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ORIGINAL PAPER

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Validation of quantitative trait loci for Ascochyta blight resistance in pea (*Pisum sativum* L.), using populations from two crosses

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Abstract Resistance to Ascochyta blight of pea was genetically characterized by mapping quantitative trait loci (QTLs) using two crosses, 3147-A26 (A26, partially resistant) × cultivar Rovar (susceptible) and 3148-A88 (A88, partially resistant) × Rovar, with the aim of developing an increased understanding of the genetics of resistance and of identifying linked molecular markers that may be used to develop resistant germplasm. Molecular linkage maps for both crosses were aligned so that the results of QTL mapping could be compared. Ascochyta blight disease severity in response to natural epidemics was measured in field trials conducted in Western Australia and New Zealand. Eleven putative QTLs for Ascochyta blight resistance were identified from the A26 × Rovar population and 14 putative QTLs from the A88 × Rovar population. Six QTLs were associated with the same genomic regions in both populations. These QTLs reside on linkage groups II, III, IV, V, and VII (two QTLs). The severity of Ascochyta blight disease symptoms on pea increases during field epidemics as plants mature; therefore, QTLs for plant reproductive maturity were mapped. Six QTLs were detected for plant maturity in the A26 × Rovar population, while five plant maturity QTLs were mapped in the A88 × Rovar population. QTLs for plant maturity coincide with Ascochyta blight resistance QTLs in four genomic regions, on linkage groups II (two regions), III, and V. The plant maturity and Ascochyta blight resistance QTLs on III were linked in repulsion phase. Therefore, the coincidence of these QTLs may be explained by linkage of distinct loci for the two traits. The QTLs on linkage groups II and V were linked in coupling phase; therefore, linked QTLs for resistance and maturity may be present in these regions, or the Ascochyta blight resistance QTLs detected in these regions are the result of pleiotropic effects of plant-maturity genetic loci.

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Introduction

Ascochyta blight is a major disease of pea (*Pisum sativum* L.) crops around the world that can result in serious declines in yield and quality (Beasse et al. 1999). The disease is caused by a complex that may consist of three fungal pathogens: *Mycosphaerella pinodes* (Berk. and Bloxham) Verstergren, *Phoma medicaginis* Malbr. & Roum var. *pinodella* (L. K. Jones) Boerema, and *Ascochyta pisi* Lib. The present paper concerns disease epidemics in environments where *M. pinodes* and *P. medicaginis* var. *pinodella* have been detected (Timmerman-Vaughan et al. 2002). The symptoms of infection by *M. pinodes* and *P. medicaginis* var. *pinodella* include foot rot and necrotic spots on leaves, stems, and pods (Bretag and Ramsey 2001). Pod disease can lead to infection of the seed, which produces seed staining, reducing seed quality, and subsequent economic value, and most importantly results in seed transmission of the disease.

Development of pea cultivars resistant to *M. pinodes* and *P. medicaginis* var. *pinodella* would assist in controlling this disease and minimizing its damage. Consequently, a number of researchers have examined

pea germplasm for Ascochyta blight resistance (Clulow et al. 1991; Onfroy et al. 1999; Wroth 1998, 1999; Xue et al. 1998). In all cases to date, the germplasm characterized provided partial resistance to *M. pinodes* and *P. medicaginis* var. *pinodella*. Resistance to *Ascochyta pisi* is also present in pea germplasm, and quantitative trait loci (QTLs) for resistance to Race C have been mapped (Dirlewanger et al. 1994).

QTLs for Ascochyta blight resistance were identified previously using an F_2-derived population of a cross between the partially resistant breeding line A88 and the susceptible cultivar Rovar (Timmerman-Vaughan et al. 2002). Thirteen putative QTLs were detected, each explaining only a small fraction of the phenotypic variation. In the field, Ascochyta blight epidemics become more severe as the pea crop matures (Kraft et al. 1998). This may be explained if genes for resistance and late maturity are linked, or if increased susceptibility to Ascochyta blight is physiologically associated with maturation of the plant. Using the A88 × Rovar population, one QTL for plant maturity was identified in association with a genomic region that also contained an Ascochyta blight resistance QTL.

For QTL mapping to be used as the basis of marker-assisted selection (MAS) in plant breeding, the estimates of the minimum number of QTLs, their effects, and association with linked markers must be as accurate as possible. However, simulation and validation studies have shown that QTL mapping experiments where a relatively large number of QTLs have been detected can overestimate the magnitude of individual QTL effects as well as the phenotypic variation (R^2) explained by individual QTLs (Beavis 1994; Melchinger et al. 1998; Utz and Melchinger 1994). Optimal strategies for MAS also require information on the genetic distance between the marker and trait loci. Usually, however, there is considerable uncertainty about the genetic distances between QTLs and associated molecular marker loci because the confidence intervals around QTL peak locations are large for typical QTL experiments that involve populations of 100–200 progeny lines (van Ooijen 1992; Visscher et al. 1996). Accurate estimates of QTL peak locations and effects are also necessary to identify the genes underlying the QTLs, using either candidate gene or positional cloning strategies.

In this paper, QTLs detected for resistance to Ascochyta blight and plant reproductive maturity, using two mapping populations using different resistant parental lines, are compared based on phenotypic assessments made in field trials in New Zealand and Western Australia. This comparison enables previous results obtained by mapping QTLs in one population in field trials in Western Australia

Table 1 Primer sequences and PCR conditions for sequence-tagged site (STS) assays

STS	Primer sequences	Primers (nM)	$MgCl_2$ (mM)	Amplification conditions
sN13-927	5′ AGCGTCACTCATGTTTG 3′	500	1.5	1 cycle of (95°C 5 min), 40 cycles of (95°C 1 min, 62°C 1 min, 72°C 1 min), 1 cycle of (72°C 8 min)
	5′ AGCGTCACTCCATGCAT 3′			
sT11-800	5′ CCGCGATAGAGCCAGAAAAGCTAC 3′	200	2.5	40 cycles of (95°C 45 s, 60°C 1 min, 72°C 45 s), 1 cycle of (72°C 8 min)
	5′ GCTTTTTATCCCTAGTCCACCATCTTAAG 3′			
sM2P5-234	5′ CCTTGCGAAACATTACTACGG 3′	200	1.5	40 cycles of (94°C 1 min, 60°C 45 s, 72°C 1 min), 1 cycle of (72°C 8 min)
	5′ GGAGAAGGTGGAGGAAAGAC 3′			
sV06-895	5′ ACGCCCAGGTTATCAAT 3′	500	1.5	1 cycle of (95°C 5 min), 40 cycles of (95°C 1 min, 62°C 1 min, 72°C 1 min), 1 cycle of (72°C 8 min)
	5′ ACGCCCAGGTACTGCA 3′			
sD13-487	5′ GGGTGACGACATAAACAACATAATC 3′	100	1.5	40 cycles of (94°C 1 min, 62°C 1 min, 72°C 1 min), 1 cycle of (72°C 8 min)
	5′ AAAGTAGGAAACATAGGACCGTCA 3′			
sB17-509	5′ AGGAATAATGGCGTGTGGATCACT 3′	100	1.5	40 cycles of (95°C 1 min, 60°C 1 min, 72°C 1 min), 1 cycle of (72°C 8 min)
	5′ GAACGAGGTTGTTGGTAACCGAAG 3′			
sO12-581	5′ AGTGCTGTGGAAGTATGAGCATT 3′	100	1.5	40 cycles of (95°C 1 min, 60°C 1 min, 72°C 1 min), 1 cycle of (72°C 8 min)
	5′ AGTGCTGTGTGTTTAGTGTTAGGG 3′			
sM2P2-304	5′ TATGCATATTTAGGTGGATGGAGA 3′	100	2.0	40 cycles of (95°C 1 min, 60°C 30 s, 72°C 30 s), 1 cycle of (72°C 8 min)
	5′ GATTTATTCTTTCCGATTGTTTGG 3′			

(Timmerman-Vaughan et al. 2002) to be substantiated and independent estimates of QTL effects and peak locations to be obtained. In addition, by carrying out phenotypic assessment in a different environment (New Zealand) and by using the A26 × Rovar population, new genomic regions associated with QTLs determining Ascochyta blight resistance and plant reproductive maturity are identified.

Materials and methods

Population development

A population of 148 F_2 lines was produced by pollinating flowers of a single plant of the partially Ascochyta blight resistant blue pea breeding line 3146-A26 (A26, Crop and Food Research, Lincoln, New Zealand) with pollen from Rovar (Cebeco, Lelystadt, The Netherlands), an Ascochyta blight susceptible blue pea cultivar. Development of the 3148-A88 (A88) × Rovar population was described previously (Timmerman-Vaughan et al. 2002). F_3 seed representing F_2 families of both populations was produced by growing individual F_2 plants in the field at Lincoln, New Zealand. To produce F_4 seed representing F_2 families, at least 5 F_3 plants were grown either in the greenhouse or the field, and the resulting seed was bulked.

DNA marker methods

DNA extraction, random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP) methods were described previously (Timmerman-Vaughan et al. 2002). AFLPs are labeled according to the primers used for amplification and the size of the resulting PCR product. For example, M3P8-120 was produced using primers MseP3 and PstP8 and was estimated to be 120 bp by comparison with the 30–330-bp AFLP DNA Ladder (Invitrogen). RFLP probe c206 was obtained from Noel Ellis (John Innes Institute, Norwich, UK). Pea R-gene analogue (RGA) probes were described by Timmerman-Vaughan et al. (2000), and the other RFLP probes were described by Gilpin et al. (1997). Primer sequences and assay conditions for markers sP2P5 and sY16-1121 were described by Timmerman-Vaughan et al. (2002).

Seven new polymorphic sequence-tagged site (STS) loci were developed as described by Frew et al. (2002). PCR reactions were conducted in 25-µl reactions as described by Gilpin et al. (1997), using the primers and conditions detailed in Table 1. The sN13-927 and sV06-895 assays included a hot start, using Fast Start Polymerase (Roche). The sequence polymorphisms detected by the sB17-509, sO12-581, and sM2P2-304 STS assays were analyzed by SSCP gel electrophoresis (McCallum et al. 2001) in gels composed of 0.5× Serdogel (SERVAS, Heidelberg, Germany) and stained with SYBR Green II (Roche). DNA fragments for the remaining STS assays were analyzed by electrophoresis in 1% agarose, 1% Agarose-1000 (Invitrogen) gels, and stained with ethidium bromide.

Field trials

Field trials to evaluate the A26 × Rovar and A88 × Rovar progeny lines were conducted on an irrigated site at Medina, Western Australia, in 1997 and 1998, and on an unirrigated site near Winton, Southland, New Zealand, during the 1997–1998 and 1998–1999 southern summers. These environments have been designated as WA97, WA98, NZ98, and NZ99, respectively. In every trial, one plot of each progeny line was planted because of a limit on seed and field space. A number of replicate check line plots (A26 and/or A88, Rovar and Dundale) were distributed throughout the trials to allow assessment and adjustment of field variation. Dundale is a dun pea cultivar that is susceptible to Ascochyta blight. The check plots were distributed based on a modified Latin-square design to give roughly even placement of the lines across the rows and columns of each trial.

The planting and management of the WA97 and WA98 trials and the layout for the A88 × Rovar progeny were described previously (Timmerman-Vaughan et al. 2002). The WA97 trial also contained 225 A26 × Rovar progeny plots, in a 15- by 20-plot layout that was planted alongside the A88 × Rovar progeny plots. There were 25 plots of each check line (A26, Rovar, and Dundale). The 225 progeny plots in the trial included the 148 lines used for linkage and QTL mapping described in this paper, and an additional 77 progeny lines were produced by crossing a different A26 plant (plant no. 6) with Rovar. The WA98 trial also contained 197 A26 × Rovar progeny lines in a 15- by 18-plot layout. There were 26, 23, and 24 check plots, containing A26, Rovar, and Dundale, respectively. The 197 progeny plots included 131 lines from the cross being used for linkage and QTL mapping, and an additional 66 lines from the A26 plant no. 6 × Rovar cross.

The NZ98 trial was sown on 27 Sept 1997. The trial contained 153 plots from the A88 × Rovar population in a 15- by 14-plot layout. This was adjacent to 111 plots from the A26 × Rovar mapping population and 52 plots from the A26 plant no. 6 × Rovar population not being used for mapping, intermingled in a 15- by 14-plot layout. In the entire trial, there were 14 check plots each of A88 and A26, and 28 check plots each of Rovar and Dundale, 11 filler plots containing the cultivar Primo (Cebeco), and nine containing OSU442-15 (Baggett and Hampton 1977). The NZ99 trial was sown on 8 October 1998 in a 15- by 29-plot layout. The trial contained 150 plots of A88 × Rovar progeny, 138 plots of the A26 × Rovar mapping population, and 70 plots of the A26 plant no. 6 × Rovar population. Rovar, A88, and A26 were included as check plots with 39, 16, and 24 plots, respectively. Both trials were sown as described for the Medina, Western Australia, trials (Timmerman-Vaughan et al. 2002). The

Table 2 Changes in nomenclature used for amplified fragment length polymorphisms on the A88 × Rovar linkage map in Timmerman-Vaughan et al. (2002) and this report (Fig. 1)

Linkage group	Previous locus name	Locus name this paper
I	AFP3_P8f	M3P8-120
I	AFP3_P8i	M5P8-186
I	AFP5_P4i	M5P4-121
II	sP2P5	sM2P5
II	AFP5_P4a	M5P4-335
III	AFP2_P2j	M2P2-129
III	AFP5_P8a	M5P8-335
III	AFP5_P8f	M5P8-232
III	AFP5_P8j	M5P8-154
V	AFP2_P2b	M2P2-338
V	AFP2_P2k	M2P2-125
V	AFP3_P2bc	coM3P2-478/458
V	AFP5_P4g	M5P4-156
V	AFP2_P5d	M2P5-234
V	P3P8bM09	coM09
V	AFP2_P2e	M2P2-205
V	sAFP2P2c	sM2P2-304
V	AFP2_P2d	M2P2-236
V	AFP3_P2e	M3P2-304
VI	AFP3_P8e	M3P8-139
VI	AFP5_P4b	M5P4-304
VI	AFP2_P5b	M2P5-320
VII	AFP2_P2hl	coM2P2-193/115
VII	AFP5_P8l	M5P8-110

standard agronomic practices used included herbicide applications to control weeds.

The WA97 and WA98 field trials were evaluated for Ascochyta blight and plant developmental stage using the ordinal scales described previously (Timmerman-Vaughan et al. 2002). Briefly, Ascochyta blight was scored separately on stems (0 = no lesions, 1 = flecks, 3 = a few large lesions, 5 = many large lesions, 7 = stem girdled with lesions, and 9 = plant dead), leaves (1 = no lesions, 3 = lesions up one quarter of the plant height with only a trace of disease apparent, 5 = lesions up one half of the plant height with several diseased areas, 7 = lesions up three quarters of the plant height with several diseased areas, and 9 = lesions to the top of the plant with severe disease apparent) and pods (0 = no lesions, 2 = a few pinpoint lesions, 4 = many pinpoint lesions, 6 = many pinpoint lesions and a few coalesced and sunken lesions visible, 8 = large coalesced and sunken lesions present, and 10 = pods nearly completely blackened). Plant developmental stage was scored using a scale based on the reproductive stages described by Knott (1987) e.g., 2 = visible buds, 3 = first open flower, 4 = pod set, 5 = flat pod, 6 = pod swell, 7 = pod fill, and 8 = green wrinkled pod. Developmental stages were scored on the lowest pods apparent on the main stems. Intermediate scores were used. The NZ98 and NZ99 trials were evaluated on 2–3 February 1998 and 10–12 February 1999.

Spatial analysis of field trends

Most commonly, field trials to assess progeny trait values for detection of QTLs involve replication of progeny lines. An alternative approach for increasing the precision of trait measurement is to use experimental designs containing single plots of progeny families in combination with highly replicated check plots and analysis methods that characterize local trends as well as broad effects (e.g., row and column effects and gradient variations) and then to develop models that can be used to adjust trait values (Cullis et al. 1989; Kempton and Gleeson 1997; Moreau et al. 1999). Field trials were designed to permit analysis of spatial trends in trait scores using a residual maximum likelihoods (REML) analysis (Patterson and Thompson 1971). Within each trial, each progeny family was represented in a single plot. All trials included highly replicated check plots containing resistant and susceptible lines to assist with assessing spatial variation. The use of these design strategies and REML analysis to map Ascochyta blight resistance QTLs is discussed more fully in Timmerman-Vaughan et al. (2002).

The analysis followed that described previously (Timmerman-Vaughan et al. 2002). Data were examined separately for the four trials and for each trait. Initially, field trends were examined graphically. The raw scores and the residuals from a null analysis were plotted in a two-dimensional array, using colors to represent the value for each plot, and the average score across the rows (or columns) was plotted against row (or column) number. REML methods (Genstat Committee 1997, 2002) were then used to model the trends using the general approach described by Gilmour et al. (1997). The first model fitted, the null model, made no adjustment for trends, with other models allowing for general patterns across the rows or the columns, auto-correlation between adjacent plots in either direction, or for smooth trends, modeled with smoothing splines (Verbyla et al. 1999). Mean scores for each line were then predicted from the final model chosen, and these adjusted scores were used in further analyses.

Linkage and QTL mapping

A molecular linkage map of the A26 × Rovar cross was constructed using MAPMAKER/EXP, version 3.0 (Lincoln et al. 1992), as described previously (Gilpin et al. 1997; Timmerman-Vaughan et al. 2002). The threshold for assigning markers to linkage groups was LOD = 5.0. Final marker orders were tested using the *ripple* command (LOD = 2.0). A number of codominant loci were generated by joining haplotypes for linked dominantly inherited markers that were in opposite phases and showed no crossovers between the A26/A26 and Rovar/Rovar genotypes. The resulting codominant loci (and their contributing dominantly inherited markers) were coS13-770 (S13-700 and S13-430), coP10-700 (P10-711 and P10-538), coE12-1K (E12-1150 and E12-1000), coM2P5-176/122 (M2P5-176 and M2P5-122), coO09-800 (O09-

820 and O09-770), coS13-840 (S13-840 and S13-830), and coM09 (M09-2400 and M09-830). These loci mapped to the same map intervals as the respective dominant markers (which may or may not be allelic) and increased the support for the map orders obtained.

QTLs were mapped by composite interval mapping (CIM), using QTL Cartographer, version 2.0 software (Wang et al. 2002). Forward-backward regression was used to select up to five cofactors (P=0.1) to control the genetic background for CIM conducted using model 6, with the window size set at 20 cM. Chromosome-wise significance thresholds (α=0.05) for QTL detection were determined by conducting permutation tests (Churchill and Doerge 1994) on individual linkage groups with QTL Cartographer and 1,000 shuffles of the trait data. QTL peaks have been reported that have significant likelihood ratio test statistics under H_3/H_0 and H_3/H_1. These three hypotheses are H_0: a=0, d=0; H_1: $a\neq0$, d=0; and H_3: $a\neq0$, $d\neq0$ (a, additive effect; d, dominance effect). QTL peaks that occurred mid-interval and were not supported by significant associations with flanking markers were not reported.

Results and discussion

Linkage maps for the A26 × Rovar and A88 × Rovar populations

The linkage map of the A26 × Rovar population of 148 F_2 progeny was constructed using 99 loci and covers about 930 cM of the pea genome on 13 linkage groups (Fig. 1). The marker loci used for map construction include RAPDs (44), RFLPs (29), AFLPs (17), and STSs (9). There are 36 codominant loci. The average distance between markers is 10.8 cM. The markers used for map construction were chosen from 159 segregating markers to maximize the number of codominant loci, minimize missing data, and permit alignment with the A88 × Rovar map (Timmerman-Vaughan et al. 2002). Eleven of the A26 × Rovar linkage groups were aligned with the pea genome consensus map (Weeden et al. 1998) by mapping anchor loci (Fig. 1). Linkage group V maps were identified by mapping sY16_1121, M2P5-234 and M2P2-304 between *gp* and *Pgdc* (data not presented) in the JI1794 × Slow reference population (Weeden et al. 1993). In addition, the small linkage groups containing P346 were assigned to IV by mapping RGA2.75 in the JI1794 × Slow population (Timmerman-Vaughan et al. 2000).

Alignment of the A26 × Rovar and A88 × Rovar maps is also shown in Fig. 1. There are 49 loci in common between these two maps. To permit alignment of these two maps, additional markers have been placed on the A88 × Rovar map that were not mapped previously (Timmerman-Vaughan et al. 2002). The nomenclature used for AFLPs has been changed since publication of the A88 × Rovar map to include estimates of the amplified fragment lengths (Table 2), which should facilitate the comparison of maps obtained from different pedigrees. The maps are colinear except for the inversion of the Q407–Adh1 order on linkage group I and a segment of linkage group V where the group of markers between V06-895 and M2P5-234 is inverted with respect to M2P2-236.

Fig. 1 Linkage and quantitative trait locus (QTL) maps of the A26 × Rovar and A88 × Rovar populations. The loci shared between these maps are indicated in *boldface*. Anchor loci that relate these linkage groups to the consensus pea map are *underlined*. The loci in *brackets* help align the two maps, and their most likely placement is shown but does not reach the LOD = 2.0 threshold for map order. The scale represents centiMorgans (Haldane units). The 1 LOD confidence intervals for QTL peaks are shown with *vertical bars*. Peak locations are indicated with *closed diamonds*. Ascochyta blight resistance QTLs detected in Western Australia are indicated with *black bars*, in New Zealand with *grey bars*, and plant-maturity QTLs are represented with *hatched bars*

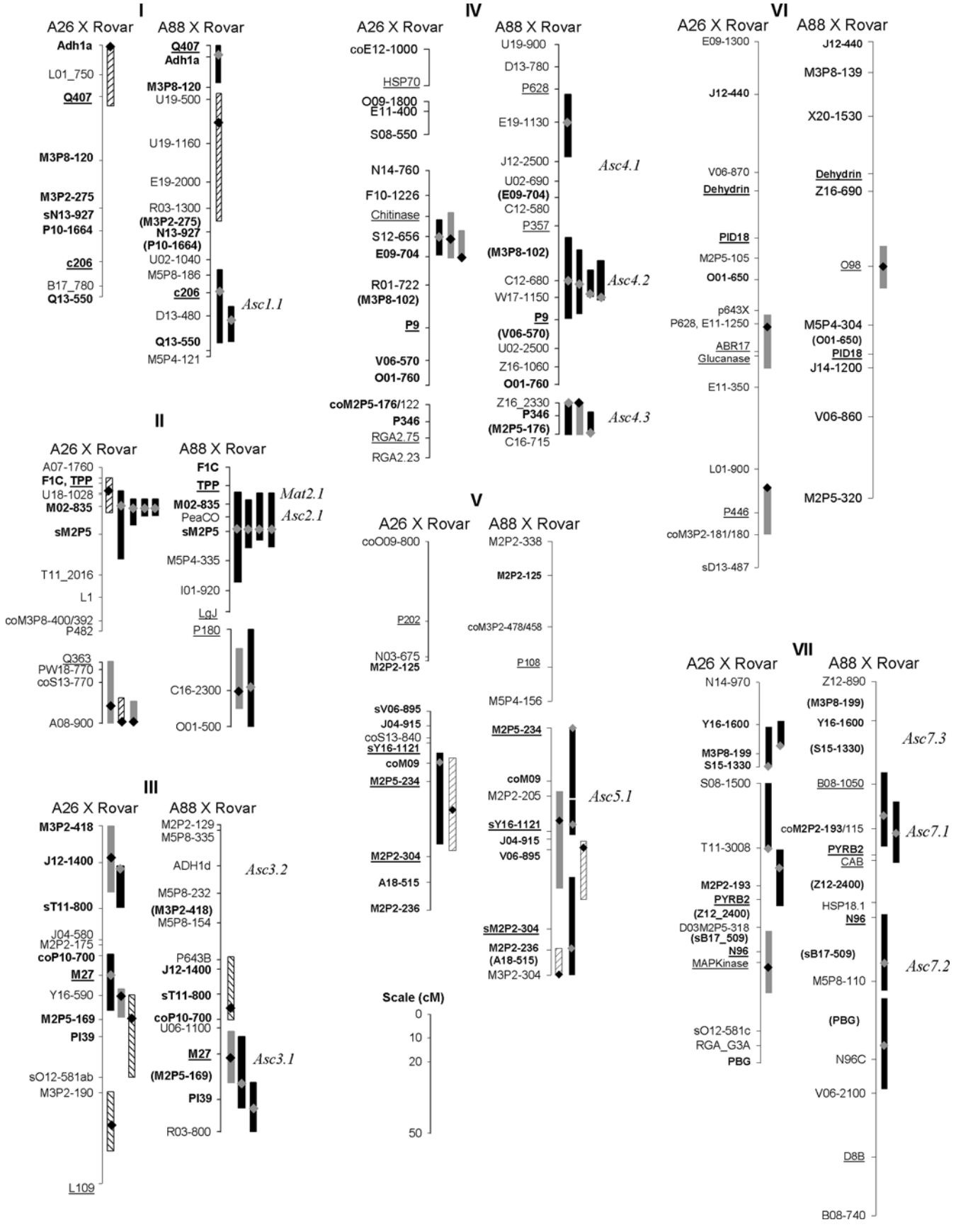
Assessment of phenotypic variation

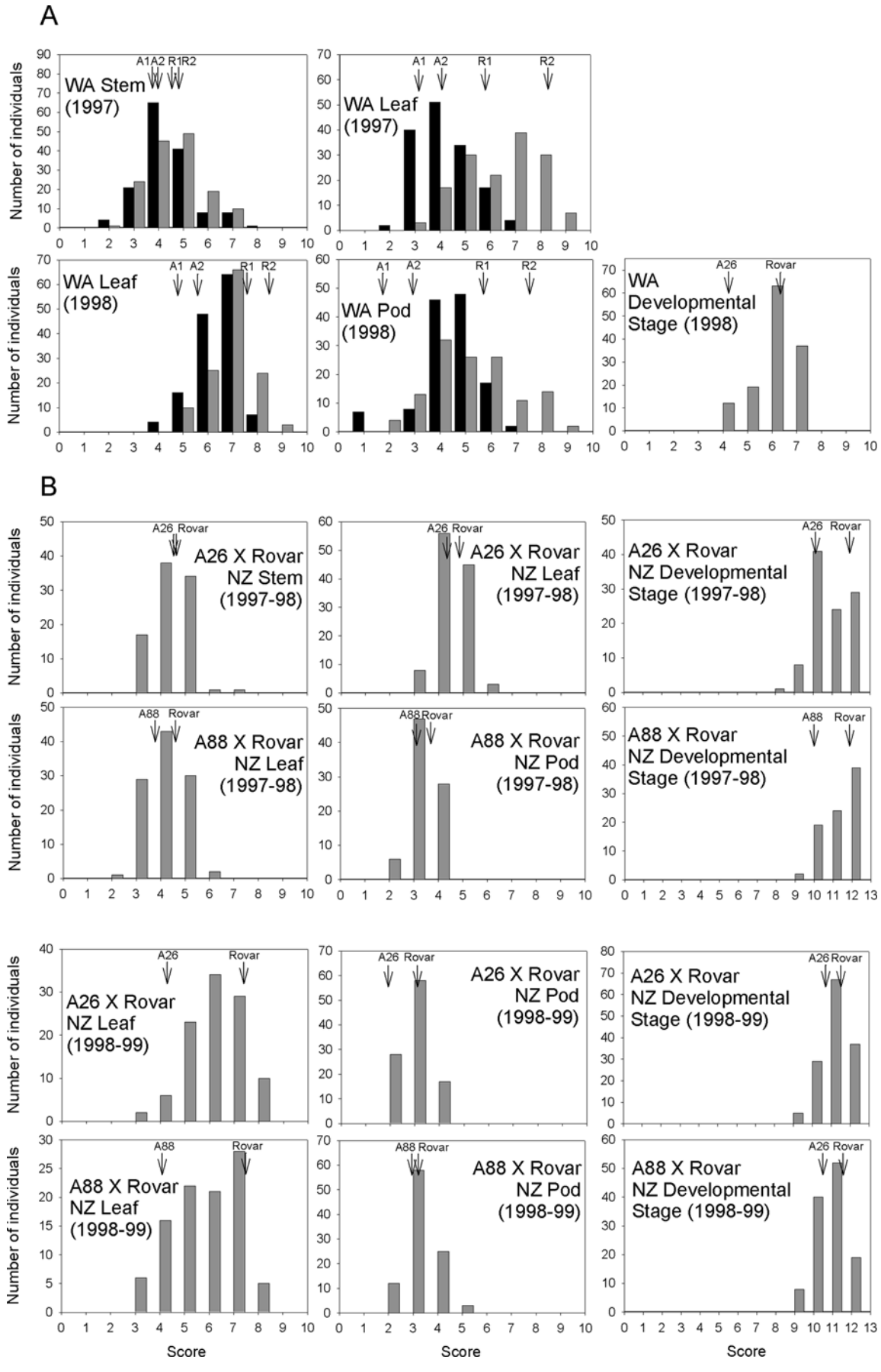
Variation in Ascochyta blight resistance and plant maturity among the progeny families was assessed in four field trials, two held near Medina, Western Australia (WA97 and WA98), and two held near Winton, New Zealand (NZ98 and NZ99). Each site-by-year combination is treated as a separate environment. For most of the trait data from these four environments, the field trends were not strong (data not presented). The adjustments made to trait values resulted in only minor alterations to the raw scores, with little effect on the distribution of progeny trait values. The exception was in the Maturity (NZ99) trait, where a larger field trend was observed. QTL analysis was carried out using raw and adjusted scores with high similarity between the results. Consequently, results from QTL mapping of raw scores are presented.

The frequency distributions obtained from both the A26 × Rovar and A88 × Rovar populations for the disease severity and reproductive stage (maturity) traits in field trials that were used for QTL mapping are shown with parental means in Fig. 2. For some disease scores, the phenotypic range among the progeny families was narrow (less than three scores between highest and lowest values). Therefore, these were not used for QTL mapping. The scores not used for the A26 × Rovar population were Stem1 (WA98) and Stem2 (WA98), Pod (NZ98), and Stem (NZ99); and for the A88 × Rovar population were Stem1 (WA97), and Stem (NZ99). Transgressive segregation was observed for many of the traits (Fig. 2).

Ascochyta blight resistance QTLs in the A26 × Rovar population

Eleven putative QTLs for Ascochyta blight resistance were identified using CIM (Fig. 1; Table 3) on linkage groups II, III, IV, V, VI, and VII. Seven putative QTLs were detected using ≥ two trait scores or ≥ two environments. QTLs on linkage group III (associated with M3P2-418 and J12-1400) and VII (associated with S15-1330 and M3P8-199), detected using multiple trait


I
A26 X Rovar
A88 X Rovar
Adh1a
L01_750
Q407
M3P8-120
M3P2-275
sN13-927
P10-1664
c206
B17_780
Q13-550
Q407
Adh1a
M3P8-120
U19-500
U19-1160
E19-2000
R03-1300
(M3P2-275)
N13-927
(P10-1664)
U02-1040
M5P8-186
c206
D13-480
Q13-550
M5P4-121
Asc1.1
II
A26 X Rovar
A88 X Rovar
A07-1760
F1C, TPP
U18-1028
M02-835
sM2P5
T11_2016
L1
coM3P8-400/392
P482
Q363
PW18-770
coS13-770
A08-900
F1C
TPP
M02-835
PeaCO
sM2P5
M5P4-335
I01-920
LgJ
P180
C16-2300
O01-500
Mat2.1
Asc2.1
III
A26 X Rovar
A88 X Rovar
M3P2-418
J12-1400
sT11-800
J04-580
M2P2-175
coP10-700
M27
Y16-590
M2P5-169
PI39
sO12-581ab
M3P2-190
L109
M2P2-129
M5P8-335
ADH1d
M5P8-232
(M3P2-418)
M5P8-154
P643B
J12-1400
sT11-800
coP10-700
U06-1100
M27
(M2P5-169)
PI39
R03-800
Asc3.2
Asc3.1
IV
A26 X Rovar
A88 X Rovar
coE12-1000
HSP70
O09-1800
E11-400
S08-550
N14-760
F10-1226
Chitinase
S12-656
E09-704
R01-722
(M3P8-102)
P9
V06-570
O01-760
coM2P5-176/122
P346
RGA2.75
RGA2.23
U19-900
D13-780
P628
E19-1130
J12-2500
U02-690
(E09-704)
C12-580
P357
(M3P8-102)
C12-680
W17-1150
P9
(V06-570)
U02-2500
Z16-1060
O01-760
Z16_2330
P346
(M2P5-176)
C16-715
Asc4.1
Asc4.2
Asc4.3
V
A26 X Rovar
A88 X Rovar
coO09-800
P202
N03-675
M2P2-125
sV06-895
J04-915
coS13-840
sY16-1121
coM09
M2P5-234
M2P2-304
A18-515
M2P2-236
M2P2-338
M2P2-125
coM3P2-478/458
P108
M5P4-156
M2P5-234
coM09
M2P2-205
sY16-1121
J04-915
V06-895
sM2P5-304
M2P2-236
(A18-515)
M3P2-304
Asc5.1
Scale (cM)
0
10
20
50
VI
A26 X Rovar
A88 X Rovar
E09-1300
J12-440
V06-870
Dehydrin
PID18
M2P5-105
O01-650
p643X
P628, E11-1250
ABR17
Glucanase
E11-350
L01-900
P446
coM3P2-181/180
sD13-487
J12-440
M3P8-139
X20-1530
Dehydrin
Z16-690
O98
M5P4-304
(O01-650)
PID18
J14-1200
V06-860
M2P5-320
VII
A26 X Rovar
A88 X Rovar
N14-970
Y16-1600
M3P8-199
S15-1330
S08-1500
T11-3008
M2P2-193
PYRB2
(Z12_2400)
D03M2P5-318
(sB17_509)
N96
MAPKinase
sO12-581c
RGA_G3A
PBG
Z12-890
(M3P8-199)
Y16-1600
(S15-1330)
B08-1050
coM2P2-193/115
PYRB2
CAB
(Z12-2400)
HSP18.1
N96
(sB17-509)
M5P8-110
(PBG)
N96C
V06-2100
D8B
B08-740
Asc7.3
Asc7.1
Asc7.2


A
WA Stem (1997)
A1 A2 R1 R2
WA Leaf (1997)
A1 A2 R1 R2
WA Leaf (1998)
A1 A2 R1 R2
WA Pod (1998)
A1 A2 R1 R2
WA Developmental Stage (1998)
A26 Rovar
Number of individuals
B
A26 X Rovar NZ Stem (1997-98)
A26 Rovar
A26 X Rovar NZ Leaf (1997-98)
A26 Rovar
A26 X Rovar NZ Developmental Stage (1997-98)
A26 Rovar
A88 X Rovar NZ Leaf (1997-98)
A88 Rovar
A88 X Rovar NZ Pod (1997-98)
A88 Rovar
A88 X Rovar NZ Developmental Stage (1997-98)
A88 Rovar
A26 X Rovar NZ Leaf (1998-99)
A26 Rovar
A26 X Rovar NZ Pod (1998-99)
A26 Rovar
A26 X Rovar NZ Developmental Stage (1998-99)
A26 Rovar
A88 X Rovar NZ Leaf (1998-99)
A88 Rovar
A88 X Rovar NZ Pod (1998-99)
A88 Rovar
A88 X Rovar NZ Developmental Stage (1998-99)
A26 Rovar
Number of individuals
Score

◀ **Fig. 2** Distributions of Ascochyta blight disease and reproductive stage (maturity) scores in mapping families. **a** Distributions of scores for the F_2-derived families from the A26 × Rovar cross assessed at Medina, Western Australia, in 1997 and 1998. Means of the two parents, A26 (*A*) and Rovar (*R*) for the first (*A1* or *R1*) and second (*A2* and *R2*) scoring dates are shown. *Black* and *grey bars* represent the first and second scoring dates, respectively. **b** Distributions of scores for the A26 × Rovar and A88 × Rovar F_2-derived families at Winton, New Zealand, in 1997–98 and 1998–99. Parental mean scores are shown

scores, were not reported in the A88 × Rovar population and have been named *Asc*3.2 and *Asc*7.2, respectively. A further four putative QTLs were detected using only single trait scores.

The percent of variance (R^2) explained by the individual QTLs ranged from 5.7% to 21.2%. Each individual trait detected only a subset of the total QTLs discovered. The Leaf (NZ99) scores detected four QTLs, the most identified by a single-trait score in a single environment. Other trait scores detected one or two QTLs.

The resistance (low-score) alleles of the QTLs detected on linkage groups II, V, and VII were associated with marker alleles derived from the resistant parent, A26 (Table 3). In these cases, a negative value was observed for *a* estimated under the H_3 hypothesis ($a \neq 0$, $d \neq 0$) by QTL Cartographer. In contrast, the susceptibility alleles of QTLs detected on linkage groups IIb, III, IV, and VI were associated with A26 marker alleles, and a positive value was estimated for *a*. The transgressive segregation observed (Fig. 2) may be explained if the susceptible parent contributes resistance alleles or if epistatic (gene × gene) interactions occur. For QTLs detected using trait scores showing transgressive segregation—the Stem (WA97), Stem (NZ98), Leaf (NZ98), and Pod (NZ99) traits—resistance (low-score) trait means were associated with marker alleles from the susceptible parent Rovar (Table 3).

Ascochyta blight resistance QTLs in the A88 × Rovar population

Using disease scores obtained in Western Australia (Timmerman-Vaughan et al. 2002) and New Zealand field trials (new data this paper), 14 putative QTLs for Ascochyta blight resistance have been mapped using the A88 × Rovar population (Fig. 1, Table 3). Eight of these QTLs were detected using ≥ two trait scores or environments, while the remaining six were identified using only one trait score. Putative QTLs were detected on all seven linkage groups.

The New Zealand environment QTLs on linkage groups III, IV, and V were associated with the genomic regions containing QTLs *Asc*3.1, *Asc*4.3, and *Asc*5.1 previously identified in Western Australian environments. Data from NZ98 and NZ99 have resulted in one additional putative QTL being identified and add support to four of the QTLs detected in the Western Australian environments. The peak for the New Zealand environment QTL on linkage group II (associated with C16-2300) coincides with a QTL peak detected using the Stem1 (WA98). The putative QTL on linkage group VI associated with the RFLP marker O98 was only detected in NZ99. The resistance alleles of the QTLs detected in NZ98 and NZ99 were associated with marker alleles derived from the resistant parent, A88 (Table 3). The New Zealand environment QTLs explain between 9.4% and 34.2% of R^2 observed.

Comparison of Ascochyta blight resistance QTLs detected using two populations

QTL mapping using the A26 × Rovar population confirmed QTLs *Asc*2.1, *Asc*3.1, *Asc*5.1, and *Asc*7.1 previously identified using the A88 × Rovar population (Timmerman-Vaughan et al. 2002). *Asc*4.1 may have been detected using both mapping populations, but was associated with a genomic region where these two maps are poorly integrated (Fig. 1). Additional QTLs have been named on linkage groups II and VII (*Asc2.2* and *Asc7.2*). *Asc2.2* and *Asc7.2* were reported previously but were not named, because they were detected using only single-trait scores. *Asc*1.1, *Asc*4.2, and *Asc*4.3 have not been detected using the A26 × Rovar population.

The estimated magnitudes of *a* for *Asc*2.1, *Asc*5.1, *Asc*7.1, and *Asc*7.2 were similar for the QTLs detected using both populations (Table 3). In both populations, the resistance alleles at all four QTLs were associated with marker alleles derived from the resistant parent. QTL mapping experiments can overestimate the magnitude of QTL effects when experiments involve relatively small progeny numbers and low heritabilities (Beavis 1994; Melchinger et al. 1998; Utz and Melchinger 1994). Validation using the A26 × Rovar population has produced similar estimates of *a* and R^2 for these four QTLs. However, since only a maximum of four QTLs has been detected using any given disease score, it remains a possibility that *a* and R^2 values are overestimates.

In contrast, the *Asc*3.1 map location has been confirmed, but the estimated *a* differs for the A26 × Rovar versus the A88 × Rovar QTLs. In the A26 × Rovar population, the susceptible (high-score) allele was associated with A26 marker alleles. The magnitude of *a* at *Asc*3.1 for traits measured in A88 × Rovar was variable, but the resistant (low-score) allele was generally associated with A88 marker alleles. This suggests that the QTL allelic values at *Asc*3.1 may differ in A26 and A88. Another explanation is that the allelic values in these two breeding lines are similar, but that a type III error may have occurred. A type III error occurs when a QTL is correctly declared but the phase of the marker-trait association is not identified correctly (Dudley 1993).

Resistance QTLs have also been detected in both populations on linkage group IIb associated with Q363 (in A26 × Rovar) and P180 (in A88 × Rovar) (Fig. 1). The maps are not integrated for these segments, and attempts to integrate the maps have not succeeded. Gilpin et al. (1997)

Table 3 Summary statistics for Ascochyta blight resistance and plant reproductive stage QTL peaks discovered using two mapping populations

LG	CentiMorgans	Trait	Environment	LOD	R^2 (%)	*a*	*d*/*a*
QTLs detected using the A26 × Rovar population							
I	0	Maturity	NZ98	3.59	27.5	0.515	−1.36
II	11	Maturity	WA98	5.55	17.7	−0.538	−0.13
II	15	Pod1	WA98	3.70	12.4	−0.133	1.06
II	16	Leaf1	WA98	3.20	10.4	−0.441	0.36
II	16	Leaf2	WA98	7.13	19.6	−0.584	0.81
II	16	Pod2	WA98	3.98	13.2	−0.816	0.64
IIb	18	Leaf	NZ98	2.77	20.6	0.252	−2.24
IIb	24	Maturity	WA98	2.48	21.2	0.086	5.87
IIb	24	Pod1	WA98	2.59 (H_1:H_3)	12.3	−0.185	−4.44
III	14	Leaf	NZ99	3.45	11.3	−0.082	6.12
III	18	Stem1	WA97	3.97	16.1	0.569	−0.64
III	62	Stem2	WA97	2.58	7.8	0.404	−0.90
III	73	Stem	NZ98	3.87	16.3	0.234	1.86
III	83	Maturity	NZ98	2.60	8.2	−0.466	−0.44
III	127	Maturity	NZ99	4.59	22.3	−0.400	1.02
IVb	21	Pod2	WA98	2.98 (H_1:H_3)	5.7	0.244	−3.32
IVb	23	Leaf	NZ99	5.04	17.2	0.263	−2.49
IVb	28	Leaf	NZ98	2.49	9.6	−0.250	0.59
Vb	20	Leaf1	WA97	3.12	9.6	−0.388	0.73
Vb	40	Maturity	NZ99	2.59	18.8	−0.255	−2.41
VI	108	Pod	NZ99	2.84	12	0.119	−3.60
VI	166	Leaf	NZ99	3.69	11.1	0.332	−1.22
VIIa	23	Leaf2	WA97	2.97	7.8	−0.512	−0.86
VIIa	34	Leaf2	WA98	2.60	15.9	−0.543	0.72
VII	24	Leaf1	WA97	2.63	11.9	−0.344	1.56
VII	35	Stem1	WA97	3.12	11.9	−0.503	−1.01
VII	67	Leaf	NZ99	2.59	7.5	−0.167	2.66
QTLs detected using the A88 × Rovar population							
I	5	Stem1	WA98	4.40	19.4	0.180	3.48
I	30	Maturity	WA98	2.79	37.4	0.504	1.63
I	114	Stem2	WA98	5.26	35.9	−0.273	−2.39
I	102	Stem1	WA98	3.27	10.1	−0.250	1.58
II	22	Leaf2	WA97	4.48	13.0	−0.561	0.51
II	22	Leaf1	WA98	5.87	16.2	−0.521	0.89
II	22	Leaf2	WA98	5.42	15.2	−0.472	0.88
II	22	Pod2	WA98	3.24	10.4	−0.705	0.63
IIb	24	Pod	NZ98	2.99	34.2	−0.383	−1.35
IIb	22	Stem1	WA98	2.61	19.6	−0.272	−2.32
III	75	Maturity	NZ99	2.82 (H_1:H_3)	4.6	−0.126	−2.73
III	93	Pod	NZ99	4.58	14.0	0.044	11.6
III	106	Leaf1	WA98	3.03	10.3	−0.182	−3.43
III	117	Leaf1	WA97	5.19	16.9	−0.606	−0.25
IV	32	Leaf2	WA98	2.84	9.7	−0.458	0.29
IV	95	Stem2	WA98	2.69	8.8	−0.054	6.11
IV	99	Leaf2	WA97	2.84	10.6	−0.083	8.81
IV	101	Pod2	WA98	2.96 (H_1:H_3)	7.3	−0.328	2.55
IV	103	Stem1	WA97	2.90 (H_1:H_3)	5.8	−0.073	3.22
IVc	0	Leaf1	WA98	2.73	8.8	−0.407	−0.57
IVc	0	Leaf	NZ99	2.56	11.8	−0.391	−1.86
IVc	11	Pod1	WA98	3.21	12.7	−0.149	−6.97
Vb	0	Stem1	WA98	2.50	10.2	−0.143	−3.34
Vb	35	Leaf	NZ98	2.63	11.7	−0.430	−0.20
Vb	37	Pod2	WA98	2.69	10.6	−0.397	−2.56
Vb	48	Maturity	NZ99	2.72	8.3	−0.309	−0.78

Table 3 (continued)

LG	CentiMorgans	Trait	Environment	LOD	R^2 (%)	*a*	*d*/*a*
Vb	88	Pod2	WA98	2.41	8.1	0.334	2.53
Vb	98	Maturity	WA98	2.72	28.0	0.262	3.46
VI	84	Leaf	NZ99	3.65 (H_1:H_3)	9.4	−0.142	−5.77
VII	51	Leaf2	WA97	2.95	13.9	−0.504	−0.94
VII	61	Stem2	WA97	2.81	9.2	−0.261	−0.18
VII	110	Leaf2	WA98	2.71 (H_1:H_3)	12.8	−0.136	5.08
VII	145	Leaf1	WA98	3.38	12.1	−0.485	0.59

showed that Q363 and P180 define a relatively short region of linkage group II (then called IA/II).

QTLs for plant maturity

Ascochyta blight severity increases during field epidemics as pea plants mature (Kraft et al. 1998). Consequently, late maturing progeny lines or cultivars may receive a deceptively low disease score compared with early maturing lines when disease is assessed on a single date. At the genetic level, this correlation may be explained if resistance and plant-maturity genes are linked, or if a low disease score is a pleiotropic effect of late maturity. Therefore, QTLs have been mapped for reproductive maturity in the A88 × Rovar and A26 × Rovar populations.

QTLs for reproductive maturity were detected in the A26 × Rovar population on linkage groups I, II, IIb, III (two putative QTLs), and Vb (Fig. 1; Table 3). The putative maturity QTLs were all identified using single-maturity scores. The late-maturity (low-score) phenotype was associated with A26 marker alleles for the linkage group II, III, and Vb QTLs. The late-maturity phenotype for the linkage group I and IIb QTLs were associated with Rovar marker alleles.

In the A88 × Rovar population, QTLs for maturity were detected on linkage groups I, III, and V (two QTLs) (Fig. 1; Table 3). A QTL on linkage group II (*Mat*2.1) was reported previously using maturity scores from the WA99 environment and multiple-environment mean scores (Timmerman-Vaughan et al. 2002). *Mat2.1* was associated with the same genomic regions in both populations. The QTLs on linkage group I and V (associated with M3P2-304) were detected using the maturity (WA98) scores, but were not reported previously, because the LOD scores obtained from the CIM analysis conducted previously did not exceed the α=0.05 chromosome-wise threshold determined by permutation testing. Based on the analysis for the current paper, these QTLs have been declared as significant. The difference has occurred because CIM was conducted using slightly different parameters for cofactor selection (control parameters 20 cM window, cofactors selected by forward backward regression) versus the parameters used for the earlier analyses (control parameters 10 cM window, cofactors selected by forward regression).

Flowering time in pea is a quantitatively inherited character. The role of major single genes in controlling flowering time has been well characterized (Weller et al. 1997). Of the maturity QTLs mapped, two were found in regions of the genome containing previously characterized flowering-time genes. *Mat2.1* maps to the same linkage group II genomic region as *Ppd* (photoperiod, Murfet and Taylor 1999). On linkage group III, the QTL in the A88 × Rovar population associated with sT11-800 maps to the same genomic region as *dne* (day neutral, Weeden et al. 1998). The remaining maturity QTLs were not associated with genomic regions containing genes known to control flowering time.

Association of disease resistance with plant maturity

Ascochyta blight of pea is one of a number of plant diseases where a correlation is observed between resistance in the field and agronomic characters such as late maturity. QTL mapping offers one means of dissecting these correlations and in particular, of determining whether they are due to linkage or to pleiotropic effects. Specific examples include late blight of potato, where major QTLs for disease resistance and foliage maturity are coincident (Visker et al. 2003); Fusarium head blight of wheat, where QTLs for flowering day and plant height coincide with disease resistance QTLs (Gervais et al. 2003); and Fusarium head blight of barley, where resistance QTLs coincide with QTLs for a number of agronomic traits (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000). In some cases, linkage is suggested when associated QTLs are linked in repulsion phase, as was observed for the presence of awns and Fusarium head blight in wheat (Mesterhazy 1995) and for foliage maturity and late blight of potato (Visker et al. 2003).

The LOD peaks for the A26 × Rovar plant-maturity QTLs on linkage groups II, IIb, III (one of the QTLs), and V overlap with QTLs for Ascochyta blight resistance (Fig. 1). The resolution of these experiments was not sufficient to distinguish pleiotropy from genetic linkage by map location for overlapping QTLs. As an alternate approach, *a* and degree of dominance (*d*/*a*) statistics were examined (Table 3). Pleiotropy may explain the QTL associations on linkage groups II, IIb, and Vb, because the maturity and resistance QTLs show similar *d*/*a* statistics (IIb) or similar additive effects and *d*/*a* statistics (II and Vb). On linkage group III, however, linkage was

suggested, since disease resistance and maturity QTLs were in repulsion. In this case the resistance (low-score) phenotype at this QTL was associated with Rovar marker alleles (Stem2 WA97, a=0.404; Stem2 NZ98, a=0.234), while the late-maturity (low-score) phenotype at the coincident maturity QTL was associated with A26 marker alleles (Maturity NZ98, a=−0.466).

The peaks for the two putative maturity QTLs detected on linkage group V coincide with Ascochyta blight resistance QTL peaks. Estimates of the a and d/a support the hypothesis that Ascochyta blight resistance explained by these QTLs may be a pleiotropic effect of genetic loci that affect plant maturity (Table 3), since the magnitude and phase of the additive effects and estimates of dominance deviation were similar for both sets of coincident QTL peaks. Following the same reasoning, *Asc2.1* may be a pleiotropic effect of *Mat2.1* (Timmerman-Vaughan et al. 2002).

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