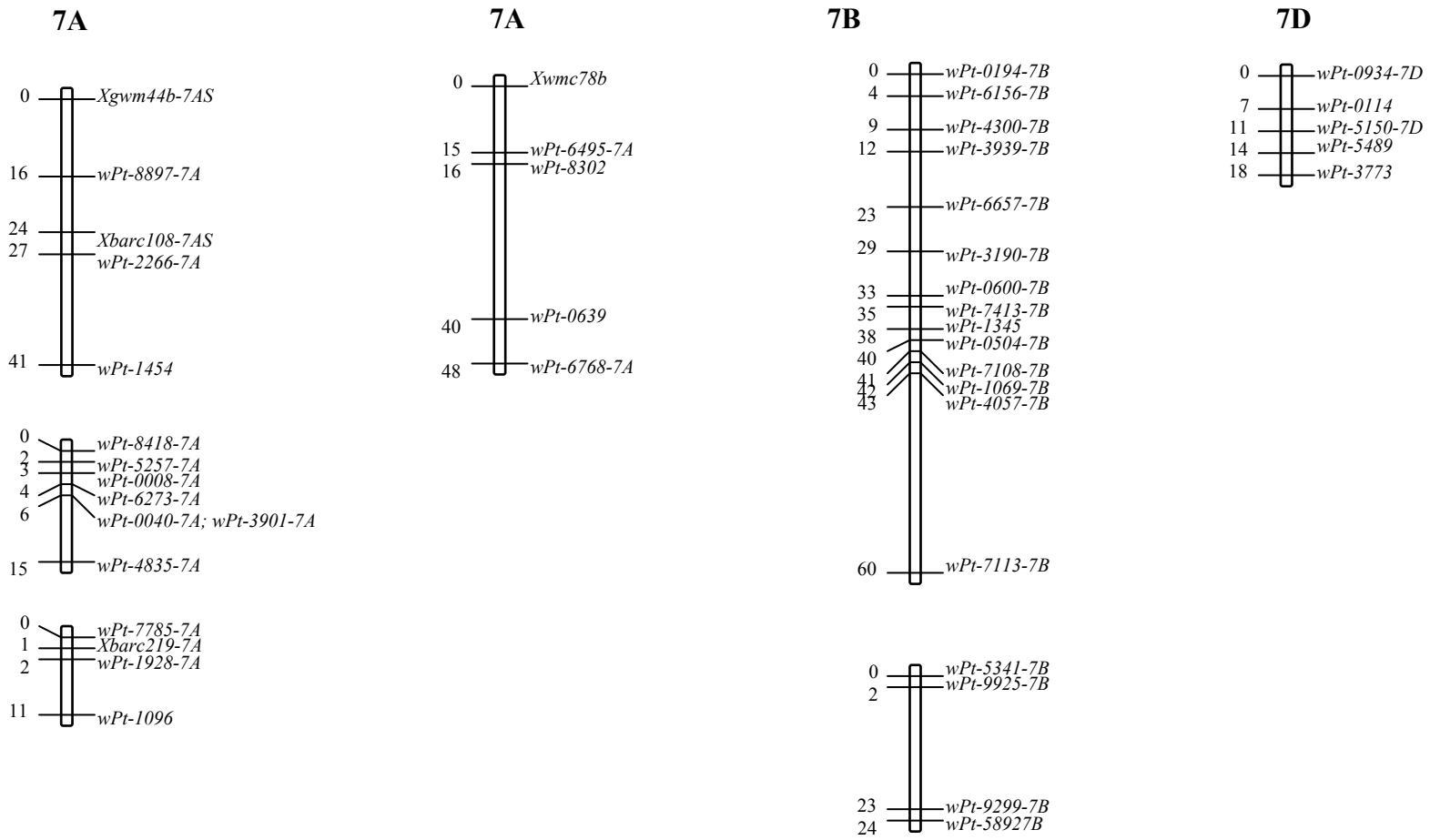


Figure 4.4 continued. Genetic marker map of the Claire x Lemhi cross

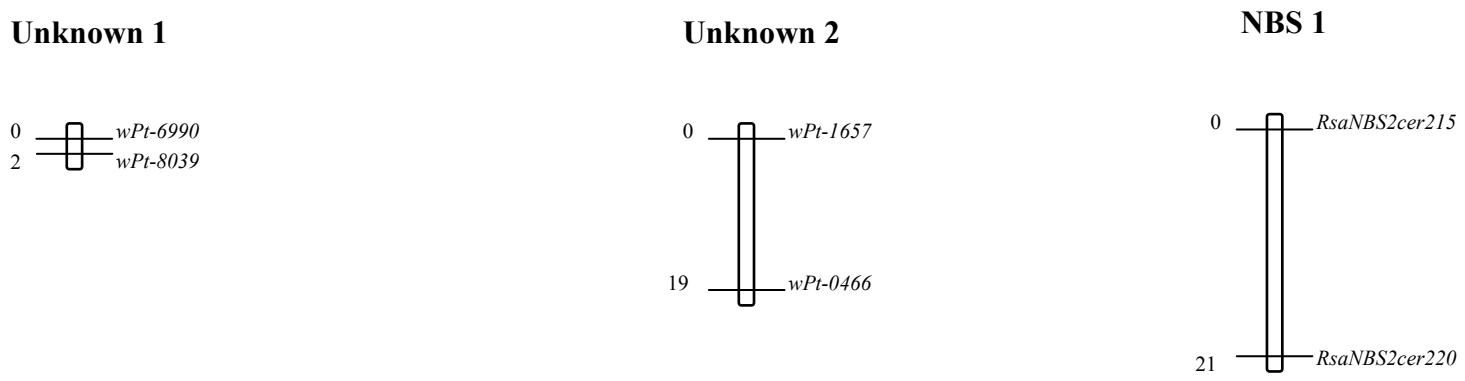


Figure 4.4 continued. Genetic marker map of the Claire x Lemhi cross

Variation was observed in the number of marker loci, map length and marker density between the chromosomes of the A, B and D genomes (Table 4.7). Marker loci number and density were highest in the B genome (138 loci and 4.5cM marker^{-1}), while the D genome had the lowest number of marker loci (66 loci) and lowest marker density (6.02cM marker^{-1}). The B genome contained the highest number of DArT marker loci (50% of the mapped DArT markers), while the D genome had the highest number of SSR marker loci (36.2% of those mapped), EST-derived marker loci (60% of those mapped) and AFLP marker loci (66.7% of those mapped). The D genome (397 cM) was shorter than the A (503 cM) and the B (618 cM) genomes even though the D genome contained the highest number of mapped SSR, EST-derived and AFLP marker loci.

Table 4.7 Comparison of map length, marker loci number and density across the genomes.

Genome	Marker loci number	Map length	Marker Density (cM marker $^{-1}$)
A	95	503	5.3
B	138	618	4.5
D	66	397	6.02

The SSR marker loci order was compared with published maps for all 21 hexaploid wheat chromosomes, on the wheat consensus map (Somers *et al*., 2004), the wheat composite map (http://shigen.nig.ac.jp/wheat/komugi/maps/markermap.jsp) and the ITMI map (Song *et al*., 2005). The majority of SSR marker loci mapped to their predicted chromosomes as determined by comparison with published wheat consensus maps (Somers et al., 2004, Roder *et al*., 1998), the two exceptions being *Xwmc*154 (mapped to 3BS) and *Xgwm*357 (mapped to 5BL/7BL translocation) where the expected locations were 2BS and 1AL, respectively. The CMAP program on the Graingenes website was used to compare these contradictory locus orders between the comparative maps. The differences found when comparing genetic maps is not unexpected as genetic mapping only gives an indication of the SSR marker locus order relative to one another (Sourdille *et al*., 2004). Three linkage groups could not be assigned a chromosomal location because of the absence of a SSR or known DArT marker locus within these linkage groups. All three unknown linkage

groups contained just two loci and varied from 2 to 21 cM in length. Two of the linkage groups contained only DArT markers with unknown chromosomal locations, while the third unknown group consisted of two linked NBS loci.

4.4 Discussion

A pre-requisite for effective and accurate QTL analysis is the construction of a genetic map that covers a high proportion of the genome. This is a difficult task in wheat because it is a hexaploid and has large genome of 16 million kb haploid $cell^{-1}$ (Liu and Tsunewaki, 1991, Bennett, 1982, Bennett and Smith, 1976). DArT and SSR markers were used to create a framework map covering all 21 wheat chromosomes. This allowed for preliminary QTL analysis to be carried out identifying regions that could be associated with seedling and APR for yellow rust in the cultivar Claire. A previous study, using a Claire x Lemhi $F_{2:3}$ population identified two AFLP markers to be highly associated with APR. One of these AFLP loci (S15/M32-155) mapped in the Claire x Lemhi DH population to 2DL, while the other remained unlinked (S15/M45-190). Preliminary QTL analysis found similar results as Lewis (2006), identifying a yellow rust APR QTL on 2DL associated with the AFLP locus S15/M32-155. This region of the genome was subsequently density increased with EST-derived markers and further SSR in order to precisely map the yellow rust resistance QTL.

A small percentage of marker loci (9.6%) had segregation ratios that differed significantly from the expected 1:1 ratio and were therefore removed from genetic map construction. Segregation distortion is a common trend in wide crosses and has been frequently reported in wheat. Liu and Tsunewaki (1991), Mantovani *et al*. (2008), Peleg *et al*. (2008) and Messmer *et al*. (1999) found the proportion of distorted loci to be 10, 26.5, 30 and 35% respectively, much higher than in the current study. The mapping population in the current study was derived from a cross between a UK winter and an American spring wheat which may explain the proportion of distorted loci identified, even though the percentage was low in comparison to other studies. Semagn *et al*. (2006c) reported considerable segregation distortion (20.4%) in the ArNK population, which was also developed from a cross between a winter and a spring wheat.

Early wheat genetic maps were comprised of hybridisation based RFLP markers. These were superseded when PCR based markers were introduced. Recently SSR markers have become the preferred marker for genetic mapping in wheat, particularly for use in MAS. The large numbers of DArT markers now available have made obtaining a good coverage of the wheat genome achievable in a relatively short time frame. These anonymous genetic markers are not developed from genes themselves, but provide the tools to localise the genes of agronomically important traits. Over the last few years wheat genomic research has resulted in a large accumulation of EST sequence data. These databases permit the development of a new type of marker which targets the expressed region of the genome (Chapter 3). It has been estimated that there is 1 SNP for every 540bp of wheat EST sequence (Somers *et al*., 2003). The extensive EST databases provide a unique opportunity to identify SNP within these expressed regions of the genome, allowing the potential to develop functional markers. Polymorphisms identified within an EST sequence can therefore be used to directly map gene functional regions of the genome.

The 550 marker loci screened in 70 DH lines was large enough to construct a genetic map of the Claire x Lemhi cross. Gupta *et al*. (1999) suggested that 300-400 markers are required to adequately cover the entire hexaploid wheat genome. However, the ideal number of DH lines required to establish an accurate genetic map is debatable (Ferreira *et al*., 2006, Vales *et al*., 2005, Jansen *et al*., 2001). The power of map construction and subsequent QTL analysis is augmented by increased marker density and population size (Van Ooijen and Voorips, 2001). A small mapping population can create mapping inaccuracies, producing fragmented linkage groups and inaccurate locus order, increasing the size of the mapping population enables a more precise genetic map to be achieved (Semagn *et al*., 2006). A population of 500 to 1000 genotypes provides an accurate genetic map, but this is not always viable due to the increase in cost and labour required (Ferreira *et al*., 2006). Saturated wheat genetic linkage maps have been constructed with populations consisting of less than 100 genotypes (Akbari *et al*., 2006, Semagn *et al*., 2006c, Quarrie *et al*., 2005, Suenaga *et al*., 2003). Akbari *et al*. (2006) produced a genetic map covering all 21 wheat chromosomes from 90 DH individuals of a Cranbrook x Halbred cross. RFLP, AFLP and SSR marker loci initially created a framework map spanning 2534cM,

subsequent DArT markers were added which increased the map length to 2937cM. A genetic linkage map of 93 DH lines derived from a cross between a winter wheat Arina and a spring wheat breeding line NK93604 produced a credible map of 2595cM from 624 marker loci (Semagn *et al*., 2006c). Both these studies analysed the map length, marker distribution and effect of DArT marker technology on the production of genetic maps. Suenaga *et al*. (2003) on the other hand identified various QTL associated with leaf and yellow rust resistance from a map of 107 DH lines which provided adequate genome coverage for the identification of QTL. Quarrie *et al*. (2005) produced a genetic map from 95 individuals of a Chinese Spring x SQ1 (breeding line) DH population allowing localisation of QTL associated with grain yield. It would be interesting to determine whether the population size severely alters the marker order, map size and the number of linkage groups for this Claire x Lemhi DH population. Further study could be done by doubling, tripling and quadrupling the number of DH lines to allow for a comparative study of the effect population size has on these key factors in genetic map construction. Using a DH population has advantages over the F_2 population used by Lewis (2006). DH lines are completely homozygous, with all alleles fixed, allowing repetition of phenotyping which facilitates the selection of quantitative characters. F_2 generations have a loose genetic identity in comparison, containing a high proportion of heterozygous loci, making selection for quantitative characters less reliable.

The 21 wheat chromosomes were represented by 49 linkage groups in the Claire x Lemhi DH population. The large number of linkage groups suggests that the molecular markers are not distributed evenly across all chromosomes, gaps without marker loci being present in the genetic map. If the average length of a wheat chromosome is assumed to be 200cM (Messmer *et al*., 1999) the total estimated map length of wheat would be 4200cM. This suggests that the Claire x Lemhi DH genetic map (1560cM) covers 37% of the wheat genome, leaving 63% of the genome undetected. The percentage coverage of the wheat genome is some what less than that of other published wheat maps. The crosses Guardian x Avocet S (Melichar *et al*., 2008), CS x T.spelta (Liu and Tsunawaki, 1991) and Forno x Oberkulmer (Messmer *et al*., 1999) have lengths of 1238, 1801 and 2469cM, covering 30%, 43% and 59% of the wheat genome, respectively. No large distances were found between two consecutive marker loci, the exception being chromosome 5AS having the

largest distance of 32cM. Other wheat genetic maps have found distances of over 50 cM (Mantovani *et al*., 2008) and 37cM (Melichar *et al*., 2008) between marker loci. Several regions of the genome had no associated linkage groups (1DL, 4AS, 4BS, 4DS and 5AL) other areas of the genome had only two marker loci representing the chromosomal region (3DS, 3DL and 6D), while 5D had three marker loci. The presence of large gaps within the genome and low marker densities has been described in several other wheat maps. The poor coverage of 4AS, 4D and 5AL is consistent with other published wheat maps. Akbari *et al*. (2006) and Semagn *et al*. (2006) did not report any DArT markers mapping on chromosome 4AS or 4D and only 3 DArT markers mapping to 5A over a total of several hundred successfully mapped loci. Mantovani *et al*. (2008) also found that no markers mapped to these particular regions. The short arms of 4B and 4D and the long arm of 5D were also found to be under represented in the ArNK genetic map (Semagn *et al*., 2006), this being mainly attributed to the lack of a sufficient number of polymorphic SSRs. Paillard *et al*. (2003), Sourdille *et al*. (2003) and Cadalen *et al*. (1997) reported the short arms of chromosomes 4A, 4B and 4D to be lacking in marker loci. Paillard *et al*. (2003) found all of the short arms of group 4 chromosomes to be undetected by marker loci. In a durum wheat map the short arm of 4A was only partly covered and 4BS was not covered by marker loci (Peleg *et al*., 2008). Chromosomal areas with insufficient coverage within the Claire x Lemhi genetic map seem to coincide with other published wheat maps. These specific genomic areas could be regions of high recombination and/or low sequence variation, resulting in insufficient polymorphic loci (Somers *et al*., 2004).

Most wheat SSR markers are chromosome specific and amplify only one locus on the A, B or D genome (Roder *et al*., 1998a, b). SSRs were selected according to their known positions (Song *et al*., 2005, Somers *et al*., 2004) to ensure good coverage and avoid closely linked multiple loci when developing the Claire x Lemhi genetic map. Four SSRs amplified homoeologous loci. One SSR primer pair amplified two loci located on the same chromosome and two SSR primer pairs amplified loci from non-homoeologous regions within the wheat genome. Whilst the remaining four SSR primer pairs that amplified multiple loci did not map within the population. It is not uncommon for SSR to identify homoeologous loci, Pestova *et al*. (2000) found that 14% of SSRs specifically developed to amplify loci on the D

genome (GDM), also amplified secondary loci on either the A or the B homoeologous chromosome. SSR are developed from repeat DNA sequences within the genome. Ancestral transposable elements make up a large proportion of Gramineae genomes (Roder *et al*., 1998a, b). SSR picking up secondary loci on non-homoeologous chromosomes could be due to the SSR residing within this repetitive element of the genome.

A large number of DArT markers were found to cluster, this occurred across the whole genome, but seemingly more towards the telomeric ends. A high proportion of these DArT markers were therefore excluded from the final map (43.9%) as they provided no unique loci information, leaving one informative marker at each locus. The occurrence of DArT clusters in distal-telomeric regions of chromosome arms has been observed in other wheat (Mantovani *et al.*, 2008, Akbari *et al*., 2006, Semagn *et al*., 2006) and barley mapping studies (Wenzel *et al*., 2004). It has been reported that DArT markers have a tendency to map to gene-rich telomeric regions of the genome (Vuylsteke *et al*., 1999). Gill *et al*. (1996a, b) found that in wheat more than 85% of genes physically span only 5-10% of the chromosomal regions. The clustering of the DArT markers towards the telomeric ends of the chromosomes may have contributed to the high number of linkage groups, an uneven distribution of marker loci across the genome resulting in gaps.

The use of different types of markers in the production of a genetic map should provide a diverse spread of markers, with different markers targeting different genomic regions. The use of DArT and AFLP allows for the production of hundreds of anonymous marker loci rapidly, without the need for sequence information. EST-derived and NBS markers target specific functional regions of the genome. SSR markers offer the ability of pre-selection to allow for a targeted approach, mapping specific regions of the genome. NBS markers were adopted in order to sample the genetic variation in and around potentially functional R-genes within the Claire x Lemhi cross, with the aim of identifying potential yellow rust resistance loci. It was not as successful as other reports (Mantovani *et al*., 2006, Calenge *et al*., 2005, van der Linder *et al*., 2004), with only two NBS loci forming a genetic linkage group, the remaining NBS loci remaining unlinked despite reducing the LOD threshold and none were found to be linked to yellow rust resistance (Chapter 6). Clustering of

NBS loci is not uncommon (Syed *et al*., 2006, Calenge *et al*. 2005), even though cloned NBS loci have shown significant similarity with known NBS-LRR R-genes (Mantovani *et al*., 2006, van der Linden *et al* 2004) the polymorphic bands identified here may not be derived from R-genes or RGAs. Fewer NBS marker loci were identified than with other marker systems adopted. This is not surprising as in principle each locus should correspond to a member of a NBS-LRR resistance gene analogue gene family which are found in much lower abundance than random AFLP fragments. Syed *et al*. (2006) found that less than 50% of NBS-LRR bands identified resembled identifiable RGAs.

Cross genome comparisons showed the D genome to have the shortest length and also the lowest number of loci in comparison to the A and B genomes, even though 2DL was saturated with markers to allow for fine mapping of the yellow rust resistance QTL found on this chromosome arm. The density of DArT markers was also higher in the A and B genome. The lack of markers on the D genome can be attributed to the low level of polymorphism found in the D genome which has been well documented in other studies (Perovic *et al*., 2009, Wicker *et al*., 2009, Balfourier *et al*., 2007, Akbari *et al*., 2006, Chalmers *et al*., 2001, Roder *et al*., 1998a, b). Consequently, poor marker coverage of chromosomes 3D, 5D and 6D was obtained in the Claire x Lemhi genetic map. There was a lack of DArT marker loci on these D genome chromosomes and as a consequence many of the SSRs that were found to be polymorphic on these chromosomes remained unmapped.

A genetic linkage map has been produced for the Claire x Lemhi cross, that while giving a reasonable coverage of the wheat genome, 1560cM consisted of 49 linkage groups. This would indicate a large proportion of gaps, where insufficient marker loci and/or high levels of recombination prevented linkage groups from being joined together. However, this inability to join linkage groups may also be a consequence of the small size of the Claire x Lemhi DH population available. Further improvement of the Claire x Lemhi genetic map may be achieved by adding additional markers, however additional DH lines may prove more effective.

Chapter 5

Development of EST-derived markers targeted to yellow rust resistance QTL

5.1 Introduction

Expressed sequence tags (ESTs) are partial cDNA sequences derived from the mRNA of transcribed genes (Adams, 1991). The International *Triticeae* Mapping Initiative (http://wheat.pw.usda.gov/ITMI) includes a vast amount of wheat EST data stored on the GrainGenes public database. EST data provides a rich resource for genome mapping, providing a link to genetic information in plant species that are less suitable for whole genome sequencing such as wheat. Over one million ESTs have been generated from hexaploid and tetraploid wheat and closely related species, all of which are publicly available through the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html).

The hexaploid nature of wheat means that it is able to withstand the loss of whole chromosomes, chromosome arms and segments (Sears and Sears 1978, Sears 1969). As a result, a collection of overlapping deletion lines for all chromosome arms has been developed (Endo and Gill, 1996). The breakpoints on each chromosome arm define physical segments for that particular arm, more commonly referred to as deletion bins. Sears developed a series of nullisomic-tetrasomic lines for each of the seven homoeologous chromosome groups, where the removed chromosome pair is compensated by the presence of an additional pair of one of the homoeologous chromosomes (Sears and Sears 1978, Sears 1969). Similarly, ditelosomic lines include all 21 chromosome pairs, but have one particular chromosome pair that contains only the short or long arm. These lines help to clarify the centromere position in genetic mapping and also locate the arm position of markers (Anderson *et al*., 1992). Wheat ESTs are currently being mapped, as part of a collaborative program involving ten research laboratories, to chromosomal locations defined by these deletion line breakpoints (http://wheat.pw.usda.gov/NSF/progress.html). Nullisomic-tetrasomic, ditelosomic and the deletion lines have been used to physically map more than 18,000 EST loci to specific regions of wheat chromosomes (http://wheat.pw.usda.gov/wEST/binmaps/) (Qi *et al*., 2004).

A vast range of molecular markers have been developed over the last three decades (discussed in Section 1.4) of which most are based on genomic DNA, derived from either the transcribed or un-transcribed regions of the genome. However, over the

last few years the development of markers from the transcribed regions of the genome has become a major target. The substantial progress made in mapping wheat ESTs to specific deletion bins makes them ideal candidates for targeted marker development for fine mapping traits of interest and saturation of genetic maps. There are a range of genetic marker types which have been exploited using the EST data sets, such as EST-SSR markers (Rota *et al*., 2005, Yu *et al*., 2004a, b) and intron spanning EST-derived markers (Ishikawa *et al*., 2007 and Wei *et al*., 2005). The continued increase in genomic and functional genomic tools in model plant species, together with the efficiency of comparative genomics facilitates this. Conserved orthologue set (COS) markers or anchored reference loci are gene sequence markers that anchor common sytenic regions between related genomes, identifying putative orthologs by utilising comparative plant genomics (Fulton *et al*., 2002). COS markers have been poorly utilised in the cereals, they have however been extensively studied in dicots taking advantage of the completed genome sequence of the model plant *Arabidopsis* (Fulton *et al*., 2002). Accurate analysis of the synteny between mapped wheat ESTs with the reference genomes of rice and *Brachypodium* allows the identification of COS markers. Quraishi *et al*. (2009) successfully developed 695 conserved intron spanning primers in wheat using rice as a template. Thirty one of these COS markers were used to fine map a QTL responsible for pentosan viscosity. COS markers offer portability between wheat genotypes as they are designed to conserved regions in the exons.

Rice has long been the model plant species for the cereals, providing a useful tool for comparative genomics and facilitating the study of other important cereal crops (http://www.gramene.org/). Rice was the first grass species to have its genome completely sequenced (Goff *et al*., 2002), targeted primarily because of its small genome and global economic significance (Huo *et al*., 2009). Since its completion the use of rice as a framework for the study of other grass species has been unprecedented, particularly for those cereal species with much larger genomes or that differ in ploidy level and chromosomal number such as wheat. At the level of the genetic map colinearity between grass species is generally well conserved (Devos, 2005) and the seven chromosomes of wheat can be represented by the 12 chromosomes of rice (Figure 5.1)

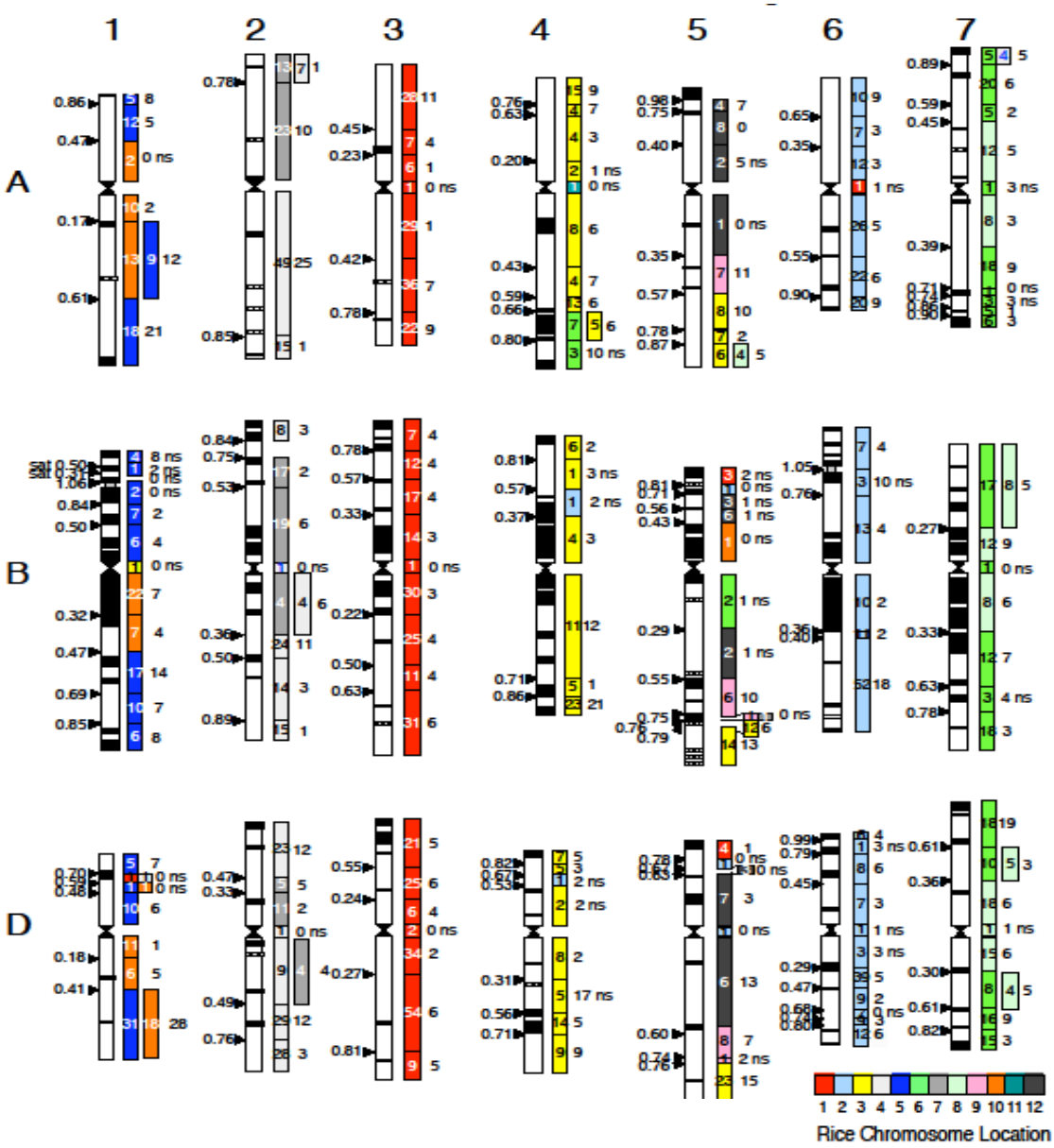


Figure 5.1 Wheat-Rice genome relationships. The rice chromosomes and the sytenic deletion bins of wheat are colour coded according to the rice chromosome location. Inside the coloured coded area is the number of mapped wheat EST, the number outside are those that mapped to other regions in rice (La Rota *et al*., 2004).

Deletion mapping of wheat ESTs together with the availability of the rice genomic sequence allowed the co-linearity between wheat and rice to be examined at the DNA level. Sorrells *et al*. (2003) constructed a high-resolution, sequence-based comparative map between wheat and rice by using mapped wheat ESTs and rice genome sequence data, demonstrating that the linear order of genes in rice and wheat is well conserved at the micro level. Both exons (coding region) and introns (non

coding region) are largely conserved among homologous genes from different species (Batzoglou *et al*., 2000). Therefore, the rice sequence has been particularly useful for predicting the intron regions within wheat ESTs (Yang *et al*., 2007). Rice genomic sequence data, together with the increasing availability of wheat ESTs is making sequence comparative mapping a key resource for the development of new PCR-based EST-derived makers.

In order to identify regions of similarity between sequences that may be a consequence of functional, structural or evolutionary relationships computational algorithms have been designed that allow sequence alignments to be made (Altschul *et al*., 1990). Computational approaches typically fall into two categories based on either global or local alignments. Global approaches optimise the overall alignment of two entire sequences by forcing the alignment to span the entire length of the query sequence. Local alignments identify regions of similarity within long sequences that are often widely divergent overall. Altschul and colleagues (1990) designed a local alignment algorithm that emphasized speed, the Basic Local Alignment Search Tool (BLAST), which enabled high throughput comparisons between both amino acid and nucleotide sequences against the constantly updated protein and DNA sequence databases. As these databases continue to grow in size they become increasingly more useful in the analysis of complex genomes such as wheat.

The abundance of wheat EST sequences, together with deletion bin mapping data and sequence alignment tools provide a valuable resource for genome analysis, comparative mapping, saturation mapping and tagging of target genes. For genetic linkage map construction EST derived markers can enhance the resolution and quality of a linkage map and as they target expressed genes they are particularly useful for QTL mapping (Eujayl *et al*., 2002). This novel approach to developing new, functional markers has been deployed in numerous studies (De Keyser *et al*., 2009, Gadaleta *et al*., 2009, Lu *et al*., 2006, Yu *et al*., 2004a, b). ESTs have also provided a new source of SSR markers that are physically associated with coding regions of the genome (EST-derived SSR). These types of markers have already proven useful in a variety of crops including rice (Cho *et al*., 2000), durum wheat (Eujayl *et al*., 2002), barley (Thiel *et al*., 2003) and hexaploid wheat (Yu *et al*.,

2004a, b, Gupta *et al*., 2003). Sequence redundancy is a major disadvantage of the EST-derived SSRs resulting in the production of multiple sets of alleles at the same locus. However, wheat EST sequences have recently been assembled into continuous unique gene sequences called unigenes (http://www.ncbi.nlm.nih.gov). Unigene databases can be systematically searched for SSRs of unique identity that are positioned in the transcribed regions of the genome (Parida *et al*., 2006). Cho *et al*. (2000) found that the level of polymorphism of SSR motifs located in exons to be considerably less than those found in the untranslated intron regions. This led to the development of EST-derived markers that targeted the polymorphism found in introns, designing primers that flanked the intron region. This strategy of marker development has been applied in rhododendron (De Keyser *et al*., 2009, Wei *et al*., 2005), a cross between ryegrass (*Lolium*) and fescues (*Festuca*) (Tamura *et al*., 2009) and in hexaploid wheat (Ishikawa *et al*., 2009 and 2007).

5.2 Aims of current study

Roder *et al*. (1998a) physically mapped the SSR marker GWM301 to the long arm of wheat chromosome 2D within the telomeric deletion bin 0.76-1.00. In this study, this SSR marker was found to be genetically linked to the AFLP S15M32-155bp (Chapter 4). Previous investigations of a Claire x Lemhi $F_{2:3}$ population found S15M32-155bp to be significantly associated with the yellow rust APR phenotype in Claire (Lewis, 2006). The objective of this chapter was to identify suitable, non-paralogous wheat ESTs within the terminal deletion bin of 2DL (0.76-1.00) to aid fine mapping of the yellow rust resistance located in this region. In order to predict the exon/intron junction sites within the selected wheat 2DL-specific ESTs sequence alignments were made with the rice genome. This allowed intron-targeting primers to be designed for the development of EST-based markers. These EST-derived markers were used in the construction of the Claire x Lemhi genetic map (Chapter 4) and the subsequent fine mapping of APR QTLs found in Claire (Chapter 6).

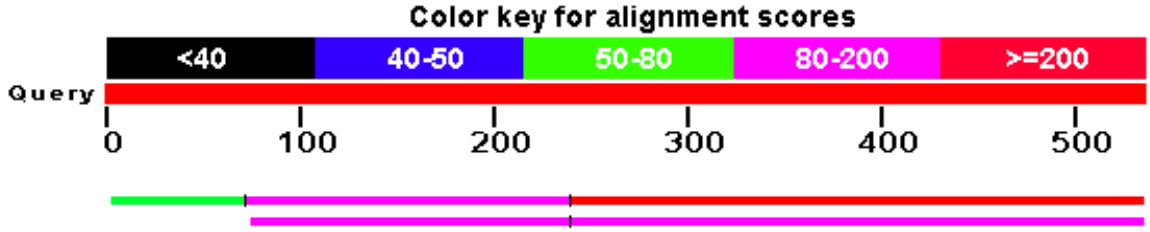
5.3 Materials and Methods

5.3.1 Strategy for developing EST-PCR markers

Wheat EST sequences which had been physically mapped to chromosome 2DL were downloaded from the GrainGenes database (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). Southern blot images for those EST which had been located to the terminal 2DL deletion bin 0.76-1.00 were analysed to identify copy number and confirm location. Low copy number ESTs were selected for further analysis. A BLASTn pairwise alignment was carried out between each wheat EST and the rice genomic nucleotide sequence, using the NCBI web interface (http://blast.ncbi.nlm.nih.gov/Blast.cgi Figure 5.2) to identify the predicted exon-intron-exon junctions within the wheat EST (Figure 5.3, Step 1).

Forward and reverse primers were designed based upon the wheat EST exon sequences that flanked the exon-intron-exon junction sites, thereby producing a PCR amplicon that spans the predicted intron (Figure 5.3, Step 2). A small region either side of the predicted intron (~10 bp) was excluded from the primer design to ensure the primer pair flanks the predicted intron. Primers were designed using the default settings of Primer 3 (http://primer3.sourceforge.net/ Rozen and Skaletsky, 2000).

a)



b) Score = 105 bits (53), E-value = 1e-19, Identities = 86/97 (88%), Gaps = 0/97 (0%)

```
Query       TGCTTTATTATTGAGTAGGTAGCAGATATCGTGGCTTGACTTGCAATTATTGCTGCAGCA   61
            |||||||||||| ||||| || |||||||| ||||| || ||||||| ||||||||| |||
Subject     TGCTTTATTATCGAGTAAGTCGCAGATATGGTGGCCTGGCTTGCAACTATTGCTGCTGCA   146211

Query       GTTGCTATAACAAAGGCTGGCCAGTATATGGCATCTG   98
            |||||||| ||||| ||||||||||||||||| |||||
Subject     GTTGCTATGACAAATGCTGGCCAGTATATGGAATCTG   146248
```

Score = 135 bits (68), E-value = 2e-28, Identities = 107/120 (89%), Gaps = 0/120 (89%)

```
Query       CTGGAATGGAGCGATAGAAGGCGTCATTCACATGTTGCTTGTTGGAAATTATATAAGCAG   155
            |||||||||||||||| || || |||   ||||| | ||||| || ||| ||||||||||
Subject     CTGGAATGGAGCGATAAAAAGCATCAGCAACATGGTCCTTGTGGGCAATGATATAAGCAG   146526

Query       CCTGCCCTGTGTATGCCAGAAGAAGGCATGGGAATACAATGAAGGTGAATGCAATCTGAA   215
            |||||||||||||||||||| |||||||||||||||||||| ||||||||||||||||||
Subject     CCTGCCCTGTGTATGCCAGTAGAAGGCATGGGAATACAATCAAGGTGAATGCAATCTGAA   146586
```

Figure 5.2 a) Graphic overview of BLAST results. The query sequence (wheat EST) is represented by the numbered red bar at the top of the figure. Database hits are shown aligned to the query, in descending order of similarity. In this case, there are two high scoring database matches that align to the query sequence. **b)** Pairwise sequence alignment from a blast report of wheat EST WHE0073_D04_H07ZS (EST 1) and rice genomic DNA homolog sequence (rice chromosome 4, BAC clone:OSJNBa0070C17, complete sequence length = 160484). Query = wheat EST, subject = rice genomic sequence. Information on the bit score, E-value, number of identical nucleotides in the alignment (identities) and the number of gaps in the alignment are also given. The numbers on the right refer to the position in the sequence. The vertical lines between the two sequences indicate identical nucleotides.

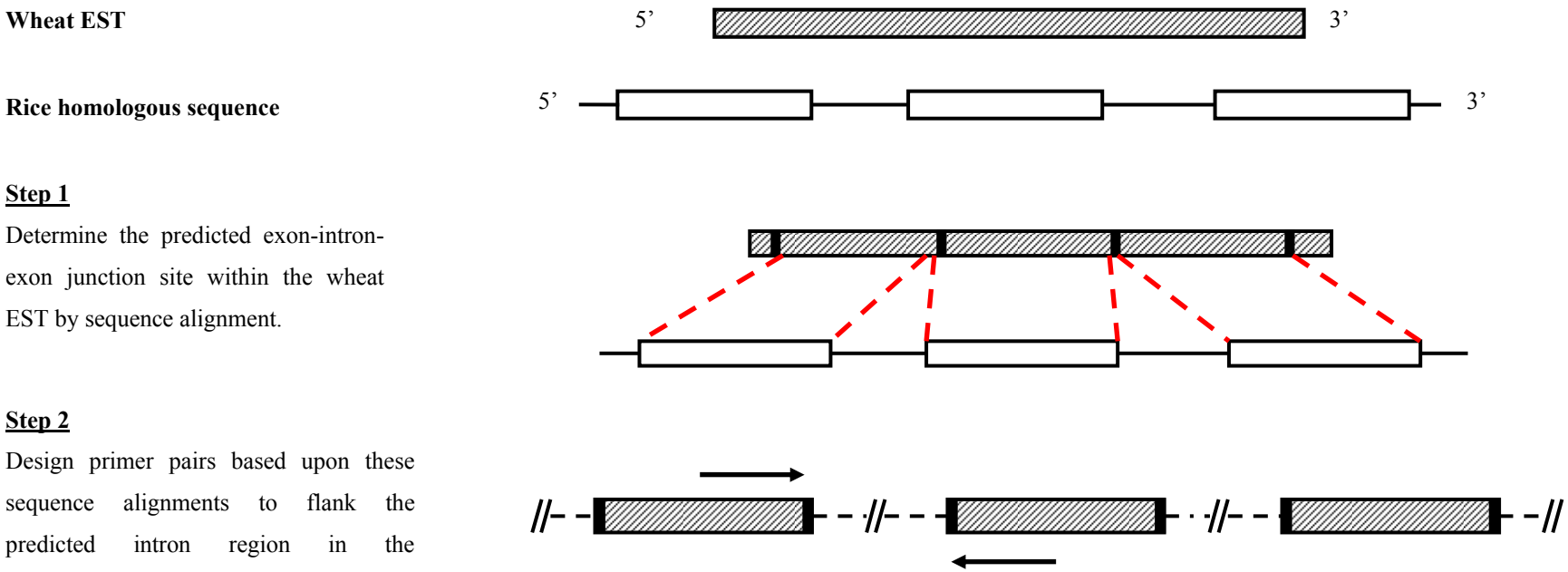


Figure 5.3 Schematic representation of EST-derived marker development. Primer design specifically flanking intron regions that will amplify PCR products in wheat genomic DNA. ▭ = rice exon, ▨ = wheat exon, ▮ = rice intron, ⎯ = predicted exon/intron junction site in wheat, ⟶ = forward and reverse primer sites.

5.3.2 EST-derived marker screen

The Claire x Lemhi DH mapping population, including both parents, was screened with the EST-derived primer pairs at the industrial case partner Advanta-Nickerson Seeds Ltd, Station Road, Docking, Kings Lynn, Norfolk, PE31 8LS, UK. Twenty-three primer pairs were fluorescently labelled and screened against the parents Claire and Lemhi in order to identify polymorphisms, with the PCR product being analysed on a DNA sequencer (DNA Analyzer Gene ReadIR 4200, Li-COR sequencing system). Polymorphic EST primer pairs were then screened against all DH lines. DNA amplifications were carried out in 10 μl reaction volumes, each containing 10 ng μl^{-1} of template DNA. The reaction mixture consisted of 0.1 μl platinum Taq DNA polymerase, 0.16 μl of 0.05-0.25 pmol of EST-derived primer pair, 0.3 μl of 1.5 mM $MgCl_2$, 0.4 μl of 0.2 mM each of dATP, dCTP, dGTP and dTTP, 1.0 μl 1 x buffer and 6.04 μl ddH_20. PCR conditions were as follows: an initial denaturing step at 94°C for three minutes followed by 35 cycles of 94°C for one minute, 60°C for one minute, 72°C for two minutes, with a final extension step at 72°C for ten minutes. An aliquot (0.8 μl) of each PCR product was denatured for 3 minutes at 94°C and cooled on ice before the fluorescently labelled samples were separated on a denaturing sequencing gel. The polyacrylamide gel (20 ml of a 6.5% KB gel matrix, 15 μl Temed and 150 μl 10% AMPS) was polymerised between two glass plates separated by 0.25 mm deep spaces. The fluorescently labelled PCR amplicons were separated on this denaturing sequencing gel buffered in 1X TBE by running at 40 W for 90 minutes. Fluorescent PCR bands were detected using an automatic DNA sequencer (DNA Analyzer Gene ReadIR 4200, Li-COR sequencing system, Li-Cor Biosciences UK Ltd, St. John's Innovation Centre, Cowley Road, Cambridge, CB4 0WS, UK) and the data analysed with the software Genescan and Genotyper (Applied Biosystems, Lingley House, 120 Birchwood Boulevard, Warrington, WA3 7QH, UK). Polymorphic EST markers were scored in each DH line. This EST marker locus data was added to the DArT, SSR, AFLP and NBS-AFLP locus information obtained in Chapter 4.

5.3.3 Sequence comparison and function classification

To determine a putative function for the EST derived markers the sequence of each EST was initially searched against the NCBI non-redundant protein sequence database using the BLASTx algorithm (http://blast.ncbi.nlm.nih.gov/blast.cgi). Secondly the corresponding unigene of each EST was determined. The EST name was used to search the GrainGenes database to give a sequence report (http://wheat.pw.usda.gov/GG2/index.shtml). The contig (unigene sequence) was then downloaded and subjected to a BLASTx search against NCBI non-redundant protein sequence database.

5.4 Results

5.4.1 EST identification

5.4.1.1 Wheat EST analysis

A total of 540 ESTs were identified as physically mapped to chromosome 2DL and 197 of these were mapped to the telomeric deletion bin 2DL 0.76-1.00 (Table 5.1). The Southern blot images of the 197 ESTs in the terminal deletion bin were downloaded from GrainGenes and 94 EST were found to represent low copy number genes and be chromosome 2-specific, based on Southern blot results. These 94 ESTs were selected for further analysis, thereby reducing the likelihood of developing EST primers for paralogous loci.

Table 5.1 Total number of deletion bin mapped ESTs on chromosome 2DL

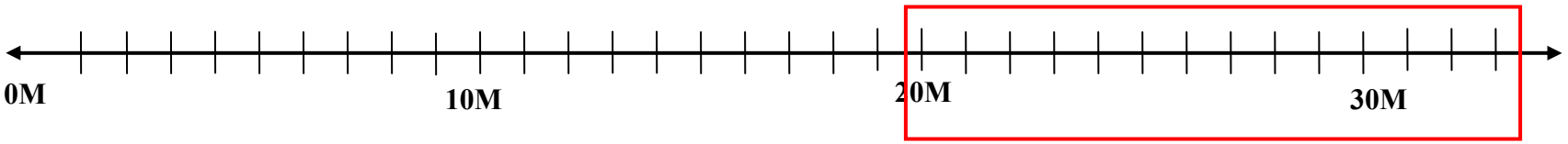
Deletion Bin	Mapped ESTs
2DL[1]	49
0-0.49	109
0.49-0.76	185
0.76-1.00	197

[1]2DL ESTs that could not be specifically mapped to a deletion bin.

5.4.1.2 Comparative genomics

The BLAST nucleotide algorithm (BLASTn) was used to identify regions of homology between the 94 wheat ESTs and the rice genomic nucleotide sequences. Following the BLASTn analysis 34 ESTs (36.1%) were predicted to have no introns. Fifteen ESTs (16%) had introns greater than 500 bp in length and eight (8.5%) had introns of less than 50 bp in length. Two ESTs (2.1%) had no significant pairwise alignment with rice and 12 ESTs (12.8%) had a low sequence similarity to rice, having e-values greater than 1e X 10^{-15}. Out of the 94 ESTs that were subjected to BLASTn against the rice genomic sequence a total of 22 ESTs (23.4%) had significant pair-wise alignments and contained suitable intronic regions to target for primer design. The comparative genomic physical position of all 22 ESTs targeted for primer design on rice chromosome four is illustrated in Figure 5.4.

a) Overview of rice chromosome four



b) Detailed positioning of wheat ESTs on rice chromosome four

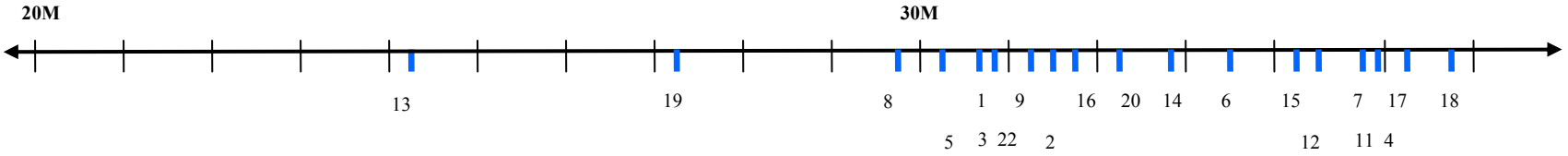


Figure 5.4 a) Overview of rice chromosome four, red box indicates the region targeted for EST sequences. **b)** Position of the 20 wheat ESTs relative to rice chromosome 4. EST 10 and EST 21 are syntenic with rice chromosome 3.

All 22 selected ESTs had rice alignments that were considered to be of high sequence similarity, with E-values less than 1e x 10^{-15}, the exception being EST 9 and EST 10 with E-values 0.12 and 1.6 respectively (Table 5.2). These ESTs were short sequences, less than 80 bp in length, the high E-values could therefore be attributed to the fact that the sequence is small. Statistically E-values do not take into account the size of the query sequence, for this reason these two EST were retained for primer design. The bit score is another statistical indicator that measures sequence similarity taking into account the length of the query sequence. Bit scores ranged from 81 to 325, with an average of 176.8. Even though the E-values of EST 9 and EST 10 were higher than the threshold set, the bit scores indicated that the alignments with the rice genomic sequence were significant. Bit scores are calculated with respect to sequence length and adjusted accordingly, the higher the bit score the more significant the match. Since bit scores are normalised with respect to the scoring system they can also be used to compare alignment scores from different searches (http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/glossary2.html).

The subsequent position of potential intron regions with the corresponding genomic copy of the wheat EST could therefore be identified based on the position of the introns in the rice homologue. Targeted introns were more than 70 bp in length, which is the required length for correct splicing from precursor mRNA, but less than 500 bp in length to generate an efficient PCR product.

Table 5.2 Summary of BLASTn results of wheat EST selected as suitable for intron flanking primer design.

EST name	Accession number	Marker code	Intron size (bp)	Bit score	E-value before intron	E-value after intron	% ID before intron	% ID after intron	Homolog rice chromosome
WHE0073_D04_H07ZS	BE423738	EST1	219	135	1.00E-19	2.00E-28	88	89	4
WHE0426_G06_M12ZS	BE403217	EST2	201	242	6.00E-61	1.00E-12	87	91	4
WHE0436_E01_J02ZS	BE403768	EST3	402	109	1.00E-20	2.00E-19	90	89	4
WHE0972_E08_I16ZS	BE499251	EST4	265	89.7	7.00E-15	2.00E-09	83	85	4
WHE1701-1704_O04_O04ZS	BE605112	EST5	377	161	3.00E-36	2.00E-24	90	93	4
WHE0964_G02_M04ZS	BE498622	EST6	200	182	5.00E-13	7.00E-43	90	89	4
WHE0363_B12_C23ZS	BE489906	EST7	277	305	7.00E-18	6.00E-80	83	90	4
WHE0802_G12_M24ZS	BE517704	EST8	290	301	1.00E-78	7.00E-46	86	90	4
WHE0839_B11_D21ZS	BF473851	EST9	337	82	2.00E-15	0.12	84	89	4
WHE0360_A04_A08ZS	BE490491	EST10	340	154	6.00E-31	1.6	90	90	3
WHE0972_E08_I16ZS	BE499251	EST11	265	95	7.00E-49	2.00E-40	70	70	4
WHE0968_F06_K12ZS	BE498892	EST12	131	99.6	3.00E-05	1.00E-17	81	80	3
WHE1105_C11_E21ZS	BE442722	EST13	83	262	6.00E-28	1.00E-66	84	86	4
WHE1057_G02_N03ZS	BE488384	EST14	108	115	8.00E-12	1.00E-22	84	86	4
WHE1806_A09_A18ZS	BE637228	EST15	130	159	1.00E-35	4.00E-32	91	94	4
WHE1124_B06_D12ZS	BE443833	EST16	122	178	2.00E-16	1.00E-41	83	87	4
WHE1201_G10_M19ZS	BE404151	EST17	112	325	4.00E-14	1.00E-85	86	83	4
WHE0363_B07_C13ZS	BE489901	EST18	127	260	7.00E-37	4.00E-66	84	90	4
WHE0467-0470_E07_E07ZS	BM136904	EST19	167	333	1.00E-25	2.00E-88	88	93	4
WHE2057_H05_P09ZS	BG313738	EST20	176	119	1.00E-13	8.00E-24	86	83	4
WHE0969_C10_F19ZS	BE499008	EST21	90	103	5.00E-19	5.00E-19	81	85	3
WHE0436_E01_J02ZS	BE403768	EST22	505	81	5.00E-15	7.00E-10	86	88	4

5.4.1.3 Primer design

Primer pairs were designed to the 22 selected EST sequences to regions of exon sequence that were predicted to flank introns within the genomic copy of the wheat EST (Table 5.3).

Table 5.3 Intron flanking EST-derived primer sequences with corresponding amplicon size (primer length plus expected intron length), intron size (determined by sequence alignment to rice) and annealing temperature (°C) for PCR reaction.

Marker code	Forward primer	Reverse primer	Predicted amplicon size (bp)	Predicted intron size based on rice (bp)	Annealing temp. (°C)
EST1	cgtggcttgacttgcaattat	tttccaacaagcaacatgtga	294	219	60
EST2	ggatggtgctttctccaaga	gcagcctctcttcaccattc	245	201	60
EST3	agctggatggcagagcaat	ttggtcagttgcagctttga	459	402	60
EST4	gggtcaagatcatggacagc	cggtactcctcgtccttgac	355	265	60
EST5	ccaaaatggagatggagagg	gcacagatcgaaccactgtt	431	377	60
EST6	gccaagtctggaggatcaag	tggagttgaagctctctgttg	258	200	60
EST7	caccgactacgactacgtcttc	gctggtcatctccaagatacg	316	277	60
EST8	cctggattcctcttagtgctg	gaaggaagctgcaagcaat	339	290	60
EST9	tgaccccgaaaatttctgat	gatagcatattctggagccaa	359	337	60
EST10	tccagcgacccctacgtc	agagggtcagctcgtcgtt	374	340	60
EST11	ggagctcagggtcaagatca	cggtactcctcgtccttgac	343	265	60
EST12	aatggggtgtgcctaatgac	ggaatctccattccaagatca	177	131	60
EST13	tgccagacagcatctcaaag	atgtgagcccgttgtagctt	103	83	60
EST14	caaccttctgcaatctggtg	tgaggctcacttcaagaaca	144	108	60
EST15	ggatttcaggcattgcatct	ctcccgcgactctaacaatg	168	130	60
EST16	aagtcatctcgcccaaagaa	gcggttgaattggatgttca	159	122	60
EST17	gtctcatgcgcagacatcac	gcctgagggagatcgaaga	182	112	60
EST18	ctacatcccggtctccttca	cttgctccaaaccaaccact	173	127	60
EST19	tgatagccacacctgtcctg	cagtgcctgaacttcaccaa	186	167	60
EST20	gtcttgctggcccctaatg	gctgctcctttgtggaactc	195	176	60
EST21	gttgatctccgggactggac	gtgctcttcatcggcaaact	144	90	60
EST22	gggaaaggcattcttcacaa	cctccccaatgcgttgttat	543	505	60

5.4.1.4 Identification of polymorphism

PCR amplification of the EST-derived primers was found to be 100% successful. The 22 2DL specific EST-derived primers were initially screened against Claire and Lemhi. Eleven of the EST-derived primer pairs were found to detect polymorphisms (50% polymorphism rate).

The eleven polymorphic EST-derived primers (EST 4, 6, 7, 8, 9, 10, 11, 12, 16, 18 and 22) were screened against the entire DH mapping population (Figure 5.5). All of the primer pairs generated multiple PCR products, potentially identifying homoeolegous loci from each of the three wheat genomes. However, only one of the multiple PCR products showed polymorphism between the resistant parent Claire and susceptible parent Lemhi, the exception being EST 18 which displayed three polymorphisms that could be mapped in the DH population.

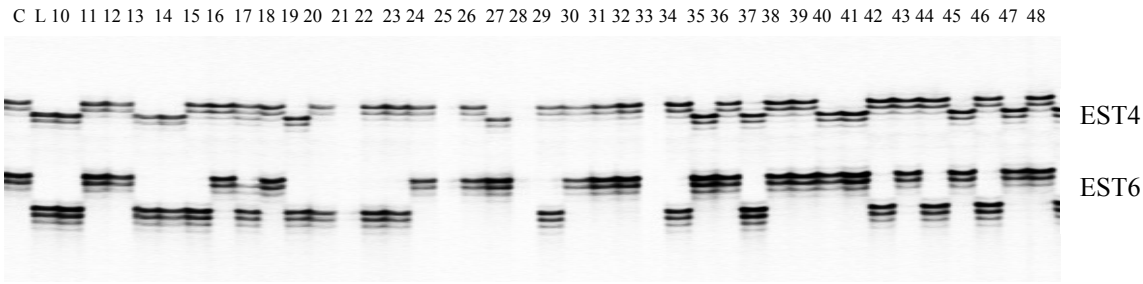


Figure 5.5 PCR products of EST 4 and EST 6 primers against parents Claire (C) and Lemhi (L) and 38 DH lines from the mapping population (DH10 – DH48) run on a DNA sequencer (DNA Analyzer Gene ReadIR 4200, Li-COR sequencing system) and analysed with the software Genescan and Genotyper (Applied Biosystems).

The thirteen polymorphic marker loci were added to the mapping data (Chapter 4), respective chromosomal locations summarised in Table 5.4.

Table 5.4 Mapping location of the polymorphic EST-derived primers.

Polymorphic EST-derived primer	Mapped Location
EST 4	U[1]
EST 6	2DL[2]
EST 7	2DL
EST 8	U
EST 9	2AL
EST 10	2DL[2]
EST 11	2DL
EST 12	2AL
EST 16	U
EST 18a	2DL
EST 18b	2AL
EST 18c	5BL/7BL
EST 22	2DL[2]

[1]Unidentified as failed to form a linkage group, [2]EST mapped to regions where QTLs for *Yr* resistance were localised.

5.4.1.5 Putative functional annotation of ESTs

All 22 ESTs selected for primer design were subjected to BLASTx similarity searches to assign a putative annotation and function (Table 5.5). The corresponding unigene of each EST was also subjected to a BLASTx search against NCBI non-redundant protein sequence database (Table 5.6). The data indicated that all 22 ESTs showed high sequence identity (86-100%), high bit scores and low E-values to sequences of mostly characterised functions. The sequence similarity searches revealed hydrolase and transferase activity, transporters, elongation factors and protein kinases. Many of the ESTs showed similarity to expressed proteins with kinase activity involved in signalling pathways such as EST 3 and EST 22. EST 4 and EST 11 showed high sequence similarity to C2 domain which could play a role in secondary messaging during defence response. The putative functions are discussed in depth in Section 5.5.

Table 5.5. Putative function of EST used for marker development

EST	Gene identification	Hit score	E-value	Identity (%)
1	Potassium transporter	6297	0	96.95
2	ABC transporter family protein	2012	4.6e-204	94.83
3	Aminotransferase	466	2e-129	
4	C2 domain containing protein	1419	38.2e-123	86.92
5	G protein coupled receptor (GPR89A)	2181	0	99.55
6[1]	Mediator of RNA polymerase II transcription subunit 7	1751	9.1e-184	98.63
7	Phosphomannomutase	1890	4.2e-173	100
8	Elongation factor Tu	2445	0	85.94
9	Tyrosine protein kinase domain	809	7.4e-110	77.16
10[1]	Expressed protein	3287	2.8e-232	99.25
11	C2 domain containing protein	1419	8.2e-123	86.92
12	Pyruvate kinase	5585	0	100
13	Leucine rich protein	4207	2.0e-185	97.96
14	Alpha-N-acetylglucosaminidase	1152	5e-66	95.4
15	Amine oxidase, flavin-containing protein	2523	0	91.99
16	Expressed protein	9316	0	99.68
17	Peroxidase precursor	3789	3.1e-166	87.37
18	Phosphate translocator	2420	2.3e-202	92.68
19	Hydrolase, NUDIX family	3447	0	99.71
20	Diacylglycerol kinase	2540	2.8e-287	100
21	F-box domain containing protein	2701	3.1e-117	99.63
22[1]	Aminotransferase	2091	9.3e-268	96.67

[1]EST mapped to regions where QTLs for *Yr* resistance were located.

Table 5.6 Putative function of the corresponding Unigene of each wheat EST used for marker development

	Unigene	Putative function	Bit Score	E-value	Identity (%)
1	Ta.14157	Potassium transporter	1685	0	85
2	Ta.56198	Multidrug resistance protein - like ABC transporter	2002	0	92
3	Ta.26668	Aminotransferase	1624	2e-178	88
4	Ta.49756	Elicitor-responsive protein/ C2 domain-containing protein	430	1e-40	60
5	Ta.10525	G coupled protein receptor GPR89A	651	2e-66	85
6[1]	Ta.55713	Cofactor required for Sp1 transcriptional	699	1e-71	85
7	Ta.3539	Phosphomannomutase	1239	5e-134	100
8	Ta.9646	Translation elongation factor eEF-1	1621	3e-178	86
9	Ta.299	Serine/threonine-protein kinase receptor precursor	906	5e-96	92
10[1]	Ta.46314	C2 domain-containing protein	598	4e-60	79
11	Ta.49756	Elicitor-responsive protein	430	1e-40	60
12	Ta.21113	Pyruvate kinase, cytosolic	2353	0	93
13	Ta.55734	Leucine-rich protein	454	1e-43	76
14	Ta.33927	Hypothetical protein	375	1e-34	64
15	No Unigene	Lysine-specific histone demethylase 1	422	5e-40	90
16	Ta.30565	Hypothetical protein	1246	8e-135	81
17	Ta.25632	Peroxidase 60 precursor	604	6e-92	75
18	Ta.43380	Glucose-6-phosphate/ phosphate translocator	1487	1e-162	81
19	Ta.8733	Xyloglucan endotransglycosylase (XET)	1409	1e-153	94
20	Ta.28081	Diacylglycerol kinase 3	738	4e-76	81
21	Ta.12732	F-box domain containing protein	1184	1e-127	79
22[1]	Ta.26668	Aminotransferase	1624	2e-178	88

[1]EST mapped to regions where QTLs for *Yr* resistance were located.

5.5 Discussion

Plant EST databases are a valuable resource from which PCR-based, gene-specific markers can be developed. The vast number of wheat ESTs physically mapped to chromosomes via Southern blot hybridisation, together with the fully sequenced rice

genome and the growing efficiency of comparative sequence alignment algorithms makes wheat ESTs ideal prospective candidates for marker development.

Lewis (2006) found the AFLP marker (S15M32-155bp) to be associated with the yellow rust APR in Claire. This AFLP was found to be linked to the SSR loci *Xgwm301*, *Xwmc817*, *Xgwm311* and *Xwmc167* (Chapter 4). Roder *et al*. (1998a) placed the SSR marker *Xgwm301* physically in the telomeric 2DL deletion bin. The 197 wheat ESTs located to the telomeric deletion bin (2DL 0.76-1.00) were therefore used to develop EST-derived markers to saturate the region of 2DL defined by the AFLP and four SSR marker loci.

The hexaploid genome of wheat often results in simultaneous PCR amplification of homoeologous genes. If paralogues of the EST also exist the number of amplified PCR products is likely to be abundant (Ishikawa *et al*., 2007). This can hinder the mapping of EST based markers to targeted regions of the wheat genome. The initial selection steps were therefore designed to target only those ESTs that were single loci and specific to 2DL deletion bin 0.76-1.00. The resulting EST primers should in theory produce a simple banding pattern, targeting polymorphisms specific to the group 2 chromosomes.

Introns, the non-coding sequences found within genomic copies of genes, tend to have greater nucleotide variability. This is attributed to the fact that little or no selection pressure acts on this non-translated region of the gene (Weining and Langridge, 1991). Exons retain a higher percentage of identity, evolving at a slower rate due to selection pressure for conserved gene function. For example, in eight varieties of rice (*Oyrza sativa*) the average number of single nucleotide polymorphisms (SNP's) per 1000 bp in introns is over three times higher to that in exons (Feltus *et al*., 2006). Jia *et al*. (2004) also reported exon lengths to be similar between genotypes in comparison to that of introns. These observations support the Neutral Theory, which predicts that gene regions of critical functional importance evolve slower than less functionally important regions (Ohta and Gillespie, 1996, Ohta, 1992, Kimura and Ohta, 1974). Primer design based upon the intron-targeting strategy applied in this study is expected to yield higher levels of polymorphism than other EST-PCR based strategies (Wei *et al*., 2005) or if only exon sequences were

used (Small *et al*., 2004). However, to date studies exploiting intron flanking markers have been restricted to a few species (De Keyser *et al*., 2009, Tamura *et al*., 2009, Wang *et al*., 2005, Wei *et al*., 2005, Choi *et al*., 2004). As EST markers target the expressed region of the genome, any marker found to be highly associated with the APR found in Claire are potential candidates for the APR genes.

As discussed in Chapter 4 the wheat D genome commonly exhibits lower levels of DNA polymorphism (Caldwell *et al*., 2004). In order to be able to fine map the yellow rust APR QTL identified on the long arm of chromosome 2D targeted D genome-specific markers derived from ESTs were developed. Identification of a high proportion of polymorphic EST-derived markers that map to the targeted location shows the success of this marker strategy. Exploiting introns in the design of EST-derived markers proved successful. Half of the primer pairs revealing polymorphism between Claire and Lemhi (50% polymorphism rate). Wei *et al*. (2005) obtained a higher rate of polymorphism when using this same strategy in wheat (63% polymorphism). In comparison, primers failed to identify polymorphisms when they did not flank intronic regions (Wei *et al*., 2005). The high polymorphism rate achieved in this study, using an intron flanking strategy for primer design, supports the assumption that introns are less conserved and prone to generate more polymorphism than exons.

The telomeric ends of wheat group 2 chromosomes show colinearity with rice chromosome 4, however the telomeric deletion bin on 2DL also show high levels of synteny to rice chromosome 3 (Sorrells *et al*., 2003). Consequently, the wheat ESTs from the telomeric deletion bin of 2DL showed high sequence alignments for rice chromosomes 3 and 4. The majority of EST markers found to be polymorphic in this study however, mapped to the long arm of chromosome 2D (55% mapped to 2DL, Chapter 4). Even though the furthest point from the centromere was targeted for EST identification, this overlap in synteny with rice chromosome 3 reported by Sorrells *et al*. (2003) did not effect mapping of EST to the targeted regions.

Wheat ESTs have also proven a useful resource for the identification of new SSR markers. Many EST contain short sequence repeats within their coding region that can be targeted for new SSR marker development. Genomic SSRs (gSSRs) are

expensive to develop, while EST-derived SSRs are a free resource derived from the constantly expanding EST databases. EST-SSRs have some intrinsic advantages over gSSRs as they show considerable scope for transferability between related species, gSSRs showing less cross species amplification (Sourdille *et al*., 2001). EST-SSRs have however been shown to be less polymorphic than gSSR markers (Eujayl *et al*., 2002, 2001).

Using the BLASTx algorithm, nucleotide sequences of ESTs used to develop markers and their corresponding unigene were compared with the non redundant protein database at NCBI in order to identify putative functions. No EST resembled any known cloned R-gene sequence. The disruption of synteny between the cereals at loci containing disease resistance gene homologs is not uncommon (Leister *et al*., 1998). Brueggeman *et al*. (2002) confirmed through synteny based cloning of the barley stem rust resistance gene (*Rpg1*) that the rice genome does not possess a *Rpg1* orthologous gene. Therefore these EST derived markers may still be associated with the APR yellow rust QTL found in Claire, even if they do not show synteny to any disease resistance loci in rice.

The putative functions linked to the annotations of the ESTs included proteins with kinase activity and involvement in signalling pathways, which could be involved in resistance signalling pathways. EST 2 showed homology to a multidrug resistance protein-like ATP-binding cassette transporter (ABC transporter). The *Lr34* gene has recently been isolated by Krattinger *et al*. (2009) and is predicted to encode a pleiotrophic drug resistance (PDR)-like ATP-binding cassette (ABC) transporter which are known to confer resistance to various drugs. *Lr34* provides durable partial APR to leaf rust, being most effective in the flag leaves, stimulating senescence like process in the tips and edges of flag leaves (Singh, 1992). It has been suggested that *Lr34* may export metabolites that affect fungal growth, similar to the proposed role of PEN3 in Arabidopsis which is a PDR-type ABC transporter protein involved in non-host resistance to barley powdery mildew (Stein *et al*., 2006). If EST 2 is found to be highly associated with the yellow rust APR found in Claire (Chapter 6) then it could be the APR gene itself.

EST 3 and EST 22 have putative functions as aminotransferases, which are an important and diverse group of proteins. Plant aminotransferases are involved in a diverse variety of processes, from metabolism, photorespiration and plant stress responses (Liepman *et al*., 2004). EST 4, EST 10 and EST 11 contain predicted C2 domains. The C2 domain is a calcium dependant membrane targeting domain, it is known that calcium ions play an important role in secondary messaging during defence response (Kim *et al*., 2008, 2003). Changes in cytosolic calcium ions are thought to be recognised by proteins with calcium binding domains (Sanders *et al*., 1999). A variety of studies have shown that pathogen attack results in a change in cytosolic calcium concentration which results in downstream signal transduction pathways via calcium binding proteins, of which most contain a C2 domain (Kim *et al*., 2008, Yang *et al*., 2006). The putative peroxidise function of EST 17 could also have a role in plant defence, as it has been shown that resistant plants exhibit higher peroxidise activity during infection than their susceptible counterparts (Flott *et al*., 1989). Peroxidases that are secreted extracellularly are thought to catalyze the generation of reactive oxygen species (ROS) transferring the extracellular signals which eventually stimulate the intracellular calcium signalling required for induction of defence responses (Kawano, 2003).

In this study the syntenic relationship between the genomes of rice and wheat was used to target intronic regions within wheat ESTs for primer design. Another, closely related model grass genome, *Brachypodium* is rapidly becoming an intermediate for comparative analyses. *Brachypodium* is a genus of temperate grasses belonging to the *Brachypodieae* tribe, a sister group of the grass tribes *Triticeae*, *Averieae*, *Poeae* and *Biomeae*. *Brachypodium* is more closely related to the temperate cereals and forage grasses than rice (Draper *et al*., 2001), with the divergence of *Brachypodium* and wheat estimated to have occurred 35-40 million years ago, whilst rice and wheat diverged more than 50 million years ago (Tang *et al*., 2006, Paterson *et al*., 2004). Therefore, *Brachypodium* has the potential to show greater gene colinearity to the genomes of temperate cereals and forage grasses and several studies have shown that the relationship between *Brachypodium* and wheat is much closer than that of rice and wheat (Huo *et al*., 2009, Griffiths *et al*., 2006, Draper *et al*., 2001). Despite the growing interest in the use of *Brachypodium* as a model species it remains to be thoroughly tested. *B. sylvativum*, a perennial outbreeding species has been used in

comparative analysis with rice and wheat. Griffiths *et al*. (2006) utilised *B. sylvativum* in mapping the wheat *Ph1* gene. Orthologous sequences were found to be more colinear with wheat compared to rice, with the probes derived from *B. sylvativum* sequences being used directly for mapping in wheat. *Brachypodium*, unlike rice, is susceptible to yellow rust, exhibiting a range of responses from no visible symptoms to pustule formation within areas of extensive necrosis (Draper *et al*., 2001). The genomic tools currently being developed in *Brachypodium* could provide further markers to fine map the QTL associated with APR found in Claire and could potentially lead to the identification of the true orthologous yellow rust APR genes.

The high success rate of intron flanking EST-derived markers developed in this study illustrates that wheat ESTs, together with the rice genome sequence provide a good resource for marker development. The polymorphic EST markers will be added to the genetic linkage map of the Claire x Lemhi cross constructed in Chapter 4, and subsequent fine mapping of the yellow rust APR QTL found to be located on the long arm of chromosome 2D.

Chapter 6

Detection and location of Quantitative Trait Loci which are influential in yellow rust APR and seedling resistance in a Claire x Lemhi doubled haploid population

6.1 Introduction

Resistance in wheat to yellow rust can be race-specific, which is usually qualitatively inherited, with phenotypes falling into discrete categories, or non-race-specific, which is usually quantitatively inherited, showing continuous variation within a segregating population. Race-specific resistance usually confers almost complete protection against disease and is expressed at all stages of development (Borner *et al*., 2000). This form of resistance follows the gene-for-gene relationship (Flor, 1955), which can be overcome by the rapid adaptation of the pathogen (Wellings, 2007). Non-race-specific resistance usually confers partial resistance that is effective from the adult plant growth stage, and is known as adult plant resistance (APR) (Zadoks, 1974). Genes that contribute a partial quantitative phenotype are termed quantitative trait loci (QTL). Wheat cultivars carrying APR QTLs display susceptible phenotypes at the seedling stage, but exhibit low disease severity at adult plant growth stages. It is difficult to study the genetics of quantitative resistance, as the effects of individual QTL are often small, with the expression being influenced by the genetic background and/or the environment (Lander and Botstein, 1989). APR is often characterised by lower pathogen infection, longer latent periods, fewer and smaller uredinia and fewer urediniospores (Liang *et al*., 2006, Chen and Line, 1995).

Currently 49 genes conferring resistance to yellow rust in wheat have been given a *Yr* designation (McIntosh *et al*., 2008). Resistance conferred by most *Yr* genes is race-specific (McIntosh *et al*., 2008, 2005, 2003 and 1995). While race-specific, seedling resistance usually confers complete immunity (Eriksen *et al*., 2004), partial additive APR genes are now more favoured by breeders due to their perceived durability (Lin and Chen, 2009, Lu *et al*., 2009, Mallard *et al*., 2005, Singh *et al*., 2005, Navabi *et al*., 2004 and Ramburanan *et al*., 2004). The focus of wheat yellow rust resistance breeding efforts in recent years has been towards cultivars that display evidence of field durability (Table 6.1).

Table 6.1 Identified yellow rust resistance QTL in wheat

Cultivar[1]	Chromosome[2]	Reference
Louise (HTAP[3])	2BS	Carter *et al*., 2009
Renan	2BS, 3BS, 6B	Dedryver *et al*., 2009
Libellula	2DS, 4BL, 5BL, 7DS	Lu *et al*., 2009
Strampelli	4BL, 5BL, 7DS	Lu *et al*., 2009
Alcedo	2D, 4B	Jagger, 2009
Express (HTAP)	1BL, 3BL, 6AS	Lin and Chen, 2009
Guardian	1B, 2D, 4B	Melichar *et al*., 2008
Atilla	1BL, 2BS, 7BL	Rosewarne et al., 2008
Alpowa	7BL	Lin and Chen., 2007
Camp Remy	2AL, 2BL	Boukhatem *et al*., 2002
	2A, 2BL, 2DS, 5B, 5BL	Mallard et al., 2005
Cook	7DS	Navabi *et al*., 2005
Kariega	2B, 4A 7D	Ramburan *et al*., 2004
Fukuho-komugi	7DS	Suenaga *et al*., 2003
Opata 85	2BS, 7AL	Borner *et al*., 2002
	2BS, 3DS, 5AL, 6DL, 7DS	Boukhatem *et al*., 2002
	3BS, 3DS, 4DS, 7DS	Singh *et al*., 2000
Pavon 76	1BL, 3BS, 4B, 6B	William *et al*., 2003

[1]Wheat genotype where the QTL was identified, [2]chromosomal location of identified QTL, [3]High Temperature Adult Plant resistance.

QTL analysis is used to identify, locate and determine the effects and interactions of contributing genes. Two fundamental components are required for QTL analysis, a genetic marker map with high levels of genome coverage and phenotypic data segregating for the trait of interest. QTL analysis detects associations between genotype and phenotype using molecular markers. Genotypes are grouped depending on the allele present at each marker locus and each genotype group compared for differing mean phenotypes (Tanksley *et al*., 1993). Several statistical methods are used to detect QTL, the simplest being single marker regression analysis. As this analysis is based upon single markers a genetic map is not required. Single marker analysis is often used to provide an approximate QTL location. The further away a marker is located from the

potential QTL the higher the potential recombination frequency between the marker loci and the QTL, reducing the likelihood of detection. Common statistical methods used for single marker regression are t-test, ANOVA and linear regression (coefficient of determination R^2). However, linked marker loci can be analysed simultaneously by a statistical process referred to as interval mapping (IM), providing more accurate estimate of QTL location (Lander and Botstein, 1989). Phenotypic data associated with linked markers along a chromosome is analysed to predict the likelihood of a QTL residing between those markers (Van Ooijen, 1999). The likelihood of a QTL is determined based on a strong statistical association between markers and QTL, as opposed to the QTL occurring by chance (Jansen and Stam, 1994). Those associations that exceed a specified significance level indicate probable sites of QTL. Composite interval mapping (CIM) uses interval mapping but includes additional genetic marker loci within the statistical model, to produce a more precise and effective analysis (Jansen and Stam, 1994, Jansen, 1993). QTL cartographer (Bateson *et al*., 2001, 1994) MapQTL (Van Oojen, 2004), MapManager QTL (Manly *et al*., 2001), QGene (Manly *et al*., 2001), MapManager QTX (Nelson *et al*., 1997) and Map Maker QTL (Lander and Botstein, 1989) are the most frequently used software tools for QTL analysis.

6.2 Aims of the current study

The genetic linkage map (Chapter 4) comprised of DArT, SSR, AFLP, NBS-AFLP and EST-derived (Chapter 5) markers of a DH mapping population from a cross between wheat cultivars Claire x Lemhi, together with yellow rust resistance phenotypic data sets (Chapter 3) were used to locate QTL associated with yellow rust resistance found in the winter wheat Claire. Initially single marker analysis was used to give an indication of QTL location and to identify whether any unlinked marker loci were highly associated with the resistance. IM and CIM were used to determine the precise location and contribution of each yellow rust resistance QTL. All QTL analysis was conducted using the computer package MapQTL.

6.3 Materials and Methods

All QTL analyses were carried out as described in Chapter 2 using the genetic map (Chapter 4) and phenotypic data (Chapter 3) previously reported. The infection type (IT) nominal and percentage infection (PI) adult plant data sets consisted of predicted means across two replicates, taken at two score dates from field data collected in 2006, two replicates, taken at three score dates from field data collected in 2007 and for three replicates obtained from a glasshouse trial in 2008. In total six PI data sets (LOGIT+ transformed) and six IT data sets (log transformed) were analysed (Table 6.2).

Table 6.2 Adult plant datasets used in the QTL analyses

Year	Source	Score[1]	Percentage infection (PI)	Infection type (IT)
1	Field	F	PIYr1F	ITYr1F
1	Field	L	PIYr1L	ITYr1L
2	Field	F	PIYr2F	ITYr2F
2	Field	M	PIYr2M	ITYr2M
2	Field	L	PIYr2L	ITYr2L
3	Glasshouse	L	PIYr3	ITYr3
1	Field	F + L	PIYr1	ITYr1
2	Field	F + M + L	PIYr2	ITYr2

[1]F = first score date, M = middle score date, L = last score date, F + L = average of first and last score date and F + M + L = average of first, middle and last score date.

For comparative purposes and to evaluate the significance levels across the three years of phenotypic data, an average value across all score dates within each year were also used for QTL analysis.

Phenotypic data for seedling resistance responses of the Claire x Lemhi DH population was supplied by Dr. L. A. Boyd (log transformed) for QTL analysis.

Kruskall and Wallis (single marker regression) analysis was used to provide an indication of significant associations between markers and QTL. Furthermore, this

analysis was used to identify any unlinked marker loci that could be associated with yellow rust resistance. IM was used to give a precise location of QTL. Any highly significant marker loci identified were selected as co-factors preceding MQM analysis with the aim of identifying any minor QTL which would otherwise be masked by the major QTL. Genome wide significant LOD thresholds were determined for each quantitative trait data set by conducting a permutation test (statistical significance test) with 1000 permutations in order to determine the significance threshold.

6.3 Results

6.3.1 Adult Plant QTL analysis

Kruskall and Wallis analysis, a non parametric statistic, of phenotypic data against marker data was used to initially screen the genome for markers associated with the resistance phenotype. This one way ANOVA of six PI and six IT data sets identified markers showing significant associations with the resistant phenotype (Table 6.3). Regions on chromosomes 2B and 2D were found to be associated with the yellow rust APR, all originating from the resistant parent Claire and a significance probability of $p \leq 0.001$. No unlinked markers were found associated with yellow rust resistance at this level of significance.

Seven markers on chromosome 2DL exhibited an association with the yellow rust resistance phenotypes (linkage group designated 2Da), where Claire donated the resistant allele (Table 6.3). The highest level of significance within this linkage group was for marker loci *wPt-7408* and *S15M32-155* ($p = 0.0001$), using all field data sets collected within the first year (PIYr1F, PIYr1L, ITYr1F and ITYr1L). The glasshouse data (PIYr3 and ITYr3) was also significance with these marker loci. However, year two field data (PIYr2F, PIYr2M, PIYr2L, ITYr2F, ITYr2M and ITYr2L) showed a low significance with these markers associated on linkage group 2Da ($p = 0.05$).

Three markers on linkage group 2Db were associated with the yellow rust resistant phenotypes using all 12 data sets (Table 6.3). The DArT marker *wPt-4413* was

significant at a level of p = 0.005 using all PI and IT datasets. The SSR marker *Xgwm539* was significant at a level of p = 0.005 for PI and IT from the first and third year of datasets, whilst with the second year data this significance level dropped to p = 0.05. In all cases Claire contributed the allele associated with resistance.

Marker locus *wPt-0950* had the highest level of significance on the 2B linkage group (p = 0.0005) for PI over all score dates within the second year (Table 6.3). This associated level of significance was less for IT in year two (p = 0.005 for ITYr2F and p = 0.01 for ITYr2M and ITYr2L). The DArT markers *wPt-9190* and *wPt-7200* and SSR marker *Xwmc175* both displayed a higher level of significance for PI within the second year (p = 0.005 – p = 0.001) than for IT (p = 0.05 – p = 0.0005). Claire contributed the resistant allele in this linkage group. However, the markers *wPt-0950*, *wPt-9190*, *wPt-7200* and *Xwmc175* on linkage group 2B associated with the resistant phenotype were not significant for the PIYr3 and ITYr3 data sets. The marker *Xwmc175* and *wPt-9190* had a low significance level (p = 0.1 and p = 0.05 respectively) for all phenotypic data sets within year one, whereas *wPt-7200* and *wPt-0950* were not significantly associated with the yellow rust APR found in Claire.

Table 6.3 Kruskall and Wallis analysis of significant QTL for the yellow rust APR segregating in the Claire x Lemhi DH population. Percentage infection (PI) and infection type (IT) phenotypic datasets were used.

Chromosome[1]	Locus[2]	Parent[3]	Percentage Infection (PI)						Infection type (IT)					
			PIYr1F	PIYr1L	PIYr2F	PIYr2M	PIYr2L	PIYr3	ITYr1F	ITYr1L	ITYr2F	ITYr2M	ITYr2L	ITYr3
2B	*wPt-9190*	Claire	0.05	0.01	0.005	0.001	<0.001	ns	0.005	0.01	0.005	0.05	0.01	ns
2B	*wPt-7200*	Claire	ns	ns	0.005	0.001	0.001	ns	ns	ns	0.005	0.05	0.05	ns
2B	*wPt-0950*	Claire	ns	ns	<0.001	<0.001	<0.001	ns	ns	ns	0.005	0.01	0.01	ns
2B	*Xwmc175*	Claire	ns	0.05	0.005	0.001	0.001	ns	ns	ns	0.005	0.05	0.05	ns
2Da	*Xwmc167*	Claire	<0.001	0.001	0.05	ns	ns	0.005	<0.001	0.005	0.01	ns	ns	0.01
2Da	*Xwmc311a*	Claire	0.001	<0.001	ns	ns	ns	0.005	<0.001	<0.001	ns	ns	ns	0.005
2Da	*EST18a*	Claire	0.001	<0.001	ns	ns	ns	0.005	<0.001	<0.001	ns	ns	ns	0.005
2Da	*wPt-7408*	Claire	<0.001	<0.001	0.05	0.05	0.05	<0.001	0.001	<0.001	0.05	ns	ns	<0.001
2Da	*S15M32-155*	Claire	<0.001	<0.001	ns	0.05	0.05	<0.001	<0.001	<0.001	0.05	ns	ns	<0.001
2Da	*Xwmc301a*	Claire	0.001	<0.001	0.05	0.05	ns	<0.001	<0.001	<0.001	0.05	ns	ns	<0.001
2Db	*wPt-4413*	Claire	0.01	0.005	0.05	0.005	0.01	0.005	0.005	0.005	ns	0.005	0.005	0.005
2Db	*Xgwm539*	Claire	0.005	0.005	ns	0.05	0.05	0.005	0.005	0.005	ns	0.05	0.05	0.05
2Db	*EST22*	Claire	0.01	0.005	0.05	0.01	0.05	<0.001	0.005	0.005	ns	0.005	0.05	0.005

[1]Chromosomes containing the significant loci, [2]marker loci associated with the significant QTL, [3]parental allele associated with the APR, ns = not significant.

When the PI and IT data for each year was averaged across score dates the chromosomal locations identified by Kruskall and Wallis analysis as associated with the yellow rust APR phenotype did not alter (Table 6.4).

Table 6.4 Kruskall and Wallis analysis of marker loci significantly associated with APR detected in the Claire x Lemhi DH population within each of the three years (average over the score dates within each year) for percentage infection (PI) and infection type (IT) phenotypic datasets using MapQTL v 5.0 (Van Ooijen, 2004).

Chromosome[1]	Locus[2]	Parent[3]	Percentage Infection (PI)			Infection type (IT)		
			PIYr1	PIYr2	PIYr3	ITYr1	ITYr2	ITYr3
2B	*wPt-9190*	Claire	0.05	0.001	ns	0.005	0.005	ns
2B	*wPt-7200*	Claire	ns	0.001	ns	ns	0.005	ns
2B	*wPt-0950*	Claire	ns	<0.001	ns	ns	0.005	ns
2B	*Xwmc175*	Claire	ns	0.001	ns	ns	0.005	ns
2Da	*Xgwm301c*	Claire	0.05	ns	0.001	0.01	ns	0.01
2Da	*Xwmc301a*	Claire	<0.001	0.05	<0.001	<0.001	0.05	<0.001
2Da	*S15M32-155*	Claire	<0.001	ns	<0.001	<0.001	ns	<0.001
2Da	*wPt-7408*	Claire	<0.001	0.05	<0.001	<0.001	0.05	<0.001
2Da	*Xwmc311a*	Claire	0.001	ns	0.005	<0.001	ns	0.005
2Da	*EST18a*	Claire	0.001	ns	0.005	<0.001	ns	0.005
2Da	*Xwmc817a*	Claire	0.005	ns	0.05	0.001	ns	0.05
2Da	*Xwmc167*	Claire	<0.001	ns	0.005	<0.001	0.05	0.01
2Db	*EST22*	Claire	0.05	0.01	<0.001	0.05	0.05	0.005
2Db	*Xgwm539*	Claire	0.005	0.05	0.005	0.001	ns	0.05

[1]Chromosome containing the significant loci, [2]marker loci associated with the significant QTL, [3]parental allele associated with the APR, ns = not significant.

To precisely identify where the APR QTL are located and assess the contribution of each QTL to the yellow rust resistance segregating in the Claire x Lemhi cross IM was conducted (Table 6.5 – Table 6.9). A number of QTL significantly associated with yellow rust APR were identified, with individual linkage group LOD thresholds ranging from 1.0 – 1.8 depending on the phenotypic data set used. Permutation tests gave genome wide LOD thresholds of 2.9 to 3.4 at a 5% significance level. Due to the variability of the genome wide LOD scores, individual linkage group thresholds were used.

IM identified a QTL peak located near *S15M32-155* on the 2Da linkage group positioned on the long arm of chromosome 2D, designated *QTLAPR.2Da*. Claire was

the parental allele contributing the resistant phenotype for each significantly associated marker. MQM mapping confirmed that Claire was the source of the yellow rust resistance QTL. *QTLAPR.2Da* was found to be significant with all the datasets used with IM mapping. The LOD scores obtained in year 1 and year 3 were higher than those obtained in year 2. For PI data sets the LOD scores ranged from 4.7 to 5.1 in year 1 (Table 6.5), 0.7 to 1.0 in year 2 (Table 6.6) and 4.1 in year 3 (Table 6.7). The percentage phenotype variation explained by *QTLAPR.2Da* was found to be higher in year 1 (26.8% to 28.7%) and year 3 (28.4%) in comparison to year 2 (4.9 % to 8.6%). For IT data sets in year 1 the LOD scores ranged from 5.3 to 5.8 (Table 6.8), 0.3 to 1.4 in year 2 (Table 6.9) and 4.5 in year 3 (Table 6.7). The percentage IT phenotypic variation explained by *QTLAPR.2Da* ranged from 30.7% to 31.8% (year 1), 2.5% to 10.0% (year 2) and 26.6% (year 3).

A second QTL from the resistant parent Claire was positioned near *EST22* and was also located on the long arm of chromosome 2D, designated *QTLAPR.2Db*. This QTL was significant across all data sets analysed. For the PI data sets the LOD scores ranged from 2.2 to 3.0 in year 1 (Table 6.5), 1.2 to 2.2 in year 2 (Table 6.6) and 3.2 in year 3 (Table 6.7). The percentage of PI phenotypic variation explained by *QTLAPR.2Db* ranged from 16.0% to 21.3% in year 1, 8.7% to 16.1% in year 2 and 21.6% in year 3. The IT LOD scores ranged from 3.0 to 3.4 in year 1 (Table 6.8), 1.0 to 2.0 in year 2 (Table 6.9) and 2.9 in year 3 (Table 6.7). The explained percentage variation of the IT phenotype ranged from 21.6% to 23.4% (year 1), 7.6% to 14.6% (year 2) and 13.1% to 21.4% (year 3).

Minor QTL was located near the DArT marker *wPt-9190*, located on the long arm of chromosome 2B and again originating from the resistance parent Claire (*QTLAPR.2B*). This QTL was significant across all data sets in year 1 (Table 6.5 and 6.8) and year 2 (Table 6.6 and Table 6.9) but not significant in year 3 (glasshouse trial). For PI data sets the LOD scores ranged from 1.4 to 1.7 in year 1 and 2.4 to 3.1 in year 2. The percentage phenotype variation explained by *QTLAPR.2B* ranged from 8.5% to 10.5% in year 1 and 18.7% to 23.8% in year 2. For the IT datasets the LOD ranged from 1.6 to 1.8 in year 1 and 1.5 to 2.6 in year 2. The percentage phenotype variation explained by *QTLAPR.2B* for IT data sets ranged from 10.4% to 11.1% in year 1 and 12.1% to 19.6% in year 2.

A second, small effect QTL, significant across all data sets, was also located on the long arm of 7B. Again originating from Claire this QTL was designated *QTLAPR.7B*. LOD scores varied from 1.5 to 1.6 in year 1 (Table 6.5), 1.3 to 1.7 in year 2 (Table 6.6) and 1.8 in year 3 (Table 6.7). The explained percentage variance varied across the three years of data sets, for PI in year 1 ranged from 9.2% to 10.0%, year 2 11.7% to 12.7% and 11.8% in year 3. For IT the explained percentage variance ranged from 7.9% to 11.2% in year 1 (LOD 1.2 to 1.8), 4.9% to 12.6% in year 2 (LOD 0.6 to 1.7) and 6.7% in year 3 (LOD 1.0).

Table 6.5 Yellow rust resistance QTL detected in the Claire x Lemhi cross using percentage infection (PI) data sets from year 1.

				PIYr1F					PIYr1L				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl. % variance[6]**	**Phenotypic means[7]**		**LOD threshold**	**LOD**	**Expl. % variance**	**Phenotypic means**	
							C	**L**				**C**	**L**
IM	2B	Claire	*wPt-9190*	1.5	*	8.5	4.0	12.4	1.5	1.7	10.5	3.7	14.6
	2Da	Claire	*S15M32-155*	1.4	4.7	26.8	2.8	20.4	1.3	5.1	28.7	2.7	24.5
	2Db	Claire	*EST22*	1.5	2.2	16.0	3.8	17.5	1.6	3.0	21.3	3.3	22.7
	7B	Claire	*wPt-1069*	1.6	*	9.2	3.8	12.5	1.8	*	10.0	3.6	14.0
MQM	2B	Claire	*wPt-9190*	1.6	*	8.5	4.0	12.5	1.5	1.7	10.5	3.7	14.6
	2Da	Claire	*S15M32-155*	1.4	4.7	26.5	3.4	20.6	1.3	5.1	28.7	3.3	23.6
	2Db	Claire	*EST22*	1.5	2.2	16.0	4.7	15.5	1.6	3.0	21.3	4.2	20.8
	7B	Claire	*wPt-1069*	1.6	*	9.2	4.7	14.3	1.8	*	10.0	5.1	16.4

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Genome wide threshold PIYr1F = 3.2, PIYr1L = 3.1. [5]Maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi, * = LOD value of QTL did not exceed LOD threshold for the linkage group.

Table 6.6 Yellow rust resistance QTL detected in the Claire x Lemhi cross using percentage infection (PI) data sets from year 2.

				PIYr2F					PIYr2M					PIYr2L				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl. % var.[6]**	**Phenotypic[7] means**		**LOD threshold**	**LOD**	**Expl. % var.**	**Phenotypic means**		**LOD threshold**	**LOD**	**Expl. % var.**	**Phenotypic means**	
							C	**L**				**C**	**L**				**C**	**L**
IM	2B	Claire	*wPt-0615*	1.4	2.4	18.7	0.5	3.7	1.5	3.1	21.7	0.9	10.1	1.5	3.1	23.8	1.7	23.7
	2Da	Claire	*S15M32-155*	1.4	*	8.6	0.8	2.9	1.5	*	4.9	1.9	6.1	1.5	*	5.0	4.6	15.7
	2Db	Claire	*EST22*	1.6	*	8.7	0.8	3.3	1.5	1.6	13.3	1.5	9.3	1.5	2.2	16.1	2.8	23.7
	2Dc	Claire	*wPt-1079*	1.2	1.6	16.4	0.7	4.0	1.3	1.5	14.9	1.1	8.6	1.1	1.6	16.6	1.5	15.8
	3B	Claire	*wPt-4412*	1.1	1.7	12.2	0.6	3.3	1.2	1.2	8.9	1.5	6.8	0.8	*	4.0	4.5	12.8
	5D	Claire	*wPt-2526*	1.4	*	11.1	0.6	3.1	1.3	*	9.3	1.3	6.4	1.3	1.9	20.6	1.9	22.8
	7B	Claire	*wPt-4057*	1.6	1.6	12.7	0.6	3.1	1.6	*	11.7	1.4	6.6	1.6	1.7	12.6	2.6	18.1
MQM	2B	Claire	*wPt-9190*	1.4	2.4	18.7	0.5	3.2	1.5	2.9	18.9	0.7	8.3	1.5	3.1	23.8	1.8	21.8
	2Da	Claire	*S15M32-155*	1.4	*	6.6	0.7	3.4	1.5	*	4.9	2.7	7.0	1.5	*	5.0	6.8	17.9
	2Db	Claire	*EST22*	1.6	*	8.7	0.9	4.0	1.5	1.6	13.3	1.6	10.7	1.5	1.7	13.9	3.0	28.2
	5D	Claire	*wPt-2526*	1.4	*	11.1	0.6	3.0	1.3	*	10.3	1.3	6.4	1.3	1.9	20.6	2.0	22.8
	7B	Claire	*wPt-4057*	1.6	1.6	11.5	0.8	4.2	1.6	*	9.4	1.7	10.1	1.6	*	9.9	4.1	25.3

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Genome wide LOD threshold PIYr2F = 3.2, PIYr2M = 3.4, PIYr2L = 3.2, [5]maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7] phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi, * = LOD value of QTL did not exceed LOD threshold for the linkage group.

Table 6.7 Yellow rust resistance QTL detected in the Claire x Lemhi cross using percentage infection (PI) and infection type (IT) data sets from year 3.

				PIYr3					ITYr3				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl. % variance[6]**	**Phenotypic means[7]**		**LOD threshold**	**LOD**	**Expl. % variance**	**Phenotypic means**	
							C	**L**				**C**	**L**
IM	2Da	Claire	*S15M32-155*	1.4	4.1	28.4	2.0	16.5	1.3	4.5	26.6	0.3	0.5
	2Db	Claire	*EST22*	1.6	3.2	21.6	3.0	18.0	1.6	2.9	21.4	0.3	0.5
	7B	Claire	*wPt-9925*	1.3	1.8	11.8	2.8	11.0	1.2	*	6.7	0.3	0.4
MQM	2Da	Claire	*S15M32-155*	1.4	3.9	22.9	3.3	17.5	1.3	4.5	25.7	0.3	0.5
	2Db	Claire	*EST22*	1.6	3.1	19.2	3.4	16.1	1.6	2.8	17.0	0.3	0.5
	7B	Claire	*wPt-9925*	1.3	*	2.9	4.1	11.9	1.0	*	3.3	0.3	0.35

[1]Chromosome location of the QTL, [2] parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Genome wide threshold PIYr3= 3.2, PIYr3 = 3.1, [5]maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi, * = LOD value of QTL did not exceed LOD threshold for the linkage group.

Table 6.8 Yellow rust resistance QTL detected in the Claire x Lemhi cross using infection type (IT) data sets from year 1.

				ITYr1F					ITYr1L				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl. % variance[6]**	**Phenotypic means[7]**		**LOD threshold**	**LOD**	**Expl. % variance**	**Phenotypic means**	
							C	**L**				**C**	**L**
IM	2B	Claire	*wPt-9190*	1.6	1.8	11.1	0.3	0.5	1.6	1.6	10.4	0.3	0.5
	2Da	Claire	*S15M32-155*	1.4	5.8	31.8	0.3	0.5	1.4	5.3	30.7	0.3	0.5
	2Db	Claire	*EST22*	1.6	3.0	21.6	0.3	0.5	1.6	3.4	23.4	0.3	0.5
	7B	Claire	*wPt-1069*	1.6	1.8	11.2	0.4	0.5	1.6	*	7.9	0.4	0.5
MQM	2B	Claire	*wPt-9190*	1.6	1.8	11.1	0.4	0.5	1.6	1.6	10.4	0.3	0.5
	2Da	Claire	*S15M32-155*	1.4	5.8	31.7	0.3	0.5	1.4	5.3	29.6	0.3	0.5
	2Db	Claire	*EST22*	1.6	2.9	21.6	0.3	0.5	1.6	3.4	23.5	0.3	0.5
	7B	Claire	*wPt-1069*	1.6	1.8	11.2	0.4	0.5	1.6	*	7.9	0.4	0.5

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Genome wide threshold ITYr1F = 3.1, ITYr1L = 3.1, [5]maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi, * = LOD value of QTL did not exceed LOD threshold for the linkage group.

Table 6.9 Yellow rust resistance QTL detected in the Claire x Lemhi cross using infection type (IT) data sets from year 2.

				ITYr2F					ITYr2M					ITYr2L				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl. % var.[6]**	**Phenotypic[7] means**		**LOD threshold**	**LOD**	**Expl % var.**	**Phenotypic means**		**LOD threshold**	**LOD**	**Expl. % var.**	**Phenotypic means**	
							C	**L**				**C**	**L**				**C**	**L**
IM	2B	Claire	*wPt-9190*	1.5	2.6	19.6	0.2	0.4	1.4	1.7	13.4	0.3	0.4	1.5	1.5	12.1	0.3	0.4
	2Da	Claire	*S15M32-155*	1.4	1.4	10.2	0.2	0.4	1.3	0.3	2.5	0.3	0.4	1.4	*	5.3	0.4	0.4
	2Db	Claire	*EST22*	1.6	*	7.6	0.2	0.3	1.6	1.7	13.3	0.3	0.4	1.6	2.0	14.6	0.3	0.4
	2Dc	Claire	*wPt-1079*	1.3	1.4	15.7	0.2	0.3	1.1	2.4	17.6	0.3	0.4	1.2	1.9	14.8	0.3	0.4
	3B	Claire	*wPt-4412*	1.2	1.7	12.2	0.2	0.3	0.9	0.2	1.7	0.3	0.4	0.8	*	2.0	0.3	0.4
	5D	Claire	*wPt-2526*	1.2	1.9	19.3	0.2	0.3	1.2	1.0	8.6	0.3	0.4	0.9	0.9	9.2	0.3	0.4
	7B	Claire	*wPt-1069*	1.3	*	4.9	0.2	0.3	1.7	1.6	11.9	0.3	0.4	1.6	1.7	12.6	0.3	0.4
MQM	2B	Claire	*wPt-9190*	1.5	2.6	19.6	0.2	0.4	1.4	1.7	13.0	0.3	0.4	1.5	1.5	12.1	0.3	0.4
	2Da	Claire	*S15M32-155*	1.4	*	8.2	0.3	0.3	1.3	*	2.5	0.3	0.3	1.4	*	5.3	0.3	0.4
	2Db	Claire	*EST22*	1.6	*	7.3	0.3	0.3	1.6	1.7	13.3	0.3	0.4	1.6	*	11.6	0.3	0.4
	5D	Claire	*wPt-2526*	1.2	1.9	19.3	0.2	0.4	1.2	*	8.6	0.3	0.4	1.2	*	9.2	0.3	0.4
	7B	Claire	*wPt-1069*	1.7	*	4.9	0.3	0.3	1.7	1.6	11.9	0.3	0.4	1.6	1.7	12.3	0.3	0.4

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Genome wide LOD threshold ITYr2F = 3.1, ITYr2M = 2.9, ITYr2L = 2.9, [5]maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi, * = LOD value of QTL did not exceed LOD threshold for the linkage group.

To detect any minor QTL contributing to the yellow rust resistance marker loci close to the QTL on linkage groups 2Da and 2Db were used as cofactors in MQM mapping. The AFLP marker *S15M32-155* (associated with *QTLAPR.2Da*) and EST marker *EST22* (associated with *QTLAPR.2Db*) were selected as co-factors in MQM mapping. The backward elimination process of automatic cofactor selection in MapQTL was used to confirm the choice of cofactors. The selection of co-factors had no effect on the QTL associated with these marker loci. Additional marker loci, *wPt-9190* closely linked to the minor QTL on 2BL and *wPt-1069* closely linked to the QTL on 7BL were also used as cofactors in MQM analysis. The MQM results presented in Tables 6.5 to 6.9 are obtained from using all the co-factors described above and subsequently used for construction of QTL plots (Figure 6.1). QTL plots obtained from MQM mapping results without the selected co-factors are presented in Appendix Figure 1.

With MQM mapping *QTLAPR.2Da* was highly significant with year 1 and 3 datasets. With the PI data sets *QTLAPR.2Da* explaining between 22.9% to 28.7% of the phenotypic variation in year 1 and 3, in year 2 however this decreased to 4.9% to 6.6%. IT data sets showed the same pattern explaining 25.7% to 31.7% of the phenotypic variation in year 1 and year 3 whereas in year 2 the variation was reduced to 2.5% to 8.2%. The *QTLAPR.2Da* peak is positioned between the DArT marker *wPt-7408* and the AFLP marker loci *S15M32-155*, this linkage group is found centromeric of the long arm of chromosome 2D (Figure 6.1a (i)).

With MQM mapping *QTLAPR.2Db* was also highly significant in year 1 and year 3 data sets, having a lower level of significance in year 2. With the PI data sets *QTLAPR.2Db* explained 16.0% to 21.3% of the total phenotypic variation found in years 1 and 3, this percentage decreased to 8.7% to 13.9% in year 2. The percentage of phenotypic variation explained by *QTLAPR.2Db* was similar for IT scores, ranging from 17.0% to 23.5% for years 1 and 3, whereas in year 3 the total variation explained was 7.3% to 13.3%. The *QTLAPR.2Db* peak is positioned between the SSR marker *Xgwm539* and the EST marker loci *EST22*, this linkage group is found at the distal end of the long arm of chromosome 2D (Figure 6.1b (i)).

With MQM mapping *QTLAPR.2B* was highly significant for PI and IT data sets from year 2 explaining 18.7% to 21.8% of the PI phenotypic variation and 12.1% to 19.6% of the IT phenotype variation. In year 1 the explained percentage variation decreased significantly to 8.5% to 10.5% for PI and 10.4% to 11.1% for IT data sets. This QTL was not detected with the PI or IT data sets in year 3. The QTL peak occurred between the DArT markers *wPt-9190* and *wPt-7200* (Figure 6.1c (i)).

With MQM mapping *QTLAPR.7B* explained 9.2% to 11.5% of the total phenotypic variation for PI in year 2, for IT the percentage was more variable, ranging from 4.9% to 12.3%. The percentage of phenotypic variation explained by *QTLAPR.7B* was similar for PI and IT scores in year 1 ranging from 7.9% to 11.2%. In year 3 the explained phenotypic variation for both PI and IT was minimal, ranging from 2.9% to 3.3%. The QTL peak occurred between the DArT markers *wPt-9190* and *wPt-7200* (Figure 6.1d (i)).

When the PI and IT data for year 1 and year 2 was averaged across score dates the number of QTL significantly associated with the resistance phenotype did not alter. Following MQM mapping the LOD scores decreased for all identified QTL. However, the explained percentage variance did not seem to be affected (Table 6.10). *QTLAPR.2Da* (Figure 6.1a (ii)) and *QTLAPR.2Db* (Figure 6.1b (ii)) explained the highest level of percentage variance in year 1 PI and IT data sets. Whilst *QTLAPR.2B* (Figure 6.1c (ii)) explained the highest level of percentage variance in year 2 PI and IT data sets. The percentage of phenotypic variation explained by *QTLAPR.7B* was highest in year 2 (Figure 6.1d (ii)).

Table 6.10 Yellow rust resistance QTL detected in the Claire x Lemhi DH population within year 1 and 2 of an average over score dates for percentage infection (PI) and infection type (IT) data sets.

				Year 1 PI					Year 1 IT				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl. % variance[6]**	**Phenotypic means[7] C**	**L**	**LOD threshold**	**LOD**	**Expl. % variance**	**Phenotypic means C**	**L**
IM	2B	Claire	*wPt-9190*	1.0	1.5	7.8	5.4	11.1	0.8	1.2	9.2	0.3	0.4
	2Da	Claire	*S15M32-155*	1.6	5.2	29.0	2.8	22.5	1.2	5.8	33.3	0.3	0.4
	2Db	Claire	*EST22*	1.3	2.3	19.4	3.5	19.0	1.3	3.3	23.5	0.3	0.4
	7B	Claire	*wPt-1069*	1.0	1.5	9.5	3.9	13.2	1.1	1.6	10.1	0.3	0.4
MQM	2B	Claire	*wPt-9190*	1.0	1.6	10.1	3.4	13.5	0.8	1.8	11.4	0.3	0.4
	2Da	Claire	*S15M32-155*	1.6	5.2	29.0	2.3	22.5	1.2	6.0	32.5	0.3	0.4
	2Db	Claire	*EST22*	1.3	2.3	19.3	3.5	19.5	1.3	3.0	23.4	0.3	0.4
	7B	Claire	*wPt-1069*	1.0	1.5	9.5	2.9	12.9	1.1	1.6	10.1	0.3	0.4

				Year 2 PI					Year 2 IT				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl. % variance[6]**	**Phenotypic means[7] C**	**L**	**LOD threshold**	**LOD**	**Expl. % variance**	**Phenotypic means C**	**L**
IM	2B	Claire	*wPt-9190*	0.9	2.4	19.9	1.0	9.2	0.9	1.8	15.6	0.3	0.4
	2Da	Claire	*S15M32-155*	1.3	1.6	12.3	1.4	7.7	1.1	*	6.3	0.3	0.4
	2Db	Claire	*EST22*	1.3	1.8	13.4	1.4	8.8	1.2	1.5	10.8	0.3	0.4
	5D	Claire	*wPt-5078*	1.0	1.4	14.5	1.2	8.0	1.1	1.5	15.5	0.3	0.4
	7B	Claire	*wPt-1069*	1.0	1.5	12.0	1.4	7.4	1.0	1.3	9.8	0.3	0.4
MQM	2B	Claire	*wPt-9190*	1.0	1.8	11.4	0.9	8.7	0.9	2.5	18.6	0.3	0.4
	2Da	Claire	*S15M32-155*	1.3	*	5.5	2.1	6.6	1.1	*	6.3	0.3	0.4
	2Db	Claire	*EST22*	1.3	*	9.8	1.7	7.7	1.2	*	9.0	0.3	0.4
	5D	Claire	*wPt-5078*	1.0	1.4	14.5	1.2	8.0	1.1	1.5	15.5	0.3	0.4
	7B	Claire	*wPt-1069*	1.0	1.5	10.8	1.4	7.3	1.0	2.4	18.9	0.2	0.4

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Genome wide LOD threshold PI Year 1 = 2.7, PI Year 2 = 2.8, IT Year 1 = 2.8, IT Year 2 = 2.7, [5]maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi, * = LOD value of QTL did not exceed LOD threshold for the linkage group.

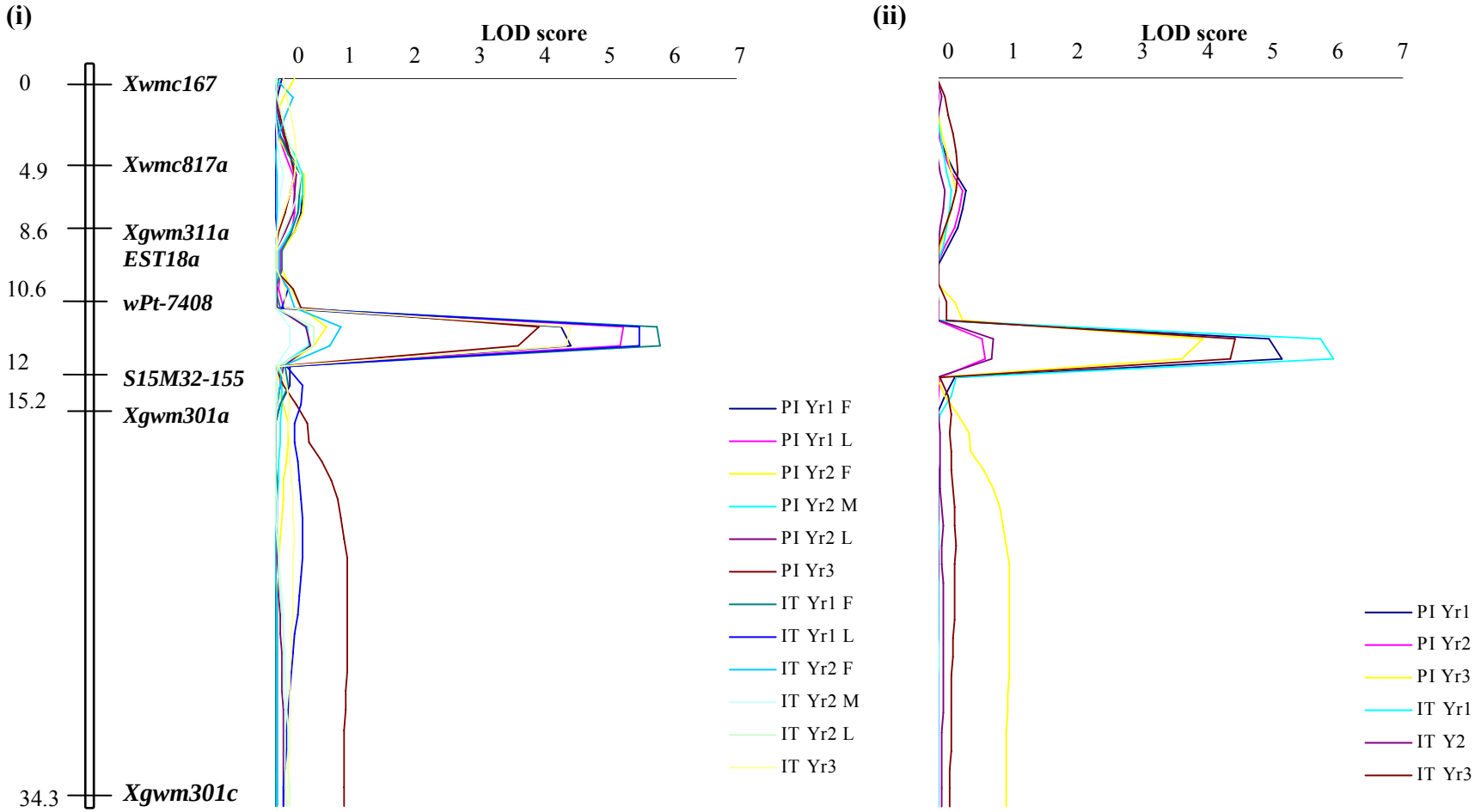


Figure 6.1a QTL plots obtained from MQM mapping using cofactors as described in Section 6.3.1 for the percentage infection (PI) and infection type (IT) datasets. The corresponding linkage groups are shown with the relevant marker loci and the map distances in cM (Kosambi) for each plot (a) *QTLAPR.2Da* (b) *QTLAPR.2Db* (c) *QTLAPR.2B* (d) *QTLAPR.7B* LOD thresholds see Table 6.5 to Table 6.9. QTL graph (i) represents all score dates within each data set, (ii) average across each year. For QTL plots obtained from MQM mapping without the selection of cofactors see Appendix Figure 1.

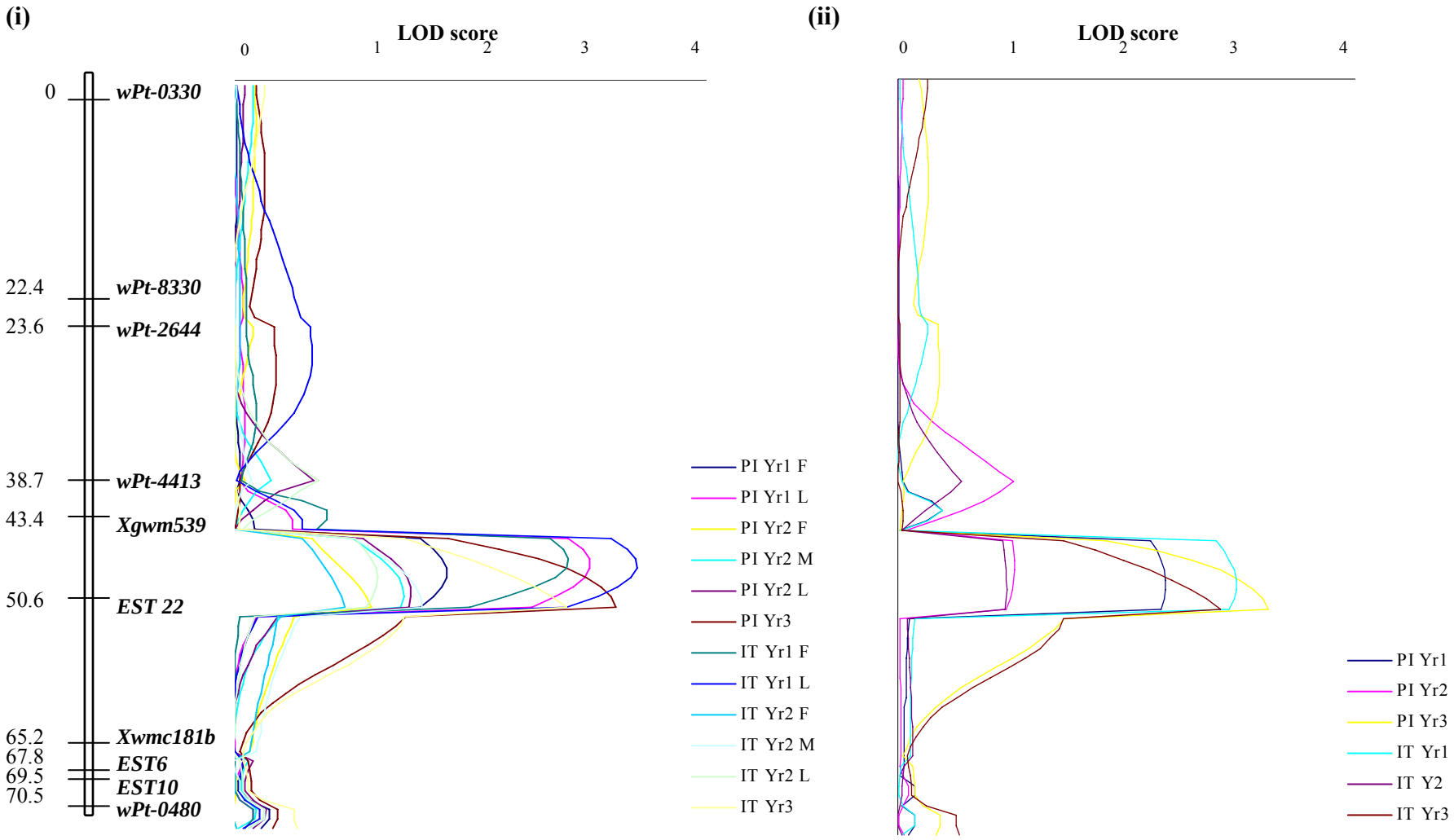


Figure 6.1b *QTLAPR.2Db*

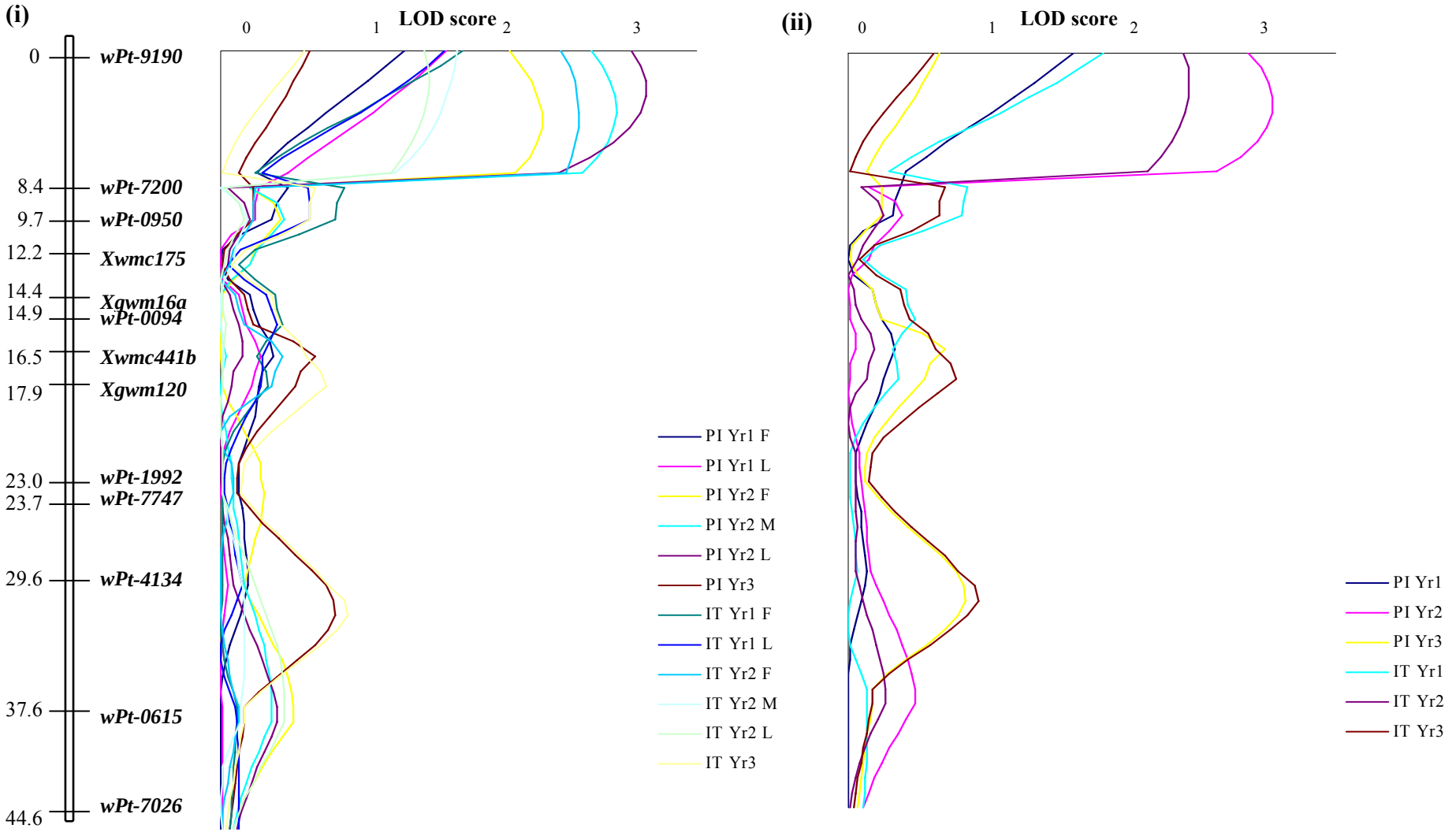


Figure 6.1c *QTLAPR.2B*

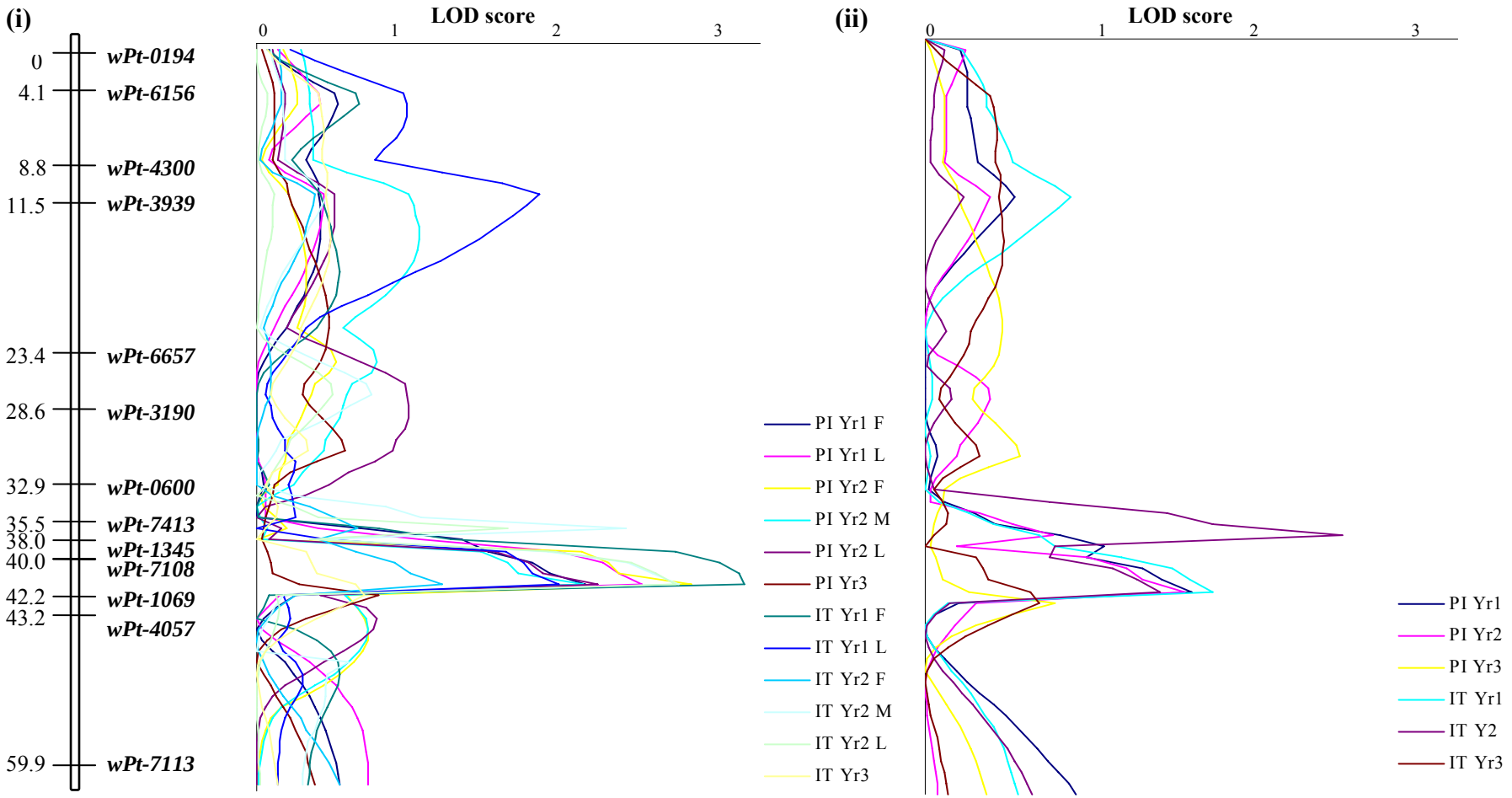


Figure 6.1d *QTLAPR.7B*

The DH lines were divided into 16 genotypes based upon the four significant QTLs detected with the PI and IT data sets (Table 6.11). The closest linked marker loci were used for identification (*QTLAPR.2Da = wPt-7408* and *S15M32-155*, *QTLAPR.2Db = Xgwm539* and *EST22*, *QTLAPR.2B = wPt-9190* and *QTLAPR.7B = wPt-1069*) and the mean PI and IT score for each genotypic group was calculated (Figure 6.2 and 6.3).

Table 6.11 The genotypic groups each of the DH lines are divided.

Genotype	DH line
No identified QTL	9, 59, 74
QTLAPR.2Da	11, 53
QTLAPR.2Db	53, 58, 62, 66, 69
QTLAPR.2B	12, 17, 20, 26, 32, 45, 63
QTLAPR.7B	38, 43, 44
QTLAPR.2Da, QTLAPR.2Db	6, 33, 42, 46
QTLAPR.2Da, QTLAPR.2B	61, 68, 75
QTLAPR.2Da QTLAPR.,7B	18, 35, 40, 41, 56, 65
QTLAPR.2Db, QTLAPR.2B	21, 34, 50
QTLAPR.2Db, QTLAPR.7B	10, 37, 73
QTLAPR.2B, QTLAPR.7B	19, 39
QTLAPR.2Da, QTLAPR.2Db, QTLAPR.2B	8, 14, 16, 22, 23, 27, 36, 49, 51
QTLAPR.2Da, QTLAPR.2Db QTLAPR.,7B	29, 52, 55, 71
QTLAPR.2Da, QTLAPR.2B QTLAPR.,7B	4, 30, 64, 76
QTLAPR.2Db, QTLAPR.2B, QTLAPR.7B	28, 31, 57, 67, 70, 72
QTLAPR.2Da, QTLAPR.2Db, QTLAPR.2B, QTLAPR.7B	13, 24, 47, 48

When none of the QTLs identified in this study are present the highest mean PI values obtained across score data was 65% in year 1, 85% in year 2 and 74.4% in year 3 (Figure 6.2). When *QTLAPR.2Da* was present alone the mean PI was reduced to a maximum of 20.6%, 25.7% and 23.8% in year 1, 2 and 3 respectively, while *QTLAPR.2Db* alone reduced the mean PI to a maximum of 20.5% in year 1, 25.5% in year 2 and 23.6% in year 3. A similar pattern was observed for IT. When none of the QTLs were present the maximum IT nominal score obtained was 0.8 in year 1, 0.7 in year 2 and 0.7 in year 3 (Figure 6.3). The QTLs on 2Da and 2Db had a comparable effect on IT, reducing the maximum IT to 0.4 across all three years of

data. DH lines carrying just *QTLAPR.2Da* or *QTLAPR.2Db* exhibited a partial resistant phenotype for both PI (Figure 6.2) and IT (Figure 6.3) data sets.

When *QTLAPR.2B* is present alone the highest mean PI values obtained across score data was 62.5% in year 1, 81% in year 2 and 68.9% in year 3. When *QTLAPR.7B* is present alone the highest mean PI values obtained across score data was 47.5 % in year 1, 72.5% in year 2 and 52.5% in year 3. When these QTL are combined there is a clear additive effect, reducing the maximum PI to 20.5%, 25.6% and 23.7% for each year respectively. *QTLAPR.2B* has a greater effect on IT than *QTLAPR.7B*, with a maximum average IT of 0.4 across all three years, whilst 7B QTL ranges from 0.5 to 0.7. The additive effect was not seen for IT as was evident for PI as *QTLAPR.2B* had a greater effect on IT score than it did for PI. The average IT scores when these two QTLs are combined was 0.4. The two major QTLs on 2Da and 2Db, in combination with either of the two minor QTLs on 2B or 7B appear to have an additive effect on PI, with a small additive effect on IT. An additive effect is evident when all four QTLs are present, as seen for both the PI and IT scores. A greater additive effect is observed in PI than IT.

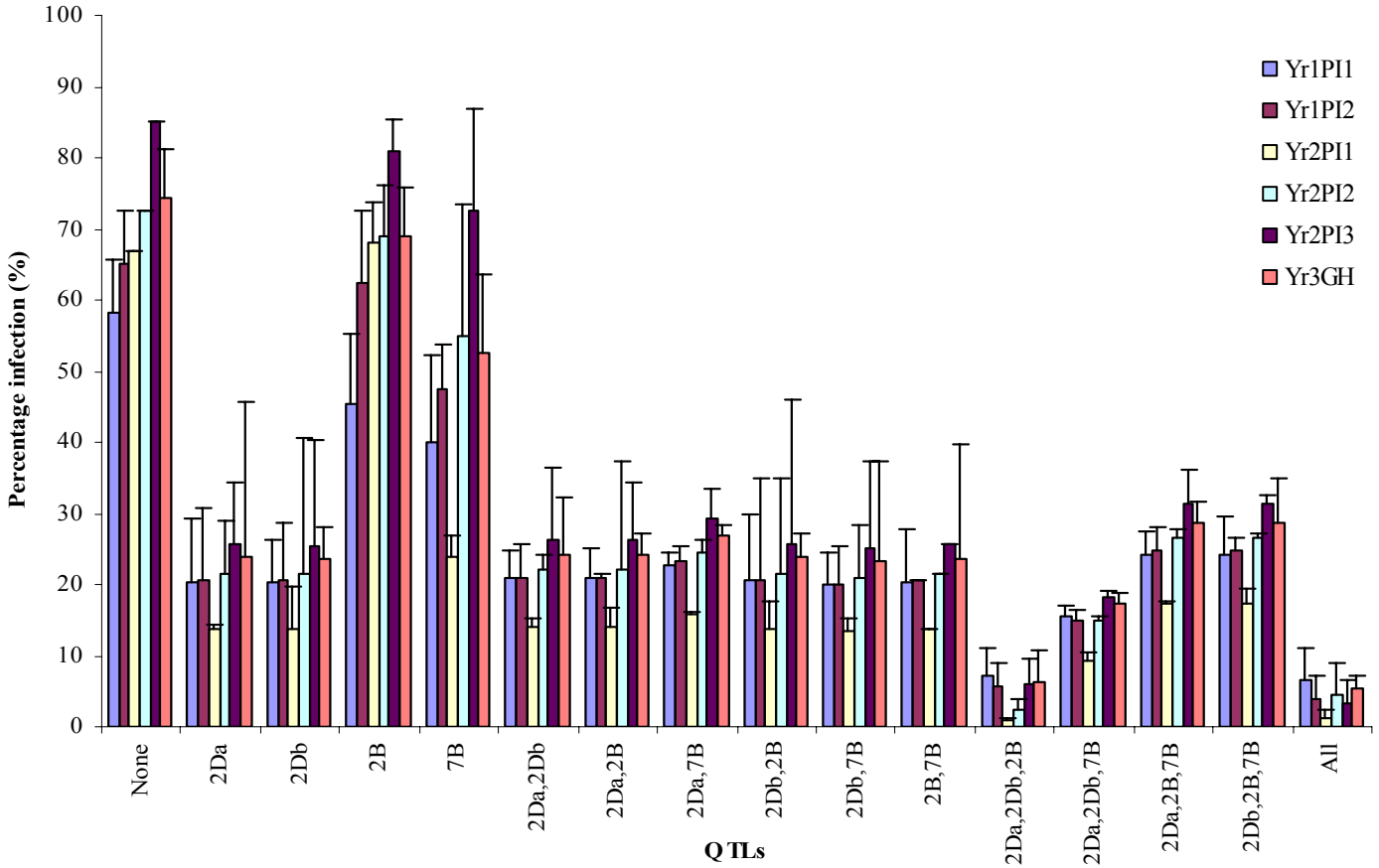


Figure 6.2 Mean percentage infection (PI) for the DH line grouped according to genotype. The X axis identifies which QTL are present: none = no QTL, 2Da = *QTLAPR.2Da*, 2Db = *QTLAPR.2Db*, 2B = *QTLAPR.2B* and 7B = *QTLAPR.7B*. Error bars represent standard error.

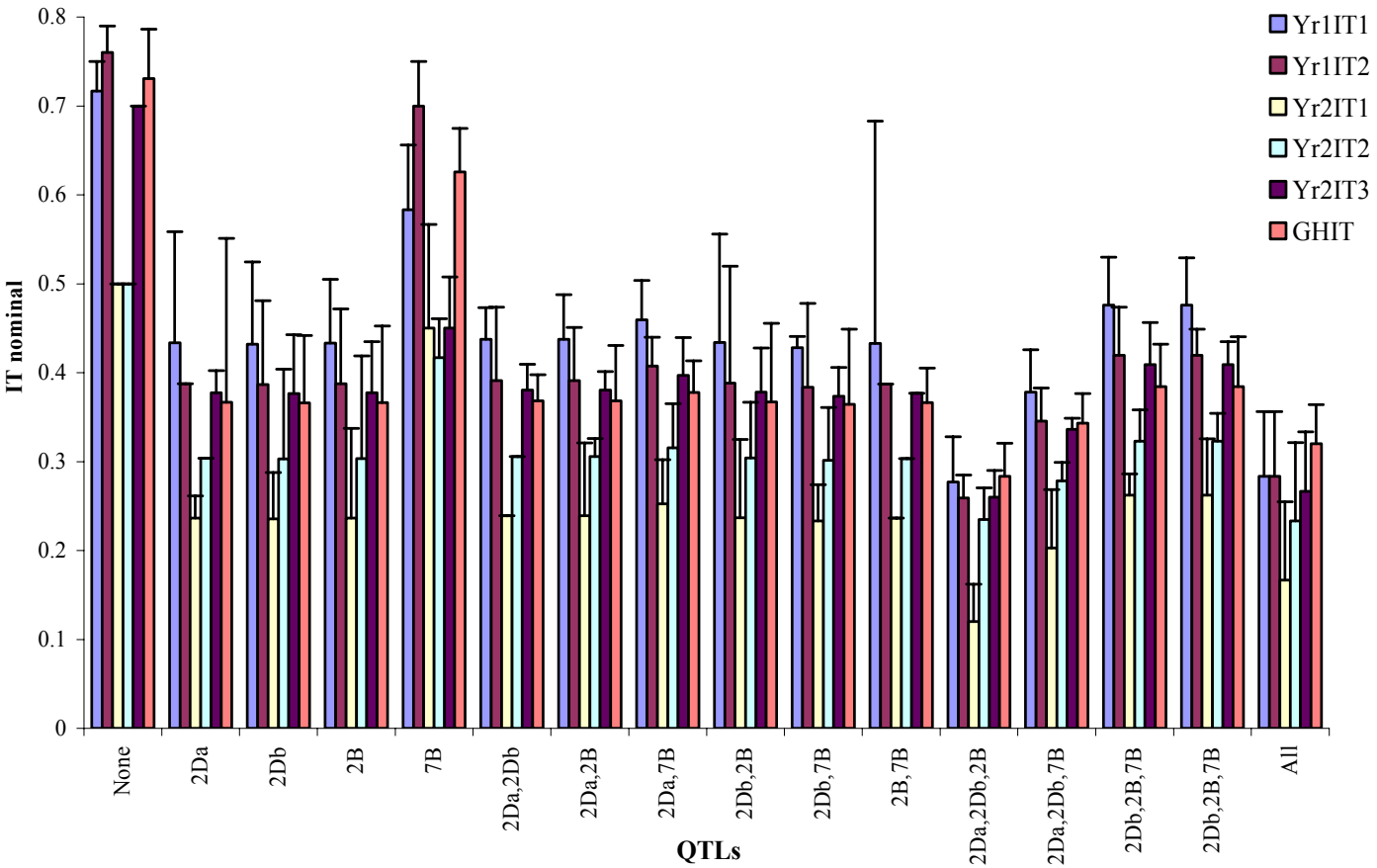


Figure 6.3 Mean infection type nominal (IT) for the DH lines grouped according to genotype. The X axis identifies which QTL are present: none = no QTL, 2Da = *QTLAPR.2Da*, 2Db = *QTLAPR.2Db*, 2B = *QTLAPR.2B* and 7B = *QTLAPR.7B*. Error bars represent standard error.

Previous work by Luke Jagger within the group of my supervisor had identified a yellow rust APR QTL from the cultivar Alcedo at the same location as *QTLAPR.2Da* (Jagger, 2009). However, unlike in this study this QTL from Alcedo alone conferred complete immunity. Thirty seven DH lines contained *QTLAPR.2Da* in varying combinations with the other three QTLs identified. These 37 DH lines divided into 7 genotypes, based upon the presence of the other three significant QTLs detected. These genotypic groups were subdivided into two categories, based upon phenotype, lines which had complete resistance and lines which exhibited partial resistance. Eighteen of the *QTLAPR.2Da* containing DH lines had a partial yellow rust resistance phenotype, while 19 were fully resistant. It was subsequently hypothesized that a suppressor of resistance could be segregating within the DH population which was specifically affecting the resistance associated with *QTLAPR.2Da*.

To compare between the two phenotypic groups the mean PI and IT score was calculated (Figures 6.4 and 6.5).When *QTLAPR.2Da* is present with the hypothesized suppressor the mean PI values obtained across score data was 41.1 % (Figure 6.4), with the mean IT score being 0.5 (Figure 6.5). No DH line contained *QTLAPR.2Da* minus the hypothesized suppressor. When *QTLAPR.2Db* is present with *QTLAPR.2Da* in the presence of the hypothesized suppressor the mean PI is 20.6% with an IT of 0.4, whilst without the suppressor the mean PI is 0.31% with an IT of 0.15. The same pattern merges when *QTLAPR.2Da* is present with the minor QTLs, in the presence of the hypothesized suppressor a higher PI and IT is seen in comparison to the hypothesized suppressor being absent.

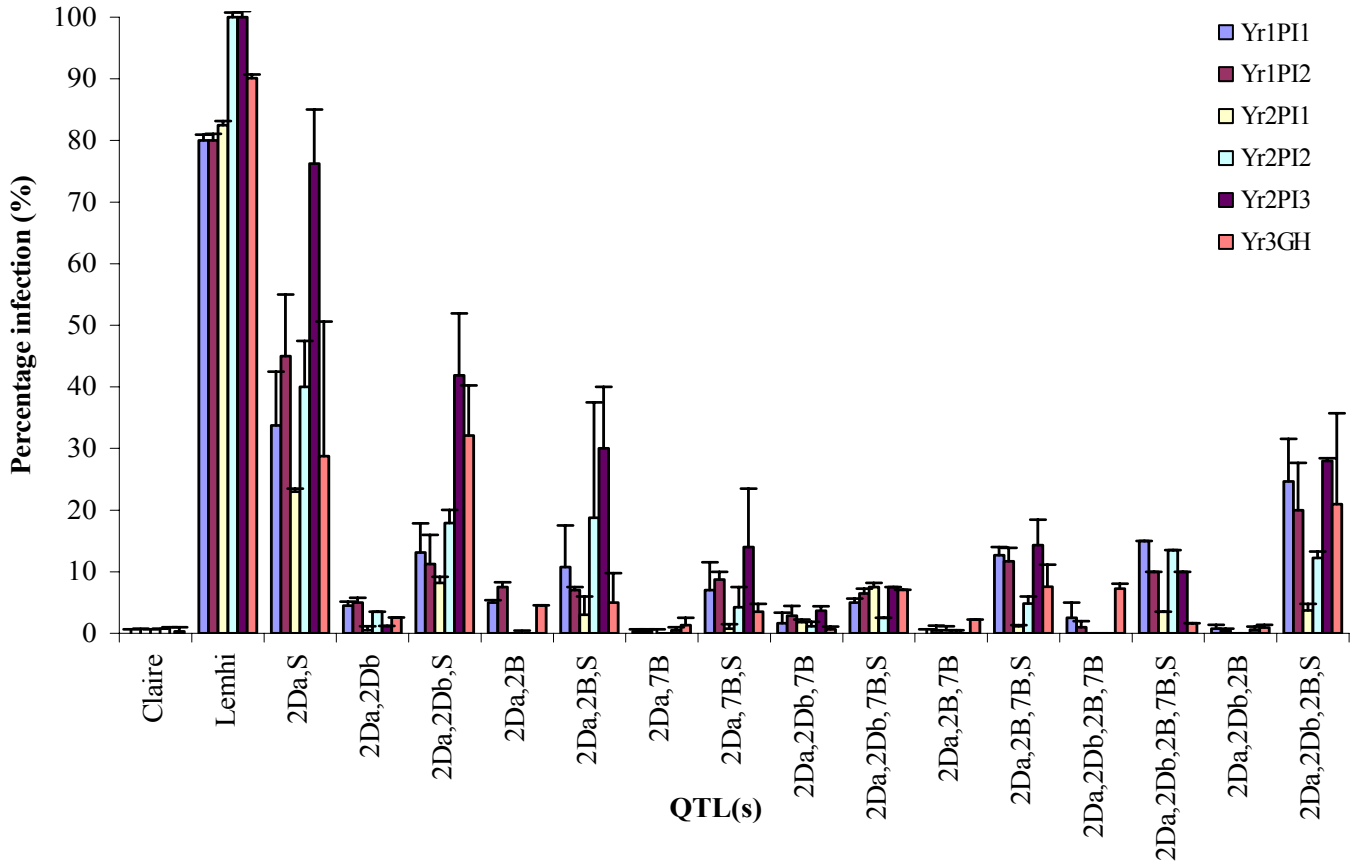


Figure 6.4 Mean percentage infection (PI) for the DH lines grouped according to genotype and then phenotype. The X axis identifies which QTL are present: none = no QTL, 2Da = *QTLAPR.2Da*, 2Db = *QTLAPR.2Db*, 2B = *QTLAPR.2B,* 7B = *QTLAPR.7B,* S = hypothesized suppressor. No DH line with genotype *QTLAPR.2Da* minus hypothesized suppressor. Error bars represent standard error.

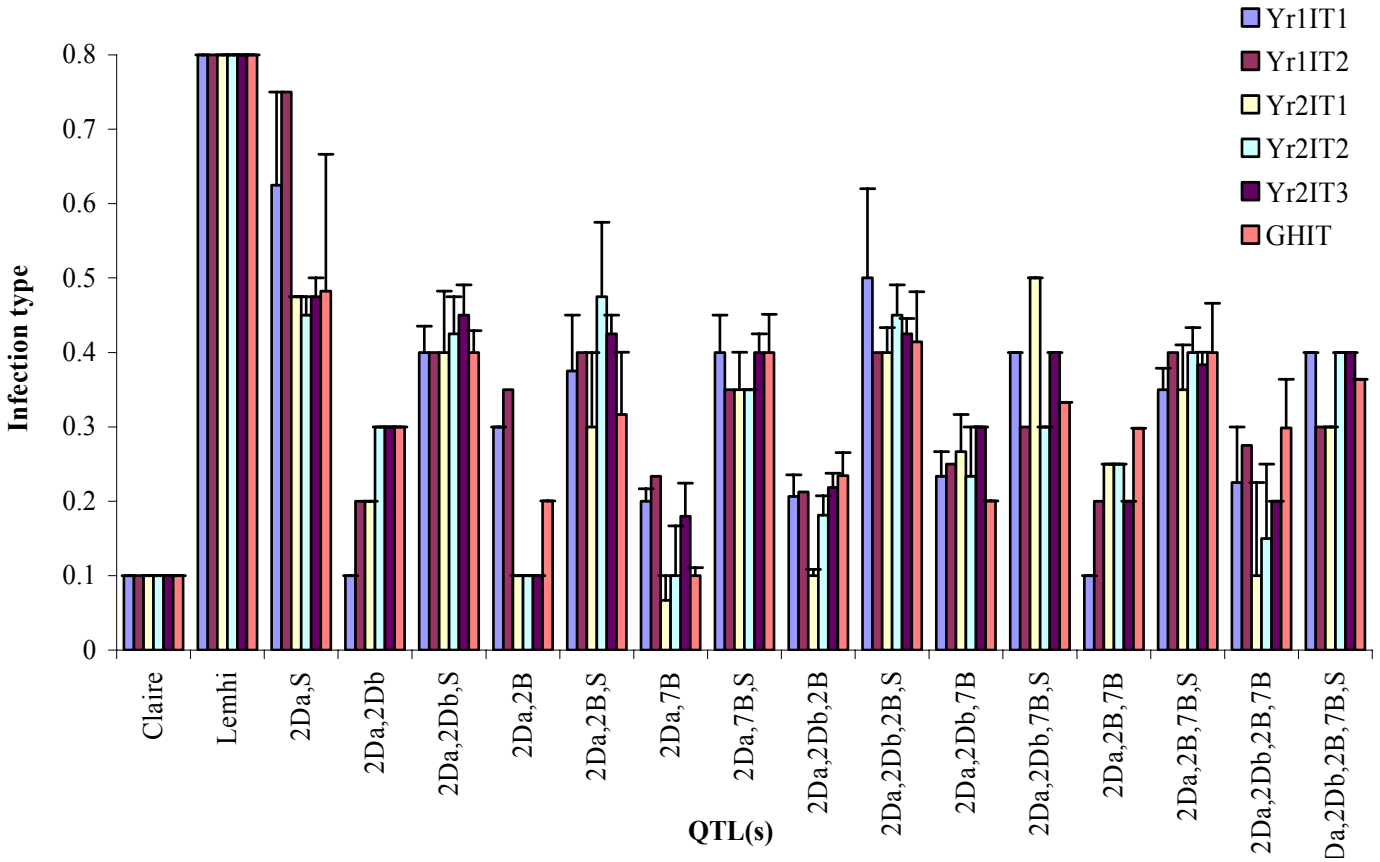


Figure 6.5 Mean infection type nominal (IT) for the DH lines grouped according to genotype and then phenotype. The X axis identifies which QTL are present: none = no QTL, 2Da = *QTLAPR.2Da*, 2Db = *QTLAPR.2Db*, 2B = *QTLAPR.2B,* 7B = *QTLAPR.7B,* S = hypothesized suppressor. No DH line with genotype *QTLAPR.2Da* minus hypothesized suppressor. Error bars represent standard error.

6.3.2 Flag leaf data

Under glasshouse conditions the first four flag leaves were scored separately for PI. Kruskall and Wallis analysis of the phenotypic data against the marker data identified regions on chromosome 2B and 2D to be highly associated with the yellow rust APR, originating from the resistant parent Claire (Table 6.12).

Table 6.12 Kruskall and Wallis analysis of significant marker associations with APR detected in the Claire x Lemhi DH population for first to fourth flag leaf for percentage infection (PI) phenotypes calculated in MapQTL v 5.0 (Van Ooijen, 2004).

Chromosome[1]	Locus[2]	Parent[3]	Percentage Infection (PI)[4]			
			Flag 1	Flag 2	Flag 3	Flag 4
2B	*wPt-9190*	Claire	0.005	0.005	ns	ns
2B	*wPt-7200*	Claire	0.05	0.05	0.05	ns
2B	*wPt-0950*	Claire	0.01	0.05	ns	ns
2B	*Xwmc175*	Claire	0.05	0.05	ns	ns
2Da	*Xwmc167*	Claire	0.005	0.05	ns	ns
2Da	*Xwmc817a*	Claire	0.01	0.05	ns	ns
2Da	*EST18a*	Claire	0.005	0.01	0.05	ns
2Da	*Xwmc311a*	Claire	0.005	0.05	0.05	ns
2Da	*wPt-7408*	Claire	0.005	0.005	0.01	0.05
2Da	*S15M32-155*	Claire	0.005	0.01	0.05	0.05
2Da	*Xwmc301a*	Claire	0.05	0.05	0.05	ns
2Db	*EST6*	Claire	0.005	0.01	0.05	0.05
2Db	*wPt-2781*	Claire	0.005	0.005	0.01	0.05
2Db	*Xwmc181*	Claire	0.05	0.05	0.01	0.05
2Db	*EST22*	Claire	<0.001	<0.001	<0.001	<0.001
2Db	*Xgwm539*	Claire	<0.001	<0.001	0.05	0.05
2Db	*wPt-4413*	Claire	<0.001	0.005	0.005	0.01

[1]Chromosome containing the significant loci, [2]marker loci associated with the significant QTL, [3]parental allele associated with the APR, ns = not significant.

IM and MQM mapping found the same seven markers associated with *QTLAPR.2Da*, three markers associated with *QTLAPR.2Db* and four markers associated with *QTLAPR.2B* were identified with the resistant yellow rust phenotype on the first four flag leaves under glasshouse conditions (Table 6.13). The same co-factors as described in Section 6.3.1 were selected for MQM mapping, but the co-factors had no effect on the QTL associated with these marker loci. Three QTLs

(*QTLAPR.2Da* (Figure 6.6a) *QTLAPR.2Db* (Figure 6.6b) and *QTLAPR.2B* (Figure 6.6c) were found to be highly associated with the yellow rust resistance following MQM. Out of the three identified QTLs *QTLAPR.2B* had the highest level of explained phenotypic variance between flag leaves, ranging from 2.3% on the fourth flag leaf to 19.5% on the first. The phenotypic variation explained by *QTLAPR.2Da* ranged from 11.7% to 18.1%, while *QTLAPR.2Db* explained the highest levels of phenotypic variation, ranging from 13.9% to 28.7%.

Table 6.13 Yellow rust resistance QTL detected using percentage infection (PI) data for 1st, 2nd, 3rd and 4th flag leaves.

Analysis	1st flag leaf							2nd flag leaf				
	Chromosome[1]	Parent[2]	Locus[3]	LOD threshold[4]	LOD[5]	Expl. % var.[6]	Phenotypic means[7] C	L	LOD threshold	LOD	Expl. % var.	Phenotypic means C	L
IM	2B	Claire	*wPt-9190*	0.9	2.9	19.5	0.2	1.6	0.8	2.1	13.8	0.2	1.9
	2Da	Claire	*S15M32-155*	1.3	1.9	11.7	0.3	1.6	1.2	2.6	16.3	0.3	2.4
	2Db	Claire	*EST22*	0.7	5.1	34.1	0.2	2.7	1.3	5.4	32.4	0.2	4.1
MQM	2B	Claire	*wPt-9190*	0.9	2.9	19.5	0.2	1.6	0.8	2.2	13.8	0.2	1.9
	2Da	Claire	*S15M32-155*	1.3	3.0	18.1	0.3	1.6	1.2	2.6	15.6	0.3	2.4
	2Db	Claire	*EST22*	1.3	4.6	28.7	0.2	2.4	1.3	5.4	25.4	0.2	3.7

Analysis	3rd flag leaf							4th flag leaf				
	Chromosome[1]	Parent[2]	Locus[3]	LOD threshold[4]	LOD[5]	Expl. % var.[6]	Phenotypic means[7] C	L	LOD threshold	LOD	Expl. % var.	Phenotypic means C	L
IM	2B	Claire	*wPt-9190*	0.8	2	12.6	0.3	1.9	0.9	*	2.3	0.6	1.4
	2Da	Claire	*S15M32-155*	1.2	2.7	16.8	0.3	2.7	1.3	3.0	18.1	0.3	2.7
	2Db	Claire	*EST22*	1.2	4.0	27.0	0.2	4.1	1.3	2.9	19.7	0.3	3.3
MQM	2B	Claire	*wPt-9190*	0.8	2.0	12.6	0.3	1.9	0.9	*	2.3	0.6	1.4
	2Da	Claire	*S15M32-155*	1.2	2.6	15.6	0.3	2.4	1.3	1.9	11.7	0.3	2.7
	2Db	Claire	*EST22*	1.2	4.0	19.4	0.2	3.7	1.3	2.9	13.9	0.4	3.1

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4] LOD threshold based on a p-value of 0.05. Genome wide LOD threshold 1st Flag = 2.7, 2nd Flag = 2.7, 3rd Flag = 2.8, 4th Flag = 2.7, [5]maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi, * = LOD value of QTL did not exceed LOD threshold for the linkage group.

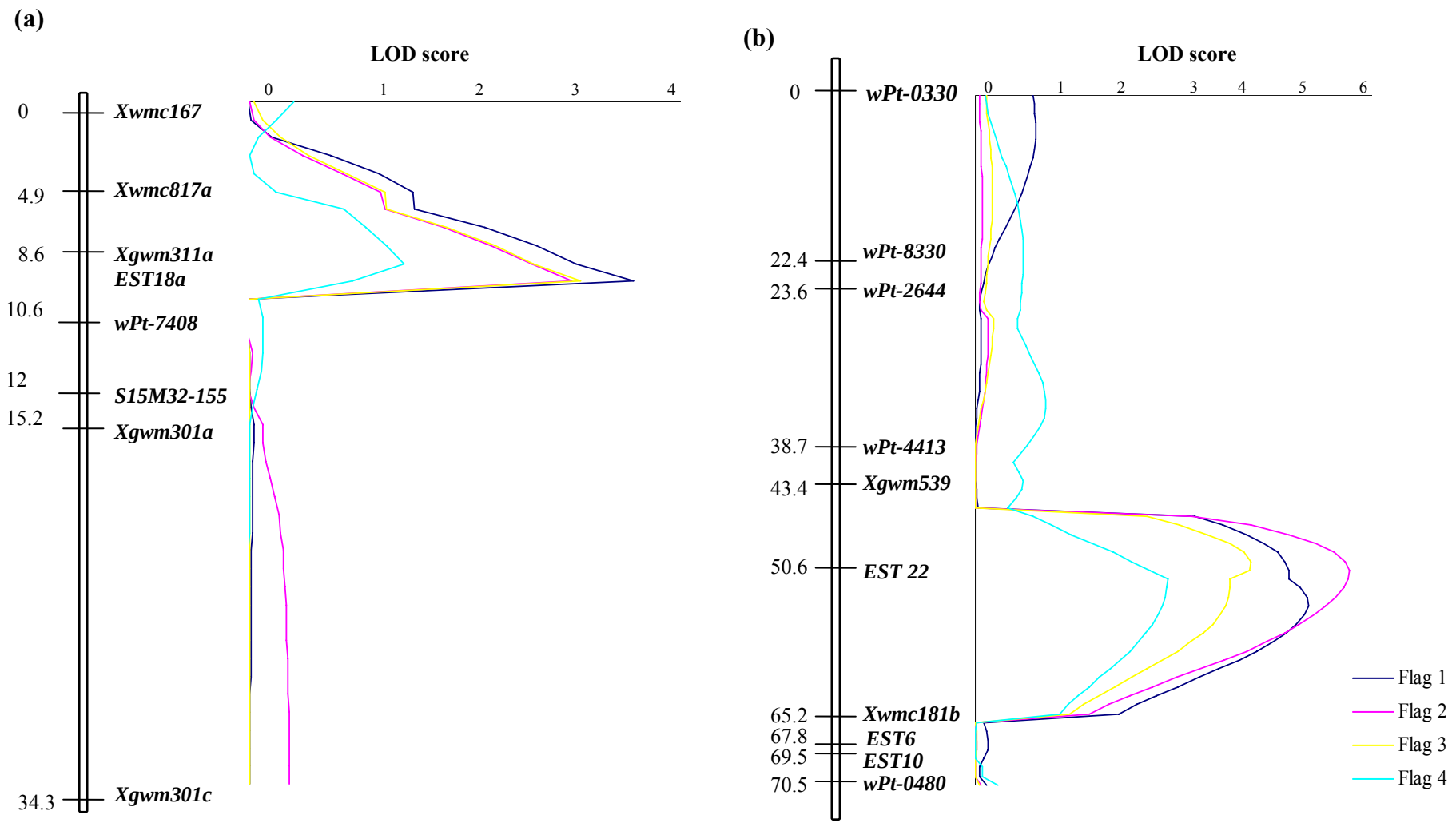


Figure 6.6 (a) *QTLAPR.2Da* **(b)** *QTLAPR.2Db* obtained from MQM mapping using cofactors as described in Section 6.3.1 for percentage infection (PI) flag leaf data set from the glasshouse trial. The corresponding linkage group is on the left, with the relevant marker loci and the map distances in cM (Kosambi).

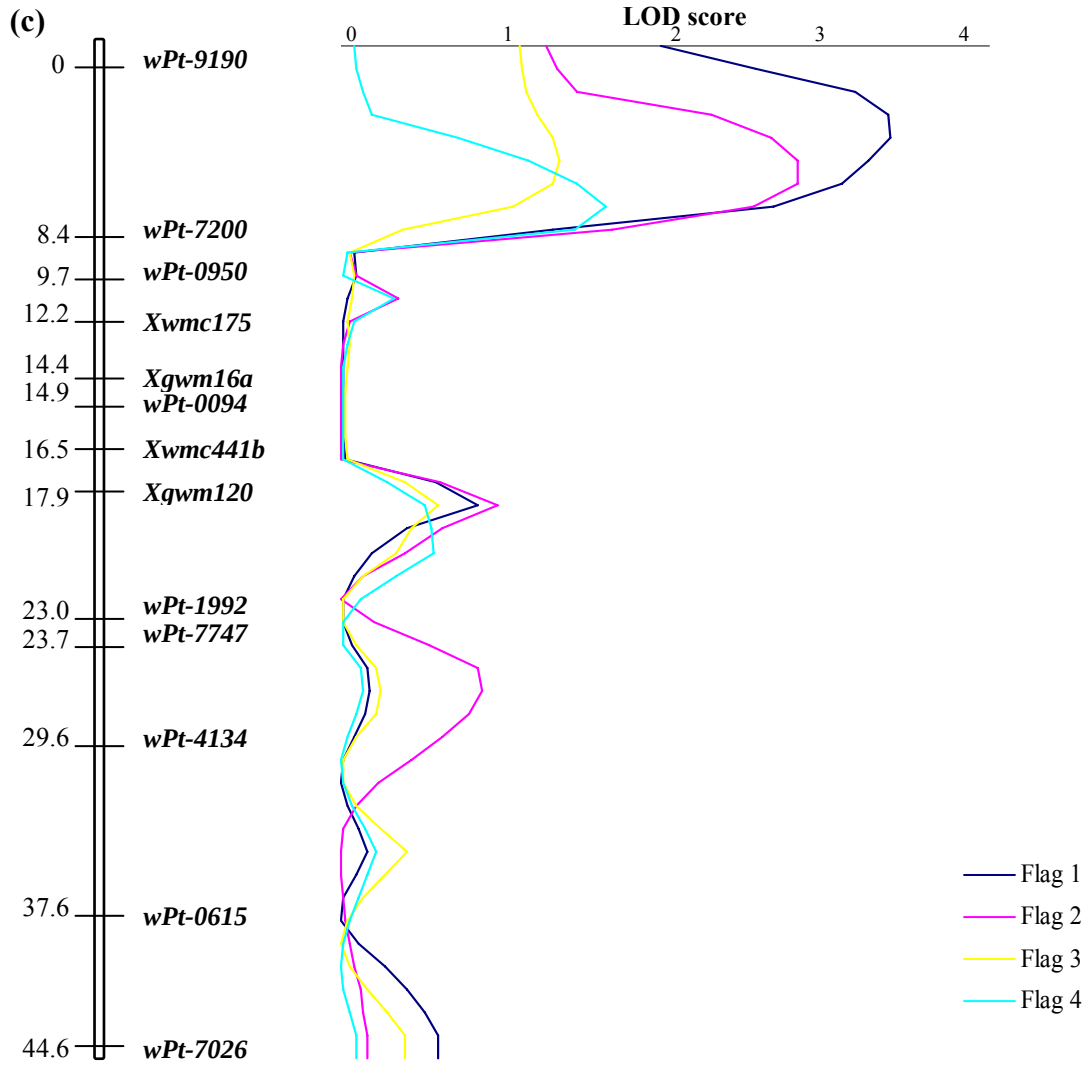


Figure 6.6 (c) *QTLAPR.2B* obtained from MQM mapping using cofactors as described in Section 6.3.1 for percentage infection (PI) flag leaf data set from the glasshouse trial. The corresponding linkage group is on the left with the relevant marker loci and the map distances in cM (Kosambi).

6.3.3 Height

Claire carries the semi dwarfing gene *Rht2*, whilst Lemhi has a tall phenotype. In order to map the *Rht2* gene segregating within the DH population and to observe whether it has any association with yellow rust resistance, plant height of all three replicates within the glasshouse trial was measured. Kruskall and Wallis analysis of the height data set identified a region on chromosome 4DL (*QTLRht.4D*) associated with the semi-dwarfing gene segregating within the population, originating from the

resistant parent Claire (Table 6.14). Following IM the LOD score was 4.44 (Table 6.15). Permutation tests gave genome wide LOD thresholds of 1.0 at a 5% significance level. The DArT marker *wPt-0710* was chosen as a cofactor which was associated with *QTLRht.4D*, the choice of co-factor was confirmed using the backward elimination package of the automatic cofactor selector in MapQTL. Following MQM analysis the LOD score increased to 21.1, with *QTLRht.4D* explaining 75.4% of the phenotypic variation (Figure 6.7).

Table 6.14 Kruskall and Wallis analysis of significant marker loci associated with semi dwarfing genes detected in the Claire x Lemhi DH population from glasshouse trial calculated in MapQTL v 5.0 (Van Ooijen, 2004).

Chromosome[1]	Locus[2]	Parent[3]	Height
4D	*Xwmc457*	Claire	<0.001
4D	*wPt-0431*	Claire	<0.001
4D	*wPt-0710*	Claire	<0.001
4D	*wPt-0472*	Claire	<0.001
4D	*wPt-0877*	Claire	<0.001
4D	*wPt-8836*	Claire	<0.001

[1]Chromosome containing the significant loci, [2]marker loci associated with the significant QTL, [3]parental allele associated with the APR.

Table 6.15 Semi-dwarfing QTL detected in the Claire x Lemhi cross using plant height data sets from year 3.

Analysis	Chromosome[1]	Parent[2]	Locus[3]	LOD threshold[4]	LOD[5]	Expl % var.[6]	Phenotypic[7] means	
							C	L
IM	4D	Claire	*wPt-0710*	1.0	4.44	39.2	87.6	98.7
MQM	4D	Claire	*wPt-0710*	1.0	21.1	75.4	54.7	93.7

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi.

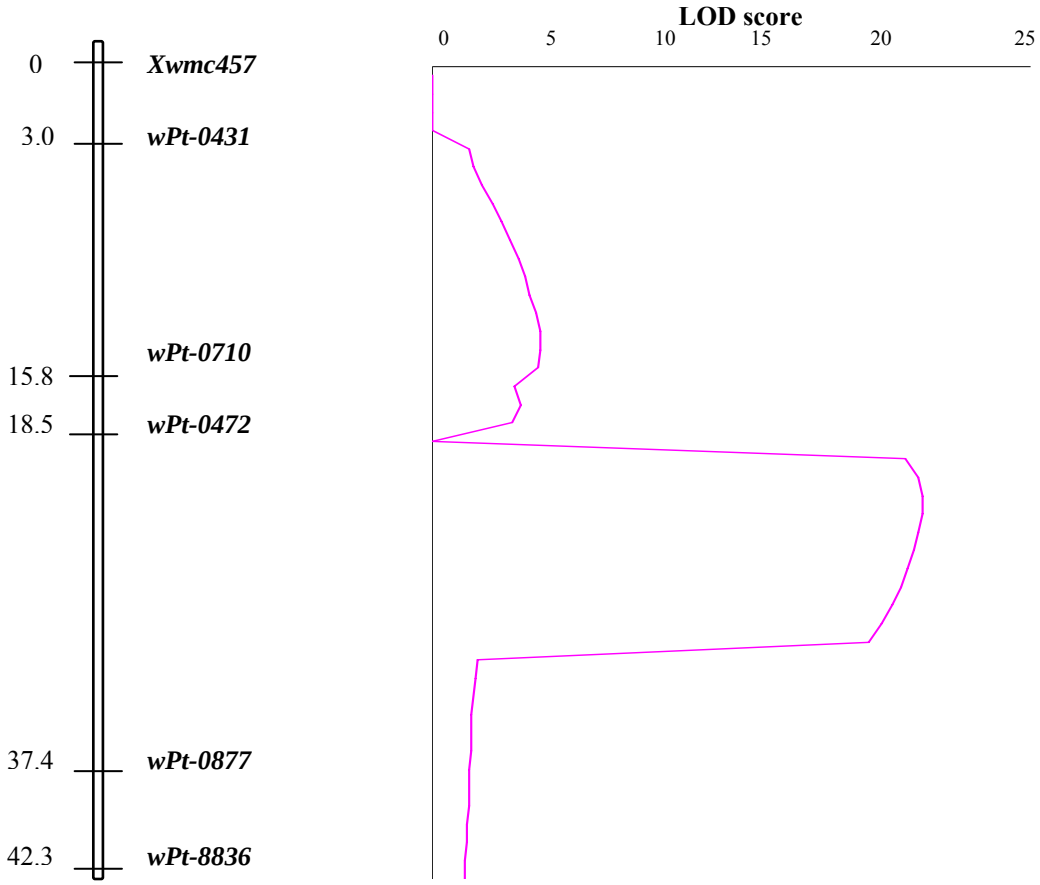


Figure 6.7 QTL plots obtained from MQM mapping using the height data set from the glasshouse trial. The corresponding linkage group is on the left with the relevant marker loci and the map distances in cM (Kosambi).

To examine whether *Rht2* has any association with yellow rust resistance plant height was compared against PI and IT for each DH line. No correlation between the plant height and resistance or susceptibility to wheat yellow rust was observed for PI or IT scores (Table 6.16).

Table 6.16 Correlation coefficient (R^2) between height and percentage infection (PI) either infection type (IT) under glasshouse conditions.

Phenotypic score	R^2
PI	0.014
IT	0.39

6.3.4 Seedling resistance

Seedling phenotypic data sets for the wheat yellow rust isolates, WYR76-10 (virulent on *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr8* and *Yr17*) and WYR68-2 (virulent on *Yr1*, *Yr2*, *Yr3*, *Yr6* and *Yr7*) identified different regions of the genome associated with seedling resistance. Kruskall and Wallis analysis using the WYR 76-10 data set identified a region on chromosome 3B to be highly associated with seedling resistance, originating from the resistant parent Claire (Table 6.17). IM confirmed the QTL location which was designated *QTLSR.3B* (Figure 6.8), the LOD score was 99.9, with *QTLSR.3B* explaining 100% of the phenotypic variation (Table 6.18a).

Table 6.17 Kruskall and Wallis analysis of significant QTL associated with seedling resistance detected in the Claire x Lemhi DH population from glasshouse trial calculated in MapQTL v 5.0 (Van Ooijen, 2004).

Chromosome[1]	Locus[2]	Parent[3]	Isolate	
			WYR 76-10	WYR 68 -2
3B	*wPt-8845*	Claire	<0.001	ns
3B	*wPt-4412*	Claire	<0.001	ns
3B	*wPt-1311*	Claire	<0.001	ns
3B	*wPt-2416*	Claire	<0.001	ns
3B	*wPt-9368*	Claire	0.005	ns
4A	*wPt-9406*	Claire	ns	0.01
4B	*Xgwm368*	Claire	ns	0.01
7A	*wPt-5257*	Claire	ns	0.005
7A	*wPt-3901*	Claire	ns	0.01

[1]Chromosome containing the significant loci, [2]marker loci associated with the significant QTL, [3]parental allele associated with the APR, ns = not significant.

Kruskall and Wallis analysis using the WYR 68-2 data set identified three regions on chromosomes 4A, 4B and 7A to be highly associated with seedling resistance, all originating from the resistant parent Claire (Table 6.18b). IM confirmed the QTL locations and the QTLs were designated *QTLSR.4A* (Figure 6.9a), *QTLSR.4B* (Figure 6.9b) and *QTLSR.4A* (Figure 6.9c). The DArT marker *wPt-9406* (associated with *QTLSR.4A*), the SSR marker *Xgwm368* (associated with *QTLSR.4B*) and the DArT

marker *wPt-8418* (associated with *QTLSR.7A*) were selected as co-factors in MQM mapping, which were confirmed by the backward elimination package of automatic cofactor selection. The selection as co-factors had no effect on the QTL associated with these marker loci and no minor QTLs were identified.

Table 6.18 Seedling QTL detected in the Claire x Lemhi cross. (a) Isolate WYR 76-10 (b) Isolate WYR 68-2

(a)				**Isolate WYR 76-10**				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl % var.[6]**	**Phenotypic means[7]**	
							C	**L**
IM	3B	Claire	*wPt-4412*	0.9	99.9	100.0	1.0	4.0

(b)				**Isolate WYR 68-2**				
Analysis	**Chromosome[1]**	**Parent[2]**	**Locus[3]**	**LOD threshold[4]**	**LOD[5]**	**Expl % var.[6]**	**Phenotypic means[7]**	
							C	**L**
IM	4A	Claire	*wPt-9406*	1.0	1.2	11.0	2.0	3.0
	4B	Claire	*Xgwm368*	1.1	1.3	10.4	1.9	2.9
	7A	Claire	*wPt-8418*	1.0	1.5	11.0	1.9	2.9
MQM	4A	Claire	*wPt-9406*	1.0	1.2	11.0	2.1	3.0
	4B	Claire	*Xgwm368*	1.1	1.3	10.4	1.9	2.9
	7A	Claire	*wPt-8418*	1.0	1.4	10.5	2.0	2.9

[1]Chromosome location of the QTL, [2]parent the QTL originated from, [3]closest marker to the QTL peak, [4]LOD threshold based on a p-value of 0.05. Genome wide LOD threshold WYR 76-10 = 3.0, WYR 68-2 = 3.0, [5]maximum LOD score associated with the QTL peak, [6]percentage phenotype explained, [7]phenotypic means of each allelic class at marker locus closest to QTL peak. C = cultivar Claire, L = cultivar Lemhi.

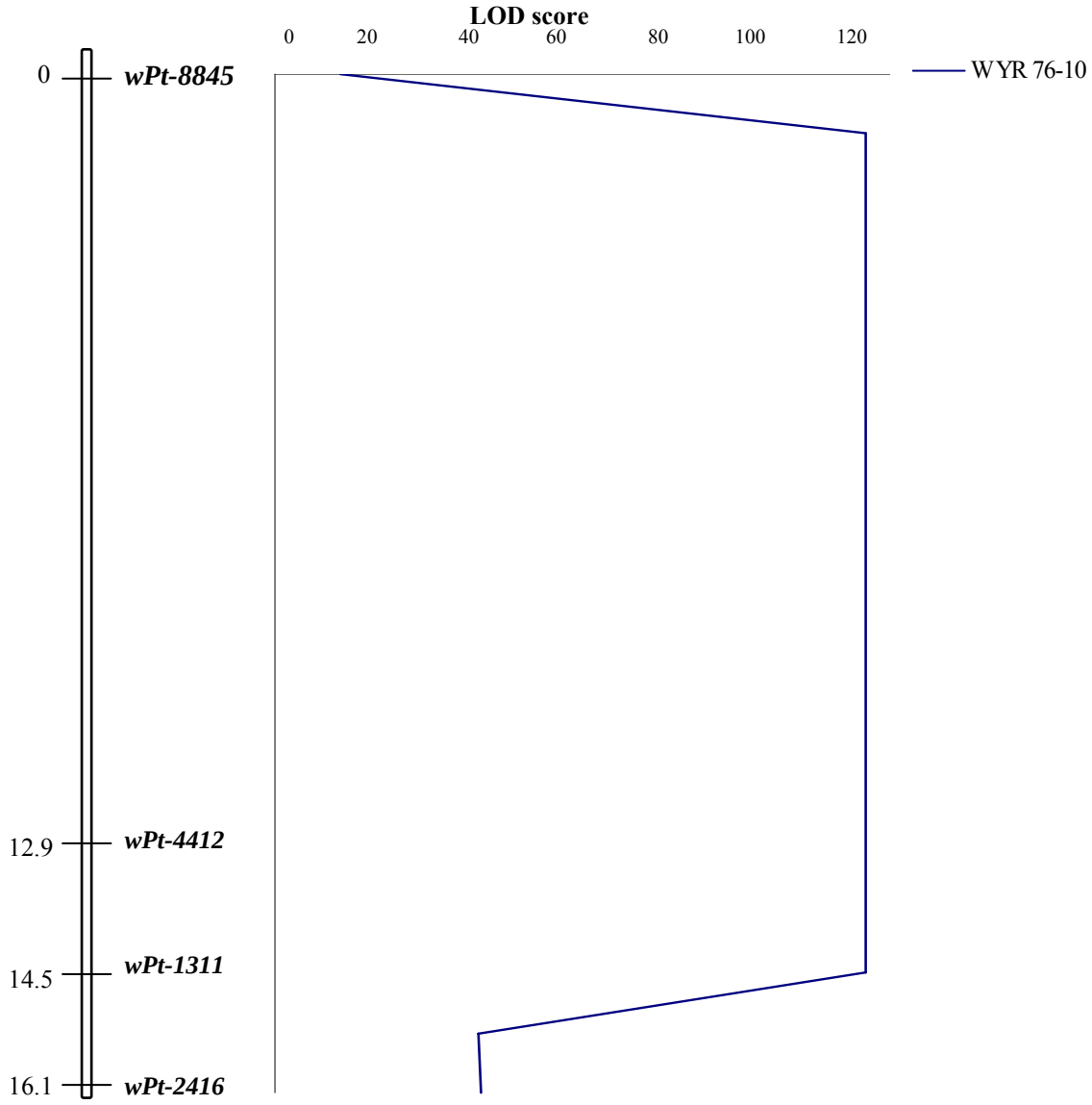


Figure 6.8 *QTLSR.3B* plots obtained from IM mapping using WYR 76-10 seedling data. The corresponding linkage group is on the left with the relevant marker loci and the map distances in cM (Kosambi).

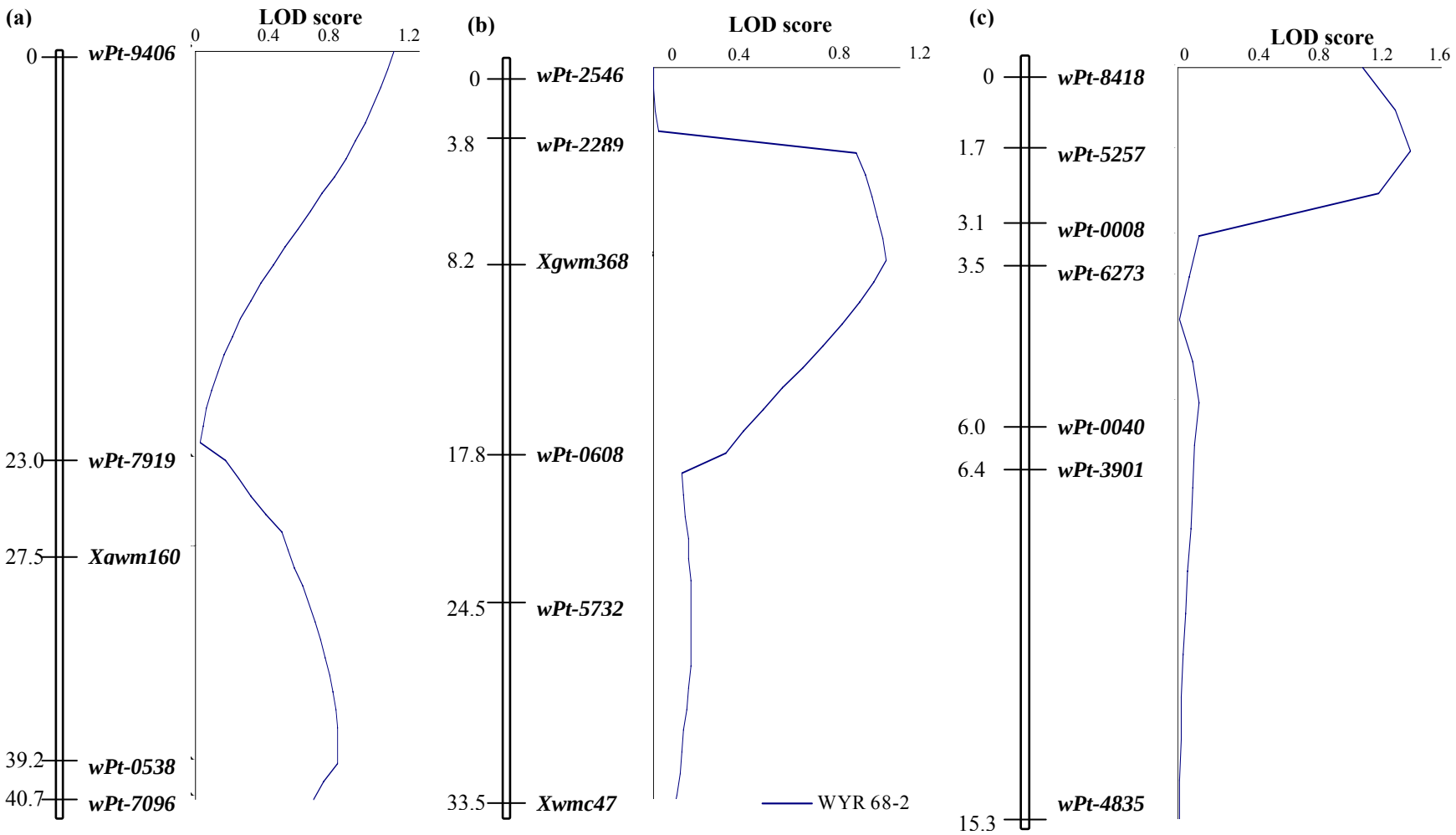


Figure 6.9 QTL plots obtained from MQM mapping using WYR 68-2 isolate seedling data. **(a)** *QTLSR.4A* **(b)** *QTLSR.4B* **(c)** *QTLSR.7A*. The corresponding linkage group is on the left with the relevant marker loci and the map distances in cM (Kosambi).

6.4 Discussion

APR genes with minor, but additive effects on resistance towards yellow rust are common in wheat (Jagger, 2009, Melichar *et al*., 2008, Mallard *et al*., 2005, Suenaga *et al*., 2003, Borner *et al*., 2002 and Boukhatem *et al*., 2002)**.** Due to the polygenic nature of this kind of resistance, APR to yellow rust remains largely uncharacterised. Cultivars that have adequate levels of APR frequently carry at least two or more genes with small to intermediate additive effects on the resistance phenotype (Singh *et al*., 2000, Bariana and McIntosh, 1995). Claire is alleged to have Carsten V within its pedigree (Lewis, 2006). The partial APR found in Carsten V is still effective in the UK and is thought to be present in many European cultivars that show field resistance to yellow rust, potentially a source of durable resistance. The phenotypic distribution of yellow rust PI and IT in the Claire x Lemhi DH population (Section 3.4.1) indicates the quantitative nature of the yellow rust in Claire. Regression analysis and IM using the molecular map information from Chapter 4 and the phenotypic data sets from Chapter 3 confirmed the quantitative nature of the resistance found in Claire. The two kinds of resistance assessment (PI and IT) gave comparable results, detecting QTLs in the same genomic regions, with approximately the same LOD scores and explaining a similar proportion of the phenotypic variance. However, differences were observed across the three years of data. Two years of field data and one year of glasshouse data indicated that the yellow rust APR is conferred by two major QTL, both located on chromosome 2D, together with two minor QTL located on chromosomes 2B and 7B, all originating from the resistant parent Claire. These results are in agreement with previous studies on the inheritance of APR, indicating that a few additive genes can confer complete APR to yellow rust (Rosewarne *et al*., 2008, Ramburan *et al*., 2004, Bariana *et al*., 2001).

The SSR marker locus *Xgwm301* maps to the long arm of chromosome 2D in the Alcedo x Brigadier genetic map and is associated with a QTL for yellow rust APR known to give complete resistant to the disease (Jagger, 2009). The same SSR marker is associated with the major QTL located to chromosome 2D (*QTLAPR.2Da*). Based on the association between marker locus *Xgwm301* and *QTLAPR.2Da* and the knowledge

that the wheat cultivar Alcedo is in the pedigree of Claire (Figure 1.7) it is likely that *QTLAPR.2Da* originates from Alcedo. However, unlike the QTL associated with marker locus *Xgwm301* on chromosome 2D from Alcedo that conferred a completely resistant phenotype, a varying degree of yellow rust invasion was seen on the Claire x Lemhi DH lines containing *QTLAPR.2Da*. A gene that suppresses the resistant phenotype conferred by *QTLAPR.2Da* could be segregating within the DH population, which when present is preventing this QTL from providing complete resistance. The hypothesized suppressor is investigated and discussed in Chapter 7.

Two major yellow rust resistance QTLs, arising from different sources, have been identified on chromosome 2BL in Camp Remy and Opata 85 which lie between the markers *Xgwm501* and *Xgwm47* (Boukhatem *et al*., 2002). From reference maps *Xgwm47* is 5.2 cM from *Xgwm120* on chromosome arm 2BL (http://wheat.pw.usda.gov/cgi-bin/graingenes/quickquery.cgi?query=nearbyloci&arg1=Xgwm120-2B&arg2=10) and *Xgwm120* is 11.7 cM away from *QTLAPR.2B*. The marker *Xgwm501* lies about 23 cM away from the centromere of chromosome 2BL (Boukhatem *et al*., 2002). A cluster of three race-specific resistance genes (*Yr5*, *Yr7* and the stem rust resistance gene *Sr9*) have been mapped 15 cM away from the centromere of chromosome 2BL (Hart *et al*., 1993). Camp Remy carries *Yr7* (De Vallavieille-Pope *et al*., 1990), which has been shown to be allelic to *Yr5* (Zhang *et al*., 2009), and is known to be in the Claire pedigree. Disease resistance genes are not randomly distributed in plant genomes and frequently occur in clusters (Islam *et al*., 1989). In wheat, complexes that confer resistance to more than one rust disease are not uncommon; *Lr46*/*Yr29* (Singh *et al*., 2003) and *Sr2*/*Lr27*/*Yr30* (Spielmeyer *et al*., 2003). This strongly suggests that the resistance QTL located on 2BL could be part of the *Yr5*, *Yr7* and *Sr9* cluster, although an adult expressed gene, rather than the seedling resistance shown by *Yr5*, *Yr7* and *Sr9*.

The French cultivar Camp Rémy is believed to have inherited several yellow rust APR genes from Cappelle-Deprez which has been extensively used for yellow rust breeding in Europe (Angus, 2001), and is thought to be the source of the durable yellow rust

resistance in Camp Rémy (Mallard *et al*., 2005). Claire shares similar ancestry to this French cultivar, with Cappelle-Deprez also being in Claire's pedigree. The partial APR gene *Yr16* maps to chromosome 2D and is responsible for part of the yellow rust resistance in Cappelle-Deprez. *Yr16* maps 5.7cM away from the SSR marker *Xgwm539* (Worland, unpublished) in Cappelle-Deprez. Camp Rémy also has a yellow rust resistance QTL on chromosome 2D that is believed to be *Yr16* inherited from Cappelle-Desprez (Mallard *et al*., 2005). The map location of *QTLAPR.2Db* and the pedigree of Claire strongly suggest that *QTLAPR.2Db* is *Yr16*.

Several factors affect the accuracy of the estimation of QTL positions and significance, including the population size and marker coverage. For reproducible QTL mapping in wheat the optimum marker density is one marker every 10 cM (Hyne *et al*., 1995). When marker density is reduced to <10 markers per linkage group QTL resolution is compromised (Hyne *et al*., 1995). The number of individuals in a mapping population also has an effect on the quality of the genetic map (as discussed in Chapter 4) and subsequent QTL detection. By increasing the 77 DH individuals to 200 DH individuals QTL resolution would improve (Hyne *et al*., 1995). Rodrigues *et al.* (2004) used a wheat genetic map with an average of 6.4 markers per linkage group (over 18 linkage groups) to successfully map two major and two minor QTLs involved in non host resistance in wheat against barley yellow rust (*Puccinia striiformis* f. sp. *hordei*). The Claire x Lemhi mapping population used in this study had a fairly small population size of 77 DH individuals. However, the genetic map on average had 10 markers per linkage group (over 49 groups) which helps to compensate for the small population size. Several measures can be taken to reduce the confidence interval, including increased replications, increasing the number of molecular markers to improve map saturation and increasing the number of individuals in the population (Kearsey, 1998). The genetic map did however have adequate genome coverage to enable the detection of *Rht2* and the major seedling resistance gene *QTLSR.3B*, both with a corresponding high explained variance.

The genetic properties of QTLs can influence the precision of mapping. If two QTL are closely linked at a genetic distance of 20 cM or less they are likely to be detected as single QTL (Tanksley, 1993), or their effects are combined to give 'ghost' QTL which map between them. If one QTL contributes to the resistance phenotype and the other subtracts from the resistance phenotype the effects of each QTL will be cancelled out by the other (Kearsey, 1998). QTL detection can also be affected by the proportion of their explained phenotypic variation. Minor QTL, explaining less than 10% of the phenotypic variation often remain undetected as the magnitude of their effect falls below the significance threshold (Collard *et al*., 2005).

QTLAPR.2Da and *QTLAPR.2Db* had a stronger effect on both disease reaction phenotypes (PI and IT) in year 1 and year 3 compared to year 2. However, *QTLAPR.2B* had a stronger effect on the disease reaction phenotypes in year 2 with a minimal effect in year 1 and year 3. This indicates that the yellow rust resistance in adult plants grown under different environmental conditions is compromised, indicating the sensitivity of the APR genes to environmental conditions. It has been widely reported that the effects of some resistance genes may not be stable across different environments (Lin and Chen, 2009, Rosewarne *et al*., 2008, Suenaga *et al*., 2003). Boukhatem *et al*. (2002) reported that QTLs that contribute less than 10% of the phenotypic variance are difficult to detect across years and environments. *QTLAPR.2B* explained less than 10% of the phenotypic PI and IT variance in year 1, with no significance in year 3. *QTLAPR.2Da* and *QTLAPR.2Db* explained a high level of PI and IT in year 1 and 3, therefore the minor effect coming from *QTLAPR.2B* may have been difficult to detect, being masked by the two main QTL. In the second year field trial the two main QTL explained less of the phenotypic variance than compared with year 1 and 3. This could have been due to a rapid onset of yellow rust within the field in year 2, such that at the time of scoring these two resistance QTL may not have been effective, resulting in the minor QTL found on chromosome 2B having a stronger effect on the phenotype. Environmental variation was also observed in the Opata 85 x synthetic reference population studied in Belgium (Boukhatem *et al*., 2002), Germany (Borner *et al*., 2002) and Mexico (Singh *et al*., 2000). Eight chromosomal locations were found to be associated with yellow rust

resistance, but no single QTL was detected in common, at all three locations. The lack of consistency highlights the environmental influence on QTL detection. The discrepancy found across the three locations could be attributed to the pathogen isolates present at each location having different virulence profiles. Although in this study the same mixture of virulent isolates were used across the three years of datasets. External sources of different virulent isolates migrating into the field trials from the environment can not be excluded. Small effect QTL can be detected across different environments as demonstrated by Lin and Chen (2009) who were able to detect two QTL across different environments even though they explained only 5% of the phenotypic variance. *QTLAPR.7B* was detected across all three environments, explaining only 2.9% to 12.3% of the phenotypic variance across the three years of data.

Flag leaf PI data detected *QTLAPR.2Da*, *QTLAPR.2Db* and *QTLAPR.2B* located in the same genomic regions. Despite no significant differences found within the four flag leaves of each DH line (Section 3.4.2.3). All three QTLs had varying LOD scores and explained proportion of phenotypic variance within the four flag leaves. The highest level of explained phenotypic variation observed for *QTLAPR.2B* was on the first flag leaf, the fourth flag leaf explaining the lowest. This high degree of variation across flag leaves may explain why *QTLAPR.2B* was not detected in year 3 whole plant glasshouse data. The stronger resistant phenotype displayed by the first flag leaf may indicate that the expression of resistance conferred by *QTLAPR.2B* is only more effective in older plant tissues. *QTLAPR.2Da* and *QTLAPR.2Db* also showed a decrease in phenotypic variance from flag leaves 1 to 4, however the levels of explained phenotypic variance were similar to that found with the whole plant PI dataset. These results suggest that older flag leaves express higher levels of yellow rust resistance.

Claire carries both adult plant and seedling expressed yellow rust resistance genes, although in the UK all the seedling resistance genes have been overcome. Seedling inoculations using *P. striiformis* f. sp. *tritici* isolates with known virulence profiles previously suggested that the seedling resistance of Claire was conferred by at least one gene, *Yr1*, inherited from the cultivar Galahad (Paul Fenwick, personal communication,

Advanta-Nickerson Seeds Ltd). Lewis (2006) found Galahad to posses *Yr1* but Claire was postulated to contain *Yr2*, *Yr3*, *Yr4*, *Yr25*, *YrHVII* and another unknown seedling resistance gene, not *Yr1*. It is not unexpected that Claire could contain *Yr2*, *Yr3* and *Yr4* as these seedling resistance genes are common in many European wheat cultivars (Stubbs, 1985). Carsten V is thought to be the origin of some of the seedling and APR genes found in Cappelle-Deprez (Boukhatem *et al*., 2002) and both these cultivars are in the Claire pedigree. Calonnec *et al*., (2002) reported Carstens V to carry two genes for seedling resistance, most probably *Yr25* and *Yr32* (Eriksen *et al*., 2004). Lewis (2006) found evidence indicating that Carsten V carries *Yr3*, *Yr4*, *Yr25*, *Yr32*, *YrCV* and *YrSd*, all possible sources of the seedling resistance found in Claire.

A significant QTL, explaining 100% of the phenotypic variance associated with resistance to isolate WYR 76-10 (virulent on *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr8* and *Yr17*) was identified on chromosome 3B. *Yr4* has been located to chromosome 3B (Worland, 1988), however this gene could not be that identified by WYR 76-10 as this isolate is virulent against *Yr4*. Isolate WYR 68-2 identified three significant QTL on chromosomes 4AL, 4BS and 7B. *QTLSR.4A* could be *YrHVII* postulated to be present in Claire and supported by isolate WYR 68-2 not being virulent against this particular resistance gene. It has been reported that seedling expressed, race-specific resistance genes may confer residual resistance in adult plants. Singh *et al*. (2002) identified a seedling yellow rust resistance gene *Yr28* on chromosome 4DS that also contributed to HTAP resistance. The seedling resistance genes identified here did not have any residual resistance in adult plants, the seedling and APR QTLs locating to different positions in the wheat genome. Therefore the two types of resistance must be controlled by different QTL.

No association was found between plant height and yellow rust severity. Wagoire *et al*. (1998) reported that plants showing resistance to yellow rust from an 8 x 8 diallel cross of eight different bread wheat varieties exhibiting a broad set of responses to the fungus were significantly taller than their susceptible counterparts. However, no genetic basis for this association was discovered. Jafary *et al*. (2008) found that non host resistance to

non-adapted rusts was not associated with height in barley. The lack of association between plant height and yellow rust infection is not unexpected, given that its dispersal is air borne.

Claire exhibits a quantitative APR controlled by a number of loci, which act additively to achieve a resistance that has remained effective since the release of Claire in 1999. The results in this chapter confirm two main QTL on chromosome 2D and two minor QTL on chromosomes 2B and 7B that contribute to the APR in Claire. Analysis of both phenotypic traits associated with resistance, PI and IT, identified the same QTLs suggesting that there is a link between the resistance mechanisms that underlie each phenotype.

Chapter 7

Genetic analysis and transcription profiling of the hypothesized suppressor phenotype associated with *QTLAPR.2Da*.

7.1 Introduction

APR genes in general confer only a partial resistant phenotype (Boyd *et al*., 2006, 2005). A disease free phenotype is however conferred by an APR QTL from the cultivar Alcedo, present on the long arm of chromosome 2D. The complete immunity conferred by this Alcedo yellow rust APR QTL makes it different to most previously reported wheat APR genes (Jagger, 2009). Alcedo is known to be in Claire's pedigree (Simon Berry, personal communication). The markers defining the Alcedo 2D APR QTL indicate that it co-locates with the *QTLAPR.2Da* in Claire, suggesting that Claire may carry the Alcedo 2D QTL. Jagger (2009) conducted a microscopy study to elucidate the mechanisms involved in the yellow rust resistance conferred by the Alcedo 2D QTL. The fungus never developed past the formation of the initial infection site, with primary cell death visible as early as 36 hours post inoculation (hpi). However, rather than a completely resistant phenotype, as observed in Alcedo, varying degrees of yellow rust infection were seen on the Claire x Lemhi DH lines containing *QTLAPR.2Da* (Chapter 6). This suggests that gene(s) could be segregating within the population that could be affecting the phenotypic expression of the complete resistance conferred by *QTLAPR.2Da*.

The term suppressor of resistance or susceptibility factor has been used to define genes that appear to favour the establishment of infection (Boyd *et al*., 2006, Kema *et al*., 1995, Ma *et al*., 1995). The resistance phenotype is altered by the interaction of the resistance gene and susceptibility factor, resulting in a suppression of the resistance response. Conversely an increase in pathogen infection may result from the presence of a host susceptibility factor that is required for pathogen development. In extreme cases a gene may completely suppress the effect of a resistance gene (Kema *et al*., 1995). Suppressors have been identified for stem rust resistance in the wheat cultivars Medea (Knott, 2000), Canthatch (Kerber *et al*., 1999, 1995, 1991, 1980) and Chinese Spring (Bai and Knott, 1992) and suppression of yellow rust resistance has been observed following the production of synthetic hexaploid wheat, *T. turgidum* x *T. tauschii* (Ma *et al*., 1995) and *T.turgidum* x *A.squarrosa* (Kema *et al*., 1995).

Successful invasion of a host plant relies on the pathogen overcoming the plants defence mechanisms both passive, preformed physical and chemical barriers limiting pathogen penetration and active defence reactions (Heath, 2000). Jagger (2009) found that the yellow rust APR conferred by Alcedo produced a hypersensitive cell death reaction similar to that observed in race-specific seedling interaction between yellow rust and wheat (Bozkurt *et al*., 2007). The reduced phenotypic expression of *QTLAPR.2Da* in Claire suggests that a suppressor could be affecting the active defence mechanism conferred by this QTL.

In plant-pathogen interactions, gene transcription profiling has provided unprecedented insight into the mechanisms underlying plant defence responses. Transcription profiling has facilitated the determination of gene function by assessing the level of change in gene transcription in response to specific treatments. Several methodologies, including suppression subtractive hybridisation (SSH, Diatchenko *et al*., 1996), representational difference analysis (RDA, Hubank and Schatz, 1994) and differential display (DD, Liang and Pardee, 1992) have been used to identify transcripts that are differentially expressed between treatments. Recently microarray technology has been developed enabling researchers to simultaneously profile thousands of genes for differential expression giving a global picture of cellular transcription. The scientific principle underlying microarray technology is complementary hybridization of the total transcriptome of the test sample to a complete expressed genome array. A number of methodologies are available including Affymetrix (www.affymetrix.com) and Agilent (www.chem.agilent.com).

Transcription profiling using microarrays has become a powerful and widely implemented tool in understanding plant-pathogen interactions (Wise *et al*., 2007), providing a wealth of information about biological processes. In wheat, transcriptome analysis has been used to identify transcripts involved in the responses of this crop to both compatible and incompatible pathogen interactions including wheat yellow rust (Coram *et al*., 2010, 2008a, 2008b), wheat brown rust (Bolton *et al*., 2008, Hulbert *et al*., 2007) and blast (*Magnaporthe oryzae*, Tufan *et al.,* 2009). The induction of transcripts specific to given interactions, as well as those in common, will help to elucidate the overall defence mechanism underlying resistance.

7.2 Aims of the current study

The quantitative APR found in Claire (Chapter 6) is controlled by two major QTL (*QTLAPR.2Da* and *QTLAPR.2Db*), together with at least two minor QTL (*QTLAPR.2B* and *QTLAPR.7B*) which act additively to achieve durable yellow rust resistance. Evidence suggests *QTLAPR.2Da* originates from the old German cultivar Alcedo, known to give complete resistance to yellow rust (Jagger, 2009). Varying degrees of yellow rust resistance were observed within the Claire x Lemhi DH population (Chapter 3), even between DH lines containing *QTLAPR.2Da* (Chapter 6) indicating the possible presence of a suppressor. The computer packages MapQTL and QTL cartographer were used to identify and locate the hypothesized suppressor. Additionally a trancriptomics-based analysis was used to profile the effect the hypothesized suppressor may have on the transcription of basal defence-related genes and elucidate the defence pathway/mechansim associated with *QTLAPR.2Da* conferred resistance.

7.3 Materials and Methods

7.3.1 Genetic analysis to identify location of hypothesized suppressor

QTL analysis was repeated using MapQTL version 5 (van Ooijen, 2004) and QTL Cartographer version 1.16 (Bateson *et al*., 1997) with only phenotypic and genotypic data from the DH lines containing *QTLAPR.2Da*. QTL Cartographer was also used to identify epistatic interactions within the DH population. Confirmation of identified QTL (*QTLAPR.2Da*, *QTLAPR.2Db*, *QTLAPR.2B* and *QTLAPR.7B*), using all phenotypic data sets was also conducted.

Analysis of covariance (ANCOVA) was conducted in Genstat 9th edition (Lawes Agricultural Trust (Rothamsted Experimental Station)) on PI and IT data sets to identify the phenotypic variance contributed by each QTL and remove variance not due to *QTLAPR.2Da*. QTL analysis using MapQTL version 5 (van Ooijen, 2004) was then repeated using the adjusted PI and IT data sets which only account for the phenotypic variance contributed by *QTLAPR.2Da*.

Based upon phenotype the DH lines containing *QTLAPR.2Da* were divided into two groups; those which contained the hypothesized suppressor (displaying intermediate resistance) and those that did not contain the hypothesized suppressor (completely resistant). For the two groups all mapped marker loci were analysed to identify any specific regions that showed a clustering of Lemhi alleles within the DH lines that contained the hypothesized suppressor and a clustering of Claire alleles in those DH lines that did not.

7.3.2 Transcription profiling

7.3.2.1 Plant Material

Two plants of each parent and selected DH lines (Table 7.1) were grown to adult plants and inoculated as described in Section 2.6.2. DH lines were chosen based upon QTL genotype and phenotype. Control plants of each line were inoculated with talcum powder only, serving as a mock inoculated control for comparative purposes.

Leaf samples (2-3 cm) were collected 48 hpi from the first flag leaf of each plant and immediately frozen in liquid nitrogen. Samples were stored at -80˚C. The time point was chosen based on the expected timing of expression of *QTLAPR.2Da* resistance. Jagger (2009) reported primary cell death as early as 36 hpi and defence responses were clearly seen 48 hpi in lines containing the Alcedo 2D QTL. All DH lines were scored for PI and IT 17 dpi as described in Section 2.7.2 to confirm success of the yellow rust inoculation.

Table 7.1 DH lines selected for transcriptome profiling.

DH line	*QTLAPR.2Da*	*QTLAPR.2Db*	*QTLAPR.2B*	*QTLAPR.7B*	Hypothesized suppressor[1]
4	X		X	X	X
10		X		X	
20			X		
21		X	X		
23	X	X	X		X
24	X	X	X	X	
31		X	X	X	
32			X		
38				X	
40	X			X	X
42	X	X			
46	X	X			X
47	X	X	X	X	X
51	X	X	X		
52	X	X		X	X
56	X			X	
58		X			
68	X		X		X
71	X	X		X	
76	X		X	X	X

[1]DH line contains identified QTL/suppressor.

7.3.2.2 RNA extraction

Leaf tissue was ground under liquid nitrogen to a fine powder. Total RNA was extracted using a QIAquick RNeasy Plant Extraction Kit (Qiagen, Hilden, Germany). RNA concentrations were quantified using a Picodrop™ spectrophotometer (Picodrop Limited, PO Box 188, Saffron Walden, CB10 9BA, UK).

RNA integrity was checked by denaturing formaldehyde gel (1.2% agarose in 1 x MOPS buffer) electrophoresis (Figure 7.1). The gel was prepared by dissolving 1.2g of agarose in 72 ml DEPC-MQ-water and 10 ml 10 x MOPS by gentle heating in the microwave. After cooling 18 ml of 37% formaldehyde was added and mixed thoroughly before being poured into a casting tray with combs. MOPS gel

electrophoresis employs a MOPS running buffer (50g MOPS premixed buffer (Sigma) to 500ml H_2O and 0.5 ml DEPC which was autoclaved after being left overnight). Total RNA (500 ng) was added to 20 µl of RNA loading buffer (50 µl 1 x MOPS, 250 µl 50% formamide, 88 µl 6.5% formaldehyde, 100 µl loading dye and 5 µl of 10 µg/ml ethidium bromide). The 10 x RNA loading dye consists of 8.33 ml of 50% glycerol, 20 µl of 1 mM EDTA pH 8.0, 500 µl of 0.1% bromophenol blue and 1 ml of DEPC-MQ-water. All RNA samples were run on the denaturing formaldehyde gel together with 4 µl RNA ladder (0.24 -9.5kb) (Invitrogen). Immediately prior to loading RNA samples were denatured by heating at 65˚C for 5 minutes, then kept on ice. The gel was pre-run for 10 minutes at 70 V in 1 x MOPS buffer. Samples were loaded and run at 70 V for approximately 1 hour and visualised under UV illumination using GeneGenius (SynGene, Nuffield Road, Cambridge, CB4 1TF, UK).

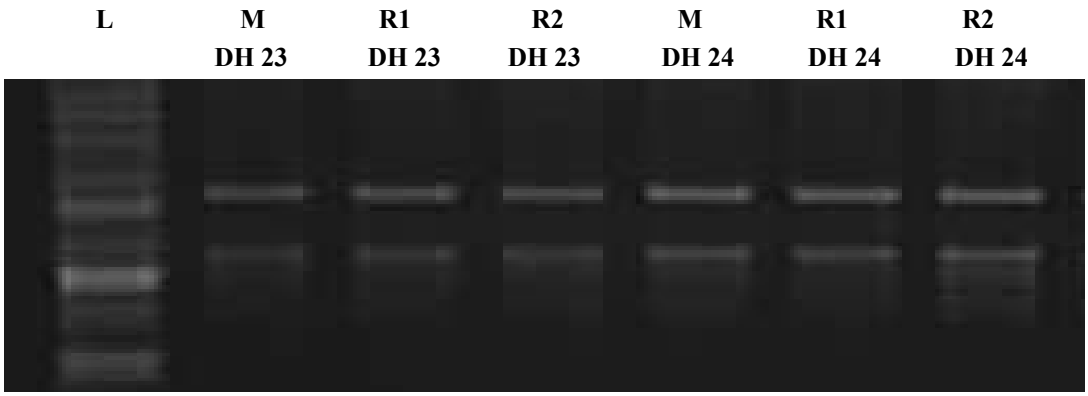


Figure 7.1 Denaturing formaldehyde gel visualised under UV light. L = ladder, M = mock inoculated, R1 = first replication yellow rust inoculated, R2 = second replication yellow rust inoculated.

To ensure all RNA extractions were free from DNA each sample was treated with Ambion TURBO DNAse 1. A 20 µl aliquot of each RNA sample was mixed with 2.5 µl of reaction buffer and 1.5 µl DNAse. After incubation at 37˚C for 30 minutes a further 1.5 µl DNAse was added and the incubation step repeated. To inactivate the DNAse enzyme 3 µl of inactivation agent was added and the sample incubated at room temperature, with intermittent mixing for 2 minutes. Following centrifugation at 10,000 x g for 1.5 minutes the supernatant containing DNase-free RNA was removed and quantified using a Picodrop^{TM} spectrophotometer.

7.3.2.3 cDNA synthesis

cDNA for quantitative reverse transcription PCR (qRT-PCR) analysis was synthesised from 1 µg total RNA using the Superscript III First-Strand Synthesis System for RT-PCR kit (Invitrogen) primed with random hexamers. JumpStart Sybr Green Taq Ready mix (Sigma) and gene specific primers (Table 7.2) were used to amplify the cDNA for qRT-PCR expression analysis.

Table 7.2 Primers used for qRT-PCR

Affymetrix probe ID	Forward primer	Reverse primer	bp	Annotation
Elongation factor 1 α	agattcccaccaagcccat	acaccaacagccacagtttgc	101	
GAPDH	ccttccgtgttcccactgttg	atgcccttgaggtttccctc	124	
Ubiquitin	ccttcacttggttctccgtct	aacgaccaggacgacagacaca	136	
Ta.28562.1.A1_at	gggagcaacatcgaggac	ctgcatcaagttcaggcaaa	127	Lignin Cinnamyl
Ta.97.1.S1_at	gccgcacagtttatggatgt	cataaggtggtgcgtgacag	154	WIR1B
Ta.21556.1.S1_at	cagcatcgaatcatcagcat	tggttagggtcgttgctacc	167	WIRB
Ta.7022.1.S1_at	agtgatgaacggcaagccagagta	tgctttggcttcatcaatgggtcg	168	PAL
Ta.92008.1.A1_s_at	ggttcatggcaacgaacaccttgt	aggagcttggaacggagtacttga	84	PAL
Ta.28.1.S1_at	cagaacaacgggctgacctacac	ctctcggaaatcaccaccttcac	110	PR 2
Ta.1174.1.S1_x_at	aagcactttgggctgttcaatccg	ccaggcagcttattcgaacgcaaa	103	PR 2
TaAffx.108556.1.S1_at	ctggacctggacttcgacac	cgcttgagcacgggttaat	138	PR 4
Ta.27762.1.S1_x_at	aagtcctgacatgggagtgc	aagttgttcggcatcatcct	141	PR 5
Ta.5385.1.S1_at	caaggtgaactcgtgatgga	ttgaggattcaaccgtcgtt	176	PR 9
Ta.22619.1.S1_x_at	acgcgtccggaagaagattgaaca	acttggaccggagatcacatggtt	86	PR 10

7.3.2.4 qRT-PCR expression analysis

Based upon previous reports (McGrann *et al*., 2009, Tufan *et al*., 2009) three reference genes, ubiquitin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and elongation factor 1α were selected for normalisation of qRT-PCR data. The published primer sequences (Table 7.2) for GAPDH and elongation factor 1α were originally designed to barley genes (McGrann *et al*., 2009), but had perfect matches to wheat sequences (Tufan *et al*., 2009). geNorm programme v3.5 (http://medgen.ugent.be/jvdesomp/genorm/; Vandesompele *et al*., 2002) was used to test the stability of these reference genes under the conditions of these experiments, providing a normalisation factor (NF) for each gene. All three reference genes had a

high level of stability across the DH lines and were therefore deemed suitable for use in normalisation of the expression data. Gene specific primers were designed for specific candidate transcripts using Primer 3 software. All primer pairs were validated using a cDNA dilution series ranging from 1:10 to 1:10,000. The threshold cycle for each dilution (C_T values) were plotted and primer efficiency calculated (10^1/(slope) x 100). Primers with efficiency > 80% were deemed suitable for qRT-PCR expression analysis, variation in primer efficiencies are accounted for in the expression calculation (Vandesompele *et al*., 2002).

qRT-PCR was carried out using the DNA engine Opticon2 Continuous Fluorescence Detector (MJ Research Inc., Alameda, CA, USA). The PCR cycling conditions consisted of an initial activation step of 10 minutes at 95˚C, followed by 40 cycles of 30 seconds at 95˚C, 1 minute 60˚C and 45 seconds at 72˚C. Data was analysed using Opticon Monitor analysis software v2.02 (MJ Research Inc.). In order to monitor primer dimer formation and the amplification of specific gene products a melt curve was produced at the end of each reaction. For each gene of interest the C_T value of DH lines inoculated with yellow rust and mock inoculated were calculated. Gene transcription was calculated based on the ΔC_T following application of the NF (Vandesompele *et al*., 2002).

7.4 Results

7.4.1 Genetic analysis to identify location of hypothesized suppressor

With all phenotypic data sets QTL Cartographer version 1.16 (Bateson *et al*., 1997) confirmed the location of *QTLAPR.2Da*, *QTLAPR.2Db*, *QTLAPR.2B* and *QTLAPR.7B* (Table 7.3). No new QTL or epistatic interactions between the four QTL were identified. QTL analysis using MapQTL of the phenotypic and genotypic data for the DH lines that only contained *QTLAPR.2Da* failed to identify any additional QTLs. All phenotypic data, for all DH lines was then adjusted by ANCOVA to take out the phenotypic variation ascribed to *QTLAPR.2Db*, *QTLAPR.2B* and *QTLAPR.7B*. This provided a complete phenotypic data set for the whole DH population for only the *QTLAPR.2Da* effect. Subsequent QTL analysis, with all DH lines, identified *QTLAPR2Da* as the only significant QTL.

Table 7.3 Yellow rust resistance QTL detected in the Claire x Lemhi DH population for percentage infection (PI) and infection type (IT) phenotypic data sets using QTL Cartographer (Bateson *et al*., 1997).

Phenotype	*QTLAPR.2Da*		*QTLAPR.2Db*		*QTLAPR.2B*		*QTLAPR.7B*	
	LOD	**Expl. % variance**	**LOD**	**Expl. % variance**	**LOD**	**Expl. % variance**	**LOD**	**Expl. % variance**
PI Yr1 F	3.5	14.7	1.8	8.8	*	5.3	*	4.5
PI Yr1 L	3.3	25.6	4.01	21.9	*	5.4	*	5.2
PI Yr2 F	*	5.5	*	7.6	1.6	10.1	1.4	9.6
PI Yr2 M	*	4.5	1.4	9.8	1.7	15.6	*	8.7
PI Yr2 L	*	4.6	1.5	10.6	1.7	18.2	*	8.5
PI Yr3	4.8	22.7	3.1	23.2	*	-	*	2.1
IT Yr1 F	6.3	26.5	1.6	19.8	1.3	8.8	1.2	7.8
IT Yr1 L	5.8	25.2	2.8	21.6	1.3	9.0	*	6.5
IT Yr2 F	*	4.8	*	6.8	1.8	15.6	*	2.1
IT Yr2 M	*	3.5	*	11.2	1.5	12.1	1.3	6.8
IT Yr2 L	*	3.2	*	10.6	1.5	12.9	1.4	7.9
IT Yr3	4.1	20.6	3.7	24.6	*	-	*	-

*LOD value of QTL did not exceed LOD threshold for linkage group.

Visual inspection of all mapped marker loci identified a large proportion of Lemhi alleles (Table 6.4) at two distinct genetic regions on the long arms of chromosome 2A (Figure 7.2 (a)), 4D (Figure 7.2 (b) and Table 7.4) in the DH lines carrying the hypothesized suppressor effects.

Table 7.4 Potential marker loci associated with the hypothesized suppressor on genetic linkage groups located on the long arm of chromosome 2A and 4D.

Marker Loci	Location	Claire alleles[1]	Lemhi alleles	Percentage skewed to Lemhi alleles (%)
wPt-3281	2AL	3	15	83.3
wPt-3179	2AL	4	13	76.5
wPt-6687	2AL	3	11	78.6
Xgwm311b	2AL	4	11	76.5
Xwmc457	4DL	6	11	65
wPt-0431	4DL	5	12	70.6

[1]Allele frequency obtained from DH lines containing the hypothesized suppressor.

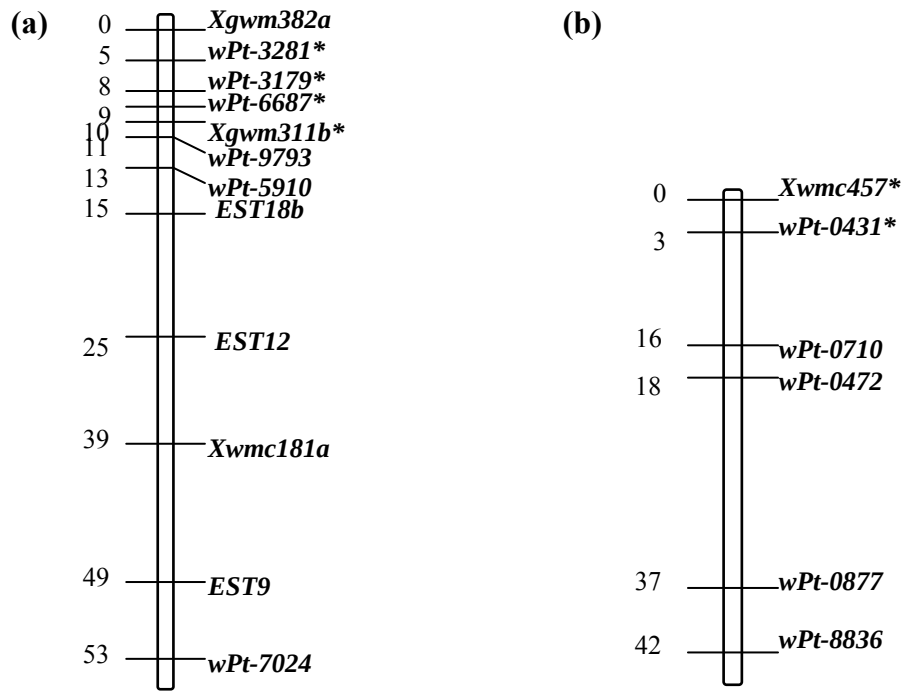


Figure 7.2 Potential location of hypothesized suppressor on genetic linkage groups located on the long arm of chromosome **(a)** 2A and **(b)** 4D. Map distances in cM (Kosambi) on the left with corresponding marker loci to the right. Those marker loci carrying the Lemhi allele in DH lines carrying the hypothesized suppressor effect are indicated by an asterisk (*).

7.4.2 Transcription profiling

7.4.2.1 Phenotypic scoring of selected DH lines

Disease progression was monitored on all mock and yellow rust inoculated DH lines 17 dpi (Table 7.5). All mock inoculated plants and Claire showed no phenotypic signs of disease. Heavy sporulation was observed on the susceptible parent Lemhi (90 S, denoting 90% infection and susceptible) and on DH 20 which contained the minor yellow rust APR-associated QTL on chromosome 2B (*APRQTL.2B*). In all DH lines containing *QTLAPR.2Da*, without the hypothesized suppressor (based on field phenotypes), little sporulation was observed on the leaf surface ranging from 0 - 2%, with infection type scores ranging from resistant to moderately resistant. DH lines containing *QTLAPR.2Da* together with the hypothesized suppressor showed an increased level of susceptibility, ranging from 5 – 40% PI and IT ranging from moderately resistant (MR) to moderately susceptible (MS) (Figure 7.3).

Table 7.5 Percentage infection (PI) and infection type (IT) scores from DH lines selected for transcriptome profiling.

DH line	QTL(s)[1]	PI		IT	
		R1	R2	R1	R2
Claire	2Da 2Db 2B 7B	0	0	0.1	0.1
Lemhi		90	90	0.8	0.8
24	2Da 2Db 2B 7B	0	0	0.1	0.1
71	2Da 2Db 7B	0	0	0.1	0.1
51	2Da 2Db 2B	0	0	0.1	0.1
56	2Da 7B	0	0	0.1	0.1
42	2Da 2Db	0	2	0.1	0.2
47	2Da 2Db 2B 7B S	20	2	0.5	0.5
23	2Da 2Db 2B S	2	23	0.4	0.4
52	2Da 2Db 7B S	10	10	0.4	0.3
4	2Da 2B 7B S	5	5	0.2	0.2
76	2Da 2B 7B S	8	20	0.4	0.4
46	2Da 2Db S	40	30	0.7	0.4
68	2Da 2B S	11	10	0.2	0.2
40	2Da 7B S	20	10	0.4	0.4
21	2Db 2B	3	5	0.2	0.2
31	2Db 2B 7B	9	6	0.4	0.4
10	2Db 7B	50	40	0.7	0.5
58	2Db	5	15	0.3	0.3
32	2B	20	15	0.4	0.4
20	2B	60	60	0.7	0.7
38	7B	30	30	0.5	0.4

[1]2Da = *APRQTL.2Da*, 2Db = *APRQTL.2Db*, 2B = *APRQTL.2B*, 7B = *APRQTL.7B*, S = hypothesized suppressor.

(a) *QTLAPR.2Da, QTLAPR.2Db, QTLAPR.2B*

(b) *QTLAPR.2Da, QTLAPR.2Db, QTLAPR.2B* and hypothesized suppressor.

Figure 7.3 Phenotypes observed under glasshouse conditions of **(a)** DH51 phenotype - 0% coverage of uredinia, resistant, genotype – *QTLAPR.2Da, QTLAPR.2Db* and *QTLAPR.2B* **(b)** DH16 phenotype – 50.3% coverage of uredinia, moderately resistant/moderately susceptible phenotype, genotype – *QTLAPR.2Da*, *QTLAPR.2Db*, *QTLAPR.2B* plus hypothesized suppressor.

7.4.2.2 Transcript selection

Gene lists were compared from published wheat microarray data identified during *Yr5* (Coram *et al*., 2008b) and *Yr1* (Bozkurt *et al*., 2010) R-gene mediated race-specific resistance and *Yr39* (Coram *et al*., 2008a) broad spectrum race-non-specific resistance to yellow rust, together with two studies of the broad spectrum race-non-specific resistance gene *Lr34* (Bolton *et al*., 2008 and Hulbert *et al*., 2007) to leaf rust (*P. triticina*). Of the five published differentially expressed gene lists compared a degree of commonality was identified (Table 7.6).

Table 7.6 The overlap of differentially up-regulated transcripts during *Yr1* (Bozkurt *et al*., 2010), *Yr5* (Coram *et al*., 2008b), *Yr39* (Coram *et al*., 2008a), *Lr34* (Bolton *et al*., 2007) and *Lr34* (Hulbert *et al.,* 2007) mediated resistance. Transcripts were classed as differentially expressed using selection criteria outlined in the specific publication.

Affymetrix ID	*Yr1*	*Yr5*	*Yr39*	*Lr34*[1]	*Lr34*[2]	Annotation	Functional classification
Ta.1174.1.S1_x_at*		X	X			PR protein	Defence and stress
Ta.13.1.S1_at	X		X			WIR1A protein	Defence and stress
Ta.21281.1.S1_at			X	X		ABC transporter	Defence and stress
Ta.21297.1.S1_at		X	X			PR protein	Defence and stress
Ta.21342.1.S1_x_at	X				X	Chitinase	Defence and stress
Ta.21354.1.A1_at		X	X			PR protein	Defence and stress
Ta.21354.1.A1_x_at		X	X			PR protein	Defence and stress
Ta.21556.1.S1_at*	X		X			WIR1B protein	Defence and stress
Ta.21556.1.S1_x_at	X		X			WIR1B protein	Defence and stress
Ta.22482.1.S1_s_at		X	X			Putative resistance protein	Defence and stress
Ta.22619.1.S1_at*		X	X			PR 10	Defence and stress
Ta.22619.1.S1_x_at	X	X	X			PR 10	Defence and stress
Ta.2278.3.S1_x_at				X	X	Chitinase (PR 3)	Defence and stress
Ta.25053.1.S1_at	X				X	Thaumatin-like (PR 5)	Defence and stress
Ta.26048.1.S1_x_at		X	X			PR protein	Defence and stress
Ta.27762.1.S1_x_at*	X	X				PR protein	Defence and stress
Ta.28.1.S1_at*	X		X			PR 2	Defence and stress
Ta.28.1.S1_x_at	X		X			PR 2	Defence and stress
Ta.28562.1.A1_at*		X		X		Lignin Cinnamyl	Defence and stress
Ta.30501.1.S1_at	X	X				PR protein	Defence and stress
Ta.30731.1.S1_at	X	X				UDP-glycosyltransferase	Defence and stress
Ta.5385.1.S1_at*	X	X				Peroxidase (PR 9)	Defence and stress
Ta.62.1.S1_x_at	X		X			PRB1-2	Defence and stress
Ta.7022.1.S1_at*		X	X			PAL	Defence and stress
Ta.7022.1.S1_s_at		X	X			PAL	Defence and stress
Ta.7022.1.S1_x_at		X	X			PAL	Defence and stress
Ta.9588.2.S1_a_at			X	X		Putative latex protein	Defence and stress
Ta.959.1.S1_at			X		X	Thaumatin-like (PR 5)	Defence and stress
Ta.97.1.S1_at*	X		X	X	X	WIR1B	Defence and stress
Ta.97.2.S1_x_at	X		X	X	X	WIR1A	Defence and stress
TaAffx.103209.1.S1_at			X	X		NB-ARC domain	Defence and stress
TaAffx.108556.1.S1_at*	X				X	PR 4	Defence and stress
TaAffx.108556.1.S1_x_at	X			X		PR4	Defence and stress
TaAffx.108743.2.S1_at				X	X	Beta-1,3-glucanase (PR 2)	Defence and stress
TaAffx.108908.1.S1_x_at				X	X	Thaumatin-like (PR 5)	Defence and stress
TaAffx.110196.1.S1_s_at	X		X			Beta-1,3-glucanase	Defence and stress
TaAffx.15327.1.S1_at	X				X	Beta-1,3-glucanase (PR 2)	Defence and stress
TaAffx.15880.1.S1_at			X	X		Germin-like protein	Defence and stress
TaAffx.24475.1.S1_x_at				X	X	Beta-1,3-glucanase (PR 2)	Defence and stress
TaAffx.83275.1.S1_at				X	X	PR 1	Defence and stress
TaAffx.92008.1.A1_s_at*		X	X			PAL	Defence and stress
Ta.22615.1.S1_at	X			X	X	Cytochrome P450	Metabolism
Ta.23309.1.S1_at			X	X		Uridine 5-monophosphate	Metabolism
Ta.24934.3.S1_at		X		X		Gibberellin oxidase	Metabolism
Ta.29534.1.S1_x_at				X	X	Cysteine proteinase	Metabolism
Ta.30739.2.S1_at				X	X	Cytochrome P450	Metabolism
Ta.4815.1.S1_at		X	X			Aminotransferase	Metabolism

[1]*Lr34* - 3 and 7 dpi (Bolton *et al*., 2007), [2]*Lr34* - 48hpi (Hulbert *et al*., 2007), *probe sets chosen for qRT-PCR analysis.

Table 7.6 continued. The overlap of differentially up-regulated transcripts during *Yr1* (Bozkurt *et al*., 2010), *Yr5* (Coram *et al*., 2008b), *Yr39* (Coram *et al*., 2008a), *Lr34* (Bolton *et al*., 2007) and *Lr34* (Hulbert *et al.,* 2007) mediated resistance. Transcripts were classed as differentially expressed using selection criteria outlined in the specific publication.

Affymetrix ID	*Yr1*	*Yr5*	*Yr39*	*Lr34*[1]	*Lr34*[2]	Annotation	Functional Classification
Ta.5654.1.S1_at		X	X			Blue copper-binding protein	Metabolism
Ta.8262.1.S1_at		X		X		Cytochrome P450	Metabolism
Ta.8346.1.A1_at		X	X	X		Cytochrome P450 51	Metabolism
Ta.8390.1.S1_at				X	X	Cytochrome P450	Metabolism
Ta.8447.1.S1_a_at	X	X				Cytochrome P450	Metabolism
Ta.8447.1.S1_x_at		X		X		Cytochrome P450	Metabolism
Ta.8674.1.A1_at				X	X	Cycloartenol synthase	Metabolism
Ta.9332.1.S1_x_at			X	X		Cytochrome P450	Metabolism
TaAffx.107507.1.S1_at		X		X		Cytochrome P450	Metabolism
TaAffx.107979.1.S1_at		X		X		Cytochrome P450 51	Metabolism
TaAffx.28047.1.S1_at		X		X		Cytochrome P450 51	Metabolism
TaAffx.53952.1.S1_at				X	X	Cytochrome P450	Metabolism
TaAffx.55612.1.S1_at		X	X			Blue copper-binding protein	Metabolism
TaAffx.81099.1.S1_at				X	X	Cycloartenol synthase	Metabolism
Ta.10084.1.S1_at		X	X			Phosphate transporter	Transport
Ta.12517.1.S1_at		X	X			Glucose transporter	Transport
Ta.18203.1.S1_at			X	X		Blue copper-binding protein	Transport
Ta.27329.1.S1_at		X		X		Glucose transporter	Transport
Ta.29523.1.S1_at			X	X		Integral membrane protein	Transport
Ta.3869.1.S1_at			X	X		Histidine transporter	Transport
Ta.8228.1.S1_at		X		X		Coumaroyltransferase	Transport
Ta.13991.1.S1_x_at		X	X				Unknown
Ta.14231.1.S1_x_at		X	X				Unknown
Ta.1454.3.S1_at		X	X				Unknown
Ta.14903.1.S1_at				X	X		Unknown
Ta.16165.1.S1_at		X	X	X			Unknown
Ta.21314.1.S1_at	X	X					Unknown
Ta.23327.1.S1_at	X				X		Unknown
Ta.23340.2.S1_at	X	X					Unknown
Ta.27882.1.S1_s_at		X	X				Unknown
TaAffx.107538.1.S1_x_at		X		X			Unknown
TaAffx.109709.1.S1_at		X	X				Unknown
TaAffx.26815.1.S1_at		X		X			Unknown
TaAffx.28836.1.S1_at				X	X		Unknown
TaAffx.53805.1.S1_at		X	X				Unknown
TaAffx.55533.1.S1_at		X	X	X			Unknown
TaAffx.7302.1.S1_at		X	X				Unknown
TaAffx.84007.1.S1_at			X	X			Unknown

[1]*Lr34* – 3 and 7 dpi (Bolton *et al*., 2007), [2]*Lr34* – 48hpi (Hulbert *et al*., 2007).

Transcripts were grouped into functional categories based on the annotation of each probe set assigned by Affymetrix (http://www.affymetrix.com). Functional classification of the differentially expressed transcripts identified in each of these experiments indicated that similar types of genes were being regulated during each interaction (Figure 7.4). The three categories with the highest level of up-regulated probe sets were cellular metabolism, structure and maintenance, defence and stress and those that were functionally uncharacterised. General defence related genes were

universally up-regulated within two or more of the data sets compared, including the cell wall defence related wheat induced gene WIR1, β-1,3- glucanases (*PR2*) and chitinases (*PR3*) and other PR proteins. Other probe sets commonly up-regulated encoded enzymes such as phenylalanine ammonia-lyase (PAL) and cinnamyl-alcohol dehydrogenase involved in the production of secondary metabolites of the phenylpropanoid pathway. Based upon the commonality between the up-regulated transcripts, 11 defence and stress related probe sets (Table 7.2) were selected to identify whether the presence of the hypothesized suppressor alters the general basal defence of the host plant.

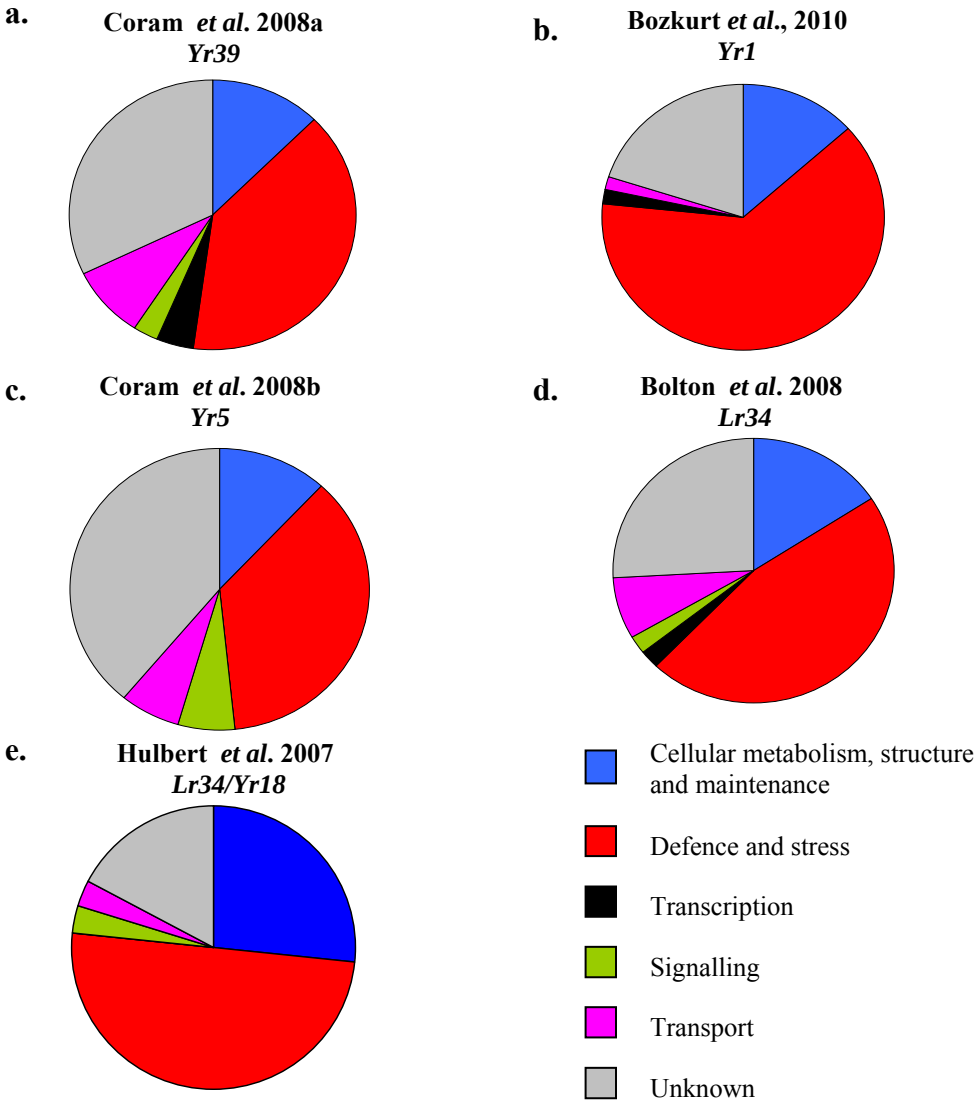


Figure 7.4 Functional classification of probe sets differentially expressed during **a)** *Yr39* (Coram *et al*., 2008a), **b)** *Yr1* (Bozkurt *et al*., 2010), and **c)** *Yr5* (Coram *et al*., 2008b) resistance to yellow rust, **d)** *Lr34* (Bolton *et al*., 2008) and **e)** *Lr34* (Hulbert *et al*., 2007) resistance to leaf rust.

7.4.2.3 qRT-PCR expression analysis

Flag leaves were inoculated and taken for subsequent transcription analysis 48 hpi. Transcriptional changes were monitored in yellow rust inoculated and mock inoculated DH lines containing different QTL with and without the hypothesized suppressor (Table 7.1). Data was normalised using geNorm with the yellow rust inoculated transcription data being compared with the mock inoculated transcription data of each DH line to identify differentially expressed transcripts attributed to the yellow rust inoculation and differing DH genotypes. If the fold change in the yellow rust inoculated plant compared to the mock inoculated was greater than two, then the transcript was considered differentially expressed.

The defence related transcripts *WIR1* (Ta.97.1.S1_at and Ta.21556.1.S1_at; Figure 7.5 (a) and (b)), PAL (Ta.92008.1.A1_s_at and Ta.7022.1.S1_at; Figure 7.5 (c) and (d)) and cinnamyl-alcohol dehydrogenase (Ta.28562.1A1_at; Figure 7.5 (e)) showed no consistent pattern of differential expression between the DH lines.

During host plant pathogen interactions PR proteins are often up-regulated as part of the basal defence response. Of the six PR protein transcripts investigated (*PR2*:Ta.1174.1.S1_x_at and Ta.28.1S1_at, *PR4*:Ta.108556.1.S1_at, *PR5*:Ta.27762.1S1_x_at and *PR10*:Ta.22619.1S1_x_at Figure 7.5 (g – k)) none were found to be differentially transcribed in response to yellow rust infection in the resistant parent Claire. *PR2* (Ta.1174.1S1_x_at Figure 7.5 (f)) and *PR9* (Ta.5385.1.S1_at (Figure 7.5 (j)) were found to be differentially up-regulated in the susceptible parent Lemhi. Transcript levels, following yellow rust inoculation relative to the mock inoculation of all five PR proteins varied across the DH lines, irrespective of the presence of QTL(s) or the hypothesized suppressor present.

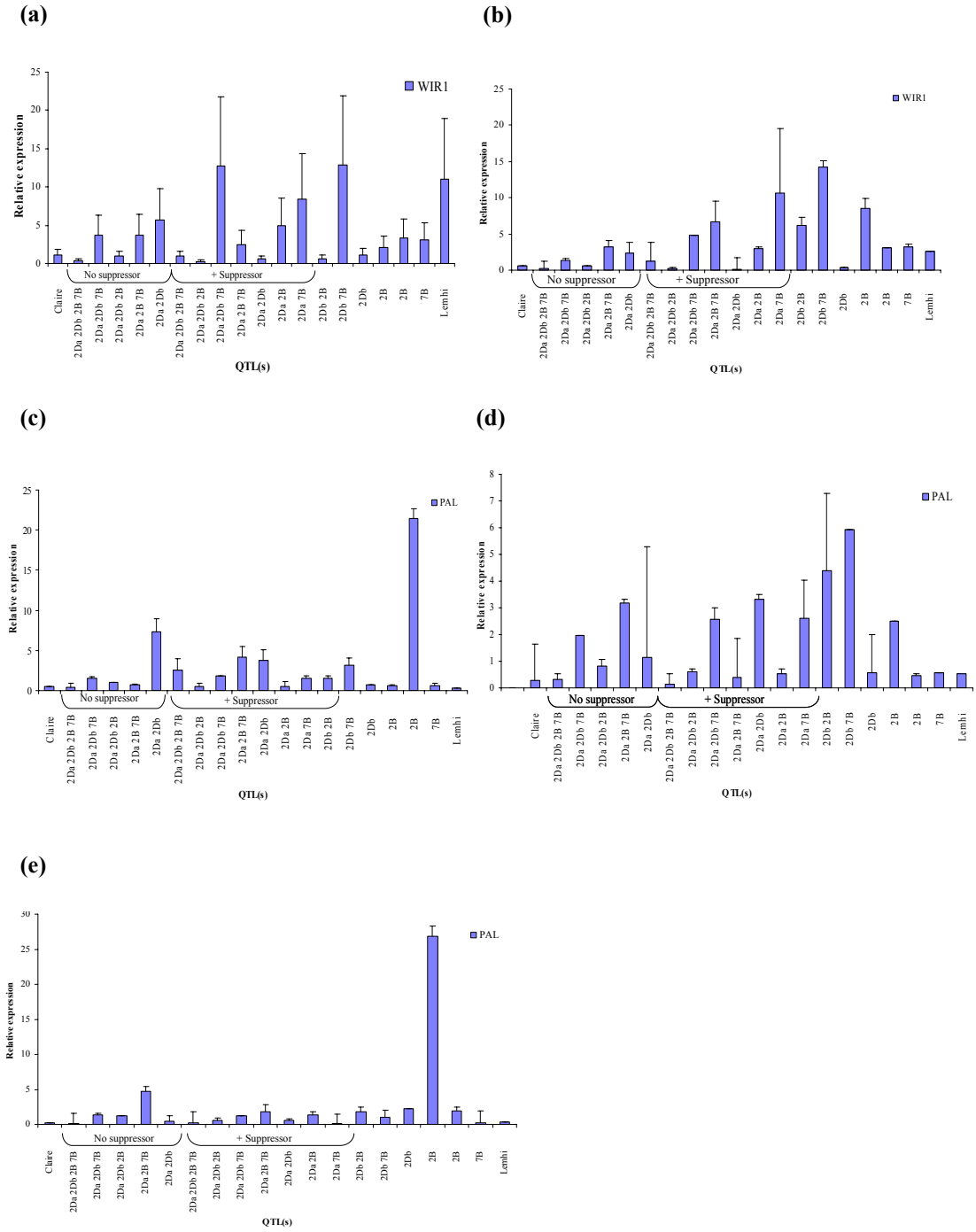


Figure 7.5. qRT-PCR analysis of DH lines containing different QTL with and without the hypothesized suppressor 48 hpi with yellow rust. Expression of selected array transcripts is shown as fold change relative to mock inoculated DH lines. Transcripts analysed were **(a)** WIR (Ta.97.1.S1_at), **(b)** WIR (Ta.21556.1.S1_at), **(c)** PAL (Ta.92008.1.A1_s_at), **(d)** PAL (Ta.7022.1.S1_at), **(e)** Cinnamyl-alcohol dehydrogenase (Ta.28562.1.A1_at).

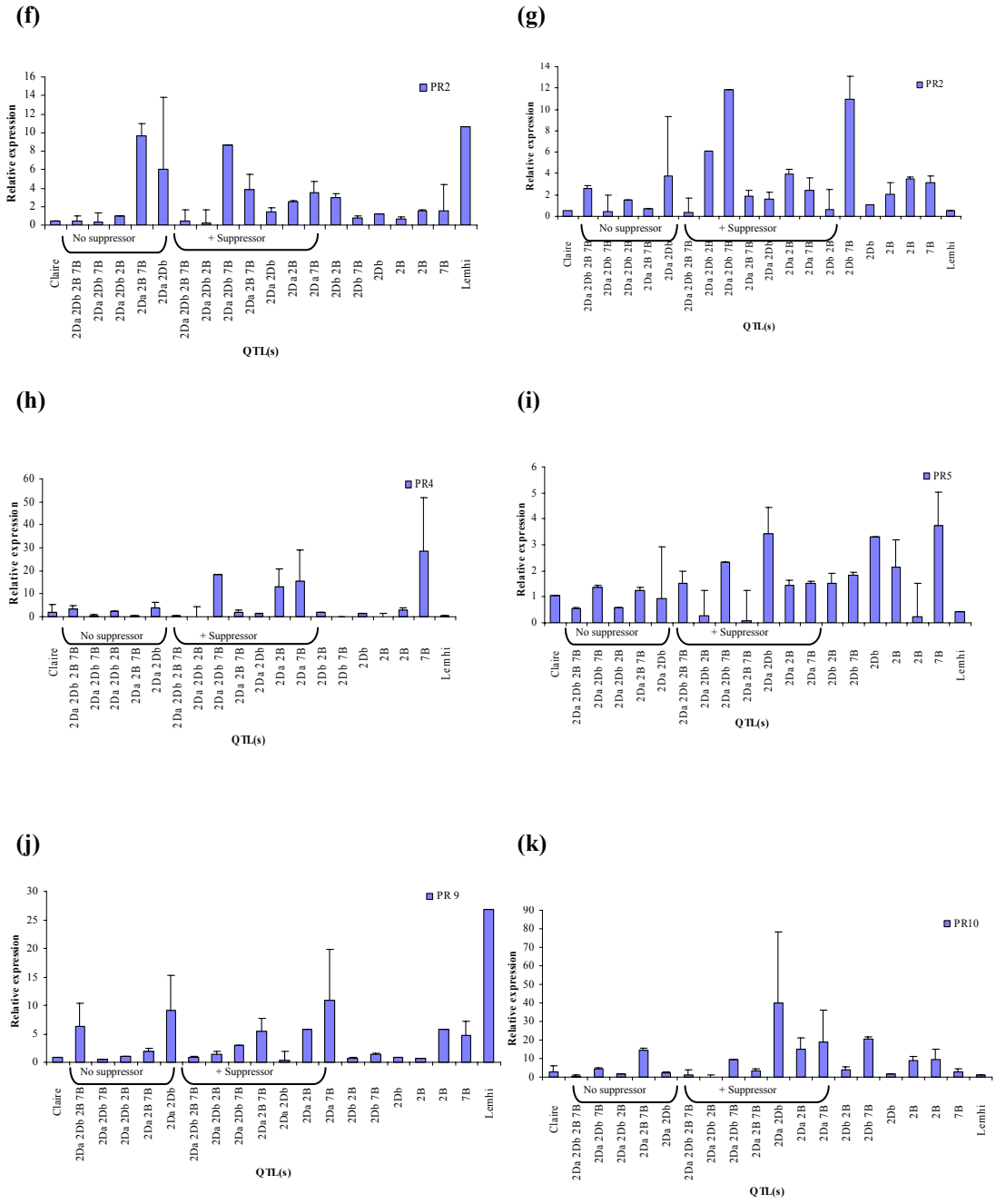


Figure 7.5 continued. qRT-PCR analysis of DH lines containing different QTL with and without the hypothesized suppressor 48 hpi with yellow rust. Expression of selected array transcripts is shown as fold change relative to mock inoculated DH lines. Transcripts analysed were **(f)** PR 2 (Ta.1174.1.S1_x_at), **(g)** PR 2 (Ta.28.1.S1_at), **(h)** PR 4 (Ta.108556.1.S1_at), **(i)** PR 5 (Ta.27762.1S1_x_at), **(j)** PR 9 (Ta.5385.1.S1_at) **(k)** PR 10 (Ta.22619.1.S1_x_at).

7.4 Discussion

QTL cartographer confirmed the location of the four QTL identified with MapQTL in Chapter 6 (*QTLAPR.2Da*, *QTLAPR.2Db*, *QTLAPR.2B* and *QTLAPR.7B*). QTL analysis using only the DH lines containing *QTLAPR.2Da* failed to identify the suppressor effect. This may in part be due to the small number of DH lines containing *QTLAPR.2Da* available for this analysis, but also because the suppressor, when present alone, has no effect on the plant phenotype. Visual inspection of the genotypic data located two potential regions for the hypothesized suppressor one on the long arm of chromosome 2A and another on 4D.

Transcription profiling has been used to analyse race-specific resistance (Bozkurt *et al*., 2010, Coram *et al*., 2008b) and APR to yellow rust (Coram *et al*., 2008a) and APR to leaf rust (Bolton *et al*., 2008, Hulbert *et al*., 2007). Comparison of this published microarray data (Bozkurt *et al*., 2010, Bolton *et al*., 2008, Coram *et al*., 2008a, 2008b, Hulbert *et al*., 2007) identified differences in the genes induced, but a core set of defence related genes implying a common defence mechanism is involved in both race-specific R-gene mediated and non race-specific broad spectrum resistance. The phenylpropanoid and shikimate pathways are commonly differentially induced by many plant-pathogen systems (Dixon *et al*., 2002). The induction of these pathways can lead to the production of anti-microbial phenolics and lignin, which act as part of the resistance response to strengthen plant cell walls (Hückelhoven *et al.*, 2007). Lignification is associated with yellow rust resistance in wheat (Moldenhauer *et al*., 2008, Moerschbacher *et al*., 1990). However, DH32 (containing *QTLAPR.2B*) was the only genotype to show a differential expression of cinnamoyl alcohol dehydrogenase, suggesting that the phenylpropanoid pathway was not induced at 48 hpi. To determine whether lignification is involved in yellow rust resistance in Claire staining with phloroglucinol/HCL needs to be carried out. PAL is a central enzyme in the phenylpropanoid pathway, controlling the biosynthesis of defence compounds such as anthocyanins, lignin and phytoalexins (Dixon *et al*., 2002). An increase in activity of the phenylpropanoid pathway during APR in wheat has been reported (Coram *et al*., 2008a). Induction of PAL transcripts was observed across the genotypes in this study, but no distinct pattern emerged relative to the presence of QTL(s) or the hypothesized suppressor.

In wheat, *WIR1* is a commonly expressed transcript induced in response to both biotrophic and necrophic pathogens (Bozkurt *et al*., 2010, Tufan *et al*., 2009, Bolton *et al*., 2008, Coram *et al*., 2008a, 2008b, Hulbert *et al*., 2007). *WIR1* genes were seen to be expressed in a number of the DH lines, but no clear pattern linking gene transcript to the genotype emerged. During pathogen attack *WIR1*, a small membrane associated protein, is proposed to help maintain the plasma membrane cell wall integrity (Bull *et al*., 1992). *WIR1* could be involved in the prevention of extensive cell-to-cell damage in regions undergoing a hypersensitive cell death (HCD).

Plants accumulate several kinds of PR proteins in response to infection by pathogens, in both compatible and resistant interactions. The expression pattern of *PR2* (β-1-3 glucanase), *PR4* (chitinase), *PR5* (thaumatin-like proteins), *PR9* (peroxidase) and *PR10* (ribonucleases) transcripts showed no deciphering pattern between genotypes differing in QTL(s) and the hypothesized suppressor. Induction of *PR* transcripts were reported in both *Yr5* race-specific resistance (Coram *et al*., 2008b) and *Lr34* (Bolton *et al*., 2008, Hulbert *et al*., 2007) and *Yr39* APR (Coram *et al*., 2008a), seemingly a common response of wheat to plant pathogens. Hulbert *et al*. (2007) and Coram *et al*. (2008a, 2008b) reported high levels of peroxidase transcripts during race-non-specific resistance, suggesting the occurrence of an oxidative burst response. *PR9* was highly expressed in the susceptible parent Lemhi indicating the induction of the oxidative burst, however no other genotype had differential expression of this transcript.

The 48 hpi time point was chosen for this qRT-PCR analysis based on the expected timing of expression of *QTLAPR.2Da*. Primary cell death was seen as early as 36 hpi and a defence response was clearly seen 48 hpi in lines containing the Alcedo QTL (Jagger, 2009). Furthermore, Coram *et al*. (2008a) identified differentially expressed transcripts involved in *Yr39*-mediated APR 48 hpi with yellow rust. Therefore, by 48 hpi differing transcript levels associated with *QTLAPR.2Da* were expected. As no deciphering pattern emerged between genotype and differential transcript levels for the genes selected a more detailed time course analysis would be required, possibly coupled with a cytological examination of pathogen development to determine any affect of the hypothesized suppressor on defence gene transcription and defence responses.

Although stringent criteria were applied to the selection of genotypes and probe sets, the experimental design of the transcription profiling investigation may have been a limiting factor. Due to seed availability only two replications were possible, with significant variation in transcript levels being observed between replications. Repetition of the transcriptomic analysis and the inclusion of a larger number of genotypes may provide a better picture of the pattern of defence gene expression in relation to the resistance QTLs present. Variation in the coverage of yellow rust infection on the flag leaf sampled for RNA extraction will also influence transcript levels.

Suppressors of yellow, leaf and stem rust have been identified in wheat (Nelson *et al*., 1997, Kema *et al*., 1995, Bai and Knott, 1992, Williams *et al*., 1992, Kerber, 1991) and are thought to occur more often than reported (Douglas Knott, personal communication, University of Saskatchewan, Canada). Chinese Spring has been reported to carry three complementary genes on chromosomes 1D, 2D and 4D which suppress stem rust resistance on the A and B genomes in three accessions of *T. turgidum var. dicoccoides* (Bai and Knott, 1992). The durum wheat cultivar Medea was reported to contain a suppressor of stem rust resistance located on the A or B genome (Knott, 2000). Canthatch, a hexaploid wheat cultivar, is susceptible to several races of stem rust however Tetra Canthatch (the tetraploid component) is resistant. The D genome again suppressing expression of resistance found on the AABB genomic component (Kerber *et al*., 1999). Although suppression has been reported in a variety of studies little has been done to elucidate the genetic mechanism behind the suppression phenomenon. Genetic studies have been conducted to locate these suppressor genes. Kerber *et al*. (1999) induced a non-suppressing mutation on chromosome arm 7DL of Canthatch and replaced 7D with the short arm of Chinese Spring, containing *Lr34* resulting in the expression of resistance. The leaf rust resistant gene *Lr2*3 is located on chromosome 2BS, while a suppressor, most likely its homoallele is located on chromosome 2DS (Nelson *et al*., 1997). In hexaploid wheat, if suppressor genes are homoalleles of the resistance gene the two homoeologous arms are likely to possess suppressors. Visual inspection of the marker data identified a potential region on the long arm of chromosome 2A which could prospectively be the homoallele of *QTLAPR.2Da*.

The challenge faced now in plant breeding is how to exploit these suppressor genes together with resistance genes to obtain a durable resistance. Evolution has maintained suppressor genes within plant genomes even though they promote pathogen proliferation and establishment of disease, meaning they must be of some advantage to the host plant. In the absence of rust pathogens some resistance genes may confer a fitness penalty, suppression would reduce or remove this fitness cost (Knott, 2000). Besides being involved in plant pathogen interactions suppressor genes or susceptibility factors maybe required for fundamental biological plant function. For example the recessive rice allele *xa13* confers resistance to bacterial blight, while the dominant allele *Xa13* is required for bacteria growth and plant pollen development (Chu *et al*., 2006). *Xa13* therefore appears to act as a susceptibility factor as described by Boyd *et al*. (2006), Kema *et al*. (1995) and Ma *et al*. (1995) favouring the establishment of bacterial blight.

In this chapter the eleven transcripts investigated represent general, basal defence responses in wheat against pathogens (Bozkurt *et al*., 2010, Tufan *et al*., 2009, Bolton *et al*., 2008, Coram *et al*., 2008a, 2008b, Hulbert *et al*., 2007). No deciphering pattern emerged between transcript levels and the QTL(s) and hypothesized suppressor genotypes in response to yellow rust inoculation. It can not be ruled out that these general basal defence genes are not affected by the presence of different QTL and the hypothesized suppressor since other factors maybe affecting transcription, as discussed. To elucidate the defence mechanism behind each resistance QTL and the action of the hypothesized suppressor further investigation will be required.

Chapter 8
General Discussion

The development of cultivars with effective and durable rust resistance has been a global goal in wheat breeding for many years. The recent spread of the stem rust fungus *Puccinia graminis* f.sp *tritici* isolate Ug99 (MacKenzie, 2008), threatening wheat crops and possible famine across Africa, Asia and the Middle East has highlighted the importance of continued development of new resistant germplasm. In the UK several winter wheat cultivars saw the revision of their HGCA yellow rust resistance rating following a disease outbreak in 2009 (Impey, 2009). However, the yellow rust resistance in Claire remained effective even though it has been grown widely since its release in 1999. Durable resistance has been attributed to a combination of quantitative and seedling race-specific resistance (Boukhatem *et al*., 2002), or multiple quantitative APR genes (McIntosh, 1992, Johnson, 1983). Genetic characterisation of potentially durable sources of resistance is fundamental to the effective use of those resistance genes.

In Claire two major QTL, one centromeric (*QTLAPR.2Db*) and the other telomeric (*QTLAPR.2Da*) on the long arm of chromosome 2D, confer APR to yellow rust. The location of *QTLAPR.2Db* suggests that it may be the partial APR gene *Yr16*, supporting the theory that modern wheat cultivars have inherited durable yellow rust APR genes from Cappelle-Deprez (Mallard *et al*., 2005, Angus, 2001). Evidence suggests that *QTLAPR.2Da* represents the Alcedo 2D APR QTL known to confer complete resistance (Jagger, 2009). However, DH lines derived from the Claire x Lemhi cross containing *QTLAPR.2Da* exhibited varying degrees of yellow rust resistance, suggesting that a gene suppressing *QTLAPR.2Da* resistance could be segregating within the Claire x Lemhi population. Suppressor genes effective against specific yellow rust resistance genes have been identified in wheat, especially on the D genome (Kerber *et al*., 1999, Singh *et al*., 1996, Kema *et al*., 1995, Bai and Knott, 1992).

The semi dwarfing gene *Rht-D1* mapped to the long arm of chromosome 4D between the DArT markers *wPt-0472* and *wPt-8836* (Chapter 6), which was also one of the potential locations of the hypothesized suppressor (Chapter 7). *Rht-D1* is an orthologue of the Arabidopsis Gibberellin Insensitive (*GAI*) mutant (Peng *et al*., 1999) known as DELLA (Navarro *et al*., 2008). Srinivasachary *et al*. (2009) found *wPt-0472* to be closely linked to *Rht-D1* and in repulsion with a major fusarium

head blight (FHB) resistance QTL. FHB susceptibility has been found to be associated with *Rht-D1*, as taller varieties show less FHB symptoms compared to shorter varieties (Drager *et al*., 2007, Steiner *et al*., 2004). It is thought that *Rht-D1* may promote cell death (Paul Nicholson, personal communication, JIC), which in turn increases FHB susceptibility as *Fusarium* is a necrophic pathogen. If *Rht-D1* was associated with yellow rust resistance then shorter DH lines will be more resistant as yellow rust is a biotroph, however no association was found between plant height and either yellow rust phenotype. However it has been reported that DELLA proteins promote susceptibility to virulent biotrophs by repressing salicylic acid (SA) defence responses (Bari and Jones, 2009, Navarro *et al*., 2008) and promoting the expression of genes encoding ROS detoxification enzymes, thereby reducing ROS levels (Achard *et al*., 2008). Whether DELLA proteins are involved in the hypersensitive response conferred by *QTLAPR.2Da*, leading to suppression of the resistant phenotype require further investigation. Either way, the identification of susceptibility factors or regulators of defence responses in plant breeding could be a key target for future research.

Many QTLs for yellow rust resistance that confer a minor phenotype have been identified, but pyramided together these minor QTLs can still provide complete immunity (Rosewarne *et al*., 2008, Boukhatem *et al*., 2002, William *et al*., 2002, Singh *et al*., 2001, Johnson, 1983). Two new minor QTLs, *QTLAPR.2B* and *QTLAPR.7B* were identified in Claire. *QTLAPR.2B* had a strong effect on the disease reaction phenotype in the year 2 field trial, but was undetected in the glasshouse trial. However, expression of this QTL was identified from individual flag leaf scores within the same glasshouse trial. The resistant phenotype was expressed to varying degrees depending on the age of the flag leaf, with younger leaves supporting more disease. Consequently *QTLAPR.2B* was detected using the yellow rust scores from the first and second flag leaves.

The Alcedo 2D QTL (*QTLAPR.2Da*) expresses resistance at earlier growth stages (Jagger, 2009). Early expression of major APR QTL play an important role in protecting a cultivar in the field from early exposure to yellow rust infection (Ma and Singh, 1995). A fundamental aspect of the durability of yellow rust resistance in

Claire may be attributed to the timing of QTL expression throughout the growth stages of the host plant.

The microphenotype of the Claire resistance response may provide an insight into why the resistance in Claire has remained effective since its release in 1999. Additional molecular and cytological examination would provide the first steps towards understanding the wheat yellow rust interaction in the presence/absence of QTL(s) and the hypothesized suppressor. The developmental stage of pathogen arrest, whether it is pre- or post-haustorial and the defence mechanisms of the plant are important aspects that can be characterised at the microscope level. In the cultivar Cappelle-Deprez (Mares and Cousen, 1977) and for the APR genes *Yr18* (Moldenhauer *et al*., 2008) and *Yr29* (Rosewarne *et al*., 2006) a hypersensitive cell death is not believed to play a role in resistance, but instead slows the rate of pathogen development. The major 2D QTL in Alcedo confers a resistance typical of a seedling race-specific defence response, rapidly inducing cell death and preventing the majority of infection sites developing runner hyphae. Characterisation of the different QTLs identified in Claire may help to explain the durability. Each QTL may confer resistance by a different mechanism, either slow rusting or race-specific, that combined together may confer a more durable source of resistance.

Gene expression analysis of selected transcripts found no genotype specific expression patterns (Chapter 7). Microphenotyping, coupled with transcription profiling using commercially available platforms (e.g. Affymetrix or Agilent microarrays) may help to elucidate the defence pathways and resistance mechanisms involved in each QTL response. Such a study would help to answer questions about the timing of resistance and why Claire's resistance has remained effective. Characterising the phenotype conferred by each resistance QTL at the microscopic level would allow resistance genes that act at different developmental stages of pathogen infection and confer resistance via potentially different defence mechanisms to be deployed together in new cultivars. The incorporation of individual QTLs into near isogenic backgrounds would provide the ideal genetic material to elucidate the specific defence mechanisms of each QTL. Near isogenic lines would also allow the suppressor effect against *QTLAPR.2Da* to be shielded in isolation of other resistance factors.

Despite the importance of APR genes and their potential durability, to date few such genes have been cloned. Cloning of the APR genes found in Claire would offer a unique opportunity to understand the molecular nature of these resistance QTLs. Cloning APR genes could have significant implications for the long term control of yellow rust. Map-based cloning in hexaploid wheat is now possible and is supported by the complete genomic sequence of rice and *Brachypodium* (Harmon *et al*., 2010, Goff *et al*., 2002). *Lr10*, a race-specific resistance gene belonging to the NBS-LRR group was the first resistance gene to be cloned from wheat (Feuillet *et al*., 2003, 1997). Recently the HTAP resistance gene *Yr36* (Fu *et al*., 2009) and APR gene *Lr34* (Krattinger *et al*., 2009) have been cloned. *Lr34* was found to encode a pleiotrophic drug resistance (PDR)-like ATP-binding cassette (ABC) transporter (Krattinger *et al*., 2009), highlighting that different resistance mechanisms may be responsible for durable APR compared to seedling race-specific resistance. Singh (1992) previously reported *Lr34* to be completely linked to the resistance gene *Yr18*. It has now been confirmed that the same cloned gene is responsible for the resistance to both pathogens. Cloning resistance genes will help to uncover at the molecular level the host-pathogen interaction, helping to determine the differences between race-specific and non-race-specific resistance mechanisms.

Recently, in New Zealand, in the Canterbury Plain area yellow rust has been observed on Claire in the field (Swallow and Abam, 2007). This would indicate that the major, race-specific-like *QTLAPR.2Da* (Jagger, 2008) and possibly the other resistance QTLs in Claire, has been overcome. However, the yellow rust resistance in Claire still remains effective in the UK and as yet this new virulent race has not been reported here. Many breeders use Claire as the basis of new varieties, the resistance breakdown in New Zealand would have obvious consequences on the confidence of the durability of Claire's resistance.

The ability to pyramid durable resistance genes into high yielding cultivars that can be grown over many years and remain durable even under high pressure is required to keep ahead of the plant-pathogen race (Boyd *et al*., 2005, Koebner and Summers, 2003). The genetic characterisation and identification of marker loci closely associated with the resistance QTLs found in Claire is invaluable within a breeding program, meaning that sources of potentially durable resistance can be easily

selected and incorporated into new cultivars. Marker loci associated with the yellow rust resistance QTLs in Claire can be used to screen current UK cultivars in order to catalogue the genetic diversity of this yellow rust resistance within UK winter wheat cultivars. The assumption that Cappelle-Deprez, Carstens V and Alcedo are the source of yellow rust resistance in modern European cultivars can also be investigated.

Plant disease resistance is a complex phenomenon, involving a multitude of genes and many interconnected signalling pathways. The identification of two major and two minor resistance QTL in Claire and a potential suppressor in Lemhi that affects *QTLAPR.2Da* will serve as a precursor to elucidating the mechanisms of the yellow rust resistance in Claire. The increasing global human population requires that higher crop yields are produced under sustainable agricultural systems. Improved control of yellow rust in new wheat cultivars is critical to achieving this goal through an effective, economical and environmentally sustainable strategy.

Appendix

Appendix Table 1 List of SSR primers (5' - 3')

SSR	Forward primer	Reverse primer
BARC24	cgcctcttatggaccagcctat	gcggtgagccatcgggttacaaag
BARC108	gcgggtcgtttcctggaaattcatctaa	gcgaaatgattggcgttacacctgttg
BARC148	gcgcaaccacaatgtatgct	ggggtgttttcctatttctt
BARC159	cgcaatttattatcggttttaggaa	cgcccgatagtttttctaatttctga
BARC171	gcggggtcatcttagtaactcaaata	actgtcaacgttggttcacattca
BARC212	ggcaactggagtgatataaataccg	caggaagggaggagaacagagg
BARC219	gcgatcccacaatgcatgacaacttc	ggacgtccgatcgaattggttt
BARC228	ccctcctctctttagccatcc	gcacgtactattcgccttcactta
BARC59	gcgttggctaatcatcgttccttc	agcaccctacccagcgtcagtcaat
BARC68	cgatgccaacacactgaggt	agccgcatgaagagataggtagagat
BARC77	gcgtattctccctcgtttccaagtctg	gtgggaatttcttgggagtctgta
CFD21	cctccatgtaggcggaaata	tgtgtcccattcactaaccg
GDM98	ccatccatgaaatggcg	gcccttcactagccttcatg
GWM189	aggagcagcggaacgaac	agaaatacggaaacccaccc
GWM301	gaggagtaagacacatgccc	gtggctggagattcaggttc
GWM311	tcacgtggaagacgctcc	ctacgtgcaccattttg
GWM120	gatccaccttcctctctctc	gattatactggtgccgaaac
GWM132	taccaaatcgaaacacatcagg	catatcaaggtctccttcccc
GWM153	gatctcgtcacccggaattc	tggtagagaaggacggagag
GWM157	gtcgtcgcggtaagcttg	gagtgaacacacgaggcttg
GWM16	gcttggactagctagagtatcatac	caatcttcaattctgtcgcacgg
GWM160	ttcaattcagtcttggcttgg	ctgcaggaaaaaaagtacaccc
GWM161	gatcgagtgatggcagatgg	tgtgaattacttggacgtgg
GWM179	aagttgagttgatgcgggag	ccatgaccagcatccactc
GWM190	gtgcttgctgagctatgagtc	gtgccacgtggtacctttg
GWM205	cgacccggttcacttcag	agtcgccgttgtatagtgcc
GWM296	aattcaacctaccaatctctg	gcctaataaactgaaaacgag
GWM297	atcgtcacgtattttgcaatg	tgcgtaagtctagcattttctg
GWM304	aggaaacagaaatatcgcgg	aggactgtggggaatgaatg
GWM312	atcgcatgatgcacgtagag	acatgcatgcctacctaatgg
GWM320	cgagatactatggaaggtgagg	atctttgcaaggattgccc
GWM333	gcccggtcatgtaaaacg	tttcagtttgcgttaagctttg
GWM349	ggcttccagaaaacaacagg	atcggtgcgtaccatcctac
GWM357	tatggtcaaagttggacctcg	aggctgcagctcttcttcag
GWM368	ccatttcacctaatgcctgc	aataaaaccatgagctcacttgc
GWM369	ctgcaggccatgatgatg	accgtgggtgttgtgagc
GWM382	gtcagataacgccgtccaat	ctacgtgcaccaccattttg
GWM383	acgccagttgatccgtaaac	gacatcaataaccgtggatgg
GWM413	tgcttgtctagattgcttggg	gatcgtctcgtccttggca
GWM44	gttgagcttttcagttcggc	actggcatccactgagctg
GWM458	aatggcaattggaagacatagc	ttcgcaatgttgatttggc
GWM469	caatcagtgctcacacaacg	cgataaccactcatccacacc
GWM526	caatagttctgtgagagctgcg	ccaacccaaatacacattctca
GWM539	ctgctctaagattcatgcaacc	gaggcttgtgccctctgtag
GWM540	aggcatggatagaggggc	tctcgctgtgaaatcctatttc

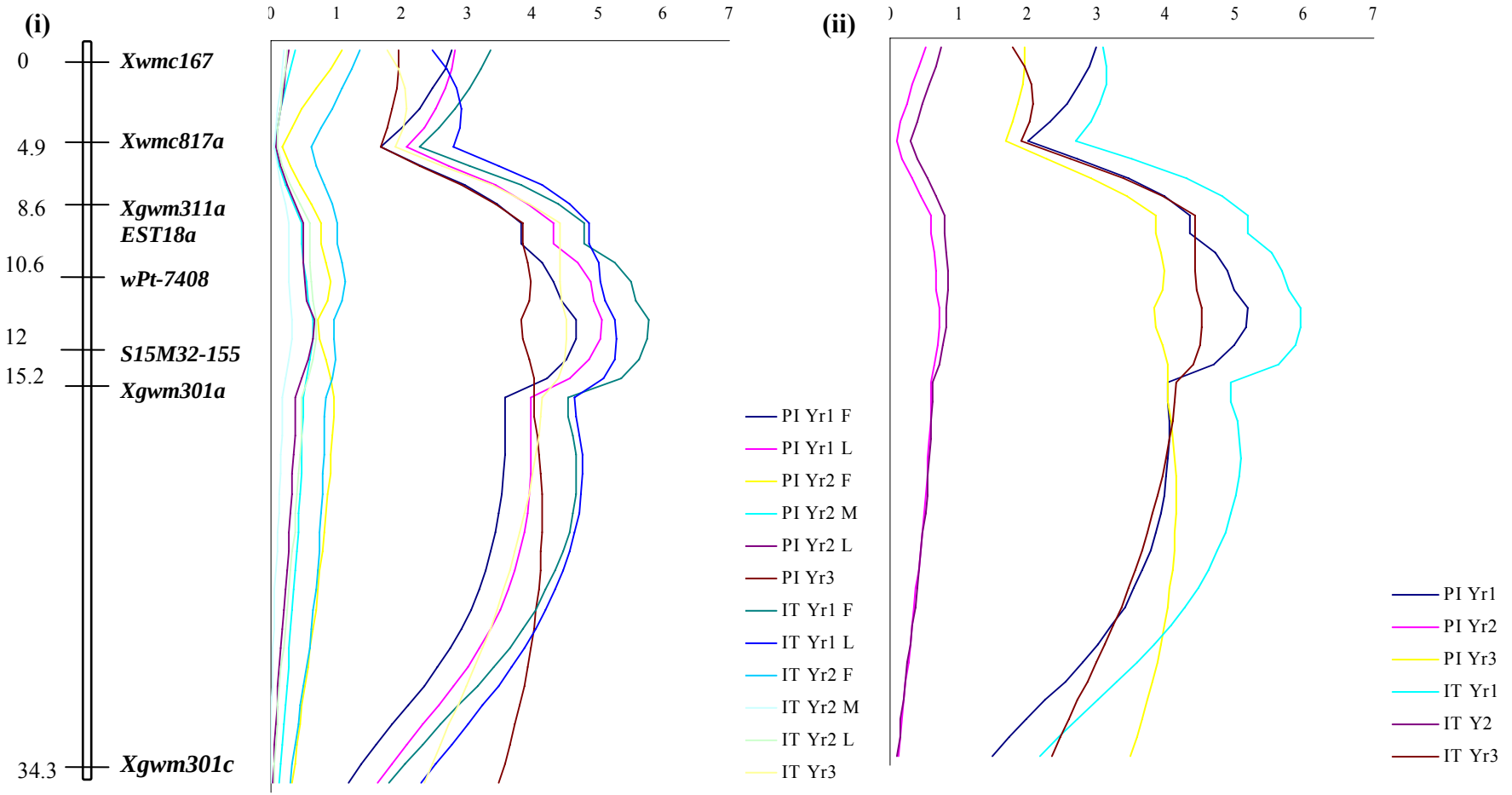
Appendix Table 1 continued

SSR	Forward primer	Reverse primer
GWM570	tcgccttttacagtcggc	atgggtagctgagagccaaa
GWM601	atcgaggacgacatgaaggt	ttaagttgctgccaatgttcc
GWM608	acattgtgtgtgcggcc	gatccctctccgctagaagc
PDP2151.3	accctaaaccctaaaccctac	gaagacacgttgccgacaaga
PSP3001	gcagagagatgagggcacc	ctctgctcccttaacttctg
PSP3039	gcatccaaatccctaaaccg	agcatgtgtgagatagacgg
PDP3047	tctgcaacattccccaacag	ccgttcataggccaatttcg
WMC154	atgctcgtcagtgtcatgtttg	aaacggaacctacctcactctt
WMC167	agtggtaatgaggtgaaagaag	tcggtcgtatatgcatgtaaag
WMC174	aggaacaaatgctccgcc	gcacgcatgcacgcaccc
WMC175	gctcagtcaaaccgctacttct	cactactccaatctatcgccgt
WMC179	catggtggccatgagtggaggt	catgatcttgcgtgtgcgtagg
WMC181	tccttgacccccttgcactaact	atggttgggagcactagcttgg
WMC222	aaaggtgcgttcatagaaaattaga	agaggtgtttgagactaatttggta
WMC25	tctggccaggatcaatattact	taagatacatagatccaacacc
WMC322	cgccccactatgctttg	cccagtccagctagcctcc
WMC407	ggtaattctaggctgacatatgctc	catatttccaaatccccaactc
WMC41	tccctcttccaagcgcggatag	ggaggaagatctcccggagcag
WMC441	tccagtagagcacctttcatt	atcacgaagataaacaaacgg
WMC441	tccagtagagcacctttcatt	atcacgaagataaacaaacgg
WMC457	cttccatgaatcaaagcagcac	catccatggcagaaacaatagc
WMC47	gaaacagggttaaccatgccaa	atggtgctgccaacaacataca
WMC54	tattgtgcaatcgcagcatctc	tgcgacattggcaaccacttct
WMC754	atccacatgaacctcaacttatgg	ggcattgttgttgtactgcagtc
WMC78	agtaaatcctcccttcggcttc	agcttctttgctagtccgttgc
WMC817	tgacggggatgatgataacg	cggtgagatgagaaaggaaaac

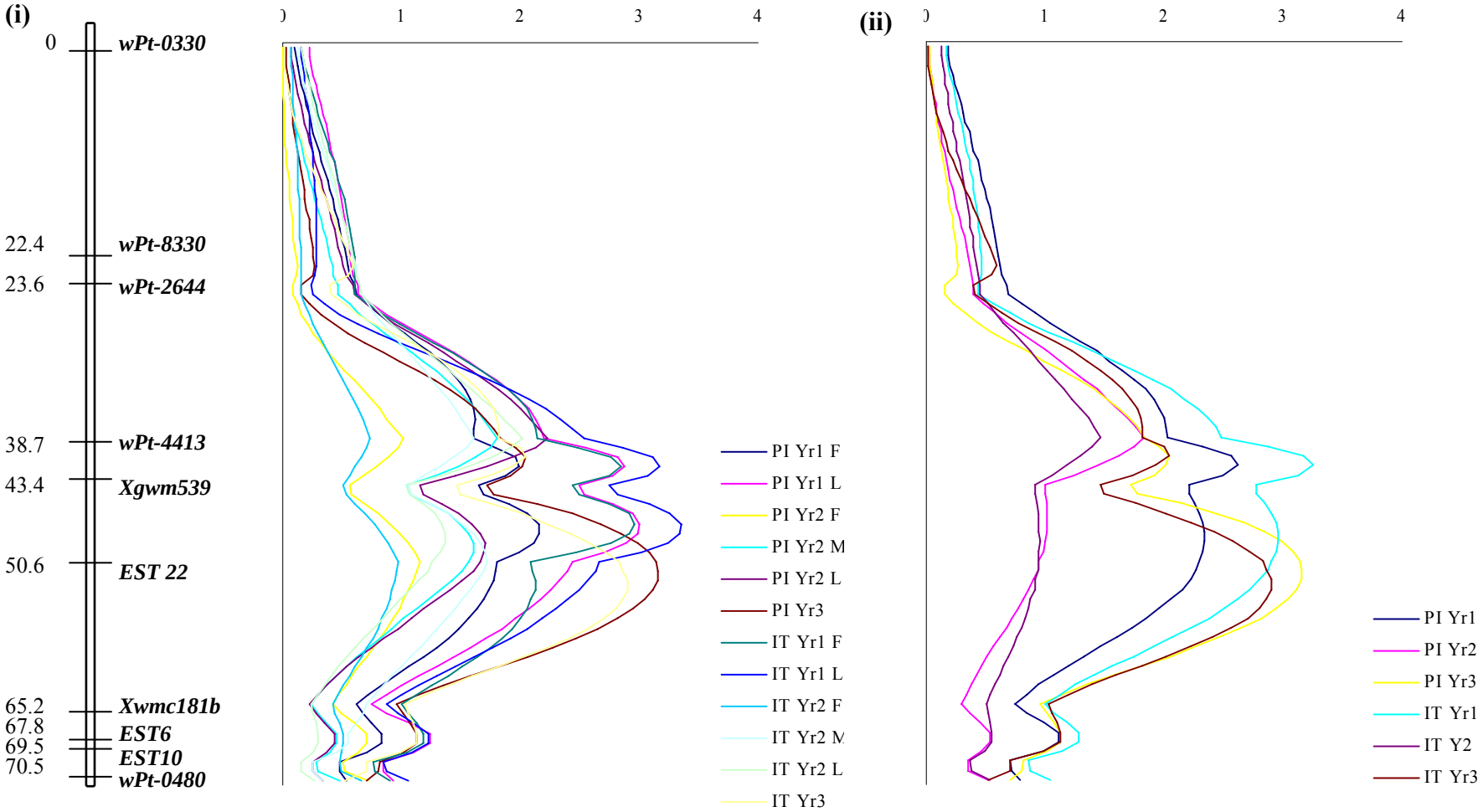
Appendix Table 2 List of AFLP and NBS primer sequences

Primer name	Sequence
S12	agactgcgtacatgcaggac
S15	agactgcgtacatgcaggca
M32	gatgagtcctgagtaaac
M45	gatgagtcctgagtaatg
M00	gacgatgagtcctgagtaa
P00	gtagactgcgtacatgcag
NBS2	gtwgtytticcyraiccisscat
NBS2cer	gtigtytticchriicchsc
Pigtail	gtttactcgattctcaacccgaaag
*Mse*I lower	tatgggatctatactt(3ac7)
*Mse*I upper	actcgattctcaacccgaaagtatagatccca

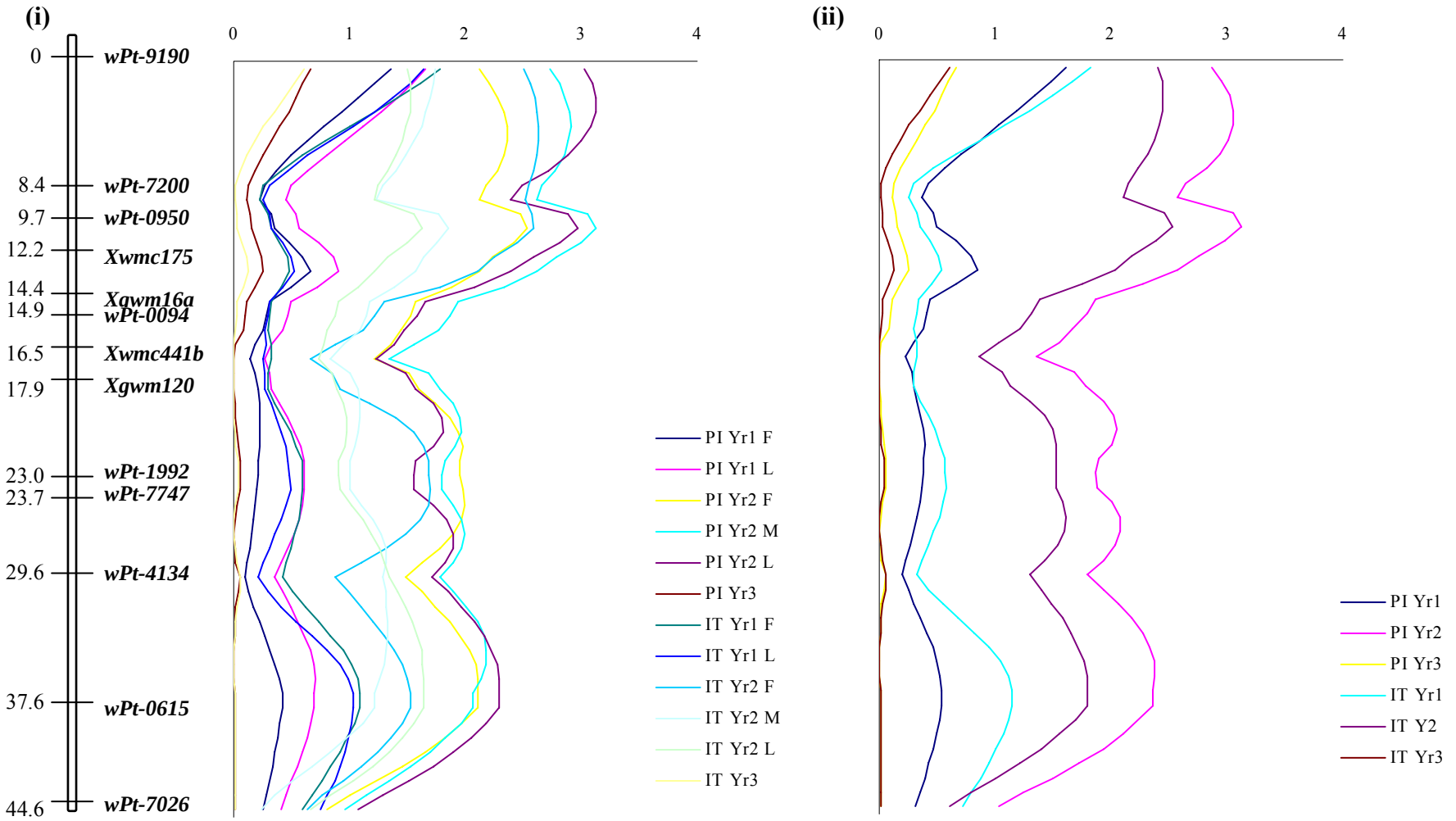
w = trypotopan (TGG), y = tyrosine (TAY), i = isoleucine (ATH), r = arginine (CGN and AGY), s = serine (TCN and AGY), h = histidine (CAY)



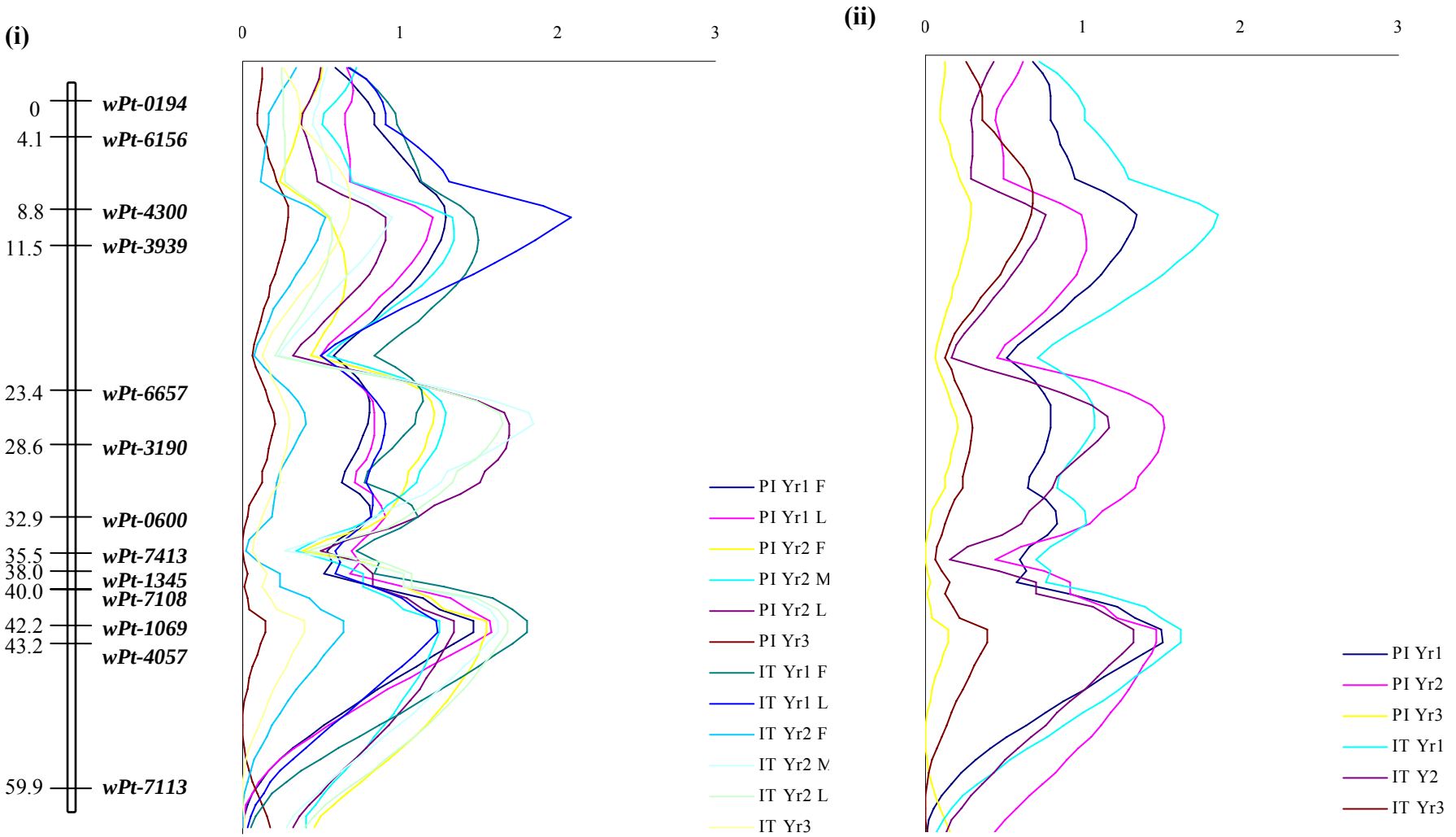
Appendix Figure 1a QTL plots obtained from MQM mapping using percentage infection (PI) and infection type (IT) datasets. The corresponding linkage groups are shown with the relevant marker loci and the map distances in cM (Kosambi) for each plot (a) *QTLAPR.2Da* (b) *QTLAPR.2Db* (c) *QTLAPR.2B* (d) *QTLAPR7B*. QTL graph (i) represents all score dates within each data set, (ii) average across each year.



Appendix Figure 1b *QTLAPR.2Db*



Appendix Figure 1c *QTLAPR.2B*



Appendix Figure 1d *QTLAPR.7B*

List of Abbreviations

ABC	ATP binding cassette
AFLP	amplified fragment length polymorphism
ANCOVA	analysis of covariance
ANOVA	analysis of variance
APR	adult plant resistance
APS	ammonium persulphate
ATP	adenosine 5’ triphosphate
AUDPC	area under the disease progression curve
avr	avirulence
BLAST	basic local alignment search tool
bp	base pair(s)
BSA	bulked segregate analysis
C	courtot
CC	N terminal coiled-coil
cDNA	complementary deoxyribonucleic acid
CI	coefficient of infection
CIM	composite interval mapping
cm	centimetre
cM	cenimorgans
COS	conserved orthologue set
CS	chinese spring
C_T	threshold cycle
dATP	adenine triphosphate
dCTP	cytosine triphosphate
dGTP	guanine triphosphate
dd.H_2O	double distilled water
ddNTP	dideoxynucleoside triphosphate
DD	differential display
DArT	diversity array technology
DH	doubled haploid
DNA	deoxyribonucleic acid
dpi	day(s) post inoculation

DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid disodium salt
EST	expressed sequence tag
FHB	fusarium head blight
f. sp.	*formae specialis*
g	gram
μg	microgram
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GLM	general linear model
GLR	general linear regression
gSSR	genomic simple sequence repeat
HGCA	Home Grown Cereals Authority
hpi	hour(s) post inoculation
HMC	haustorial mother cell
HTAP	high temperature adult plant (resistance)
HCD	hypersensitive cell death
IH	infection hyphae
IGC	international grains council
IM	interval mapping
IMTI	International Triticeae Mapping Initiative
IT	infection type
Kb	kilobase
KW	Kruskall and Wallis
L	litre
μl	microlitre
LRR	leucine rich repeat
LZ	leucine zipper
M	molar
MAS	marker assisted selection
mg	milligram
mL	millilitre
mM	millimolar
mRNA	messenger Ribonucleic Acid
MgAc	magnesium actetate

MQM	multiple QTL mapping
NBS	nucleotide binding site
NCBI	National Centre for Biotechnology Information
NF	normalisation factor
PAL	phenylalanine ammonia-lyase
PCR	polymerase chain reaction
PDR	pleiotrophic drug resistance
PI	percentage infection
PR	pathogenesis related
qRT-PCR	quantitative reverse transcription PCR
QTL	quantitative trait loci
RAPD	random amplification of polymorphic DNA
RFLP	restriction fragment length polymorphism
RDA	representational difference analysis
RGA	resistance gene analogue
R-gene	resistance gene
RH	runner hyphae
RNA	ribonucleic acid
ROS	reactive oxygen species
SA	salicylic acid
SNP	single nucleotide polymorphism
SSCP	single strand conformational polymorphism
SSH	suppression subtractive hybridisation
SSR	simple sequence repeat
SSV	sub-stomatal vesicle
Temp.	temperature
TIR	toll and interleukin 1 receptor
Tris	tris (hydroxymethyl) aminomethane
UK	United Kingdom
USA	United States of America
UV	ultra violet
V	volts
W	watt
Yr	yellow rust

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