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Development of a genetic composite map of *Vicia faba* using F_2 populations derived from trisomic plants

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Abstract Seven F_2 families of faba bean descendent from plants trisomic for chromosomes 3, 4, 5 and 6 were analyzed for isozyme markers and two of these were also studied for morphological and RAPD markers and seed-protein genes. Linkage analysis revealed 14 linkage groups, 8 of which were unambiguously assigned to specific chromosomes. Several QTLs for seed weight were identified, the most important of which, located on chromosome 6, explained approximately 30% of the total phenotypic variation. Comparison of results from *Vicia faba* with the maps of the related species *Pisum sativum* L. and *Cicer arietinum* L. revealed one possible new case of linkage conservation. A composite linkage analysis based on 42 markers analyzed in this and previous studies, where line Vf 6 was also used as the female parental, allowed the new assignment of previously independent linkage groups and/or markers to specific chromosomes. Thus, the number of linkage groups was reduced to 13, each comprising an increased number of markers. No contradictory results were detected, indicating the suitability of the statistical procedure and methodology used so far in the development of the map of this species.

Key words *Vicia faba* · Genetic map · Trisomics · RAPD · Seed-protein genes · QTLs

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Introduction

The importance of combining information obtained from separate studies to obtain the development of more complete genetic linkage maps has been clearly outlined for different major crops (Beavis and Grant 1991; Kianian and Quiros 1992; Causse et al. 1996). The composite map has some general advantages over one obtained from a single population. Composite analysis may increase the number of loci mapped and the statistical power of linkage detection between them. The accuracy of the specific localization of the markers and their internal order is improved since data from a large number of progeny derived from different segregating populations are considered. In addition to this, the common markers may be used as consensus markers controlling any discrepancy in the alignment of the linkage groups identified in different crosses. Another interesting approach is to not only combine data from different segregation populations but also to consider data from both codominant and dominant markers. The combination of physically localized markers with others not localized can be one of the first steps to the integration of genetic and physical maps in any species. Finally, primary trisomics available in *Vicia faba* have been already used to establish the chromosomal location of many genes (Torres et al. 1993, 1995, 1998; Satovic et al. 1996) and to consolidate the linkage map of this species. In any case, trisomy can be used to test results obtained from the linkage analysis. Nevertheless, all these advantages depend on the use of an adequate common statistical procedure and the existence of common markers.

In spite of recent progress in *V. faba* linkage analysis (van de Ven et al. 1991, Torres et al. 1993, 1995; Ramsey et al. 1995; Satovic et al. 1996), a joined detailed map is

not yet available. In addition to this, most of the linkage groups reported so far remain unassigned to chromosomes. In the study we report genetic linkage among new morphological, isozyme and random amplified polymorphic DNA (RAPD) markers and physically localized protein genes, obtained by the analysis of three new F_2 families derived from different trisomic plants. A composite map was also developed from these results and those previously reported (Torres et al. 1995, Satovic et al. 1996), since the use of a recurrent parent allowed us to combine data by means of common markers present in the female parent.

Materials and methods

Plant material

Primary trisomics were obtained by crossing Vf 6 (female asynaptic parent) with three different pollen parents (Vf 27, Vf 17 and Vf 46), all of which belong to the collection of genetic variants at the E.T.S.I.A.M. (Escuela Técnica Superior de Ingenieros Agronomos y Montes) in Cordoba. The F_1 plants with $2n + 1$ chromosomes were identified by studying meiotic metaphase-I and characterized as proposed by Martin and Barceló (1984). Seed weight was scored only in families derived from crosses between Vf 6, an *equina* type with medium-sized flattened seeds and Vf 27, the more primitive *paucijuga* type with small round seeds. Twenty seeds per plant were counted and weighed to quantify this trait. The three F_2 families derived from cross 6X27, showing the highest rate of polymorphisms, were also scored for isozyme, morphological, RAPD and seed-protein phenotypes, whereas the remainder of the families derived from plants trisomic for chromosomes 3, 4, 5 and 6 were scored only for isozymes (Table 1).

Methods

Morphological traits

Only one morphological trait (black seed color) was considered in the study. Sjödin (1971) localized this locus (*Sc*/*sc*) in chromosome 2 of the species.

Isozymes

Nine enzyme systems (aconitase hydratate, ACO, E.C. 4.2.1.3; acid phosphatase, ACP, E.C. 3.1.3.2; aspartate aminotransferase, AAT, E.C. 2.6.1.1; alcohol dehydrogenase, ADH, E.C. 1.1.1.1; glucose-6-phosphate isomerase, GPI, E.C. 5.3.1.9; leucine aminopeptidase, LAP, E.C. 3.4.11.1; peroxidase, PRX, E.C. 1.11.1.7; phosphoglucanate dehydrogenase, PGD, E.C. 1.1.1.44; and superoxide dismutase, SOD, E.C. 1.15.1.1) revealing 10 polymorphic loci were included in the analysis. Results obtained for each isozyme locus, with respect to their genetics and chromosome location, are reported elsewhere (Torres et al. 1998).

RAPD and seed-protein gene analysis

DNA was extracted from young leaf tissue of three F_2 families: 6x27 T4, T6 and 6x27 disomic (Table 1). For RAPD analysis, approximately 20 ng of genomic DNA was used as a template in a 25-µl polymerase chain reaction (PCR). Mixture composition and reaction conditions were as described by Williams et al. (1990) with slight modifications (Torres et al. 1993). A total of 117 RAPD primers were surveyed in the parental lines involved in the crosses. Of these, 114 were purchased in commercially available kits from OPERON Technologies (Alameda, USA) and are named OP, the rest, named Mer, were chosen since they produced intense and consistent amplification products in a previous study (Torres et al. 1993). Thirty-seven of the primers revealed intense and clearly scorable bands and were chosen for the analysis of the three F_2 families.

Three seed-protein genes located in defined regions of chromosomes 1 (*USP*), 2 (legumin *B3*) and 3 (legumin *B4*) of the species were also polymorphic in the mentioned cross. Physical localization of these genes was done by PCR with sorted or micromanipulated chromosomes (Macas et al. 1993) and later also confirmed by in situ hybridization (Fuchs and Schubert 1995). The sequence and characteristics of the primers used for the detection of length polymorphisms among the mentioned genes were reported in Macas et al. (1993). Modified conditions were adopted for the amplification of these primers in order to maximize amplification and resolution of the products. In this case, each 25-µl amplification reaction contained: 20–40 ng of plant genomic DNA, buffer [50 m*M* KCl, 10 m*M* TRIS-HCl (pH 8.3), 2.0 m*M* $MgCl_2$, 0.001% gelatin], 0.2 m*M* of each dNTP, 0.2 m*M* of primers, and 1 U of *Taq* DNA polymerase (Promega). Mixtures were overlaid by mineral oil and, after an initial denaturation (94°C, 5 min), products were amplified for 30 cycles (94°C, 1 min; 55°C, 50 s, 72°C, 1 min and 30 s) in

Table 1 Parental lines used for the crosses, number of progeny tested and isozyme loci scored

Female parent	Male parent	F_1 trisomic chromosome	F_2 population size	F_2 number of trisomic plants	Segregating isozyme loci
Vf 6 (*equina*)	Vf 27 (*paucijuga*)	4[a]	59	16	*Aat-2*, *Aco-2*, *Acp-2*, *Adh-2*, *Lap-1*, *Pgd-p*, *Prx-1*, *Prx-3*, *Sod-1*
	Vf 27 (*paucijuga*)	6[a]	60	8	*Aat-2*, *Aco-2*, *Acp-2*, *Adh-2*, *Lap-1*, *Pgd-p*, *Prx-1*, *Prx-3*, *Sod-1*
	Vf 27 (*paucijuga*)	None (disomic)[a]	56	–	*Aat-2*, *Aco-2*, *Acp-2*, *Adh-2*, *Lap-1*, *Pgd-p*, *Prx-1*, *Prx-3*, *Sod-1*
	Vf 17 *(minor)*	3[b]	54	7	*Aat-2*, *Aco-2*, *Acp-2*, *Prx-1*, *Sod-1*
	Vf 17 (*minor*)	5	60	7	*Aat-2*, *Aco-2*, *Acp-2*, *Prx-1*, *Sod-1*
	Vf 46 (*major*)	4	59	4	*Aat-2*, *Acp-2*, *Adh-2*, *Gpi-2*, *Pgd-p*
	Vf 46 (*major*)	5	60	2	*Aat-2*, *Acp-2*, *Adh-2*, *Gpi-2*, *Pgd-p*
	Vf 46 (*major*)	6	60	13	*Aat-2*, *Acp-2*, *Adh-2*, *Gpi-2*, *Pgd-p*

[a] F_2 families also scored for morphological, seed-protein and RAPD markers
[b] F_2 family also scored for seed-protein loci

a Perkin Elmer DNA thermal cycler 480. Cycling was concluded with a final extension at 72°C for 10 min. As for the RAPD markers the amplified products were electrophoresed on 1% agarose, 1% Nu-Sieve agarose, 1xTBE gels, and visualized by ethidium bromide staining.

Quantitative trait locus (QTL) analysis

We followed the linear regression approach to map QTLs which consists of an examination of the relationship between performance for the quantitative trait (seed weight) and the genotypes at the marker locus. If there is a statistically significant association between the trait performance and the marker locus genotype, one infers that a QTL is located near the marker locus. An analysis of variance was performed for each locus separately. We could not follow the interval mapping approach since the present *V. faba* map is still not sufficiently saturated.

Statistics

In order to create a composite map of the *Vicia faba* genome, we followed the statistical procedure described previously in Satovic et al. (1996). This method considers the results of the joint segregation analysis in families derived from trisomic plants and the results of gene localization. Goodness-of-fit to the normal codominant 1 : 2 : 1 or the dominant 3 : 1 ratio was tested by χ^2 analysis. When the marker under study was located on the chromosome in trisomy, the normal codominant ratio was modified due to the presence of the extra chromosome. Various critical ratios expected in an F_2 population derived from a trisomic plant were calculated depending on (1) type of marker segregation (codominant vs. dominant), (2) parent carrying the dominant allele and (3) trisomic segregation model [Random Chromosome Association (RCA) vs. Random Complete Chromatid Association (RCCA)]. Moreover, male transmission rate was assumed to be equal to zero, as demonstrated by González (1985). Thus, according to Hermsen (1970) segregation in disomic plants is independent of the segregation type considered (4 : 4 : 1), whereas in the trisomic group a ratio of 2 : 7 : 0 or 12 : 32 : 1 is expected for RCA or RCCA, respectively. These critical ratios were distinguished from the normal ratios by means of χ^2 tests.

In order to create a composite map, we assumed: (1) the genes encoding morphological and isozyme phenotypes were orthologous in different families if the resultant phenotypes were similar, (2) amplification products with the same molecular weight (MW) obtained by each primer in families derived from the same cross were identifying the same chromosomal locations and (3) since in all of the crosses the same line (Vf 6) was used as a female parent, the amplification products detected in this line corresponded to products with the same MW detected in all the plants, even if they were obtained in different crosses.

Pooling data

Contingency tables (Mather 1957) were employed to check the homogeneity of segregation ratios for the loci analyzed in more than one family, and the significance was determined by the χ^2 test. Data from different families were pooled when homogeneous and multilocus linkage groups were constructed using MAPMAKER V 2.0 (Lander et al. 1987). Recombination fractions were justified by using a LOD score of 3 as threshold and genetic distances in centimorgans (cM) were inferred by Kosambi's mapping function. When a linkage between a given pair of loci was significant (LOD > 3) considering the data pooled from more than one family, the estimates of the recombination fraction were calculated from data for each family separately. Subsequently, these estimates were tested for homogeneity with the statistics proposed by Morton (1956, recently reviewed by Beavis and Grant 1991). For linkage groups in families where the most likely gene order was not consistent with that obtained in the composite map, the log-likelihood of the most likely gene order in each population was compared with the log-likelihood of the most likely gene order from the composite population. If the difference was not significant (difference in LOD < 3), the gene order obtained from the composite map was also used for the population.

Results and discussion

The morphological marker (*Sc/sc*) and the physically localized seed-protein genes (*B3*, *B4* and *USP*) segregated in agreement with normal Mendelian ratios in the three families analyzed (6x27 T4, T6 and diploid). This was a logical result since it was already known that none of these markers maps on any of the chromosomes previously mentioned. However, linkage analysis of the 175 F_2 individuals revealed a strong linkage between *B3* and *B4* (8.8 cM, LOD 39.62) that clearly suggests their localization on the same chromosome. This outcome was unexpected and contrasts with the results reported by Macas et al. (1993) and Fuchs and Schubert (1995), where *B3* and *B4* were clearly located on chromosomes 2 and 3, respectively. In order to confirm our results, a new F_2 family derived from a plant trisomic for chromosome 3 (6x17 T3) and segregating for both genes was analyzed (Table 1). The position of both genes on chromosome 3 was confirmed since their segregation ratio perfectly fit the trisomic segregation and the χ^2 for disomic ratio was strongly significant in both cases (Table 2). One possible explanation for these different results is that the primers selectively amplified *B3* and *B4* in the translocation lines used in the previous studies (Macas et al. 1993, Fuchs and Schubert 1995), whereas the *B3* primers may have cross-reacted with *B4* alleles in the parental lines of the present analysis, thus producing bands segregating with the *B4* genes. In order to test this hypothesis, the nucleotide sequence of the fragment amplified in the 6x27 families using *B3* PCR primers was determined. However, the fragment (1008 bp) showed the highest homology (94%) with the legumin *B3* gene and much less homology with *B4* and other legumin genes (data not shown). In comparison with *B3* the fragment differed by several deletions (the largest one included the first intron) which made it shorter than both the *B3* genes amplified by Macas et al. (1993) and the other fragments produced in this study.

This surprising result may reflect evolutionary divergences between our material and the lines used for physical localization of *B3* and *B4* legumin genes. The missing intron and part of the coding sequence suggest that the polymorphic band represents a pseudogene derived from legumin *B3* genes. Since legumin *B3* pseudogenes were not detected in the field bean lines

Table 2 Goodness-of-fit to disomic and trisomic ratios of the loci located on a specific chromosome by trisomic segregation

Locus	Trisomic family	Genotype of female parent	Population size	χ^2 heterogeneity	χ^2 disomic ratio	χ^2 trisomic ratio	
						2n	2n + 1
$OPA113_{615}$	6x27 T4	AA	57	0.2397	0.474	0.04	0.159
	6x27 T6		57		6.368		
	6x27 D		52		0.000		
$OPD151_{1471}$	6x27 T4	AA	57	0.2294	0.287	0.785	0.182
	6x27 T6		54		4.173		
	6x27 D		54		0.025		
$OPI12_{1272}$	6x27 T4	aa	59	1.7877	0.141	1.316	3.571
	6x27 T6		60		5.689		
	6x27 D		54		3.000		
$OPI162_{584}$	6x27 T4	AA	59	0.0477	2.040	0.088	0.182
	6x27 T6		59		8.593		
	6x27 D		54		1.210		
$OPJ93_{1028}$	6x27 T4	AA	57	0.0433	0.146	0.431	0.159
	6x27 T6		56		9.524		
	6x27 D		52		0.410		
$OPJ131_{1227}$	6x27 T4	AA	56	1.2851	4.667	1.653	0.364
	6x27 T6		57		0.053		
	6x27 D		52		1.641		
$OPK162_{661}$	6x27 T4	AA	55	0.0041	0.006	0.040	0.182
	6x27 T6		58		6.644		
	6x27 D		50		0.027		
$OPL81_{991}$	6x27 T4	AA	55	1.1092	2.188	0.010	0.182
	6x27 T6		60		7.200		
	6x27 D		53		0.006		
$OPL181_{1140}$	6x27 T4	AA	57	0.3732	0.988	0.022	0.182
	6x27 T6		59		6.921		
	6x27 D		54		0.025		
$OPL183_{448}$	6x27 T4	AA	56		4.667	1.476	0.341
	6x27 T6		–		–		
	6x27 D		36		2.370		
$Mer33_{867}$	6x27 T4	AA	56	0.9216	1.524	0.094	0.182
	6x27 T6		56		6.095		
	6x27 D		51		0.007		
B3	6x17 T3	AA	48		5.44	0.01	0.11
B4	6x17 T3	aa	48		27.0	3.36	0.91
Aat-2	6x17 T3		54		16.63	2.84	1.72
Pgd-p	6x27 T6		60	6.30 ($df = 4$)	12.63	0.39	1.5
	6x46 T6		60		10.23		
Prx-1	6x17 T5		60		40.93	10.00[a]	0.25
Sod-1	6x27 T6		60		21.43	2.00	0.44

[a] Data already explained in previous publications (Torres et al. 1998)

used in the previous study (Macas et al. 1993), no data about their physical location is available. Structural rearrangements, such as translocations involving chromosomes 2 and 3, may have shifted some of the genes to different loci in certain lines but not in others. In order to investigate this hypothesis, investigators should cross the lines used in the different studies to visualize the presence of chiasmata in the F_1 generation.

Results of the genetics and inheritance of isozyme markers obtained in the same study have been reported elsewhere (Torres et al. 1998). Nomenclature used for chromosome, linkage group designations and isozyme loci was as proposed by Satovic et al. (1995). Isozyme loci that exhibited trisomic segregation are presented in Table 2.

The 37 RAPD primers used in the analysis resolved 121 scorable polymorphic markers (3.3 RAPDs per primer on average). Eleven of these RAPDs exhibited trisomic segregation in one family (Table 2) and typical Mendelian segregation in the other family trisomic for a different chromosome (data not shown). This allowed the precise assignment of their corresponding linkage groups to specific chromosomes. A total of 135 markers (one morphological, ten isozymes, three seed-protein genes and 121 RAPDs) were considered in the linkage analysis. Forty-two of these markers (36 RAPDs and six isozyme loci) were common with a previous study (Satovic et al. 1996), thereby allowing the construction of a consistent composite map. The combined analysis revealed a total of 13 linkage groups containing one

morphological marker (*Sc*), seven isozyme loci (*Aat-2*, *Aco-2*, *Acp-2*, *Pgd-p*, *Prx-1*, *Prx-3* and *Sod-1*), 105 RAPDs and three seed-protein genes (*B3*, *B4* and *USP*). Seven of these have been clearly assigned to a specific chromosome. No discrepancies concerning preceding location and linkage of any of the common markers were detected. Moreover, no significant differences in the recombination fraction values between pairs of common markers were observed (data not shown). This approach has enabled both a reduction in the number of linkage groups reported so far and the assignment of most of them to specific chromosomes. Since a common female parent was used, we assumed that RAPDs with the same MW in different crosses marked the same locus. RAPDs may be extended to unrelated crosses by transforming those representative of specific chromosomes into standard markers such as sequence characterized amplified regions (SCARs) in order to allow the construction of a species-wide composite map. For brevity, we describe only the linkage groups assigned to specific chromosomes; complete data are reported in Vaz Patto (1997).

Linkage groups (LGs)

Chromosome 1

This chromosome included 21 RAPDs and the seed-protein gene *USP* (Fig. 1). *USP* was physically localized by Macas et al. (1993) in chromosome 1. Seven of the RAPDs were common with a previous work (Satovic et al. 1996), which enabled us to combine 3 linkage groups (XII, XX and XXIII) previously described as independent (see Fig. 1). The length of this linkage group was the longest described in the analysis, as would be expected for the largest chromosome in *V. faba*.

Chromosome 2

One morphological marker, two isozymes and 17 RAPDs constitute this linkage group. The localization of this group of markers to chromosome 2 was made possible by the presence of the morphological marker *Sc* (black seed color) assigned by Sjödin (1971) to this chromosome. Six RAPDs common with the study of Satovic et al. (1996) allowed us to combine 2 previously reported linkage groups (XXI and XXXIX) to this array (Fig. 1).

Chromosome 3

Two linkage groups (IIIa and IIIb) were assigned to chromosome 3. In the first case, the assignment was possible due the presence of the seed-protein gene marker *B4* previously assigned to this chromosome by Macas et al. (1993). In the second case, the isozyme locus *Aat-2* exhibited trisomic segregation in the family derived from plants with an extra copy of this chromosome. Both LGs shared markers with preceding studies (Satovic et al. 1996), as shown in Fig 1. The isozyme locus *Aat-2* allowed us to combine LG XXXVII and LG VIII, which had been reported as independent by Satovic et al. (1996).

Chromosome 4

The assignment of a linkage group to this chromosome was made possible by the RAPD $OPJ131_{1227}$ which clearly exhibited typical trisomic inheritance in the family derived from a plant trisomic for chromosome 4. The markers included in the sequence are presented in Fig. 1.

Chromosome 5

Two linkage groups (Va and Vb) were assigned to this chromosome (Fig. 1). The former includes the isozyme locus *Prx-1*, previously located by Torres et al. (1995) in chromosome 5. In the case of LG Vb, the assignation was made possible by the presence of a common RAPD ($OPC191_{751}$) previously located by Satovic et al. (1996).

Chromosome 6

The most consistent linkage group was assigned to this chromosome. Three RAPDs and one isozyme locus (*Sod-1*) were common with the previous map (Satovic et al. 1996). The presence of 4 RAPDs ($OPI12_{1272}$, $OPJ93_{1028}$, $OPL81_{991}$ and $OPL181_{1140}$) displaying trisomic segregation in a family derived from a plant trisomic for chromosome 6 and the previous location of *Sod-1* by Torres et al. (1995) enabled us to assign this sequence of markers to the same chromosome.

It was not possible to assign the remaining linkage groups to specific chromosomes. Three of them consisted of both isozyme and RAPD markers whereas the rest included only RAPDs (data not shown). Although more markers are needed to connect the linkage groups assigned to the same chromosomes and the ones which remain independent, the use of shared markers derived from a common female parent has proven useful in associating linkage groups established in previous maps. Thus, 19 linkage groups and 9 RAPD markers reported to be independent in Satovic et al. (1996) were joined precisely with the linkage groups established in the present study. Moreover, it was possible to assign 9 of these linkage groups and 7 of these RAPDs not located by Satovic et al. (1996) to specific chromosomes.

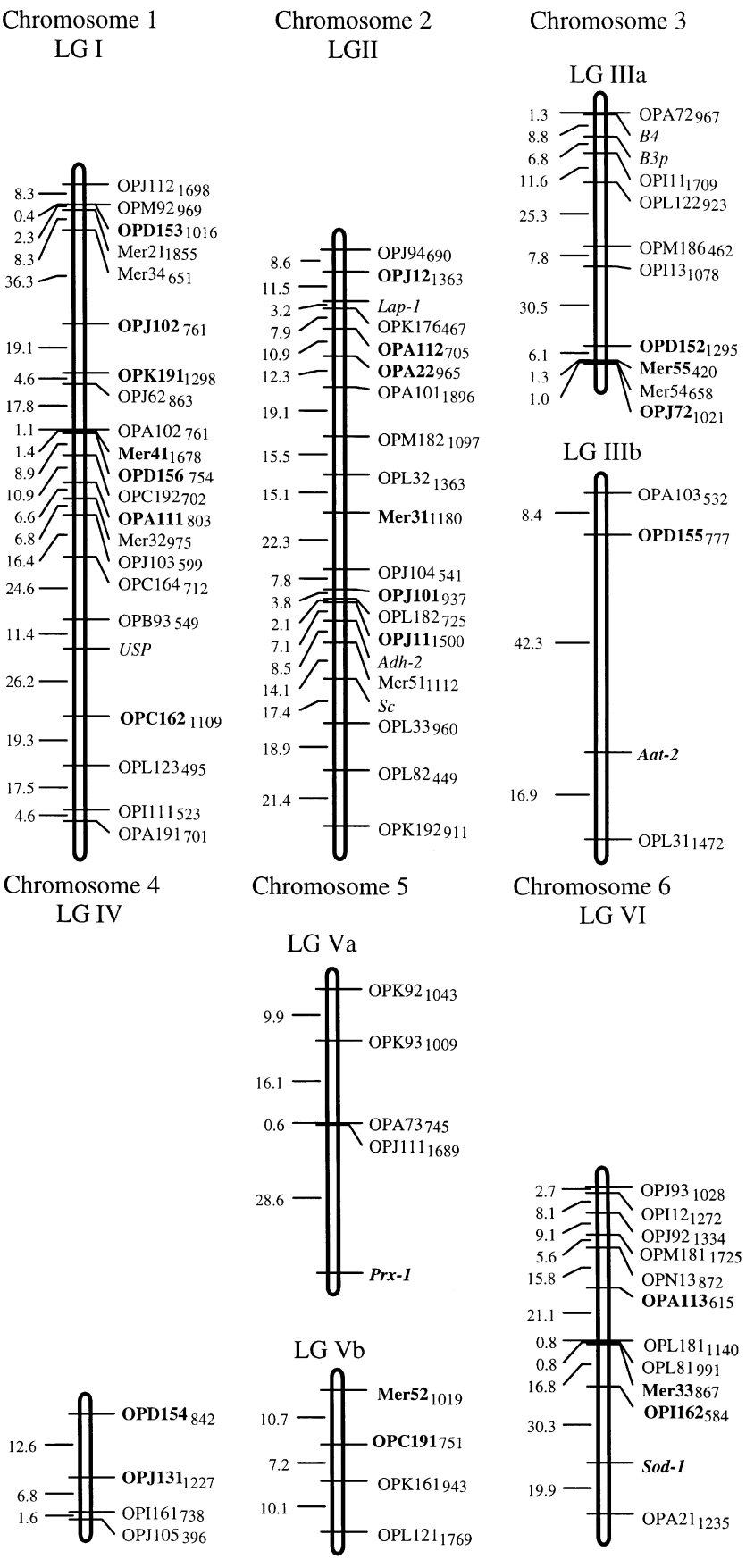

Fig. 1 Loci and linkage groups assigned to specific chromosomes of faba bean (*Vicia faba* L.). *Numbers* to the *left* of the linkage groups represent map distances in centiMorgans calculated with the Kosambi mapping function. All linkages have a LOD score ≥ 3.0. Designations to the *right* are locus names. *Italics* indicates morphological, isozyme and seed-protein loci, *Bold* indicates markers common with previous studies

Conservation of syntenic relationships

In the present study, the assignment of loci *Adh-2* and *Lap-1* to LG II assigned to chromosome 2 may provide a new example of the conservation of linkage groups between pea, chickpea and faba bean. The sequence of *Lap-2–Acp-3–Adh-1–Lap-1* described in pea (Weeden and Marx 1987) and the linkage between *Lap*, *Acp-1* and *Adh-2* detected in chickpea (Gaur and Slinkard 1990) suggests the conservation of this linkage group in the three species. It is difficult to demonstrate homology between these loci since, in the cited studies, the tissues that contained the alcohol dehydrogenase (ADH) activity were different. In pea, ADH was observed in seed extracts, in chickpea, in flower extracts, and in faba bean, in root tissues. The homology between *Lap-1* in faba bean and *Lap* in chickpea is supported by the single phenotype shared by both loci. In addition, in both cases the isozyme was expressed primarily in leaf extracts. Although homology among these loci is likely, analysis of DNA markers is needed to clearly demonstrate this.

QTLs

Seed weight is an economically important trait for faba bean production and also very significant from the taxonomic point of view, as it distinguishes between the four different botanical groups, *major*, *equina*, *minor* and *paucijuga*, that are described in the species (Muratova 1931; Hanelt 1972). This phenotypic trait is commonly considered to be controlled by multiple loci with small effects, i.e., to be a quantitative trait (Cubero 1984). The detected QTLs do not explain all of the phenotypic variance for the trait since some of the variance will be due either to environmental effects or to QTLs too small to be detected with the number of F_2 plants analyzed. The one-way analysis of variance detected significant ($P < 0.05$) associations between several markers and the phenotypic trait. A total of 20 marker loci clustered in 6 chromosomal regions were significantly associated with seed weight ($R^2 > 0.10$). The major QTL for this trait was located in chromosome 6 (close to the $OPM181_{1725}$ marker) and is responsible for 30% of the phenotypic variance (Table 3). Further expansion of the *V. faba* genetic map, including additional molecular markers in the vicinity of the detected QTL, should define the precise location of this QTL in the genome.

The present study shows that combining trisomic segregation, linkage analysis of loci detected in different populations and the use of physically localized markers allows the establishment of a more complete genetic linkage map with a wide coverage of chromosomes. The approach has allowed both a reduction in the number of linkage groups and the assignation of most of them to specific chromosomes. This is a prerequisite

Table 3 Seed weight. Analysis of variance, effect of the marker on phenotypic variation and Bartlett's test for the homogeneity of the error variance

LG	Marker	cM	6x27 T4			6x27 T6			6x27 Disomic			6x27 Total		
			P	R^{2a}	χ^{2b}	*P*	R^{2a}	χ^{2b}	*P*	R^{2a}	χ^{2b}	*P*	R^{2a}	χ^{2b}
V	$OPJ93_{1028}$													
	$OPI12_{1272}$	2.3	0.0063**	0.1511	1.45							0.0027**	0.1002	0.01
	$OPJ92_{1334}$	8.1										0.0115*	0.0810	0.70
	$OPM181_{1725}$	9.1	0.0033**	0.1768	1.49				0.0006***	0.2892	0.51	0.0001***	0.1055	0.53
	$OPN13_{872}$	5.6	0.0012**	0.2054	1.8				0.0018**	0.2578	0.01	0.0165*	0.0689	0.01
	$OPA113_{615}$	16.0	0.0273*	0.1037	0.39									
	$OPL181_{1140}$	12.5												
	$OPL81_{991}$	0.5												
	$Mer33_{867}$	0.5												
	$OPI162_{584}$	13.9												

[a] R^2: Effect of the marker on the total phenotypic variation
[b] χ^2 with 1 *df*
* $P < 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$

for an efficient and systematic mapping of QTLs in the species. The results of this study establish the present status of the *V. faba* map. Distances between markers in most of the cases were less than 20 cM covering approximately 1200 cM, thus providing a map sufficiently saturated to be used as a tool for marker assisted selection.

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