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ORIGINAL PAPER

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Quantitative trait loci for lodging resistance, plant height and partial resistance to mycosphaerella blight in field pea (*Pisum sativum* L.)

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Abstract With the development of genetic maps and the identification of the most-likely positions of quantitative trait loci (QTLs) on these maps, molecular markers for lodging resistance can be identified. Consequently, marker-assisted selection (MAS) has the potential to improve the efficiency of selection for lodging resistance in a breeding program. This study was conducted to identify genetic loci associated with lodging resistance, plant height and reaction to mycosphaerella blight in pea. A population consisting of 88 recombinant inbred lines (RILs) was developed from a cross between Carneval and MP1401. The RILs were evaluated in 11 environments across the provinces of Manitoba, Saskatchewan and Alberta, Canada in 1998, 1999 and 2000. One hundred and ninety two amplified fragment length polymorphism (AFLP) markers, 13 random amplified polymorphic DNA (RAPD) markers and one sequence tagged site (STS) marker were assigned to ten linkage groups (LGs) that covered 1,274 centi Morgans (cM) of the pea genome. Six of these LGs were aligned with the previous pea map. Two QTLs were identified for lodging resistance that collectively explained 58% of the total phenotypic variation in the mean environment. Three QTLs were identified each for plant height and resistance to mycosphaerella blight, which accounted for 65% and 36% of the total phenotypic variation, respectively, in the mean environment. These QTLs were relatively consistent across environments. The AFLP marker that was associated with the major locus for lodging resistance was converted into the sequence-characterized amplified-region (SCAR) marker. The presence or absence of the SCAR marker corresponded well with the lodging reaction of 50 commercial pea varieties.

Keywords *Pisum sativum* · Lodging resistance · Plant height · Disease resistance · Molecular mapping · Quantitative trait loci · Marker-assisted selection

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Introduction

Lodging is among the major constraints of field pea production. It enhances canopy microclimate for fungal-disease development, reduces harvest efficiency and increases harvest cost. The introduction of the semi-leafless trait (*afila* leaf morphology) into numerous pea varieties in the past 25 years has decreased the risk of lodging, as tendrils of the plants intertwine with each

other to provide mutual support. Also, the incorporation of a dwarfing gene (*le*) into many cultivars has contributed to improved lodging resistance. However, under field conditions these traits are often not enough to support upright growth. Other factors related to lodging resistance might be needed. McPhee and Muehlbauer (1999) reported variability in the stem character measured as a 'resistance to shearing' that was associated with stiffness in pea. However, no mapping information was reported.

Lodging is a quantitative trait and is highly affected by environmental conditions such as wind and rain; therefore, it is difficult to assess on a phenotypic basis. In addition, scoring for lodging resistance in the field can be inconsistent as conditions causing lodging can occur at different stages of plant development. With the development of genetic maps and the identification of the most-likely positions of quantitative trait loci (QTLs) on these maps, molecular markers for lodging resistance can be identified. Consequently, marker-assisted selection (MAS) may have potential to improve the efficiency of selection for lodging resistance in a breeding program.

A number of studies have identified QTLs for lodging and plant height in major crops. For example, in a F_2-derived soybean (*Glycine max* L.) population from the Minsoy and Noir-1 cross, Mansur et al. (1993) reported a major QTL for lodging and plant height on LG L. Using data across locations, Lee et al. (1996) identified a major locus (*Dt1*) associated with lodging and plant height in soybean. This locus accounted for 56% and 68% of the total variation for lodging and plant height, respectively. In pea, Dirlewanger et al. (1994) reported a restriction fragment length polymorphism (RFLP) marker locus (*p275*) on chromosome B that was associated with plant height in a F_2 population derived from a cross between Erygel and 661. The marker accounted for 19% of the phenotypic variation for plant height under greenhouse conditions.

To-date, no information has been reported on whether lodging and plant height are genetically associated with reaction to disease, such as mycosphaerella blight caused by the fungus *Mycosphaerella pinodes* (Berk & Bloxham) Verstergren in pea. Reaction to mycosphaerella blight is strongly affected by the morphological and the physiological characteristics of the plants, as well as the environmental conditions (Wroth 1999). Commercial varieties range in their reaction to mycosphaerella blight from highly susceptible to partially resistant (Bretag et al. 1995; Wroth 1999; Xue and Warkentin 2001). Based on the measurement of area under the disease progress curve, several pea varieties including Carneval, one of the parents used to develop the population for the current study, had low-leaf and pod areas with symptoms, and were considered partially resistant to mycosphaerella blight (Xue and Warkentin 2001). However, it is not known whether the resistance in the field is the result of physiological resistance or avoidance. A recent report by Timmerman-Vaughan et al. (2002) demonstrated a common QTL that was associated with the resistance to ascochyta blight and the plant-reproductive stage in pea. In soybean, Kim and Diers (2000) reported that two QTLs for plant reaction to sclerotinia stem rot (*Sclerotinia sclerotiorum*) were significantly associated with plant height and lodging.

Several linkage maps have been developed for pea and the most-likely positions of QTLs for important traits have been identified (Dirlewanger et al. 1994; Timmerman-Vaughan et al. 1996, 2000, 2002; Gilpin et al. 1997; McCallum et al. 1997; Laucou et al. 1998; Pilet-Nayel et al. 2002). The current study used a molecular marker-based linkage map to identify putative QTLs for lodging, plant height and reaction to mycosphaerella blight in pea. The current study provided estimates of the minimum number of QTLs affecting each trait, identified the chromosomal locations of these loci, and estimated the magnitude of the effects for each QTL. A survey of a collection of commercial pea varieties with known lodging reaction using markers associated with lodging resistance was also included in this report.

Materials and methods

The population used in this study was developed from a cross between two pea varieties, Carneval and MP1401. Both parents are semi-leafless (*afila*) and have short internodes (*le*). The lodging-resistant parent (Carneval) was developed from a cross between Bohatyr and Rigel by Svalöf Weibull Company in Sweden. Carneval is also partially resistant to *Mycosphaerella pinodes*, Berk and Bloxham Vestergren (Xue and Warkentin 2001). It has been widely grown in Western Canada over the past decade. The lodging susceptible parent (MP1401) was developed at Morden Research Station, Agriculture and Agri-Food Canada, from a cross between AC Tamor and Montana. Unselected individual F_2 plants were inbred to the F_5 generation in a greenhouse using single-seed descent. Seeds from individual 88 F_5 plants were bulked and increased in the field until the F_7 generation.

The 88 RILs and both parents were evaluated in four-row plots using a 8×12 incomplete block design in 11 environments across the provinces of Manitoba, Saskatchewan and Alberta, Canada, in 1998, 1999 and 2000 (Table 1). The plant rows were 4-m long and spaced 30-cm apart. The plant density was 75 plants per m^2. Fertility and cultivation regimes were consistent with the optimum pea-production practices for these regions. Data were collected for lodging, plant height and reaction to mycosphaerella blight under natural infection on the basis of all plants in a plot. Plant height was measured from soil level to the tip of the central stem at the end of flowering. Lodging response was assessed at maturity on a 1 to 9 scale, where 1 = upright and 9 = completely lodged. Reaction to mycosphaerella blight was visually measured at the pod-filling stage on a scale of 0 to 9, where 0 = no symptom and 9 = the whole-plant severely blighted (Xue et al. 1996).

Analyses of variance for all traits from each year and location, and a combined analysis across locations and years, were done using PROC MIXED of the SAS program (SAS Institute Incorporated, Cary, N.C., USA). Plant genotypes were considered fixed effects, while blocks and environments were random. The adjusted means of each trait across locations and years were used for the QTL analysis.

Genomic DNA was isolated from the two parents and individual recombinant inbred lines (RILs) following the hexadecyltrimethylammonium bromide (CTAB) method as described by Saghai-Maarof et al. (1984). The amplified fragment length polymorphism (AFLP) analysis was performed essentially as described in the AFLP Plant Mapping Kit protocol (Invitrogen Corporation, Carlsbad, Calif., USA). The selective amplification products were then loaded onto a 6% denaturing polyacrylamide gel. The gel was run in 1 × TBE (Tris-borate EDTA buffer) at 40 W for 4 h and silver-

Table 1 Soil types and weather conditions (1998–2000) of the experimental sites for the evaluation of 88 RILs of pea derived from a cross between Carneval and MP1401

Location	Soil type	Year	Growing season precipitation (mm)[a]	Growing season mean temperature (ºC)	Traits measured[c]
Morden, MB[b]	Clay loam	2000	300	17	Ld, Ph, Mb
Saskatoon, SK	Clay loam	2000	225	15	Ld, Ph, Mb
Floral, SK	Loam	2000	225	15	Ld, Ph, Mb
Morden, MB	Clay loam	1999	270	17	Ld, Ph
Saskatoon, SK	Clay loam	1999	275	15	Ld, Ph, Mb
Floral, SK	Loam	1999	275	15	Ld, Ph, Mb
Portage la Prairie, MB	Clay loam	1999	450	15	Ld, Ph
Grande Prairie, AB	Loam	1999	120	13	Ph
Morden, MB	Clay loam	1998	250	18	Ld, Ph
Saskatoon, SK	Clay loam	1998	150	16	Ld
Edmonton, AB	Loam	1998	180	15	Ld

[a] Growing season is from May 1st to August 31st
[b] MB = Manitoba; SK = Saskatchewan; AB = Alberta
[c] Ld = lodging; Ph = plant height; Mb = reaction to mycosphaerella blight

stained following the protocols described by Bassam et al. (1991). AFLP marker nomenclature consists of four letters and a number. The first two letters represent the *Eco*RI + 2 selective nucleotides. The second two letters represent the *Mse*I + 2 selective nucleotides. The number following these letters is the order of the polymorphic bands for a given primer pair.

The AFLP marker that accounted for the most-variation of the lodging resistance was converted into a sequence-characterized amplified-region (SCAR) marker to simplify its analysis for MAS. To facilitate cloning of an AFLP marker, the band of interest was excised with a razor blade from the stained gel and the DNA was eluted in 100 μl of sterile water for 1 h at 4°C (Chalhoub et al. 1997). Five microliters of the eluted DNA was re-amplified with the appropriate primer pair and products of re-amplification were separated on a 2% agarose gel. The fragment with the correct size was cut from the gel and eluted using the Prep-A-Gene DNA purification kit (BioRad, Hercules, Calif., USA) according to the manufacturer's instructions. The fragment was ligated into a TOPO TA cloning vector (Invitrogen Corporation, Carlsbad, Calif., USA) according to the manufacturer's instructions. DNA sequencing was done on an ABI 377 automated DNA sequencer using M13 primers. The sequence was used to design the forward and reverse primers of the SCAR marker.

The RAPD analysis was done following the protocol described by Laucou et al. (1998) with some modifications including the use of 1 unit of *Taq* DNA polymerase (Invitrogen Corporation, Carlsbad, Calif., USA) and 25 ng of genomic DNA. A set of 40 primers (Operon Technologies, Alameda, Calif., USA) selected from different LGs as described by Laucou et al. (1998) was screened for polymorphism between the parental lines. DNAs from Térèse and Torsdag, the parental lines of the mapping population used by Laucou et al. (1998), were used as references on each PCR reaction and gel electrophoresis. Seed samples of Térèse and Torsdag were kindly provided by Dr. C. Rameau of INRA, Versailles, France. Only primers that amplified bands of similar size in either Térèse or Torsdag and have been mapped in the previous population (Laucou et al. 1998) were used to characterize the current RILs. The RAPD markers were named using the Operon primer name (a letter and primer number) followed by the fragment size (in base pairs).

STS (sequence tagged site) analysis was done following the technique described by Gilpin et al. (1997). Six STSs (D8C, I7, L109, P108, P628 and Q363) were tested for polymorphism between the parental lines used in the current study.

Linkage groups of the markers were determined using the Group command of MAPMAKER/EXP program version 3.0 (Lander et al. 1987) at a LOD (logarithm of odds ratio) score of 4.0 with a maximum distance between two markers of 25 cM (Haldane map function). The order of the markers within a group was determined using the Compare command ata LOD score of 4.0. Additional markers were subsequently added using the Try command at a LOD threshold of 4.0. The best order of the markers was then verified using the Ripple command with a LOD score of 4.0.

The QTL location and effect for each trait were estimated by composite interval mapping (CIM) using the QTL Cartographer program for Windows version 1.01 (Wang et al. 1999). The standard model (Model 6) of the Zmapqtl procedure of the QTL Cartographer (Basten et al. 1994) was used in the analysis by scanning the genome every 2 centi Morgans (cM). The co-factors were specified as five marker-loci identified by stepwise regression that explained the most-variation for a given trait. The threshold levels to declare significant QTLs were empirically determined by performing 1000 permutations of the data, which maintained the chromosome-wise Type-I error rate of 0.05 (Churchill and Doerge 1994). The additive affect of the detected QTLs was also estimated by the Zmapqtl procedure.

The proportion of phenotypic variance accounted for by each detected QTL was estimated by a single-factor analysis of variance with the SAS General Linear Model (GLM) procedure on the individual marker loci closest to the QTL identified by CIM. The amount of phenotypic variation simultaneously explained by all QTLs found for a given trait was determined using a stepwise regression analysis of the closest marker locus for each QTL detected by CIM.

Results

The two parents were initially screened with 104 AFLP primer combinations to determine which combinations produced the greatest number of polymorphisms. Forty primer combinations were then selected and tested on the individual RILs. A total of 239 AFLP markers were generated from the analysis. Out of 40 RAPD primers selected from the previous map (Laucou et al. 1998), 13 primers produced bands of similar size as in Térèse or Torsdag. One (p628.HinfI) out of six tested STSs is polymorphic between Carneval and MP1401. The goodness-of-fit of the observed segregation ratio to the expected ratio indicated that 183 of the AFLP markers, all of the random amplified polymorphic DNA (RAPD) markers and the p628.HinfI STS did not significantly deviate from the expected 1:1 ratio (P>0.05).

One hundred and ninety three AFLP markers, 13 RAPD markers and one STS marker were assigned to ten

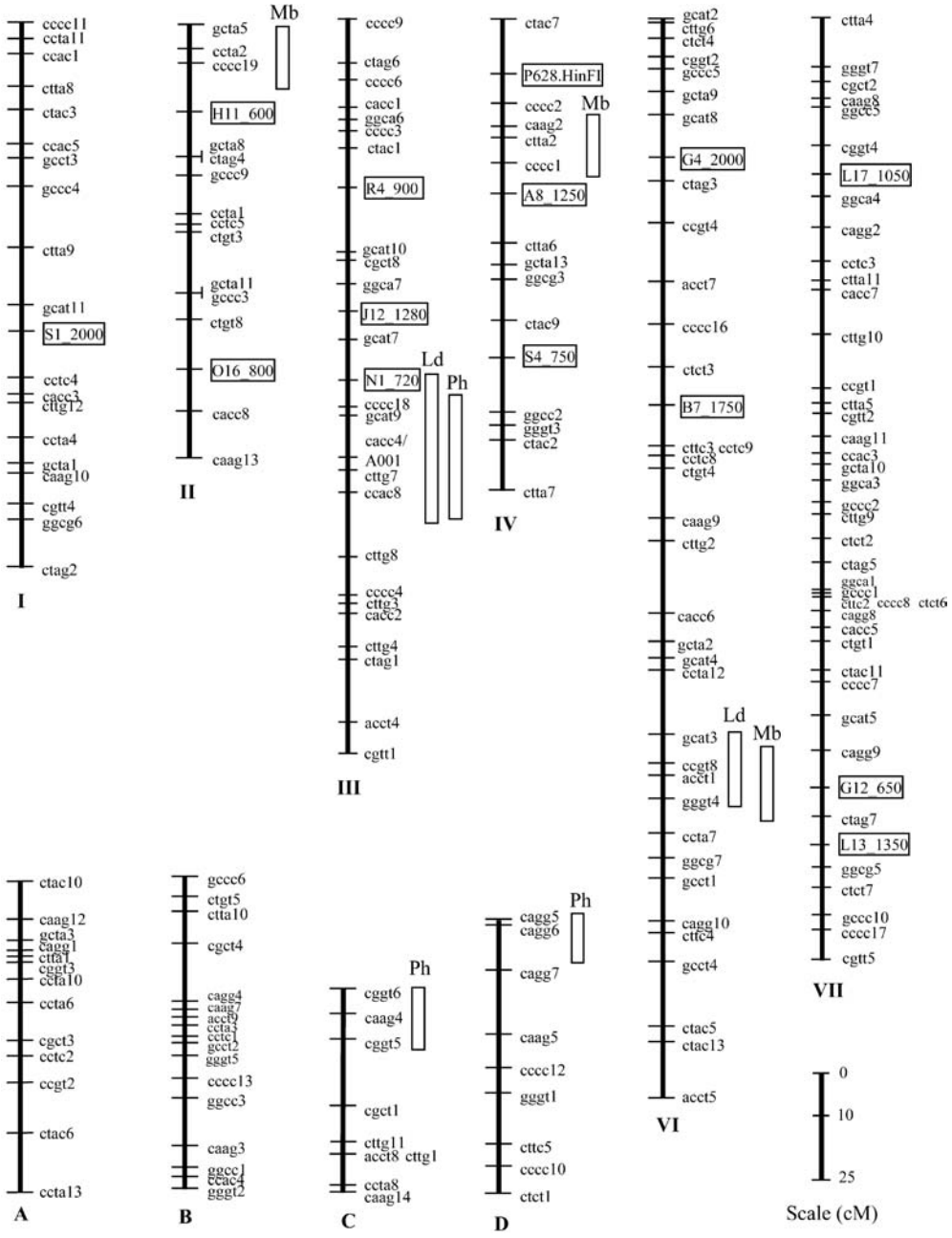

Fig. 1 Linkage map of the pea population derived from a cross between Carneval and MP1401. *Framed marker loci* indicate anchor markers used to align LGs to the previous map of pea. The scale represents centi Morgan (Haldane units). *Vertical bars* indicate the location of QTLs for a given trait with the LOD value equals or higher than the LOD threshold. *Ld* = lodging; *Ph* = plant height; *Mb* = mycosphaerella blight

linkage groups that covered 1,274 cM of the pea genome. The average linkage distance between pairs of markers was 6.2 cM (Fig. 1). Fourteen markers including 13 RAPDs and one STS were common to the previous pea genetic maps (Gilpin et al. 1997; Laucou et al. 1998). These cmmon markers allowed six (I, II, III, IV, VI and VII) of these LGs to be aligned to the previous pea linkage map, whereas four LGs (A to D) remained unassigned (Fig. 1).

The means of the parental and RILs as well as the range of RILs across locations and years are shown in Fig. 2. A correlation analysis using the mean data across locations and years showed that lodging was significantly correlated with plant height (r=–0.59; P<0.001) and with reaction to mycosphaerella blight (r=0.35; P<0.01). The correlation between plant height and reaction to mycosphaerella blight was not significant (r=0.20; P=0.07).

Mean lodging scores for Carneval and MP1401 differed significantly by a score of 2.88 [Least Significant Difference $(LSD)_{0.05}$=0.98] and the lodging scores among the 88 RILs varied from 3.02 to 6.38 (Fig. 2). Two QTLs, one each on LG III (*cacc4*) and LG VI (*acct1*), were identified for the lodging reaction (Table 2). The Carneval allele reduced lodging by 0.46 and 0.23 at the *cacc4* and *acct1* loci, respectively. The *cacc4* locus accounted for 47% of the phenotypic variation for lodging, whereas the *acct1* locus explained 26% of the phenotypic variation. A multiple QTL model showed that these two genomic

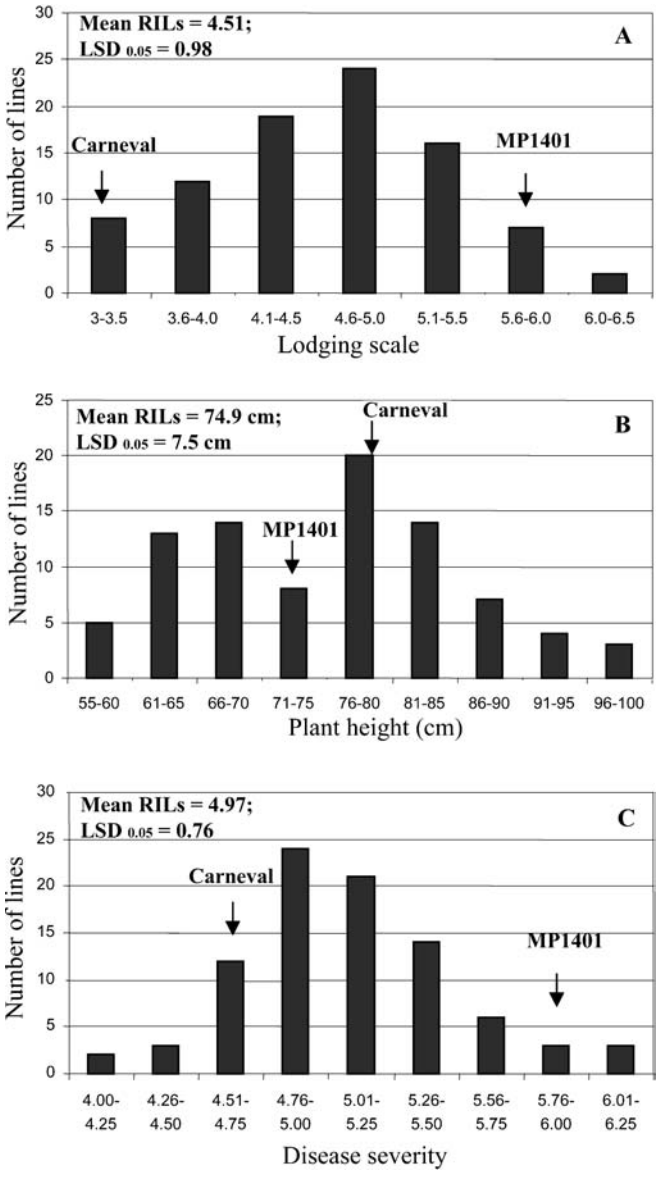

Fig. 2A–C Frequency distribution of 88 RILs for their lodging scores (**A**), plant height (**B**) and reaction to mycosphaerella blight (**C**). The scores of Carneval and MP1401as well as the mean scores of the RILs and their LSD values (least significant difference at the 5-% level) are indicated. Lodging score was on a scale of 1 (upright) to 9 (completely lodge). Reaction to mycosphaerella blight was measured on a scale of 0 (no symptoms) to 9 (plants completely blighted)

regions simultaneously accounted for 58.2% of the total phenotypic variation for lodging. The association between the *cacc4* locus and lodging reaction was maintained in seven of ten environments (Table 3). The strength of the QTL effect on lodging at this locus varied from 15.1% at Saskatoon in 2000 to 52.2% at Morden in 1998. The association between the *acct1* locus and the lodging reaction on LG VI was detected in five of the ten environments, with the largest effect (14.9%) at Morden in 1998.

The *cacc4* marker was converted into a sequence characterised amplified region (SCAR) marker, namely A001. The forward and reverse primers for the A001 marker are *5′-cttcaccatccatagtgtcg-3′* and ;*5′-cacttgcgttc-cttgtgtg-3′*, respectively. At 65°C annealing temperature these primers amplified a single-band of 300 bp in size (Fig. 3), which co-segregates exactly as the original AFLP marker.

Carneval and MP1401 differed by 4.4 cm in plant height and a wide variation (55–97 cm) occurred among the RILs (Fig. 2). The CIM procedure detected three genomic regions, one each on LGs III, C and D, that collectively explained 64.6% of the total variation for plant height. The *cttg7* locus on LG III had a major effect on plant height and accounted for 56.9% of the phenotypic variation for plant height in the mean environment (Table 2). Furthermore, the *cttg7* locus was detected as being associated with plant height at all environments (Table 3). The analyses on single environments demonstrated that the amount of phenotypic variation accounted for by the *cttg7* locus varied depending upon location and year, with the largest effect found at Saskatoon in 1999. The *caag4* (LG C) and *cagg5* (LG D) loci were identified in three and two, respectively, out of the nine environments tested. Both parents contributed alleles for plant height in the RILs. In the mean environment, the Carneval alleles at the *cttg7* and *caag4* loci increased plant height by 8.8 cm and 4.1 cm, respectively. At the *cagg5* locus on LG D, the allele from MP1401 increased plant height.

The susceptible parent (MP1401) had a mean mycosphaerella blight score of 1.1 units greater than Carneval ($LSD_{0.05}$=0.76). The mycosphaerella blight scores for the RILs varied from 4.22 to 6.12 (Fig. 2). Three genomic regions, one each on LGs II, IV and VI, were associated with the reaction to mycosphaerella blight (Table 2). Altogether these QTLs explained 35.9% of the phenotypic variation for the reaction to mycosphaerella blight. Of the three QTLs detected in the mean environment, only the *cccc1* locus is identified in all five environments (Table 3). The largest effect of this locus on the reaction to mycosphaerella blight was found at Saskatoon in 2000. The *acct1* and *ccta2* loci were associated with the reaction to mycosphaerella blight in four and one environments, respectively, out of the five environments tested. For the two regions, *acct1* and *cccc1*, which were mapped to LGs IV and VI, respectively, the alleles from MP1401 increased the susceptibility of the plants to the disease.

The potential of the A001 marker for MAS for lodging resistance was examined using a collection of 50 commercial pea varieties of diverse sources (Table 4). The presence or absence of the A001 marker corresponded well with the lodging reaction of the varieties. For example, all 16 cultivars with good-lodging resistance (scale 1–3) had the marker present, whereas eight of the nine varieties with poor lodging-reaction rating (lodging score 7 or higher) had the marker absent.

Table 2 Map location, percentage of the phenotypic variation, additive genetic effects and direction of the detected QTLs for lodging, plant height and reaction to mycosphaerella blight in 88 RILs of pea derived from a cross between Carneval and MP1401

Trait/Linkage Group	Locus with the max. LOD value	Max. LOD value	LOD threshold[a]	% Variation	Additive Genetic effect[c]	Direction[d]
Lodging						
III	*cacc4* (A001)	14.5	3.03	47.0	–0.46	MP1401
VI	*acct1*	3.5	3.03	26.4	–0.23	MP1401
			Total variation[b]	58.2		
Plant height						
III	*cttg7*	21.5	2.99	56.9	8.80	Carneval
C	*caag4*	3.3	2.99	17.2	4.07	Carneval
D	*cagg5*	3.1	2.99	6.5	–2.15	MP1401
			Total variation	64.6		
Reaction to mycosphaerella blight						
II	*ccta2*	2.9	2.87	5.0	0.12	Carneval
IV	*cccc1*	3.3	2.87	19.1	–0.12	MP1401
VI	*acct1*	3.1	2.87	16.8	–0.14	MP1401
			Total variation	35.9		

[a] The threshold levels were empirically determined by performing 1,000 permutations of the data, which maintained the chromosome-wise Type-I error rate of 0.05
[b] The amount of phenotypic variation simultaneously explained by all markers found for a given trait, which was determined using a stepwise regression analysis
[c] The value associated with the Carneval allele. A negative value means that the Carneval allele decreases the value of the trait
[d] Direction of response is the parent whose additive value of a marker allele increased the value of the trait

Table 3 LOD value, amount of phenotypic variation accounted for and additive genetic effect of QTLs for lodging, plant height and reaction to mycosphaerella blight in 88 RILs of pea derived from a cross between Carneval and MP1401 for each location and year

LG/marker locus	Parameter	2000			1999					1998		
		Morden	Floral	Saskatoon	Grande Prairie	Morden	Portage La Prairie	Floral	Saskatoon	Edmonton	Morden	Saskatoon
Lodging												
III/*cacc4*(A001)	LOD	12.4	3.1	3.0	–	13.6	5.1	ns[a]	ns	13.4	13.6	ns
	R2	47.3	17.8	15.1	–	50.8	25.8	nd	nd	48.5	52.2	nd
	Additive[b]	–0.81	–0.45	–0.53	–	–0.86	–0.48	nd	nd	–1.46	–0.76	nd
VI/*acct1*	LOD	3.0	3.8	ns	–	ns	ns	3.0	3.0	ns	3.1	ns
	R2	14.4	12.6	nd	–	nd	nd	13.1	12.8	nd	14.9	nd
	Additive	–0.46	–0.53	nd	–	nd	nd	–0.38	–0.36	nd	–0.44	nd
	Total R^2 [c]	53.1	27.0	15.1	–	50.8	25.8	13.1	12.8	48.5	57.2	nd
Plant height												
III/*cttg7*	LOD	11.4	5.8	5.9	11.3	11.3	4.1	12.9	11.2	–	6.9	–
	R2	37.8	18.3	29.3	41.3	34.6	15.1	33.3	43.8	–	34.1	–
	Additive	7.96	4.30	6.15	8.04	8.54	6.68	7.90	9.83	–	8.71	–
C/*caag4*	LOD	ns	5.0	ns	ns	ns	ns	ns	3.1	–	3.7	–
	R2	nd	14.3	nd	nd	nd	nd	nd	14.8	–	13.9	–
	Additive	nd	5.12	nd	nd	nd	nd	nd	5.86	–	6.71	–
D/*cagg5*	LOD	2.86	ns	ns	ns	ns	2.88	ns	ns	–	ns	–
	R2	12.7	nd	nd	nd	nd	12.3	nd	nd	–	nd	–
	Additive	–3.86	nd	nd	nd	nd	–3.94	nd	nd	–	nd	–
	Total R2	44.0	34.4	29.3	41.3	34.6	24.8	33.3	46.8	–	37.5	–
Reaction to mycosphaerella blight												
II/*ccta2*	LOD	2.92	ns	ns	–	–	–	ns	ns	–	–	–
	R2	13.7	nd	nd	–	–	–	nd	nd	–	–	–
	Additive	0.32	nd	nd	–	–	–	nd	nd	–	–	–
IV/*cccc1*	LOD	2.88	2.96	2.88	–	–	–	2.86	2.88	–	–	–
	R2	8.6	13.2	14.2	–	–	–	10.4	12.5	–	–	–
	Additive	–0.27	–0.29	–0.28	–	–	–	–0.32	–0.38	–	–	–
VI/*acct1*	LOD	ns	4.2	2.88	–	–	–	3.1	3.8	–	–	–
	R2	nd	12.8	10.3	–	–	–	12.4	14.3	–	–	–
	Additive	nd	–0.44	–0.32	–	–	–	–0.32	–0.42	–	–	–
	Total R2	18.5	19.4	19.6		–	–	18.7	22.6	–	–	–

– = no trial
[a] ns = Not significant, i.e. the LOD value at a given marker locus is smaller than the LOD threshold; nd = not determined
[b] The value associated with the Carneval allele. A negative value means that the Carneval allele decreases the value of the trait
[c] The amount of phenotypic variation for a trait simultaneously explained by all markers with the LOD value higher than the threshold. The value was calculated using a stepwise regression analysis

Table 4 Survey of the presence or absence of the A001 marker on a collection of 50 pea varieties recommended for production in Saskatchewan, Canada (2002 Varieties of Grain Crops. Saskatchewan Agriculture, Food and Rural Revitalization)

No.	Cultivar	Breeding institution[a]	Cotyledon colour	1,000 seed weight (g)	Leaf type[b]	Plant height (cm)	Lodging reaction rating[c]	A001 marker[d]
1	AC Advantage	AAFC Morden, Canada	Green	270	SL	60	P	–
2	AC Melfort	AAFC Morden, Canada	Yellow	240	SL	70	F	–
3	Alfetta	Cebeco Zaden, The Netherlands	Yellow	290	SL	72	F	–
4	Atomic	DLF Trifolium, Denmark	Green	282	SL	70	F	+
5	Baccara	Florimond Desprez, France	Yellow	280	SL	65	P	–
6	Badminton	Florimond Desprez, France	Yellow	250	SL	65	P	–
7	Carneval	Svalof Weibull, Sweden	Yellow	232	SL	75	G	+
8	Carrera	Cebeco Zaden, The Netherlands	Yellow	274	SL	55	P	–
9	Cascade	N.Z. Crop & Food, New Zealand	Green	200	SL	75	F	+
10	CDC April	U of S—CDC, Canada	Olive	140	SL	53	P	–
11	CDC Handel	U of S—CDC, Canada	Yellow	220	SL	75	F	+
12	CDC Minuet	U of S—CDC, Canada	Yellow	190	SL	70	F	–
13	CDC Montero	U of S—CDC, Canada	Green	230	SL	80	F	+
14	CDC Mozart	U of S—CDC, Canada	Yellow	230	SL	70	F	+
15	CDC Verdi	U of S—CDC, Canada	Green	200	SL	75	F	+
16	CDC Vienna	U of S—CDC, Canada	Olive	170	SL	61	P	–
17	CDC Winfield	U of S—CDC, Canada	Yellow	260	N	62	F	–
18	Cobra	Danisco Seeds, Denmark	Yellow	240	SL	75	F	+
19	Cresta	Sharpe's Int. Seed Ltd., Canada	Yellow	270	SL	70	F	–
20	Croma	Cebeco Zaden, The Netherlands	Yellow	300	SL	70	F	–
21	Delta	Cebeco Zaden, The Netherlands	Yellow	250	SL	72	F	+
22	DS Admiral	Danisco Seeds, Denmark	Yellow	240	SL	80	G	+
23	DS Dominator	Danisco Seeds, Denmark	Green	230	SL	65	G	+
24	DS Stalwarth	Danisco Seeds, Denmark	Yellow	240	SL	80	G	+
25	Eclipse	Cebeco Zaden, The Netherlands	Yellow	250	SL	80	F	–
26	Eiffel	Danisco Seeds, Denmark	Yellow	290	SL	67	G	+
27	Espace	Cebeco Zaden, The Netherlands	Green	230	SL	75	G	+
28	Grande	Svalof Weibull, Sweden	Yellow	260	N	90	F	–
29	Highlight	Svalof Weibull, Sweden	Yellow	210	SL	66	F	+
30	Integra	Cebeco Zaden, The Netherlands	Yellow	280	SL	75	G	+
31	Logan	Agricore, Canada	Green	180	SL	75	G	+
32	Majoret	Svalof Weibull, Sweden	Green	250	SL	59	G	+
33	Miami	Sharpes Int. Seed Ltd., Canada	Yellow	245	SL	80	G	+
34	Millenium	Mansholt, The Netherlands	Green	260	SL	65	F	–
35	Nicole	Advanta Seeds, UK	Yellow	260	SL	65	F	–
36	Nitouche	DLF Trifolium, Denmark	Green	250	SL	75	G	+
37	Passat	Cebeco Zaden, The Netherlands	Yellow	260	SL	65	F	–
38	Pekisko	Agricore, Canada	Green	210	SL	75	F	+
39	Princess	Wilbur Ellis Co., USA	Green	200	N	58	P	+
40	Radley	Sharps-Columbia Seeds	Green	210	SL	57	F	+
41	Scuba	Advanta Seeds, UK	Green	230	SL	80	F	+
42	SW Bravo	Svalof Weibull, Sweden	Yellow	260	SL	75	G	+
43	SW Parade	Svalof Weibull, Sweden	Green	220	SL	70	F	+
44	Swing	Cebeco Zaden, The Netherlands	Yellow	250	SL	75	G	+
45	Toledo	Cebeco Zaden, The Netherlands	Green	280	SL	70	G	+
46	Trapper	AAFC Morden, Canada	Yellow	140	N	95	P	–
47	Venture	Axel Toft, Denmark	Green	220	SL	75	G	+
48	Victoria	Svalof Weibull, Sweden	Yellow	190	N	84	P	–
49	Whero	Challenge Seeds	Yellow	210	N	110	P	–
50	MP1101	AAFC Morden, Canada	Yellow	200	SL	75	G	+

[a] AAFC = Agriculture and Agrifood Canada; U of S = University of Saskatchewan; CDC = Crop Development Centre
[b] SL = Semi-leafless; N = normal leaf
[c] Lodging reaction rating:
G = Good, typically 1–3 on 1–9 scale (1 = upright; 9 = completely lodged)
F = Fair, typically 4–6 on 1–9 scale
P = Poor, typically 7–9 on 1–9 scale
[d] + = Marker is present; – = marker is absent

Discussion

Several linkage maps have been developed for pea (Ellis et al. 1992; Dirlewanger et al. 1994; Timmerman-Vaughan et al. 1996; Gilpin et al. 1997; Laucou et al. 1998; Weeden et al. 1998; Pilet-Nayel et al. 2002). In order to fully benefit from the existing maps, some alignments among the maps are required. Sharing primer sequences for PCR-based markers or probes for RFLP analysis are a means to align maps. The current map

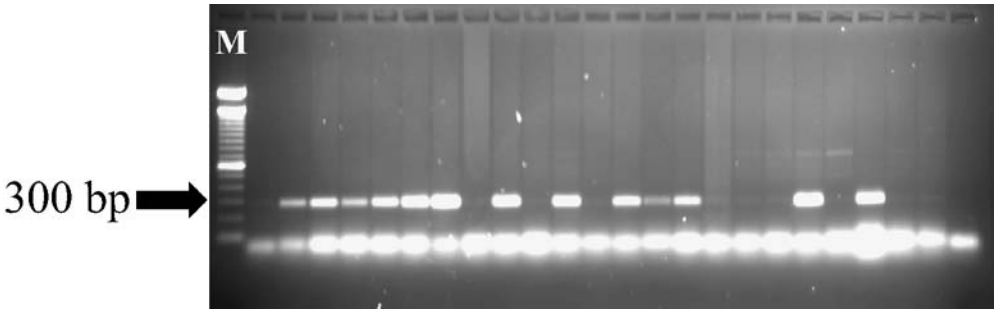

Fig. 3 Segregation of the A001 SCAR marker (300 bp) derived from *cacc4* AFLP band for a subset of 24 RILs. Amplification was done at an annealing temperature of 65°C. The forward and reverse primers for the A001 marker are 5′-*cttcaccatccatagtgtcg*-3′ and 5′-*cacttgcgttccttgtgtg*-3′, respectively. *M*=100 bp DNA ladder

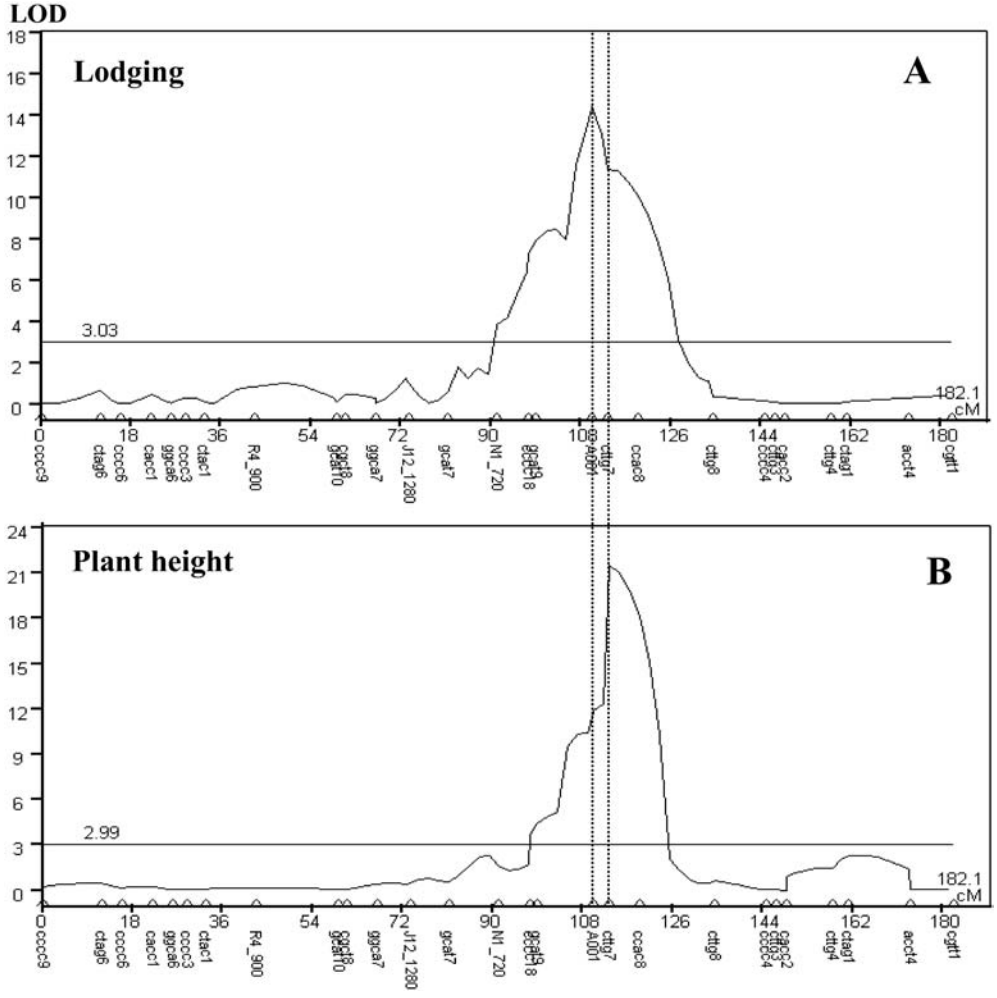

Fig. 4A, B QTL likelihood plots for lodging (**A**) and plant height (**B**) on LG III. *Vertical axes* indicate the LOD score based on CIM using the QTL Cartographer for Windows version 1.01. *Horizontal lines* indicate the LOD thresholds for lodging (3.03) and plant height (2.99), which were empirically determined by performing 1,000 permutations of the data

shares 14 markers with the previous pea linkage map (Gilpin et al. 1997; Laucou et al. 1998). These markers that proved to be transferable to the present population were distributed among six LGs (I, II, III, IV, VI and VII) of the ten LGs identified in the current study, and allowed them to be aligned with the previous pea linkage map. Previously, Pilet-Nayel et al. (2002) also used a subset of markers developed by Laucou et al. (1998) as anchors, and demonstrated the transferability of these markers between the two populations. There was no common marker located on LGs A, B, C and D of the current map; therefore, no alignment was made between the latter LGs with the previous map.

Pea contains seven pairs of chromosomes (2n=2*x*=14); consequently only seven LGs would be expected from the current linkage map. The presence of three additional LGs

in the current map suggests that fragments linking these groups are missing. The current map spans approximately 1,274 cM that is comparable with the previous map (1,330 cM) developed using a combination of AFLPs, RAPDs and RFLPs (Gilpin et al. 1997). However the current map is still larger than the estimated size of pea linkage map (700–800 cM) which was based on the frequency of chiasmtaa per meiosis (Hall et al. 1997). Several factors including population size and the number of markers used in the analysis may contribute to the differences in map coverage on different populations. Laucou et al. (1998) suggested that the difference in linkage intensity among different crosses may also be responsible for the differences in map coverage.

The current study identified major loci associated with lodging resistance and plant height on LG III. A number of morphological and physiological characters that affect stem characters have previously been mapped on LG III. These included the dwarfing gene (*le*) and the flowering gene (*Dne*; Rameau et al. 1998). The dwarfism is caused by the *le* mutation that impaired the conversion of GA_{20} to the active GA_1, resulting in short internodes (Sherriff et al. 1994). The *Dne* gene also affects the branching pattern, such as the day neutral plants usually produced single stems, whereas photoperiod-sensitive plants tend to produce basal lateral branches under short days (Murfet and Reid 1993). In the previous map, the *Le* locus was located at the far end of LG III (Rameau et al. 1998), whereas in the current map the loci that were strongly associated with lodging resistance and plant height, respectively, were located relatively in the middle of LG III. Furthermore, the current map also suggested that the major loci for lodging resistance and plant height were located separately from the *Dne* locus, which in the previous map was located close to anchor marker J12_1280 (Rameau et al. 1998). It may be possible that the markers identified in the current study are linked to a different gene(s) that affects stem properties resulting in improved lodging resistance. More common markers between the two populations are needed to determine the exact orientation of *le* or *dne* loci on LG III of the current map.

Lodging, plant height and reaction to mycosphaerella blight are highly correlated in the current population. In general, the correlation values obtained in the current study were comparable to those reported previously for pea. For example, Dirlewanger et al. (1994) reported significant correlation between plant height and the number of nodes (r=0.55; P<0.001), and between number of nodes and resistance to *Ascochyta pisi* (r=–0.26; P<0.01). The current study revealed a negative correlation between lodging and plant height (r=–0.59; P<0.001). The lodging-resistant parent (Carneval) is also taller than the other parent (MP1401). The CIM procedure identified closely linked loci for lodging and plant height on LG III. Lodging had a QTL with the maximum logarithm of odds ratio (LOD) value at the *cacc4* locus, whereas plant height had the maximum LOD value at the *cttg7* locus (Fig. 4). The MP1401 parent contributed the allele to increased lodging at this region, whereas Carneval contributed the allele that increased plant height. These conditions may be responsible for the negative correlation between lodging and plant height.

Lodging and reaction to mycosphaerella blight were positively correlated (r=0.35; P<0.01), which suggested that increased resistance to the disease might be obtained with genotypes that are less-prone to lodging. A common QTL at the *acct1* locus was found to be associated with both lodging and reaction to mycosphaerella blight on LG VI (Fig. 1; Table 2). The MP1401 allele increased lodging and susceptibility to mycosphaerella blight at the *acct1* locus. This locus may contribute to the correlation between lodging and reaction to mycosphaerella blight. A similar finding was reported in soybean for which a marker locus on LG C2 was significantly associated with the reaction to sclerotinia stem rot, plant height and greater lodging (Kim and Diers 2000).

A larger number of QTLs for reaction to ascochyta blight in a F_2 population of pea, derived from a cross between a moderately resistant breeding line 3148-A88 and a susceptible cultivar Rovar, were recently reported by Timmerman-Vaughan et al. (2002). These QTLs were distributed over seven LGs including I, II, III, IV, V, VII and A. A QTL with moderate effect was located near the anchor locus p628 on LG IV (Timmerman-Vaughan et al. 2002). In the current population, one of the three QTLs, detected for the reaction to mycosphaerella blight was also located on LG IV on the region relatively close to the p628.HinFI locus (Fig. 1). It is possible that there is a common QTL for resitance to mycosphaerella blight complex on LG IV in the two populations. Recently, Pilet-Nayel et al. (2002) also reported a major QTL for resistance to Aphanomyces root rot on LG IVb that explained 47% of the phenotypic variation.

The results of the current study demonstrated that the majority (seven out of eight) of the QTLs for lodging resistance, plant height and reaction to mycosphaerella blight, were detected in more than one environment. Thus, these QTLs were relatively consistent across environments. However, the strength of the effect of these QTLs as reflected by the amount of phenotypic variation they accounted for and their additive genetic effects, varied depending on the environments. For example, the *cacc4* locus that accounted for 47% of the phenotypic variation for lodging in the mean environment had the largest effects (47% to 51% of phenotypic variation) at Morden in all 3 years of trial. In contrast, none of the QTLs that were associated with lodging in the mean environments was detected at Saskatoon in 1998. This apparent anomaly may be attributed to environmental conditions such as the low precipitation during the growing season at Saskatoon in 1998, where the lodging-resistant and lodging-susceptible RILs performed similarly. In general, the results of the present study were consistent with the previous results on pea (Pilet-Nayel et al. 2002), common bean (Tar'an et al. 2002) and soybean (Lee et al. 1996) that QTLs with major effects are more readily detected under different environments than those

with small effects. It is not known if the restriction of QTLs with small effects to only one environment is related to gene expression or is indicative of sampling variation.

The current study identified the most-likely positions of QTLs for lodging resistance, plant height and reaction to mycosphaerella blight in field pea. Molecular markers linked with these QTLs were identified. These markers should be useful for breeders wishing to use molecular tools for selection. Our survey using the A001 marker for lodging resistance on a collection of commercial pea varieties showed that the presence or absence of the marker corresponded well with the known lodging reactions; therefore, selection for lodging-resistant genotypes can be done indirectly using the A001 marker.

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