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Linkage mapping of quantitative trait loci controlling seed weight in pea (*Pisum sativum* L.)

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Abstract Quantitative trait loci (QTLs) affecting seed weight in pea (*Pisum sativum* L.) were mapped using two populations, a field-grown F_2 progeny of a cross between two cultivated types ('Primo' and 'OSU442-15') and glasshouse-grown single-seed-descent recombinant inbred lines (RILs) from a wide cross between a *P. sativum* ssp. *sativum* line ('Slow') and a *P. sativum* ssp. *humile* accession ('JI1794'). Linkage maps for these crosses consisted of 199 and 235 markers, respectively. QTLs for seed weight in the 'Primo' × 'OSU442-15' cross were identified by interval mapping, bulked segregant analysis, and selective genotyping. Four QTLs were identified in this cross, demonstrating linkage to four intervals on three linkage groups. QTLs for seed weight in the 'JI1794' × 'Slow' cross were identified by single-marker analyses. Linkage were demonstrated to four intervals on three linkage groups plus three unlinked loci. In the two crosses, only one common genomic region was identified as containing seed-weight QTLs. Seed-weight QTLs mapped to the same region of linkage group III in both crosses. Conserved linkage relationships were demonstrated for pea, mungbean (*Vigna radiata* L.), and cowpea (*V. unguiculata* L.) genomic regions containing seed-weight QTLs by mapping RFLP loci from the *Vigna* maps in the 'Primo' × 'OSU442-15' and 'JI1794' × 'Slow' crosses.

Key words Comparative QTL mapping · RFLP · RAPD · *Pisum sativum* L. · Genetic maps

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Introduction

Seed size in grain legumes has been used as a model system for quantitative genetics since the early 1900s. The pioneering studies of Johannsen (1903) on bean seed provided the first evidence that quantitative variation arises through the interaction of genetic and environmental factors. The report by Sax (1923) of linkage between seed colour and weight in *Phaseolus* was the first observation of linkage between a genetic marker and a quantitative trait. Inheritance of seed size in pea (*Pisum sativum* L.) was studied extensively by Tschermak (cited by Wellensiek 1925) who proposed four genes, *sg-1* to *sg-4*. Seed size is an economically important trait in pea, and both the degree and uniformity of size affect crop marketability. Studies have typically indicated high heritability of seed weight in grain legumes including chickpea (Niknejad et al. 1971), mungbean (Imrie et al. 1985), cowpea (Leleji 1975), and lentil (Abbo et al. 1992). In studies of pea, Gupta et al. (1984) also estimated a high heritability for seed weight.

The availability of linkage maps based on abundant DNA markers has vastly improved the ability to identify and characterize quantitative trait loci (QTLs) (reviewed in Tanksley 1993). Seed-weight QTLs that have been identified and characterized in legume crops include genes in mungbean and cowpea (Fatokun et al. 1992), soybean (Mansur et al. 1993), and lentil (Tahir et al. 1994, Abbo et al. 1992). Conserved regions of the mungbean and cowpea genomes were found to contain seed-weight QTLs (Fatokun et al. 1992), providing the first evidence for orthologous QTLs in plants. This finding supports the notion that the development of comparative linkage maps may provide the basis for extrapolating QTL mapping results between related species. Therefore, DNA marker-QTL associations detected in one species may be of use in cultivar improvement of another species.

In the present study we report the mapping of QTLs affecting seed weight in pea based on the results of two

crosses: a wide cross between a *P. sativum* ssp. *sativum* type and a *P. sativum* ssp. *humile* type; and a cross between two cultivated types (*P. sativum* ssp. *sativum*). Furthermore, we provide evidence for the conservation of linkage relationships in genomic regions containing seed-weight QTLs between *P. sativum* and two *Vigna* species, mungbean and cowpea.

Materials and methods

Plant material

Two *P. sativum* genotypes were crossed: 'OSU442-15', a small-seeded Prussian-type (Baggett and Hampton 1977), and 'Primo' (female parent), a large seeded marrowfat (Cebeco, Lelystadt, The Netherlands). F_1 plants ($n = 89$) were grown in a greenhouse, and up to 40 selfed seeds were collected per plant. The resulting F_2 plants were grown in individual family plots in the field at Lincoln, New Zealand, in spring 1991, along with five plots of each parent, in a completely randomized design. Plants showing obvious symptoms of viral infections affecting pea seed size (alfalfa mosaic virus, cucumber mosaic virus, pea seed-borne mosaic virus) were discarded. The remaining 550 F_2 plants were individually thershed after they had dried off. Twenty seed samples were weighed after further air drying. F_3 descendants for the isolation of DNA for RFLP analysis were grown in the greenhouse.

Reciprocal crosses were made between 'Primo' and 'OSU442-15' using greenhouse-grown plants. Mean seed weights of parental:F_1 seed were determined on air-dried seeds harvested after plants had dried off. In spring 1993, reciprocal F_1 seed was sown in the field at Lincoln, New Zealand, in three blocks of 25 seeds/plot each way (six blocks total), along with blocks of 'Primo' and 'OSU442-15'. Seeds were harvested from individual plants and seed weights determined as described above.

The population of 51 single-seed-descent recombinant inbred lines (RILs) (Weeden et al. 1993b) was produced by crossing *P. sativum* ssp. *humile* accession 'JI1794' with *P. sativum* ssp. *sativum* accession 'Slow' (NFW, Cornell University). Mean seed weights were determined using seed collected from plants grown in separate pots in the greenhouse in Geneva, N.Y.

DNA marker methods

Isolation of DNA and analysis of RAPDs and RFLPs were carried out as described by Timmerman et al. (1993). AFLPs were analyzed following the methods described in the European patent application (Zabeau 1992) and according to Pickering et al. (1995), using DNA prepared for RFLP analysis. The linker-adapter and primer sequences used were as described in the patent for the *PstI*/*MseI* double-digestion example. DNA for RAPD analysis was isolated from individual field-grown F_2 plants while DNA for RFLP analysis was isolated from young leaves pooled from five F_3 descendants. RAPD primers were obtained from Operon Technologies, Inc. (Alameda, Calif. USA). Probes for RFLP analysis were either random anonymous clones from a cDNA library constructed in our laboratory using poly (A +)RNA from etiolated seedlings, or probes kindly donated by other laboratories, including genes of known function.

Bulked segregant analysis

Bulked segregant analysis (Michelmore et al. 1991) was carried out as described by Timmerman et al. (1994). DNA bulks were constructed using DNA isolated for RAPD analyses from F_2 plants with extreme heavy ($n = 16$) and light ($n = 15$) mean seed weights. The primers used in the PCR reactions were from kits A–N obtained from Operon Technologies Inc. (Alameda Calif. USA).

Linkage-map construction

Linkage maps for DNA marker loci segregating in the 'Primo' × 'OSU442-15' F_2 population and 'JI1794' × 'Slow' RILs were computed using MAPMAKER/EXP version 3.0 (Lander et al. 1987; Lincoln et al. 1992). The 'Primo' × 'OSU442-15' map was based on 102 F_2 progeny from four F_1 families, while the 'JI1794' × 'Slow' map was based on 51 single-seed-descent RILs. LOD thershold values for assigning markers to linkage groups were set at LOD $\geqslant 3.0$ for the 'Primo' × 'OSU442-15' F_2 population and at LOD $\geqslant 4.0$ for the 'JI1794' × 'Slow' RILs.

QTL mapping

QTLs in the 'Primo' × 'OSU442-15' population were detected by interval mapping using MAPMAKER/QTL version 1.1b (Lincoln et al. 1993). The significance level required to declare a QTL was set at LOD $\geqslant 2.0$. The program was run with no fixed QTLs and with unconstrained genetics. The gene action of each QTL was estimated by calculating the additive effect (a), the dominance deviation (d) and the degree of dominance (d/a) using MAPMAKER/QTL software (Paterson et al. 1991). In the RILs, putative seed-weight QTLs were predicted using single-marker analysis with significant associations ($P < 0.01$) detected by t-tests. Empirical threshold values for declaring QTLs were calculated using permutation tests (Churchill and Doerge 1994), carrying out 1000 genome scans using permuted trait data. This number of permutations is the number recommended for estimating critical values at $\alpha = 0.05$. The comparison-wise threshold is a 95% critical value for testing for a QTL at a particular point on the map, obtained from the $100(1-\alpha)$ percentile of the ranked test statistic scores at that point for all genome scans using permuted data. The experiment-wise threshold is a more conservative 95% critical value which is obtained from the $100(1-\alpha)$ percentile of the maximum test statistics obtained from each genome scan. The experiment-wise threshold value, therefore, is the critical value for QTL detection anywhere in the genome while keeping the overall type-I error rate equal to α or less.

Results

Seed-weight phenotype analysis

The seed-weight frequency distributions for the 'Primo' × 'OSU442-15' and 'JI1794' × 'Slow' populations are shown in Fig. 1. The seed collected from F_2 progeny of the 'Primo' × 'OSU442-15' cross displayed a normal distribution, suggesting polygenic inheritance. The population included plants that produced seeds whose mean weight values were as large as the heavy seeded parent and as small as the light-seeded parent. The seed collected from the 'JI1794' × 'Slow' RILs also produced a continuous frequency distribution (Fig. 1), indicative of polygenic inheritance.

The effect of maternal genotype on mean seed weight was investigated using F_1 seed collected from maternal plants after reciprocal crosses were made between 'Primo' and 'OSU442-15'. The results (Table 1) show a strong maternal effect on F_1 seed weight. The mean weights of F_1 seed and selfed seed obtained from the same parental line were very similar, although a slight influence of the male parent on seed-weight phenotype was apparent. This bias was significant ($P < 0.001$) for the F_1 seed produced from the cross 'OSU442-15' (♀) 'Primo' (♂). When reciprocal F_1 plants were grown out,

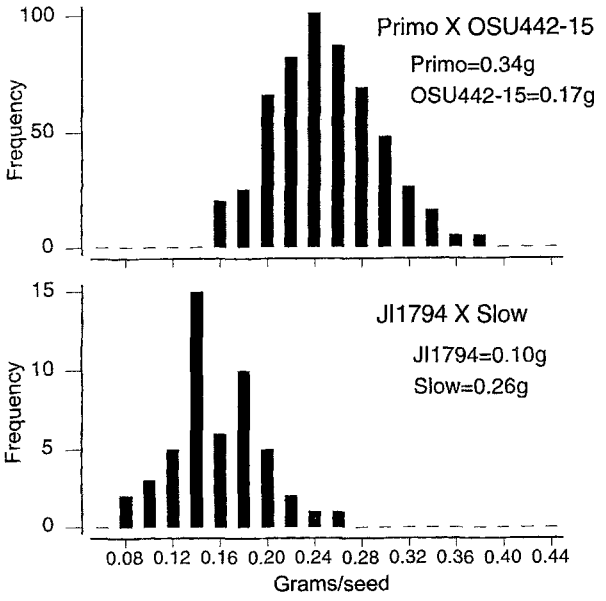

Fig. 1 Frequency distributions for mean seed weight for F_2 progeny of the 'Primo' × 'OSU442-15' cross and RILs from the 'JI1794' × 'Slow' cross. Parental means are indicated

Table 1 Influence of maternal genotype on mean seed weight for 'Primo' × 'OSU442-15' F_1 progeny

Seed source	*n*	Mean[a]	SD[b]
'Primo' (selfed)	110	0.440	0.096
'OSU442-15' (selfed)	338	0.208	0.046
'Primo'♀ × 'OSU442-15' (F_1)	75	0.429	0.081
'OSU442-14'♀ × 'Primo' (F_1)	134	0.227	0.037

[a] Grams/seed
[b] Standard deviation

and the seed from these plants were collected and weighed, no significant differences were observed in the mean seed weight of the resulting F_2 seed ($P = 0.64$ by a two-tailed *t*-test, variances not assumed equal). Davies (1975) also reported a strong materal effect on seed weight in reciprocal crosses of *P. sativum*.

Linkage-map construction

The 'Primo' × 'OSU442-15' map was constructed using 102 F_2 plants. This map consists of 14 linkage groups, containing 101 RFLP, 58 RAPD and 40 AFLP markers. The 14 groups include three small unassigned linkage groups. There were 11 unlinked loci. The total length of the map is 1510 cM (Haldane units). Markers displaying skewed segregation ratios were included in the map. These mapped to two regions: linkage group V in the vicinity of the *r* locus (detected using probe pJAM425) and linkage group VI in the vicinity of GS185 (*Gs-p*). The skewed segregation ratios for markers in the vicinity of GS185 resulted from the elimination of plants showing obvious symptoms of infection by pea seed-borne mosaic virus (PSbMV) in this field-grown population. The 'Primo' × 'OSU442-15' population is segregating for the *sbm-1* locus which conditions resistance to PSbMV.

The 'JI1794' × 'Slow' linkage map consists of 14 linkage groups containing RFLP, RAPD and AFLP markers as well as morphological and isozyme markers. The 14 linkage groups consist of 235 loci and there are 35 unlinked loci. Twelve of the fourteen linkage groups have been assigned to linkage groups on the pea map (Weeden et al. 1993a), and two groups are unassigned. The total length of this map is 1289 cM (Haldane units).

Linkage groups from the 'Primo' × 'OSU442-15' map were identified either by mapping anchor loci that are generally accepted as group-specific markers (Weeden et al. 1993a, b), by mapping RFLPs linked to group-specific markers in another pea map (Ellis et al. 1992), or by demonstrating the linkage of markers on the 'Primo' × 'OSU442-15' map to an anchor locus in the second mapping population, the 'JI1794' × 'Slow' RILs. The 'JI1794' × 'Slow' cross is polymorphic for a number of group-specific anchor loci including morphological markers and allozymes, permitting alignment of both the 'Primo' × 'OSU442-15' and 'JI1794' × 'Slow' maps with the "classical" pea linkage map. As a result, we have identified linkage groups corresponding to all groups of the *P. sativum* genetic map (Weeden et al. 1993a). The details of map construction and linkage group identification will be published elsewhere. One of the linkage groups making up the 'Primo' × 'OSU442-15' map contains markers which have been assigned to both linkage groups III and IV, suggesting that a translocation between the two is present in this cross (data not presented). This linkage group, therefore, has been designated III/IV. Although the 'Primo' × 'OSU442-15' map contains 199 loci at an average saturation of 7.5 cM/marker, some genomic regions are not represented in the map. These were identified by comparing the 'Primo' × 'OSU442-15' map, the 'JI1794' × 'Slow' map, and the *P. sativum* genetic map (Weeden et al. 1993a). In particular, the 'Primo' × 'OSU442-15' map is missing the arm of linkage group I containing the *I* locus, a large segment of linkage group VI between GS185 (*Gs-p*) and RE80 (*Fed-1*), and the distal end of the arm of linkage group VII containing rDNA2. These regions may be represented by the unassigned linkage groups or unlinked loci. Therefore, seed-weight QTLs located in the genomic regions missing from the map may not be detected by the 'Primo' × 'OSU442-15' experiment.

Interval mapping of seed-weight QTLs using 'Primo' × 'OSU442-15' progeny

Three QTLs for dry seed weight were predicted on the basis of interval mapping (LOD $\geqslant$ 2.0) using the population of 102 F_2 plants for which DNA marker genotype/phenotype data were available (Table 2). LOD

Table 2 Summary statistics for seed-weight QTLs detected by interval mapping using 'Primo' × 'OSU442-15'

Linkage group	Interval	LOD	Variance explained	d/a	Mode[a]
III/IV	M27–B08_1250	4.6	26%	−0.003	A
IV	A09_1250–A15_1580	3.2	23%	−0.042	A
V	P445–E07_1100	2.1	13%	−0.685	RA

[a] Possible modes of gene action for each QTL are indicated, based on an analysis of dominance deviation (*d*), additive effect (*a*), and ratio of dominance to additivity (*d*/*a*), as described by Paterson et al. (1991). "A" indicates that the mode of action is most likely to be additive, while "RA" indicates that recessiveness was most likely but that additivity could not be eliminated

plots for these QTLs are presented in Fig. 2. Interval mapping localized seed-weight QTLs to linkage groups III/IV, IV, and a small group, designated Va, identified as containing group-V markers. The critical threshold values needed to declare these QTLs (95% confidence interval) were determined by calculating comparison-wise and experiment-wise empirical threshold values, as described by Churchill and Doerge (1994). The experiment-wise empirical threshold value was LOD = 3.70. This value represents the critical LOD for detecting the presence of a QTL while controlling the overall type-I error rate over the entire genome to $\alpha = 0.05$ or less. The LOD score for the QTL detected on linkage group III/IV (Table 2) exceeded this threshold value. All three QTLs exceed the average comparison-wise critical value of LOD = 2.0.

Together, these three QTLs accounted for 62% of the variance observed in this population of 102 plants. The *d*/*a* ratios (unconstrained genetics) indicated that the QTLs on groups III/IV and IV probably displayed additive modes of inheritance, while the *d*/*a* ratio for the QTL on linkage group Va suggested a recessive mode of inheritance for the 'OSU442-15' allele (Table 2). Single-marker analyses did not reveal significant associations ($P < 0.05$) between seed weight and unlinked loci (data not presented).

Marker identification using extreme-phenotype individuals

Bulked segregant analysis (BSA) was carried out as another strategy to identify genomic regions linked to seed-weight QTLs. DNA bulks contained DNA samples from the 16 heaviest (heavy extreme 2.9%) and 15 lightest (light extreme 2.7%) seeded plants in the 550-

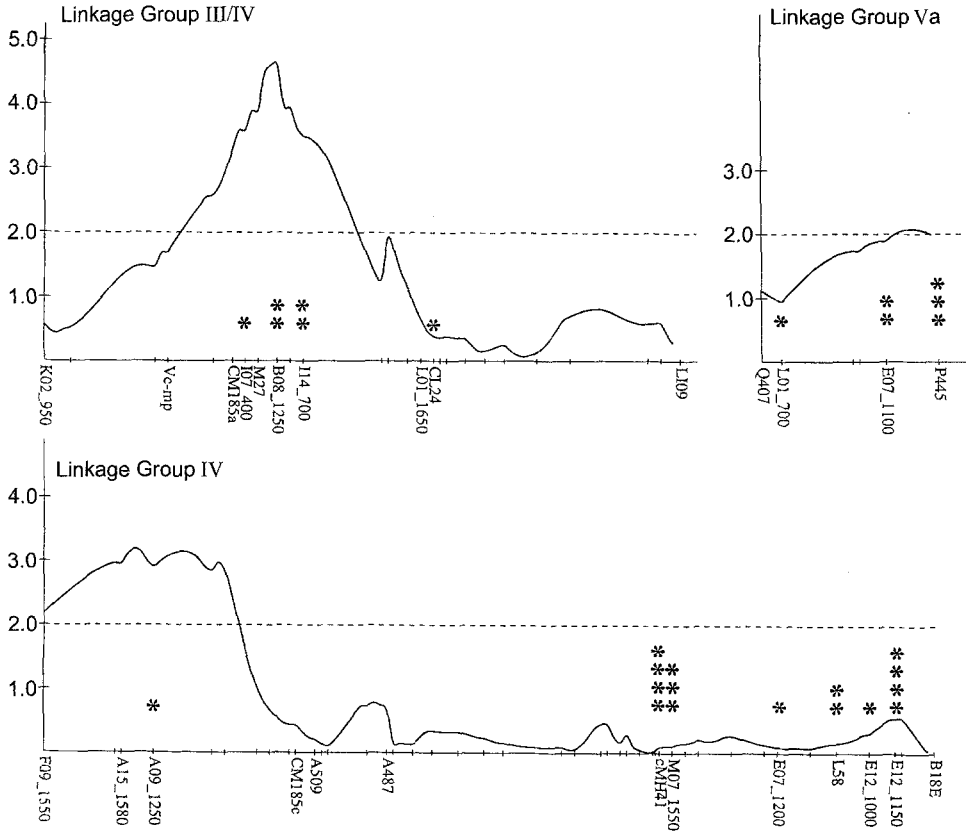

Fig. 2 LOD plots for seed-weight QTLs in the F_2 population. LOD scores were calculated by MAPMAKER/QTL, as described in Materials and methods. The linkage-map lengths (in Haldane units) are: III/IV, 166 cM; IV, 230 cM; and Va, 49 cM. *, **, *** and **** indicate significant effects ($P < 0.01$, 0.001, 0.0001 and 0.00001, respectively) at individual markers based on *t*-tests of marker segregation in seed-weight distributional extremes

member F_2 population. Using 280 primers, 14 RAPD products were identified with differences in band intensity between the high and low seed-weight DNA bulks. Eleven of the fourteen RAPD products gave reproducible and easily scored polymorphisms (Table 3). The correlation between RAPDs and seed-weight phenotypes for these 11 RAPD markers was investigated further by selective genotyping of F_2 plants with extreme heavy ($n = 39$) and light ($n = 40$) seed-weight phenotypes. The plants tested individually included those contributing DNA to the bulks. The significance of the associations between the RAPD and seed-weight phenotypes were tested by conducting χ^2 tests using 2×2 contingency tables. All except one were shown to be significant ($P < 0.01$; Table 3).

The RAPD markers identified by BSA were mapped using the F_2 mapping population (102 plants). These markers mapped to four regions of the genome, on three linkage groups (Table 3 and Fig. 2). Three of these genomic regions coincided with the regions predicted to contain seed-weight QTLs by interval mapping. The fourth region, which also occurred on linkage group IV, was not predicted by the earlier analysis.

To obtain further support for the four QTLs predicted either by interval mapping or by BSA, selective genotyping was carried out for five RFLPs genetically linked to regions identified as containing putative seed-weight QTLs. The significance of the marker genotype and the seed-weight phenotype associations were tested by conducting χ^2 tests using 3×2 contingency tables (Table 4). With RFLP marker cMH41 (Table 4), this analysis provided further support for the second QTL on linkage group IV, which was detected by BSA using RAPD markers but not by interval mapping. In addition, a very significant association was observed between the P445 genotype and the seed-weight phenotype (Table 4). This result strengthens the support for the QTL on linkage group Va, which was predicted by interval mapping, though only at LOD = 2.1 (Table 2, Fig. 2), and by BSA (Table 3).

Identification of seed-weight QTLs using 'JI1794' × 'Slow' RILs

To identify genomic regions associated with seed-weight QTLs in the RILs, single-marker analyses were carried out. The results (Table 5) indicated four regions associated with significant effects ($P < 0.01$) on linkage groups I, III and VII, and also a significant association

Table 3 Segregation of RAPD marker phenotypes in progeny from the seed-weight distributional extremes for RAPDs identified using bulked segregant analysis

RAPD marker	Linkage group	Heavy seed		Light seed		Band-absent genotype	$\chi^{2\,c}$	P^d
		Present[a]	Absent[b]	Present	Absent			
I07_400	III/IV	22	17	28	3	Primo	9.7	*
B08_1250	III/IV	20	17	30	2	Primo	13.6	**
I14_660	III/IV	24	15	30	2	Primo	12.9	**
E12_1150	IV	16	22	25	4	Primo	15.3	***
E12_1000	IV	31	7	15	17	OSU442-15	9.3	*
E07_1350	IV	29	10	14	17	OSU442-15	6.2	
M07_1550	IV	19	20	33	4	Primo	16.2	***
L18_900	IV	19	18	25	4	Primo	8.9	*
A09_1250	IV	22	17	32	6	Primo	7.1	*
E07_1100	Va	19	19	27	4	Primo	10.5	**
L01_730	Va	34	4	15	17	OSU442-15	9.3	*

[a] Number of plants in the indicated seed-weight phenotypic class with the band-present phenotype
[b] Number of plants in the indicated seed-weight phenotypic class with the band-absent phenotype
[c] Results of analysis using 2×2 contingency tables
[d] *,**,*** indicates significance at the 0.01, 0.001 and 0.0001 levels, respectively

Table 4 Segregation of the genotypes of RFLP markers linked to seed-weight QTLs in progeny from the seed-weight distributional extremes

RFLP marker	Linkage group	n	Heavy seed			Light seed			χ^{2d}	P^e
			A[a]	H[b]	B[c]	A	H	B		
CL24	III/IV	79	14	18	7	5	16	19	9.9	*
B18E	IV	78	14	15	10	4	19	16	7.4	
L58	IV	79	17	12	10	2	19	19	16.2	**
cMH41	IV	79	19	18	2	3	17	20	26.3	****
P445	Va	39	10	8	1	0	7	13	20.3	***

[a] Number of plants homozygous for the 'Primo' allele
[b] Number of heterozygotes
[c] Number of plants homozygous for the 'OSU442-15' allele
[d] Analyzed using 3×2 contingency tables
[e] *,**,***,**** indicates significance at the 0.01, 0.001, 0.0001 and 0.00001 levels, respectively

Table 5 Seed-weight QTLs detected in 'JI1794' × 'Slow' RILs using single-marker analyses

Linkage group	Interval	Marker	Genotypic means		
			μ'Slow'	μ'JI1794'	t-value[a]
I	TPPb–B526d	B526d	1.72	1.32	4.17***
III	B540a–AFP2y	AFPIx	1.68	1.35	3.14*
III	cMH95–B519a	B08_1250	1.41	1.78	3.48**
VII	*Pep-3*–B526c	B526c	1.64	1.34	3.01*
-	B499w	B499w	1.37	1.67	2.62*
-	AFP5q	AFP5q	1.35	1.67	2.93*
-	AFP6n	AFP6n	1.66	1.32	3.25*

[a] *, **, and *** indicate significance at the 0.01, 0.001 and 0.0001 levels, respectively

with three unlinked RAPD markers. Linkage maps showing the individual loci associated with seed-weight QTLs are presented in Fig. 3. Loci used to identify the linkage group are also shown. The comparison-wise threshold t-values for QTL detection averaged 2.0 ($\alpha = 0.05$) for marker loci associated with the seed-weight phenotype. These values compared well with the corresponding t-distribution critical values. In all cases, the t-test statistic exceeded the comparison-wise critical t-value at the analysis point. The experiment-wise critical t-value was 4.21 ($\alpha = 0.05$). This value was greater than the t-values describing the association of seed weight and marker loci in the RILs, except for the QTL-marker association detected on linkage group I. Therefore, experiment-wise empirical threshold analysis indicates that the power of the RIL experiment is borderline for detecting QTLs for seed weight while controlling the type-I error to $\alpha = 0.05$ over the entire genome.

Only one genomic region was predicted to contain QTLs for seed weight in both the 'Primo' × 'OSU442-15' and 'JI1794' × 'Slow' crosses. This was a region of linkage group III in 'JI1794' × 'Slow' and of III/IV in 'Primo' × 'OSU442-15' (Figs. 2 and 3). The QTL mapped to group VII in the RILs was localized in a genomic region not represented in the 'Primo' × 'OSU442-15' map (data not presented). In the RILs, other putative QTLs were predicted on groups I and III (the second QTL on this linkage group), however corresponding QTLs were not detected in the 'Primo' × 'OSU442-15' progeny. Likewise, no QTL in the RILs corresponded to the QTLs detected on linkage groups IV and Va using the 'Primo' × 'OSU442-15' population.

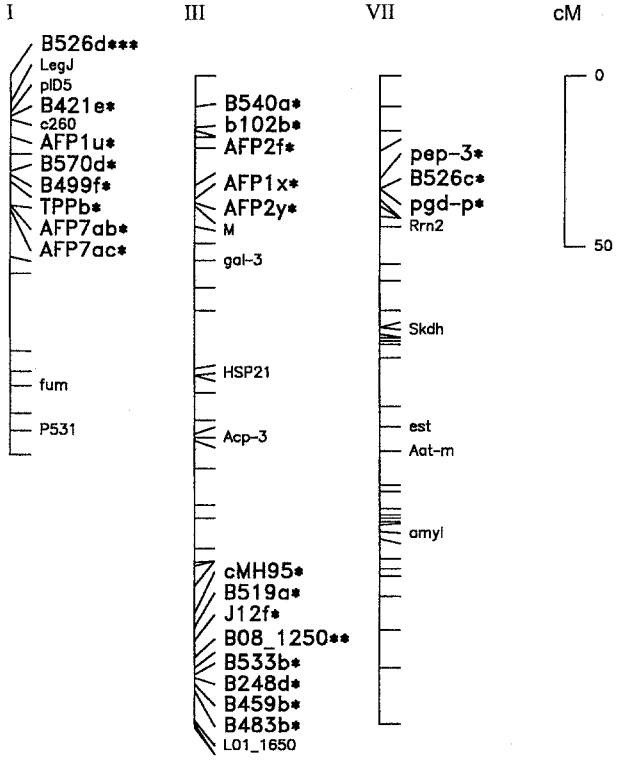

Fig. 3 Genetic map of three linkage groups containing seed-weight QTLs in the 'JI1794' × 'Slow' cross. Significant associations between marker genotypes and seed-weight phenotypes were calculated using t-tests. *, **, *** indicate significance at the $P < 0.01$, 0.001 and 0.0001 levels, respectively. Other loci shown were used for alignment of the 'JI1794' × 'Slow' map with either the 'Primo' × 'OSU442-15' map or with the pea linkage map (Weeden et al. 1993a)

Linkage conservation involving seed-weight QTLs in peas and *Vigna* species

Fatokun et al. (1992) found evidence that the major seed-weight QTLs in mungbean (*V. radiata*) and cowpea (*V. unguiculata*) reside on orthologous genomic segments. To test whether seed-weight QTLs have been conserved between *Vigna* spp. and *Pisum*, eight RFLP markers linked to the major *Vigna* seed-weight QTLs were tested for polymorphism in 'Primo' and 'OSU442-15'. Three of these probes detected polymorphisms (A487, A509, and CM185), and linkage-map locations were determined for the loci labelled using these probes. The RFLP patterns produced by the A487 probe suggested that two copies of these sequences reside on the pea genome; however, only one was polymorphic with the restriction enzymes tested. The A509 probe labelled only a single locus. The polymorphic A487 and A509 loci mapped to linkage group IV, and were separated by a distance of approximately 20 cM. The CM185 homologues differed in copy number for 'Primo' (four copies) and 'OSU442-15' (eight copies), resulting in eight polymorphic bands which showed dominant inheritance of the band-present phenotype. The hybridization intensities were equivalent for the different copies within each line (data not presented). In the F_2 progeny, the polymorphic bands segregated as four pairs; therefore, each pair was mapped as a single locus. The CM185 loci mapped to linkage groups III/IV, IV (Fig. 2), V and VI (data not presented). Two CM185 loci were linked to seed-weight QTLs, on groups III/IV and IV. The CM185 locus on linkage group IV is linked to both A487

and A509 (Fig. 2) and all three loci are adjacent to the region containing a seed-weight QTL. On linkage group III/IV, the CM185 locus and a seed-weight QTL are strongly associated (LOD = 3.6). In the 'JI1794' × 'Slow' RILs, A487 and A509 also mapped to linkage group IV; however, this region was not associated with a seed-weight QTL. CM185 was not mapped in 'JI1794' × 'Slow'.

Discussion

Interval mapping and selective genotyping

Interval mapping using the 'Primo' × 'OSU442-15' cross identified three QTLs for seed weight. BSA using DNA bulked from plants with extreme mean seed-weight phenotypes revealed a fourth QTL (Fig. 2, Tables 3 and 4). Selective genotyping provided support for all four QTLs. For example, interval mapping identified a QTL on linkage group Va, though with weak support (LOD = 2.1). Selective genotyping using the linked RFLP marker P445 (Table 4) demonstrated a statistically significant association of this marker with seed weight ($P < 0.0001$). The QTL on linkage group IV in the interval P357–F09_1550 was only detected by BSA and was not supported by interval mapping (LOD = 0.6). Selective genotyping using the linked RFLP marker cMH41 demonstrated that the association of this genomic region with seed weight was highly significant ($P < 0.00001$). These results, therefore, demonstrated that BSA and selective genotyping are cost-effective and efficient strategies for detecting or confirming QTLs, including QTLs with minor effects that are not identified by conventional interval mapping using smaller populations.

Parental allelic contributions and modes of inheritance

For all four seed-weight QTLs detected using the 'Primo' × 'OSU442-15' cross, the heavy allele was contributed by 'Primo', the heavy seeded parent (Table 4 and data from interval analysis, not presented). The mode of inheritance for the two major QTLs (on linkage groups III/IV and IV, explaining 49% of the variation) best fitted an additive model, and the QTL on group Va (accounting for 13% of the variation) best fitted a recessive model (Table 2). These effects are consistent with the normal frequency distribution observed in the 'Primo' × 'OSU442-15' progeny.

For three of the seven seed-weight QTLs detected in the 'JI1794' × 'Slow' RILs, the heavy allele was contributed by 'JI1794', the light-seeded parent (Table 5). This included the QTL on linkage group III and two QTLs associated with unlinked loci. The presence of a number of QTLs with allelic effects opposite to the parental phenotype might be expected to produce transgressive individuals (deVicente and Tanksley 1993); however, this was not observed in the RILs (Fig. 1). Possible explanations include the small size of the RIL population and the possibility of epistatic interactions among the seed weight QTLs. This latter possibility has not been tested.

Different genomic regions contain QTLs in the two crosses examined

Only one genomic region contained QTLs for seed weight in both the 'Primo' × 'OSU442-15' and 'JI1794' × 'Slow' populations, although both experiments identified a number of seed-weight QTLs. The 'Primo' × 'OSU442-15' cross detected QTLs on groups III/IV, IV (two regions) and Va, while the 'JI1794' × 'Slow' cross found QTLs on groups I, III(two regions) and VII, plus associations with three unlinked markers. As discussed above, the region of group VII containing the seed-weight QTL in the 'JI1794' × 'Slow' cross is not represented in the 'Primo' × 'OSU442-15' map. The combined results of mapping seed-weight QTLs in these two crosses indicate that as many as 7–10 genomic regions could carry QTLs determining seed weight in these field-pea genotypes.

Evidence of seed-weight QTLs conserved in the legumes

Fatokun et al. (1992) provided evidence for orthologous seed-weight QTLs in two *Vigna* species, cowpea and mungbean. Using probes linked to these *Vigna* QTLs, we obtained data suggesting that genomic segments containing seed-weight QTLs identified in *Vigna* are also conserved in pea. A schemaatic diagram of the comparative mapping results supporting this conclusion is shown in Fig. 4. Three RFLP loci (A487, A509 and CM185) were linked in both pea and cowpea, although the linkage order was different. The mungbean map of this region contained only two of the three loci, and lacked the CM185 locus (Menancio-Hautea et al. 1993). This genomic region may, therefore, have undergone rearrangements during the evolution of these species, yet has retained identifiable linkage-block conservation.

In both mungbean and pea, a CM185 locus is linked to a second seed-weight QTL (Fig. 4). In pea, linkage between the seed-weight QTL on group III/IV and locus CM185a was detected using the 'Primo' × 'OSU442-15' population. A seed-weight QTL maps to the same segment of linkage group III using the 'JI1794' × 'Slow' RILs; although CM185 loci were not mapped in the RILs. In an attempt to obtain better support for this second possible example of conserved linkage involving a seed-weight QTL, probes which showed linkage to mungbean locus CM185a and this second mungbean seed-weight QTL were examined. Unfortunately, these were not polymorphic in our crosses.

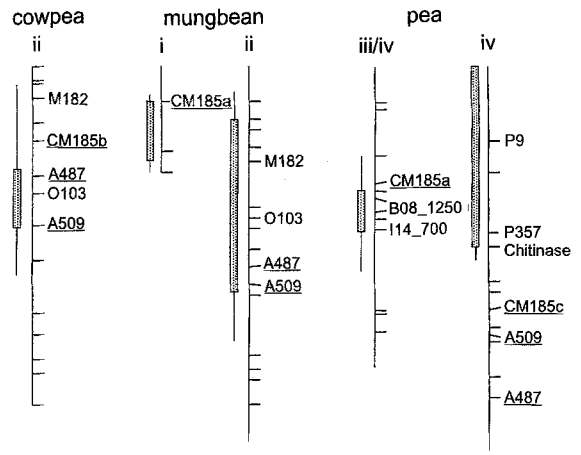

Fig. 4 Comparative linkage mapping of seed-weight QTLs in cowpea (*V. unguiculata*), mungbean (*V. radiata*), and pea. The cowpea and mungbean maps and the QTL locations were drawn based on Fatokun et al. (1992) and Menancio-Hautea et al. (1993). *Underlined loci* (A487, A509 and CM185) were mapped in all three species. *Boxes* to the left of the linkage maps indicate 1-LOD (10-fold) confidence intervals for the location of seed-weight QTLs, and *lines* extending beyond the boxes indicate 2-LOD (100-fold) confidence intervals

Probe CM185 labelled multiple loci in all three species under comparison. In pea, the differences in copy number between parental lines, the uniformity of hybridization intensity, and the dispersal of dominantly inherited CM185 loci through out the genome all suggest that CM185 sequences have undergone recent duplication in this species. A similar behaviour was observed for three other cDNA probes during development of the 'Primo' × 'OSU442-15' map (data not presented) and has been reported for RFLP markers in other plant species such as rice (McCouch et al. 1988) and lentil (Weeden et al. 1992). Therefore, in view of its apparently recent duplication, CM185 alone is of limited value for confirming conservation of linkage involving seed-weight QTLs, or indeed any trait, in peas.

Although Fatokun et al. (1992) provided the first evidence for orthologous seed-weight QTLs, our results introduce evidence that seed-weight QTLs may have been conserved more widely during legume evolution. Conserved linkage relationships between *Pisum* and *Vigna* have not otherwise been reported. The genera *Pisum* and *Vigna* are both members of the large and diverse legume sub-family Papilionoideae, but are members of different tribes, the Viceae and the Phaseolinae, respectively.

Comparative mapping studies have identified shared regions of linkage-group homology in peas and lentil (*Lens culinaris* Medik.) (Weeden et al. 1992; Simon et al. 1993). QTLs for seed weight have been investigated in lentil (*L. culinaris* and *L. orientalis*) and single-marker associations with a number of isozyme and morphological markers have been detected (Abbo et al. 1992; Tahir et al. 1994). Many of these markers have been placed on the linkage map of lentil, and some (*Gs, Pgm-p, Gal-1, Skdh, Gal-2, Pgm-c*; Tahir et al. 1994) are associated with segments showing a conservation of linkage relationships between the pea and lentil genomes (Weeden et al. 1992; Simon et al. 1993). Only one of the pea genomic regions identified in our study as containing seed-weight QTLs corresponded with a lentil genomic region associated with seed weight. This was the seed-weight QTL mapped to linkage group VII in the 'JI1794' × 'Slow' cross (Fig. 3, Table 5) and associated with markers *Pep-3*, B526c and *Pgd-p*. *Pep-3* and *Pgd-p* share a conserved linkage block with the *Pgm-c* isozyme locus in both pea (Weeden et al. 1993a) and lentils (Weeden et al. 1992; Simon et al. 1994). Tahir et al. (1994) showed that *Pgm-c* was significantly associated with seed weight in a lentil cross. Therefore, the seed-weight QTLs mapping to similar regions of linkage group VII in pea and lentil may represent another example of seed-weight QTL conservation in legumes. Pea and lentils are both members of the Viceae.

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