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IDENTIFICATION, VALIDATION, AND PYRAMIDING OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO CROWN ROT IN WHEAT

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Southern Queensland

BY

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ABSTRACT

Crown rot (causal organism: *Fusarium pseudograminearum*) is a significant disease affecting wheat in Australia. Although first reported over 60 years ago, the disease has become more prevalent in recent years due to the adoption of minimum tillage and stubble retention practices. Breeding for resistance to crown rot is difficult – phenotypic selection, which is usually done at harvest, is time-consuming, expensive, and subject to between year variability due to sensitivity to environmental conditions. For these reasons, the coupling of molecular techniques with conventional plant breeding (marker-assisted selection) has the potential to more rapidly and reliably identify genomic regions that contribute to resistance. The objective of this study was to identify, validate, and pyramid quantitative trait loci (QTL) for resistance to crown rot present in a W21MMT70 x Mendos doubled haploid wheat population.

Replicated seedling trials were conducted in 2001, 2003, and 2005. In each seedling trial, W21MMT70 displayed partial resistance to crown rot whereas Mendos seedlings were susceptible. A bulked segregant analysis (BSA), using 390 simple sequence repeat (SSR) markers chosen for their coverage of the wheat genome, was initially conducted based upon the 2001 seedling trial data in an attempt to rapidly identify genomic regions associated to resistance. The BSA did not reveal any markers associated with resistance to crown rot. As a result, a full mapping study was conducted. One hundred and twenty eight (128) SSR markers were mapped across the population to produce a framework map. Previously screened AFLP markers were added to the map. Composite interval mapping revealed eight QTL associated with resistance. Of these, three (located on chromosomes 2B, 2D, and 5D) were consistently detected in each of the three seedling trials. Two QTL (on chromosomes 1A and 3B) were detected in two of the three trials. The 2D, 3B, and 5D QTL were inherited from W21MMT70, whereas the 1A and 2B QTL were inherited from Mendos.

Two software programs were used to identify epistatic interactions between QTL. While the results of the two programs differed markedly, both programs detected a highly significant interaction between the W21MMT70 inherited 5D

QTL and a locus on chromosome 2D inherited from Mendos. The overall effect of the epistatic interactions was not as great as the additive effects of non-epistatic QTL. Nonetheless, the presence of epistasis may indicate that, particularly in the case of 5D, the effect of this QTL may be dependent on the background into which it is introgressed.

Validation of three W21MMT70-inherited QTL (on chromosomes 2D, 3B, and 5D) was conducted on three F_2 populations with W21MMT70 as one of the parents. While the 5D QTL was validated in two of the three crosses, neither the 2D nor the 3B QTL were detected in any of the F_2 validation populations. It is likely that the size of the F_2 populations (the largest composed of 94 individuals), in conjunction with the variability that is inherent when screening for resistance to crown rot, precluded validation of these regions. Validation of the 2B Mendos-inherited QTL was conducted on a Sunco x Batavia doubled haploid population because Sunco possesses the same *Triticum timopheevi* 2B introgression that is present in Mendos. This validated QTL (designated *Q.CR..usq-2B2*) explained 11 % of the phenotypic variance in the Sunco x Batavia population.

To assess the effectiveness of pyramiding QTL for resistance to crown rot, a 2-49 x W21MMT70 population was examined. A number of lines of this population performed significantly better than each of the parents in the replicated seedling trial that was conducted. Four QTL, located on chromosomes 1A, 1D, 2D, and 3B, were detected. The 1A and 1D QTL were inherited from 2-49 whereas the 2D and 3B QTL were inherited from W21MMT70. The 1A QTL from 2-49 has not been previously validated, and this QTL has been designated *QCr.usq-1A1*. The 3B QTL (designated *QCr.usq-3B1*) had the highest effect (LRS 42.1; explaining 21.0 % of the phenotypic variance) in the 2-49 x W21MMT70 population. The 2D QTL (*QCr.usq-2D1*) was shown to have a minor effect. The 5D QTL that was inherited from W21MMT70 in the W21MMT70 x Mendos population was not detected in the 2-49 x W21MMT70 population. A number of possible explanations for the inability to detect this QTL in the 2-49 x W21MMT70 population are discussed.

CERTIFICATION OF DISSERTATION

I certify that the ideas, experimental work, results, analyses, and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any other award, except where otherwise acknowledged.

____________________________ ____________________________

Signature of Candidate Date

ENDORSEMENT

____________________________ ____________________________

Signature of Principal Supervisor Date

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My knowledge of general molecular biology techniques was raw at the commencement of this work – I am thankful to Dr. Raechelle Grams for "teaching me the ropes". Dr. Bert Collard's enthusiasm instilled a real interest for QTL mapping in me (even though he himself may still be a sceptic), and Dr. Anke Lehmensiek's German efficiency helped me to appreciate the difference between a map and a good map. The majority of the phenotyping presented in this thesis was conducted under the supervision of Dr. Graham Wildermuth and Mr. Matt Davis, and I am grateful for their leadership in this area.

I have spent more years than I care to admit trying to complete a study worthy of receiving a PhD. I have no doubt that I would not have made it to the end without the community feel that is generated by the Biological and Physical Sciences Department at USQ. There are too many people to name, but Cassy Percy, Bene Watson, Grant Daggard, Eric Storlie, Joan Vickers, Vic Schultz and Pat McConnell deserve special mentions.

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I briefly mentioned two distractions that came into my life during the course of this study - but really there were three. My wife, Jessica, has been the greatest distraction of them all. I know that I haven't always been easy (and you know my finishing this PhD probably won't make that much difference), but I know that I'm a happier person with you. Thankyou Jess - your support, encouragement, and optimism were, and continue to be, a source of inspiration.

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PUBLICATIONS AND CONFERENCE PROCEEDINGS ARISING FROM THIS DISSERTATION

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Bovill WD, Ma W, Ritter K, Collard BCY, Davis M, Wildermuth GB, and Sutherland MW (2006) Identification of novel QTL for resistance to crown rot in the doubled haploid wheat population 'W21MMT70' x 'Mendos'. *Plant Breeding* 125: 538-543.

Sutherland MW, Lehmensiek A, Bovill W, Bovill J, Collard B, Daggard G, Christopher M, and Mace E (2006) Mapping markers for priority traits in the northern grains region of Australia. *Joint Meeting of the International Triticeae Mapping Initiative and the Australian Centre for Plant Functional Genomics Symposium*, Victor Harbor, August 27 -31.

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Collard BCY, Grams RA, Bovill WD, Percy CD, Jolley R, Lehmensiek A, Wildermuth GB and Sutherland MW (2005) Development of molecular markers for crown rot resistance in wheat: mapping of QTLs for seedling resistance in a 2-49 x Janz population. *Plant Breeding* **124**: 1-6.

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Chapter 1. Background and Literature Review

1.1 Introduction

Experimental research in the plant sciences provides humankind with tools to understand the complex interactions between plants and their physical and biotic environments, and thus the potential to manipulate aspects of these interactions for sustainable agriculture. This type of research is crucial in a world where human populations are expanding and where undeveloped arable land is becoming increasingly scarce. Excluding adverse environmental conditions, such as drought, salinity, and soil nutrient degradation, disease is perhaps the greatest threat to plants and therefore the products that are harvested from them.

Social disaster struck in Ireland in 1845 when prolonged changes in weather conditions led to the spread of the potato blight fungus *Phytophthora infestans*, which destroyed the crop and led to the starvation of many Irish farmers (Schumann, 1991). In 1970, a corn leaf blight epidemic caused a loss of over $1 billion in the US corn crop (Scheffer, 1997). The social and economic losses associated with plant disease are many - in order to reduce such losses, plant breeders seek to produce varieties that are better able to cope with both present and future disease pressures. The production of new varieties however, is laborious, time consuming, and reliant on careful phenotypic selection (Kumar, 1999). Through conventional breeding strategies, the production of a new wheat variety can take up to 14 years (P. Banks, pers. comm.). The pressure to produce new varieties more rapidly is increasing. Indeed, in 2006, global consumption of wheat was forecast to exceed supply for the second year running, with ending stocks forecast to reach their lowest level in 25 years (United States Department of Agriculture, 2006).

Biotechnology offers plant breeders new methods that have the potential to increase both the rate at which new wheat varieties are bred, and improve the characteristics that are desired for their end-use. Molecular marker technology is a powerful tool that can be used to increase the understanding and subsequent manipulation of the genetics of both simple and complex traits (Dubcovsky, 2004). With simple traits, such markers, when very tightly linked to the gene of

interest, can be used to indirectly select for the desirable allele (Anderson et al., 1989; Peng et al., 1999; Beecher et al., 2002). For more complex traits, genetic linkage maps composed of molecular markers can be used to identify regions of the genome that contribute to phenotypic variation (Parker et al., 1999; Chartrain et al., 2004; Chen et al., 2006). This "quantitative trait loci" (QTL) mapping technique can be used to more rapidly incorporate desirable regions into agronomically superior genotypes.

Crown rot of wheat provides a perfect example of the complex interplay between host, pathogen, and environment. In Australia, this disease (predominantly caused by the fungus *Fusarium pseudograminearum*) has been estimated to cost the cereals industry 56 M dollars per year (Brennan and Murray, 1998). Although first reported in the 1950's (McKnight and Hart, 1966), the disease has become more prevalent in recent years due mainly to the trend in farming practices towards stubble retention (Dodman and Wildermuth, 1989). The disease is more severe when plants are exposed to moisture stress late in the growing season (Wildermuth et al., 1997). Breeding for resistance to crown rot is difficult. Phenotypic evaluations, which are normally conducted at maturity, are time-consuming, expensive, and subject to environmental variation (G. Wildermuth pers. comm.). The identification of molecular markers that are tightly linked to disease resistance loci has the potential to assist breeders in the development of resistant cultivars. This literature review and subsequent chapters focus on wheat, crown rot, and the use of molecular markers as tools for the production of crown resistant materials.

1.2 The Origin of Cultivated Wheat

Wheat belongs to the family Gramineae, and the genus Triticum. It is arguably the most important cereal crop for humankind – in the form of bread, wheat provides more nutrients to the world population than any other single food source (Pena, 2002). Wheat has three levels of ploidy (number of copies of the basic number of chromosomes): diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42). Of the three ploidy levels, the tetraploid and hexaploid wheats are the most commonly grown in present day cultivation.

An area known as the Fertile Crescent is considered to be the origin of the wild progenitors of cultivated wheats (Feldman, 2001). Two valid biological species (and their subspecies) exist at each ploidy level (Table 1-1). The hybridization events that lead to the formation of the tetraploid and hexaploid wheats are complex, with various *Aegilops* species contributing significantly to the makeup of polyploid wheats (Chantret et al., 2005).

Genetic studies have shown that there are two different lineages of the polyploid wheats (Gill and Friebe, 2002), with *Triticum turgidum* (AABB) and *T. aestivum* (AABBDD) comprising one lineage, and *T. timopheevii* (AAGG) and *T. zhukovskyi* (AAA^mA^mGG) forming the other. It is widely accepted that *T. urartu* contributed the A genome to both lineages (Dvorak et al., 1993; Akhunov et al., 2005). *T. aestivum* arose from hybridization between tetraploid *T. turgidum* ssp. *dicoccum* (AABB) and diploid *Ae. tauschii* spp. *strangulata* (the D genome donor). *T. zhukovskyi* (AAA^mA^mGG) arose from hybridization between *T. timopheevii* (AAGG) and *T. monococcum* (A^mA^m), while the other set of the A genome was contributed by *T. urartu* (Huang et al., 2002a). The G genome donor is believed to be *Ae. speltoides* (Nath et al., 1984). The identity of the B genome donor is still the subject of much debate, however Provan et al. (2004) have shown that it is most closely related to *Ae. speltoides*. The elusiveness of identifying the donor has been described as being the result of either: the diploid progenitor not yet being discovered; the extinction of the donor; or the B genome rapidly evolving (through rearrangements and introgressions of chromosomal segments from other species; Levy and Feldman, 2004).

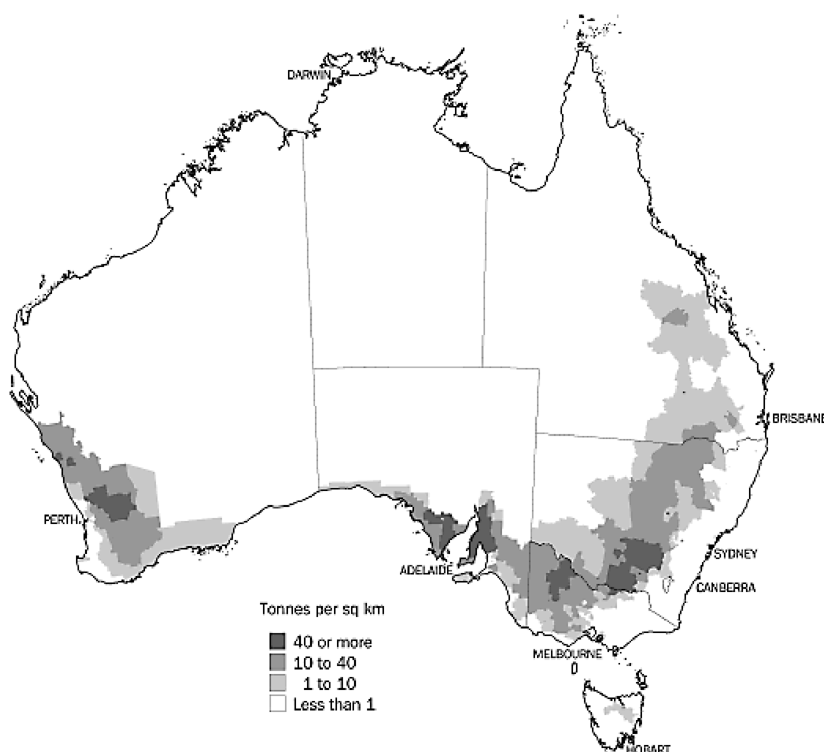
Table 1-1. The biological species of wheat that exist at each ploidy level (subspecies are not shown)

Ploidy	Species	Genetic Composition
Diploid	*Triticum urartu*	AA
(2n=2x=14)	*Triticum monococcum* L.	A^mA^m
Tetraploid	*Triticum timopheevii*	AAGG
(2n=4x=28)	*Triticum turgidum*	AABB
Hexaploid	*Triticum zhukovskyi*	AAA^mA^mGG
(2n=6x=42)	*Triticum aestivum* L.	AABBDD

1.3 Wheat Production in Australia

Although Australia produces only about 3% of the 570 million tonnes produced worldwide, wheat is Australia's largest crop (Grains Council of Australia, 2005). Excluding the Northern Territory, wheat is grown in all states. On the mainland, wheat is grown in a narrow area referred to as the wheat belt (Figure 1-1). Western Australia and New South Wales are the major wheat producing states, each accounting for approximately one third of national production. During the 10 years from 1995 – 2004, Australia produced an average of 19.7 million tonnes of wheat, from an area of 11.0 million hectares at an average yield of 1.8 tonnes/hectare (Australian Bureau of Statistics, 2006a). Of the annual production, approximately 5 million tonnes is consumed by the domestic market (for human and industrial consumption, feed, and seed) while the remainder is exported (Australian Bureau of Agricultural and Resource Economics, 2005). Over 60% of Australia's wheat is exported to six countries: Iran, Iraq, Indonesia, Japan, Egypt, and South Korea (Grain Growers Association, 2004).

Figure 1-1. Wheat production areas of Australia with productivity for the 2000-2001 season (Australian Bureau of Statistics, 2006b).



1.31 Production Limitations

The most severe constraint to wheat production in Australia is water availability. Most wheat is grown in an area that receives between 250 to 650mm of rainfall per year. In the northern grain growing region (northern New South Wales, southern Queensland and central Queensland) summer rainfall is predominant, whereas in the southern region (Western Australia, South Australia, western Victoria and south-western New South Wales) winter rainfall is predominant. Rainfall levels can be highly variable, and droughts place considerable strain on the industry. As previously mentioned, average wheat yields in Australia are estimated at 1.8 tonnes/hectare. The significant effect of drought is highlighted by the 2002-2003 season, where severe drought reduced yields by an average 50%, to 0.9 tonnes/hectare (Australian Bureau of Statistics, 2006a).

Even when rainfall is not a limiting factor, wheat yields in Australia are low. For comparison, wheat yields in New Zealand are in the order of 6.0 tonnes/hectare (New Zealand Ministry of Agriculture and Forestry, 2006), more than 300% higher than in Australia. Soil fertility provides a major environmental barrier to wheat cultivation in Australia (Curtis, 2002). As a result of soil nutrient deficiencies, nitrogen and phosphorous are regular additives that induce increased yield responses.

Disease is a further limitation to production. The six major diseases of wheat in Australia in order of potential economic losses are common bunt ($269 million), take-all ($214 million), stripe rust ($181 million), crown rot ($160 million), *Septoria tritici* blotch ($152 million) and *Septoria nodorum* blotch ($147 million). When diseases of wheat are considered in order of average annual losses per year they are *Septoria nodorum* blotch ($58 million), crown rot ($56 million), take all ($52 million), yellow spot ($49 million), cereal cyst nematode ($37 million), and root lesion nematode ($36 million) (Brennan and Murray, 1998).

1.4 Crown Rot

1.41 Economic Importance

In terms of potential economic losses, crown rot is the fourth most devastating disease of the Australian cereals industry. In terms of annual losses, crown rot ranks second only to *Septoria nodorum* blotch (Brennan and Murray, 1998). It is clear, therefore, that crown rot causes serious economic losses for the Australian cereals industry and management of this disease is of considerable importance.

1.42 Early Research

Crown rot of wheat caused by *Fusarium graminearum* Schwabe was first recorded in Queensland in 1951; although similar disease symptoms were reported on the Darling Downs as early as 1940 without the identification of the causal agent (McKnight and Hart, 1966). In their study, McKnight and Hart (1966) examined the effects of cropping history, soil factors, weather, seed treatment, and variety planted on crown rot disease severity. They concluded that: the disease was less severe in crops following a long fallow and when rotations to resistant crops were carried out; that the disease was more severe on heavier soils; that below-average rainfall during the growing period resulted in greater severity; that seed treatment had no effect on yield in badly infested soils; and that the varieties Gala, Lawrence, Gabo, Cailloux, and Puglu displayed a relatively low incidence of deadheads.

Purss (1966) also demonstrated varietal differences in reaction to crown rot, with Gala and Mengavi showing a reasonable level of field resistance. Purss (1966) concluded that differences between varieties were due to a differential rate of development of the disease rather than any difference in infection, and that none of the varieties tested displayed true resistance. Seedling tests were also carried out in this study and the author was not able to demonstrate any correlation between seedling blight in these tests and field reaction (Purss, 1966).

Purss (1969) investigated the relationship between strains of *Fusarium graminearum* Schwabe that caused crown rot of various gramineous hosts

(wheat, canary grass, barley, wild oats, *Phalaris paradoxa*, *Agropyron scabrum*, *Danthonia linkii*, and *Bromus unioloides*) and stalk rot of maize. From the results of cross-inoculation tests, Purss (1969) concluded that there were two distinct pathogenic forms of *Fusarium graminearum* Schwabe – one responsible for the crown rot disease of the various gramineous hosts, and the other affecting maize. In this paper, Purss (1969) also described the perfect state of the fungus, and identified it as *Gibberella zeae*.

In a follow-up confirmatory study, Purss (1971) examined pathogenic specialization of *Fusarium graminearum* Schwabe isolated from crown rot of wheat, stalk rot of maize, and head blight of wheat. From this work, he concluded that all were capable of causing head blight of wheat and stalk rot of maize; however, only isolates from crown rot of wheat were able to cause this particular disease syndrome in wheat. As a result he confirmed his earlier hypothesis that different pathogenic forms of the fungus exist, and concluded that a specialized form of *F. graminearum* was responsible for crown rot of wheat.

Wildermuth and Purss (1971) undertook a search for further sources of resistance to crown rot by screening approximately 400 cultivars from Australia, the 1965 International Spring Wheat Rust Nursery, and various overseas sources by conducting multi-year, multi-site field trials. The resistance of Gala and Mengavi originally identified by Purss (1966) was consistent over the range of seasonal conditions. Although no lines from the International Spring Wheat Rust Nursery performed consistently better than Gala, Wildermuth and Purss (1971) recommended that lines such as 497, 527, and 538 should be considered for inclusion in a hybridization program. These authors concluded that: there was no specific resistance (complete) identified; that variation due to site and seasonal conditions make selection of promising sources of resistance difficult; and that "there is no doubt, however, that the levels of resistance encountered offer a worthwhile means of combating the disease" (Wildermuth and Purss, 1971).

The purpose of this brief overview of some early research on crown rot is to provide context for, and a means of comparison to, the research that has been

carried out over the following years. Some of these studies are pertinent to the results of this thesis, and will be further discussed in later sections.

1.43 Symptoms of Disease

The symptoms of crown rot disease are well characterized. McKnight and Hart (1966) reported that both in the laboratory and in the field the fungus produces a pre-emergence rotting and a seedling blight. Infected adult plants show a typical honey-brown discolouration of the subcrown internode extending up into the crown, and the basal leaf sheaths and stem show a brown necrosis (McKnight and Hart, 1966). The discolouration is also found beyond the second internode, and the fungus has been re-isolated from as high as the sixth node (Purss, 1966). On the roots, two distinct types of infection have been reported – the most common is directly associated with the sub-crown internode, and rarely, other lesions occur as discrete entities on seminal and secondary roots (Purss, 1966). In plants with severely infected crowns, the roots may ultimately collapse. Purss (1966) has suggested that this is a result of infection spreading down into the roots from the crown. The most conspicuous symptom of the disease is the formation of whiteheads (heads that contain little or no grain; Klein et al., 1991). It is believed that this results from disruption of the translocation stream at the base of the plant leading to premature ripening and death (Burgess et al., 2001).

1.44 Causal Organism

In Australia, the predominant causal organism of crown rot is *Fusarium pseudograminearum*, although other species can contribute to the disease. Backhouse et al. (2004) conducted a survey of *Fusarium* species in the eastern states of Australia and reported that *F. pseudograminearum* was almost the only species isolated from northern New South Wales and southern Queensland. They also found that *F. pseudograminearum* was the most common species in Victoria and South Australia (winter dominant rainfall regions), although *F. culmorum* was also frequently isolated in these states. *F. avenaceum*, *F. crookwellense*, and *F. graminearum* were isolated infrequently (Backhouse et al., 2004). In a less geographically diverse study, Akinsanmi et al. (2004) surveyed

Fusarium species associated with crown rot and head blight from northern New South Wales and Southern Queensland, and confirmed the predominance of *F. pseudograminearum* (48% of all isolates sampled). However, in contrast to the results of Backhouse et al. (2004), 28% of all isolates were *F. graminearum*, while *F. crookwellense*, *F. avenaceum*, were also isolated at lower levels. This difference is likely a result of the sampling from both heads and crowns carried out by Akinsanmi (2004). Internationally, reports of crown rot caused by *Fusarium pseudograminearum* have come from Italy (Balmas, 1994), South Africa (Marasas et al., 1988; Lamprecht et al., 2006), New Zealand (Monds et al., 2005) and the Pacific Northwest of the United States (Paulitz et al., 2002; Smiley et al., 2005). In Section 1.42 (Early Research) *Fusarium graminearum* was described as the crown rot causing organism. The remainder of this section outlines the evolution of the nomenclature which took place in order to arrive at the current species name of *Fusarium pseudograminearum*.

In the early work of McKnight and Hart (1966) and Purss (1966) *Fusarium graminearum* Schwabe was described as the crown rot causing organism. The perfect stage (teleomorph) of the fungus was identified as *Gibberella zeae* (Purss, 1969). From pathogenicity testing, Purss (1971) concluded that a specialized form of *Fusarium graminearum* was responsible for causing crown rot of wheat.

In a survey of Fusaria associated with crown rot of wheat in Eastern Australia, Burgess et al. (1975) found isolates that were of the morphological type described by Purss as responsible for causing the severe crown rot syndrome in Queensland, but also obtained isolates from wheat stem bases that were normally found to be associated with stalk rot of maize and head blight of wheat. As a result, they separated the isolates into *Fusarium graminearum* Group 1 (causing crown rot of wheat) and *Fusarium graminearum* group 2 (causing stalk rot of maize and head blight of wheat). A distinguishing characteristic was that single spore cultures of Group 2 readily formed perithecia on a variety of substrates, whereas members of Group 1 did not – Francis and Burgess (1977) concluded that members of Group 1 are probably heterothallic and/or poorly fertile, or infertile.

With the advent of more modern molecular techniques, the taxonomic differentiation of *Fusarium graminearum* Group 1 and Group 2 underwent further scrutiny. Aoki and O'Donnell (1999) carried out morphological and molecular characterizations of *Fusarium graminearum* Group 1. By analyzing DNA sequences of the β-tubulin gene introns and exons, they concluded that the Group 1 family was a phylogenetically distinct species and renamed it *Fusarium pseudograminearum*. Further studies have supported the taxonomic split proposed by Aoki and O'Donnell. Benyon et al. (2000) used Restriction Fragment Length Polymorphism (RFLP) analysis of genomic and mitochondrial DNA to produce clusters of *Fusarium* isolates which corresponded with previously described morphological characteristics. Interestingly, *Fusarium crookwellense* and *Fusarium culmorum* shared closer genetic affinity with *Fusarium graminearum* rather than with *Fusarium pseudograminearum*, although ecological and phytopathological similarities between *F. crookwellense*, *F. culmorum*, and *F. pseudograminearum* suggest these species would share a greater genetic similarity than was demonstrated (Benyon et al., 2000).

In order to develop a rapid diagnostic assay to determine the species responsible for symptoms of crown rot disease, Williams et al. (2002) developed primers for use in polymerase chain reaction (PCR) assays. The primers used were able to detect *Fusarium* spp. present in single or mixed inoculation of seedlings, however, they did not distinguish between the individual *Fusarium* species tested. Tan and Niessen (2003) attempted to overcome this problem by analysing rDNA internal transcribed spacer (ITS) sequences; this technique was able to distinguish between *Fusarium pseudograminearium* and *Fusarium graminearum* however it could not distinguish between the more closely related head blight causing species *F. graminearum*, *F. culmorum*, and *F. cerealis*. In each of the studies carried out by Williams et al. (2002) and Tan and Niessen (2003), the identities, based on morphological characteristics, of a few of the isolates examined were not supported by the molecular evidence, thus showing the difficulty in distinguishing certain *Fusarium* species based upon culture morphology.

1.45 Environmental Factors Affecting Disease Development

Various environmental factors have been reported to affect levels of disease. The major factors include: rainfall patterns; soil moisture; crop nutrition; and stubble retention. Other factors (such as such as field topography and time of planting) also influence disease development, but to a lesser degree than those which are discussed below.

1.45.1 Rainfall Patterns

In Australia, crown rot is most prevalent on the cracking grey clay and black earth soils that are present in the central and northern regions of New South Wales and in southern Queensland (Liddell and Burgess, 1985). Although the prevalence of crown rot on these soil types may imply they are conducive to disease development, Burgess et al. (2001) point out that crown rot can also be severe on lighter soils such as those found in southern Australia and in the Pacific Northwest of the U.S.A. In the northern region of the Australian wheat belt (which encompasses northern New South Wales and southern Queensland), rainfall occurs predominantly through the summer months. McKnight and Hart (1966) reported that in the nine seasons from 1951 to 1959, the four seasons of severe incidence of crown rot were characterized by below average rainfall, whereas, in the five seasons of slight incidence, in-crop rainfall was approximately doubled. Numerous studies have supported this link between rainfall patterns and crown rot severity (Wildermuth et al., 1997; Felton et al., 1998; Swan et al., 2000), with wet starts (which encourage infection) and dry finishes (which produce moisture stress) considered conditions that increase the incidence and severity of crown rot.

1.45.2 Soil Moisture

A number of studies have been conducted to assess the relationship between both soil and plant water potential, and infection and development of crown rot through the growing season. Using wax layers for partitioning soil moisture zones, Liddell and Burgess (1985) were able to keep the subsoil moist while being able to manipulate the water potential of the top-soil. These authors

showed that infection of wheat seedlings was strongly inhibited by dry soil, and that moist, but not wet, soil was most conducive to initial colonization. Using a refined model of the wax partitioning technique, Beddis and Burgess (1992) further characterized the plant water relations that affect colonization. These authors concluded that low seedling water potential predisposes wheat seedlings to colonization by the fungus.

1.45.3 Crop Nutrition

Sparrow and Graham (1988), have demonstrated a link between zinc-deficiency and crown rot severity in glasshouse trials, such that the extent of colonization by *Fusarium pseudograminearum* above the inoculation point was greater in zinc-deficient plants than in non-deficient plants. Grewal et al. (1996) further investigated this relationship by examining the resistance of genotypes that were more efficient at extracting zinc from low zinc soils and comparing this to genotypes with poor zinc extraction efficiency. The zinc-efficient cultivar Excalibur had the greatest resistance to crown rot in zinc deficient soil. These authors conclude that growing zinc-efficient cultivars of wheat along with judicious use of zinc fertilizer in zinc-deficient areas will sustain wheat production by reducing the severity of the disease as well as increasing plant vigour (Grewal et al., 1996).

As mentioned previously, yields of Australian wheats are low compared to other international wheat cropping areas such as New Zealand. To combat the effects of poorly fertile, nutrient depleted soils, nitrogen and phosphorous are regular additives. Ironically however, the incorporation of nitrogen can lead to greater crown rot severity (Felton et al., 1998; Kirkegaard et al., 2004). This phenomenon is believed to be caused by rapid vegetative growth early in the season. Due to a lack of winter rainfall and subsequent dry finish, the increased biomass cannot be supported by a challenged vascular system, and hence, a greater proportion of diseased tillers and deadheads occur (Burgess et al., 2001).

1.45.4 Stubble Retention

Burgess and co-workers have been systematic in assessing stubble management regimes and their relationship with crown rot incidence and severity (Klein et al., 1988; Summerell and Burgess, 1988; Summerell et al., 1989; Summerell et al., 1990; Burgess et al., 1993; Swan et al., 2000). Undoubtedly, this environmental factor deserves the most consideration due to its profound effect on disease levels (Wildermuth et al., 1997). Indeed, Dodman and Wildermuth (1989) have attributed the increase in severity of crown rot in the western and south-western wheat areas of Queensland to the retention of stubble.

The relationship between stubble retention and increasing crown rot severity is due to the ability of the fungus to over-season on infected stubble (Wearing and Burgess, 1977), and then infect subsequent plantings. In a study aimed at determining the length of time *Fusarium pseudograminearum* survives on wheat straw, Burgess and Griffin (1968) found that the fungus could still be recovered after two years. More recently, the increase in stubble retention and minimum tillage practices has resulted in a build-up of inoculum in land managed in this way. This has been further exacerbated by the planting of susceptible cultivars – Dodman and Wildermuth (1989) estimated that between 1981 and 1987 between 58 to 76 percent of the area planted in Queensland was with susceptible cultivars.

1.46 Management

1.46.1 Stubble Management

Stubble management is critical for the control of crown rot because the incidence and severity of infection is directly related to inoculum build-up and persistence in stubble (Burgess et al., 2001). Various stubble management practices can also affect the site of penetration of wheat by the crown rot fungus. Summerell et al. (1990) have shown that when stubble is retained, penetration occurs principally through the crown and basal stem, but when stubble is incorporated into the soil, penetration occurs through the scutellum, subcrown internode, and lower crown regions. Regardless of the site of penetration however, basal regions were colonized to a similar extent at harvest (Summerell et al., 1990).

Stubble management practices affect the length of time the fungus is able to survive on wheat residues. Summerell and Burgess (1988) compared recovery of the fungus from retained stubble, from stubble incorporated into the soil by rotary hoeing, and from stubble that was buried in nylon mesh bags. When stubble was retained or incorporated, *Fusarium pseudograminearum* was still recovered after 104 weeks. In comparison, when buried in the nylon mesh bags, very low levels were recovered after only eight weeks, and not at all at 104 weeks. The authors suggest that the decline in the survival of the fungus is closely correlated with stubble decomposition (Summerell and Burgess, 1988).

The observation that there was no difference between incorporated and retained stubble in the study by Summerell and Burgess (1988) has been confirmed in a long-term study by Burgess et al. (1993). In this study, the effect of burning stubble was also investigated. Of the three stubble management regimes, stubble burning reduced the incidence of infection in some years, with the lack of reduction in years when burning was ineffective being attributed to susceptible weed hosts and poor burns. Dodman and Wildermuth (1989) also show that crown rot of wheat is less severe when stubble of the previous crop was burned rather than retained.

Simpfendorfer et al. (2005b) have also shown that burning reduces the incidence of crown rot, however, these authors strongly recommend against burning as a means of control. Their research showed that, although crown rot inoculum loads decreased, other important diseases (such as common root rot and take-all) became more prevalent. Furthermore, the authors point out that burning decreases soil organic carbon, soil water storage, and the activity of soil biota, while at the same time increasing the risk of soil erosion by wind and rain (Simpfendorfer et al., 2005b). Thus, the burning of stubble may not be an effective component of management strategies designed to reduce crown rot severity.

Infection by *Fusarium pseudograminearum* is initiated when plants come in contact with infected stubble (Backhouse pers. comm.). In order to decrease the

amount of contact between plants and stubble, Simpfendorfer et al. (2005a) examined the use of precision row placement and its effect on crown rot incidence and severity. By planting between previous cereal rows, the number of plants infected with *F. pseudograminearum* was reduced by 52% and disease severity was reduced by 60%. It was noted that this approach relies on minimal disturbance to previous rows in order to be effective (Simpfendorfer et al., 2005a).

1.46.2 Crop Rotation

Crop rotation is also an important management practice for controlling crown rot because of the range of cereal and other grass hosts the pathogen infects, and also due to its ability to survive for a number of years on infected stubble (Burgess and Griffin, 1968). Crop rotation is effective in lowering levels of inoculum by starving the pathogen of a suitable host (Burgess et al., 2001) and encouraging stubble breakdown.

Felton et al. (1998) examined crown rot of wheat and the disease-break effect of chickpea and reported that grain yields were about 1 tonne/hectare greater in a chickpea-wheat system compared to wheat following wheat. Furthermore, crown rot incidence for wheat after wheat was 16% compared with 2% for wheat after chickpea. Kirkegaard et al. (2004) compared the effectiveness of chickpea, canola, and mustard as break-crops. Their results showed that all three break crops were beneficial in reducing levels of crown rot infection (by 3.4-41.3%) and increasing yield of wheat (by 0.24-0.89 tonnes/hectare). The *Brassica* crops were found to be more effective than chickpea in reducing crown severity in a highly susceptible durum wheat, although this trend was less apparent in a more tolerant bread wheat (Kirkegaard et al., 2004). Apart from chickpea, canola, and mustard, other common break crops include: sorghum, mungbean, and dryland cotton in summer; and chickpeas, faba beans, field peas, and canola in winter (Burgess et al., 2001).

1.46.3 Biological Control

There appear to be only a few studies on the use of biological control agents to control crown rot of wheat. Huang and Wong (1998) assessed the usefulness of the bacterium *Burkholderia cepacia* as a biological control agent to combat crown rot. In a laboratory study where *Fusarium pseudograminearum* was paired with *Burkholderia cepacia* on the same agar plate, a clear zone of inhibition was seen. Furthermore, in glass house and field studies, *Burkholderia cepacia* significantly reduced crown rot symptoms (Huang and Wong, 1998). These promising results appear not to have been further investigated; this is presumably due to the association between the bacterium and increased mortality among, in particular, cystic fibrosis sufferers (Holmes et al., 1998; Fauroux et al., 2004).

Wong et al. (2002) examined the effectiveness of *Trichoderma* species in controlling *Fusarium pseudograminearum*. In laboratory studies, where infected wheat straw was sprayed with spores of *Trichoderma* species, the survival of *Fusarium pseudograminearum* was significantly reduced. These authors suggest that it may be possible for *Trichoderma* species to substantially reduce the inoculum of *F. pseudograminearum* during the 6-month fallow that is common in the summer-dominant rainfall areas of Australia. Cleary, these results need to be confirmed in a field situation, and studies of this nature are currently being carried out by New South Wales Agriculture and the University of Western Sydney (S. Simpfendorfer pers. comm).

1.46.4 Tolerant Varieties

Complete resistance to infection by *Fusarium pseudograminearum* has never been observed in a wheat host. However, potentially useful differences in cultivar reaction have been demonstrated in a number of studies (McKnight and Hart, 1966; Purss, 1966; Wildermuth and Purss, 1971), and some of the tolerant germplasm identified has the potential to minimize yield losses caused by crown rot. The selection of such partially resistant materials has been the subject of intense research. This selection often occurs in field situations, with either seed

inoculation (Purss, 1966; Wildermuth and Purss, 1971), inoculum added to the soil in the form of infected plant material (Dodman and Wildermuth, 1987), or by planting trials in fields where disease was high in the previous year (Dodman and Wildermuth, 1987, 1989). Unfortunately, assessment of suitably tolerant material is time-consuming. The technique used by Dodman, Wildermuth and co-workers (Dodman and Wildermuth, 1987, 1989; Wildermuth et al., 1997; Wildermuth et al., 2001) involves growing plants to maturity and harvesting the individual whole plants from the ground. This is followed by separation of individual tillers, and rating a large number of these tillers for the level of honey-brown discolouration. Experienced personnel are able to score approximately only 12-15 lines per day (G. Wildermuth, pers. comm.). Obviously, field screening is restricted to only one growing cycle per year. Furthermore, with the expression of disease severity strongly dependent on levels of in-crop rainfall and the degree of moisture stress late in the growing season, variation between years is problematic (Dodman and Wildermuth, 1987).

Liddell et al. (1986) tried to overcome some of the issues of field screening by examining a technique designed to reproduce crown rot infection in the field in greenhouse screenings. Using galvanized bins and various quantities of inoculum spread as a thin layer midway between the seed and the soil surface, they examined disease symptoms in the partially tolerant cultivar Cook, and the susceptible cultivar Songlen. After harvest (130 days after planting), symptoms and yield loss were similar to levels observed in the field. The authors conclude that the "technique is useful for studies on infection, colonization, and tolerance of various lines of wheat" (Liddell et al., 1986), however, there have been no reports of the use of the technique in later literature. This may, in part, be due to the amount of greenhouse space that would be required to conduct such an assay on a large scale. It should also be noted that the study was only carried out on two wheat cultivars, and testing lines of intermediate tolerance may have added more strength to an argument for the routine use of the technique.

As a result of the difficulties associated with field screening, significant effort has been concentrated on devising a seedling test that is a reliable indicator of resistance in the field. It should be noted that Purss (1966) was not able to

demonstrate any correlation between seedling blight in the seedling tests he conducted and field reaction. As Liddell et al. (1986) point out however, Purss (1966) used inoculated seed for his seedling test, and the plants usually died at an early post-emergent stage. Furthermore, the fungus is not normally seed borne and has been reported not to cause serious seedling death in the field (Liddell et al., 1986).

Klein et al. (1985) have reported on the development of an assay for testing tolerance of wheat to crown rot in replicated seedling trials. By using colonized grain as a source of inoculum, and spreading this across the surface of the soil, these authors were able to demonstrate differences in tolerance to crown rot in eight cultivars. Disease ratings were taken at various time-intervals (up to 102 days post-inoculation), and seedling tolerance was correlated with adult plant tolerance in six of the eight cultivars expressed. The results were consistent across two seedling trials carried at different locations and by different operators, demonstrating the robustness of the technique (Klein et al., 1985).

Subsequently, Wildermuth and McNamara (1994) developed a seedling test for assessing tolerance to crown rot by examining factors such as temperature and different means of inoculation (banded, whereby a layer of inoculum is placed between the seed and the soil surface; and dispersed, whereby inoculum is dispersed within the soil matrix). They found that growing the seedling at 25˚C decreased the time needed for assessment of tolerance, and that the banded inoculum increased the likelihood of an emerging seedling contacting the inoculum. This test was completed in a three week period, and a relatively high correlation (R^2=0.6) was shown between seedling reaction and field reaction between the 28 genotypes examined (Wildermuth and McNamara, 1994).

Wallwork et al. (2004) have argued that only the most tolerant sources of resistance can be detected in seedlings, whereas other more intermediate sources, which may be useful for breeding programs, can be lost. As a result of this perceived problem, Wallwork et al. (2004) have developed an improved method for screening adult plants for resistance to crown rot. This method involves growing plants in open-ended tubes (100mm long x 50 mm) set in galvanized

baskets which are then placed outdoors on a sand base (the "Terrace"). Potting mix is used to fill the tubes up to three quarter level, the seed is placed on this soil, and the remainder of the tube filled with potting mix with *Fusarium pseudograminearum* inoculum dispersed. The method allows for screening of a greater number of plants than the conventional technique, however, the variability that is associated with field trials still exists (Wallwork et al., 2004).

Mitter et al. (2006) have recently published a high-throughput glasshouse bioassay for determining resistance to crown rot in wheat. This technique involves the placement of a droplet from a macroconidia suspension directly onto the base of the stem of seedlings. The results show a good correlation between seedling resistance and field resistance (as previously determined by the Queensland Department of Primary Industries), although only a few genotypes were used in this comparison. The authors acknowledge this limitation and state that a broader range of genotypes need to be tested in order to confirm preliminary results (Mitter et al., 2006). A potential problem with this assay is that it does not make any effort to mimic infection processes that occur in the field – infection with conidia is not believed to be an important aspect in infection in the field (Wildermuth pers. comm.). The authors are critical of the seedling test developed by Wildermuth and McNamara (1994) and believe that the addition of inoculum to the soil adds to variability due to uneven distribution. While this may be the case in other assays, whereby inoculum is distributed through the soil profile, the method of Wildermuth and McNamara (1994) uses a banded inoculum that will decrease such variability. An advantage of the Wildermuth and McNamara (1994) technique is that the seedlings grow through a chaff that is infected with hyphae – a closer representation to the field situation compared to the Mitter et al. (2006) method. Nevertheless, if the relationship between seedling and field resistance is further examined and shown to be positive, a high-throughput assay would be desirable for future studies on crown rot resistance.

1.47 Gene Expression of Tolerant Varieties

Only one study has been conducted to explore gene expression in response to infection with crown rot. Desmond et al. (2006) analysed the expression of various defence genes including a number of pathogenesis related proteins, peroxidase, and germin-like protein. This work was conducted using the inoculation procedure described by Mitter et al. (2006) and examined the responses of seedlings of the wheat cultivars Kennedy (referred to as susceptible by the authors) and Sunco (referred to as partially resistant by the authors). The authors state that seedling inoculations did not clearly demonstrate greater crown rot resistance in Sunco compared to Kennedy (Desmond et al., 2006). This is not surprising given that it is widely recognized that Sunco does not exhibit seedling resistance to crown rot – the partial resistance claimed for Sunco has been determined from rating adult plants in field trials (Wildermuth and McNamara, 1994), a point the authors concede in their discussion. The study also examined a potential role for systemic acquired resistance by pre-treating plants with methyl jasmonate prior to infection. Not surprisingly, pre-treatment with methyl jasmonate equally delayed the development of necrotic symptoms for two weeks in both the wheat cultivars Sunco and Kennedy. Our understanding of the mechanisms of resistance to crown rot remains poor, and thoughtful, further studies are needed on this subject.

1.5 The Wheat Genome

Langridge et al. (2001) suggest that there are three features of wheat that add greatly to the complexity of breeding and selection. These are: the wide range of end uses; the genome size; and the level of polymorphism. A discussion of the wide range of end uses is beyond the scope of this review, but genome size and level of polymorphism will be considered below, since these greatly affect the efficacy of molecular markers as selection tools.

The size of the complex hexaploid bread wheat genome is approximately 16,000 Mb (Gill et al., 2004) – this is roughly 7 times larger than the maize genome (Palmer et al., 2003), and 40 times larger than the rice genome (Sasaki and Burr,

2000). Wheat chromosomes have regions of high gene density interspersed by large regions of repetitive DNA. Based on a sample of 3025 gene loci, Erayman et al. (2004) reported that 29% of the wheat genome contains 94% of the genes, with 60% of the genes concentrated in only 11% of the genome. Regardless of size, the molecular unravelling of the wheat genome has been further confounded by its composition. Wheat is an allopolyploid, formed from the hybridization and subsequent chromosome doubling of two (durum wheat) or three (bread wheat) diploid donors. Each of the diploid donors are different species, however, there is a large degree of similarity between these donors. Based on molecular studies, the divergence of the diploid donors from a common progenitor is believed to have occurred 2.5-4.5 million years ago (Huang et al., 2002a), and it is because this divergence is relatively recent that there is a high degree of synteny between the three genomes of bread wheat. For molecular studies, the close relationship between each of the genomes can make the assignment of markers to chromosomes difficult. Furthermore, the generally low level of polymorphism in wheat compared to other grass species means that a large number of markers need to be screened in order to identify polymorphisms (Langridge et al., 2001).

There is, however, an advantage gained from the synteny between genomes. The pioneering work of Sears (1954) exploited this synteny, in which he developed a set of aneuploid lines within which homoeologous chromosomes are able to compensate for the absence of others. For example, the line nullisomic 5A / tetrasomic 5B has lost both copies of 5A but has four copies of 5B. Sears and Sears (1978) produced ditelosomic lines, whereby one arm has been lost from a chromosome. Endo and Gill (1996) characterised a set of deletion lines, in which segments of individual chromosome are missing. The aneuploid (nulli-tetrasomic and deletion) lines have been used to identify the chromosomal location of genes and markers (Gill et al., 1993; Huang et al., 2000; Qi et al., 2003), and are thus powerful tools for unravelling the genetics of wheat.

1.6 Genetic Markers

The selection of superior genotypes by conventional plant breeding is time consuming and often dependent upon environmental conditions. As a result, plant breeders are interested in improved techniques that will make the selection of better varieties more reliable and timelier. Marker technology offers a wide range of novel approaches for improving the efficiency of selection (Langridge et al., 2001).

Genetic markers can be classified into three broad groups: 1) morphological markers; 2) biochemical markers; and 3) molecular markers. Genetic markers represent diversity between individuals; often they do so not because they are a part of the target genes themselves, but rather act as neighbouring "flags" for the genes (Collard et al., 2005a). Morphological and biochemical markers are referred to as "classical" markers but only a few are routinely used due to drawbacks such as their limited number and frequent dependence on environmental factors or developmental stage of the plant. Furthermore, they often act in dominant-recessive fashion, which makes it impossible to identify heterozygous individuals (Kumar, 1999). Molecular markers are currently the most widely used type of marker as they are potentially unlimited in number and unaffected by environmental conditions.

1.61 Molecular Marker Types

A number of molecular marker systems have been used to detect sequence variation between individuals (Langridge et al., 2001). These include (but are not limited to): random amplified polymorphic DNA (RAPD); restriction fragment length polymorphism (RFLP); amplified fragment length polymorphism (AFLP); simple sequence repeats (SSRs, commonly referred to as microsatellites); expressed sequence tags (ESTs) with SSRs in their sequence (EST-SSRs); and single nucleotide polymorphisms (SNPs; Table 1-2). RAPD technology, although technically simple and able to amplify multiple loci, has lost favour with molecular biologists due to the poor reproducibility that is a

result of using short, random primers in conjunction with low annealing temperatures (Jones et al., 1997). The RFLP technique is robust, but compared with PCR based techniques, it suffers in that it is technically demanding, requires large quantities of DNA and detects only a limited amount of polymorphism (particularly between closely related genotypes). AFLP is a reliable technique that is based upon restriction digestion in conjunction with PCR (Kumar, 1999). The AFLP technique results in the production of numerous fragments and detects high levels of polymorphism, however, disadvantages (such as being dominant markers and having a low level of transferability between mapping populations) limit their usefulness.

Table 1-2. Advantages and disadvantages of commonly used molecular marker types (adapted from Collard et al. (2005) and Langridge et al. (2001)).

Marker Type	Advantages	Disadvantages
RAPD	• Technically simple • Inexpensive • Amplify multiple loci	• Unreliable • Dominant
RFLP	• Reliable • Co-dominant • Target specific regions	• Technically difficult • Requires large amounts of DNA • Limited polymorphism
AFLP	• Reliable • High levels of polymorphism • Amplify multiple loci	• Dominant • Technically difficult • Random
SSRs	• Reliable • Technically simple • Target specific regions • Co-dominant • Transferable between mapping populations	• High development cost
EST-SSRs	• Functional • Developed at no cost • High level of transferability	• Lower polymorphism level than SSRs
SNPs	• Functional • Extremely abundant • High throughput genotyping	• Potentially high development cost

In recent years, the marker of choice for many laboratories is, by far, the SSR. SSRs are genomic regions that consist of a mono-, di-, tri- or tetrameric sequence

repeated multiple times in a tandem array for which the level of repetition may vary between genotypes (Hearne et al., 1992). The advantages of SSRs over other marker types are many – they are reliable; they target specific genomic regions but are also dispersed throughout the genome; the assays are technically simple to conduct; and the products are generally co-dominant. Furthermore, they are generally transferable between mapping populations. The disadvantage of the technique results from the expense and effort required to identify suitable primer sequences which flank these regions (Collard et al., 2005b). However, in recent years, the sequences for large numbers of primers for a number of organisms have been made publicly available (http://wheat.pw.usda.gov/GG2/index.shtml), and, as a result, smaller laboratories are able to harness the power provided by this marker type.

EST-SSRs are derived from ESTs. ESTs are typically unedited, automatically processed single-read sequences produced from cDNAs (small DNA molecules reverse-transcribed from cellular mRNA, Rudd, 2003). Because ESTs are reverse-transcribed from mRNA, they provide a "snap-shot" of the transcribed region of the genome. There is a wealth of EST sequence information - as at January 26 2007, there are over 855,000 EST sequences present in public databases. Through bioinformatics approaches, it is possible to identify SSRs within EST sequences (Gupta et al., 2003). The advantages of EST-SSRs are that they are functional (and thus may enhance the role of genetic markers by assaying variation in known function genes); and that development costs are very low (simple scripts can be written to search the database for repetitive sequences). Their major disadvantages are that they are not as polymorphic as SSRs (Eujayl et al., 2002; Peng and Lapitan, 2005).

SNPs are single base-pair changes at specific sites in the genome (Langridge et al., 2001). The same bioinformatics strategy for identifying EST-SSRs has been used to identify SNPs, and a pilot study has shown that one SNP is present for every 540 bp of wheat EST sequence (Somers et al., 2003). Alternative methods of detection can, however, be expensive (Gupta et al., 2001). SNPs are not routinely used markers, however, due to their abundance, a major project funded by the NSF is currently mapping SNPs in tetraploid and hexaploid wheat

(http://rye.pw.usda.gov/snpworld/Search) and it envisaged that this marker type will dominate wheat genetics studies in the future (Rafalski, 2002).

1.62 Applications of Molecular Markers

1.62.1 Germplasm Diversity Analysis

Molecular markers are useful tools for assessing diversity within germplasm collections (Langridge et al., 2001). The information gathered from such studies provides insights into the relatedness of genotypes within a collection. For example, by analyzing genetic diversity among 998 accessions of hexaploid bread wheat using a set of 24 SSR markers, Huang et al. (2002b) showed that accessions from the Near East and Middle East exhibited more genetic diversity than those from other regions. Roussel et al. (2005) used SSR markers to study allelic diversity changes in 480 European bread wheat cultivars released from 1840 to 2000. The results of this study have shown that, when seven successive periods of release were considered, the total number of alleles was quite stable until the 1960's, after which time it regularly decreased (Roussel et al., 2005). These authors conclude that European breeders should increase their exchange of genetic resources in order to expand material and improve cultivars (Roussel et al., 2005). Fu et al. (2006) used 37 EST-SSRs in their study of the germplasm diversity of Canadian hard red spring wheat and also concluded that recent breeding efforts have reduced genetic diversity in hard red spring wheat. In contrast to the results of Roussel et al. (2005) and Fu et al. (2006), Parker et al. (2002) using a set of 19 SSR markers, found that, in Australia, the older varieties were genetically less diverse than the newer varieties – this was attributed to the much broader range of genetic material available to and utilized by breeders in recent years. The information provided from such studies is thus useful, and can be used by plant breeders to make more informed decisions when selecting parents to be included in a crossing program.

1.62.2 Construction of Genetic Linkage Maps

A major use of molecular markers is in the construction of genetic linkage maps (Korzun, 2002). Genetic linkage maps indicate the position and distance

between markers, thereby giving a graphical representation of the arrangement of markers along chromosomes (Collard et al., 2005a). For plant breeding programs, the most important use of linkage maps is the identification of regions of the genome which contribute to a phenotype. Three major steps are involved in the construction of a genetic linkage map: 1) production of a mapping population; 2) polymorphism assessment; and 3) linkage analysis.

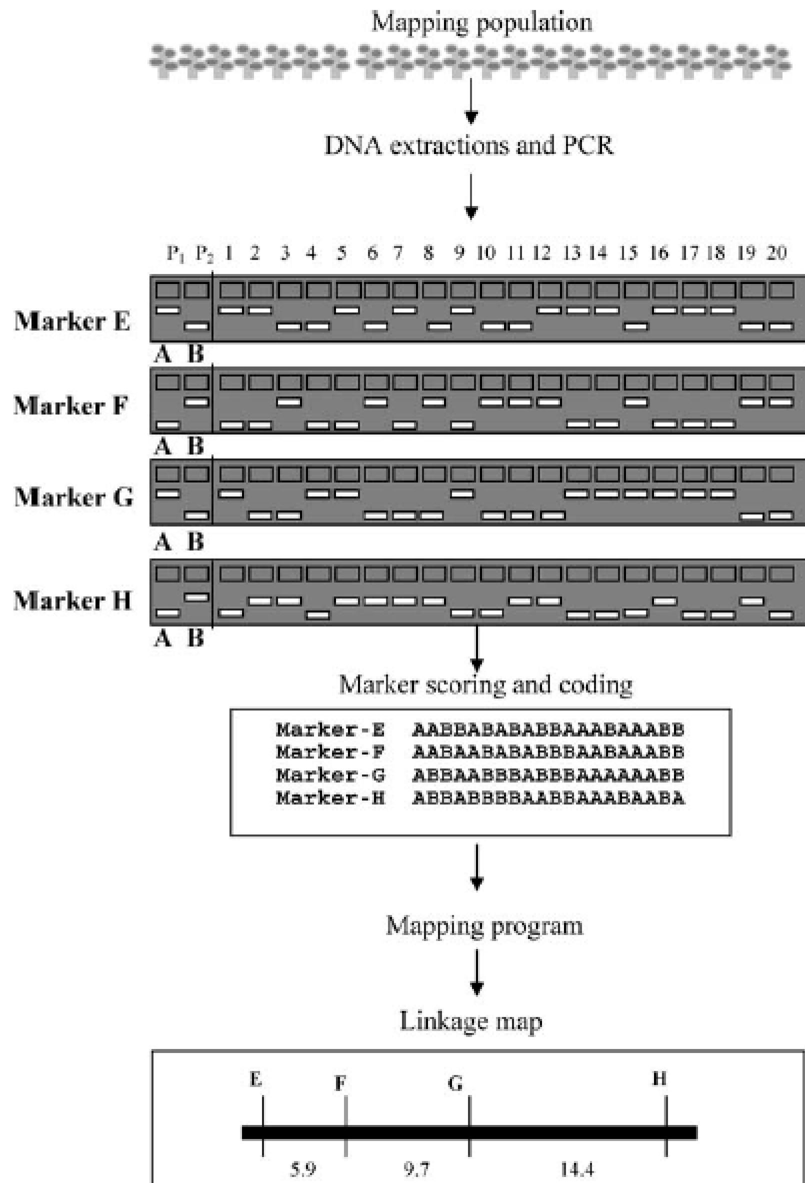
Kumar (1999) suggests that the most important step of linkage map construction lies with the selection of appropriate parental lines. It is critical that the parents chosen for population construction are genetically diverse so as to exhibit enough polymorphism for the construction of a map (Young, 1996). It is possible to undertake a "molecular screen" of the parental genotypes to confirm sufficient polymorphism, although, more commonly, parent lines are chosen based upon how much they differ for the phenotype of interest. The parents are then crossed to produce a segregating population - such as an F_2 population; backcross population; recombinant inbred population; or doubled haploid population (for example).

Following the production of a suitable mapping population, the parental lines are screened for polymorphism. The markers that are shown to have different size alleles (i.e. polymorphic) between the parents are then screened across the entire population to generate the marker data required for linkage analysis.

Linkage analysis is conducted on the genotypic data that is produced by screening polymorphic markers across the population of interest. This step involves coding data for each marker on each individual (Collard et al., 2005a), and using appropriate computer software to determine the most likely position and order of markers on a map. While it is possible to manually determine position and distance between markers for a small number of markers, the large number of markers used to create linkage maps renders the use of computer programs a necessity. A suite of programs can perform linkage analysis, and these include for example: MapMaker/EXP (Lander et al., 1987); JoinMap (Stam, 1993); MapManager QTX (Manly et al., 2001); and RECORD for marker

ordering (Van Os et al., 2005). With the exception of JoinMap, these computer programs are available freely over the internet.

Figure 1-2. An overview of the steps involved in linkage map construction. DNA is extracted from the mapping population and PCR conducted. Markers are scored on individuals to reflect which parent has contributed the region in each of the individuals. The data is entered into a mapping software program to calculate distances between the markers. Image reproduced with permission from Collard et al. (2005a).



1.62.2.1 Wheat Genetic Linkage Maps

Early wheat genetic linkage maps were composed of RFLP markers (Chao et al., 1989; Devos et al., 1992; Nelson et al., 1995), but with the advent of the PCR-based techniques, these were superseded by maps composed of RAPD (Williams et al., 1990; Devos and Gale, 1992), AFLP (Vos et al., 1995), and SSR markers (Roder et al., 1998; Pestova et al., 2000; Gupta et al., 2002). The wheat genetic maps that have been constructed vary considerably in length. Chalmers et al. (2001) reported map distances of a Cranbrook x Halberd doubled haploid (DH) population at 4110cM, a CD87 x Katepwa DH population at 3484cM, and a Sunco x Tasman DH population at 3164cM. Paillard et al. (2003) reported a distance of 3086cM for a linkage map based upon a cross between two Swiss winter wheat varieties. The map generated from a cross between Courtot and Chinese Spring by Sourdille et al. (2003) is 3685cM. Somers et al (2004) produced a consensus SSR map by joining data from four independent genetic maps, and the 1235 SSR markers incorporated into the map produced a final map distance of 2569 cM. While the genetic distance of the maps varies considerably, the common ground between these maps is that they are all composed of large numbers of markers. This is highly desirable, because the construction of a genetic linkage map is often the first step to identifying regions of the wheat genome that contribute to the expression of quantitative traits.

1.62.3 QTL Mapping

Many traits of interest to breeding programs show a continuous range of values (for example yield and quality) rather than forming distinct classes. Such traits are under the control of several genes (and the environment) that are referred to as polygenes or quantitative trait loci (QTL) (Tanksley, 1993). Molecular markers are useful for identifying loci that control quantitative traits (Langridge et al., 2001) because markers which tend to be transmitted with specific values of the trait are likely to be close to a gene affecting the trait (Doerge et al., 1997).

1.62.3.1 Methods to Detect QTL

The simplest methods to detect QTL are carried out with statistical tests such as ANOVA and linear regression (Hackett, 2002), likelihood analyses (Doerge et al., 1997) or t-tests (Collard et al., 2005a). For a doubled haploid population, the process involves scoring the marker data of the population, and calculating and comparing phenotypic means of the two genotypic classes to identify significant differences. If a significant difference is found, it is determined that the marker is linked to a gene affecting the trait of interest. These types of analyses do not require a genetic linkage map, and are referred to as single point or single marker analysis. The main disadvantage of single point analysis is that the further away the marker is from the gene, the less likely it is to be detected statistically due to recombination between the marker and the gene (Tanksley, 1993).

To overcome the problems associated with single marker analysis, Lander and Botstein (1989) devised the method of interval analysis (or interval mapping). This method requires a genetic linkage map. Interval mapping builds upon single point analysis by compensating for recombination between the marker and the gene affecting the trait of interest by using linked markers for the analysis (Lander and Botstein, 1989). As Tanksley (1993) points out, this method is of maximum benefit when linked markers are fairly far apart (because of a large number of recombination events), but when markers are more dense, the single point analysis gives similar results to interval mapping.

Composite interval mapping (Zeng, 1994) and multiple QTL mapping (Jansen, 1993) are further methods that may be used for QTL detection. Interval mapping assesses the likelihood of a single QTL at each location on the genome – however QTL located elsewhere on the genome can have an interfering effect (Jansen, 1993). By combining interval mapping with multiple regression (using markers associated with other QTL as cofactors), these techniques are superior to interval mapping and increase the accuracy and precision of QTL detection (Hackett, 2002).

1.62.3.2 Considerations for QTL Analysis

Each of the methods to detect QTL (single marker, interval mapping, composite interval and multiple QTL mapping) are able to be carried out using a range of computer programs – however as Asins (2002) aptly concludes: "QTL mapping is much more than running a programme". Asins (2002) points out other factors that should be considered, including: population size; the heritability of the trait; the number of QTL; their interaction; and the reliability of the marker order of the linkage map.

The size of the population and the heritability of the trait (the proportion of phenotypic variance that is genetic (Lynch and Walsh, 1998)), are the most important factors that affect QTL mapping studies (Collard et al., 2005a). Tanksley (1993) suggests that, with typical population sizes used for QTL mapping (100-250 individuals), only QTL with large effect are likely to be identified, and furthermore, the effect of QTL that are identified can be over-inflated. In a theoretical assessment, Lande and Thompson (1990) showed that the proportion of the genetic variance explained by the QTL is inversely related to the product, h^2N, (where h^2 is the narrow-sense heritability of the trait and N is the population size), such that, in traits with low heritability, only QTL with large effects will be identified with typical population sizes. Studies using both simulated and experimental data have confirmed these hypotheses. In a simulation study, Beavis (1994) reported that phenotypic variances associated with QTL are greatly overestimated, and that this is most pronounced if only small populations (e.g. 100 individuals) are evaluated. Beavis (Beavis, 1994) suggested that the actual phenotypic variance explained by QTL can only be accurately estimated from populations of between 500 and 1000 individuals. Furthermore, if 10 loci affect a trait of 30% heritability and a population of 100 F_2 is used for mapping, each true QTL will be identified as significant with only 9% probability, and the variance explained overestimated by 5.6 times the true value (Beavis, 1994, 1998). Melchinger et al. (1998) confirmed the low power of QTL detection and large bias of QTL effects by comparing population sizes of N = 344 and N = 107 for detection of QTL controlling various agronomic traits

in maize. With the larger population, 107 QTL were detected. With the smaller population, 39 QTL were detected. Only 20 QTL were in common between the different sized populations. Melchinger et al. (1998) concluded that QTL effects need to be estimated in an independent population before they can be used with any reliability in marker-assisted selection schemes (see section 1.62.4 Marker-Assisted Selection). It should be noted that Lande and Thompson (1990), in 1990, also suggested that a way to obtain unbiased estimates of QTL effects was to map QTL in one cross and then confirm the effects of the detected QTL in another population (so called "marker validation"). It is likely that the recommendations of Lande and Thompson (1990) were infrequently followed because of the cost of genotyping in the past. More recently however, the cost of genotyping has decreased substantially, and validation studies are becoming a more frequent, companion component to QTL detection studies.

From the above paragraph it should be clear that the heritability of the trait and the size of the mapping population will have an effect on the number of QTL that can be detected. With smaller population sizes, fewer QTL (and only those with large effects) will be identified. Kearsey and Farquhar (1998) point out that, because only QTL with significant effects are reported in the literature, the phenotypic effects of the reported QTL will be biased towards larger values. These biases are larger with QTL of small effect, and thus imply that studies will tend to underestimate the true number of QTL, but exaggerate their effect (Kearsey and Farquhar, 1998).

Carlborg and Haley (2004) suggest that interactions between QTL (epitasis) are often neglected in studies of complex traits. The lack of studies demonstrating epistatic interactions between QTL has been attributed to: the low statistical power in small population sizes that are typically used in QTL mapping (Tanksley, 1993); the availability of suitable molecular evidence to assess such interactions (Carlborg and Haley, 2004) and; the availability of appropriate statistical tests to assess their significance (Cordell, 2002). However, with the production of a now large number of "whole-genome" molecular maps (see for example Chalmers et al., 2001; Sourdille et al., 2003), investigations of the importance of epistasis are now becoming more common. In rice, for example,

epistatic interactions have been shown to be important in a range of phenotypes such as panicle number (Liao et al., 2001), yield components (Xing et al., 2002; Zhuang et al., 2002), and heterosis (Yu et al., 1997). In wheat, epistatic interactions appear important for the effects of glutenin loci on dough rheological properties (Ma et al., 2005). Each of these studies have found that the contribution to phenotypic variance of main effect QTL is larger than that of epistatic QTL – nonetheless, the apparent ubiquity of epistatic interactions warrants further investigation of this phenomenon in QTL mapping studies.

The ordering of loci within linkage groups is of great importance for robust QTL detection. Wu et al. (2003) have suggested that even if QTL detection methods are appropriate, QTL identified may be incorrect if marker order is inaccurate. Indeed, in a recent study investigating the effect of thorough map curation, Lehmensiek et al. (2005) found that reordering of marker loci not only improved QTL resolution, but also affected the magnitude of QTL effect. In contrast to the findings of Wu et al. (2003) and Lehmensiek et al. (2005), Dodds et al. (2004), using simulation techniques to assess the effects of incorrect map order on QTL detection, found that, provided no markers are assigned to an incorrect linkage group, the accuracy of the map had little or no impact on the detection of QTL. The findings of Dodds et al. (2004) are surprising given the number of reports that emphasise the importance of an accurate marker order for QTL mapping (Asins, 2002; Wu et al., 2003; Lehmensiek et al., 2005; Van Os et al., 2005; Van Os et al., 2006). The simulation study of Dodds et al. (2004) may not have found limitations of marker order because the study only investigated the effects of one or two markers being ordered incorrectly- had more markers been "misplaced" a greater effect of marker order may have been identified.

1.62.3.4 Bulked Segregant Analysis – A Shortcut to Detecting QTL?

Bulked-segregant analysis (BSA; also referred to as distributional extreme analysis) is a technique devised by Michelmore et al. (1991) that involves pooling the DNA of individuals at the extremes of a phenotypic distribution and then using molecular markers to identify differences between the "bulks". Clear polymorphisms between the two bulks are derived from regions of the genome

that are common between individuals of each pool, but different between each pool (Langridge et al., 2001), and therefore indicate linkage between the marker and the trait of interest. Most successful reports of using BSA to identify molecular markers linked to traits of interest have involved qualitative traits such as powdery mildew resistance (Xie et al., 2004), common bunt resistance (He and Hughes, 2003), and leaf rust resistance (Cherukuri et al., 2003). When used in studies on quantitative traits, the BSA technique appears most useful at identifying loci with large phenotypic effects (Cook et al., 2004). Thus, although the technique may appear to provide a more efficient mechanism to find markers linked to genes of interest, only loci with large effects are likely to be identified, and thus, for quantitative traits, construction of complete genetic linkage maps and QTL mapping is likely to identify a more complete set of contributing QTL.

1.62.4 Marker-Assisted Selection

Conventional plant breeding has relied upon careful phenotypic selection of superior progenies from segregating populations for advancement through breeding programs. This approach has been very successful (Kumar, 1999), but with the advent of molecular marker technologies, there exists the potential to improve the efficiency of breeding for desirable phenotypes. Marker-assisted selection (MAS) is a technique that involves the selection of plants carrying genomic regions of interest through the use of molecular markers (Babu et al., 2004). The perceived advantages of MAS to breeding programs are many. These include:

- increasing the efficiency of backcross breeding strategies;
- combining (pyramiding) genes for traits of interest; and
- incorporating target QTL into breeding programs (Collard et al., 2005a; Francia et al., 2005).

The success of MAS will depend upon the location of the marker with respect to the gene contributing to the quantitative trait. Markers located within the gene of interest are the most sought after but these usually require the target gene to be cloned (Francia et al., 2005). Generally, markers are not located within the target

gene and tightly linked flanking markers are required to accurately locate the QTL controlling a trait of interest. Markers located closely either side of QTL are used to minimise the chance of double recombination events between the QTL and both flanking markers (Doerge, 2002).

1.62.4.1 Backcross Breeding

Backcross breeding is used to transfer genes from a donor genotype into the genetic background of a recipient genotype (Frisch, 2004). Most commonly, this process involves a target trait that is controlled by a single gene. Compared to conventional backcrossing, the use of MAS improves the efficiency of the backcrossing process in a number of ways. Firstly, for traits that are difficult to phenotype, selection for a marker allele close to the target gene can increase the efficiency and accuracy of selection (Langridge and Chalmers, 2004). An example of such a trait is resistance to barley yellow dwarf virus. Resistance to this aphid vectored virus is extremely difficult to screen for (Ayala et al., 2001), and the identification of tightly linked molecular markers for resistance has allowed the successful introgression of the resistance gene into a wheat breeding program (Zhang et al., 2004). Secondly, in the case of recessive genes, the use of markers overcomes the additional selfing generations needed and thus decreases the time it takes to achieve the desired outcome (Francia et al., 2005). Thirdly, markers can be used to select backcross progeny with the least amount of donor chromosome flanking a target locus on the 'carrier chromosome" thus reducing linkage drag (retention of unwanted segments of donor DNA). Chen et al. (2000) used this approach in the improvement of an elite restorer line to bacterial blight resistance, and were able to produce an improved version of the restorer line that differed only in a 3.8 cM fragment from the donor parent. Finally, by selecting markers on "non-carrier chromosomes" (i.e. those which do not contain target loci) the recovery of the recurrent parent can be greatly accelerated. This is called 'background selection', and simulation studies suggest that two or three generations can be saved by using markers compared to conventional backcrossing (Frisch et al., 1999).

1.62.4.2 Gene Pyramiding

MAS is particularly well suited to the pyramiding of disease resistance genes (Feuillet and Keller, 2004). This approach has mainly been used to combine major genes into a single genotype in order to provide more durable resistance. Such a goal can be difficult to achieve in conventional breeding, because it is difficult to select multiple resistance genes based on phenotype alone as the action of one gene may mask the action of another (Mohler and Singrun, 2004). There are a number of successful examples of the use of marker-assisted selection for gene pyramiding. Hittalmani et al. (2000) combined three major genes for blast resistance in rice, and found that the two- and three-gene pyramids conveyed enhanced resistance compared to effects of the individual genes. Datta et al. (Datta et al.) successfully pyramided the genes *Xa21* (resistance to bacterial blight), *Bt* (resistance to insects), and a chitinase (broad-spectrum fungal resistance-associated enzyme) by crossing transgenic parental lines transformed independently with the different genes. The resultant pyramid showed resistance to bacterial blight, resistance to yellow stem borer, and high tolerance to sheath blight caused by *Rhizoctonia solani* (Datta et al., 2002). More recently, Zhang et al. (2006) combined two genes (*Xa7* and *Xa21*) for resistance to bacterial blight into an elite hybrid rice restorer line. The two gene pyramid that was constructed provided a higher level of resistance to bacterial blight, with the authors concluding that the combining of major dominant resistance genes is a useful approach for improving bacterial blight resistance in hybrid rice (Zhang et al., 2006). These examples thus show the effectiveness of MAS to achieve goals of pyramiding to provide enhanced resistance to diseases.

1.62.4.3 Incorporating QTL into Breeding Programs

MAS has been touted as having the potential to revolutionise plant breeding and lead to the occurrence of another "Green Revolution" (Naylor and Manning, 2005). Presently however, and as was described in previous sections, MAS is routinely used in plant breeding programs only for selecting alleles with large effects on traits with simple inheritance (Holland, 2004). While these activities provide evidence of the value of MAS, many traits of agricultural importance

(such as yield, quality and resistance to certain diseases) are under polygenic control (Tanksley, 1993), and successful application of MAS for such polygenic traits is highly desirable, but not common. Indeed, Mohler and Singrun (2004) suggest that the incorporation of loci that contribute to quantitative traits (quantitative trait loci; QTL) into breeding programs is the principle task of MAS.

MAS for QTL can theoretically be achieved simply by selecting for the presence of specific marker alleles that are tightly linked to, or flank, favourable QTL alleles. However, despite an explosion in the reports on the identification of QTL for various traits, MAS for quantitative traits is often unsuccessful (Langridge and Chalmers, 2004). Francia et al. (2005) have described a number of reasons as to why this is the case. These include: uncertainty of the QTL position; deficiencies in QTL analysis leading to under-estimation or over-estimation of the number and magnitude of effects of QTL; an inability to detect a QTL-marker association in divergent backgrounds; the possibility of losing target QTL due to recombination between marker and QTL; difficulty in evaluating epistatic effects; and difficulty in evaluating QTL x environment interactions (Francia et al., 2005).

Many of the problems of adopting MAS for QTL that were highlighted by Francia et al. (2005) relate to deficiencies in the original QTL mapping experiments, and these were discussed in a previous section (see section 1.62.3.2). It is worth noting that MAS for QTL is highly desirable because phenotypic selection for some quantitative traits is often unreliable. In some instances, this is because the phenotypic tests themselves do not accurately reflect the intricacies of a certain trait, and in these cases, MAS for QTL may remain unsuccessful even if the problems Francia et al. (2005) highlight are overcome. Holland (2004) describes this as the “catch-22” of MAS for quantitative traits: if phenotypic data are poor indicators of genotypic values, it is difficult to accurately map QTL to implement MAS, but if phenotypic data are reliable, MAS may not be needed to make genetic improvement unless phenotypic screening is expensive or slow. Nonetheless, MAS for QTL remains

an active area of research due to the potential benefits the application of the process can bring to breeding programs.

1.62.4.4 Cost-Benefit Analyses

The cost of conducting MAS compared to conventional breeding will have an impact on the choice of method a plant breeder may employ. Researchers from CIMMYT have provided results of a case study highlighting their experience when comparing two MAS strategies with two conventional breeding strategies aimed at incorporating a single elite allele into a single elite maize line (Dreher et al., 2003; Morris et al., 2003). These results showed that the conventional breeding strategies were more cost effective, but that the MAS strategies were completed in less time. These authors concluded that ultimately the best strategy depends on operating capital – if operating capital is abundant then the best strategy is that which maximises the net present value (i.e MAS; Morris et al., 2003).

Kuchel et al. (2005) provided a more comprehensive cost-benefit analysis by examining the point at which molecular markers are applied in a selection strategy that integrated both restricted backcrossing and doubled haploid technology. This computer simulation study was based on defect elimination in the high yielding cultivar Stylet, due to a rust pathotype which possessed virulence against this cultivar. Four selection strategies were examined: A) no MAS; B) allele enrichment in the BC_1F_2 population; C) allele enrichment at the BC_1F_2 stage and screening of haploid regenerates to ensure that all haploids undergoing chromosome duplication were of semi-dwarf phenotype, carried the desired rust resistance genes, and had the potential to make high quality end products (through selection for desirable glutenin alleles); and D) the same as C) but doubled haploids were screened with random markers to eliminate individuals that carried less than 30% of the Stylet genome. This simulation showed that strategy D) was the most effective in terms of delivering a high frequency of desired outcomes and at combining the favourable rust resistance, end use quality and grain yield alleles. However, when costs were incorporated, strategy C) was identified as the optimal strategy, and not only did this strategy increase genetic gain over the phenotypic alternative but actually reduced the

overall cost by 40%. In the no MAS strategy, haploid lines not meeting selection criteria for rust resistance and dough strength were subjected to chromosome doubling as there was no opportunity for phenotypic selection between haploid and doubled haploid phases. As a result, all doubled haploids were included in grain yield experiments, disease nurseries, and end use quality. Kuchel et al. (2005) conclude that these results highlight the potential impact of MAS not just as an aid or replacement for phenotypic selection, but rather as a tool used to focus the allocation of resources in late generations of germplasm with a much greater probability of success.

There are hundreds of studies that have identified QTL in wheat for traits ranging from aluminium tolerance (Raman et al., 2005) to yellow spot resistance (Cheong et al., 2004). A detailed analysis of these QTL mapping studies is beyond the scope of this review. The final section of this review will focus on the use of molecular markers for identifying genomic regions which contribute to resistance to crown rot in wheat.

1.7 Molecular Markers for Resistance to Crown Rot

At the commencement of this PhD study, no QTL conditioning partial resistance in wheat against crown rot had been identified. Since then two independent studies have reported progress on this front. Wallwork et al. (2004), using a bulked-segregant analysis (BSA), identified a QTL located on chromosome 4B in a doubled haploid population produced from a cross between what the authors refers to as the "moderately resistant" cultivar Kukri, and the susceptible cultivar Janz. It should be noted however, that the "moderately resistant" cultivar Kukri is described as moderately susceptible to crown rot in the Australian Wheat Board grower guide and performs poorly in this regard in the Northern Grains region (G. Wildermuth, pers. comm). The identification of a single QTL is consistent with the limitations of using BSA to unravel the genetics of quantitative traits (Cook et al., 2004). The 4B QTL was highly significant and explained a large percentage of the phenotypic variance (up to 48%) within the population. Collard et al. (2005b) used a more rigorous mapping approach to reveal five QTL for resistance to crown rot inherited from line 2-49.

Collectively, these QTL explained 40.6% of the phenotypic variance. A third report (Bovill et al., 2006), arising from the work in this dissertation has also been published and will be discussed in detail in the chapters that follow.

1.8 Rationale for the Current Study

Research on crown rot in Australia first began over 40 years ago (McKnight and Hart, 1966; Purss, 1966). Some of the earliest studies reported that: the disease was less severe in crops following a long fallow and/or when rotations to resistant crops were carried out (Purss, 1966); the disease was more severe on heavier soils and that below-average rainfall during the growing period resulted in greater severity; that seed treatment had no effect on yield in badly infested soils (Purss, 1966); that varietal differences in tolerance are present (Purss, 1966; Wildermuth and Purss, 1971); and that the fungus can survive for a lengthy time (up to 2 years) on infected stubble (Burgess and Griffin, 1968). Since then, these factors have been investigated in more detail and with more sophisticated techniques – these more recent findings have confirmed the earlier research but the disease still remains a major issue for the Australian cereals industry. As a result of the continuing problem, the Grains Research and Development Corporation (GRDC) is investing considerable resources into finding solutions. A number of initiatives have been funded, including a component of the Australian Winter Cereals Wheat Molecular Marker Project (AWCMMP).

The AWCMMP is a nationally coordinated program, and its goals are to identify markers for priority traits and to incorporate these markers into breeding programs. One of these traits is resistance to crown rot. Wildermuth and Purss, in 1971, recommended a variety of lines that should be included in breeding programs aimed at producing cultivars that were tolerant to crown rot (Wildermuth and Purss, 1971). These lines included an entry from the International Wheat Spring Rust Nusery: line 497. Other lines of promise identified in this and other studies, were Gala and line 2-49 (a selection from a Gluyas Early/Gala cross). It is interesting to note that members from the AWCMMP based at the University of Southern Queensland have only recently published results identifying molecular markers for partial resistance to crown rot

from line 2-49 (Collard et al., 2005b). Another source of resistance that is currently undergoing marker analysis is line IRN497 (Bovill et al. unpublished data), the same line from the International Wheat Spring Rust Nursery that was recommended for breeding by Wildermuth and Purss (1971). That these lines were identified over 35 years ago and still have not been successfully incorporated into breeding programs highlights the difficulties encountered by breeders in the selection of partially resistant materials.

During his PhD studies, Ma (2000) produced a molecular map (composed largely of AFLP and RAPD markers) of a W21MMT70 x Mendos doubled haploid wheat population for a study on wheat quality attributes. Due to the nature of the markers, chromosome locations of the linkage groups were largely unknown. In an Honours research project, Ritter (2001) showed that the population segregated for resistance to crown rot, with W21MMT70 displaying a comparable level of resistance to line 2-49. Ritter (2001) conducted a seedling trial on the population and used the mapping data produced by Ma (2000) in an attempt to identify QTL associated with resistance to crown rot. From this study, Ritter (2001) was able to identify a single putative QTL, derived from the susceptible parent "Mendos", on a linkage group of unknown chromosomal location.

This PhD study progresses forward from the initial results obtained by Ma (2000) and Ritter (2001). There are eight objectives of this study. These are:

1. To conduct a bulked-segregant analysis based upon the phenotypic data produced in a seedling test that was conducted by Ritter (2001);
2. To conduct further detailed seedling trials of the W21MMT70 x Mendos doubled haploid population;
3. To produce a framework map composed of SSR markers to be used for the incorporation of AFLP markers produced by Ma (2000), into linkage groups of known chromosomal location
4. To use the methods of marker-regression, simple interval mapping, and composite interval mapping to identify QTL for resistance to crown rot in the W21MMT70 x Mendos population;

5. To evaluate the usefulness of advanced software for the construction of genetic linkage maps;
6. To assess the importance of epistatic interactions involved in resistance to crown rot by comparing the results of two software packages;
7. To validate identified QTL in a range of genetic backgrounds; and
8. To assess the potential of pyramiding QTL for resistance to crown rot to enhance resistance to this disease.

Chapter 2. Seedling Trials, Bulked-Segregant Analysis, and Genetic Map Construction

2.1 Introduction

Crown rot (causal organism *Fusarium pseudograminearum*) is a significant fungal disease of wheat in Australia (Backhouse et al., 2004) and elsewhere (Marasas et al., 1988; Balmas, 1994; Paulitz et al., 2002), particularly in production regions where stubble of previous cereal crops is retained and water stress late in the growing season is common. It has been estimated that losses due to crown rot cost the Australian cereals industry $56 million annually (Brennan and Murray, 1998).

Crown rot is a difficult disease to manage. *F. pseudograminearum* survives between wheat crops on infected wheat stubble or grass weeds (Wildermuth et al., 1997). Due to the evolution of farming systems towards stubble retention practices, the disease has become more prevalent in the past decade. The majority of cultivars currently grown in Queensland are moderately or highly susceptible to the disease (Wildermuth et al., 2001). Current control methods focus on crop rotation and the planting of partially resistant varieties such as Sunco, Baxter, and Lang (Wildermuth and Morgan, 2004). However, even partially resistant cultivars can suffer yield losses if planted in soil where the level of disease was high in a previous crop, and when environmental conditions favour the pathogen (G. Wildermuth pers. comm.).

As a result of this significant problem, breeding programmes are aiming to produce elite varieties that reliably exhibit improved resistance in the field in a range of environmental conditions. Phenotypic disease assessments of genetic variation in field trials, which are generally made at harvest, are time-consuming, labour intensive and suffer from significant environmental effects on disease expression. For these reasons, the coupling of molecular techniques with conventional breeding (marker-assisted selection) has the potential to more rapidly and reliably identify genomic regions from various sources that contribute to resistance, and should greatly increase the efficiency of selecting such resistance sources.

The first aim of the work in this chapter was to conduct extensive phenotypic testing of the W21MMT70 x Mendos DH population, and to use this data to identify microsatellite markers in the resistant parent W21MMT70 linked to resistance to crown rot using bulked-segregant analysis. The second aim was to produce a framework map of the population based on microsatellites and to incorporate previously screened AFLP markers.

2.2 Materials and Methods

2.21 Plant Materials

A wheat x maize induced doubled haploid population consisting of 95 lines was produced from a cross between W21MMT70 and Mendos by Kammholz et al. (1998). The W21MMT70 parent is a Western Australian experimental line. The pedigree of the line is reported to be Cranbrook*2/HIP SP#7. The HIP SP#7 parent is a reputed high protein line that is described as "CIMMYT SEL (Klepper)" and is accessible from the Australian Winter Wheat Collection (accession - AUS20890). Mendos is an Australian cultivar of the pedigree: Eureka / CItr12362 /2/ 2*Gabo /3/ Mentana / 6*Gabo /4/ Spica / Koda /2/ Gabo (as per the Graingenes website: http://wheat.pw.usda.gov). The cultivar has not been widely grown since 1969, when *Sr36* and certain other additional genes it possessed for resistance to stem rust were overcome (Zwer et al., 1992). Line W21MMT70 displays partial seedling and adult plant resistance to crown rot, whereas Mendos is susceptible in seedling trials, but displays partial adult resistance (G. Wildermuth, unpublished results).

2.22 Seedling Disease Assessment

Three seedling trials were carried out in a growth cabinet (25˚C, 60% humidity, 12-hour photoperiod) at the University of Southern Queensland in 2001, by an Honours student (Ritter, 2001) and in glasshouse tests at the Leslie Research Centre in 2003 and 2005 by myself in collaboration with Queensland Department of Primary Industries and Fisheries (QDPI&F) staff. Phenotyping was carried out as per the method of Wildermuth and McNamara (1994). The inoculum was prepared by colonizing 200 g of wheat:barley grain (1:1) in Erlenmeyer flasks

with Czapek Dox agar inoculated with *Fusarium pseudograminearum*. After 21 days (at 25˚C) the grain was air dried and ground to pass through a 2 mm sieve. The soil used was a shallow, brown clay-loam that, before use, was moistened to 35% (w/w) and heated at 70˚C with a steam-air mixture for 30 minutes, and air-dried. Fine soil was produced by passing the soil through a 6.25 mm sieve. Inoculum production and soil preparation was carried out by QDPI&F staff.

The layered pot design of Wildermuth and McNamara (1994) was used. Two hundred and ninety-five (295) g of coarse soil was first added to the pots, and 13 seeds were distributed upon this layer. Fine dry soil (160 g), was then added, and the inoculum (0.45 g) was evenly spread across this layer. A final layer of dry fine soil (40 g) was then added to each pot. Daily watering to field capacity was delayed for 7 days to allow seedlings to become established prior to the activation of the inoculum by wetting. After 21 days, each of the first three leaf sheaths from 10 seedlings per pot were rated for disease severity using a five point scale whereby: 0 = no infection; 1 = 0-25%; 2 = 25-50%; 3 = 50-75%; and 4 = 75 – 100%. The values obtained for each leaf sheath were added to give an overall score out of 12. Due to space constraints in the cabinet, each of the 2001 trials contained only single pot entries of each genotype, but was repeated three times over a four month period. In the 2003 and 2005 trial entries were replicated twice and four times respectively in single trials. All trials included the susceptible check cultivar Puseas, and disease severity ratings of the doubled-haploid lines were converted to a % Puseas scale. Narrow-sense heritability based upon line-mean in each trial was calculated from the estimates of genetic (σ^2_G) and residual (σ^2_E) variances derived from the expected mean squares of the analysis of variance: $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_E/k)$ where k is the number of replications (Sohal and Rohlf, 1995). Data from seedling trials were analysed using SPSS version 12.0.1.

2.23 DNA Extraction and Quantification

DNA was extracted from 3-5 leaves of 14-day-old seedlings as described by Cakir et al. (2003). Briefly, the collected leaf material (approximately 200 mg) was frozen in liquid nitrogen and ground with a mortar and pestle. The resulting

powder was placed into a 10 mL centrifuge tube, and 4 mL of DNA extraction buffer (1% sarcosyl; 100 mM Tris-HCl; 100 mM NaCl; 10 mM EDTA; pH adjusted to 8.5) was added. The tubes were incubated in a water bath at 65ºC for 30 min with periodic disruption. After 30 min, 4 mL of a phenol:chloroform:isoamyl-alcohol (25:24:1) solution was added and the tubes were mixed vigorously. The tube was then centrifuged at 3,750 rpm for 10 min. The aqueous phase was transferred to a fresh centrifuge tube and another 4 mL of phenol:chloroform:isoamyl-alcohol (25:24:1) was added. After mixing, the tube was centrifuged for another 10 min (3,750 rpm). The aqueous phase was transferred to a fresh 10 mL centrifuge tube, and 400 µL of 3 M sodium acetate (pH 5.2) and 4 mL of pre-chilled (4˚C) absolute ethanol was added. The tube was then mixed gently by inversion, and placed in a freezer for 30 minutes. After 30 min, the tube was centrifuged (3,750 rpm for 10 min) in order to pellet the DNA. The pellet was washed with 2 mL of 70% ethanol. A final centrifugation was carried out (3,750 rpm for 10 min), the ethanol was discarded, and the DNA pellet allowed to air-dry overnight. The DNA was resuspended in 200 µL of TE buffer and concentration determined by agarose gel electrophoresis. DNA concentration was adjusted to 10 ng/µL prior to use in PCR.

2.24 Bulked Segregant Analysis and Genotyping

A bulked-segregant analysis (Michelmore et al., 1991) was initially conducted to determine putative crown rot resistance-associated markers. Two DNA bulks were constructed by combining equal amounts of DNA from 15 resistant or 14 susceptible lines based upon phenotypic results obtained from the 2001 seedling trial (the 2003 and 2005 data were unavailable at the time of BSA). Bulks were included when screening for polymorphism between parental lines. Three hundred and ninety (390) microsatellite (SSR) primer pairs from published sources (Roder et al., 1998; Pestova et al., 2000; Gupta et al., 2002; Song et al., 2002) were used to determine polymorphism between parents and bulks. Additional sequences were obtained from the GrainGenes website (http://wheat.pw.usda.gov). Primers were synthesized by Invitrogen (Mount Waverley, Victoria, Australia). PCR was conducted in a 10 µL reaction

containing: 500 nM of each primer; 1.5 mM $MgCl_2$; 200 μM of each dNTP; 1 x PCR buffer; and 0.5 U *Taq* DNA polymerase. Thermocylcing was carried out in MJ Research PT-100 machines, with an initial 5 min 94˚C denaturation step, followed by 30-45 cycles of: 94˚C for 30 s or 1 min; 50-60˚C for 30 s or 1 min; and 72˚C for 30 s or 1 min. A final 10 min extension (72˚C) was also performed.

Electrophoresis was carried out with Bio-Rad Sequi-Gen GT Sequencing Cells. A gel mix composed of 15 mL of 40% acrylamide/bis-acrylamide (29:1; Astral Scientific), 15 mL of 40% (w/v) urea, and 6 mL of 10 x TBE (890 mM Tris, 890 mM boric acid, and 20 mM EDTA) was made up to a final volume of 60 mL with MilliQ water. To the gel mix, 600 μL of ammonium persulfate (10% w/v) and 60 μL of TEMED was added. The gel (0.4 mm thick) was poured between two glass plates – one previously treated with bind silane and the other with Rainex. From each PCR sample, 5 μL of product were loaded onto the gel, and the gel was run at 60 W for 1 h 30 min. DNA was visualized with silver-staining essentially as per Sourdille et al. (1998). Briefly, the gel was fixed for 10 min in 7.5% glacial acetic acid followed by 3 rinses (2 min each rinse) with MilliQ water. The gel was stained for 30 min in a solution containing 0.1% sliver nitrate and 0.05% formaldehyde. After a quick rinse (approximately 10 s) development was achieved by adding a solution of 3% sodium carbonate, 0.05% formaldehyde, and 2 mg/L sodium thiosulphate. The developing reaction was stopped by the addition of 7.5% glacial acetic acid. The gel was then rinsed in MilliQ water, allowed to dry, and finally scored and scanned for preservation of the image.

2.25 Genetic Map Construction

Ma (2000) produced a linkage map of the W21MMT70 x Mendos population consisting of a total of 407 markers including AFLP (331), RAPD (59), SSR (14), and phenotypic markers (3). Because chromosomal locations of these markers were largely unknown, a framework microsatellite map consisting of 128 SSR, one sequence-tagged-site (STS66-3B), and four phenotypic markers (*Sr36* {data kindly provided by Dr. Harbans Bariana}, awns, *GluB3*, and *GluD3*) was produced using the program Map Manager QTX (Manly et al., 2001) with a

stringency of p=0.01. The previously screened AFLP markers were manually added to the framework map and their best location was determined by using the links report generated by Map Manager QTX. Due to the much reported problem of reproducibility of RAPD markers (see for example Jones et al., 1997) these were not included for mapping.

2.3 Results

2.31 Seedling Disease Assessment

Means were calculated from the phenotypic data from each of the seedling trials (Figure 2-1). In each trial, the W21MMT70 parent showed a greater level of resistance than the susceptible parent Mendos.

Figure 2-1. Histograms of mean crown rot severity ratings of the W21MMT70 x Mendos wheat population from the 2001, 2003, and 2005 seedling trials. The disease severity rating of the parents are indicated by filled (W21MMT70) and unfilled (Mendos) arrows.

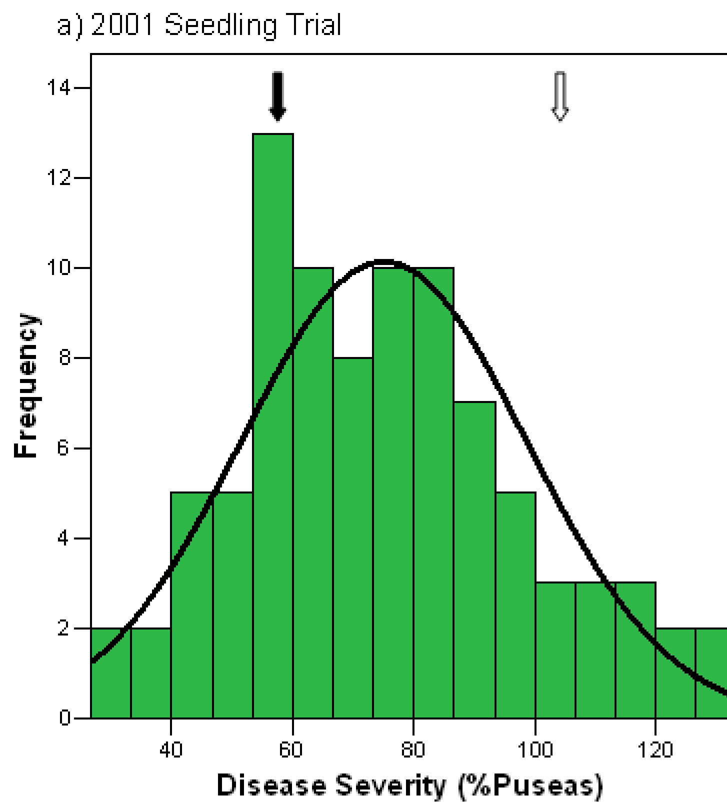
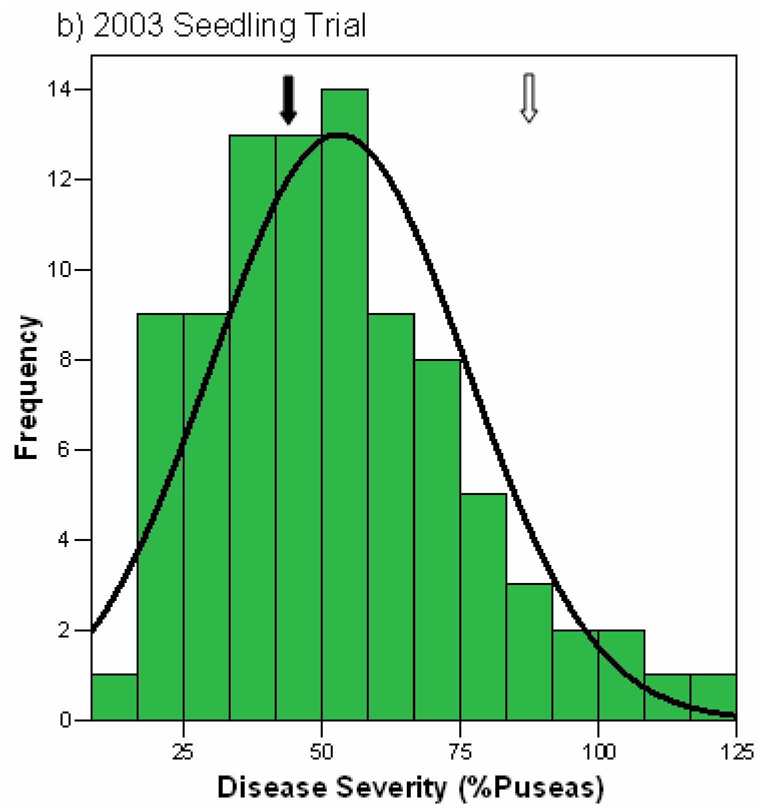
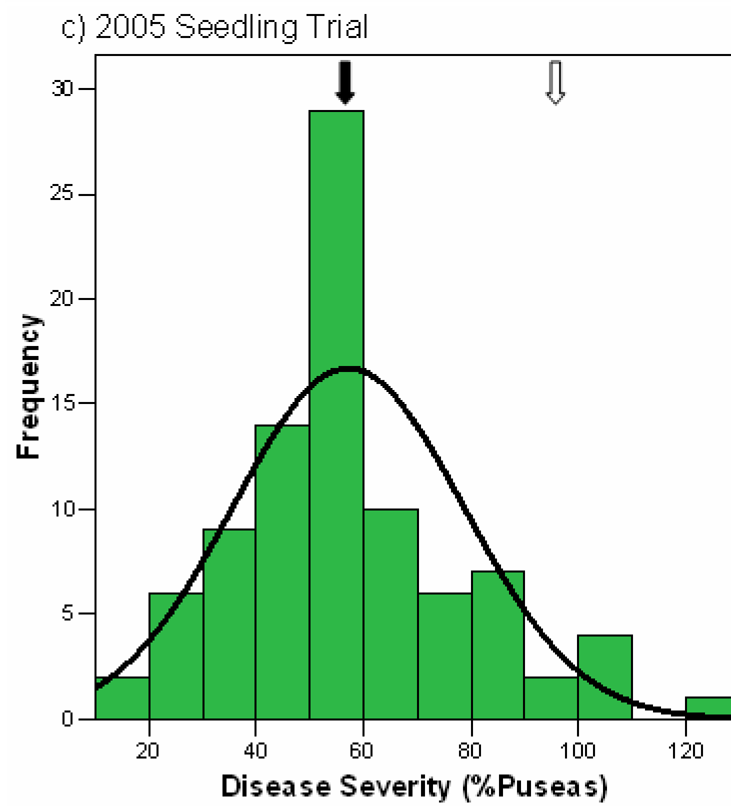


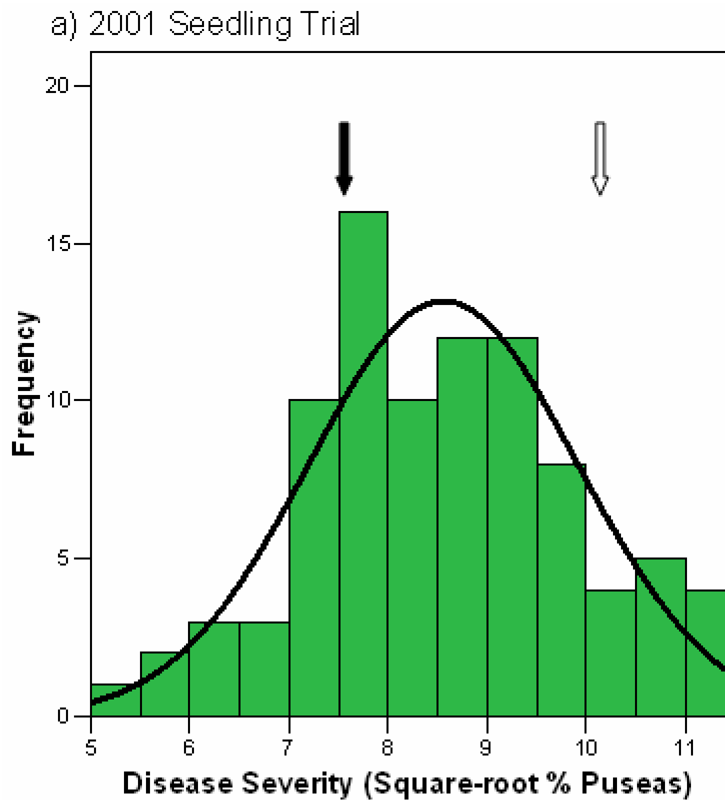
Figure 2-1. Continued.





The Shapiro-Wilk test for normality was carried out within SPSS. Results showed that only means for the 2001 trial were normally distributed. Both the 2003 ($p<0.05$) and the 2005 ($p<0.01$) trials were not normally distributed and in both instances were skewed towards resistance. In order to satisfy the assumption of normality for ANOVA between the seedling trials, the data was subjected to a square root transformation. After transformation, all seedling trials were normally distributed (Shapiro-Wilk test, $p>0.1$). Histograms of the transformed data are shown in Figure 2-2.

Figure 2-2. Histograms of the three seedling trials following square-root transformation. Disease severity (square-root % Puseas) of the parents are indicated by filled (W21MMT70) or unfilled (Mendos) arrows.



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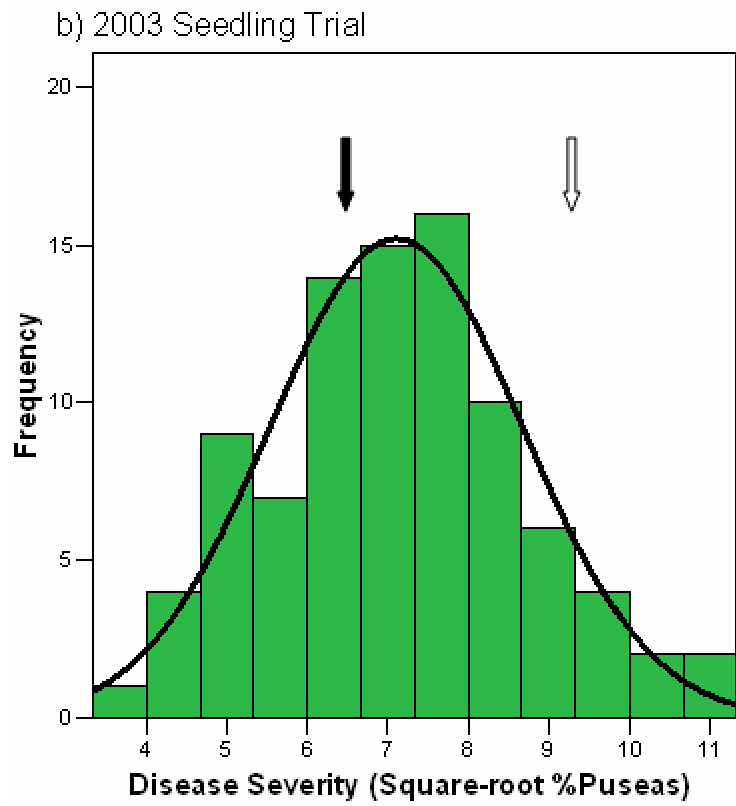
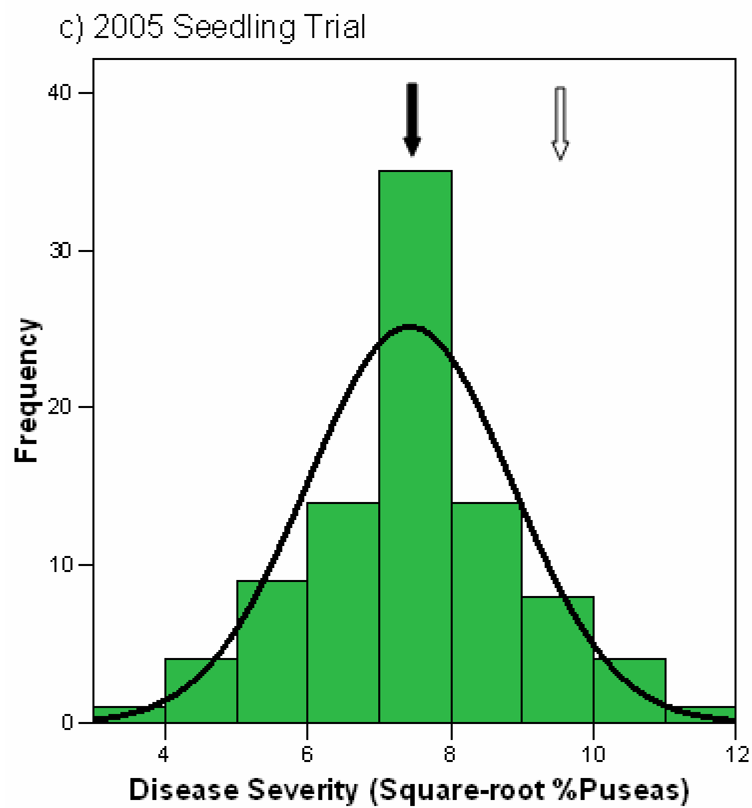




A one-way ANOVA was used to compare means of each of the seedling trials. Levene's test for homogeneity of variance was not significant ($p=0.3$). The ANOVA showed that the phenotypic data obtained from the seedling trials were significantly different ($p<0.001$). The Tukey honestly significant difference (HSD) procedure revealed that the two glasshouse seedling trials were not significantly different to each other, but were both significantly different to the 2001 growth cabinet trial (Table 2-1). With the exception of the 2001 trial, line 2-49 (included for the purpose of comparison) performed better than both W21MMT70 and Mendos.

Table 2-1. Means and ranges for crown rot disease severity for parental lines and doubled haploids. Population means sharing the same letter are not significantly different (Tukey HSD, $p>0.05$).

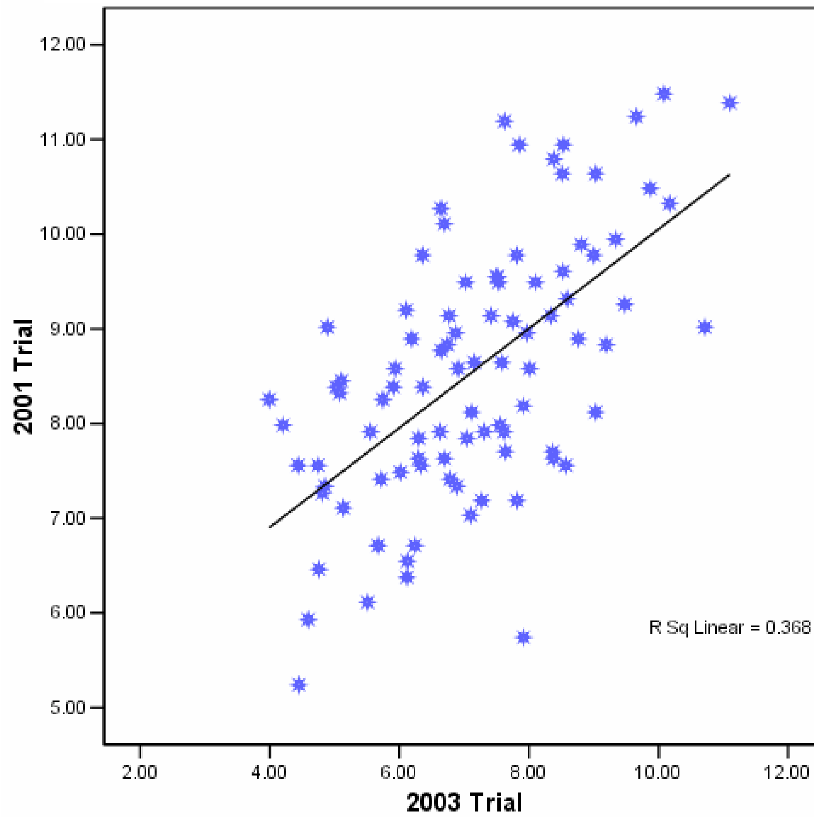
Year	Disease Severity (square-root % Puseas)				
	W21MMT70	Mendos	2-49*	Population Mean	Population Range
2001	7.56	10.22	6.63	8.56^{a}	5.24-11.48
2003	6.62	9.40	6.72	7.11^{b}	3.99-11.10
2005	7.62	9.65	6.12	7.42^{b}	3.82-11.38

*Line 2-49 was also included in all trials and its score is provided for comparison.

Correlations between the seedling trials are shown in Figure 2-3. The correlation coefficient (r) between the seedling trials ranged from 0.573 for the 2001 vs 2005 trials (Figure2-3b), to 0.638 for the 2003 vs 2005 trials (Figure 2-3c). All possible correlations between trials were significant ($p<0.01$).

Figure 2-3. X-Y scatterplots showing correlations between the 3 seedling trials.

a) Correlation between the 2001 and 2003 trials



b) Correlation between the 2001 and 2005 trials

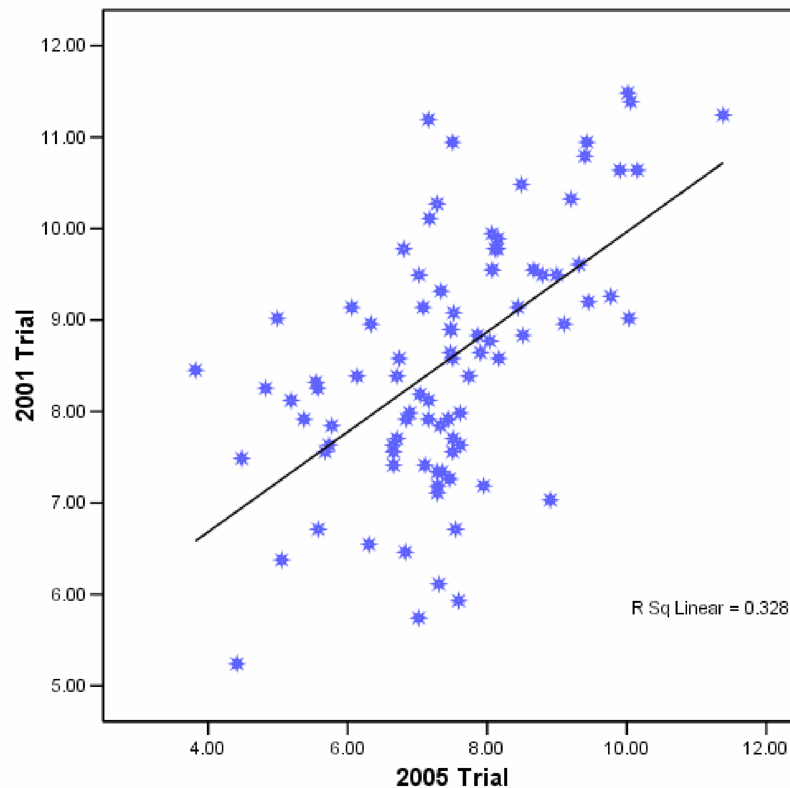
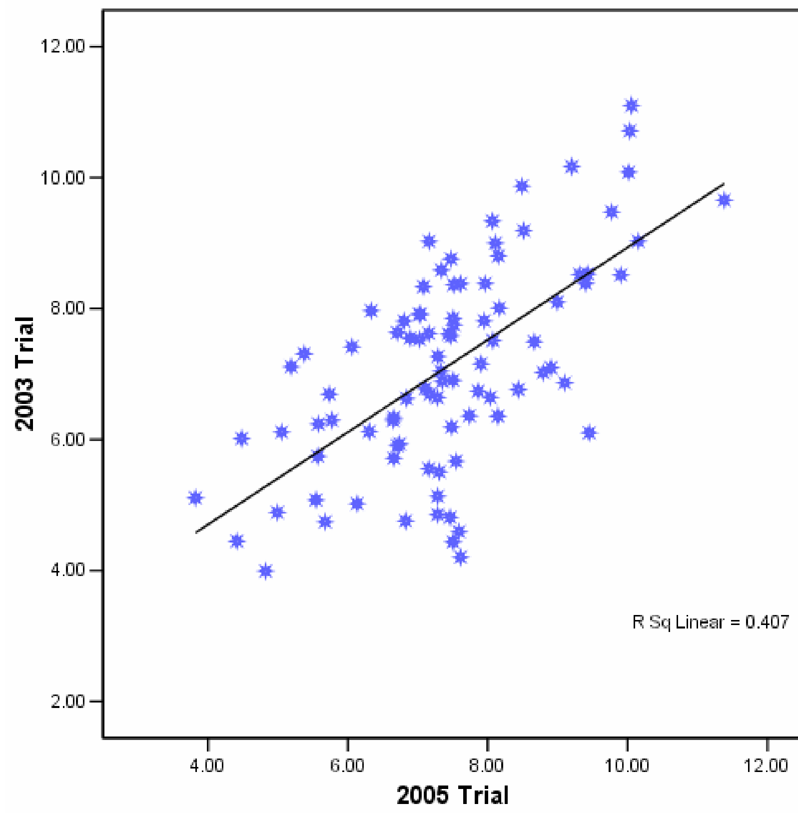


Figure 2-3. Continued.

c) Correlation between the 2003 and 2005 trials



Analysis of variance showed significant genotypic differences within each seedling trial (Table 2-2). Narrow-sense heritability estimates based upon line-mean ranged from 0.77 for the 2003 seedling trial, to 0.89 for the 2005 seedling trial.

Table 2-2. One-way ANOVA for each seedling trial with estimated heritability (+/- standard error) values.

Source	df	Sum of Squares	Mean Square	F	Sig.	Heritability
2001						
Between	91	520.723	5.722	4.569	.000	0.78
Within	177	221.666	1.252			
Total	268	742.389				
2003						
Between	91	437.093	4.803	4.455	.000	0.77
Within	92	99.413	1.081			
Total	183	536.506				
2005						
Between	90	745.377	8.282	9.465	.000	0.89
Within	273	238.877	0.875			
Total	363	984.254				

2.32 Bulked-Segregant Analysis

In an attempt to rapidly identify molecular markers linked to crown rot resistance a bulked-segregant analysis was conducted. Bulks were chosen based upon the results of the 2001 seedling trial (data from the 2003 and 2005 trials were not available prior to conducting BSA). The lines chosen for BSA and their disease severity rating are shown in Table 2-3. The average disease severity for the resistant bulks was 45.4% - much lower than the average disease severity of the susceptible bulks (115.9%).

Table 2-3. Lines included in bulks and their disease severity (% Puseas) rating.

Resistant Bulk		Susceptible Bulk	
DH Line	Severity (% Puseas)	DH Line	Severity (% Puseas)
2	52.8	4	106.6
3	50.5	12	119.8
8	53.8	13	131.9
14	37.3	20	119.8
57	45.1	31	126.4
90	49.5	39	113.2
114	51.6	40	102.2
126	45.1	42	116.5
138	51.6	74	113.2
162	57.1	80	102.2
175	27.5	127	125.3
177	33.0	133	105.5
181	48.3	155	129.7
188	42.9	224	109.9
193	35.2		
Mean	45.4	Mean	115.9

A total of 390 microsatellite markers were screened across parents and bulks. Of these, 163 (41.9%) identified polymorphisms between W21MMT70 and Mendos. The microsatellites were selected for their genome coverage, with 52 (31.9%) present in the A genome, 64 (39.3%) present in the B genome, and 47 (28.8%) present in the D genome. Eleven primer pairs showed banding patterns in the bulks that suggested they might be associated with resistance. However, marker analysis of the individuals within the bulks did not indicate any consistent linkage with crown rot resistance. For example, SSR marker gwm350 (located on chromosome 7A) exhibited a pattern whereby the allele size of the resistant bulk was the same size as W21MMT70 and the allele size of the susceptible bulk was the same as Mendos. However, when this marker was assayed on the individuals in the bulk, only five of the 15 individuals in the resistant bulk had W21MMT70 alleles, and six of the 14 individuals in the susceptible bulk also contained W21MMT70 alleles. As the BSA approach proved unsuccessful for identifying genomic regions associated with crown rot resistance, the entire population was framework mapped in order to identify quantitative trait loci that confer partial resistance in this population.

2.33 Framework Mapping

113 SSR and one sequence-tagged site (STS) marker were used to genotype the entire DH population. Ma (2000) also generated data for 14 Xpsp SSR markers on the W21MMT70 x Mendos population. Phenotypic data from markers of known chromosomal location (*Sr36*, awnedness, *Glu3B* and *Glu3D*) were combined with genotypic data from the SSR markers to generate a framework map. Using the "make linkage groups" command in Map Manager, the 131 marker loci were placed into linkage groups at a threshold of p=0.01. The linkage groups were assigned to chromosomes on the basis of consensus maps. In order to determine the best possible location of the SSR markers, links reports were generated and the markers placed in such an order that gave the smallest map distance.

SSR markers mapped to all chromosomes (Figure 2-4) with the exception of chromosomes 6B and 6D, although only one marker reported to reside on each of these chromosomes was mapped. The number of markers per chromosome ranged from two (chromosomes 1B, 3B, 4A, and 4D) to fourteen (chromosome 5D). Several chromosomes were split into two (chromosomes 1D, 2D, 3B, 5A, and 7A) or three (chromosome 2A) linkage groups at the stringency used. Sixteen (16) markers were unlinked (Table 2-4), and one linkage group, composed of two markers (STS66 and Xwmc011), was unable to be assigned to a chromosome. The total distance of the framework map was 1272.7cM.

Table 2-4. Unlinked SSR markers and chromosomal locations (Chr.) they have been reported to map to (Appels, 2003; Somers et al., 2004).

SSR	Chr.	SSR	Chr.	SSR	Chr.	SSR	Chr.
Xgwm268	1B	Xwmc179	2A,2B,4A,6A	Xgwm165	4A,4B,4D	Xgwm219	6B
Xwmc230	1B	Xgwm614	2B	Xgwm192	4B,5D	Xgdm98	6D
Xgdm126	1B,1D	Xgwm183	3B	Xpsp3065	5B	Xgwm428	7D
Xgdm111	1D	Xgdm99	3D,5D	Xgwm272	5D	Xwmc506	7D

Figure 2-4. Framework SSR and phenotypic marker map of the W21MMT70 x Mendos doubled-haploid population. Chromosomal designation following markers (in parentheses) indicate previously reported map locations (Appels, 2003; Somers et al., 2004).

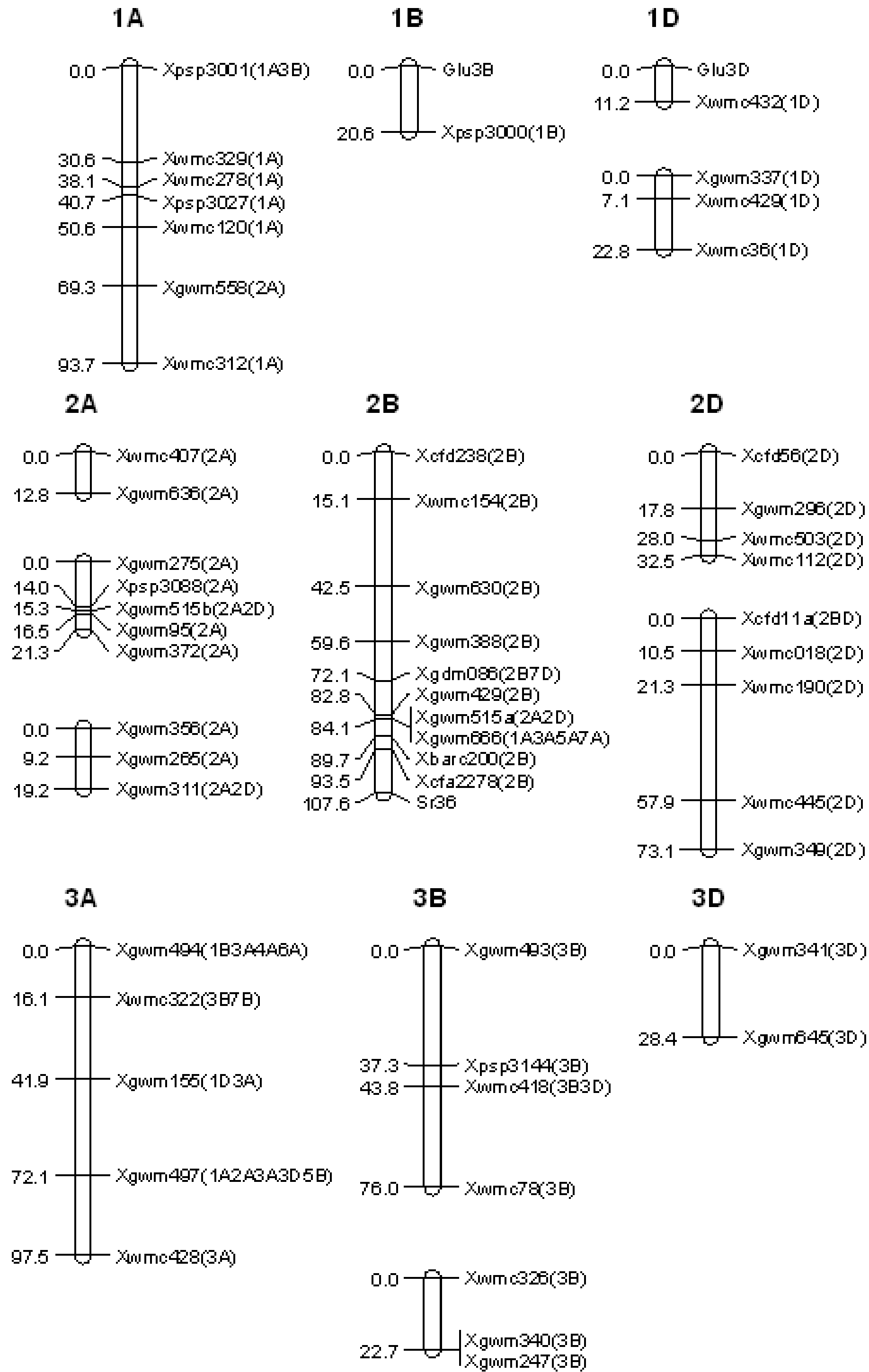


Figure 2-4. Continued.

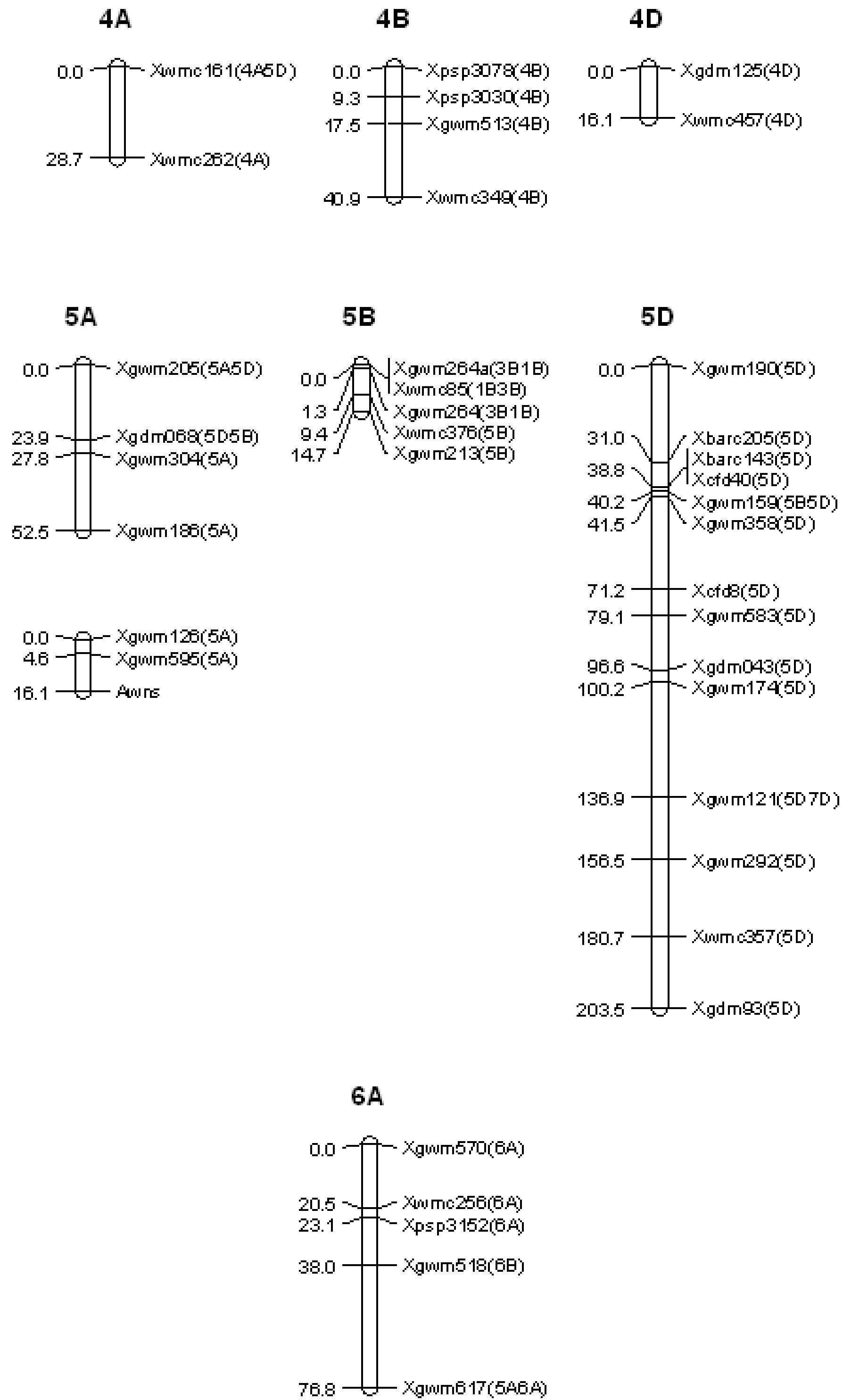
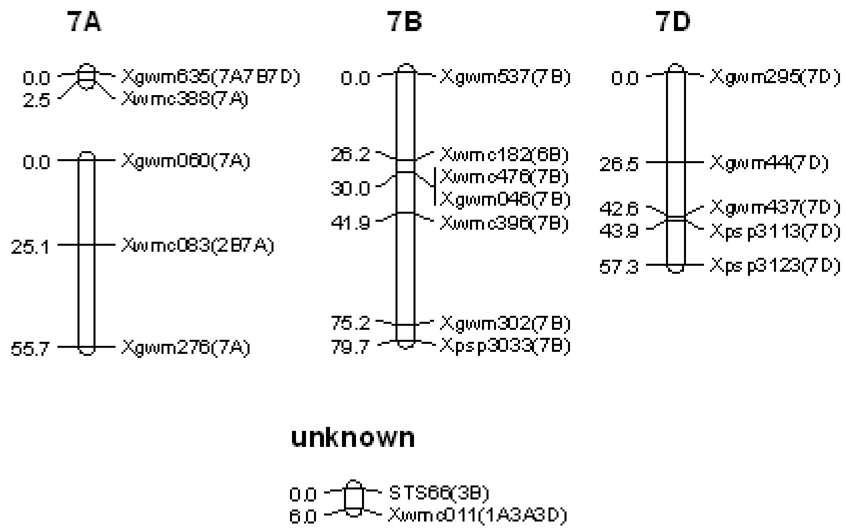


Figure 2-4. Continued



2.34 Addition of AFLP Markers from Ma (2000)

Unlinked markers from 6B and 6D were placed into separate linkage groups in Map Manager. Linkage groups of the same chromosome were placed together into a single chromosome before adding the AFLP markers. The AFLP markers were firstly added to the framework map by using the “distribute” command in Map Manager with a stringency of p=0.001.

Of the 331 AFLP markers, 223 (67.4%) were distributed amongst the framework map, while 108 (32.6%) did not link to the framework map with the stringency used. The number of markers per chromosome ranged from two (chromosomes 3D and 4D) to 90 (chromosome 2B, shown in Figure 2.5) and the total length of each chromosome ranged from 16.1cM (4D) to 728.6cM (2B; Table 2-5). Four AFLP markers linked to the unknown SSR linkage group. The total map distance was 4908.9cM.

Table 2-5. Number of markers and chromosome length after addition of AFLP markers to the framework map.

Chromo-some	A		B		D	
	Markers	Length(cM)	Markers	Length(cM)	Markers	Length(cM)
1	16	166.0	7	83.3	8	92.3
2	25	222.1	90	728.6	37	430.0
3	10	216.2	21	215.8	2	28.4
4	6	99.1	9	111.1	2	16.1
5	10	107.1	11	83.5	14	203.5
6	20	332.9	5	54.3	3	52.8
7	23	371.0	11	87.7	7	78.6

The distribute function in Map Manager appeared unable to cope with the large amount of data, particularly in the case of chromosome 2B. This was evidenced by, for example, the ripple function in Map Manager being unable to change the order of markers. Also, the markers were often not in the best position because by manually moving the markers the map distances were frequently made smaller on many chromosomes. As a result, markers were added manually to the SSR framework map. Links reports were generated for each AFLP marker to

determine the most likely interval in which the marker should reside. If, after addition, the AFLP marker significantly increased the distance between its flanking markers, the marker was discarded. After all AFLP markers were added to the framework map, double cross-overs between loci less than 30 cM apart were removed and scored as missing data. The number of markers per linkage group, the length of the map prior to double cross-over removal, the number of double cross-overs removed, and the map distance after removal of the double cross-overs are shown in Table 2-6. Linkage maps are displayed in Figure 2-5.

Table 2-6. Number of markers per chromosome, map distance, number of double cross-overs, and map distance after removal of double cross-overs from the framework map after addition of AFLP markers.

Chromosome	Number of Markers	Map Distance (cM)	Number of double cross-overs	Map distance after removal of double cross-overs (cM)
1A	15	122.6	35	49.3
1B	8	138.0	4	128.6
1D	8	92.3	5	80.5
2A	14	90.6	19	48.9
2Ai	6	33.1	7	15.9
2Aii	5	52.0	9	29.6
2B	27	203.6	57	68.2
2D	32	350.6	35	272.2
2Di	5	33.6	0	33.6
3A	10	216.2	31	143.0
3B	7	83.1	0	83.1
3Bi	14	132.6	29	91.9
3D	2	28.4	0	28.4
4A	6	99.1	8	79.8
4B	9	111.1	10	87.0
4D	2	16.1	0	16.1
5A	6	54.6	9	36.3
5Ai	4	51.8	3	45.0
5B	18	223.5	22	198.8
5D	14	203.5	6	188.1
6A	20	332.9	41	241.4
6B	5	54.3	0	54.3
6D	3	52.8	3	45.0
7A	11	148.2	39	57.4
7Ai	12	153.9	29	86.9
7B	11	87.7	4	77.9
7D	7	78.6	8	59.7
Unknown	4	42.9	1	40.8
Unknown	2	19.9	0	19.9
Unknown	2	20.7	0	20.7
Unknown	3	41.6	4	33.2
Unknown	11	54.5	10	27.3
Unknown	2	25.6	0	25.6
Unknown	4	31.7	2	27.4
Unknown	4	24.4	4	15.9
Unknown	2	13.5	0	13.5
Unknown	2	4.6	0	4.6
Unknown	4	45.3	4	35.9
TOTAL	**321**	**3569.5**	**438**	**2611.7**

Figure 2-5. Linkage groups after addition of AFLP markers. Linkage groups comprised solely of AFLP markers that could not be assigned to chromosomes are not shown.

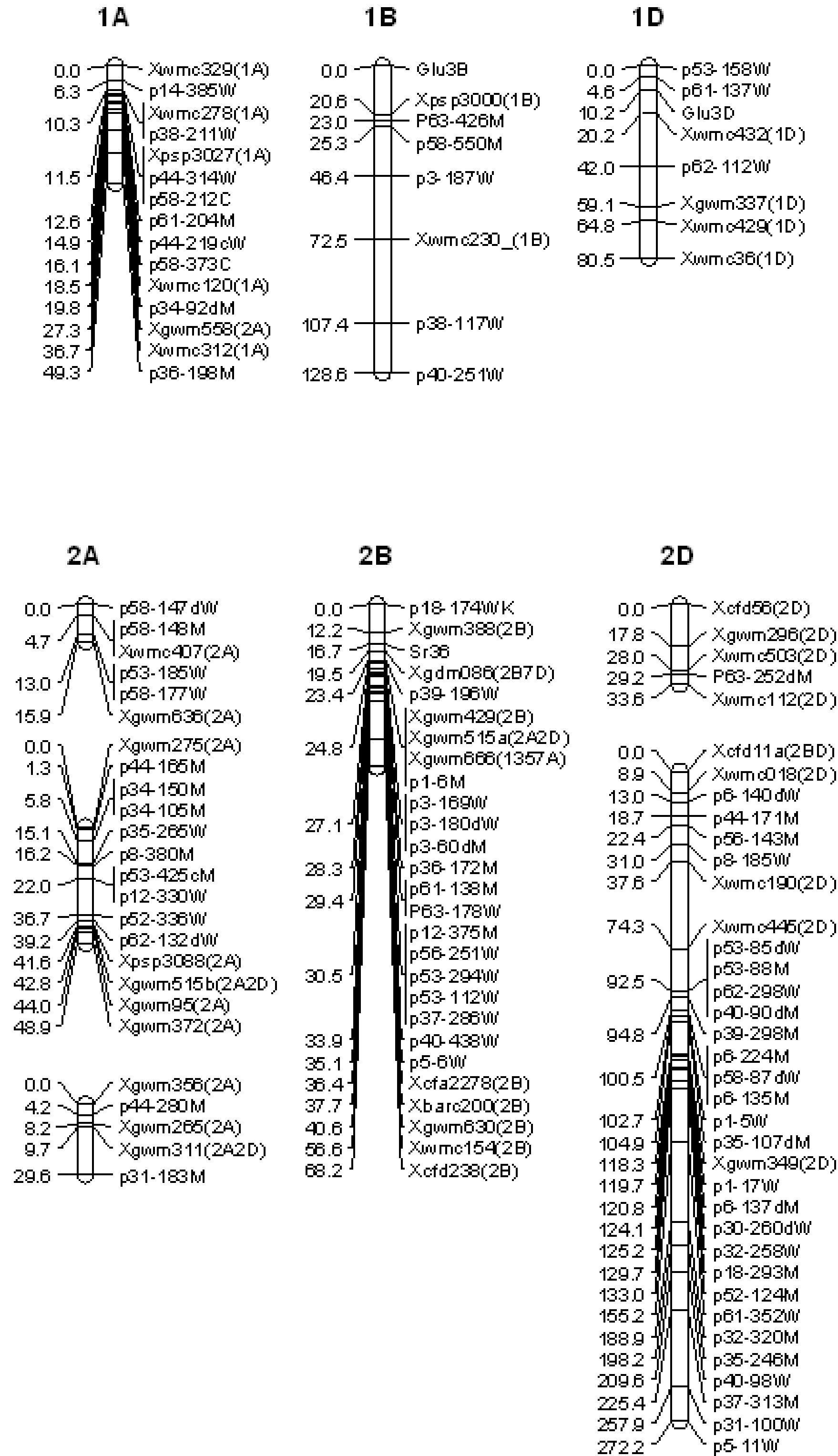


Figure 2-5. Continued.

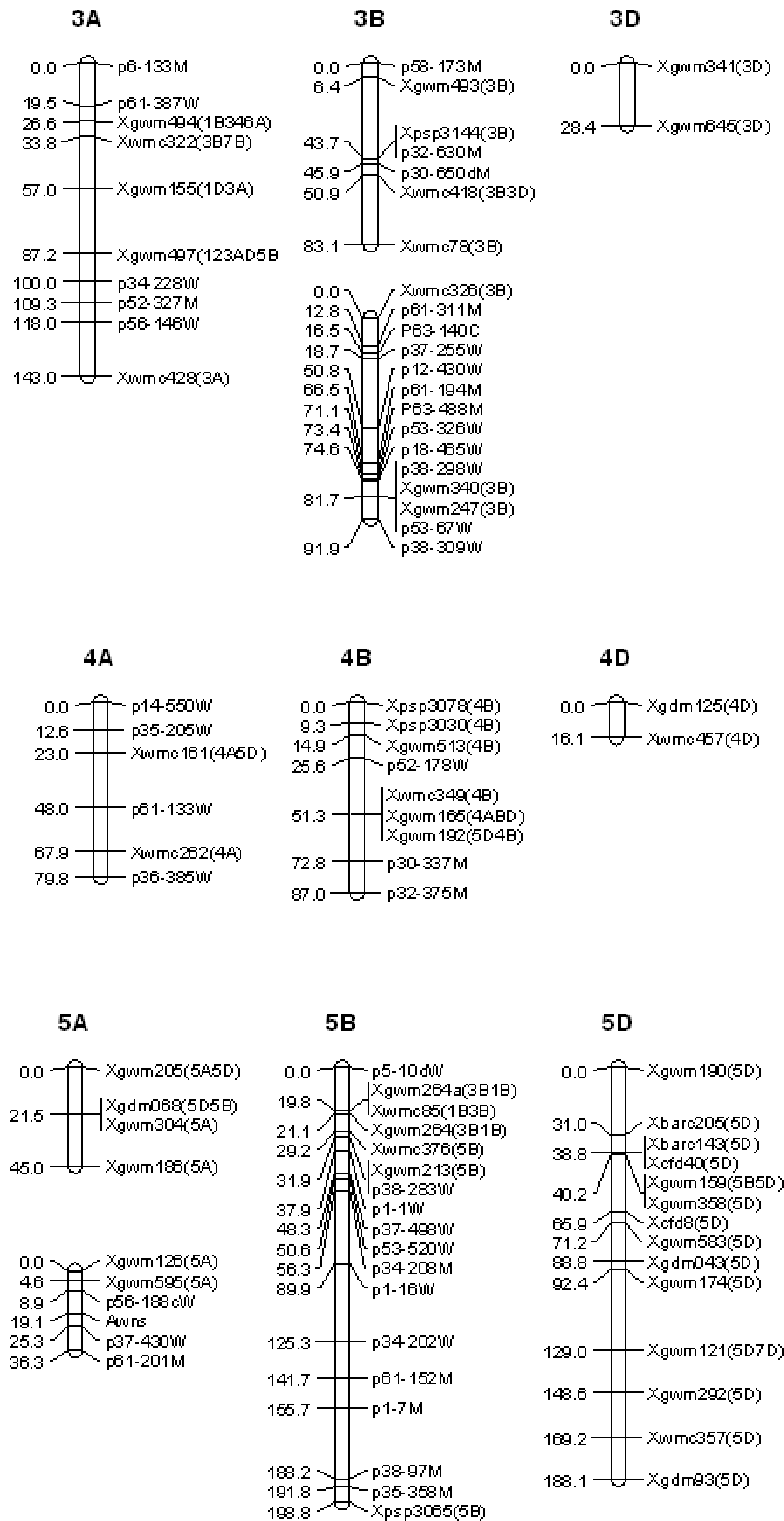
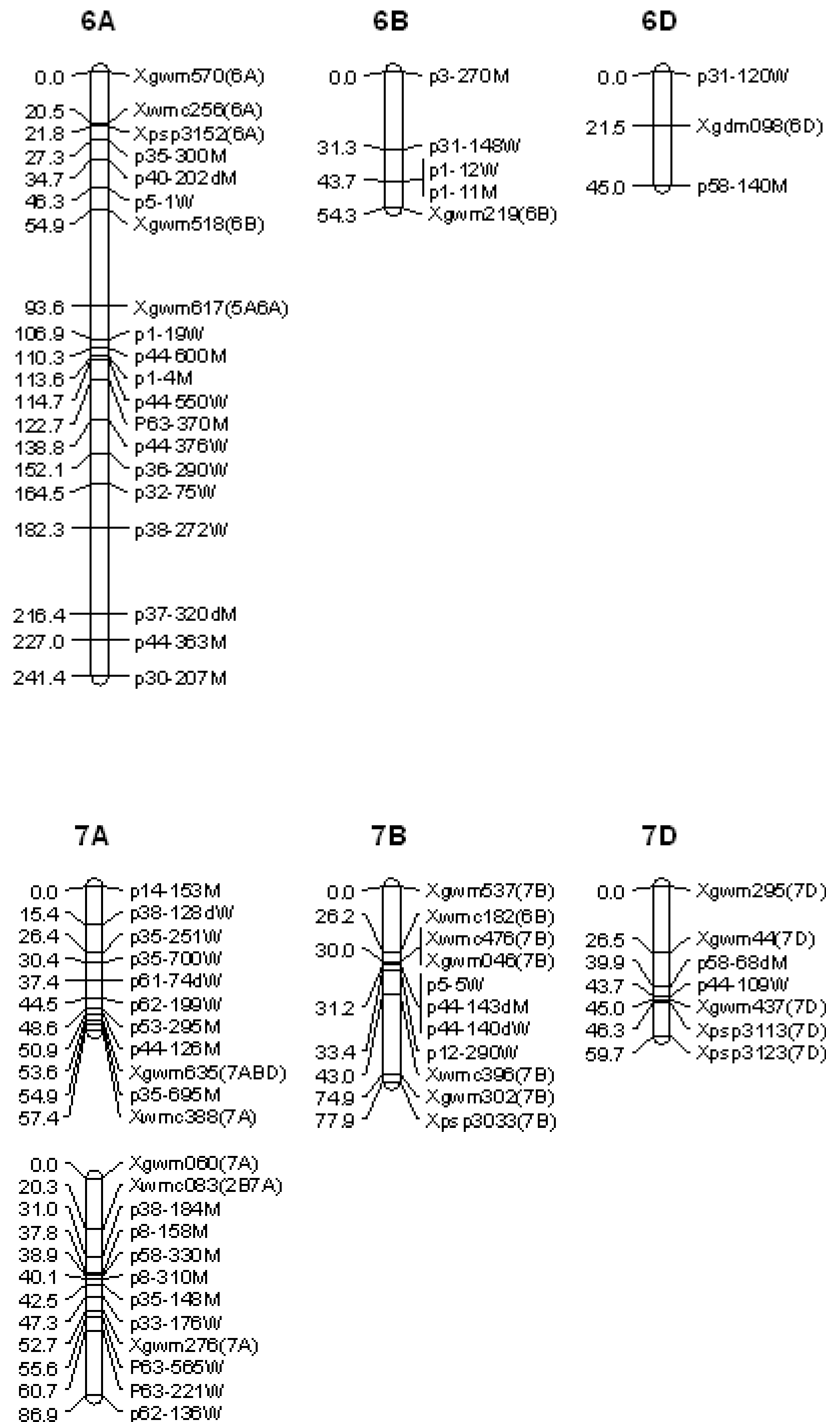


Figure 2-5. Continued.



2.35 Segregation Distortion

Segregation distortion was observed for 13.5% of the markers. Of these, 64.9% were AFLP markers. Deviation from the expected 1:1 ratio at $p<0.05$ was shown in 13 of the 27 linkage groups. With exception of some markers on 2B and 5D, the distorted loci were not clustered. In the case of chromosome 2B, the distorted loci favoured the Mendos parent, whereas for chromosome 5D, distorted loci favoured the W21MMT70 source.

2.4 Discussion

In each seedling trial, disease severity showed a wide range of variation. In all cases, the W21MMT70 parent displayed partial resistance to crown rot, whereas the Mendos parent was significantly more susceptible that W21MMT70, but less so than Puseas. The presence of transgressive segregants within each of the trials suggests that the susceptible parent Mendos contributed to the expression of resistance in those lines that performed better than the partially resistant parent W21MMT70. This observation is supported by early work by Purss (1966) who concluded that Mengavi (a progenitor of Mendos) displayed a reasonable level of tolerance to crown rot infection in the field.

The continuous distribution of disease severity ratings from all three seedling trials, as well as the presence of transgressive segregants, supports the observation that crown rot resistance is a quantitative trait. The population mean for disease severity in the growth cabinet trial was higher than each of the glasshouse trials, which may indicate that this environment was more conducive to development of disease symptoms. In addition to the inclusion of the check cultivar Puseas, line 2-49 was also included in each of the seedling trials (data not shown). Line 2-49 is recognized as one of the best available sources of resistance to crown rot (Wildermuth and McNamara, 1994). In comparison to line 2-49 (mean disease severity of 42.2% across the 3 trials), W21MMT70 showed a mean severity rating of 53%. Thus, W21MMT70 possesses a level of resistance that should be useful to breeding programs, particularly if it should prove additive to that present in 2-49.

Significant correlations were found between each of the trials. The highest correlation was found between the two glasshouse trials, although the correlation between the growth cabinet and each of the two glasshouse trials was still strong ($r = 0.61$ for the 2001-2003 trials, and $r = 0.57$ for the 2003-2005 trials). Although significant, the correlations were not as high as one might expect given the care used to set up and maintain each of the experiments. Indeed, these correlations are not as high as that reported by Wildermuth and McNamara (1994) for comparison between seedling and field trial scores ($r = 0.78$) of 28

different genotypes of wheat. The seedling trials were conducted in different environments (growth cabinet vs. glasshouse), in different years, and at different times of the year, and factors such as differences in temperature and humidity may have played a role in increasing the variation between the trials. Other factors, such as aggressiveness of inoculum between years, may have also contributed to the variation. Putative differences in aggressiveness are not due to a pathogen race structure, as Percy et al. (unpublished results) have shown, that although a range of isolates collected from across Australia differ in their aggressiveness across a differential set of wheat genotypes, the resistance ranking of each of the genotypes does not change when challenged by each of the different isolates.

In an attempt to rapidly and efficiently identify genomic regions associated with seedling resistance to crown rot, BSA was carried out using microsatellites of known location in the wheat genome. The lines chosen for BSA were identified from the 2001 growth cabinet trials (data from 2003 and 2005 were unavailable at the time of BSA). While this approach identified a number of potential candidate locations, when the individuals within the bulks were analysed, linkage to crown rot resistance could not be confirmed. Most successful reports of using BSA to identify molecular markers linked to traits of interest have involved qualitative traits such as powdery mildew resistance (Xie et al., 2004), common bunt resistance (He and Hughes, 2003), and leaf rust resistance (Cherukuri et al., 2003). When used in studies on quantitative traits, the BSA technique appears most useful at identifying loci with large phenotypic effects (Cook et al., 2004). It is possible that the inability to find markers associated with crown rot resistance by a bulked segregant analysis approach in this study is the result of choosing too large a number of lines (15 in the resistant bulk and 14 in the susceptible bulk – almost 1/3 of the population in total) for inclusion in the bulks. Furthermore, if the 2003 and 2005 data was available, different lines may have been included in the bulks perhaps making the use of the technique more effective. For example, of the 15 lines chosen for the resistant bulk based upon 2001 data, less than 50% were ranked in the most resistant 15 lines of the 2003 trial, and only 20% were ranked in the most resistant 15 lines of the 2005 trial. Of the lines chosen for the susceptible bulks, less than 50% ranked in the most

susceptible 14 lines of the 2003 trial, and less than 60% ranked in the most susceptible 14 lines of the 2005 trial. The mapping approach was thus used as an alternative means to identify the QTL for crown rot resistance.

One hundred and thirty one (131) SSR and phenotypic markers were used to construct the framework map that covered a total distance of 1278.7 cM. Initially, it was planned that 2-3 markers per arm be mapped in order to locate the AFLP data. However, some chromosomes showed poor marker coverage with none (chromosomes 6B and 6D) or only two SSR markers. In these instances, the lack of coverage was caused by a lack of available polymorphic SSRs in these regions. The purpose of the framework map was not only to provide a scaffold for the addition of AFLP markers produced by Ma (2000), but also so that the AFLP markers could be used to bridge gaps between SSR linkage groups. For this reason, it was decided that no further SSR markers needed to be added to the scaffold.

The order of the SSR markers is, overall, largely consistent with those of the consensus maps of Appels (2003) and Somers et al. (2004). There are some discrepancies, and these are most notable in the case of chromosomes 2B and 2D. The susceptible parent in this study (Mendos) does possess an introgression from *Triticum timopheevi* on chromosome 2B, and this is likely to have an impact on the order of markers on this chromosome. However, there is also poor agreement between the two consensus maps for both chromosome 2B and chromosome 2D, making comparisons of these chromosomes between maps difficult.

In all instances, gaps in chromosomes that were composed of two linkage groups (chromosomes 1D, 2D, 5A, and 7B) were consistent with the consensus maps in that large distances between the markers at the ends of each linkage group in the current map were present in the consensus maps. It should be highlighted that distances between markers that are calculated by map construction software such as Map Manager are estimates only, and each distance has a standard error associated with it (Manly et al., 2001). Estimates of standard errors of recombination frequency decrease with population size (Ferreira et al., 2006),

and the relatively small population size used in this study (95 lines) will thus affect the distance between markers on the linkage groups.

Prior to the addition of the AFLP markers, the total distance of the framework map was 1278.7cM. After their addition, the length of the map increased to 2611.7cM – more than twice the length of the framework map. Densely mapped populations from several groups, suggests that the size of the wheat genome is over 3000cM. For example, Chalmers et al. (2001) reported map distances of a Cranbrook x Halberd DH population at 4110cM, a CD87 x Katepwa DH population at 3484cM, and a Sunco x Tasman DH population at 3164cM. Paillard et al. (2003) reported a distance of 3086cM for a linkage map based upon a cross between two Swiss winter wheat varieties. The map generated from a cross between Courtot and Chinese Spring by Sourdille et al. (2003) is 3685cM. More recently, Liu et al. (Liu et al., 2005) using SSR and TRAP (Targeted Region Amplified Polymorphism) markers, reported a map distance of 3045cM based on an intervarietal cross. Clearly, the map presented in the current study is shorter than the examples provided.

There are a number of reasons to explain the shorter distance of the current map compared to other published maps. Firstly, a number of chromosomes were composed of more than one linkage group. Clearly, the addition of markers to bridge the gaps between linkage groups would have increased the length of the map. Secondly, some chromosomes were poorly covered with only a few SSR and AFLP markers present. Had polymorphic SSR markers been available at the time of map construction, the addition of such markers would also have increased the distance of the map. Finally, the process of removing double-crossovers between markers that were closer than 30 cM apart and replacing these data as missing values, also played a large role in decreasing the length of the map.

Prior to the removal of double-crossovers, the distance of the SSR-AFLP map was 3569.5 cM. After removal of the 438 double-crossovers, the map distance decreased to 2611.7 cM. Although 438 may appear a large number of presumed genotyping errors, these represent only 1.4% of all marker data, thus showing

that significant inflation of map distances can be the result of a relatively small amount of genotyping errors. Indeed, Lehmensiek et al. (2005) found almost identical results (around 1% of the total) by using this strategy for the curation of the wheat maps previously produced by Chalmers et al. (2001). The advantage of removing such genotyping errors is further supported by simulated data of Hacket and Broadfoot (2003), who showed that missing values had less of an effect than genotyping errors on ordering of markers within linkage groups.

A number of markers (13.5%) displayed segregation distortion. Often in reports on the construction of linkage maps, distorted loci are removed prior to map construction (Román et al., 2002; Ubi et al., 2004). Distorted markers have been reported to cause inaccuracies in the linkage distances calculated between markers (Cloutier et al., 1997), hence the decision to often exclude them. However, Hacket and Broadfoot (2003) have provided evidence from simulation studies that suggest distorted segregation ratios have little effect on map construction. Distortion was observed in the regions of chromosomes 2B (in favour of the Mendos allele) and 5D (in favour of the W21MMT70 allele). Segregation distortion on chromosome 2B has been previously reported in a Sunco x Tasman mapping population (Kammholz et al., 2001). The authors explain the distortion as being caused by the presence of an alien introgression from *Triticum timopheevi* that is present in Sunco. As mentioned, Mendos also contains this introgression, and this may be the cause of the distortion in this population as well. Faris et al. (1998) reported the occurrence of three regions of segregation distortion on chromosome 5D in an *Aegilops tauschii* (the D genome donor in bread wheat) cross, although comparison between these regions and that of the current study are difficult to make due to a lack of common markers. Most of the other distorted markers were not clustered as was the case with 2B and 5D, and it is acknowledged that markers displaying mild segregation distortion may simply be the result of less than optimal numbers of individuals for genotyping.

The use of the framework SSR map for the distribution of the AFLP markers produced by Ma (2000) proved an effective strategy for designating linkage groups to chromosomes. Although it was envisaged that the addition of the AFLP markers would bridge gaps between SSR linkage groups, this only

occurred in the case of chromosome 1D. The original AFLP map of Ma (2000) was composed of large numbers of linkage groups of unknown location, and Ritter (2001), using that map, identified a region of an unknown linkage that was inherited from the susceptible parent Mendos as contributing to resistance to crown rot. By using the map produced in this study, it was found that this unknown linkage group was chromosome 2B. Although BSA of this region was conducted in the current study, linkage to crown rot resistance could not be confirmed for reasons previously addressed (see Section 2.42). The map produced forms the basis for identifying QTL for resistance to crown rot.

Chapter 3. QTL detection

3.1 Introduction

Deployment of crown rot resistant cultivars is a major objective of Australian wheat breeding programs. In the Northern grain growing region of Australia, the disease is more devastating than in the South and West, with yield losses as high as 90% reported (D. Herde, pers. comm.). Conventional breeding has had limited success in producing crown rot resistant materials. To date, the most resistant cultivar (Sunco) will still suffer yield losses when moisture stress occurs at the end of the season (G. Wildermuth pers. comm.). The cultivation of genetically resistant cultivars remains the most effective means of control of this disease.

Genetic variation for resistance to crown rot has been well documented (Purss, 1966; Wildermuth and Purss, 1971). Despite these efforts, transferring such resistance sources into adapted breeding lines has proven challenging. The use of molecular markers has provided a new tool to study the genetics of resistance to crown rot. Two studies have reported the identification of molecular markers for resistance to crown rot in wheat. Wallwork et al. (2004) identified a single QTL on chromosome 4B from the moderately resistant cultivar Janz. This study was based upon BSA and subsequent mapping of the region identified by BSA. More recently, Collard et al. (2005b), using a framework mapping strategy, identified up to six QTL for resistance to crown rot in a cross between wheat line 2-49 and the cultivar Janz. Only one major QTL was identified by BSA in the work of Collard et al. (2005b) thus highlighting the need for constructing linkage maps to identify loci of smaller effect.

Although the markers identified in line 2-49 (Collard et al., 2005) are being used in national and international (R. Trethowan, CIMMYT, pers. comm.) breeding programs, additional resistance genes are needed to avoid complete reliance on a single source. Furthermore, crown rot resistance transferred from line 2-49 may not be high enough to avoid economic loss in seasons when disease pressures are

great. The objective of this chapter is to identify QTL for resistance to crown rot in the W21MMT70 x Mendos DH population, by performing marker regression, interval mapping, and composite interval mapping.

3.2 Materials and Methods

3.21 Marker-Trait Associations

Molecular Map. The molecular map described in Chapter 2 (with a combination of SSR and AFLP markers) was used for identifying QTL. Markers that were deemed redundant were not included in the analysis.

QTL Detection. Marker regression was carried out using MapManager (QTXb20) with only markers showing a significance of $p<0.01$ being investigated. One thousand (1000) permutation tests at 2cM intervals were carried out to determine significance thresholds for QTL detection for all trials. Simple and composite interval mapping for seedling resistance to crown rot was carried out using Windows QTL Cartographer version 2.0 (Wang et al., 2001-2004). Simple interval mapping (SIM) was conducted with a walkspeed of 2 cM. For composite interval mapping (CIM), model 6, with a 10 cM window, forward regression for selection of five background markers, and 2 cM walk-speed was employed.

3.22 Leaf Sheath Specific QTL

Traditionally, SIM and CIM are conducted on data produced by adding the disease severity scores of each of the first three leaf sheaths together, dividing this value by the score of the susceptible cultivar Puseas, and converting thus value to obtain the final percent Puseas score for each of the individual lines. SIM and CIM were also conducted on data from each individual leaf sheath in order to detect any putative leaf sheath specific QTL.

3.3 Results

3.31 Marker Regression

Marker regression was carried out within the Map Manager program with a significance threshold of $p<0.01$ for the 2001 (Table 3-1), 2003 (Table 3-2), and 2005 (Table 3-3) seedling trials. In 2001, three chromosomes (chromosomes 1A, 2B, and 5D) contained markers that had a significant influence on the trait values. As indicated by the additive regression coefficient values, the 1A and 2B marker-trait associations were inherited from the susceptible parent Mendos, whereas the 5D region was inherited from the W21MMT70 parent. Chromosome 5D had by far the strongest effect on the trait, with the highest LRS (46.4) at SSR marker Xbarc143. The AFLP marker p61-138M on chromosome 2B had an LRS of 25.3, while the strongest 1A marker (Xgwm558) had an LRS of only 6.8.

In 2003 (Table 3-2), six chromosomes contained markers that putatively influenced the trait. These included chromosomes 1A, 2B, and 5D as was found in the 2001 trial. In this trial, more markers on chromosome 1A had significant affects on the trait, with AFLP maker p44-314W having the highest LRS of 10.6. The AFLP marker p61-178W on chromosome 2B had an LRS of 19.5, and for chromosome 5D, SSR marker Xbarc205 had the highest LRS of 13.8. As well as the chromosomes consistent between the 2001 and 2003 trials, markers present on chromosomes 2Di, 5B, and 6A were also shown to have a significant ($p<0.01$) effect based on the 2003 data. The highest LRS of markers in each of these regions (2Di, p58-87dW, LRS 7.6; 5B, p34-208M, LRS7.5; 6A, Xpsp3152, LRS 7.9) were lower than those that were consistent (on chromosomes 1A, 2B, and 5D) between both the 2001 and 2003 trials.

In 2005 (Table 3-3), chromosomes 1A (Xpsp3027, LRS 13.7), 2B (Xgwm515a, LRS 23.5), and 5D (Xbarc143, LRS13.8) were once again shown to have a significant effect on the trait. As was the case with the 2003 data, a marker on

chromosome 2Di (p56-143M) had a significant effect on the trait (LRS 7.2). No markers on chromosome 1D were shown to be associated with the trait in either 2001 or 2003, however with the 2005 data, AFLP marker p62-112W (LRS 8.5) appeared to have an effect.

The additive regression coefficient values of the 2003 and 2005 trials show that the 1A and 2B regions that are associated with resistance are inherited from the susceptible parent Mendos, whereas the 5D region is inherited from the partially resistant parent W21MMT70. Of the other chromosomes, the 1A, 1D, and 5B regions were inherited from Mendos, whereas the 2Di and 6A regions are inherited from W21MMT70.

Table 3-1. Significant markers (p<0.01) from the marker regression report (Map Manager) based upon 2001 seedling trial data.

Chromosome	Marker	LRS[1]	%V.E.[2]	P	Add[3]
1A	Xgwm558	6.8	7	0.00918	-6.76
2B	Xgwm388	9.7	10	0.00189	-8.1
2B	Sr36	10.1	10	0.00146	-8.03
2B	Xgdm086	11.7	12	0.00064	-8.68
2B	p39-196W	17	17	0.00004	-9.84
2B	Xgwm429	9.9	10	0.00166	-8.35
2B	Xgwm515a	17.9	17	0.00002	-10.21
2B	Xgwm666	12.5	13	0.00042	-9.15
2B	p1-6M	18.7	18	0.00002	-10.41
2B	p3-169W	13.7	14	0.00022	-9.2
2B	p3-180dW	14.5	14	0.00014	-9.35
2B	p3-60dM	14.8	15	0.00012	-9.48
2B	p36-172M	21.2	20	<0.00000	-11.08
2B	p61-138M	25.3	24	<0.00000	-11.82
2B	P63-178W	25.1	24	<0.00000	-12.19
2B	p12-375M	19.6	19	0.00001	-10.6
2B	p56-251W	17.7	17	0.00003	-10.19
2B	p53-294W	19.4	19	0.00001	-10.7
2B	p53-112W	19.1	19	0.00001	-10.73
2B	p37-286W	19.7	19	0.00001	-10.81
2B	p40-438W	18	18	0.00002	-10.51
2B	p5-6W	16.2	16	0.00006	-10.14
2B	Xcfa2278	13.3	14	0.00027	-9.05
2B	Xbarc200	15.8	16	0.00007	-10.5
2B	Xgwm630	12.1	13	0.00051	-8.84
5D	Xgwm190	12.1	13	0.0005	8.45
5D	Xbarc205	34.4	32	<0.00000	14.29
5D	Xbarc143	46.4	40	<0.00000	16.26
5D	Xcfd40	26.6	26	<0.00000	13.05
5D	Xgwm159	34.9	32	<0.00000	14.28
5D	Xgwm358	38.9	35	<0.00000	14.82

[1] LRS – Likelihood ratio statistic

[2] %V.E. – The percentage of phenotypic variance explained

[3] Add – The additive regression coefficient; positive if the presence of the paternal allele tends to increase the trait.

Table 3-2. Significant markers ($p<0.01$) from the marker regression report (Map Manager) based upon 2003 seedling trial data.

Chromosome	Marker	LRS	%V.E.	P	Add
1A	p14-385W	7.2	8	0.00744	-6.43
1A	p38-211W	7.9	8	0.005	-6.62
1A	Xpsp3027	9.3	10	0.00223	-7.32
1A	p44-314W	10.6	11	0.00113	-7.58
1A	p58-212C	8.4	9	0.00378	-6.82
1A	p61-204M	7.8	8	0.00532	-6.53
2B	Xgwm388	8.1	8	0.00445	-7.29
2B	Sr36	7.4	8	0.00638	-6.75
2B	Xgdm086	11.4	12	0.00072	-8.4
2B	p39-196W	16.5	16	0.00005	-9.54
2B	Xgwm429	10.4	11	0.00128	-8.46
2B	Xgwm515a	13.4	14	0.00025	-8.76
2B	Xgwm666	8.3	9	0.00398	-7.41
2B	p1-6M	13	13	0.0003	-8.65
2B	p3-169W	11.6	12	0.00065	-8.29
2B	p3-180dW	12.8	13	0.00035	-8.58
2B	p3-60dM	10.8	11	0.00101	-7.96
2B	p36-172M	14.6	15	0.00014	-9.11
2B	p61-138M	18.2	18	0.00002	-9.97
2B	P63-178W	19.5	19	0.00001	-10.67
2B	p12-375M	14.2	14	0.00017	-8.92
2B	p56-251W	12.5	13	0.0004	-8.49
2B	p53-294W	14.8	15	0.00012	-9.25
2B	p53-112W	15.5	16	0.00008	-9.52
2B	p37-286W	15.7	16	0.00008	-9.5
2B	p40-438W	11.7	12	0.00062	-8.4
2B	p5-6W	11.4	12	0.00073	-8.4
2B	Xcfa2278	9.8	10	0.00171	-7.71
2B	Xbarc200	9.7	10	0.00184	-8.2
2B	Xgwm630	9.4	10	0.00221	-7.81
2B	Xwmc154	8.2	9	0.00408	-7.19
2B	Xcfd238	9.3	11	0.0023	-7.56
2Di	p6-224M	7.5	8	0.00609	6.45
2Di	p58-87dW	7.6	8	0.0057	6.58
2Di	p6-135M	7	7	0.00825	6.19
5B	p34-208M	7.5	8	0.00612	-6.48
5D	Xgwm190	8.5	9	0.00364	7.03
5D	Xbarc205	13.8	14	0.0002	9.45
5D	Xbarc143	13.2	14	0.00028	9.32
5D	Xcfd40	9.1	10	0.00252	7.93
5D	Xgwm159	11.6	12	0.00067	8.73
5D	Xgwm358	12.7	13	0.00037	8.97
6A	Xwmc256	7.8	8	0.00533	6.67
6A	Xpsp3152	7.9	8	0.005	7.06
6A	Xgwm518	7	7	0.00822	6.44

Table 3-3. Significant markers (p<0.01) from the marker regression report (Map Manager) based upon 2005 seedling trial data.

Chromosome	Marker	LRS	%V.E.	P	Add
1A	p14-385W	7.7	8	0.00563	-6.35
1A	Xwmc278	8.4	9	0.00379	-6.79
1A	p38-211W	11.8	12	0.0006	-7.65
1A	Xpsp3027	13.7	14	0.00022	-8.36
1A	p44-314W	12.8	13	0.00034	-7.92
1A	p58-212C	13	13	0.00031	-8.02
1A	p61-204M	13.4	14	0.00025	-8.07
1A	p44-219cW	9.5	10	0.00209	-6.98
1A	p58-373C	10.2	11	0.00139	-7.2
1A	p34-92dM	9.3	10	0.00231	-7.1
1A	Xgwm558	9.9	10	0.00163	-7.44
1D	p62-112W	8.5	9	0.00348	-6.64
2B	Xgwm388	10.4	11	0.00126	-7.71
2B	Sr36	11.2	12	0.00081	-7.69
2B	Xgdm086	12.9	13	0.00033	-8.29
2B	p39-196W	18.6	18	0.00002	-9.42
2B	Xgwm429	19.4	19	0.00001	-10.59
2B	Xgwm515a	23.5	23	<0.00000	-10.58
2B	Xgwm666	16.5	17	0.00005	-9.58
2B	p1-6M	20.4	20	0.00001	-9.93
2B	p3-169W	19	19	0.00001	-9.74
2B	p3-180dW	18.2	18	0.00002	-9.44
2B	p3-60dM	17.7	18	0.00003	-9.38
2B	p36-172M	22.1	22	<0.00000	-10.3
2B	p61-138M	28.2	27	<0.00000	-11.32
2B	P63-178W	27.8	26	<0.00000	-11.66
2B	p12-375M	23	22	<0.00000	-10.39
2B	p56-251W	21.8	21	<0.00000	-10.24
2B	p53-294W	21.3	21	<0.00000	-10.2
2B	p53-112W	21	21	<0.00000	-10.22
2B	p37-286W	23.1	22	<0.00000	-10.58
2B	p40-438W	18.4	18	0.00002	-9.66
2B	p5-6W	16.7	17	0.00004	-9.39
2B	Xcfa2278	18.9	19	0.00001	-9.9
2B	Xbarc200	17.7	18	0.00003	-10.31
2B	Xgwm630	13.5	14	0.00023	-8.84
2Di	p56-143M	7.2	8	0.00712	6.34
5D	Xbarc205	7.8	8	0.00533	6.84
5D	Xbarc143	13.8	15	0.0002	9.05
5D	Xcfd40	8.2	9	0.00426	7.15
5D	Xgwm159	11.7	12	0.00064	8.32
5D	Xgwm358	11.7	12	0.00064	8.18

3.32 Simple Interval Mapping

Simple interval mapping was carried out within QTL Cartographer. In order to determine thresholds for QTL, permutation tests were first carried out for each of the seedling trials. 1000 permutations at 2cM intervals were used to determine significance thresholds (Table 3-4).

Table 3-4. Thresholds for QTL detection based upon permutation tests (10000 permutations at 2cM intervals).

Year	Suggestive	Significant	Highly Significant
2001	7.7	13.8	22.3
2003	7.7	13.8	21.6
2005	7.7	13.6	22.0

QTL Cartographer carries out SIM across all traits (or trials) and gives the user the option of displaying the results together for ease of comparison. Using the previously described permutation test results, QTL Cartographer was employed to carry out SIM across all three trials.

Chromosome 1A had a suggestive QTL in all three seedling trials in the interval between Xgwm558 and p36-198M (Figure 3-1a). In 2001, this QTL had a maximum LRS of 9.1 and explained 10.4% of the phenotypic variance; in 2003, this QTL had a maximum LRS of 8.3 and explained 8.9% of the phenotypic variance; and in 2005, the QTL had a maximum LRS of 9.5 and explained 10.3% of the phenotypic variance. For the 2005 trial, the results of the SIM may indicate the presence of a second suggestive QTL on 1A in the interval between p14-385W and p58-373C (LRS of 10.8, 11.1% phenotypic variance explained).

Suggestive QTL were identified on chromosome 1D in the 2003 and 2005 trials (Figure 3-1b). The peaks of these QTLs are in different regions of chromosome 1D. In 2003, the interval between Xwmc429 and Xwmc36 (maximum LRS of 9.3; 13.1% phenotypic variance explained) was identified. In 2005, the peak of the QTL was at AFLP marker p62-112W (maximum LRS 8.3; 8.8% of the

phenotypic variance explained). In 2001 the LRS value of the 1D QTL was below the significance of the thresholds determined by the permutation tests.

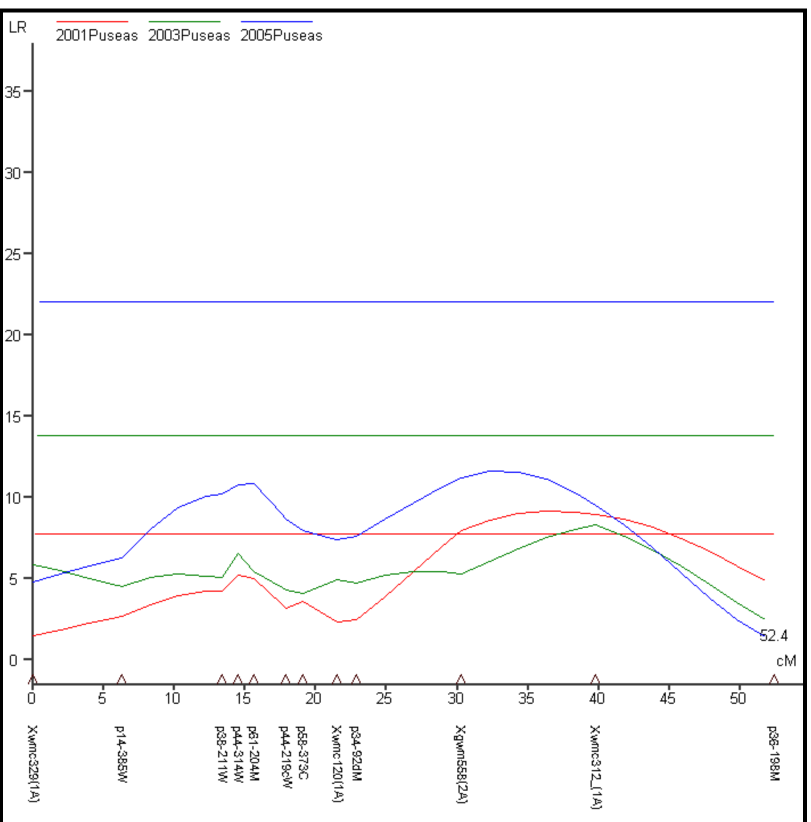
A large region of chromosome 2B was either significant (2003 trial) or highly significant (2001 and 2005 trials). In each seedling trial, the peak of the QTL was located at AFLP marker p61-138M (Figure 3-1c). This QTL had maximum LRS values of 22.8 (explaining 21.6% of the phenotypic variance), 16.2 (explaining 16.0% of the phenotypic variance), and 26.8 (explaining 25.2% of the phenotypic variance) for the 2001, 2003, and 2005 trials respectively.

Highly significant (2001 data), significant (2003 data), and suggestive QTL (2005 data) were identified in a region of chromosome 5D (Figure 3-1d). In 2001, this QTL had a maximum LRS of 40.3 and explained 36.2% of the phenotypic variance; in 2003 this QTL had a maximum LRS of 17.6 and explained 25.7% of the phenotypic variance; and in 2005 this QTL had a maximum LRS of 11.6 and explained 12.2% of the phenotypic variance.

Table 3-5 provides an overview of the QTL detected in each year by simple interval mapping.

Figure 3-1. QTL identified by simple interval mapping using the program QTL Cartographer in three seedling trials. Horizontal red, green, and blue lines indicated thresholds for suggestive, significant, and highly significant QTL respectively.

a) Chromosome 1A



b) Chromosome 1D

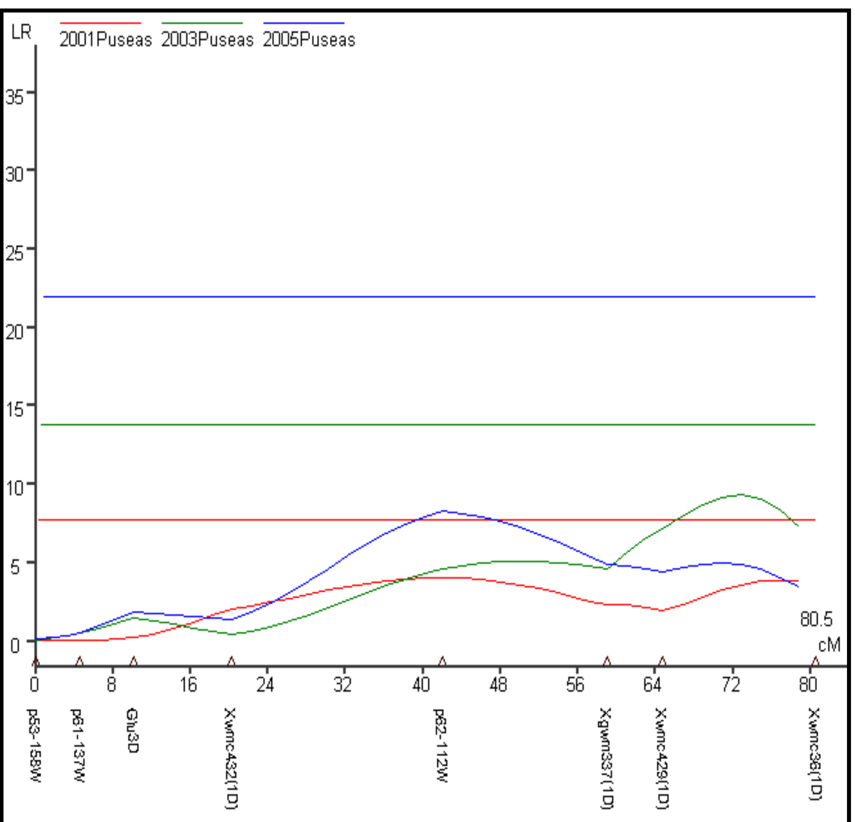
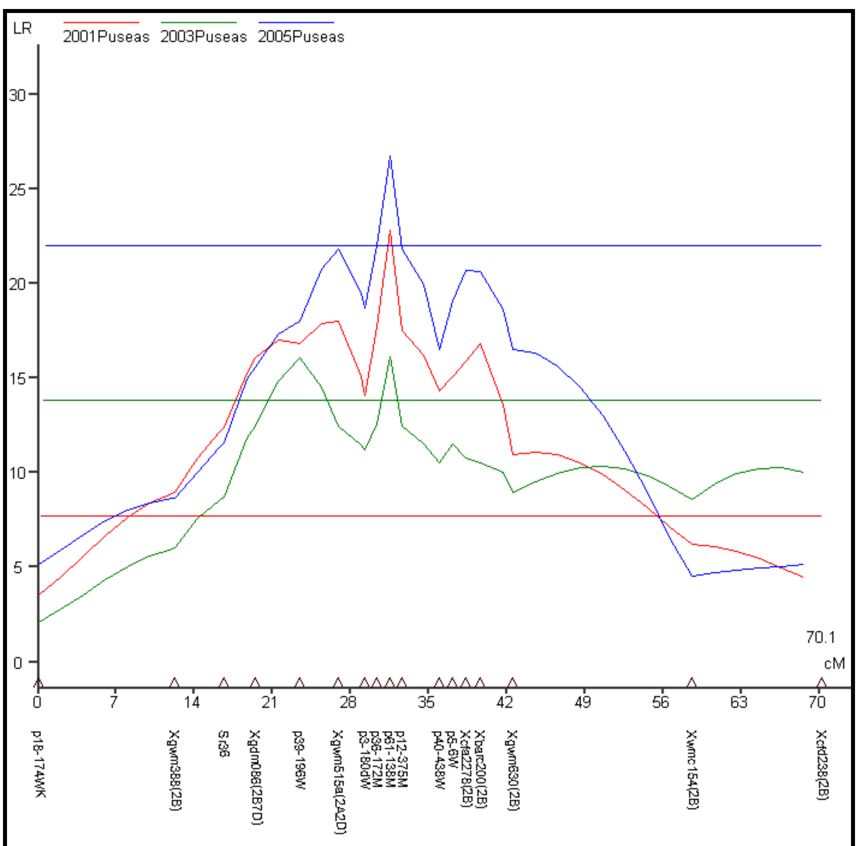


Figure 3-1. Continued.

c) Chromosome 2B



d) Chromosome 5D

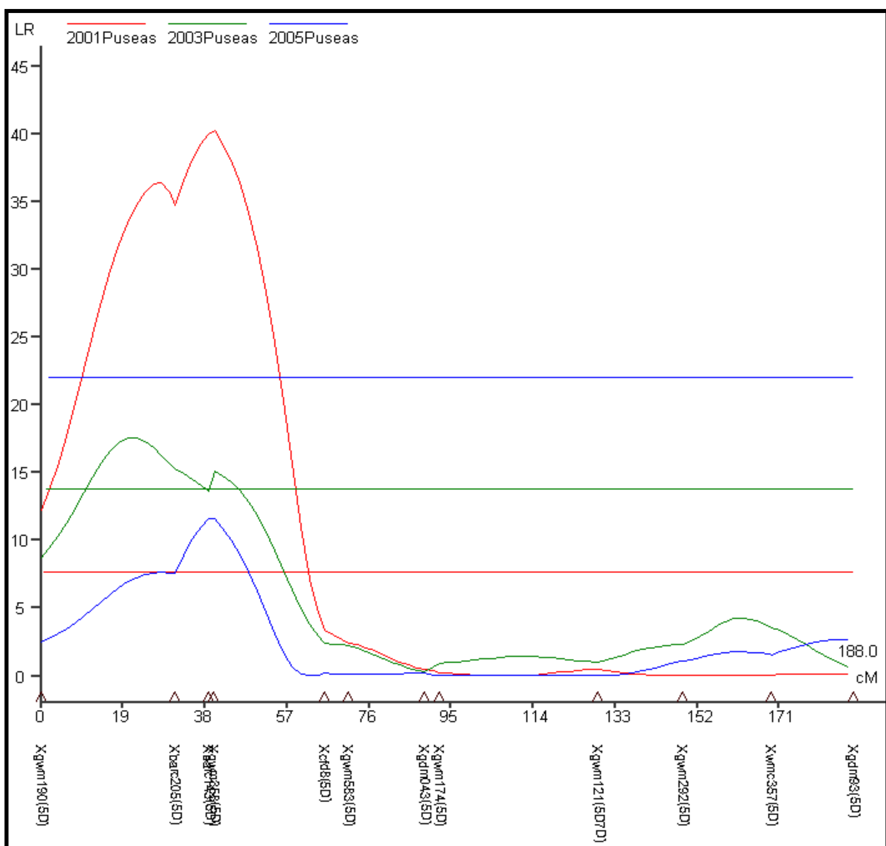


Table 3-5. QTL for crown rot resistance detected using simple interval mapping in three seedling trials. Chromosome location, LRS value, percent phenotypic variance explained (%VE), significance level (SL), and the parent contributing the resistance allele (Source) are shown.

Chromosome	2001			2003			2005			Source
	LRS	%VE	SL[1]	LRS	%VE	SL	LRS	%VE	SL	
1A	9.1	10.4	Sg	8.3	8.9	Sg	9.5	10.3	Sg	Mendos
1D	3.9	5.2	NS	9.3	13.1	Sg	8.3	8.8	Sg	Mendos
2B	22.8	21.6	HS	16.2	16.0	S	26.8	25.2	HS	Mendos
5D	40.3	36.2	HS	17.6	25.7	S	11.6	12.2	Sg	W21MMT70

[1]Significance levels were determined by permutations (1000 permutations at 1 cM intervals). Suggestive (Sg), significant (S), and highly significant (HS) LRS thresholds respectively for each seedling trial are: 2001 – 7.7, 13.8, 22.3; 2003 – 7.7, 13.8, 21.6; 2005 – 7.7, 13.8, 22.6. Data for corresponding regions that were not significant (NS) in particular seedlings trials are shown for comparison.

3.33 Composite Interval Mapping

Using composite interval mapping, eight QTL for seedling resistance to crown rot were found (Table 3-6). Of these eight QTL, only three (on chromosomes 2D, 2B, and 5D) were consistent in all three seedling trials. The remaining QTL were identified in only one (chromosomes 4A, 5A, and 6A) or two (chromosomes 1A and 3B) of the three seedling trials. Of the QTL observed in all three seedling trials, the 5D and 2D QTL alleles were inherited from the resistant parent W21MMT70, whereas the 2B QTL alleles were inherited from the susceptible parent Mendos.

Interval maps and LRS plots for the 2B, 2D, and 5D QTLs are shown in Figure 3-2. The 2B QTL (Figure 3-2a) had maximum LRS values of 26.0 (2001 trial) 18.9 (2003 trial) and 31.6 (2005) trial. This QTL explained between 13.2% and 19.9% of the phenotypic variance. The 2D QTL (Figure 3-2b) was suggestive in both the 2001 and 2003 trials (LRS of 10.7 and 11.3 respectively), and significant in the 2005 trial (LRS 17.0). This QTL explained 10.2% of the phenotypic variance in 2005. The 5D QTL (Figure 3-2c) was highly significant in 2001 (LRS 43.5, explaining 28.1% of the phenotypic variance), significant in 2003 (LRS 13.6, explaining 13.8% of the phenotypic variance), and only suggestive based upon the 2005 data (LRS 8.1, explaining 4.8% of the phenotypic variance).

The effect of various combinations of alleles at the 2B, 2D, and 5D QTL are shown in Figure 3-3. The doubled-haploid lines with all 3 resistance alleles (the 2B allele from Mendos, and the 2D and 5D alleles from W21MMT70) had a mean severity rating of 44.4%. This value is 28.4% lower than the population mean of 62%, and 54.5% lower than lines having susceptible alleles at all three loci (97.6% Puseas).

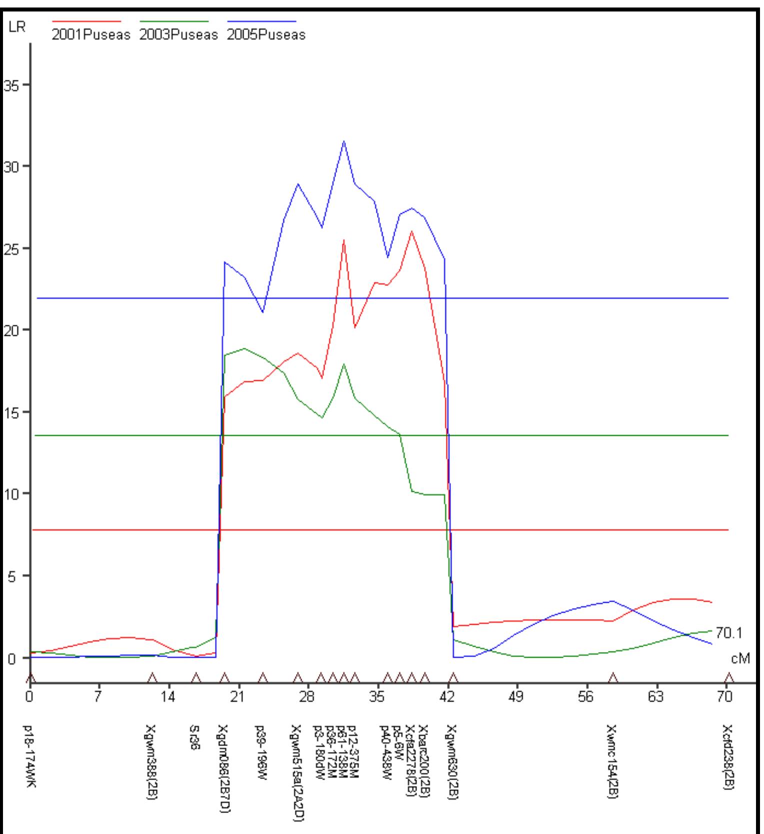
Table 3-6. QTL for crown rot resistance detected using composite interval mapping in three seedling trials. Chromosome location, LRS value, percent phenotypic variance explained (%VE), significance level (SL), and the parent contributing the resistance allele (Source) are shown.

Chromo-some	2001			2003			2005			Source
	LRS	%VE	SL[1]	LRS	%VE	SL	LRS	%VE	SL	
1A	7.8	4.9	Sg	3.3	2.2	NS	8.3	4.8	Sg	Mendos
2B	26.0	13.2	HS	18.9	13.0	HS	31.6	19.9	HS	Mendos
2D	10.3	4.8	Sg	11.3	7.7	Sg	17.0	10.2	S	W21MMT70
3B	1.8	0.8	NS	8.2	5.0	Sg	13.7	8.1	Sg	W21MMT70
4A	1.9	0.8	NS	0.8	0.6	NS	9.3	5.2	Sg	Mendos
5A	9.9	6.2	Sg	3.5	2.5	NS	0.9	0.7	NS	Mendos
5D	43.5	28.1	HS	13.6	13.8	S	8.1	4.8	Sg	W21MMT70
6A	3.4	1.6	NS	9.3	5.9	Sg	0.8	0.4	NS	W21MMT70

[1]Significance levels were determined by permutations (1000 permutations at 1 cM intervals). Suggestive (Sg), significant (S), and highly significant (HS) LRS thresholds respectively for each seedling trial are: 2001 – 7.7, 13.8, 22.3; 2003 – 7.7, 13.8, 21.6; 2005 – 7.7, 13.8, 22.6. Data for corresponding regions that were not significant (NS) in particular seedlings trials are shown for comparison.

Figure 3-2. QTL identified by composite interval mapping in all three trials. Horizontal red, green, and blue lines indicated thresholds for suggestive, significant, and highly significant QTL respectively.

a) Chromosome 2B



b) Chromosome 2D

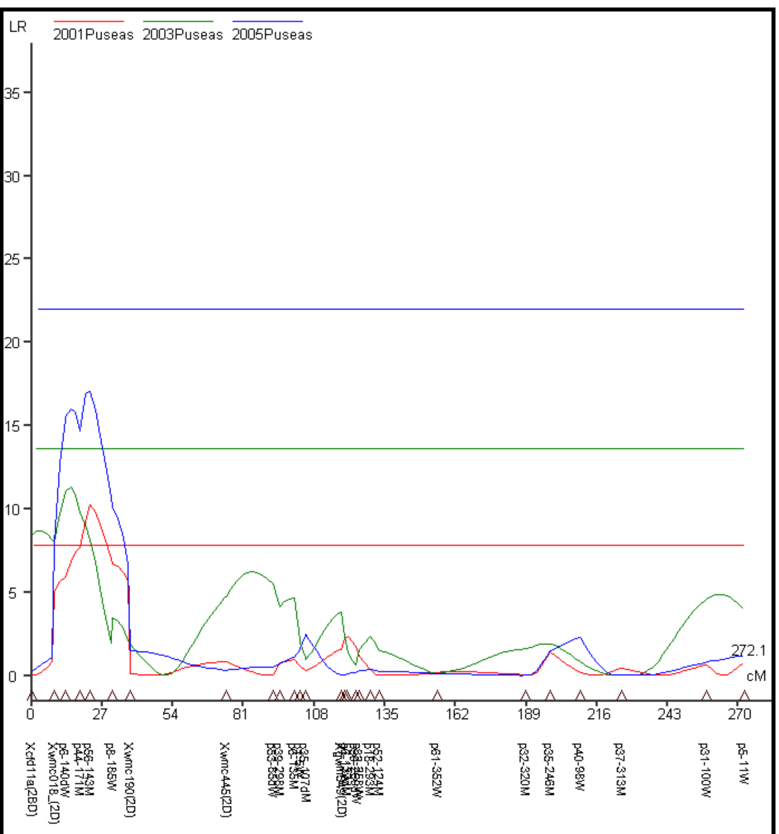


Figure 3-2. Continued.

c) Chromosome 5D

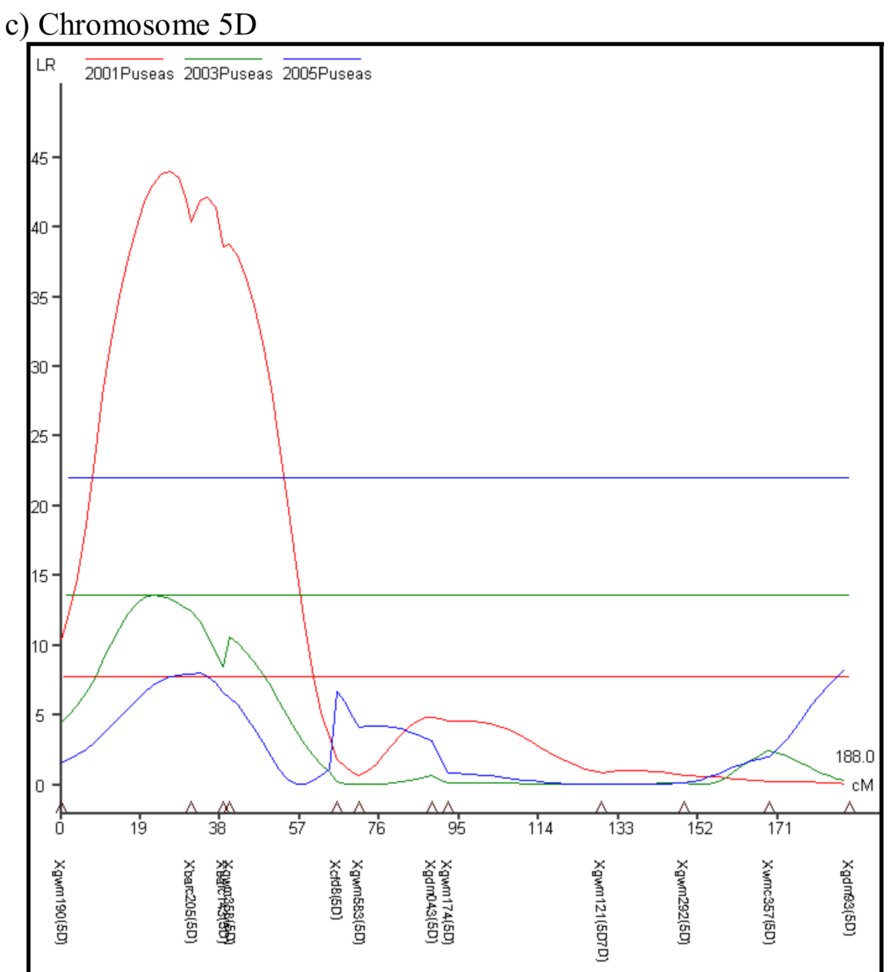
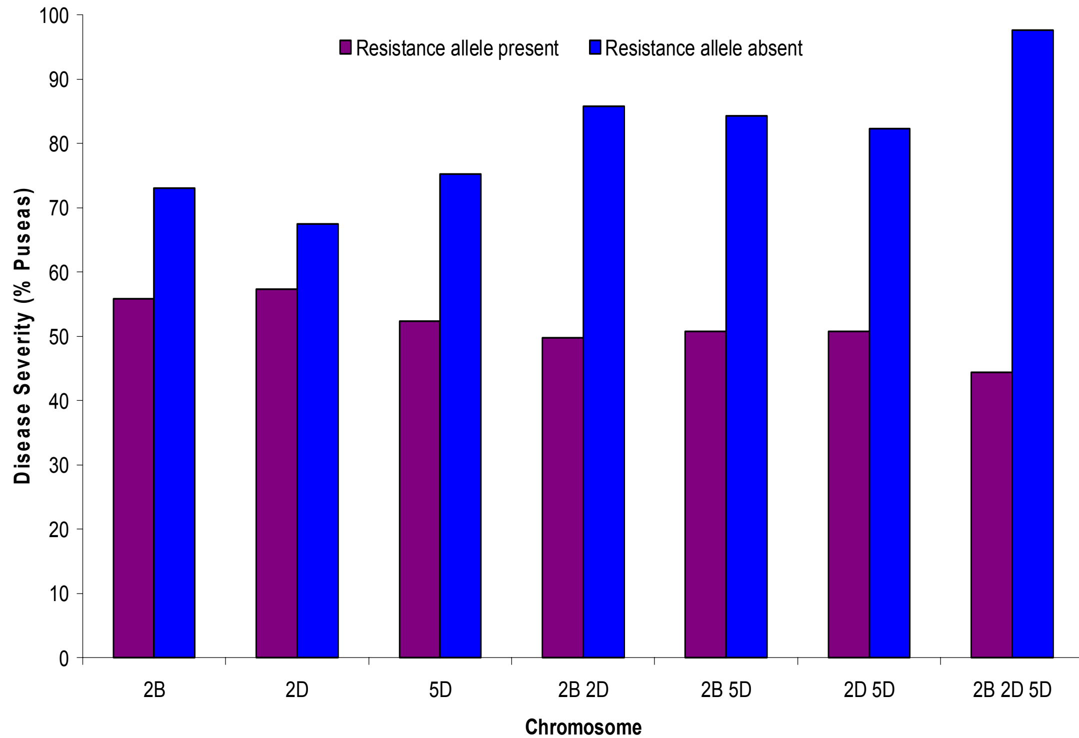


Figure 3-3. Mean disease severity (% of 'Puseas') of doubled-haploid lines with combinations of alleles from the three QTL regions. In all instances, differences were significant between lines carrying a QTL contributing to resistance and those without (Students t-test, $p<0.05$).



3.34 Leaf Sheath Specific QTL

QTL analysis was conducted on data from each of the individual leaf sheaths, rather than upon the final score of the three leaf sheaths added together, in order to identify any potential differences in QTL detected when the disease severity scores of the individual leaf sheaths were analysed. Each of the QTL that were detected based on the total leaf sheath data were detected for at least one of the individual leaf sheaths (Figure 3-4). Four further QTL were detected however, located on chromosomes 2A, 3A, 4B, and 7A. Of these four QTL, only one, on chromosome 4B was detected in more than one of the three trials.

Of the QTL that were identified on combined leaf sheath data, the 1A, 1D, and 3B QTL were only detected with leaf sheath one data. In two of three trials, the 2D QTL was detected regardless of the leaf sheath analysed. The 2B QTL was

only detected with leaf sheath one data in one of the trials (2003); in each of the other trials, this QTL was only detected with leaf sheath two and three data. The 5D QTL generally had greatest effects with the second and third leaf sheath data.

Figure 3-4. Identification of leaf sheath specific QTL detected by QTL Cartographer using CIM for the a) 2001 seedling trial; b) 2003 seedling trial; and c) 2005 seedling trial. The percentage phenotypic variance explained for QTL detected for the individual leaf sheaths (LS1 – leaf sheath 1; LS2 – leaf sheath 2; LS3 – leaf sheath 3) on each chromosome is plotted.

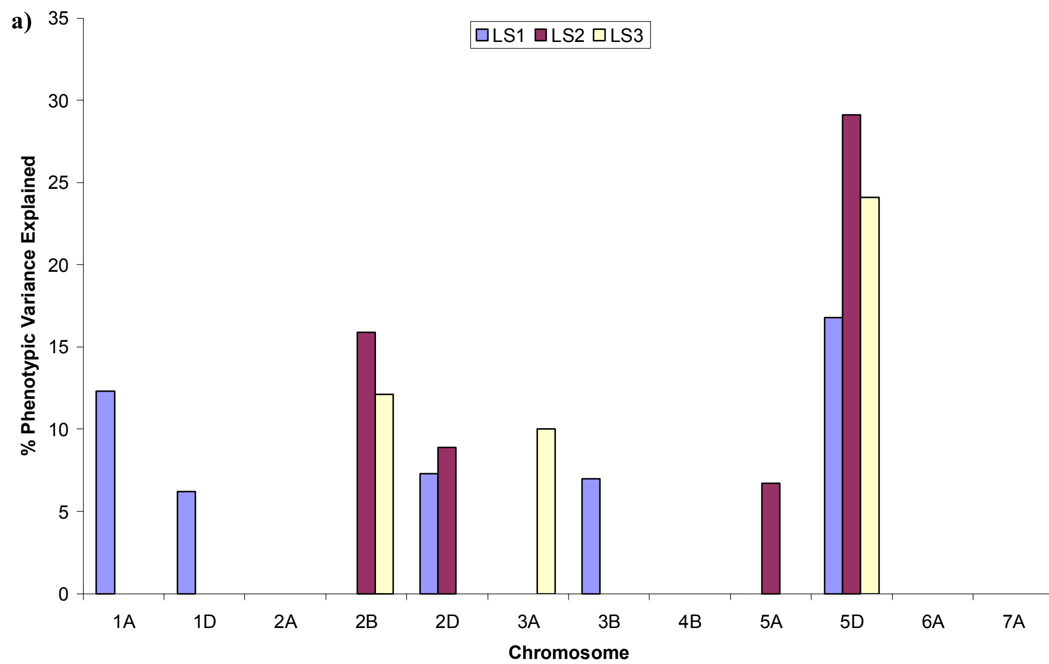
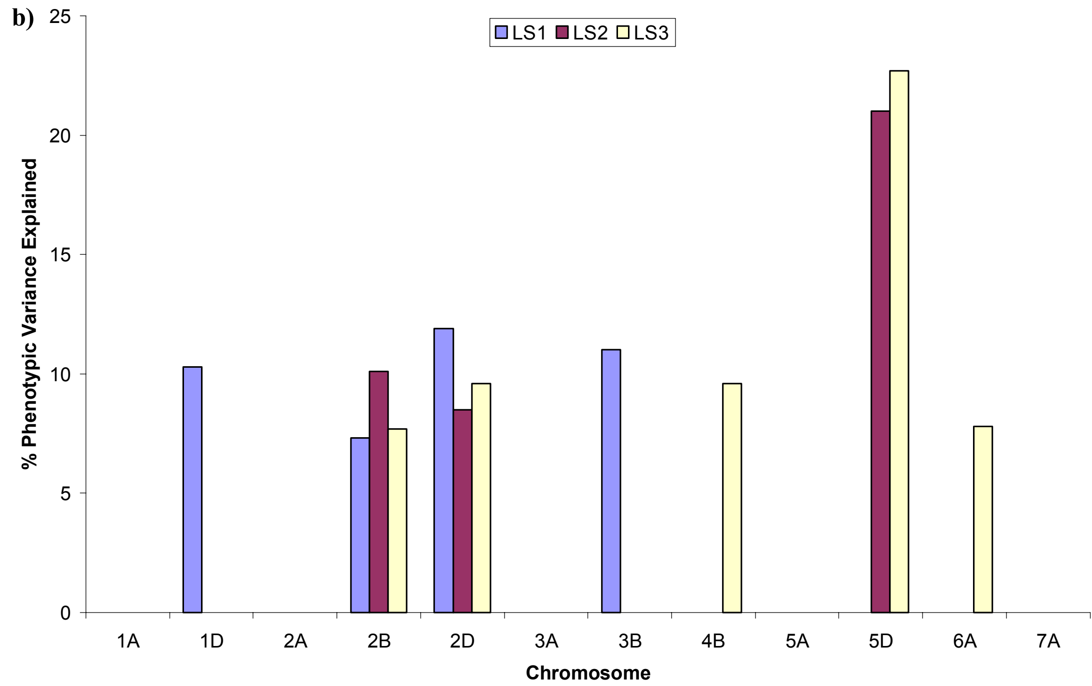
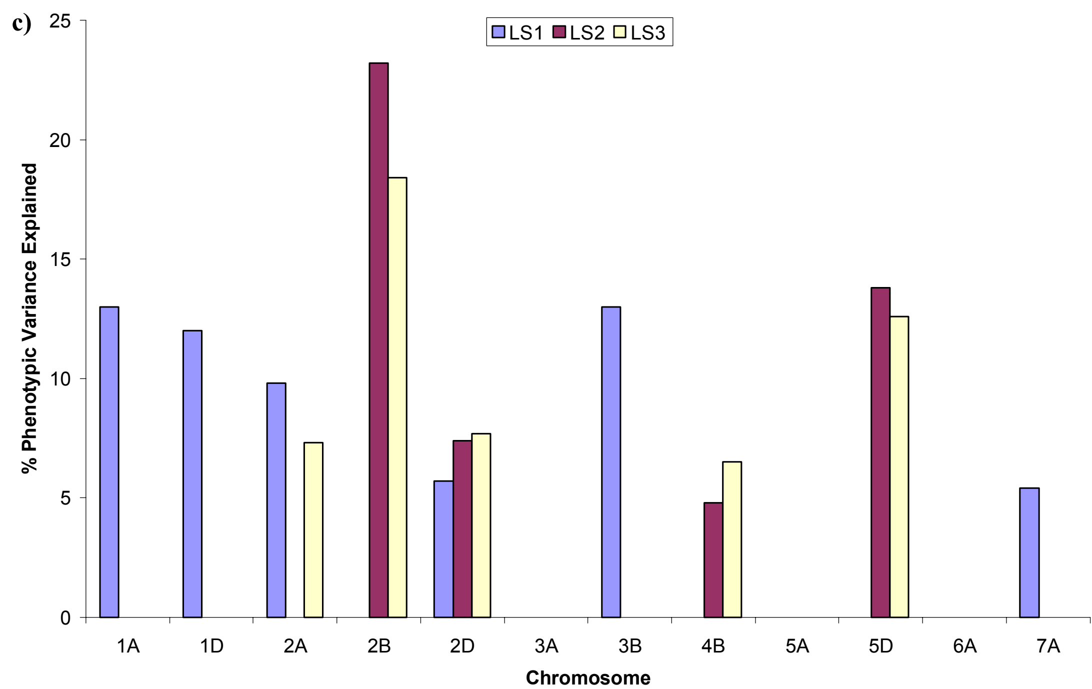


Figure 3-4. (Continued)





3.4 Discussion

Marker regression provides a "first-glance" analysis of genomic regions associated with traits of interest. In the current study, marker regression identified large numbers of markers that were associated with partial resistance to crown rot. Three (3) chromosomes were consistently detected with marker regression over the three seedling trials – chromosomes 1A, 2B, and 5D. Other putative associations were only detected in one or two of the three seedling trials. It is well established however, that the main disadvantage of this type of analysis is that the further the away the marker is from the gene(s), the less likely it is to be detected statistically due to recombination between the marker and the gene(s) (Tanksley, 1993). Simple interval (Lander and Botstein, 1989) and composite interval mapping (Zeng, 1994) are two widely used methods to overcome the disadvantages of marker regression. Simple interval mapping (SIM) builds upon regression analysis by using linked markers for the analysis to assess the statistical likelihood of a QTL occurring in the interval between two markers. Composite interval mapping (CIM) is considered more statistically powerful than simple interval mapping because this method attempts to account for the effect of QTL located elsewhere on the genome that may have an interfering effect (Jansen, 1993), thereby increasing the accuracy and precision of QTL detection (Hackett, 2002).

Although the SIM and CIM techniques often produce similar results, differences have been reported by other researchers (Falak et al., 1999; Cai and Morishima, 2000; Collard et al., 2005b) and were also apparent in the current study. The QTL on chromosome 1A that was detected in all three trials by SIM was only detected in two of the three trials by CIM. The 2D QTL that was detected in all three trials by CIM was not detected at all by SIM. Furthermore, the CIM method detected three other QTL (on chromosomes 3B, 4A, and 6A) that were not detected by SIM. The width of the QTL peaks that were consistent between both SIM and CIM analysis (located on chromosomes 2B and 5D) were better defined using the CIM approach (particularly in the case of chromosome 2B),

which is consistent with the greater precision that is offered by this technique (Doerge, 2002).

Of the eight QTL identified by CIM, only three (the 2B, 2D, and 5D QTL) were consistent across all seedling trials. This finding highlights the significant environmental variation exhibited when screening for resistance to crown rot, and supports the role of molecular markers as valuable tools to aid breeding programs in the selection of resistant materials. The other five QTL identified were only minor in their effect, and as these QTL were only identified in one or two of the three seedling trials, further studies need to be carried out in order to confirm their putative correlation with crown rot resistance. Given the results for SIM, the region on 1A should be of particular interest.

During her Honours project, Kimberley Ritter (2001) phenotyped the W21MMT70 x Mendos DH population for seedling resistance to crown rot, and used the molecular map of Ma (2000) to identify QTL for resistance. In the previous chapter, the chromosomal location of markers identified by Ritter was confirmed as chromosome 2B. Of the QTL identified in all three seedling trials by CIM, neither the 2D nor the 5D QTL were detected in this early study. Ritter (2001) used SIM for her study, and, as in the current study, this method did not detect the 2D QTL that was contributed by W21MMT70. The 5D QTL is composed solely of SSR markers, and as the map of Ma (2000) used by Ritter did not cover this chromosome 5D region, it would have been undetectable. This demonstrates that the production of a framework SSR map and the incorporation of AFLP markers onto it, has proven a successful strategy for locating QTL for partial resistance to crown rot.

The identification of the QTL on chromosome 2B from the susceptible parent (Mendos) is consistent with the observation of trangressive segregants in the seedling trials. Indeed, other studies on disease resistance have shown that significant QTL may be inherited from susceptible parents (Steiner et al., 2004; Collard et al., 2005b). The QTL for crown rot resistance on chromosome 2B is

in close proximity to Sr36, which indicates that this QTL is located on the introgression from *Triticum timopheevi* – an introgression that contains a number of important disease resistance genes (Tao et al., 2000; Bariana et al., 2001; Brown-Guedira et al., 2003). Strong evidence has been provided for the clustering of disease resistance genes within chromosomes (Dilbirligi et al., 2004), and the close association of the 2B crown rot QTL with Sr36 may indicate that this QTL is positioned within such a cluster. As is the case for black-point resistance in wheat (Lehmensiek et al., 2004), screening for Sr36 resistance may also be a promising strategy for identifying a useful level of crown rot resistance in pedigrees that contain this *T. timopheevi* introgression.

The analysis of the individual leaf sheath data has provided some interesting results that show that, as the pathogen progresses through the leaf sheaths, different QTL are detected. For example, the 1A, 1D, and 3B QTL are expressed only when the data from leaf sheath one are analysed. The 2B QTL is only detected in one of three trials based upon leaf sheath one data, but has a strong effect in leaf sheaths two and three. The 5D QTL has a minor effect on leaf sheath one in one trial, and becomes increasingly important as the pathogen passes through the leaf sheaths. Schroeder and Christensen (1963) proposed two types of resistance to head scab of wheat caused by *Fusarium graminearum*: type I (resistance to initial infection) and type II (resistance to spread within the spike). The results of the QTL analysis of crown rot resistance in the different leaf sheaths suggests the possibility that similar types of resistance may be displayed in response to infection by *F. pseudograminearum*. For example, it may be possible that the 1A, 1D, and 3B QTL govern resistance to initial penetration (as they are only expressed in the first [outer] leaf sheath), whereas the 2B and 5D QTL govern resistance to pathogen spread (as they are expressed as the pathogen spreads through the tissue). This result suggests that QTL for resistance to crown rot may be important at different stages of the disease process, and detecting their expression may be dependent upon when phenotyping is conducted.

Only two other studies have reported the discovery of molecular markers associated with resistance to crown rot. Wallwork et al. (2004) have identified a QTL on chromosome 4B that explained up to 48% of the phenotypic variance from a cross between the moderately resistant cultivar Kukri and the susceptible cultivar Janz. Collard et al. (2005b) reported two major QTL derived from 2-49 on chromosomes 1A and 1D as well as up to four potential minor QTL, including a 4B locus in the same region as that identified by Wallwork et al. (2004). The molecular map produced in the current study was sufficient to enable comparisons with the QTL reported in Wallwork et al. (2004) and Collard et al. (2005b). Of the loci identified in this current study, only the minor 1A QTL appears to coincide with any previously identified region. A comparison of flanking markers from Collard et al. (2005b) and the current study reveals that this QTL is located in a similar region of chromosome 1A as that found in line 2-49. As this marker-trait association is only suggestive in two of the three seedling trials in the W21MMT70 x Mendos DH population, further work is required to confirm its significance.

At the date of submission, this is the second mapping study of molecular markers linked to partial crown rot resistance in seedlings. Three consistent QTL were identified on chromosomes 2B, 2D, and 5D in each of the three seedling trials conducted. These QTL differ from the major QTL previously described by Collard et al. (2005b), and thus represent potential for pyramiding QTL for the improvement of wheat affected by this economically important disease.

Chapter 4. Advances in Software for Linkage Map Construction, QTL Analysis, and Detection of Epistasis

4.1 Introduction

The ordering of loci within linkage groups is of great importance for robust QTL detection. Wu et al. (2003) have suggested that even if QTL detection methods are appropriate, QTL identified may be incorrect if marker order is inaccurate. Indeed, in a recent study investigating the effect of thorough map curation, Lehmensiek et al. (2005) found that reordering of marker loci not only improved QTL resolution, but also affected the magnitude of QTL effect. In the previous chapter, markers were manually ordered into linkage groups due to the apparent inability of MapManager QTX to link markers in an order that resulted in the shortest possible map distance – this was particularly evident in the case of chromosome 2B. While the manual ordering improved the map compared to the order produced by MapManager, new tools have become available over the progress of this dissertation. RECORD (Van Os et al., 2005) is a recently developed program that was designed to cope with the dense marker data that is being produced by a number of mapping groups. This program orders markers by minimizing the number of recombination events (Van Os et al., 2005), and has recently been used to order markers in an ultra-dense (10,000 marker) map of potato (Van Os et al., 2006).

Resistance to crown rot in wheat is a quantitatively inherited trait showing continuous distribution in all of the segregating populations that have been examined in the literature (Wallwork et al., 2004; Collard et al., 2005b; Bovill et al., 2006). Such quantitative inheritance of complex traits is considered to be the result of a combination of: i) genes with main effects; ii) their interaction with other loci (epistasis); and iii) their interaction with environments that affect trait expression (Wade et al., 2001). To date, the majority of genome mapping studies have focused on the independent effects of main effect QTL to produce a phenotype. However, as Carlborg and Haley (2004) emphasize, interactions between loci or between genes and the environment can make a substantial contribution to the phenotypic variation of complex traits. Futhermore, as

Cheverud and Routman (1995) have pointed out, based upon current physiological knowledge, interaction among gene products appears ubiquitous.

The lack of studies demonstrating epistatic interactions between loci has been attributed to both the availability of suitable molecular evidence to assess such interactions (Carlborg and Haley, 2004), and the availability of appropriate statistical tests to assess their significance (Cordell, 2002). However, with the production of a now large number of “whole-genome” molecular maps (see for example Chalmers et al., 2001; Sourdille et al., 2003), investigations of the importance of epistasis are now becoming more common. In rice, for example, epistatic interactions have been shown to be important in a range of phenotypes such as panicle number (Liao et al., 2001), yield components (Xing et al., 2002; Zhuang et al., 2002), and heterosis (Yu et al., 1997). In wheat, epistatic interactions appear important for the effects of glutenin loci on dough rheological properties (Ma et al., 2005). Each of these studies have found that the contribution to phenotypic variance of main effect QTL is larger than that of epistatic QTL – nonetheless, the apparent ubiquity of epistatic interactions warrants further investigation of their potential role in the expression of resistance to crown rot.

In order to detect epistatic interactions for resistance to crown rot in the W21MMT70 x Mendos DH wheat population, two software packages have been used. The first, Epistat (Chase et al., 1997), was originally designed for investigations of epistatic interactions for agromonic traits in soybean recombinant inbred (RI) populations (Lark et al., 1995; Chase et al., 1997; Orf et al., 1999). The program divides the homozygous RI population into sub-populations based upon genotypic combinations of allele pairs, and uses log-likelihood ratio to determine if non-additive (i.e. epistatic) interactions exist Chase et al., 1997). The second program, QTLNetwork 2.0 (Yang et al., 2005), performs a more complex mixed-linear-model analysis for simultaneous interval mapping of QTL with main (additive) or epistatic (non-additive) effects, as well as QTL x environment interactions (Wang et al., 1999; Yang and Zhu, 2005).

The objectives of this chapter are to: i) assess the usefulness of the program RECORD (Van Os et al., 2005) for ordering of marker loci; ii) compare QTL mapping results using both QTL Cartographer (Wang et al., 2001-2004) and QTLNetwork (Yang et al., 2005) with the map modified according to RECORD (Van Os et al., 2005) output; and iii) identify whether epistatic interactions contribute to crown rot resistance through a comparison of the results of QTLNework 2.0 (Yang et al., 2005) and Epistat (Chase et al., 1997).

4.2 Materials and Methods

4.21 Genetic Map Reconstruction

The effectiveness of the software program RECORD (Van Os et al., 2005) to order markers in linkage groups was assessed. As was the case in Chapter 2, the RAPD markers produced by Ma (2000) were not used for map reconstruction. The remaining markers (331 AFLP markers, 128 SSR markers, 4 phenotypic markers, and 1 STS marker) were entered into the RECORD program for ordering. As RECORD produces a marker order but does not calculate the distance between markers, Map Manager QTX (Manly et al., 2001) was used to calculate distances between markers. To provide a visual overview of major differences between maps, the program R/qtl (Browman et al., 2003) was used to produce a "heat map" of pairwise recombination fractions plotted against LOD scores.

4.22 Revised Marker Order and QTL Analysis

To confirm that QTL detected with the manually produced map were detected with the map produced with the use of RECORD, the results of CIM by QTL Cartographer reported in Chapter 3 were compared with the different versions of the map.

4.23 QTLNetwork

QTLNetwork 2.0 (Yang et al., 2005) is a recently developed software package for detection of main effect QTL, QTL x QTL (i.e. epistatic) interactions, and QTL x environment effects. The program uses a mixed-linear-model approach to detect such interactions. In contrast to QTL Cartographer (Wang et al., 2001-2004), whereby QTL x environment interactions can only be inferred by the appearance or disappearance of particular QTL in different environments, QTLNetwork provides an analysis of the level at which particular QTL are influenced by environmental conditions. A 2D genome scan was used to detect QTL with or without single-locus effects. One thousand (1000) permutations were used to calculate critical F values, and the Monte Carlo Markov Chain method with a Gibbs sample size of 20000 was used to estimate QTL effects. As

the figures drawn by QTLNetwork 2.0 are not always informative (markers are not shown on linkage groups for example), for main effect QTL, F values calculated by QTLNetwork 2.0 were taken from the output (.qnk) file and used to draw QTL using MapChart (Voorrips, 2002).

4.24 Epistat

Epistatic interactions identified by QTLNetwork 2.0 were compared with those reported by the computer program Epistat (Chase et al., 1997). The Epistat program performs whole genome searches for interactions between loci, and is able to identify those which display conditional (i.e. where the magnitude of effect of the primary QTL is dependent upon the presence of alleles at another locus) and/or coadaptive interactions (i.e. where loci have no effect alone on the trait, but when considered with other loci there appears to be an affect). The program examines pairs of markers and uses log-likelihood ratios (LLR) to determine if effects are explained by additive (where the joint effect of two loci is equal to the sum of their individual main effects) or epistatic interactions (Chase et al., 1997). Briefly, an automated, complete pairwise search was carried out to identify epistatic interactions between all pairs of loci using the arbitrarily chosen significance threshold of LLR > 7.0 (higher than the LLR of 6 as recommended by the Epistat authors). The significance of the results of this search was analyzed with a Monte Carlo program contained within the Epistat program. Only the interactions that were detected in at least two of the three seedling trials are reported.

4.3 Results

4.31 Genetic Map Reconstruction

Using the marker order determined by RECORD, a total of 375 markers could be assigned to linkage groups to span a total distance of 2588.4 cM (Table 4-1). When markers were manually ordered to produce the molecular map, the 281 markers that could be assigned to linkage groups spanned a distance of 2346.9 cM. Thus, the use of RECORD to reorder markers resulted in the addition of 94 extra markers (deemed too difficult to place in the manual version of the map) and an increase in map distance of 241.5 cM. When comparing the two versions of the map, the largest differences are seen with chromosomes 1A, 2B, 2D, and 7B. The length of chromosome 1A increased by 76.2 cM with the addition of only two markers – both of which were placed at the distal ends of the linkage group. The map distance and the number of markers on chromosome 2B increased substantially (an increase of 66 markers for a subsequent increase in map distance of 279 cM). This was the same for chromosome 7B, where the addition of 18 markers increased the map distance by 84.3 cM. The length of chromosome 2D decreased by 75.8 cM even after the inclusion of an extra 5 markers to this linkage group.

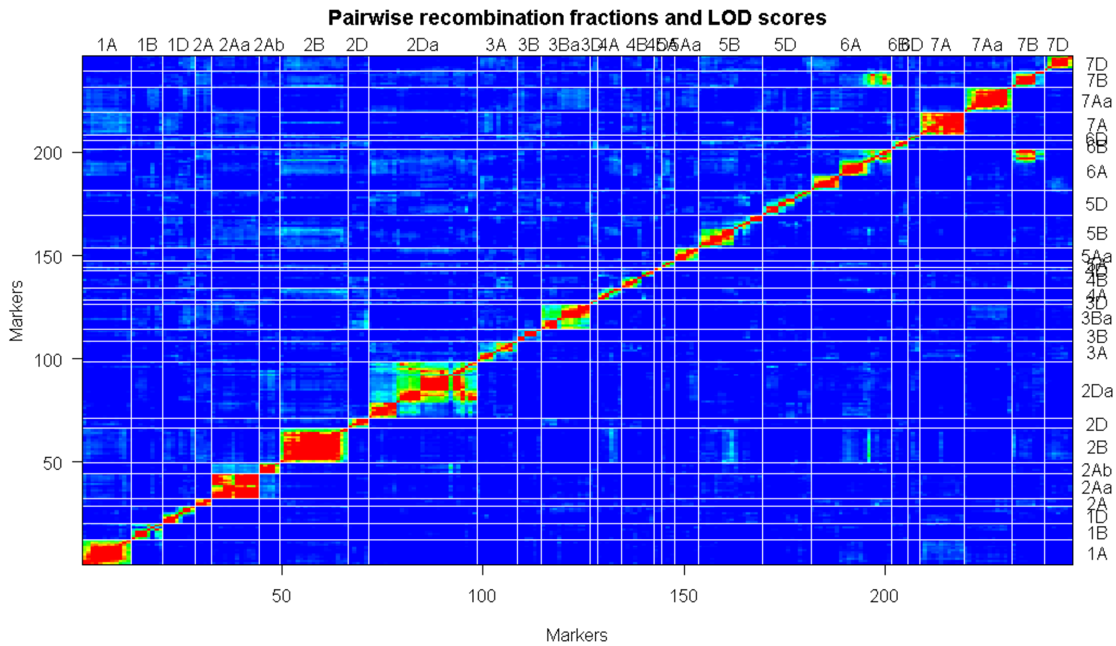
To give a graphical overview of potential problem areas/differences between the two maps, heat maps of each of the genetic maps were produced (Figure 4-1) using R/qtl (Browman et al., 2003). An aspect of this R function is the ability to estimate recombination fractions for all pairs of markers along with LOD scores for the test of r = 1/2. Red indicates a large LOD score or a small recombination fraction, while blue indicated a small LOD score and large recombination fraction. Using this function of R/qtl, it can be seen that on the manual version of the map, markers on chromosome 6A share a strong association with markers on chromosome 7B (Figure 4-1a). The apparent error was not present with the RECORD version of the map (Figure 4-1b).

Table 4-1. Summary of the reconstructed map produced using RECORD. Number of markers per chromosome, map distance, number of double cross-overs, and map distance after removal of double cross-overs are shown. For ease of comparison, linkage groups that could not be assigned to chromosome are not shown.

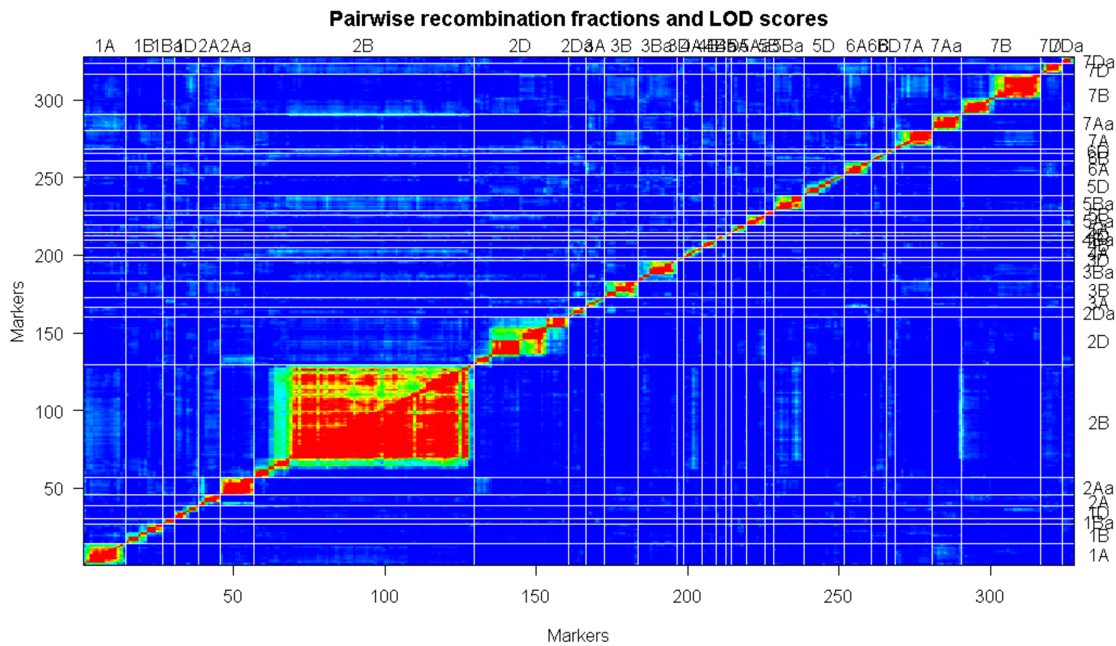
Chromosome	Number of Markers	Map Distance (cM)	Number of Double Cross-Overs	Distance after Double Cross-Over Removal (cM)
1A	17	178.5	22	125.5
1B	12	166.1	14	135.3
1Bi	4	25.8	0	25.8
1D	8	92.3	6	77.9
2A	8	57.9	4	48.9
2Ai	14	65.5	12	40.5
2B	93	704.0	165	347.2
2D	37	365.3	73	196.4
2Di	7	86.1	2	81.0
3A	6	118.1	13	84.4
3B	14	110.6	17	80.6
3Bi	14	176.1	18	137.1
3D	2	28.4	0	28.4
4A	6	99.1	12	69.5
4B	5	63.8	8	43.6
4Bi	4	47.3	5	33.5
4D	2	16.1	0	16.1
5A	5	74.3	6	59.9
5Ai	6	55.8	10	34.1
5B	3	23.4	6	10.6
5Bi	12	112.8	13	83.0
5D	14	198.9	5	187.6
6A	10	134.2	22	86.6
6B	6	86.0	0	86.0
6D	3	52.8	3	45.0
7A	12	136.4	24	82.0
7Ai	11	113.2	14	84.1
7B	29	234.2	34	162.2
7D	7	78.6	8	59.7
7Di	4	45.3	4	35.9
TOTAL	**375**	**3746.9**	**520**	**2588.4**

Figure 4-1. Heat maps produced using R/qtl (Browman et al., 2003) of the original molecular map produced through manual ordering of loci (a), and the molecular map produced through the ordering of loci by the software package RECORD (b). The pairwise recombination fractions are in the top left triangle, whereas the LOD scores between markers are in the bottom right triangle.

a)



b)



4.32 QTL Analysis and the Revised Marker Order

Composite interval mapping was conducted using QTL Cartographer in order to assess potential differences between QTL detected with the two versions of the map. Seven QTL for seedling resistance to crown rot were found using the RECORD version of the map (Table 4-2), compared to the eight QTL that were detected with the manually ordered version of the map (see Table 3-6). Using RECORD, two QTL were consistent between the three trials (on chromosomes 2B and 5D). The 2D QTL that was consistent between trials based upon the manual map was suggestive in two of the three trials (2001 and 2003), and approached the significance level for being deemed a suggestive QTL in the other trial (2005). The remaining QTL were identified in only one (chromosomes 1D, 3B, and 4A) or two (chromosome 1A) of the three seedling trials. Although the 3B QTL was only identified in one of the three trials, it approached the suggestive threshold in each of the other two trials (LRS of 5.7 and 7.1 for the 2001 and 2005 trials respectively). While only passing the significance threshold in two of the three trials (2003 and 2005), the QTL on chromosome 1A approached the suggestive threshold of LRS 8.0 in the 2001 trial (with an LRS value of 7.5). The results obtained from the two maps are largely the same, however putative QTL identified on chromosomes 5A and 6A with the manual version of the map were not detected with the RECORD version, and the putative 1D QTL detected with RECORD was not detected with the manual version of the map.

Table 4-2. QTL for crown rot resistance detected by composite interval mapping in three seedling trials with markers ordered according to RECORD. Chromosome location, likelihood ratio statistic (LRS), percent phenotypic variance explained (VE), significance level (SL), and the parent contributing the resistance allele (Source) are shown.

Chromo-some	2001			2003			2005			Source
	LRS	VE	SL[1]	LRS	VE	SL	LRS	VE	SL	
1A	7.5	4.0	NS	13.3	9.1	Sg	13.5	11.1	Sg	Mendos
1D	0.9	0.4	NS	15.3	12.1	S	0.8	2.1	NS	Mendos
2B	25.1	12.4	HS	8.2	5.2	Sg	21.0	13.4	HS	Mendos
2D	10.6	6.1	Sg	11.6	6.9	Sg	6.2	3.7	NS	W21MMT70
3B	5.7	3.2	NS	10.4	7.4	Sg	7.1	4.0	NS	W21MMT70
4A	2.7	1.2	NS	0.7	2.3	NS	8.7	6.2	Sg	Mendos
5D	46.9	27.9	HS	17.3	18.2	HS	8.1	4.9	Sg	W21MMT70

[1]Significance levels were determined by permutations (1000 permutations at 1 cM intervals). Suggestive (Sg), significant (S), and highly significant (HS) LRS thresholds respectively for each seedling trial are: 2001 – 8.0, 14.3, 21.9; 2003 – 8.2, 15.0, 24.0; 2005 – 8.0, 13.6, 23.8. Data for corresponding regions that were not significant (NS) in particular seedlings trials are shown for comparison.

4.33 QTLNetwork

Interval maps and F profile plots for each of the QTL identified by QTLNetwork are shown in Figure 4-2. Six QTL were detected by QTLNetwork – each of these QTL were also detected with QTLCartographer. Five of the six QTL were not significantly influenced by the different environments of the three seedling trials (Table 4-3). There was however, significant environmental interaction with the 5D QTL – as was found with QTL Cartographer, this QTL was greatest in effect based upon the 2001 seedling trial. The 5D QTL explained the highest amount of the phenotypic variance in each of the seedling trials (up to 12.31%), followed by the 2D (6.01%), 2B (5.53%), 3B (5.16%), 1A (5.02%), and 1D (3.24%) QTL.

Figure 4-2. QTL detected using the software program QTLNetwork. The significance threshold (F=6.7) is displayed as a vertical dotted line. Flanking markers identified by the program are displayed in bold.

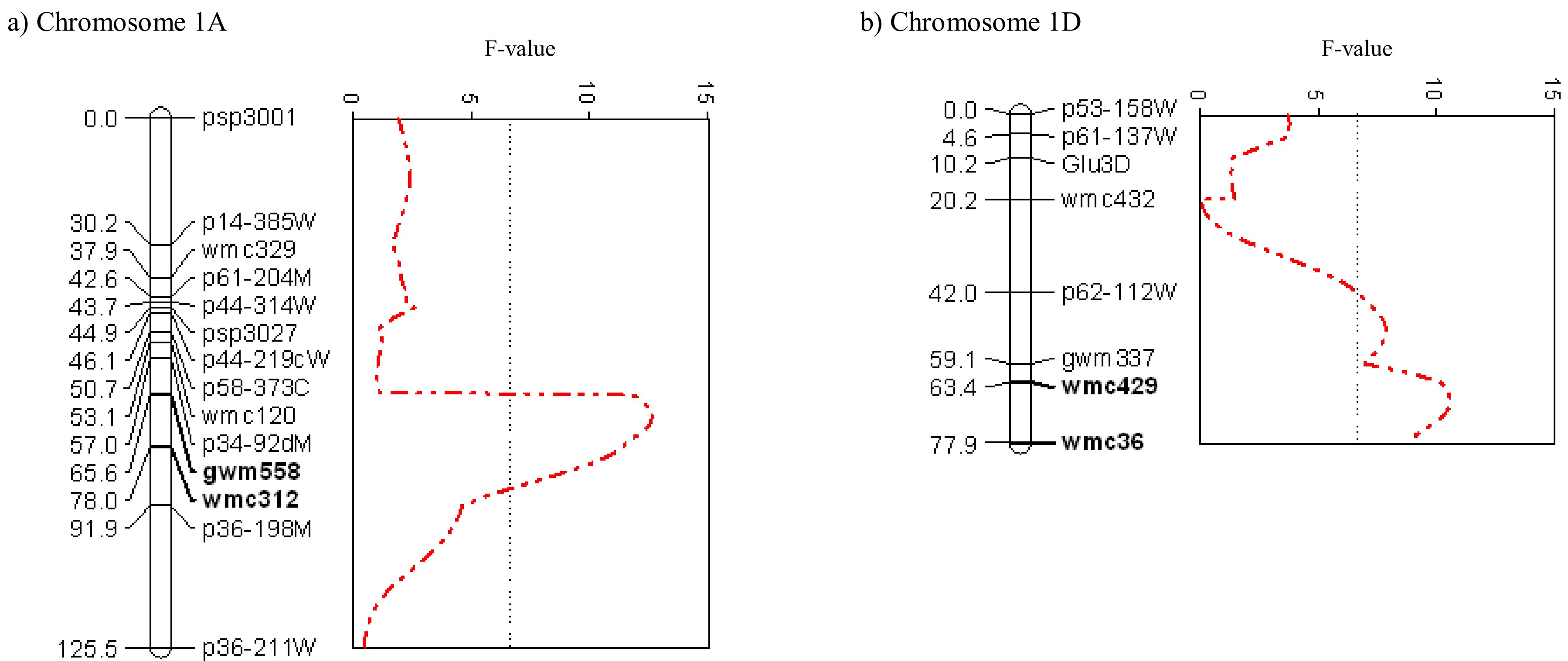
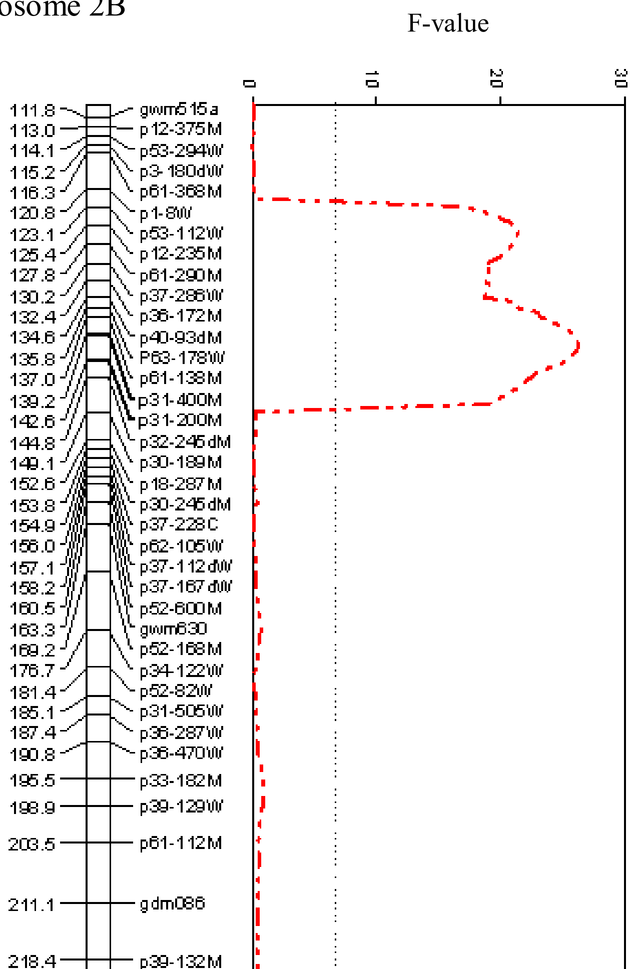


Figure 4-2. Continued.

c) Chromosome 2B



d) Chromosome 2D

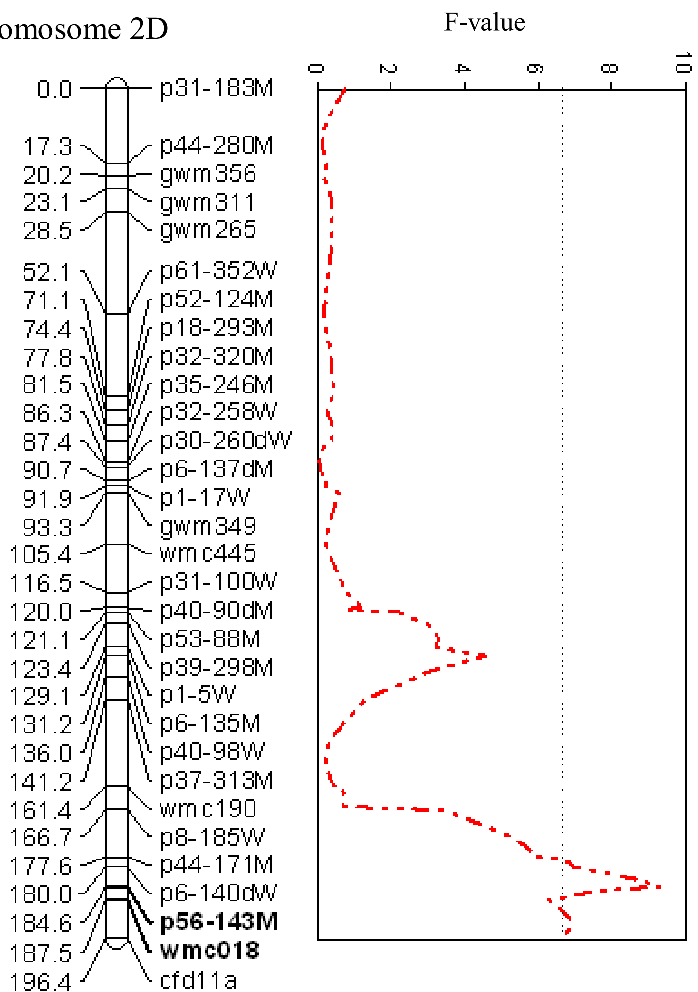
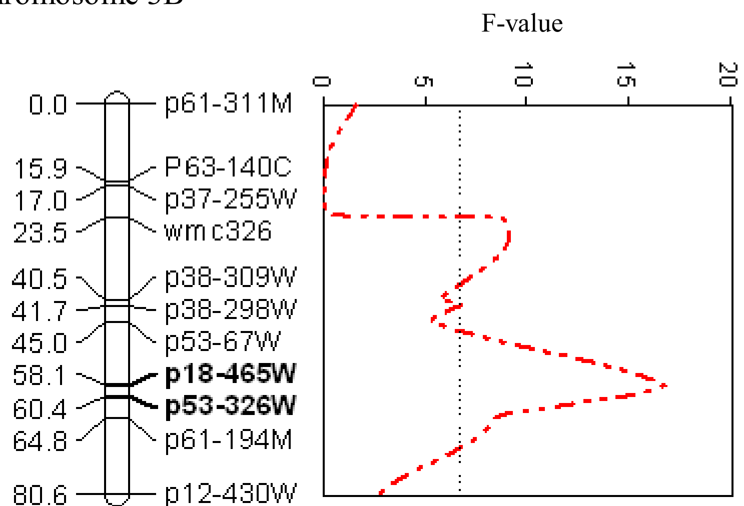


Figure 4-2. Continued

e) Chromosome 3B



f) Chromosome 5D

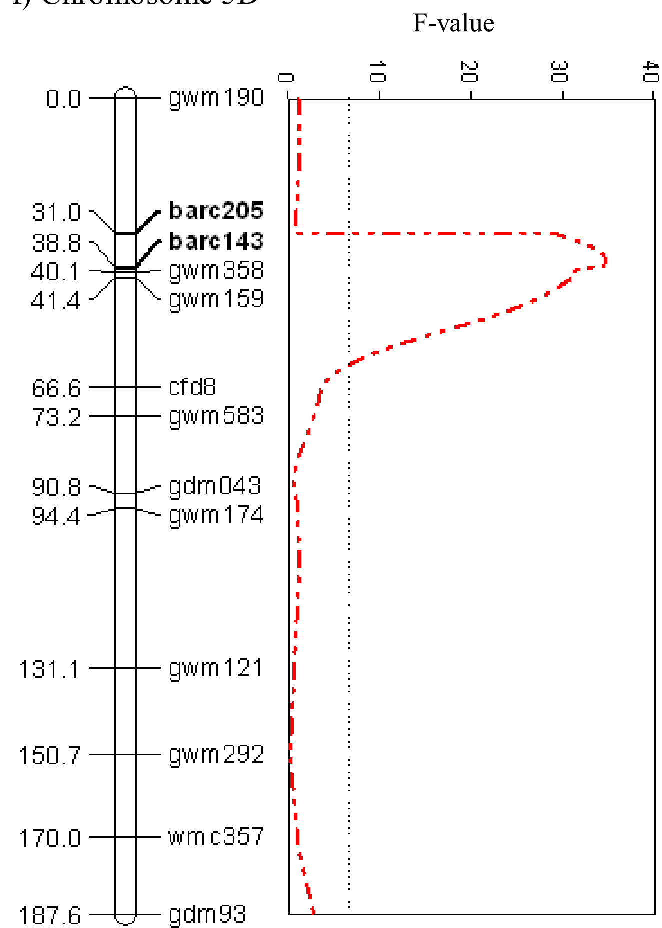


Table 4-3. Summary of QTL detected using QTLNetwork. Chromosome location (Chr.), flanking markers (Marker Interval), map location (Range), significance (P-Value), the percentage phenotypic variation explained by the QTL in each of the trials, and the parent contributing the resistance allele (Source) are shown.

Chr.	Marker Interval	Range (cM)	P-Value	Phenotypic variation explained (%)			Source
				2001	2003	2005	
1A	gwm558-wmc312	65.0-80.0	<0.00001	5.02	5.02	5.02	Mendos
1D	wmc429-Xwmc36	61.1-77.4	<0.00001	3.24	3.24	3.24	Mendos
2B	p31-400M-p31-200M	137.0-144.6	<0.00001	5.53	5.53	5.53	Mendos
2D	p56-143M-wmc018	181.0-186.6	<0.00001	6.01	6.01	6.01	W21MMT70
3B	p18-465W-p53-326W	55.0-60.4	<0.00001	5.16	5.16	5.16	W21MMT70
5D	barc205-barc143	34.0-39.8	<0.00001	12.31	9.70	10.84	W21MMT70

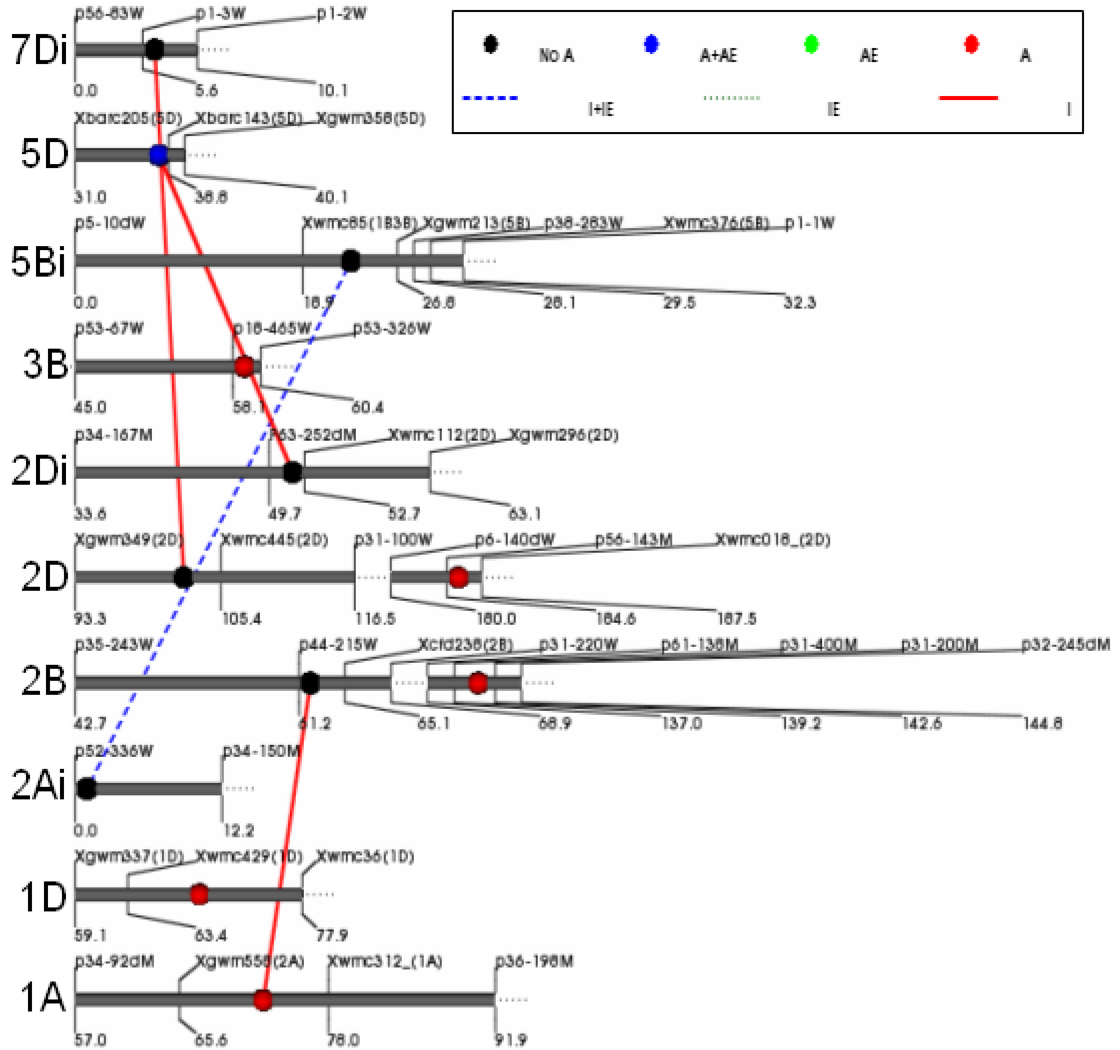
4.34 Epistasis I - QTLNetwork

QTLNetwork was also used to detect any potential epistatic interactions for resistance to crown rot. Four epistatic interactions were detected (Table 4-4). The most significant of these, between chromosomes 2Ai and 5Bi (explaining 3.97% of the phenotypic variance in the 2001 and 2003 trials, and 4.68% of the phenotypic variance in the 2005 trial), displayed both additive and additive x environment interactions. The other three interactions (between chromosomes: 1A and 2B; 2D and 7Di; and 2Di and 5D) displayed additive x additive epistasis. A graphical overview of both main effect and epistatic interactions detected by QTLNetwork is shown in Figure 4-3.

Table 4-4. Epistatic interactions detected by QTLNetwork. The chromosome location of the first marker interval (Chr_i; Interval_i), and the range of that interval (Range_i; cM), as well as the chromosome location of the second marker interval (Chr_j; Interval_j), and the range of that interval (Range_j; cM) are shown. The percent phenotypic variance explained (%V.E.) by the interactions in each year the seedling trials were conducted are given.

Chr_i	Interval_i	Range_i	Chr_j	Interval_j	Range_j	P-Value	Phenotypic variation explained (%)		
							2001	2003	2005
1A	gwm558-wmc312	65.0-80.0	2B	p44-215W-cfd238	54.7-66.1	0.003157	0.68	0.68	0.68
2Ai	p52-336W-p34-150M	0.0-7.0	5Bi	wmc85-gwm213	11.0-31.5	<0.000001	3.97	3.97	4.68
2D	gwm349-wmc445	93.3-108.4	7Di	p1-3W-p1-2W	0.0-8.6	0.000005	1.76	1.76	1.76
2Di	P63-252dM-wmc112	42.6-56.7	5D	barc205-barc143	34.0-39.8	<0.000001	2.35	2.35	2.35

Figure 4-3. A graphical representation of main effect QTL and epistatic interactions identified by QTLNetwork. The box contains a key to deciphering the interactions. Circles refer to QTL (black = no additive effect; blue = additive and additive x environment effect; green = additive x environment effect; red = only additive effect); lines refer to epistatic interactions (dashed blue = epistatic and epistatic x environment effect; dashed red = epistatic x environment effect; solid red = only epistatic main effect).



4.35 Epistasis II – Epistat

Ten (10) digenic interactions, present in at least two of the three seedling trials, were detected using Epistat (Table 4-5). Only two of these (between linkage groups 1B and 5Ai; and 2A and 5Bi) were detected in all three seedling trials. Of the ten interactions detected by Epistat, only two were detected by QTLNetwork (between linkage groups 2Ai and 5Bi; and 2Di and 5D). The majority (eight) of the interactions detected by Epistat were coadaptive – individually, neither of the loci have a significant effect on the trait; however, when considered together, the presence of particular allele combinations appears to have an effect. For example, in the case of the 1B and 7D interaction, neither locus has an effect on the trait individually (the p-value of each marker is >0.2), however, the mean of the AA subpopulation (i.e. with a W21MMT70 allele for both markers) is lower than any other possible combination. Two conditional interactions (in which the effect of a QTL with a detectable main effect appears to be dependent on the presence of a particular allele at another locus) were detected. The 1D QTL inherited from Mendos is dependent on the presence of a W21MMT70 allele on chromosome 2D. The 5D QTL inherited from W21MMT70 is dependent upon the presence of a Mendos allele on chromosome 2Di.

The effect of either the conditional or coadaptive interactions are, in many cases, quite significant. For example, when means of all possible subgroups are calculated for each interaction, and compared to the mean of the subgroup with the lowest disease severity, the percentage difference ranges from 15.9% (for the 4B – 6A interaction) to 25.4% (for the 2Di -5D interaction) lower. As these values are calculated based on the average of all years in which the interactions were identified, the effect may be greater in an individual year than has been estimated.

Table 4-5. Significant digenic interactions between loci for resistance to crown rot that were detected in at least two of the three seedling trials using the Epistat program. The years in which the epistatic interactions were detected (Year); marker loci involved in the interaction; the additive log-likelihood ratio (Additive LLR); the additive p-value; the p-value for both marker 1 and marker 2 individually; and the mean response to crown rot infection in the various subpopulations are shown. The sub-populations with the lowest disease severity are highlighted in grey.

Year	Marker 1#	Marker 2	Additive LLR	Additive p-value	Marker 1 p-value	Marker 2 p-value	AA* mean	AB* mean	BB* mean	BA* mean
2003	1A-p14-385W	3B-p37-255W	7.01	0.0012	0.0276	0.8449	66.4	53.3	56.43	40.8
2003	1A-p14-385W	3B-P63-140C	8.26	0.0004	0.02	0.5212	66.93	53.3	58.16	39.59
2003	1A-p34-92dM	3B-p61-311M	7.3	0.0012	0.0227	0.4433	67.66	51.34	53.65	38.45
2005	1A-p14-385W	3B-P63-140C	7.98	0.0006	0.0055	0.4544	70.9	58	60.17	42.34
2001	1B-p3-187W	7D-p44-109W	8.13	0.0002	0.2322	0.283	65.09	79	68.3	92.46
2001	1B-p3-187W	7D-p58-68dM	9.7	0.0002	0.6134	0.3881	65.09	78.83	66.85	90.49
2001	1B-p3-187W	7D-Xgwm437	8.54	0.0003	0.9209	0.5558	65.13	80.8	66.05	89.04
2001	1B-p3-187W	7D-Xpsp3113	10.45	0	0.4687	0.3931	65.09	79.51	68.97	93.97
2005	1B-p3-187W	7D-Xgwm437	8.17	0.0004	0.0819	0.7735	39.88	60.68	55.62	75.47
2001	1B-Xwmc230	5Ai-Awns	7.09	0.0009	0.9884	0.015	59.45	94.86	76.15	76.78
2001	1B-Xwmc230	5Ai-Xgwm595	8.79	0.0006	0.943	0.2992	61.35	91.81	71.75	81.91
2003	1B-Xwmc230	5Ai-Xgwm595	9.66	0.0004	0.5242	0.6767	36.81	62.32	47.83	60.44
2005	1B-Xwmc230	5Ai-Xgwm595	7.51	0.0008	0.7621	0.6504	46.83	65.82	50.23	67.1
2001	1D-Xgwm337	2D-p31-183M	7.21	0.004	0.1923	0.4434	88.54	67.87	80.08	67.23
2005	1D-Xgwm337	2D-p31-183M	18.76	0	0.0211	0.7969	71.7	52.83	62.86	44.64
2005	1D-Xwmc429	2D-p31-183M	12.81	0.0001	0.0947	0.9125	66.81	52.56	61.91	47.44
2005	1D-Xwmc429	2D-p44-280M	12.5	0	0.1062	0.8181	68.2	51.36	57.78	42.22

Year	Marker 1[#]	Marker 2	Additive LLR	Additive p-value	Marker 1 p-value	Marker 2 p-value	AA[*] mean	AB[*] mean	BB[*] mean	BA[*] mean
2001	2Ai-p34-150M	5Bi-p38-283W	7.25	0.001	0.5262	0.7864	82.81	62.65	83.16	64.5
2001	2Ai-p52-336W	5Bi-p38-283W	9.67	0	0.5559	0.7883	82.76	64.45	86.05	61.7
2001	2Ai-p52-336W	5Bi-Xgwm213	8.12	0.0004	0.4089	0.9709	84.91	66.14	84.29	62.42
2001	2Ai-p52-336W	5Bi-Xwmc376	8.25	0.0003	0.4479	0.8903	84.52	64.98	86.57	63.08
2001	2Ai-p8-380M	5Bi-p38-283W	7.27	0.0008	0.191	0.8221	84.51	67.3	82.23	61.3
2001	2Ai-p8-380M	5Bi-Xwmc376	7.34	0.0016	0.1307	0.8667	86.86	66.27	81.47	61.52
2003	2Ai-p52-336W	5Bi-p38-283W	7.59	0.0009	0.0657	0.3046	63.92	45.7	55.23	42.38
2003	2Ai-p52-336W	5Bi-Xwmc376	7.23	0.001	0.0169	0.1024	67.13	44.56	52.11	42.21
2005	2Ai-p8-380M	5Bi-p5-10dW	7.92	0.0006	0.6779	0.241	69.27	46.76	62.8	52.44
2001	2Di-Xwmc112	5D-Xbarc143	7.00	0.002	0.85	0	71.09	84.17	105.2	58.28
2005	2Di-Xwmc112	5D-Xbarc143	7.74	0.0004	0.7833	0.0055	60.44	54.8	74.98	45.03
2001	4B-p52-178W	6A-p58-406M	7.60	0.0007	0.9187	0.4751	82.84	67.84	82.86	63.01
2001	4B-Xgwm513	6A-p58-406M	10.99	0.0003	0.8132	0.7625	82.56	65.31	81.45	61.84
2001	4B-Xwmc349	6A-p58-406M	10.28	0.0001	0.8549	0.6067	84.33	68.32	81.12	60.55
2005	4B-p52-178W	6A-p58-406M	7.72	0.0009	0.2005	0.0902	56.94	49.17	67.45	46.39
2003	5Ai-Xgwm595	7Ai-p44-126M	7.65	0.0029	0.5957	0.9158	58.87	42.84	62.4	46.76
2005	5Ai-Xgwm126	7Ai-p35-695M	7.14	0.0014	0.9483	0.5621	62.69	52.62	68.13	45.4
2005	5Ai-Xgwm126	7Ai-p35-700W	7.26	0.0005	0.9921	0.6072	64.32	53.66	68.13	45.4
2005	5Ai-Xgwm126	7Ai-p38-128dW	7.40	0.0007	0.9539	0.9475	64.73	51.51	65.77	46.28
2005	5Ai-Xgwm595	7Ai-p44-126M	8.13	0.0005	0.7495	0.4918	65.14	51.51	67.21	47.19
2001	5A-Xgwm205	6B-Xgwm219	7.32	0.0013	0.0601	0.3925	71.96	91.28	62.8	74.88
2005	5A-Xgwm205	6B-p1-12W	7.53	0.0008	0.1578	0.5773	50.53	69.92	46.17	59.94

Year	Marker 1[#]	Marker 2	Additive LLR	Additive p-value	Marker 1 p-value	Marker 2 p-value	AA[*] mean	AB[*] mean	BB[*] mean	BA[*] mean
2005	5A-Xgwm205	6B-Xgwm219	7.58	0.0019	0.4294	0.0515	47.96	74.09	52.32	58.38
2003	6B-p31-148W	7B-p44-363M	7.53	0.0004	0.6718	0.0803	49.71	88.7	42.83	64.57
2005	6B-p31-148W	7B-Xwmc182	7.68	0.0018	0.3413	0.3257	46.09	60.96	50.86	71.25
2005	6B-Xgwm219	7B-p44-363M	7.28	0.0022	0.0465	0.3866	48.93	57.84	53.24	80.47

[#]Chromosome locations are indicated before the marker name. [*]The mean response to infection (expressed as a % of the susceptible check cultivar Puseas) of each of the following subpopulations: AA – refers to the presence of W21MMT70 alleles at both marker 1 and marker 2; AB – W21MMT70 allele for marker 1 and a Mendos allele for marker 2; BB – Mendos alleles at both marker 1 and marker 2; BA – Mendos allele at marker 1 and a W21MMT70 allele at marker 2.

4.4 Discussion

This chapter has investigated i) the usefulness of the marker ordering program RECORD; ii) the effects of using the marker order given by RECORD for QTL mapping; and iii) the occurrence of epistasis as identified by each of two software programs.

The use of the software package RECORD (Van Os et al., 2005) for ordering the markers resulted in the addition of 94 extra markers and an increase in map distance of 241.5 cM. The major differences between the manual version of the map and the RECORD version of the map were with chromosomes 1A, 2B, 2D, and 7B. For chromosome 1A, the addition of two distal markers (one on the end of each arm) increased the map distance by 76.2 cM. There were major differences between chromosomes 2B and 2D between the two versions of the map. Sixty – six (66) markers were added to chromosome 2B. When manually ordering the map, these markers were extremely difficult to place and as a result, were omitted from that version of the map. The large numbers of markers on 2D were also difficult to order manually; RECORD added five markers to this linkage group and still achieved an overall decrease in map distance, indicating the likelihood that a more correct order was achieved using RECORD. The heat map function of R/qtl (Browman et al., 2003) proved useful for graphically identifying potential location issues with markers on chromosomes 6A and 7B in the manual version of the map; once again, RECORD was able to resolve this issue. RECORD was designed for ordering large numbers of markers within individual linkage groups, and achieves this "best-possible" marker order by minimizing the number of recombination events between markers (Van Os et al., 2005). Isidore et al. (2003) using an unpublished version of RECORD, concluded that the program is particularly good in marker-dense regions – this finding is supported by the improved marker order in for example, chromosome 2B in the current study.

To assess the effect of the RECORD version of the map on detection of QTL, composite interval mapping using QTL Cartographer was carried out and

compared with the results obtained using this software with the manual version of the map. Overall, the QTL mapping results across the two versions of the map were largely consistent. The most notable difference is in the identification of differing minor QTL that were only detected in one of the three trials, for each version of the map. Two of the three QTL that were consistently detected with the manual version of the map (on chromosomes 2B and 5D) were also detected with the RECORD version of the map – the 2D QTL was, however, identified in two of the three trials and approached levels for being deemed a suggestive QTL in the other trial. Dodds et al. (2004) used simulation techniques to assess the effects of incorrect map order on QTL detection. In this study, Dodds et al. (2004) have shown that provided no markers are assigned to an incorrect linkage group, the accuracy of the map had little or no impact on the detection of QTL. These results are supported by the current study, in that although a number of changes were made between the manual and RECORD versions of the map, the QTL detected were largely the same.

QTLNetwork is a recently developed program (Yang et al., 2005) for detecting QTL, QTL x environment, and also QTL x QTL (epistatic) interactions. In the first instance, this program was used to assess main effect (additive) QTL and potential environmental interactions. Six additive QTL were detected by QTLNetwork compared to the seven detected by QTL Cartographer. The 4A QTL not detected by QTLNetwork was only deemed to be suggestive in one of the three trials using QTL Cartographer. Therefore, the two programs appear generally consistent in their detection of main effect QTL.

Of particular interest is the identification of QTL on chromosomes 1A and 1D, inherited from Mendos, by QTLNetwork. In a study of QTL for resistance to crown rot in a 2-49 x Janz DH population, Collard et al. (2005b) identified major QTL, inherited from line 2-49, located on these chromosomes. A comparison between flanking markers in the study by Collard (2005b) and in the current study, reveal that these QTL are located in similar regions. However, by comparing marker allele sizes in Mendos with those published for 2-49, (Collard et al., 2006) it is evident that the Mendos haplotype is quite different to that of line 2-49. Indeed, Mendos only has the same allele size for two of five 1A SSRs,

and one of five 1D SSRs. Haplotype analysis is becoming a common method of determining if divergent germplasm is likely to contain the same QTL for a particular trait of interest that has already been identified (McCartney et al., 2004). In the case of Fusarium head blight (FHB) resistance, haplotype analysis is actively being used to prioritize which sources of resistance should undergo further characterization – lines with different haplotypes to Sumai 3 have been presumed to contain different QTL for resistance and therefore warrant further investigation (Liu and Anderson, 2003). However, Ma et al. (2006) in a study of FHB resistance in a recombinant inbred population found that, although alleles sizes of linked markers were different in their resistant parent compared to Sumai 3, a QTL was still detected in the same region of chromosome 3B. The result of Ma et al. (2006) and those presented in this chapter may suggest that, while the haplotyping approach may increase the likelihood of identifying novel QTL, in some instances, a different haplotype may not necessarily be indicative of the absence of a particular QTL in the haplotyped genomic region.

Genotype x environment (or QTL x environment) interaction plays an important role governing the stability, and suitability, of varieties in different environments. Due to a lack of suitable analytical tools, studies that have compared QTL in different environments (see for example Shah et al., 1999; Huang et al., 2003b) have considered QTL that contribute differently across environments as displaying a QTL x environment interaction. In these cases, the data from each environment is analysed separately; Xing et al. (2002) suggests that such an analysis can not provide estimates regarding the amounts and relative importance of QTL x environment interactions in these data sets. Of the six QTL detected by QTLNetwork (which considers all environments in a single analysis), one (located on chromosome 5D) displayed significant QTL x environment interaction. This QTL explained the highest proportion of the phenotypic variance in 2001 (12.31 %). Analysis of the individual trials by QTL Cartographer also showed that this QTL had the greatest effect in 2001. In Chapter 2, it was shown that the mean disease score for lines resistant to crown rot in the W21MMT70 x Mendos DH population was higher in 2001, indicating that the growth cabinet environment was more favourable for disease development. The analysis with QTLNetwork has shown that, although this

QTL has a greater effect under conditions which favour increased disease pressure, it also played an important role in the glasshouse environments.

Two software programs were used to detect epistatic interactions. The first, QTLNetwork, which uses a linear-mixed-model approach to detect epistatic QTL, identified four digenic interactions. The phenotypic variance explained by these epistatic QTL ranged from 0.68 % to 4.68 %. Given that the most significant additive QTL explained up to 12.31 % of the phenotypic variance, the epistatic interactions detected appear to play a significant role in governing resistance.

The second program, Epistat (Chase et al., 1997), which uses log-likelihood ratios to compare epistatic and additive models, detected 10 digenic interactions. The majority of these were co-adaptive (i.e. neither QTL had an effect on their own, but particular combinations displayed a phenotypic effect); however, of particular interest, two conditional interactions (i.e. QTL with both main and epistatic interactions) were also detected. These conditional interactions were displayed between the 1D QTL inherited from Mendos and a W21MMT70 modifying locus on chromosome 2D; and also with the 5D QTL inherited from W21MMT70 and a Mendos modifying locus on chromosome 2Di. This finding is of importance to the use of such QTL in breeding programs, as it indicates that the effect of main effect QTL may vary depending upon the genetic background into which they are transferred. In a study on rice yield components, Zhuang et al. (2002) found similar additive and additive by additive QTL effects, and concluded that such QTL may display both types of effects or a single type of effect depending on genetic background.

From a total of 14 epistatic interactions detected by both programs, only two interactions were in common between the software packages (the 5D W21MMT70 – 2Di Mendos interaction, and the 2Ai Mendos – 5Bi W21MMT70 interaction). The majority of publications that do report epistatic interactions tend to do so based upon the results of one statistical approach for detecting epistasis (see for example Liao et al., 2001; Jia et al., 2005). The results

presented here suggest that variable outcomes can be found depending on the method used to detect the interactions. This finding supports the views of Cordell (2002) who suggests that the degree to which statistical modelling can elucidate the underlying biological mechanisms is limited, and that confirmation of biological interactions may be better answered via molecular, rather than statistical, investigation.

The epistatic interactions reported in this chapter are based upon a doubled-haploid mapping population from which only additive x additive and additive x additive x environment interactions can be measured - additive x dominant and dominant x dominant (and their potential environmental interactions) cannot be determined with this population structure (Wade, 2002). This size of the population is 95 doubled haploid lines, and this relatively small population size may result in an inability to detect both main effect and epistatic interactions of smaller effect (Beavis, 1998). Because of these factors, it is likely that the total amount of epistasis has been underestimated and that epistasis may play an important role in determining resistance to crown rot.

In summary, the use of RECORD improved the order of the linkage map. In spite of the changes, only minor differences in the QTL detected were apparent when the RECORD version was used for analysis rather than manual version of the map. QTLNetwork detected largely the same QTL that were detected with QTL Cartographer, but provided a more robust analysis of QTL x environment interaction. Finally, epistasis was found to play a role in resistance to crown rot. In some instances, main effect QTL also displayed additive epistatic interactions which may impact on their usefulness in different genetic backgrounds. The following chapter will focus on the validation of QTL detected in different genetic backgrounds.

Chapter 5. Validation and Pyramiding of QTL for Resistance to Crown Rot

5.1 Introduction

The previous chapters have provided a detailed analysis of QTL for resistance to crown rot in the W21MMT70 x Mendos population. Regardless of the statistical significance of the QTL and their putative interactions, their true worth, for breeding purposes, lies in their ability to have an effect in different genetic backgrounds. QTL validation refers to the process of testing and confirming the effectiveness of previously identified QTL in other backgrounds (Langridge et al., 2001; Sharp et al., 2001). The process is of extreme importance, primarily due to the observation of what is now referred to as "the Beavis effect". In a simulation study, Beavis (1994) reported that phenotypic variances associated with QTL were greatly overestimated if only 100 individuals were evaluated, and suggested that the actual phenotypic variance explained by QTL can only be accurately estimated from populations of between 500 and 1000 individuals. Furthermore, if 10 loci affect a trait of 30% heritability and a population of 100 F_2 is used for mapping, each true QTL will be identified as significant with only 9% probability, and the variance explained overestimated by 5.6 times the true value (Beavis, 1994, 1998). Thus, the predicted efficiency of using unvalidated QTL detected from typical population sizes (100-300 individuals) for later marker-assisted selection may be considerably overestimated (Holland, 2004). Therefore, the primary aim of this chapter is to validate the QTL identified earlier, across a range of genetic backgrounds.

The second aim of this chapter is to assess the effectiveness of pyramiding QTL from different sources to increase resistance to crown rot. Pyramiding of QTL, for disease resistance, has been seen as perhaps the most valuable use of molecular markers linked to QTL (Dekkers and Hospital, 2002). However, despite the identification of QTL for many disease resistances, there are only a handful that report on pyramiding of QTL. For example, a search of Current Contents Connect (conducted 09/02/07) for "QTL pyramiding" reveals 54 hits. Of these 54, only two (Castro et al., 2003; Richardson et al., 2006) actually report on the outcomes of QTL pyramiding – one report is a review (Ashikari

and Matsuoka, 2006); one reports on mapping previously uncharacterised QTL from a cross between two resistant parents (Tabien et al., 2002); and the remainder suggest that the QTL that were identified in the study will be useful for pyramiding. Of the two that do report on the outcome of QTL pyramiding (Castro et al., 2003; Richardson et al., 2006), both relate to pyramiding of resistance to barley stripe rust. Obviously, the search described is not exhaustive; however, it does highlight the lack of reports on successful QTL pyramiding outcomes. To assess the effectiveness of pyramiding QTL for crown rot resistance, a population created from a cross between W21MMT70 and the well characterized 2-49 source of crown rot resistance has been analysed.

5.2 Materials and Methods

5.21 Validation

5.21.1 Plant Materials and Phenotyping

The three W21MMT70-inherited QTL (on chromosomes 2D, 3B, and 5D) that were detected with both versions of the molecular map were subjected to validation studies. The validation of these QTL was conducted on material provided by Dr. Damian Herde from the Queensland Department of Primary Industries and Fisheries (QDPI&F) at the Leslie Research Centre (LRC). Dr. Herde is conducting a GRDC funded project aimed at elucidating the genetics of resistance to crown rot. His project involves the production and analysis of a half-diallel – W21MMT70 is one of the lines present in the half-diallel and F_2 progeny from selected W21MMT70 crosses have been used for validation in this chapter. Three crosses were chosen. The first is a cross between W21MMT70 and the highly susceptible cultivar Puseas. The second is a cross between W21MMT70 and line IRN497 – QTL from line IRN497 have been identified in an IRN497 x Janz doubled haploid population, and a major QTL has been located on chromosome 3B in the same region as was identified in the W21MMT70 x Mendos population (Bovill et al., unpublished data). The third is a cross between W21MMT70 and line QT10162 (a sister line of EGA Wylie). The experiments were set-up as described in Section 2.22 but the scale used for rating disease severity was slightly different – instead of a 0-4 scale for each of the first three leaf sheaths being added to give a final cumulative score, each leaf

sheath was assessed for percent of infection (to the nearest 5 %) with the final score calculated by adding the values of each of the leaf sheaths together.

A Sunco x Batavia doubled haploid population (213 lines) was used for the validation of the 2B QTL that was inherited from Mendos. This population was generated for an unrelated study, and was chosen for validation primarily because the cultivar Sunco contains the same 2B *Triticum timopheevi* introgression that is present in Mendos. The resistance Sunco possesses is not able to be detected in the seedling trial of Wildermuth and McNamara (1994) and can only be detected in the field. As a result, this population was phenotyped in the field by staff at the QDPI&F (LRC) under the supervision of Dr. Graham Wildermuth.

5.22 Pyramiding of QTL

5.22.1 Plant Materials and Phenotyping

The potential for pyramiding QTL for resistance to crown rot was assessed on a 2-49 x W21MMT70 doubled haploid population of 207 lines. Line 2-49 is widely recognized as one of the best sources of resistance to crown rot (Wildermuth pers. comm.), and QTL for resistance to crown rot inherited from line 2-49 have been previously identified (Collard et al., 2005b; Collard et al., 2006). Phenotyping of this population was carried out as per Section 2.22.

5.23 DNA Extraction

DNA from the W21MMT70-derived F_2 plants, the Sunco x Batavia doubled haploid population, and the 2-49 x W21MMT70 doubled haploid population was extracted in 96-well plate format using a Qiagen TissueLyser. A Wizard genomic DNA purification kit (Promega) was used to extract DNA as per the manufacturer's instructions. DNA was diluted 1:10 prior to use in PCR.

5.24 Genotyping and Data Analysis

Markers identified in the original W21MMT70 x Mendos mapping study as flanking the QTL were initially screened across parents of each of the crosses. In instances where markers were not polymorphic in particular crosses, consensus maps were used to identify alternative closely linked polymorphic markers. Polymorphic markers were assayed across the individuals from the crosses on a Corbett3000 Gelscan instrument. When polymorphisms between parents were not able to be resolved on the Corbett3000, attempts were made to improve resolution by running product on BioRad mini-sequencing gels that were visualised with silver staining (described in Section 2.24).

Genotypic data was entered into MapManager QTX (Manly et al., 2001) and, for the F_2 populations, marker regression or interval mapping was carried out in order to determine the significance (based upon 10000 permutations at 2 cM intervals) of the marker-trait associations. For the 2-49 x W21MMT70 doubled haploid population, composite interval mapping was conducted using both QTLNetwork (Yang et al., 2005) and QTL Cartographer (Wang et al., 2001-2004).

5.3 Results

5.31 Validation of W21MMT70 QTL

In order to determine appropriate markers to genotype each of the F_2 populations, polymorphism screens were conducted on parental genotypes and a subset of four individuals from each population (Table 5-1). With the exception of chromosome 3B in the Puseas x W21MMT70 population, sufficient polymorphism was available to construct partial linkage maps of chromosome regions of interest in each of the populations.

Table 5-1. Polymorphism assessment for suitable markers to be screened on each of the W21MMT70-derived QTL in each F_2 population. Chromosome location (Chr.), SSR marker name, and the location of the markers (Map) on the consensus map of Appels (2003) are shown. Polymorphic markers are denoted P; markers that were not polymorphic are denoted NP; and markers with putative polymorphism but that were deemed too difficult to score with the gel-based assays that were used are denoted P*. Markers used to construct partial linkage maps in each population are highlighted.

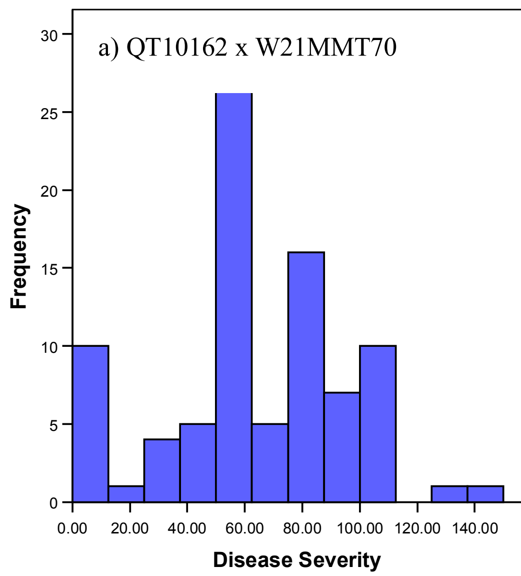
Chr.	Marker	Map	F_2 Population		
		(cM)	Puseas x W21	IRN497 x W21	QT10162 x W21
2D	cfd11	18	P*	P*	P
2D	wmc18	46	NP	P	P
2D	wmc190	47	P	P	P
3B	wmc326	74	NP	P	NP
3B	gwm299	88	NP	P	NP
3B	gwm340	98	NP	P	P
3B	gwm181	99	NP	P	P
3B	gwm547	100	NP	P	P
3B	gwm247	101	NP	P	P
5D	gwm190	5	P	P	P
5D	cfa2104	10	NP	P	NP
5D	barc205	11	P*	NP	NP
5D	gwm358	13	P	P	P
5D	cfd67	14	NP	P	P
5D	cfd78	15	NP	NP	NP
5D	barc143	16	P*	P	P
5D	cfd40	17	P	P*	NP
5D	gdm68	18	P	P	P
5D	cfd8	35	NP	NP	NP
5D	gdm43	43	P	P*	P

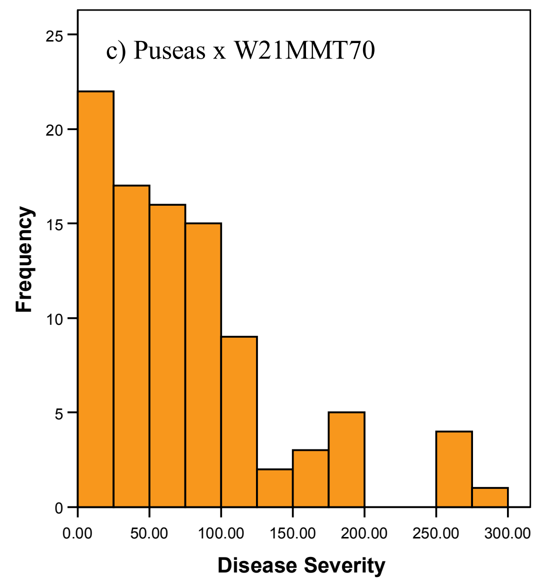
Descriptive statistics and histograms of the three populations chosen for validation of the W21MMT70 QTL are displayed in Table 5-2 and Figure 5-1 respectively. All three populations were significantly skewed towards resistance, although this was most pronounced in the Puseas x W21MMT70 and IRN497 x W21MMT70 populations. One F_2 individual from the QT10162 x W21MMT70 population and two F_2 individuals from the Puseas x W21MMT70 population displayed no infection. In contrast, 25 (27 %) individuals displayed no infection in the IRN497 x W21MMT70 F_2 population and the mean of this population was 40.6 - significantly lower than both the Puseas x W21MMT70 (74.4) and QT10162 x W21MMT70 (62.8) populations.

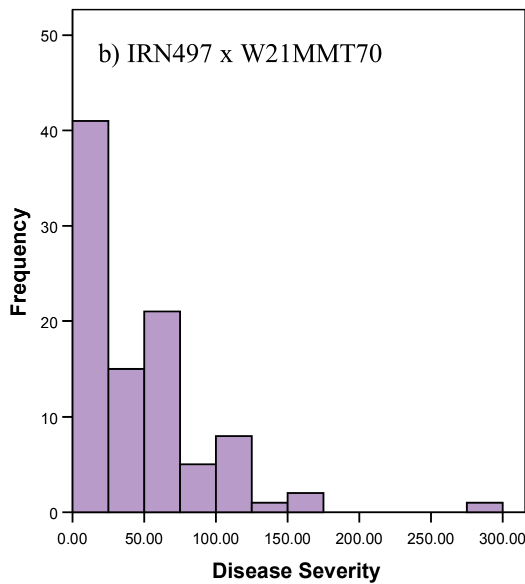
Table 5-2. Descriptive statistics of the three F_2 populations chosen for validation of W21MMT70 derived QTL. Population means for disease score (Mean) sharing the same letter were not significantly different (Tukey test, $p>0.05$).

Population	N	Mean	Median	Range	Skewness	Kurtosis
Puseas x W21MMT70	94	74.4[a]	60.0	0-290	1.4	2.0
IRN497 x W21MMT70	94	40.6[b]	30.0	0-300	2.2	8.7
QT10162 x W21MMT70	88	62.8[a]	60.0	0-150	-0.09	-0.04

Figure 5-1. Histograms of disease severity in each of the F_2 populations chosen for validation. a) QT10162 x W21MMT70; b) IRN497 x W21MMT70; and c) Puseas x W21MMT70.







Rankit's formula (SPSS Inc., Chicago, IL, USA, 1989-2003) was used to normalise the distribution of each of the populations prior to QTL analysis. Interval mapping was conducted within Map Manager QTX (Manly et al., 2001) to assess the putative effects of the QTL in each of the F_2 populations. Neither the 2D nor the 3B QTL could be validated in any of the populations (Table 5-3). The effect of the 5D QTL could not be confirmed in the QT10162 x W21MMT70 population, but was found to have a suggestive effect in the IRN497 x W21MMT70 population, and a significant effect in the Puseas x W21MMT70 population.

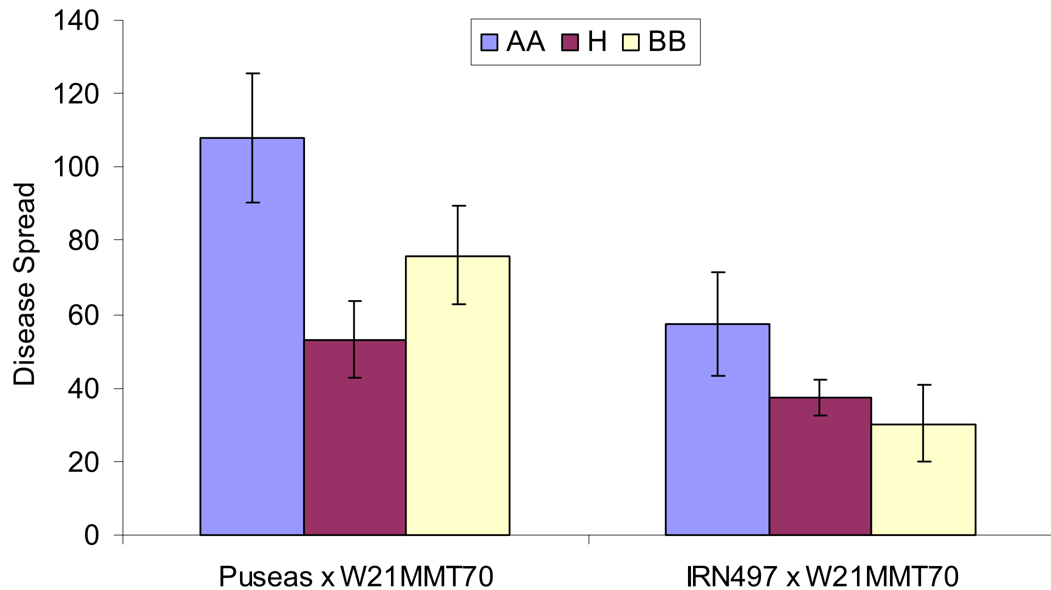
Table 5-3. Validation of W21MMT70-derived QTL in each of the F_2 populations. Likelihood ratio values (LRS), percent phenotypic variance explained (%P.E.) and significance of QTL (Sig; determined by permutations) are not shown for non-significant marker-trait associations.

Population	QTL Location								
	2D			3B			5D		
	LRS	%P.E.	Sig.*	LRS	%P.E.	Sig.	LRS	%P.E.	Sig.*
QT10162 x W21			NS			NS			NS
IRN497 x W21			NS			NS	4.1	4.0	Sg
Puseas x W21			NS			NS	11.1	13.0	S

* NS – not significant; Sg – suggestive; S - significant

The effect of homozygous W21MMT70, homozygous QT10162 or IRN497, or heterozygote genotypes from each of the populations where the 5D QTL was validated is displayed in Figure 5-2. In the Puseas x W21MMT70 population, the spread of disease in the homozygous W21MMT70 genotypes was 29.6 % lower than the homozygous Puseas genotypes. In the IRN497 x W21MMT70, the spread of disease in the homozygous W21MMT70 genotypes was 47.1 % lower than the homozygous IRN497 genotypes.

Figure 5-2. Effect of alternative 5D alleles in both the Puseas x W21MMT70 and IRN497 x W21MMT70 F_2 populations (mean ± standard error). A disease spread value of 300 signifies complete symptoms of disease on all three leaf sheaths. AA= homozygous Puseas or IRN497; BB=homozygous W21MMT70; H = heterozygous.



5.32 Validation of Mendos QTL

For validation of the 2B Mendos QTL, a Sunco x Batavia population was examined because Sunco possesses the same 2B *T. timopheevi* introgression that is present in Mendos. The field reaction of the Sunco x Batavia population exhibited a slight skewness towards susceptibility (Figure 5-3), with an average disease severity of 90.5 % Batavia. Individuals ranged in their severity from 69.7 to 110.8 % Batavia. None of the lines of the doubled haploid population performed better than Sunco.

Polymorphic microsatellite markers on chromosome 2B were mapped on the entire doubled haploid population to produce a partial linkage map of the region where the 2B QTL inherited from Mendos was identified (Figure 5-4). Interval mapping identified the presence of a QTL in the same region as found in Mendos. This QTL had an LRS of 18.6 and explained 11% of the phenotypic variance.

Figure 5-3. Field reaction of the Sunco x Batavia doubled haploid population (expressed as a percentage of Batavia). The reaction of Sunco is indicated by the arrow.

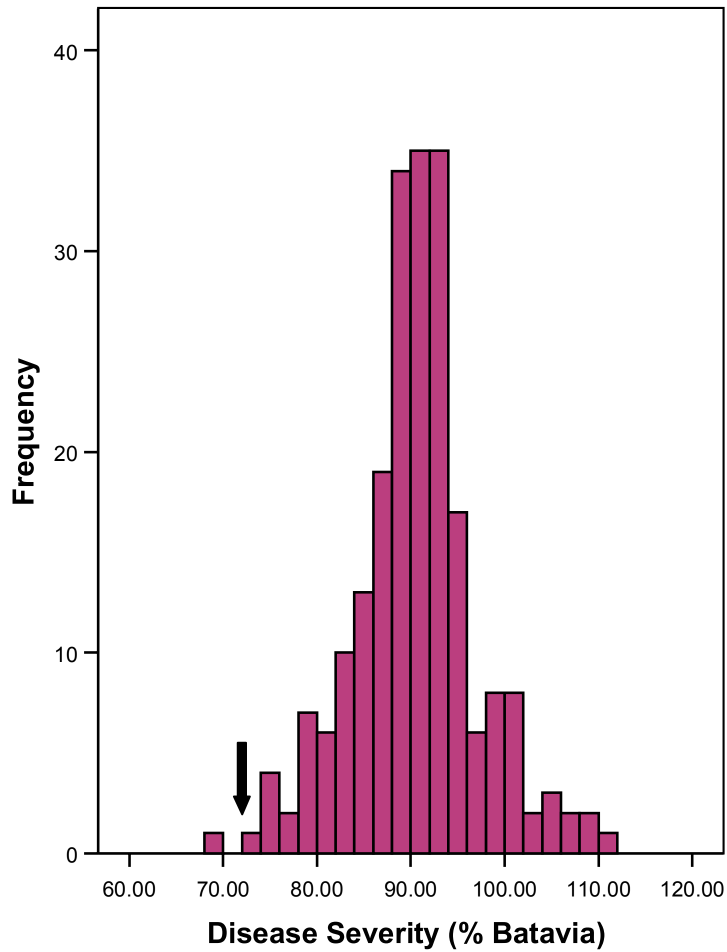
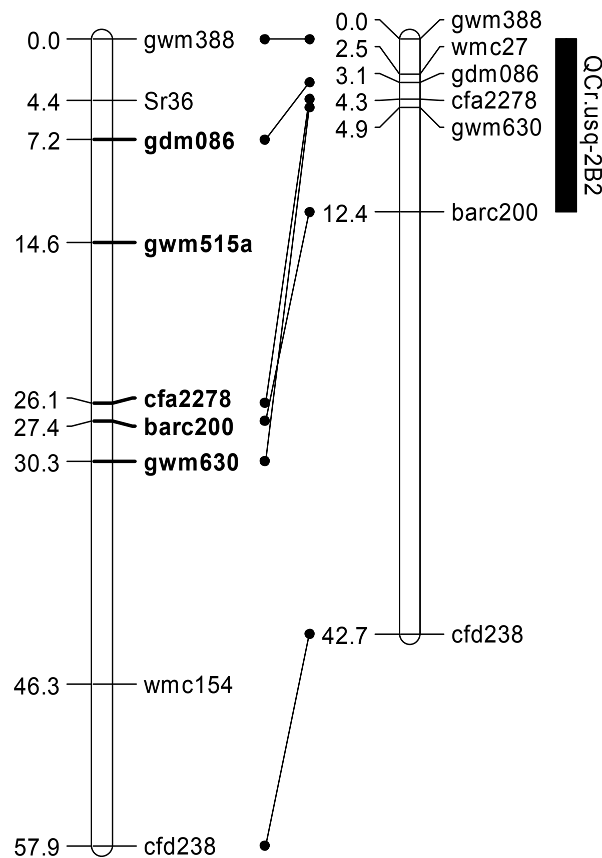


Figure 5-4. Validation of the Mendos-derived 2B QTL in a Sunco x Batavia doubled haploid population. Linkage maps for chromosome 2B of W21MMT70 x Mendos (left) and Sunco x Batavia (right) are shown. Markers associated with the 2B QTL inherited from Mendos are emphasized in bold. The region where the QTL was confirmed in the Sunco x Batavia population is indicated by the vertical bar. Markers in common between the two linkage maps are joined by lines. AFLP markers from the W21MMT70 x Mendos population are not included for the purpose of clarity.



5.33 Pyramiding of QTL for Resistance

The possibility of pyramiding QTL from diverse resistance sources was examined by analysing a doubled haploid population produced from a cross between line 2-49 and W21MMT70. One replicated seedling trial was carried out late in 2006. Histograms and descriptive statistics for combined and individual leaf sheath data for disease severity of the 2-49 x W21MMT70 doubled haploid population are displayed in Figure 5-5 and Table 5-4 respectively.

With the exception of the leaf sheath one data set, all histograms are significantly skewed towards resistance (Shapiro-Wilk test, $p<0.05$; Table 5-4). This is particularly evident with leaf sheath two and three data; 9% of lines displayed no symptoms of disease in leaf sheath two, and 70% of lines displayed no symptoms of disease in the third leaf sheath. The disease severity of leaf sheath one displayed a normal distribution (Shapiro-Wilk test, $p>0.2$). In both the combined and leaf sheath one data sets, all plants displayed at least some evidence of disease. The average disease severity was greatest in the leaf sheath one data set (77.34% Puseas) and was progressively lower for leaf sheath two (20.06%) and three (9.29%). Regardless of the data set examined (combined, or each of the leaf sheaths individually) line 2-49 displayed a greater level of resistance than W21MMT70.

Figure 5-5. Histograms of disease severity of the 2-49 x W21MMT70 doubled haploid population. a) The combined reaction of all three leaf sheaths; b) the reaction of leaf sheath one only; c) the reaction of leaf sheath two only; and d) the reaction of leaf sheath 3 only. The reaction of 2-49 and W21MMT70 are shown as filled and unfilled arrows respectively.

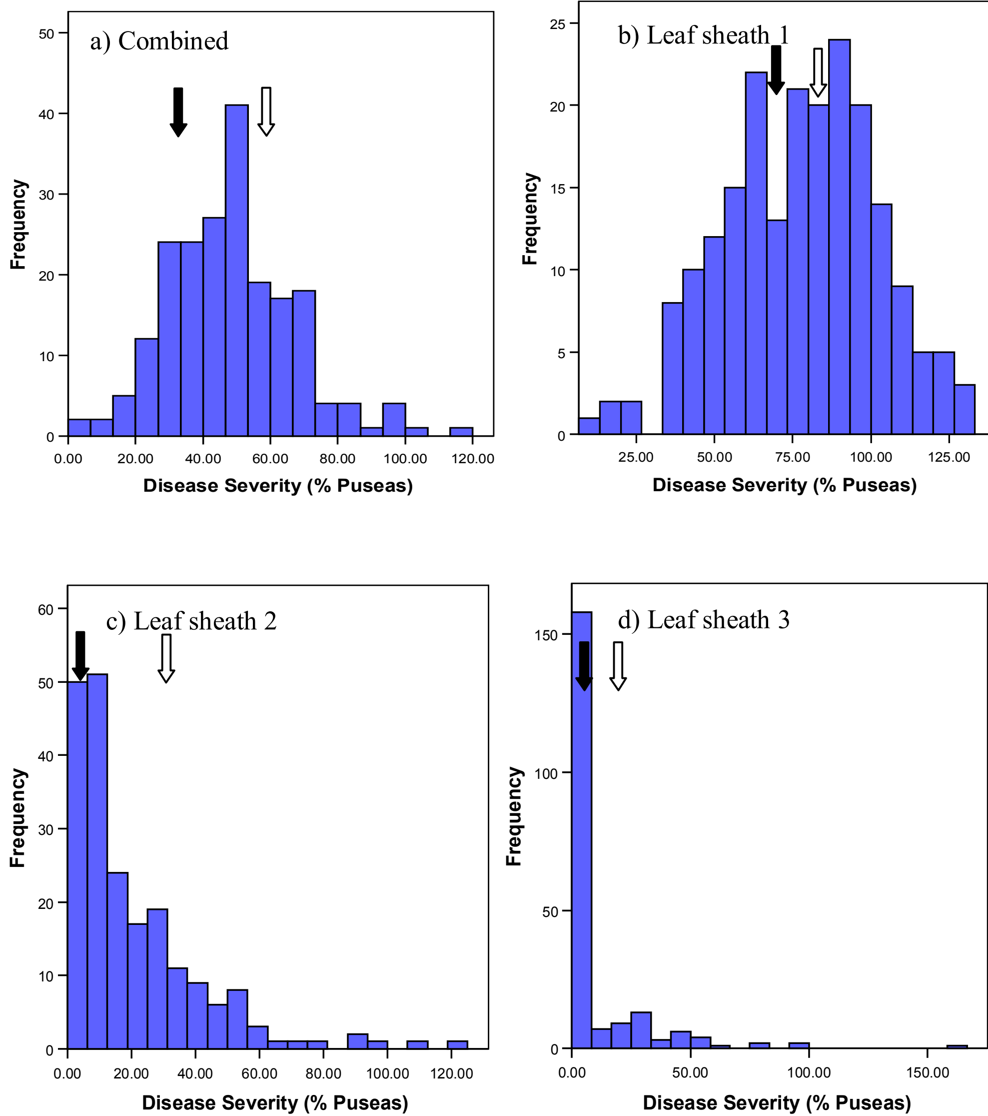


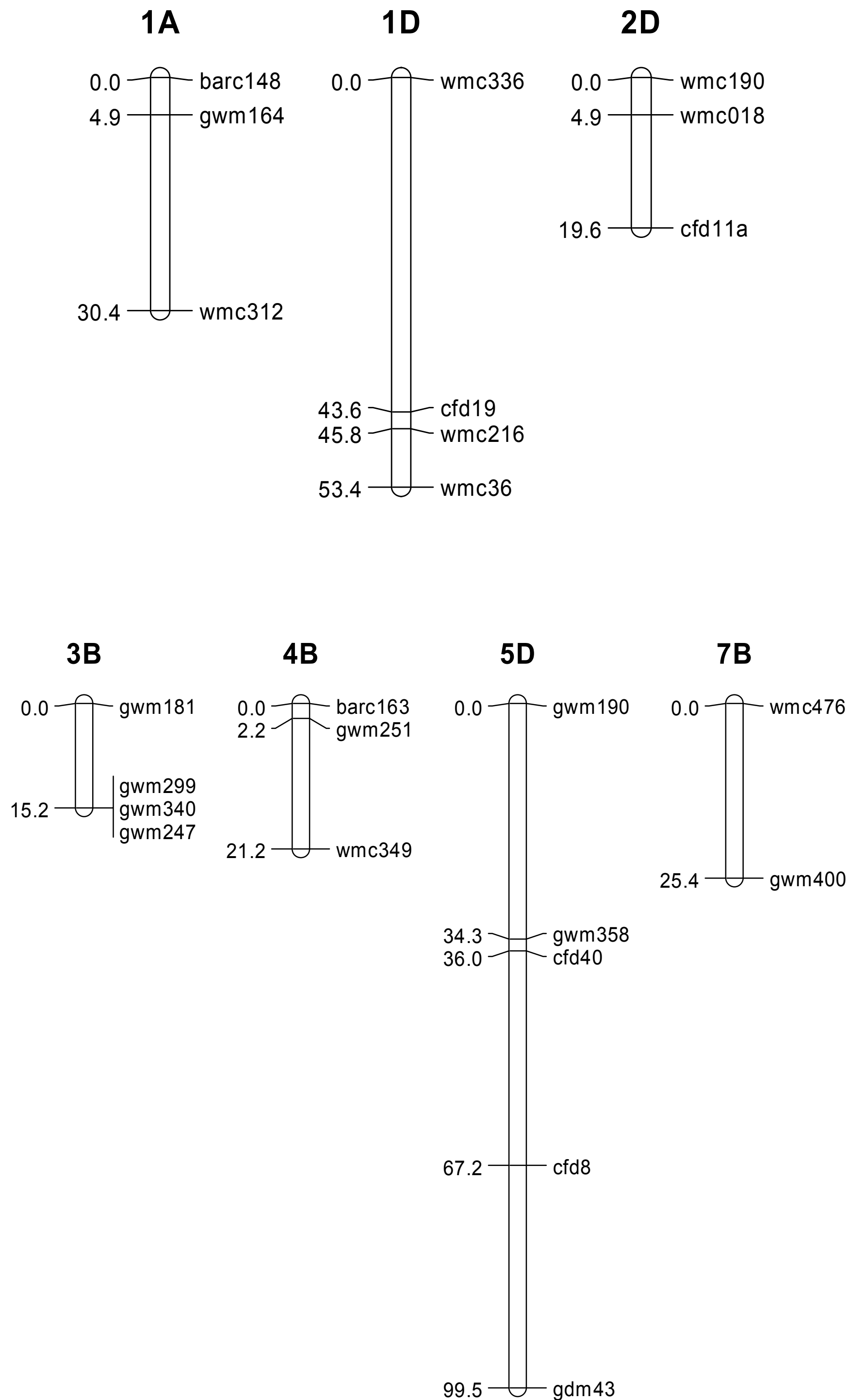
Table 5-4. Descriptive statistics of combined data, leaf sheath one data (Sheath 1), leaf sheath two data (Sheath 2), and leaf sheath three data (Sheath 3) for the 2-49 x W21MMT70 doubled haploid population.

Data	N	Range	Min.	Max.	Mean	S.E.	Skewness	Kurtosis
Combined	207	112.05	2.36	114.41	47.92	1.31	0.49	0.85
Sheath 1	207	122.18	6.90	129.08	77.34	1.72	-0.26	-0.17
Sheath 2	207	123.59	0.00	123.59	20.06	1.48	2.00	5.08
Sheath 3	207	163.04	0.00	163.04	9.29	1.44	3.55	17.34

5.33.1 Linkage Map Construction

In a previous study, Collard et al. (Collard et al., 2005b) identified QTL inherited from line 2-49 on chromosomes 1A, 1D, 4B, and 7B, in a doubled haploid wheat population produced from a cross between line 2-49 and the cultivar Janz. In order to assess the effectiveness of pyramiding QTL from the 2-49 and W21MMT70 sources of resistance, partial linkage maps of each of the chromosomes where QTL were previously identified from line 2-49 or W21MMT70 were produced (Figure 5-6). Twenty four (24) SSR markers were chosen for both their location with regards to QTL previously identified, and also for commonality between maps. For each QTL, polymorphic markers that flanked the QTL of interest were identified and used to genotype the entire 2-49 x W21MMT70 population.

Figure 5-6. Linkage map of chromosomal regions of interest from the 2-49 x W21MMT70 doubled haploid population. QTL inherited from 2-49 in a previous study were located on chromosomes 1A, 1D, 4B, and 7B (Collard et al., 2005b). The W21MMT70 QTL were located on chromosomes 2D, 3B, and 5D (Bovill et al., 2006).



5.33.2 QTL Detection

QTL detection was carried out using both QTL Cartographer and QTLNetwork in order to compare the results of the different packages. QTL mapping was conducted on the combined data set, and also on the individual leaf sheath scores (Table 5-5). The combined data set and the leaf sheath two data set were normalised using Rankit's formula (SPSS Inc., Chicago, IL, USA, 1989-2003) prior to QTL detection. The leaf sheath three data set was unable to be normalised due to the extreme skewness towards resistance. Permutation tests (10000 in 2 cM intervals) were used to assess the significance of the marker trait associations.

In the combined data set, QTL were detected on chromosomes 1A, 1D, 2D, and 3B by QTL Cartographer, whereas QTLNetwork only detected the 1D and 3B QTL. For leaf sheath one, three QTL, located on chromosomes 1A, 1D, and 3B were detected by both software packages. A QTL on chromosome 2D was detected by QTL Cartographer but not QTLNetwork with this data set. For leaf sheath two, one QTL (located on chromosome 3B), was detected by both software packages, and two other QTL (located on chromosomes 1D and 2D) were detected by QTL Cartographer. No QTL were detected with leaf sheath three data. No QTL were detected on chromosomes 4B, 5D, and 7B with any of the data sets. Overall, the 3B QTL inherited from W21MMT70 had the greatest effect on reducing levels of disease.

In order to determine whether the regions where the QTL were detected in the 2-49 x W21MMT70 doubled haploid population were the same as in either the 2-49 x Janz DH population or the W21MMT70 x Mendos DH populations, linkage maps of chromosomal regions where QTL were detected were aligned (Figures 5-7; 5-8; 5-9 and 5-10). In each instance, the regions where QTL were detected in the 2-49 x W21MMT70 pyramiding population are the same as in the original mapping populations.

Table 5-5. A comparison of results from QTL Cartographer and QTLNetwork for the chromosomal regions of interest in the 2-49 x W21MMT70 population. Non-significant marker-trait associations are not included.

Chromosome	QTL Cartographer			QTLNetwork	
	LRS	Sig.[+]	% P.E.[#]	p-value[^]	% P.E.[#]
Combined					
1A	7.4	Sg	4.1		
1D	22.6	HS	9.5	<0.00002	7.5
2D	5.7	Sg	2.5		
3B	42.1	HS	21.0	<0.00000	18.6
4B					
5D					
7B					
Leaf Sheath 1					
1A	10.3	S	6.2	0.014	1.9
1D	28.0	HS	12.0	<0.00005	10.1
2D	4.7	Sg	2.0		
3B	43.1	HS	21.5	<0.00000	16.7
4B					
5D					
7B					
Leaf Sheath 2					
1A					
1D	4.9	Sg	2.3		
2D	5.7	Sg	3.3		
3B	16.7	HS	9.1	<0.00008	8.8
4B					
5D					
7B					
Leaf Sheath 3					
1A					
1D					
2D					
3B					
4B					
5D					
7B					

[+]Significance (Sig.) level thresholds (Sg: suggestive; S: significant; HS: highly significant) were determined by permutations.
[#]%.P.E.: Percent phenotypic variation explained
[^]p-value: The p-value calculated by QTLNetwork

Figure 5-7. Comparison of the 1A QTL identified in a 2-49 x Janz population and the 2-49 x W21MMT70 population. Linkage maps for chromosome 1A of 2-49 x Janz (left) and 2-49 x W21MMT70 (right) are shown. Markers associated with the 1A QTL inherited from 2-49 in the original 2-49 x Janz study (Collard et al., 2005b) are emphasized in bold. The region where the QTL was identified in the 2-49 x W21MMT70 population is indicated by the vertical bar. Markers in common between the two linkage maps are joined by lines.

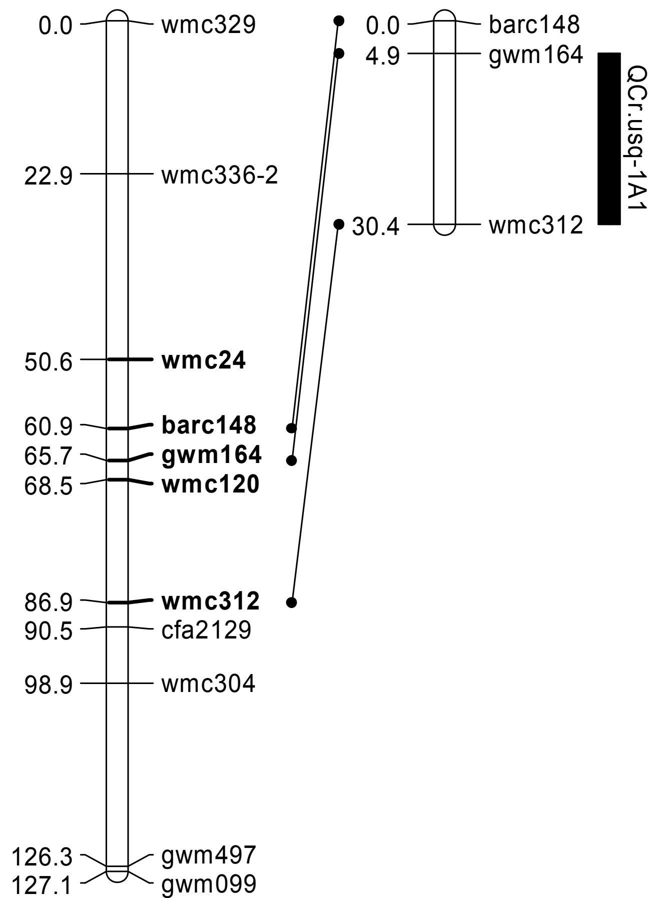


Figure 5-8. Comparison of the 1D QTL identified in a 2-49 x Janz population and the 2-49 x W21MMT70 population. Linkage maps for chromosome 1D of 2-49 x Janz (left) and 2-49 x W21MMT70 (right) are shown. Markers associated with the 1D QTL inherited from 2-49 in the original 2-49 x Janz study (Collard et al., 2005b) are emphasized in bold. The region where the QTL was identified in the 2-49 x W21MMT70 population is indicated by the vertical bar. Markers in common between the two linkage maps are joined by lines.

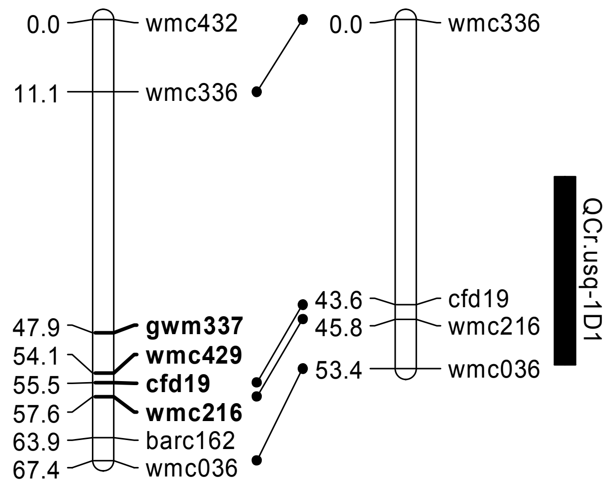


Figure 5-9. Comparison of the 2D QTL identified in the W21MMT70 x Mendos population and the 2-49 x W21MMT70 population. Linkage maps for chromosome 2D of W21MMT70 x Mendos (left) and 2-49 x W21MMT70 (right) are shown. Markers associated with the 2D QTL inherited from W21MMT70 in the original W21MMT70 x Mendos study are emphasized in bold. The region where the QTL was identified in the 2-49 x W21MMT70 population is indicated by the vertical bar. Markers in common between the two linkage maps are joined by lines.

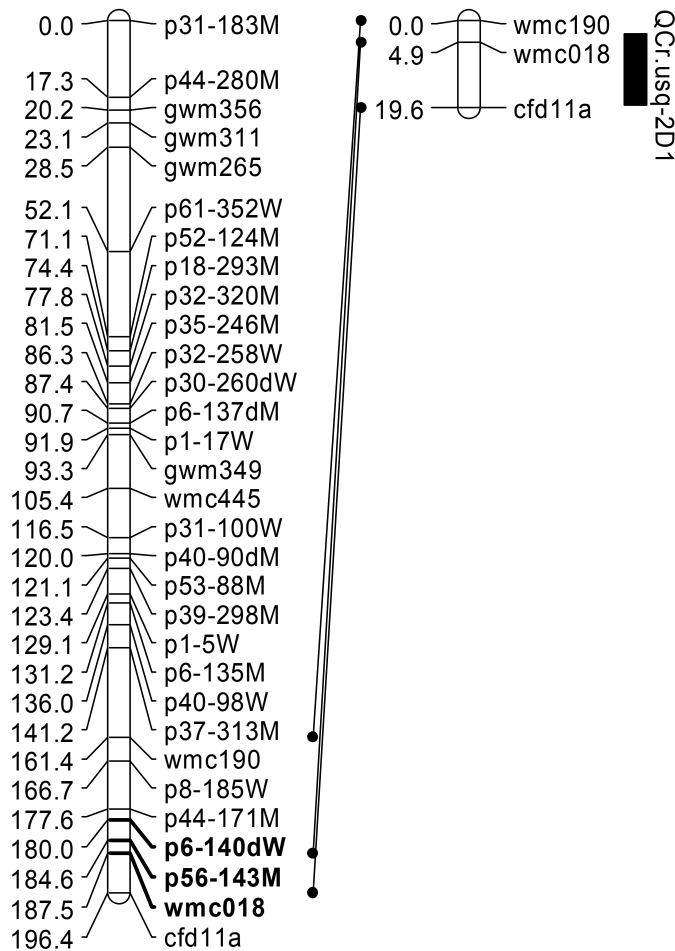
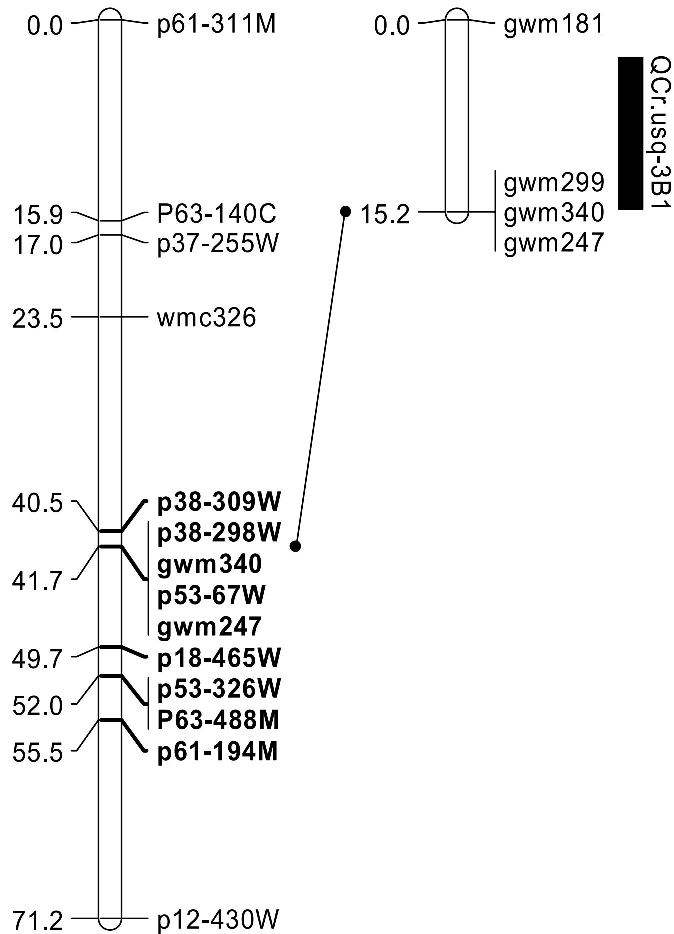
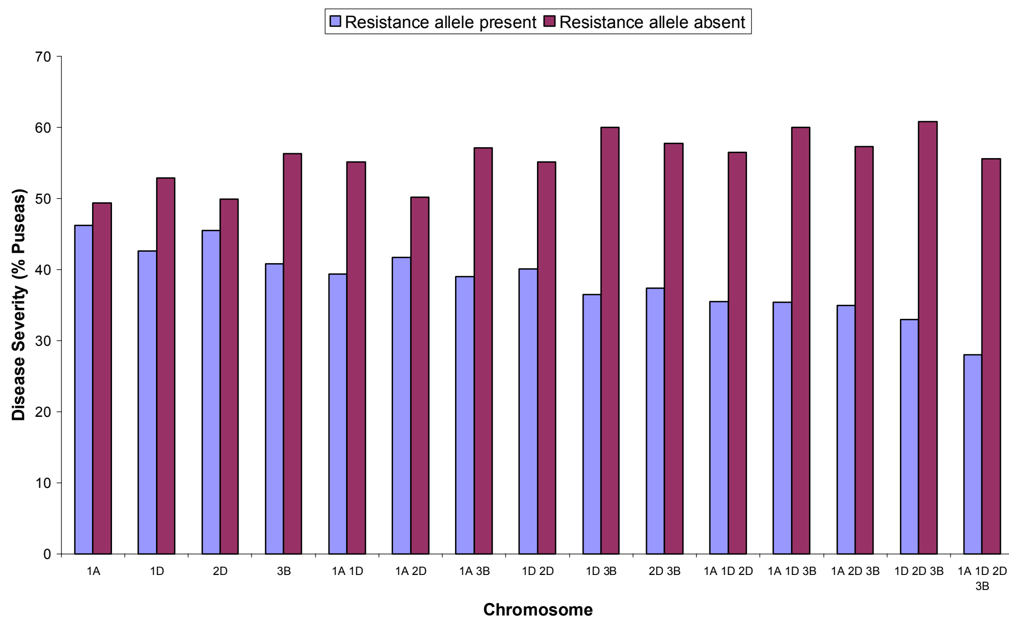


Figure 5-10. Comparison of the 3B QTL identified in the W21MMT70 x Mendos population and in the 2-49 x W21MMT70 population. Linkage maps for chromosome 3B of W21MMT70 x Mendos (left) and 2-49 x W21MMT70 (right) are shown. Markers associated with the 3B QTL inherited from W21MMT70 in the original W21MMT70 x Mendos study (Bovill et al., 2006) are emphasized in bold. The region where the QTL was identified in the 2-49 x W21MMT70 population is indicated by the vertical bar. Markers in common between the two linkage maps are joined by lines.



The effect of various combinations of alleles at the 1A, 1D, 2D and 3B QTL are shown in Figure 5-11. The doubled-haploid lines with all four resistance alleles (the 1A and 1D alleles from 2-49, and the 2D and 3B allele from W21MMT70) had a mean severity rating of 28.0%. This value is 41.5% lower than the population mean of 47.9%, and 49.6% lower than lines having susceptible alleles at all three loci (55.6% Puseas).

Figure 5-11. Mean disease severity (% of 'Puseas') of doubled-haploid lines with combinations of alleles from the four QTL regions. In all instances, differences were significant between lines carrying a QTL contributing to resistance and those without (Students t-test, $p<0.05$).



5.34 Haplotyping

Three closely linked SSR markers from the W21MMT70 inherited QTL (located on chromosomes 2D, 3B, and 5D) and the Mendos 2B QTL were chosen and used to examine allelic composition of a range of genotypes (Table 5-6). The genotypes chosen are either partially resistant or susceptible to crown rot, and many are important pedigrees in the Northern region. The allele size of the three 2B SSR markers are the same between Mendos, Lang and Sunco. Lang and Sunco possess the same *T. timopheevi* intogression that is present in Mendos. No marker was unique in being able to detect the W21MMT70 2D QTL. However, none of the other genotypes tested displayed the same size haplotype as W21MMT70 for all three 2D SSR markers. A similar trend is seen with the 3B region, except that the highly susceptible cultivar Puseas shares the same haplotype as W21MMT70 in this region. Marker gwm358 on chromosome 5D appears unique in identifying the W21MMT70 5D QTL region, and no other genotypes have the same haplotype as W21MMT70 in this QTL region.

Table 5-6. Estimated allele size (base pairs) of selected SSR markers in the regions of the 2B, 2D, 3B, and 5D QTL. Allele size of parental lines for the markers associated with the QTL of interest are underlined and highlighted. Genotypes with W21MMT70 (2D, 3B, and 5D) and Mendos (2B) marker alleles are highlighted.

Genotype	Classification*	Chromosome 2B			Chromosome 2D			Chromosome 3B			Chromosome 5D		
		cfa2278	gwm630	gwm515	wmc18	wmc190	cfd11	gwm181	gwm247	gwm547	gwm358	cfd8	barc143
W21MMT70	PR	340	null	null	226	132	300	134	162	192	164	156	228
Mendos	PR	284	160	90	220	128	340	120	158	192	160	152	226
2.49	PR	260	140	null	222	128	340	120	158	192	160	158	226
GE	PR	260	140	null	222	128	null	136	170	182	160	158	226
Gala	PR	260	140	null	228	128	340	120	158	192	160	156	228
CPI133814	PR	340	null	null	230	120	280	120,126	170	192	166	158	220
IRN497	PR	260	140	null	230	130	240	126	162	182	160	156	222
Kukri	PR	340	140	null	230	132	300	128	162	182	160	156	222
Lang	PR	284	160	90	230	132	280	120	158	192	160	152	230
Sunco	PR	284	160	90	230	132	280	120	158	192	160	152	230
Sumai3	PR	260	140	null	226	124	228	138	168	182	160	156	226
Batavia	S	340	140	null	226	128	420	120	158	192	162	160	224
CPI133872	S	340	null	null	226	120	200	124,130	150	182	144	152	220
Hartog	S	260	null	null	230	122	420	136	170	182	162	156	224
Janz	S	340	140	null	230	132	280	120	158	192	160	160	230
Puseas	S	260	null	null	226	122	300	134	162	192	160	156	226
QT8766	S	260	140	null	230	132	280	136	170	182	162	156	224
Sunbrook	S	340	140	null	230	132	280	136	170	182	162	156	230
Sunstate	S	260	140	null	230	122	null	136	170	182	162	156	224
Vasco	S	340	140	null	220	128	null	126	159	182	162	160	228

* Classification (PR, partially resistant; S, susceptible) taken from Collard et al. (2006).

5.4 Discussion

This aims of this chapter were to: i) validate both the W21MMT70 and Mendos derived QTL; and ii) investigate the potential of pyramiding QTL from different sources to increase the level of resistance to crown rot in wheat.

None of the W21MMT70 derived QTL could be convincingly validated using the F_2 populations. The 2D and 3B QTL were not detected in any of the F_2 populations. It was however expected that the 3B QTL would not be able to be validated in the IRN497 x W21MMT70 population, as recent work outside the scope of this thesis has revealed that IRN497 carries a QTL in what appears to be the same region of chromosome 3B (Bovill et al., unpublished results), and thus this QTL will not segregate in the IRN497 x W21MMT70 population. The 5D QTL had a significant effect in the Puseas x W21MMT70 population, a suggestive effect in the IRN497 x W21MMT70 population, but no effect in the QT10162 x W21MMT70 population. Such findings may indicate that these QTL are not robust; however, other factors may have contributed to the inability to conclusively validate these QTL in each of the populations examined. The most significant of these factors is probably the size of the populations used for validation - the largest population consisted of 94 individuals. This may appear sufficient when compared to the original W21MMT70 x Mendos population of 95 individuals, but when the F_2 population structure is considered this is clearly not enough. For example, each line in the W21MMT70 x Mendos population was replicated four times in 2005 (see Section 2.22), and the 2-49 x W21MMT70 doubled haploid population of 207 lines was replicated twice. It should be emphasized that each replicate consists of an average score from ten plants per pot – thus, for the W21MMT70 x Mendos trial in 2005, 3800 individual plants were rated for disease severity, and over 4000 plants (4140) were rated in the 2-49 x W21MMT70 population. Obviously, the data obtained in an F_2 population is based upon the rating of only one individual. A high degree of variability within samples is seen when phenotyping for resistance to crown rot (Wallwork et al., 2004; Collard et al., 2006). As was shown in Chapter 2, despite extensive replication, the correlation between the three seedling trials, although significant,

was certainly not absolute. Furthermore, a number of plants that were highly susceptible (i.e produced a score of 300) were not able to be leaf sampled as they had died. As there is no ability to repeat seedling trials on the F_2 individuals, the results from using such populations for QTL detection or validation studies on resistance to crown rot should be treated with caution, unless perhaps, if they are very large (Beavis, 1994, 1998).

The 2B QTL inherited from Mendos was able to be validated in a Sunco x Batavia population. This QTL has been designated *QCr.usq-2B2* to reflect that it is different to *QCr.usq-2B1* that was identified by Collard et al. (2006) in a 2-49 x Janz mapping population. In the study of Collard et al. (2006), *QCr.usq-2B1* was inherited from the susceptible cultivar Janz. Janz does not possess the *Triticum timopheevi* introgression that contributes to resistance in both Mendos and Sunco indicating that these QTL are indeed different. Other lines that utilize this introgression include (for example) Cook, Lang and Mengavi. Each of these cultivars display(ed) some level of resistance to crown rot (Purss, 1966; Klein et al., 1989; Wildermuth and Morgan, 2004), and it is probable that this introgression is contributing to the resistance these cultivars possess. However, as these cultivars do not possess the same level of resistance that is present in Sunco, it is likely that other QTL from Sunco remain undetected.

Pyramiding of major, monogenic (i.e. qualitative) resistance genes, using markers, has been successfully conducted for a number of diseases (Huang et al., 1997; Castro et al., 2003). However, reports of pyramiding genes for polygenic (i.e. quantitative) resistances, such as crown rot, are uncommon. This is because quantitative resistance genes (QTL) have a much lower effect than major loci, are environmentally sensitive, and prone to interaction with the genetic background into which they are introgressed. The analysis of the 2-49 x W21MMT70 doubled haploid population described in this chapter was conducted in order to quantify the additive effects of the QTL that had been identified in previous studies (see Collard et al., 2005b; Bovill et al., 2006). Based upon the combined data set, a number of doubled haploid lines performed significantly better than line 2-49. Line 2-49 was recognized as the "gold standard" for resistance to crown rot, and the identification of such lines is

extremely promising. Indeed, such doubled haploid lines fixed for each of the QTL detected, could be readily introgressed into elite backgrounds in order to provide some relief to this economically important disease.

Of seven QTL detected in the source mapping populations (four inherited from line 2-49 and three from W21MMT70), four were detected in the 2-49 x W21MMT70 population. Collard et al. (2006) previously validated the 1D QTL (*QCr.usq-1D1*) inherited from 2-49 in a Gluyas Early x Janz doubled haploid population. Gluyas Early is one of the parents of line 2-49. The detection of *QCr.usq-1D1* in the current study provides further evidence of the significance of this QTL. The other QTL inherited from line 2-49 (on chromosomes 1A, 4B, and 7B) were not able to be validated in the study of Collard (2006) as these were inherited from Gala – the other parent of line 2-49. In the current study, the 1A QTL (*QCr.usq-1A1*) was validated, however, the 4B and 7B QTL were not. Each of these QTL were only minor in their effect in the original 2-49 x Janz mapping study (Collard et al., 2005b), and their putative effects could not be confirmed in the 2-49 x W21MMT70 doubled haploid population.

Of the three W21MMT70 QTL identified in the source mapping population (located on chromosomes 2D, 3B, and 5D) two (*QCr.usq-2D1 and QCr.usq-3B1*) were shown to have an effect in the 2-49 x W21MMT70 population, although the effect of *QCR.usq-2D1* was only minor. There are a number of reasons that may explain the inability to detect the 5D QTL. Firstly, in the source W21MMT70 x Mendos population, this QTL was shown to have a highly significant effect in the 2001 growth cabinet trial, but significant and suggestive effects in the 2003 and 2005 glasshouse trials. The phenotyping of the 2-49 x W21MMT70 population was conducted in glasshouse trials; it may be possible that this QTL could have been detected if the trial was conducted in a growth cabinet environment. Secondly, the analysis of the individual leaf sheaths (see Chapter 3) showed that this QTL had the greatest effect in leaf sheaths two and three. The majority of offspring from the 2-49 x W21MMT70 population displayed little or no infection in leaf sheaths two and three which may have precluded the ability to detect this QTL. Finally, as shown in Chapter 4, two software packages (Epistat and QTLNetwork) reported an epistatic interaction between

the 5D QTL and a Mendos locus on chromosome 2Di. No tests for epistatic interactions were conducted on the 2-49 x W21MMT70 population (as results are available from only one trial and any firm conclusion regarding epistatic interactions could thus not be made). Liao et al. (2001) in a study on the effects of genetic background and environment on QTL and epistasis for rice panicle number, reported that in a doubled haploid population a locus was reported as a QTL, whereas in a recombinant inbred population the same locus was involved in an epistatic interaction. It may be possible that this is indeed the case with the 5D QTL, however further studies are required to investigate this possibility.

QCr.usq-3B1 had the greatest effect (LRS 42.1, explaining 21.0 % of the phenotypic variance based upon the combined disease severity rating) of any of the QTL detected in the pyramiding study. In the W21MMT70 x Mendos population, this QTL was suggestive in only one of the three seedling trials (2003), although it did approach the significance threshold for being deemed suggestive in each of the other trials. The strong effect of this QTL in the 2-49 x W21MMT70 population was unexpected. The detection of the large effect of the 3B QTL supports the need for detailed validation studies to be conducted prior to the use of markers for marker assisted selection (Langridge et al., 2001).

The phenotypic variation within lines that either did or did not carry the combination of QTL detected was large. For example, the 11 lines that possessed each of the 1A, 1D, 2D and 3B QTL ranged in disease severity from 2.36 to 51.24 % Puseas. Similarly, the 5 lines that did not possess any of the QTL detected ranged from 27.80 to 79.13 % Puseas. Miedaner et al. (2006) reported similar results when pyramiding three QTL for *Fusarium* head blight resistance into an elite European spring wheat. These researchers found that some of the genotypes without any of the target QTL still yielded some rather resistant individuals (Miedaner et al., 2006). Miedaner et al. (2006) conclude that phenotypic selection following marker-based selection is necessary to reach maximum gain from selection for resistance to *Fusarium* head blight, and it would appear wise to follow this recommendation when selecting for resistance to crown rot.

From the investigation of SSR allele sizes in 20 different genotypes, it was found that very few markers could be uniquely linked with any of the QTL regions studied based upon SSR fragment size. Only one (gwm358) on chromosome 5D appeared diagnostic for the W21MMT70 parent. Marker-assisted selection based upon the three 2B SSRs from Mendos should be successful as these markers appear diagnostic in detecting the presence of the 2B *Triticum timopheevi* which contributes to resistance in both the W21MMT70 x Mendos and Sunco x Batavia populations. No markers were diagnostic for the 2D QTL region, although none of the other genotypes tested possessed the same haplotype as W21MMT70. The finding of no polymorphism of the 3B QTL region between W21MMT70 and the highly crown rot susceptible cultivar Puseas, but, in contrast, polymorphism between IRN497 and W21MMT70 indicates that haplotyping based upon SSR fragment sizes does not unambiguously infer the presence or absence of a QTL in the haplotyped region.

SSR haplotyping has been used extensively to infer presence or absence of QTL for resistance to Fusarium head blight of wheat (Liu and Anderson, 2003; McCartney et al., 2004). Similar to the results of the current study, Ma et al. (2006) identified a QTL for resistance to Fusarium head blight on chromosome 3B in a Chinese Spring Sumai 3 disomic substitution line, but showed that the SSR haplotype of this region was different to that of Sumai 3. Spielmeyer et al. (2003), in a study on SSR markers lined to the durable stem rust resistance gene *Sr2*, identified an SSR marker (gwm533) which was diagnostic for all genotypes known to possess this resistance gene. However, some genotypes that did not possess *Sr2* displayed an identical 120 bp fragment. These authors sequenced the 120 bp SSR fragment and showed that the structure of the microsatellite repeat differed between the susceptible and resistance genotypes (Spielmeyer et al., 2003). Such “allelic homoplasy” (alleles identical by size, but not identical by descent) therefore needs to be considered before the likelihood of QTL in common can be inferred from an SSR haplotype.

In summary, this chapter has focussed on validating the Mendos 2B QTL and the 2D, 3B, and 5D QTL from W21MMT70. The Mendos 2B QTL was successfully validated in a Sunco x Batavia population. The 5D W21MMT70 QTL was able

to be validated in two of three F_2 populations, and the 2D and 3B QTL were validated in the 2-49 x W21MMT70 doubled haploid populations. Such findings indicate that some QTL may be dependent on the background into which they are introgressed for expression. The pyramiding of the 2-49 and W21MMT70 produced genotypes with extremely high levels of resistance (as low as 2.36% Puseas) and the fixed lines identified will be useful to breeding programs. Finally, the results of the haplotype analysis suggest that SSR haplotypes can not be unambiguously used to infer the presence of a QTL unless the SSR alleles are identical by descent.

Chapter 6. General Discussion

6.1 Research Outcomes

This study met the objectives that were outlined in the thesis rationale. Specifically this study has:

- Conducted a BSA on the original data set available;
- Completed two further seedling test trials in two different years;
- Generated and applied a framework SSR map to incorporate AFLP markers of unknown chromosomal location to increase marker density;
- Identified QTL for resistance that were inherited from both W21MMT70 and Mendos;
- Evaluated a range of analysis packages for their comparative ability to detect QTL;
- Confirmed a role for epistatic interactions in the expression of crown rot resistance;
- Validated the major QTL originating in W21MMT70 and Mendos in some, but not all, alternative populations; and
- Identified lines carrying the major QTL from W21MMT70 and 2-49 which express a greater level of resistance to crown rot.

Each of these outcomes has been discussed in detail in previous chapters. This general discussion will thus focus upon the contribution this work may make to the wheat breeding community and address future directions that would add value to the results presented in this dissertation.

6.2 Contribution to the Wheat Breeding Community

Crown rot resistance has proven a challenging trait for wheat breeders to incorporate into their breeding programs. This challenge relates to the difficulty of selecting partially resistant germplasm based upon phenotype alone. To increase the accuracy of phenotypic selection, a range of methods have been trialled and are in use by various research groups. However, each of the methods still display significant between year variation. For example, the terrace method

(see Section 11.46.4), although offering the advantage of higher throughout screening compared to field trials, “has not eliminated the enormous variability encountered in screening for crown rot resistance” (Wallwork et al., 2004). The seedling method of Wildermuth and McNamara (1994), as was used in the current study, was designed as a faster technique to identify crown rot resistance germplasm. Compared to adult plant screening, where resistance is based upon the level of honey-brown discolouration on internodes at harvest, this technique involves the rating of lesions on individual leaf sheaths, and an inhibition of lesion development is a clear indication of partial resistance. Using this method, the current study has revealed a range of QTL that contribute to this partial resistance in the W21MMT70 x Mendos population.

Resistance to crown rot is quantitatively inherited in all published reports that involve screening individuals from a segregating population (e.g. Wallwork et al., 2004; Collard et al., 2005b; Collard et al., 2006). Histograms for disease severity of the W21MMT70 x Mendos population showed a continuous distribution, providing further confirmation that resistance to crown rot is quantitative. Correlations between each of the three seedling trials that were conducted were significant, but the ranking of lines between trials differed. Thus, despite the use of fixed structure of the population (i.e. doubled haploid), extensive replication, and the great care that was taken in conducting the seedling assays, a degree of variability within lines was demonstrated. The variability found highlights the difficulty of selecting for crown rot resistant materials, and supports the role of molecular markers as tools to assist breeders in selecting crown rot resistant materials.

The QTL that have been detected have the potential to improve the efficiency of selecting for resistance to crown rot, and the identification of markers that flank these QTL offer breeders tools for the incorporation of the W21MMT70 source of resistance into their breeding programs. Prior to completion of this study, QTL for only two sources of resistance had been identified. Wallwork et al. (2004) identified a major QTL on chromosome 4B, identified by bulked-segregant analysis, that contributed to resistance in a Kukri x Janz population. The detection of only one QTL in this population is in agreement with the

limitations of using bulked-segregant analysis for identifying loci that contribute to quantitative traits (Cook et al., 2004). Furthermore, the resistance Kukri possesses is, at best, moderate, and breeders are unlikely to focus on deploying this source of resistance. Collard et al. (2005b) have reported a range of QTL inherited from line 2-49 – a line that is widely recognised as a superior source of resistance to crown rot. However, to date, this resistance has not been successfully incorporated into Australian breeding programs. It appears that the main reason for this is the poor agronomic characteristics (such as height) of 2-49. Through discussions with breeders, it appears that the markers have not been used for early generation selection, and by the F_4 to F_5 stage of development, the QTL for resistance have been lost because only agronomic characters have been selected for in early generations (P. Banks, pers. comm.). In contrast to the Australian situation, CIMMYT wheat breeders have used the 1D 2-49 markers for population enrichment in early generations and have reported promising results (R. Trethowan, pers. comm.).

The QTL inherited from W21MMT70 offer breeders an alternative source of resistance to incorporate into their programs. The resistance that W21MMT70 possesses approaches that offered by 2-49. Indeed, in one of the three seedling trials that were conducted, W21MMT70 displayed a marginally greater level of resistance than did 2-49. Therefore, these QTL will aid breeders in the production of crown rot resistant cultivars. The identification of a QTL on chromosome 2B, inherited from Mendos, has provided unexpected benefits. This QTL, located on a *T. timopheevi* introgression has been validated in the cultivar Sunco. Sunco is currently the best commercial source of crown rot resistance to crown rot (G. Wildermuth, pers. comm.) and this study has provided breeders with tools to assist in the selection of this source of partial resistance.

The chromosomal location of QTL detected in W21MMT70 and those previously reported from 2-49 (Collard et al., 2005b) are different, and the pyramiding of these QTL was conducted to determine if the effects of these different sources of resistance were additive. Based upon the seedling trial that has been conducted, this appears to be the case. A number of DH lines performed significantly better than the parental lines. However, this result is

based upon only one seedling trial, and further trials are needed to confirm the greater level of resistance that is present in such lines. Of seven QTL detected in the source mapping populations (four inherited from line 2-49 and three from W21MMT70), four were detected in the 2-49 x W21MMT70 population. Two were inherited from 2-49 (chromosomes 1A and 1D) and two from W21MMT70 (chromosome 2D and 3B). There are a number of reasons that may explain why the other QTL were not detected. These include: the method of detection (composite interval mapping vs. simple interval mapping and marker regression); environmental interactions; leaf sheath specificity; and background dependence. *QCr.usq-3B1* had the greatest effect (LRS 42.1, explaining 21.0 % of the phenotypic variance (based upon the combined disease severity rating)) of any of the QTL detected. In the W21MMT70 x Mendos population, this QTL was suggestive in two of the three seedling trials based upon the manual version of the map, but only in one of three seedling trials based upon the RECORD version of the map (although it did approach the suggestive threshold in the other two trials). The strong effect of this QTL in the 2-49 x W21MMT70 population was unexpected. The detection of the large effect of the 3B QTL supports the need for detailed validation studies to be conducted prior to the use of markers in marker assisted selection (Langridge et al., 2001). Nonetheless, such lines with QTL from both parents should prove valuable to breeding programs.

Two software programs were used in the current study to detect epistatic interactions. The first, QTLNetwork (Yang et al., 2005), identified four digenic interactions. The phenotypic variance explained by these epistatic QTL ranged from 0.68 % to 4.68 %. Given that the most significant additive QTL explained up to 12.31 % of the phenotypic variance (as determined by QTLNetwork), the epistatic interactions detected appear to play a significant role in governing resistance. The second program, Epistat (Chase et al., 1997), which uses log-likelihood ratios to compare epistatic and additive models, detected 10 digenic interactions. The majority of these were co-adaptive (i.e. neither QTL had an effect on their own, but particular combinations displayed a phenotypic effect); however, of particular interest, conditional interactions (i.e. QTL with both main and epistatic interactions) were also detected. The most interesting of these involved the 5D QTL inherited from W21MMT70 and a Mendos modifying

locus on chromosome 2Di. This finding is of importance to the use of such QTL in breeding programs, as it indicates that the effect of main effect QTL may vary depending upon the genetic background into which they are transferred.

Nevertheless, there is good evidence now emerging that the QTL identified above are likely to be effective across a wide range of backgrounds. Very recent work at USQ by the author, which is outside the scope of this PhD study, has characterised other sources of resistance in which a number of these same QTL appear to be effective in conditioning resistance. IRN497 has already been refered to earlier as an independent source of resistance first identified in the 1960's (Wildermuth and Purss, 1971), and Sunco is currently the best commercial source of partial resistance (G. Wilermuth, pers. comm.).

Figure **6-1** provides a comparison of the QTL that have now been identified in four doubled haploid wheat populations, including the W21MMT70 x Mendos population which has been the subject of this dissertation. Although the combinations of QTL that have been detected in each of the populations are different, there are regions that are common between populations. For example, the 1A QTL has been detected in the 2-49 x Janz, W21MMT70 x Mendos, and the Sunco x Batavia populations. The 1D QTL is present in the 2-49 x Janz population and the W21MMT70 x Mendos population. A QTL on chromosome 2B has been detected in the 2-49 x Janz, W21MMT70 x Mendos, and Sunco x Batavia populations. The 3B QTL and the 5D QTL have now been detected in the W21MMT70 x Mendos population and the IRN497 x Janz population. The 4B QTL is present in both the 2-49 x Janz population and the Sunco x Batavia population. This QTL also appears to be in the same region that Wallwork et al. (2004) identified in their Kukri x Janz population. With the exception of the 2B QTL inherited from Janz in the 2-49 x Janz population, all of the QTL that have been mentioned above are located in the same region of individual chromosomes within the different crosses.

Figure 6-1. Location of QTL for resistance to crown rot in a range of doubled haploid populations. The resistance parent in each of the crosses is underlined. The parent contributing each of the QTL is designated by the first letter of the genotype name in each chromosome where QTL were identified. Significant QTL are indicated by green boxes, whereas suggestive QTL are indicated by purple boxes.

2-49 x Janz

	A	B	D
1	2		2
2		J	
3			
4		2	
5			
6			
7		2	

W21 x Mendos

	A	B	D
1	M		M
2		M	W
3		W	
4			
5			W
6			
7			

IRN497 x Janz

	A	B	D
1			
2			
3		I	
4			
5	J		I
6			
7			

Sunco x Batavia

	A	B	D
1	S		
2		S	
3			S
4		S	
5		S	
6			
7			

Significant QTL Suggestive QTL

The effects of what appear likely to be the same QTL differs between populations. For example, the 1A and 1D QTL are significant in effect in the 2-49 x Janz population, but only suggestive in the W21MMT70 x Mendos population. The 3B QTL region was significant in the IRN497 x Janz population, but only suggestive in the W21MMT70 x Mendos population; when combined in the 2-49 background this suggestive W21MMT70 QTL is highly significant in effect. While these findings highlight the variable effect of QTL in different backgrounds they also suggest that they are expressed in a range of genetic backgrounds. Bearing in mind the unrelatedness of IRN497 (sourced from a collection from the International Spring Wheat Rust Nursery and reported to originate from Mexico), to both the 2-49 source (developed in Australia) and the W21MMT70 source (possibly derived from Canadian materials), the fact that

similar QTL are detected implies a positive independent selection for these regions across a range of environments and background germplasm.

6.3 Future Directions

Based on the findings of this study, future work on crown rot resistance should focus on the following:

1. *Working with larger populations*

The population size of 95 individuals was sufficient to identify QTL of large effect. However, many QTL of smaller effect may not have been identified. This population size is typical of those generated for mapping studies in the mid 1990's due to the expense of genotyping a large number of individuals. Costs of genotyping have now decreased significantly and data generation is many times faster, so it is now feasible to genotype larger numbers of individuals, particularly using recent developments such as diversity array technology (DArTs). Increasing the size of the population should decrease the size of confidence intervals associated with the QTL, thus providing a more accurate estimate of QTL position, as well as lead to the identification of putative QTL of smaller effect. The development of techniques for stimulating shorter generation times has also made the use of single seed descent (SSD) populations more attractive in this context, particularly as they usually involve six or more meiotic generations, leading to many more cross-over events that will assist fine mapping of regions of interest. Fine mapping of large SSD populations or selected backcross/near isogenic lines (see below) in order to discover flanking markers even more tightly linked to regions of interest leads eventually to map-based cloning to attempt to discover the genes responsible for conditioning the resistant response. This will not be a trivial exercise in the wheat genome (Huang et al., 2003; Yan et al., 2003).

2. *Conduct field trials to assess the relationship between seedling and field resistance*

The results of leaf sheath specific QTL that were identified in chapter 2 suggest that QTL for resistance are important at different stages of the disease process,

and detecting their expression may be dependent upon when phenotyping is conducted. This finding has potentially serious ramifications for the usefulness of these QTL to predict field resistance to crown rot. While seedling resistance is a good predictor of field resistance, we do not yet know whether all the QTL effective at the seedling stages also condition resistance in adult plants. The case of Sunco certainly suggests that some QTL are much more effective under field conditions. Therefore, it is clear that replicated field trials need to be conducted on relevant populations to confirm the expression of the detected seedling QTL in adult plants under field conditions. A related issue is the improvement of the cost, efficiency and accuracy of field-based and other screening techniques. Discussions between the research community and the GRDC are currently in progress with regard to ways to achieve these goals and agree on more uniform screening approaches.

3. *Use a candidate gene approach to identify genes that contribute to resistance to crown rot*

Marker-assisted selection will be more efficient if the markers used are located within the gene(s) that contribute to resistance. With the vast amount of EST sequence data accumulating for wheat (over 855,000 ESTs as at January 2007) it may be possible to identify candidate genes that contribute to resistance to crown rot. Many of the SSR markers that flank the QTL detected in this study have been bin-mapped on the wheat aneuploid stocks (http://wheat.pw.usda.gov/GG2/index.shtml). Likewise, a large number of ESTs have also been bin-mapped (Lazo et al., 2004). By conducting appropriate bioinformatics searches, it may be possible to identify genes that contribute to crown rot resistance based upon their function.

4. *Produce a set of near-isogenic lines to improve current understanding of the genetics of resistance to crown rot*

An improved understanding of the genetics of resistance to crown rot would be highly beneficial to breeding programs. A strategy to further elucidate the genetics for resistance could involve the production of a set of near-isogenic-lines (NILs) that differ only for particular QTL regions of interest. Such a

strategy would firstly involve selection of a suitable recurrent parent (ideally the parent would be a highly desirable cultivar or breeding line that would benefit from increased crown rot resistance). NILs can be produced by conducting several rounds of backcrossing using markers to select individuals that are heterozygous for the QTL region at each backcross, and background selection could also be conducted in order to increase the rate at which the recurrent parent is fixed.

A number of groups have used NILs to further elucidate the genetics of phenotypes of interest. For example, Quarrie et al. (2006) produced a set of NILs that differed for a yield QTL on chromosome 7AL, and found that the allele with positive effects increased yield per ear by greater than 20 %, and was significantly associated with higher flag leaf chlorophyll content and flag leaf width. James et al. (2006) used NILs to identify two genes (*Nax1* and *Nax2*) that improve salt tolerance through sodium exclusion in durum wheat. In this report, the use of NILs enabled a greater understanding of the physiological function of the genes involved in sodium exclusion (James et al., 2006). The benefits of using NILs include: the identification of more tightly linked markers for the QTL of interest; fewer individuals need to be genotyped and phenotyped; the materials that have the QTL of interest would be advanced breeding lines (depending on choice of recurrent parent); and the fine mapping of the region could be amenable to map-based cloning (del Blanco et al., 2003). Thus, using the NIL approach may increase our understanding of the genetics of partial resistance to crown rot.

6.4 Conclusion

This dissertation reports the identification of QTL for resistance to crown rot that have been detected following the generation of a genetic linkage map. Markers which flank QTL from the novel W21MMT70 source of resistance offer breeders tools to increase the level of resistance to crown rot in their germplasm. Furthermore, the value of the chromosome 2B *T. timopheevi* introgression, present in Mendos and Sunco, as a further source of resistance has been

demonstrated. Putative additive effects of QTL from two independent sources of resistance (W21MMT70 and 2-49) have been confirmed, and this finding is encouraging for the future of crown rot resistance breeding. The challenge however, lies in incorporating such QTL into elite adapted backgrounds where their expression can be shown to enhance commercially significant resistance against this disease.

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APPENDIX I – Genotypic and Phenotypic Data

The raw genotypic and phenotypic data is available on CD-ROM in the slip on the back cover of this dissertation.

APPENDIX II –Publications

In preparation:

Bovill, W.D. and Sutherland, M.W. Epistasis plays a role in determining resistance to crown rot in wheat.

Bovill, W.D., Herde, D., and Sutherland, M.W. Validation of W21MMT70 and Mendos-derived QTL for resistance to crown rot in wheat.

Bovill, W.D., Davis, M., Wildermuth, G.B., and Sutherland, M.W. Pyramiding of QTL increases levels of resistance to crown rot in wheat.

Attached on the following pages:

Bovill, W.D., Ma, W., Ritter, K., Collard, B.C.Y., Davis, M., Wildermuth, G.B., and Sutherland, M.W. (2006) Identification of novel QTL for resistance to crown rot in the doubled haploid wheat population 'W21MMT70' x 'Mendos'. *Plant Breeding* 125: 538-543.