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ORIGINAL PAPER

Identification of genome regions controlling cotyledon, pod wall/seed coat and pod wall resistance to pea weevil through QTL mapping

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Abstract Pea weevil, *Bruchus pisorum*, is one of the limiting factors for field pea (*Pisum sativum*) cultivation in the world with pesticide application the only available method for its control. Resistance to pea weevil has been found in an accession of *Pisum fulvum* but transfer of this resistance to cultivated pea (*P. sativum*) is limited due to a lack of easy-to-use techniques for screening interspecific breeding populations. To address this problem, an interspecific population was created from a cross between cultivated field pea and *P. fulvum* (resistance source). Quantitative trait locus (QTL) mapping was performed to discover the regions associated with resistance to cotyledon, pod wall/seed coat and pod wall resistance. Three major QTLs, located on linkage groups LG2, LG4 and LG5 were found for cotyledon resistance explaining approximately 80 % of the phenotypic variation. Two major QTLs were found for pod wall/seed coat resistance on LG2 and LG5 explaining approximately 70 % of the phenotypic variation. Co-linearity of QTLs for cotyledon and pod wall/seed coat resistance suggested that the mechanism of resistance for these two traits might act through the same pathways. Only one QTL was found for pod wall resistance on LG7 explaining approximately 9 % of the phenotypic variation. This is the first report on the development of QTL markers to probe *Pisum* germplasm for pea weevil resistance genes. These flanking markers will be useful in accelerating the process of screening when breeding for pea weevil resistance.

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Introduction

Pea weevil (*Bruchus pisorum*) is one of the most intractable pest problems of cultivated field pea in most parts of the world including North and South America (Brindley 1933; Pesho et al. 1977; Clement et al. 2009), Europe (Marzo et al. 1997; Girsch et al. 1999), the Indian sub-continent (Pajni and Sood 1975), and Australia (Hardie and Clement 2001; Newman 1932; Birks 1965). Field pea producers rely mainly on well-timed contact insecticide spray applications to control pea weevil adults in fields before females lay eggs on pods (Horne and Bailey 1991; O'Keeffe 1992; Clement et al. 2000). Australian registered insecticides provide maximum protection for 7 days in high pest pressure situations (Michael et al. 1993). Therefore, timing of chemical applications to coincide with female oviposition is difficult to achieve and several insecticide applications may be

required to control a prolonged invasion of pea weevils if weevil colonization continues for 2–4 weeks in a pea field (Michael et al. 1990). The development and utilization of pea weevil-resistant cultivars with pod and/or seed resistance would reduce pest control costs and provide an environmentally safe option for the integrated management of this serious pest (Aryamanesh et al. 2012; Clement et al. 2009).

Lack of a reliable pea weevil resistance source in *Pisum sativum* accessions led to the exploration and identification of resistant sources in the secondary gene pool of *Pisum* (Pesho et al. 1977; Hardie 1990, 1995; Clement et al. 1994, 2002; Hardie and Clement 2001) which resulted in the discovery of pod and seed resistance in *Pisum fulvum* accessions (Hardie et al. 1995, 1999; Clement et al. 2002). *P. fulvum* accession ATC113 (PI 595933) has been successfully crossed with *P. sativum* accession Pennant and produced interspecific progenies with resistant lines (Byrne 2005; Byrne et al. 2008; Hardie 1992). The introgression of pea weevil resistance into cultivated field pea was further demonstrated in advanced backcross lines of the original population (Aryamanesh et al. 2012). Therefore, an interspecific hybridization approach has potential for developing pea cultivars with resistance to pea weevil.

Currently, glasshouse bioassay is the most robust technique for phenotyping pea weevil resistance. Despite development of screening methods including glasshouse bioassay (Hardie and Clement 2001) and caesium chloride density separation method (Aryamanesh et al. 2012), no reliable DNA-based molecular marker is available for pea weevil resistance. The objectives of this study were to create an interspecific mapping population segregating for pea weevil resistance and to identify the regions in the pea genome associated with resistance to pea weevil using quantitative trait locus (QTL) mapping. To our knowledge, this is the first such report to discover novel QTLs for pea weevil resistance in the field pea genome using microsatellite markers.

Materials and methods

Plant materials

Pisum sativum cv. Pennant was supplied by The Waite Agricultural Research Institute, South Australia. *P. fulvum* accession ATC113 (PI 595933) was obtained from the Australian Temperate Field Crops Collection, Horsham, Victoria (ATFC).

The pea weevil susceptible *P. sativum* cv. Pennant was crossed with wild pea weevil-resistant *P. fulvum* accession ATC113 in a glasshouse. An interspesific population consisting of 270 F_2 individuals was produced (Byrne 2005; Byrne et al. 2008; Hardie 1992). All F_2 plants were assessed for pea weevil resistance; however, only 188 randomly selected F_2 individuals and two parental lines were used for QTL mapping.

Pea weevil resistance bioassay

All 270 F_2 plants and parental lines were evaluated for seed and pod resistance using the best available screening method, the in situ pod bioassay developed by Hardie and Clement (2001) and Clement et al. (2002) and as described by Byrne et al. (2008). Modifications are described by Aryamanesh et al. (2012) as follows: 20–25 mature pea weevils collected from Medina Field Station from both genders were kept in 1.5 L clear plastic rearing cages (200 × 100 × 75 mm) supplied with organic honey (Pure and Natural Honey, Wescobee Ltd), fresh drinking water, insecticide-free commercial bee pollen (Bee Pack Bee Pollen, Allwest Apiaries) and fresh field pea pods. The cages were maintained in a 25 ± 2 °C growth cabinet with a relative humidity of 60 ± 10 % and 12 h photoperiod. Fresh field pea pods were added to cages after 7 days as egg-laying substrates for the ovipositing females to harvest eggs. Pods were replaced regularly and used as a source of pea weevil eggs. Pennant or commercially grown pesticide-free pea pods were used as the egg-laying substrate for the duration of the in situ glasshouse experiments. Viable mature eggs were examined with a binocular microscope and used for inoculation of field pea pods (Hardie and Clement 2001).

F_2 population and parental lines were grown in a glasshouse. Pods of the F_2 population and parental lines still attached to plants were inoculated with pea weevil eggs using a water-moistened fine-tipped brush. All eggs were examined under binoculear microscope for viability (dark-colored head capsule visible through the egg chorion). Two eggs were placed adjacent to a developing seed on the surface of the pod, with an average of ten seeds (on at least three different pods) per plant inoculated. Pea weevil eggs were securely stuck to the pod and egg hatching and burrowing into the pods were monitored throughout the experiment. Pods were harvested after the plants senesced (within 1.5–2 months) and stored at room temperature (18–25 °C) for approximately 3 months to allow insect development to be completed to the adult stage.

F_2 pods and F_2-derived F_3 seeds were then examined for larval chewing entrance and exit holes. Three levels of pea weevil resistance measurements were used in this study for QTL mapping as described by Simmonds et al. (1989) and adapted by Byrne et al. (2008). These included cotyledon (seed) resistance, pod wall/seed coat resistance and pod wall resistance. Cotyledon (seed) resistance was calculated as the percentage of seeds with no exit holes but with the seed coat entry puncture holes. Pod wall/seed coat

resistance was calculated as the percentage of the number of seed coat puncture holes versus the number of pod wall puncture holes. Pod wall resistance was measured as the percentage of the number of larval puncture holes versus the number of pea weevil eggs placed on the pod. Puncture hole(s) were always given a numerical value of either 0 (absence) or 1 (presence) regardless of the number of entry holes, as only one pea weevil emerges from a single seed as described by Byrne et al. (2008).

Molecular mapping

Young leaves were collected from parental lines and F_2 plants. DNA extraction was conducted using Nucleon Phytopure Plant DNA Extraction Kit (GE Healthcare) with 0.1 mg of fresh leaf material according to the manufacturer's instructions. Microsatellite primers developed by Agrogene Inc. (Moissy-Cramayel, France) and Burstin et al. (2001) were synthesized with generic non-complementary nucleotide sequences at their 5′-end (forward primer tag 5′ ACGACGTTGTAAAA 3′and reverse primer tag 5′ CATTAAGTTCCCATTA 3′) as described by Hayden et al. (2008). Locus-specific primers were prepared by mixing equimolar amounts of appropriate forward and reverse primer in ddH_2O. Two generic tag primers (dye-labeled tagF 5′ ACGACGTTGTAAAA 3′ and unlabeled tagR 5′ CATTAAGTTCCCATTA 3′) were also synthesized which had the same sequence as the non-complementary nucleotide in locus-specific primers. The tagF primer was fluorescently labeled at its 5′-end with VIC, FAM, NED or PET dyes (Applied Biosystems, Warrington, UK). The amplification of field pea SSR markers was performed using multiplex-ready PCR as described by Hayden et al. (2008). SSR scoring was performed using Gene Mapper v3.7 software (Applied Biosystems). Dominant SSR markers that only amplified in the maternal parent were suffixed by 'NP' (null paternal) and the paternal dominant markers were suffixed by 'NM' (null maternal) as described previously by Aryamanesh et al. (2010). Markers that detected an additional locus were distinguished using the molecular weight of the field pea parent allele in parentheses at the end of the locus name.

Statistical analyses and genetic mapping

Chi square (χ^2) analyses were performed to determine segregation of SSR markers in the F_2 population for goodness of fit to the expected 1:2:1 and 3:1 ratios. Genetic linkage mapping was conducted using MapManager QTX version 0.30 (Manly et al. 2001) using the Kosambi mapping function with a minimum threshold LOD (logarithm of odds) score of 3 and a maximum recombination of 25 %. Linkage groups were assigned based on some common markers with Loridon et al. (2005). QTL mapping was carried out using MultiQTL software, version 2.5 (http://www.multiqtl.com), with parameters previously described by Aryamanesh et al. (2010) as follow. Three hypotheses (H0, H1 and H2) were tested for QTL detection including; (H0), the QTL has no effect on a trait; (H1), a single QTL on a chromosome has an effect on a trait; and (H2), two linked QTLs on a chromosome have an effect on a trait. Two sub-models for H1 including equal or unequal variance, and four sub-models for H2 including equal or unequal variance, with or without epistasis were tested by running 3,000 permutation tests. Where the models were significantly different ($P < 0.05$), the model with the highest LOD score was selected for QTL detection. Whenever there was no significant difference between models ($P > 0.05$), the simplest model with equal variance and no epistasis (in the case of two linked QTL) were selected. 5,000 bootstrap repeats were run for the selected model to estimate the parameters and their standard deviations.

Results

Pea weevil responses of parental lines and the F_2 population

Pisum sativum accession ATC113 showed 100 % cotyledon resistance with no pea weevil seed emergence or exit holes, while the cultivated parental line Pennant had a mean seed emergence of 91.3 %. The distribution of the F_2 population was continuous but skewed towards the susceptible parent Pennant with an average of 79.9 % susceptibility (Fig. 1a). Only five F_2 plants showed 100 % cotyledon resistance comparable to wild accession ATC113.

For pod wall/seed coat susceptibility, there was no significant difference between ATC113 and Pennant with 97.4 and 97.9 %, respectively ($P > 0.05$). The distribution of the F_2 population was continuous and some individuals with strong pod wall/seed coat resistance were observed in the F_2 population ranging from 0.0 to 100 % (Fig. 1b).

Pod wall susceptibility of parental lines was significantly different—ATC113 with a mean of 62.4 % (ranging from 86.2 to 100 %) compared to a mean of 74.3 % (ranging from 90.2 to 100 %) for the susceptible parent Pennant ($P < 0.01$). Transgressive segregation was observed for pod wall susceptibility in the F_2 population ranging from 0.0 to 100 % (Fig. 1c). Only four F_2 lines had less than 20 % pod wall susceptibility, while most of the lines were in the same category as parental lines.

Correlations of traits

Pod wall/seed coat susceptibility and cotyledon susceptibility were positively correlated ($r = 0.24$, $P < 0.001$).

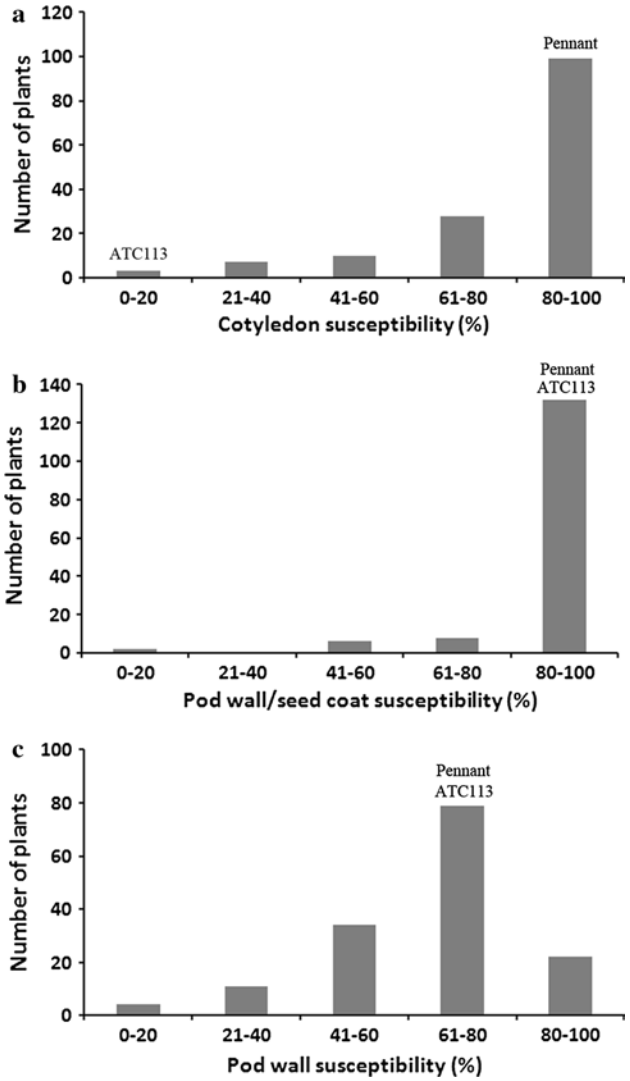

Fig. 1 Distribution of F_2 population in response to pea weevil in **a** cotyledon, **b** pod wall/seed coat and **c** pod wall levels

There was a significant negative correlation between pod wall susceptibility and pod wall/seed coat susceptibility ($r = -0.20$, $P < 0.01$). Correlation between pod wall susceptibility and cotyledon susceptibility was, also, significant ($r = 0.15$, $P < 0.05$). However, these correlations were at a very low level with best correlation accounting for less than 6 % of the variation ($r = 0.24$).

Genetic linkage map of field pea

The linkage map comprised 155 microsatellite markers in eight linkage groups covering 2,685.8 cM of the field pea genome (Fig. 2). 49 markers remained unlinked. The average spacing between microsatellite markers was 17.3 cM.

The observed and expected marker allele segregation ratios were compared using Chi square tests ($P < 0.05$). Of the 155 markers mapped in the linkage groups, 77 (49 %) had segregation ratios that significantly deviated from the expected 1:2:1 ratio for co-dominant markers and the 3:1 ratio for dominant markers in the F_2 population. Of the 77 distorted markers, 19 were distorted towards the *P. fulvum* accession ATC113 parent located on LG1, LG6 and LG8 and the rest (58 markers) were distorted towards *P. sativum* parent distributed mainly on LG7.

Mapping cotyledon resistance

Three major QTL regions (on LG2, LG4 and LG5) and five minor QTL regions (on LG7, LG3, LG4 and LG5) significantly associated with pea weevil cotyledon resistance were detected. A major single QTL (COR2) associated with pea weevil cotyledon resistance was detected on LG2 (LOD = 12.1, $P = 0.0297$) with flanking markers AA179 (4.9 cM) and AA189 (14.2 cM) explaining 42.1 % of the phenotypic variation in the population (Table 1). The second major QTL (COR4b) was found on LG4 explaining 20.9 % of the phenotypic variation with flanking markers AB28 and AA297 (Table 2). Two linked QTLs, COR5a and COR5b were found on LG5 explaining 16.7 and 7.7 % of the phenotypic variation, respectively. Another four minor QTLs were detected for cotyledon resistance on LG7 (COR7), LG3 (COR3a, COR3b) and LG4 (COR4a) explaining 1, 3.7, 1.2 and 3.2 % of the phenotypic variation, respectively. In all, the QTLs explained 96.5 % of the phenotypic variation for pea weevil cotyledon resistance. Dominance played an important role in pea weevil cotyledon resistance, in particular, for the QTL located on LG7 and LG2 (COR7 and COR2) (Table 1). Epistatic effects were also important in the control of cotyledon resistance to pea weevil in the mapping population.

Mapping pod wall/seed coat resistance

In the case of pod wall/seed coat resistance, two major QTLs were found on LG2 (LOD = 9.4, $P = 0.045$) and LG5 (LOD = 126.9, $P = 0.002$). A single QTL on LG2 (SCR2) explained 39.0 % of phenotypic variation with flanking markers AA179 (2.6 cM) and AA189 (16.6 cM) (Table 1). The linked QTL on LG5–SCR5a and SCR5b– explained 21.4 and 4.2 % of phenotypic variation, respectively (Table 2). SCR5a was located in interval 3 with flanking markers AD280 and AA399 while SCR5b was located in interval 8 with flanking markers AD160 and A5. Two other minor linked QTLs, SCR7a and SCR7b were detected on LG7 in intervals 51 and 60 explaining only 2.1 and 3.1 % of phenotypic variation (Table 2). Pod wall/seed coat resistance QTLs explained a total of 70.7 % of phenotypic variation. Both additive/dominant and epistatic effects

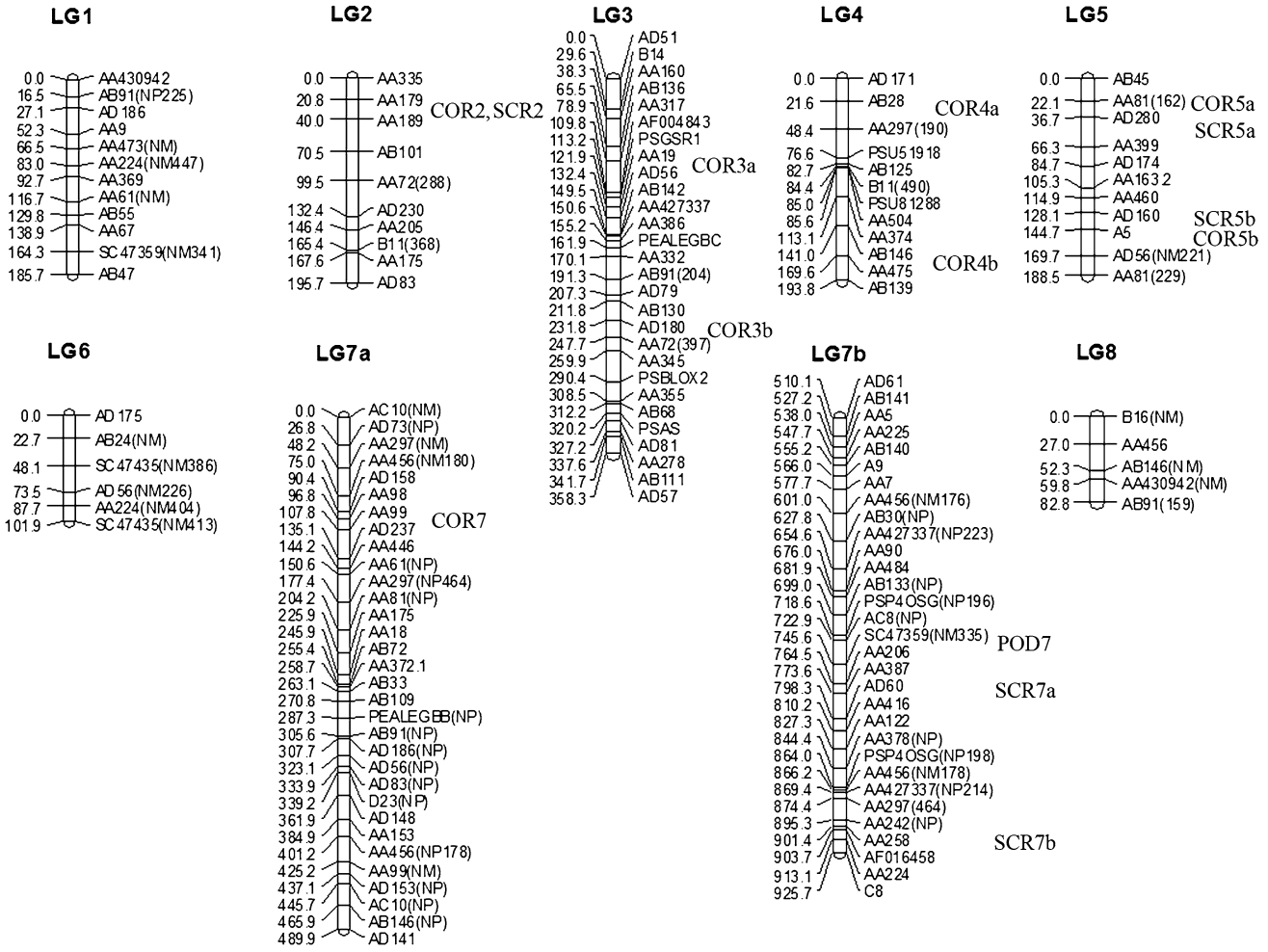

Fig. 2 The genetic linkage map of *Pisum* based on the interspecific F_2 population, derived from a cross between *Pisum sativum* (cv. Pennant) and *Pisum fulvum* (accession ATC113) using microsatellite markers. The locations of QTLs are shown in front of the markers for cotyledon resistance (COR), pod wall/seed coat resistance (SCR) and pod wall resistance (POD)

were important in the control of pod wall/seed coat resistance to pea weevil.

Mapping pod wall resistance

A single QTL was detected for pod wall resistance on LG7 (LOD = 6.4, $P = 0.0443$). This QTL (POD7) explained only 8.8 % of the phenotypic variation with flanking markers SC47359 and AA206 with 8.7 and 10.2 cM apart from the QTL region, respectively.

Discussion

In this study, we identified QTLs for pea weevil resistance that included cotyledon resistance, pod wall/seed coat resistance and pod wall resistance. This is the first mapping analysis for pea weevil resistance in field pea. We also provided evidence that cotyledon resistance and pod wall/seed coat resistance are controlled by three and two major QTLs, respectively; a few other QTLs had minor effects on the traits. There was a positive correlation between pod wall/seed coat resistance and cotyledon resistance albeit at a low level.

The polygenic inheritance for cotyledon resistance found in this study was further confirmed by three major QTLs for cotyledon resistance located on LG2, LG4 and LG5. These results agree with the work of Byrne et al. (2008) who reported three major recessive alleles controlling seed resistance. The QTL located on LG2, COR2, had a major effect on the trait, accounting for 42.1 % of phenotypic variation. QTLs COR4b and COR5a located on LG4 and LG5 explained 20.9 and 16.7 % of the phenotypic variation, respectively. All three major QTLs for cotyledon resistance (COR2, COR4b and COR5a) accounted for around 80 % of phenotypic variation. These results suggest that we retained

Table 1 Detection of QTLs for pea weevil resistance in the interspecific F_2 mapping population (*Pisum sativum* × *Pisum fulvum*)

QTL characteristics	Cotyledon resistance	Cotyledon resistance	Pod wall/seed coat resistance	Pod wall resistance
QTL name	COR2	COR7	SCR2	POD7
LG (interval)[a]	2 (2)	7a (3)	2 (2)	7b (48)
P[b]	0.0297	0.0117	0.0453	0.0443
LOD[c]	12.1(4.1)	11.8(3.8)	9.4 (6.8)	6.4 (2.6)
Position[d]	31.0 (26.4)	151.4 (55.2)	28.2 (35.1)	881.3 (35.4)
PEV[e]	0.718 (0.123)	0.747 (0.093)	0.547 (0.208)	0.625 (0.141)
PEV (additive)	0.379 (0.142)	0.012(0.058)	0.434 (0.173)	0.145 (0.161)
Response mean	58.7 (8.489)	89.2 (4.469)	88.01 (10.97)	63.24 (12.02)
Effect(additive)	42.11 (21.12)	−1.0 (8.413)	39.91 (26.24)	8.857 (23.6)
Effect(dominant)	30.12 (10.03)	−45.5 (14.46)	8.451 (11.75)	−15.94 (30.25)
Flanking markers	AA179 (304 bp) AA189 (232 bp)	AA99 (210 bp) AD237 (160 bp)	AA179 (304 bp) AA189 (232 bp)	SC47359 (335 bp) AA206 (220 bp)
Donor parent	ATC113	Pennant	ATC113	ATC113

a linkage group and interval within LG associated with the quantitative trait, *b* probability values from 3,000 permutation analyses testing the presence of a QTL, *c* maximum LOD value for the given interval, *d* position (cM) of maximum LOD value within the interval measured from the first marker in the linkage group (0 cM), *e* proportion of explained phenotypic variation by the putative QTL, *c*–*e* estimated by 5,000 bootstrap tests. Estimates obtained with MultiQTL software and corrected according to distance obtained from MapManager. Standard deviations (SD) and allele sizes are shown in parentheses

all three major genes from wild *P. fulvum* in the F_2 population. Polygenic inheritance of other *Bruchid* species has been reported in other legume species such as black gram (Souframanien et al. 2010), cowpea (Adjadi et al. 1985; Rusoke and Fatunla 1987), common bean (Kornegay and Cardona 1991) and mungbean weevil (Mei et al. 2009). The proportion of explained phenotypic variation explained by cotyledon resistance QTLs in our study was higher than other reported QTLs on black gram with around 67 % (Souframanien et al. 2010), on maize with 28–47 % (Garcia-Lara et al. 2009, 2010), on mungbean with 55 % (Mei et al. 2009). In the estimation of genetic effects, the observed decrease in variance explained by QTLs may be attributed to two confounded factors including environmental and genotypic sampling (Utz et al. 2000). As observed in soybean, QTL detection could be hampered in the population due to isolated regions of abnormal segregation (Rector et al. 2000).

LG2 seems to play an important role in pea weevil resistance since the major QTL for pod wall/seed coat resistance (SCR2) was also located in the same region explaining 21.4 % of the variation. This linkage between cotyledon resistance and pod wall/seed coat resistance was also reflected in the high correlation of phenotypic data for these two traits. The second major QTL for pod wall/seed coat resistance (SCR5a) was located on LG5 explaining 21.4 % of the phenotypic variation which was in close proximity to the QTL for cotyledon resistance, COR5a. These data suggest that pod wall/seed coat resistance and cotyledon resistance may employ similar mechanisms to resist pea weevil. All QTLs for pod wall/seed coat resistance explained around 70 % of phenotypic variation suggesting considerable environmental effects. Epistasis effect was also important for both pod wall/seed coat and cotyledon resistance as observed by Byrne et al. (2008).

Pod wall resistance was mostly influenced by environmental effects as only a single QTL (POD1) with a relatively minor effect was found (8.8 %). POD1 was flanked by an AFLP-based marker, SC47359 which was previously developed in this population using a multivariate statistical approach that was applied to bulked segregants (Byrne 2005; Byrne et al. 2002). A mechanism for pod resistance in peas appears to be supported by the neoplastic pod gene (*Np*); pea lines with this gene form callus in response to the presence of pea weevil eggs on pods that reduces larval entry into the pod (Hardie 1990; Berdnikov et al. 1992; Doss et al. 2000). Doss et al. (2000) reported that lines with the *Np* gene had a lower rate of weevil-infested seed (62.2 %) compared to that in a susceptible line (85.4 %) in a field trial. Although this gene was effective in reducing the infestation ratio by around 23 %, breeders are not certain about the value of the *Np* trait in breeding weevil-resistant cultivars (Clement et al. 2009).

For the first time, we constructed a linkage map for *Pisum* using an interspesific population derived from a cross between cultivated field pea and *P. fulvum* for the genetic study of pea weevil resistance. The total coverage of the linkage map generated in this study was 2,232.4 cM, which is considerably greater than some of the previously reported maps for field pea, which range from 450 to 2,416 cM (McCallum et al. 1997; Laucou et al. 1998; von Stackelberg et al. 2003; Tar'an et al. 2003, 2004; Loridon et al. 2005; Aubert et al. 2006;

Table 2 Detection of two linked QTLs controlling pea weevil resistance in the interspecific F_2 mapping population in field pea

Traits	QTL name	LOD (SD)	LG (interval)	Flanking markers (allele size in bp)		Donor parent	PEV		Parameters of genetic model						
							Total	Additive	M	D	H	E1	E2	E3	E4
Cotyledon resistance															
	COR3a	53.3	3 (8)	AA19 (245)	AD56 (178)	ATC113	0.721	0.259	71.9	3.7 (24.4)	10.4 (18.3)	−1.5	1.3	5.2	−7.9
	COR3b	(8.3)	3 (18)	AD180 (183)	AA72 (397)	Pennant	(0.315)	(0.244)	(12.1)	1.2 (22.5)	8.3 (19.2)	(12.5)	(19.8)	(22.2)	(24.8)
	COR4a	58.3	4 (2)	AB28 (385)	AA297 (190)	ATC113	0.85	0.339	79.1	−3.2 (18.1)	−5.4 (18.6)	−0.2	−0.4	−12.6	−2.6
	COR4b	(8.7)	4 (10)	AB146 (210)	AA475 (199)	ATC113	(0.219)	(0.36)	(9.2)	20.9 (20.9)	5.7 (12.9)	(11.8)	(15.4)	(19.8)	(27.3)
	COR5a	53.0	5 (2)	AA81 (162)	AD280 (230)	ATC113	0.808	0.328	72.1	16.4 (19.1)	−1.3 (17.5)	3.9	−2.6	−9.9	−3.6
	COR5b	(7.8)	5 (9)	A5 (360)	AD56 (221)	ATC113	(0.233)	(0.275)	(9.0)	7.7 (23.7)	14.6 (15.0)	(13.2)	(14.5)	(27.7)	(23.2)
Pod wall/seed coat resistance															
	SCR5a	126.9	5 (3)	AD280 (230)	AA399 (208)	ATC113	0.731	0.467	78.9	21.4 (25.9)	14.2 (17.5)	2.19	−9.2	−0.6	−19.8
	SCR5b	15.1	5 (8)	AD160 (250)	A5 (360)	ATC113	(0.339)	(0.374)	(13.4)	4.2 (34.9)	14.3 (19.7)	(13.9)	(17.8)	(17.5)	(29.5)
	SCR7a	119.3	7b (51)	AD60 (210)	AA416 (395)	Pennant	0.799	0.199	94.3	−2.1 (13.9)	−5.1 (12.2)	0.3	0.4	−2.3	−0.2
	SCR7b	17.0	7b (60)	AA258 (258)	AF016458 (189)	ATC113	(0.289)	(0.353)	(6.3)	3.1 (13.7)	−2.3 (11.5)	(7.8)	(11.6)	(13.1)	(25.7)

LOD maximum LOD score for the given interval, *PEV* proportion of variability explained by the putative QTLs, *M* response mean, *SD* standard deviation are shown in parentheses, *D* additive effect of the allele in the respective locus, *H* dominant effect of the allele in the respective locus

E1 effect of the interaction between loci, the epistatic interaction in Homologous QTL1 × Homologous QTL2

E2 effect of the interaction between loci, the epistatic interaction in Homologous QTL1 × Heterologous QTL2

E3 effect of the interaction between loci, the epistatic interaction in Heterologous QTL1 × Homologous QTL2

E4 effect of the interaction between loci, the epistatic interaction in Heterologous QTL1 × Heterologous QTL2

Ubayasena et al. 2010, 2011). The average distance between markers in the genetic linkage map described in this study was 14.4 cM which is relatively greater than the previously reported average distances in intraspecific populations ranging from 2 to 12 cM. The relatively high linkage map coverage and frequency of polymorphic SSR markers observed in this interspecific population may suggest a diverse genetic relationship of the parental lines.

Distortion of markers was relatively high (49 %) in this study. This phenomenon has been reported to a lesser extent in other intraspecific populations of *Pisum fulvum* (Barilli et al. 2010; De Martino et al. 2000) and interspecific populations in the *Cicer* genome (Aryamanesh et al. 2010; Collard et al. 2003; Flandez-Galves et al. 2003; Tekeoglu et al. 2002). The high number of distorted markers in this interspecific field pea population might be attributed to the higher genetic distance/diversity of cultivated pea with *Pisum fulvum* than in chickpea interspecific populations.

Current assays to screen pea weevil resistance including glasshouse bioassay (Hardie and Clement 2001) and caesium chloride density separation method (Aryamanesh et al. 2012) are laborious and time consuming. These methods have limited potential for application in breeding programs. For the first time, we report the development of mapped QTL markers to probe Pisum germplasm for pea weevil resistance genes in breeding programs. These flanking markers could accelerate the process of screening and breeding for pea weevil resistance.

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