

1 **Identification of novel QTLs for resistance to crown rot in the doubled**  
2 **haploid wheat population ‘W21MMT70’ x ‘Mendos’**

3

4 W.D. Bovill<sup>1</sup>, W. Ma<sup>3</sup>, K. Ritter<sup>1</sup>, B.C.Y. Collard<sup>4</sup>, M. Davis<sup>2</sup>, G.B.  
5 Wildermuth<sup>2</sup> and M.W. Sutherland<sup>1,5</sup>

6

7 <sup>1</sup> Centre for Systems Biology, Faculty of Sciences, University of Southern  
8 Queensland, Toowoomba 4350, Queensland, Australia;

9 <sup>2</sup> Leslie Research Centre, Queensland Department of Primary Industries,  
10 P.O. Box 2282, Toowoomba, 4350, Queensland, Australia;

11 <sup>3</sup> Western Australian Department of Agriculture and MPB CRC, State  
12 Agriculture Biotechnology Centre, Murdoch University, 6150, Western  
13 Australia, Australia;

14 <sup>4</sup> Plant Breeding, Genetics and Biotechnology Division, International Rice  
15 Research Institute, Philippines;

16 <sup>5</sup> Corresponding Author; email: marksuth@usq.edu.au

17 With 3 figures and 2 tables

18 Received January 5, 2006

19

20 **Abstract**

21 Crown rot (causal agent *Fusarium pseudograminearum*) is a fungal  
22 disease of major significance to wheat cultivation in Australia. A doubled

1 haploid wheat population was produced from a cross between line  
2 ‘W21MMT70’, which displays partial seedling and adult plant (field)  
3 resistance to crown rot, and ‘Mendos’, which is moderately susceptible in  
4 seedling tests but partially resistant in field trials. Bulk segregant analysis  
5 (BSA) based on seedling trial data did not reveal markers for crown rot  
6 resistance. A framework map was produced consisting of 128 microsatellite  
7 markers, four phenotypic markers, and one sequence tagged site marker. To  
8 this map 331 previously screened AFLP markers were then added. Three  
9 quantitative trait loci (QTLs) were identified with composite interval  
10 mapping across all of the three seedling trials conducted. These QTLs are  
11 located on chromosomes 2B, 2D, and 5D. The 2D and 5D QTLs are  
12 inherited from the line ‘W21MMT70’, whereas the 2B QTL is inherited  
13 from ‘Mendos’. These loci are different from those associated with crown  
14 rot resistance in other wheat populations that have been examined, and may  
15 represent an opportunity for pyramiding QTLs to provide more durable  
16 resistance to crown rot.

17

18 **Key words**

19 *Triticum aestivum* - *Fusarium pseudograminearum* – QTL mapping –  
20 disease resistance

1 Crown rot (causal organism *Fusarium pseudograminearum*) is a significant  
2 fungal disease of wheat in Australia (Backhouse et al. 2004) and elsewhere  
3 (Paulitz et al. 2003, Balmas 1994, Marasas et al. 1988), particularly in  
4 production regions where stubble of previous cereal crops is retained and  
5 water stress late in the growing season is common. Symptoms of the  
6 disease in seedlings include browning of the coleoptile, subcrown internode  
7 and basal leaf sheaths. In adult plants, a honey brown discoloration of the  
8 tiller bases is typical followed by whiteheads (heads containing no grain) or  
9 varying degrees of shrivelled grain (Klein et al. 1991). It has been estimated  
10 that losses due to crown rot cost the Australian cereals industry \$56 million  
11 annually (Brennan and Murray 1998).

12 Crown rot is a difficult disease to manage, as *F. pseudograminearum*  
13 survives between wheat crops on infected wheat stubble or grass weeds  
14 (Wildermuth et al. 1997). In the last 15 years the disease has become more  
15 prevalent in many Australian growing environments, due to the adoption of  
16 minimum tillage, stubble retention practices in farming systems. Evidence  
17 from the North-West United States indicates that incidence and severity of  
18 the disease is increasing there also (Smiley et al. 2005). The majority of  
19 cultivars currently grown in Queensland are moderately or highly  
20 susceptible to the disease (Wildermuth et al. 2001). Current control  
21 methods focus on crop rotation and the planting of partially resistant  
22 varieties such as 'Sunco', 'Baxter', and 'Lang' (Wildermuth et al. 2004).

1 However, even partially resistant cultivars can suffer yield losses if planted  
2 in soil where the level of inoculum on stubble residues is high, and when  
3 environmental conditions favour the pathogen.

4 Breeding programmes are seeking to develop varieties which express  
5 improved levels of crown rot resistance in the field in a range of  
6 environmental conditions. Phenotypic disease assessments of genetic  
7 variation in field trials, which are generally made at harvest, are time-  
8 consuming, labour intensive and suffer from significant environmental  
9 effects on disease expression. A seedling test is available which correlates  
10 well with the field performance of many genotypes, however there is a  
11 subset of cultivars which show moderate susceptibility in seedling trials but  
12 partial resistance as adult plants in the field. For these reasons the coupling  
13 of molecular techniques with conventional breeding (marker-assisted  
14 selection) has the potential to more rapidly and reliably identify genomic  
15 regions from various sources that contribute to resistance, and should  
16 greatly increase the efficiency of selecting such resistance sources.

17 There are currently only two previous reports of molecular markers  
18 identified for resistance to crown rot. Wallwork et al. (2004), using a  
19 bulked segregant analysis, reported markers for a crown rot resistance QTL  
20 in a cross between the moderately field resistant cultivar 'Kukri' and the  
21 susceptible cultivar 'Janz'. In a more detailed mapping study, Collard et al.

1 (2005) have recently identified both major and minor QTLs for seedling  
2 resistance to crown rot in the partially resistant wheat line '2-49'.

3 We have been screening for alternative sources of partial resistance with  
4 the goal of pyramiding additional QTLs together with those identified in '2-  
5 49' to gain improved levels of resistance. This screening has identified  
6 significant variation for seedling resistance between the parents of a  
7 'W21MMT70' x 'Mendos' doubled haploid (DH) population, originally  
8 constructed for unrelated studies of quality attributes (Kammholz et al.  
9 1998). In this report the phenotypic and molecular analysis of seedling  
10 resistance in this population indicates that several previously undescribed  
11 QTLs contribute to resistance.

12

### 13 **Materials and Methods**

14 **Plant materials:** A wheat x maize induced doubled haploid population  
15 consisting of 95 lines of wheat (*Triticum aestivum* L.) was produced at the  
16 Leslie Research Centre from a cross between 'W21MMT70' and 'Mendos'  
17 by Kammholz et al. (1998). The Western Australian experimental line  
18 'W21MMT70' displays partial seedling and adult plant (field) resistance to  
19 crown rot, whereas the Australian cultivar 'Mendos' is susceptible in  
20 seedling trials, but displays partial adult plant resistance.

21

1 **Seedling disease assessment:** Three seedling trials were carried out in a  
2 growth cabinet (25°C, 60% humidity, 12-hour photoperiod) at the  
3 University of Southern Queensland in 2001, and in glasshouse tests at the  
4 Leslie Research Centre in 2003 and 2005. Phenotyping was conducted  
5 according to the method of Wildermuth and McNamara (1994). Briefly, 13  
6 seeds of each genotype were sown in pots containing steam/air treated soil  
7 (70°C, 30 minutes) inoculated with *Fusarium pseudograminearum*. After  
8 21 days, each of the first three leaf sheaths from 10 seedlings per pot were  
9 rated for disease severity using a five point scale whereby: 0 = no infection;  
10 1 = 0-25%; 2 = 25-50%; 3 = 50-75%; and 4 = 75 – 100%. The values  
11 obtained for each leaf sheath were added to give an overall score out of 12.  
12 Due to space constraints in the growth cabinet, each repeat of the 2001 trial  
13 contained only single pot entries of each genotype, but was replicated three  
14 times over a four month period. In the 2003 and 2005 trials entries were  
15 replicated twice and four times respectively. All trials included the  
16 susceptible check cultivar ‘Puseas’, and disease severity ratings of the  
17 doubled-haploid lines were converted to a percent (%) ‘Puseas’ scale. Data  
18 from seedling trials were analysed using SPSS version 12.0.1 (SPSS Inc.,  
19 1989-2003).

20

21 **DNA extraction:** DNA was extracted from 3-5 leaves of 14-day-old  
22 seedlings as described by Cakir et al. (2003). Quantification was carried out

1 on Agarose gels, and DNA diluted to a concentration of 10ng/μL prior to  
2 use in PCR.

3

4 **Bulked segregant analysis:** Bulk segregant analysis (BSA; Michelmore  
5 et al. 1991) was initially conducted to identify putative crown rot resistance-  
6 associated markers. Two DNA bulks were constructed, one by combining  
7 equal amounts of DNA from 15 resistant lines and one by combining DNA  
8 from 14 susceptible lines. These decisions were based upon phenotypic  
9 results obtained from the 2001 seedling trial. Bulks were included when  
10 screening for polymorphism between parental lines. Three hundred and  
11 ninety (390) microsatellite (SSR) primer pairs were used to determine  
12 polymorphism between parents and bulks. The SSR sources and sequences,  
13 amplification conditions, and electrophoresis protocols are described in  
14 Collard et al. (2005).

15

16 **Molecular mapping:** Ma (2000) produced a linkage map of the  
17 ‘W21MMT70’ x ‘Mendos’ population consisting of a total of 407 markers  
18 including AFLP (331), RAPD (59), SSR (14), and phenotypic markers (3).  
19 Because chromosomal locations of these markers were largely unknown, a  
20 framework microsatellite map consisting of 128 SSR, one sequence-tagged-  
21 site (STS66-3B), the phenotypic markers of Ma (2000; awns, *GluB3* and  
22 *GluD3*) and a phenotypic marker for *Sr36* (data kindly provided by Dr.

1 Harbans Bariana), was produced using the program Map Manager QTX  
2 (Manly et al. 2001) with a stringency of  $p=0.01$ . The previously screened  
3 AFLP markers were manually added to the framework map and their best  
4 location was determined by using the links report generated by Map  
5 Manager QTX. Due to the much reported problem of reproducibility of  
6 RAPD markers (see for example Jones et al. 1997), these were not included  
7 for mapping. Chi-squared tests for segregation distortion of markers were  
8 carried out, and markers showing distortion were noted but not removed  
9 prior to map construction and QTL detection.

10

11 **QTL detection:** One thousand (1000) permutation tests at 2cM intervals  
12 were carried out to determine **likelihood ratio statistic (LRS; equal to LOD x**  
13 **4.61)** significance thresholds for QTL detection for all trials. Composite  
14 interval mapping (using default parameters) for seedling resistance to crown  
15 rot was carried out using Windows QTL Cartographer version 2.0 (Wang et  
16 al. 2001-2004). MapChart (Voorrips 2002) was used for graphical  
17 presentation of linkage groups and QTLs.

18

## 19 **Results**

### 20 **Phenotypic analysis**

21 Means of parental and doubled-haploid lines, as well as the range of the  
22 population from each seedling trial are given in Table 1. Both the 2001 and



1 2005 trials were not normally distributed (Shapiro-Wilk test  $p < 0.05$ ) and  
2 hence all trials were subject to square root transformation to satisfy  
3 assumptions of normality for ANOVA. Levene's test revealed that the  
4 requirement for homogeneity of variance was met ( $p > 0.05$ ). The Tukey  
5 honestly significant difference procedure showed that both glasshouse trials  
6 were not significantly different from each other, but were significantly  
7 different to the growth cabinet trial. The population mean was higher  
8 (greater susceptibility to crown rot) when the trials were carried out in the  
9 growth cabinet. The distribution of mean severity ratings from each of the  
10 seedling trials of the DH lines and parents are shown in Figures 1a, 1b, and  
11 1c. In each trial, 'W21MMT70' displayed partial resistance to crown rot,  
12 whereas 'Mendos' seedlings were susceptible.

13

#### 14 **Bulked segregant analysis**

15 A total of 390 microsatellite markers were screened across parents and  
16 bulks. Of these, 163 (41.9%) identified polymorphisms between  
17 W21MMT70 and Mendos. The microsatellites were selected for their  
18 genome coverage, with 52 (31.9%) present in the A genome, 64 (39.3%)  
19 present in the B genome, and 47 (28.8%) present in the D genome. Eleven  
20 primer pairs showed banding patterns in the bulks that suggested they might  
21 be associated with resistance. However, marker analysis of the individuals  
22 within the bulks, constructed based on results from the 2001 trial, did not

1 indicate any consistent linkage with crown rot resistance. Consequently a  
2 mapping approach was adopted in an attempt to identify QTLs.

3

#### 4 **Molecular map**

5 One hundred and fifteen (115) of the 163 polymorphic markers were scored  
6 on the DH population, and these were combined with phenotypic data for  
7 *Sr36*, and the SSR and phenotypic markers of Ma (2000) in order to produce  
8 a framework linkage map consisting of 133 markers. Of the 331 AFLP  
9 markers, 159 were manually distributed amongst the framework map. After  
10 addition of the AFLP markers 27 linkage groups were formed, and all were  
11 able to be assigned to chromosomes on the basis of consensus maps (Somers  
12 et al. 2004, Appels 2003). Due to gaps, chromosome 2A was separated into  
13 three linkage groups, while chromosomes 2D, 3B, 5A, and 7A were  
14 separated into two linkage groups. All 21 chromosomes contained markers,  
15 although some (particularly chromosomes 3D, 4D and 6D) were less  
16 densely mapped than others. The total map distance was 2204cM (data not  
17 shown).

18

#### 19 **Segregation distortion**

20 Segregation distortion was observed for 13.5% of the markers. Of these,  
21 64.9% were AFLP markers. Deviation from the expected 1:1 ratio was  
22 shown in 13 of the 27 linkage groups. With exception of some markers on

1 2B and 5D, the distorted loci were not clustered. In the case of chromosome  
2 2B, the distorted loci favoured the 'Mendos' parent, whereas for  
3 chromosome 5D, distorted loci favoured the 'W21MMT70' source.

4

#### 5 **QTLs for seedling resistance to crown rot**

6 Using composite interval mapping, eight QTLs for seedling resistance to  
7 crown rot were found (Table 2). Of these eight QTLs, only three (on  
8 chromosomes 2D, 2B, and 5D) were consistently present in all three  
9 seedling trials. The remaining QTLs were identified in only one  
10 (chromosomes 4A, 5A, and 6A) or two (chromosomes 1A and 3B) of the  
11 three seedling trials. Of the QTLs observed in all three seedling trials, the  
12 5D and 2D QTLs were inherited from the resistant parent 'W21MMT70',  
13 whereas the 2B QTL was inherited from the susceptible parent 'Mendos'.

14 Interval maps and likelihood ratio statistic (LRS) plots for the 2B,  
15 2D, and 5D QTLs are shown in Figure 3. The 2B QTL (Figure 3a) had  
16 maximum LRS values of 26.06 (2001 trial) 18.9 (2003 trial) and 31.6 (2005  
17 trial. This QTL explained between 13.2% and 19.9% of the phenotypic  
18 variance. The 2D QTL was suggestive in both the 2001 and 2003 trials  
19 (LRS of 10.7 and 11.3 respectively), and significant in the 2005 trial (LRS  
20 17.0). This QTL explained 10.2% of the phenotypic variance in 2005. The  
21 5D QTL was highly significant in 2001 (LRS 43.5, explaining 28.1% of the  
22 phenotypic variance), significant in 2003 (LRS 13.6, explaining 13.8% of

1 the phenotypic variance), and only suggestive based upon the 2005 data  
2 (LRS 8.1, explaining 4.8% of the phenotypic variance).

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

## 10 **Discussion**

11 The continuous distribution of disease severity ratings from all three  
12 seedling trials, as well as the presence of transgressive segregants, indicates  
13 that crown rot resistance is a quantitative trait. The population mean of the  
14 growth cabinet trial was higher than each of the glasshouse trials, and this  
15 may indicate that this environment was more conducive to development of  
16 disease symptoms. In addition to the inclusion of the check cultivar  
17 ‘Puseas’ (the cultivar against which % disease severity was expressed) line  
18 2-49 was also included in each of the seedling trials (data not shown). Line  
19 2-49 is recognized as one of the best sources of resistance to crown rot  
20 currently available (Wildermuth et al. 2001). In comparison to line ‘2-49’  
21 (mean disease severity of 42.2% across the 3 trials), ‘W21MMT70’ had a  
22 mean severity rating of 53%.

1           In an attempt to rapidly and efficiently identify genomic regions  
2 associated with seedling resistance to crown rot, BSA was carried out using  
3 microsatellites of known location in the wheat genome. The lines chosen  
4 for BSA were identified from the 2001 growth cabinet trials and prior to  
5 subsequent trials. While this approach identified a number of potential  
6 candidate locations, when the individuals of the bulks were analysed,  
7 linkage to crown rot resistance could not be confirmed. Most successful  
8 reports of the use of BSA to identify molecular markers linked to traits of  
9 interest have involved qualitative traits such as powdery mildew resistance  
10 (Xie et al. 2004), common bunt resistance (He and Hughes 2003), and leaf  
11 rust resistance (Cherukuri et al. 2003). When used in studies on quantitative  
12 traits, the BSA technique appears most useful at identifying loci with large  
13 phenotypic effects (Cook et al. 2004), although in the current study, even  
14 the 5D QTL with the largest effect based on the 2001 phenotypic data (LRS  
15 46.2) was not detected by BSA. This inability to detect QTLs by  
16 conducting BSA based on one years' data possibly reflects the between-year  
17 variability that is evident in the phenotypic data.

18           In the mapping study, a number of markers (13.5%) displayed  
19 segregation distortion. Often in reports on the construction of linkage maps,  
20 distorted loci are removed prior to map construction (e.g. Ubi et al. 2004,  
21 Román et al. 2002). Distorted markers have been reported to cause  
22 inaccuracies in the linkage distances calculated between markers (Cloutier

1 et al. 1997), hence the decision is often made to exclude them. However,  
2 Hacket and Broadfoot (2003) have conducted simulation studies that  
3 suggest distorted segregation ratios have little effect on map construction.  
4 Of particular interest, distortion was observed in the regions of  
5 chromosomes 2B (in favour of the ‘Mendos’ allele) and 5D (in favour of the  
6 ‘W21MMT70’ allele) where QTLs were found. Segregation distortion on  
7 chromosome 2B has been previously reported in a ‘Sunco’ x ‘Tasman’  
8 mapping population (Kammholz et al. 2001). The authors explain the  
9 distortion as being caused by the presence of an alien introgression from  
10 *Triticum timopheevi* that is present in ‘Sunco’. ‘Mendos’ also contains this  
11 introgression, and this may be the cause of the distortion in this population  
12 as well. Faris et al. (1998) reported the occurrence of three regions of  
13 segregation distortion on chromosome 5D in an *Aegilops tauschii* (the D  
14 genome donor in bread wheat) cross, although comparison between these  
15 regions and that of the current study are difficult to make due to a lack of  
16 common markers. Apart from those on 2B and 5D, most of the other  
17 distorted markers were not clustered, and it is acknowledged that markers  
18 displaying mild segregation distortion may simply be the result of less than  
19 optimal numbers of individuals for genotyping.

20         Of the eight QTLs identified, only three (the 2B, 2D, and 5D QTLs)  
21 were consistent across all seedling trials. This finding highlights the  
22 significant environmental variation exhibited when screening for resistance

1 to crown rot, and supports the role of molecular markers as valuable tools to  
2 aid breeding programs in the selection of resistant materials. The other five  
3 QTLs identified were only minor in their effect, and as these QTLs were  
4 only identified in one or two of the three seedling trials, further studies need  
5 to be carried out in order to confirm their putative correlation with crown rot  
6 resistance.

7         Only two other studies have reported the discovery of molecular  
8 markers associated with resistance to crown rot. Wallwork et al. (2004)  
9 have identified a QTL on chromosome 4B that explained up to 48% of the  
10 phenotypic variance from a cross between the moderately resistant cultivar  
11 ‘Kukri’ and the susceptible cultivar ‘Janz’. Collard et al. (2005) reported  
12 two major QTLs derived from ‘2-49’ on chromosomes 1A and 1D as well as  
13 up to four potential minor QTLs, including a 4B locus in the same region as  
14 that identified by Wallwork et al. (2004). The molecular map produced in  
15 the current study was sufficient to enable comparisons with the QTLs  
16 reported in Wallwork et al. (2004) and Collard et al. (2005). Of the loci  
17 identified in this current study, only the 1A QTL appears to coincide with  
18 any previously identified region. A comparison of flanking markers from  
19 Collard et al. (2005) and the current study reveals that this QTL is located in  
20 a similar region of chromosome 1A as that found in line ‘2-49’. As this  
21 marker-trait association is only suggestive in two of the three seedling trials

1 in the 'W21MMT70' x 'Mendos' DH population, further work is required to  
2 confirm its significance.

3 The identification of the QTL on chromosome 2B from the  
4 susceptible parent ('Mendos') is consistent with the observation of  
5 transgressive segregants in the seedling trials. Indeed, other studies on  
6 disease resistance have shown that significant QTLs may be inherited from  
7 susceptible parents (Collard et al. 2005; Steiner et al. 2004). 'Mendos' is an  
8 Australian wheat cultivar that has not been widely grown since 1969, when  
9 *Sr36* and certain other additional genes it possessed for resistance to stem  
10 rust were overcome (Zwer et al. 1992). The QTL for crown rot resistance  
11 on chromosome 2B is in close proximity to *Sr36*, which indicates that this  
12 QTL is located on the introgression from *Triticum timopheevi* – an  
13 introgression that contains a number of important disease resistance genes  
14 (Brown-Guedira et al. 2003, Bariana et al. 2001, Tao et al. 2000). Strong  
15 evidence has been provided for the clustering of disease resistance genes  
16 within chromosomes (Dilbirligi et al. 2004), and the close association of the  
17 2B crown rot QTL with *Sr36* may indicate that this QTL is positioned  
18 within such a cluster. As is the case for black-point resistance in wheat  
19 (Lehmensiek et al., 2004), screening for *Sr36* resistance may also be a  
20 promising strategy for identifying a useful level of crown rot resistance in  
21 pedigrees that utilize the *T. timopheevi* introgression.



1           This is the second mapping study of molecular markers linked to  
2 partial crown rot resistance in seedlings. Three consistent QTLs were  
3 identified on chromosomes 2B, 2D, and 5D in each of the three seedling  
4 trials conducted. These QTLs differ from the major QTLs previously  
5 described by Collard et al. (2005), and thus represent potential for  
6 pyramiding QTLs for the improvement of wheat affected by this  
7 economically important disease. We are currently screening individuals  
8 from a '2-49' x 'W21MMT70' DH population to test the hypothesis that  
9 these two resistance sources can be combined to produce higher levels of  
10 resistance to crown rot.

11

## 12 **Acknowledgements**

13 This research was funded by the Grains Research and Development  
14 Corporation as part of the Australian Winter Cereals Molecular Marker  
15 Program. The technical assistance of Sally Coverdale, Maria Harris, Boyd  
16 McNamara, and Tina Walters is greatly appreciated. The authors thank Dr.  
17 Anke Lehmensiek for helpful discussions on mapping.

18

19

20

## References

- Appels R., 2003: A consensus molecular genetic map for wheat – a cooperative international effort. In: N.E. Prognia (ed.), Proceedings of the 10<sup>th</sup> International Wheat Genetics Symposium. Paestum, Italy. 211-214.
- Backhouse D., A.A. Abubakar, L.W. Burgess, J.I. Dennis, G.J. Hollaway, G.B. Wildermuth, and F.J. Henry FJ, 2004: Survey of *Fusarium* species associated with crown rot of wheat and barley in eastern Australia. *Australasian Plant Pathology* 33, 255-261.
- Balmas V., 1994: Root rot of wheat in Italy caused by *Fusarium graminearum* Group 1. *Plant Disease* 78, 317.
- Bariana H., M.J. Hayden, N.U. Ahmed, J.A. Bell, P.J. Sharp, and R.A. McIntosh, 2001: Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. *Australian Journal of Agricultural Research* 52, 1247-1255.
- Brennan J.P., and G.M. Murray, 1998: Economic importance of wheat diseases in Australia. NSW Agriculture, Wagga Wagga.
- Brown-Guedira G.L., S. Singh, and A.K. Fritz 2003: Performance and mapping of leaf rust resistance transferred to wheat from *Triticum timopheevii* subsp. *armeniicum*. *Phytopathology* 93, 784-789.
- Cakir M., D. Poulsen, N.W. Galwey, G.A. Ablett, K.J. Chalmers, G.J. Platz, R.F. Park, R.C.M. Lance, J.F. Panozzo, B.J. Read, D.B. Moody, A.R. Barr, P. Johnston, C.D. Li, W.J.R. Boyd, C.R. Grime, R. Appels, M.G.K. Jones, and P. Langridge, 2003: Mapping and QTL analysis of the barley population Tallon x Kaputar. *Australian Journal of Agricultural Research* 54, 1155-1162.
- Cherukuri D.P., S.K. Gupta, A. Charpe, S. Koul, K.V. Prabhu, R.B. Singh, Q.M.R. Haq, and S.V.S. Chauhan, 2003: Identification of a molecular marker linked to an *Agropyron elongatum*-derived gene *Lr19* for leaf rust resistance in wheat. *Plant Breeding* 122: 204-208.
- Cloutier S., M. Cappadocia, and B.S. Landry, 1997: Analysis of RFLP mapping inaccuracy in *Brassica napus* L. *Theoretical and Applied Genetics* 95, 83-91.
- Collard, B.C.Y., R.A. Grams, W.D. Bovill, C.D. Percy, R. Jolley, A. Lehmensiek, G. Wildermuth, and M.W. Sutherland, 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, 532-537.

Cook, J.P., D.M. Wichman, J.M. Martin, P.L. Bruckner, and L.E. Talbert, 2004: Identification of microsatellite markers associated with a stem solidness loci in wheat. *Crop Science* 44, 1397-1402.

Dilbirligi, M., M. Erayman, D. Sandhu, D. Sidhu, and K.S. Gill, 2004: Identification of wheat chromosomal regions containing expressed resistance genes. *Genetics* 166, 461-481.

Faris, J.D., B. Laddomada, and B.S. Gill BS, 1998: Molecular mapping of segregation distortion loci in *Aegilops tauschii*. *Genetics* 149, 319-327.

Hackett, C.A., and L.B. Broadfoot, 2003: Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. *Heredity* 90, 33-38.

He, C., and G.R. Hughes, 2003: Development of RAPD markers associated with common bunt resistance to race *TI* (*Tilletia tritici*) in spelt wheat. *Plant Breeding* 122, 375-377.

Jones, C.J., K.J. Edwards, S. Castaglione, M.O. Winfield, F. Sala, C. van deWiel, G. Bredemeijer, B. Vosman, M. Matthes, A. Daly, R. Brettschneider, P. Bettini, M. Buiatti, E. Maestri, A. Malcevski, N. Marmioli, R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vazquez, and A. Karp, 1997: Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding* 3, 381-390.

Kammholz, S.J., R.A. Grams, P.M. Banks, and M.W. Sutherland, 1998: Segregation of glutenins in wheat x maize-derived doubled haploid wheat populations. *Australian Journal of Agricultural Research* 49, 1253-1259.

Kammholz, S.J., A.W. Campbell, M.W. Sutherland, G.J. Hollamby, P.J. Martin, R.F. Eastwood, I. Barclay, R.E. Wilson, P.S. Brennan, and J.A. Sheppard, 2001: Establishment and characterization of wheat genetic mapping populations. *Australian Journal of Agricultural Research* 52, 1079-1088.

Klein, T.A., L.W. Burgess, and F.W. Ellison, 1991: The incidence and spatial patterns of wheat plants infected by *Fusarium graminearum* Group 1

and the effect of crown rot on yield. *Australian Journal of Agricultural Research* 42, 399-407.

Lehmensiek, A., A.W. Campbell, P.M. Williamson, M. Michalowicz, M.W. Sutherland, and G.E. Daggard, 2004: QTLs for black-point resistance in wheat and the identification of potential markers for use in breeding programmes. *Plant Breeding* 123, 410-416.

Ma, W., 2000: Molecular mapping of wheat quality attributes in a wheat doubled haploid population W21MMT70 x Mendos. PhD Dissertation, University of Southern Queensland, Toowoomba.

Manly, K.F., R.H. Cudmore Jr., and J.M. Meer, 2001: Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome* 12, 930-932.

Marasas, W.F.O., W.G.J. Voigt, S.C. Lamprecht, and P.S. Van Wyk, 1988: Crown rot and head blight of wheat caused by *Fusarium-graminerum* Groups 1 and 2 in the Southern Cape province South Africa. *Phytophylactica* 20, 385-390.

Michelmore, R.W., I. Paran, and P.V. Kesseli, 1991: Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific regions using segregating populations. *Proceedings of the National Academy of Sciences of the United States of America* 88, 9828-9832.

Paulitz, T.C., R.W. Smiley, and R.J. Cook, 2002: Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, U.S.A. *Canadian Journal of Plant Pathology* 24, 416-428.

Román, B., A.M. Torres, D. Rubiales, J.I. Cubero, and Z. Satovic, 2002: Mapping of quantitative trait loci controlling broomrape (*Orobanche crenata* Forsk.) resistance in faba bean (*Vicia faba* L.). *Genome* 45, 1057-1063.

Steiner, B., M. Lemmens, M. Griesser, U. Scholz, J. Schondelmaier, and H. Buerstmayr, 2004: Molecular mapping of resistance to *Fusarium* head blight in the spring wheat cultivar Frontana. *Theoretical and Applied Genetics* 109, 215-224.

- Smiley, R.W., J.A. Gourlie, S.A. Easley, L.-M. Patterson, and R.G. Whittaker, 2005: Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. *Plant Disease* 85, 595-604.
- Sommers, D.J., P. Isaac, and K. Edwards, 2004: A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 109, 1105-1114.
- Tao, W., D. Liu, J. Liu, Y. Feng, and P. Chen, 2000: Genetic mapping of the powdery mildew resistance gene Pm6 in wheat by RFLP analysis. *Theoretical and Applied Genetics* 100, 564-568.
- Ubi, B.E., M. Fujimori, Y. Mano, and T. Komatsu, 2004: A genetic linkage map of rhodesgrass based on an F<sub>1</sub> pseudo-testcross population. *Plant Breeding* 123, 247-253.
- Voorrips, R.E., 2002: MapChart: Software for the graphical presentation of linkage maps and QTLs. *Heredity* 93, 77-78.
- Wallwork, H., M. Butt, J.P.E. Cheong, and K.J. Williams, 2004: Resistance to crown rot in wheat identified through an improved method for screening adult plants. *Australasian Plant Pathology* 33, 1-7.
- Wang, S., C.J. Basten, and Z.-B. Zeng, 2001-2004: Windows QTL Cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh, NC.
- Wildermuth, G.B., and R.B. McNamara, 1994: Testing wheat seedlings for resistance to crown rot caused by *Fusarium graminearum* Group 1. *Plant Disease* 78, 949-953.
- Wildermuth, G.B., G.A. Thomas, B.J. Radford, R.B. McNamara and A. Kelly, 1997: Crown rot and common root rot in wheat grown under different tillage and stubble treatments in southern Australia. *Soil and Tillage Research*, 44, 211-224.
- Wildermuth, G.B., R.B. McNamara, and J.S. Quick, 2001: Crown depth and susceptibility to crown rot in wheat. *Euphytica* 122, 397-405.
- Wildermuth, G.B., and J.N. Morgan, 2004: Genotypic differences in partial resistance to crown rot caused by *Fusarium pseudograminearum* in relation to an osmoregulation gene in wheat. *Australasian Plant Pathology* 33, 121-123.

Xie, C., Q. Sun, Z. Ni, T. Yang, E. Nevo, and T. Fahima, 2004:  
Identification of resistance gene analog markers closely linked to wheat  
powdery mildew resistance gene *Pm31*. *Plant Breeding* 124, 198-200.

Zwer, P.K., R.F. Park, and R.A. McIntosh, 1992: Wheat Stem Rust in  
Australia – 1969 – 1985. *Australian Journal of Agricultural Research* 43,  
399-431.

Table 1. Means and ranges for crown rot disease severity (expressed as the square-root percentage of the susceptible check cultivar Puseas) for parental lines and doubled haploids. Population means sharing the same letter are not significantly different (Tukey HSD,  $p > 0.05$ ).

Table 2. QTLs for crown rot resistance detected using composite interval mapping in three seedling trials. Chromosome location, likelihood ratio statistic (LRS) value, percent phenotypic variance explained (VE), significance level (SL), and the parent contributing the resistance allele (Source) are shown.

Figure 1. Histograms of crown rot severity ratings of the ‘W21MMT70’ x ‘Mendos’ wheat population from the 2001 (a) 2003 (b) and 2005 (c) seedling trials. Disease severity is expressed as the square-root percentage of the susceptible check cultivar ‘Puseas’. Parental means are indicated by filled (‘W21MMT70’) and unfilled (‘Mendos’) arrows.

Figure 2. 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,  $p < 0.05$ ).

Figure 3. Interval maps (cM) of the 2B (a), 2D (b), and 5D (c) QTL found in all three seedling trials. Chromosome regions exceeding the suggestive threshold are indicated by filled vertical bars. AFLP markers are not included for the purpose of clarity.



**TABLE 1.**

Table 1. Year	W21MMT70	Mendos	Population Mean	Population Range
2001	7.56	10.22	8.56 <sup>a</sup>	5.24-11.48
2003	6.62	9.40	7.11 <sup>b</sup>	3.99-11.10
2005	7.62	9.65	7.42 <sup>b</sup>	3.82-11.38

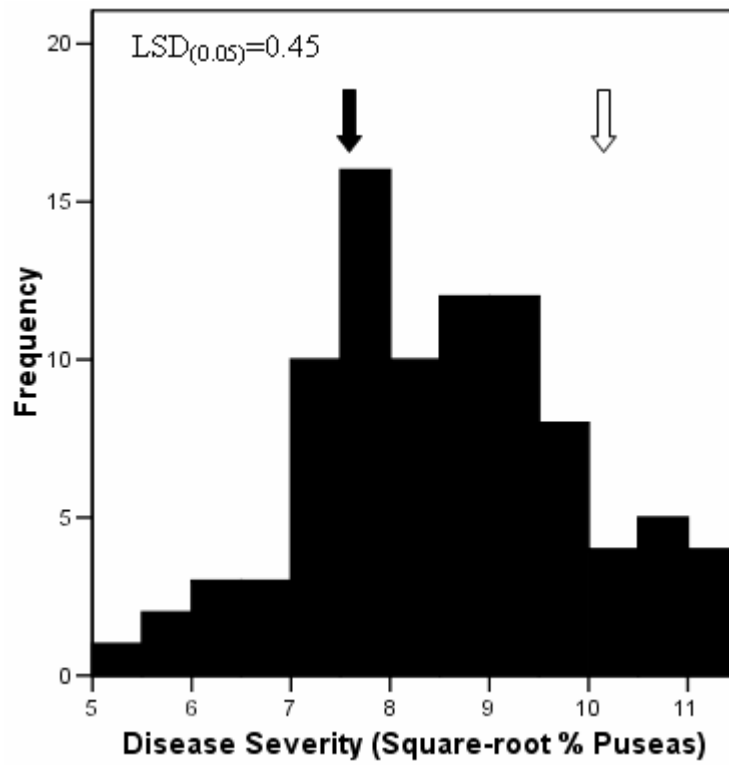
**TABLE 2.**

Chromo- some	2001			2003			2005			Source
	LRS	VE	SL <sup>1</sup>	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’

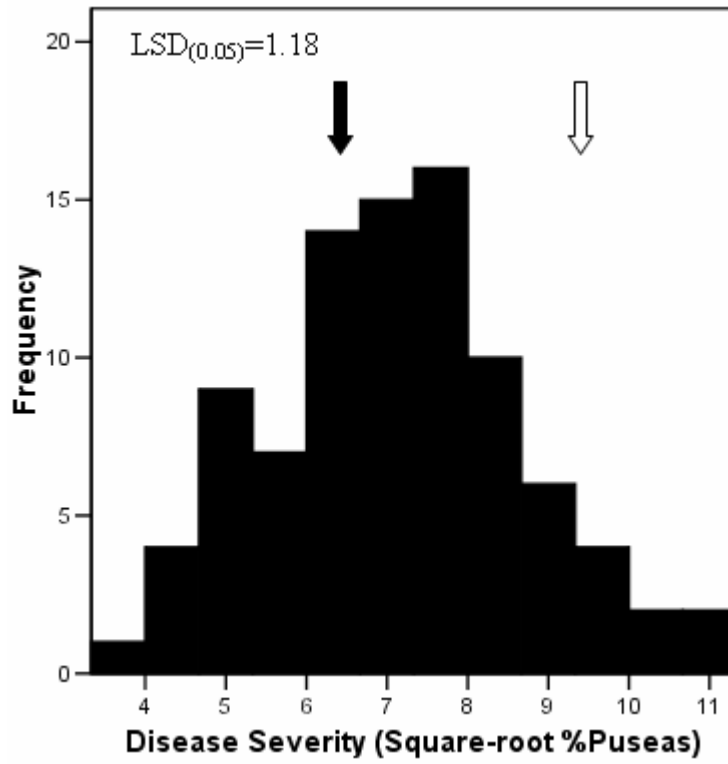
<sup>1</sup> Significance level determined by 1000 permutation tests for each seedling trial. NS – not significant; Sg – suggestive (LRS greater than 7.8, 7.9, and 7.8 for the 2001, 2003, and 2005 trials respectively; S – significant (LRS greater than 14.5, 13.5, and 18.2 for the 2001, 2003, and 2005 trials respectively; HS – highly significant (LRS greater than 20.6, 18.2, and 22.3 for the 2001, 2003, and 2005 trials respectively).

**FIGURE 1.**

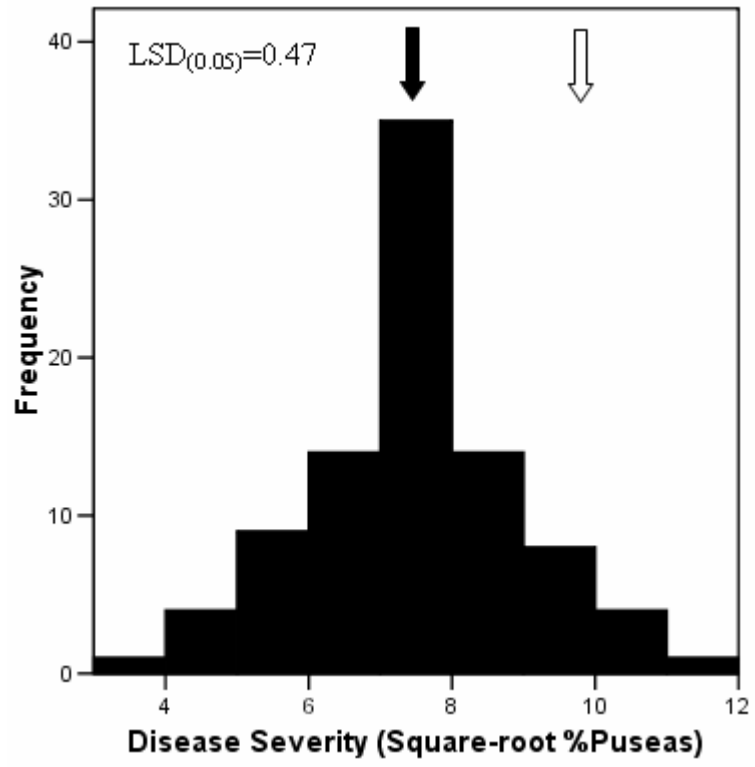
**a) 2001 Seedling Trial**



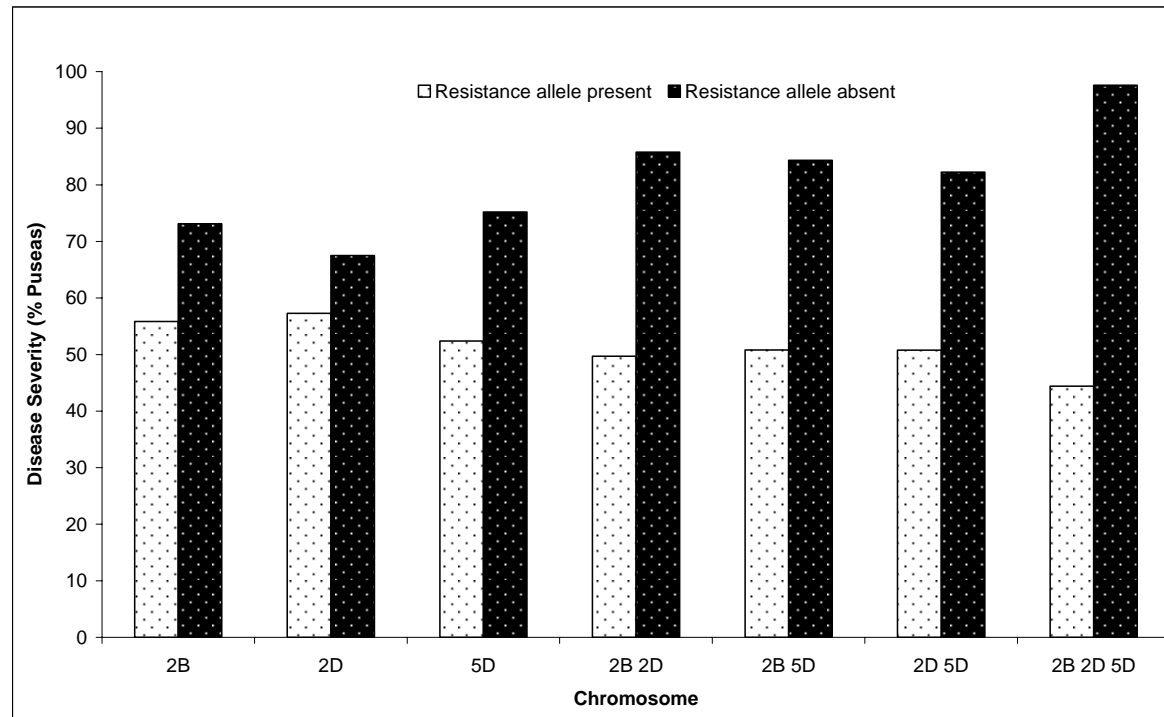
**b) 2003 Seedling Trial**



**c) 2005 Seedling Trial**

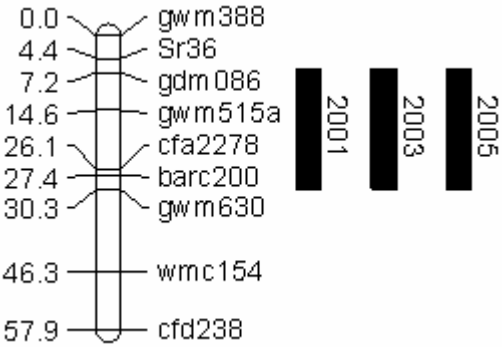


**FIGURE 2.**

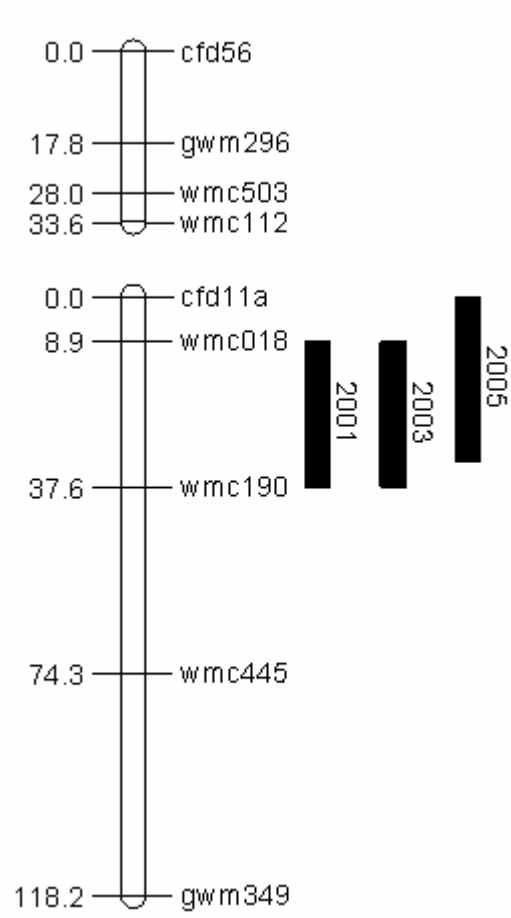


**FIGURE 3.**

**a) Chromosome 2B**



**b) Chromosome 2D**





**c) Chromosome 5D**

