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Genetic mapping of new morphological, isozyme and RAPD markers in *Vicia faba* L. using trisomics

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Abstract Thirteen F_2 families of faba bean (*Vicia faba* L.), descended from plants trisomic for chromosomes 3, 4, 5 and 6, have been analyzed for morphological, isozyme and RAPD markers. This allowed the establishment of linkage relationships among these markers as well as the assignment of some markers and/or linkage groups to their respective chromosomes. The linkage analysis of partially overlapping sets of informative genetic markers for the data pooled from 13 F_2 families has revealed 48 linkage groups, six of which have been precisely assigned to specific chromosomes. A statistical procedure to analyze the data of joint segregation analysis in families derived from trisomic plants has been developed.

Key words *Vicia faba* · Trisomic · Isozymes · RAPDs · Linkage groups

Introduction

It has been widely recognized that the construction of a linkage map in any species greatly increases the efficiency of its genetic and breeding studies. In comparison with other legume species, the faba bean (*Vicia faba* L.) has been the focus of little research in this area. It is worth mentioning the early attempts of Erith (1930), Sirks (1931) and Picard (1963) in the assessment of genetic variation and linkage studies, as well as the use of translocation stocks in the assignment of different loci to their respective chromosomes (Sjödin 1971 a). Another available tool for assigning genes and linkage groups to specific chromosomes are primary trisomics. Trisomics of *V. faba* have been obtained from different sources such as polyploids (Poulsen and Martín 1977), translocation stocks (Schubert et al. 1983) and asynaptic mutants (González and Martín 1983). After successful identification and characterization of five of the six possible primary trisomics (Martín and Barceló 1984), crosses between an asynaptic line and a normal diploid parent proved to be advantageous in localizing genes to their respective chromosomes (González 1985; Cabrera et al. 1989; Torres et al. 1995). On the other hand, preliminary analyses of genetic linkage in faba bean included the study of morphological traits (Cabrera and Martín 1989) and was followed by the establishment of linkage maps based on morphological, isozyme, RFLP and RAPD markers (Van de Ven et al. 1991; Torres et al. 1993 a, b). Additionally, a first attempt at mapping quantitative trait loci in faba bean has been recently reported (Ramsey et al. 1995).

The main aim of the present study was to take full advantage of the availability of an asynaptic line to produce easily identifiable trisomic plants and to study the genetic linkage among as many as possible morphological, isozyme and RAPD markers in order to generate a composite genetic map of linkage groups localized to their respective chromosomes.

Materials and methods

Genetic material

Primary trisomics for chromosomes 3, 4, 5, and 6 were obtained by crossing Vf6 (an asynaptic line used always as the female parent) with six different pollen parents, all of them from the collection of genetic variants at the E.T.S.I.A.M. (Escuela Técnica Superior de Ingenieros Agrónomos y Montes) in Córdoba. The F_1 plants with 2n+1 chromosomes were identified by studying meiotic metaphase-1 and characterized following Martín and Barceló (1984). Seven F_2 families derived from these trisomic plants were scored for morphological, isozyme and RAPD phenotypes, and six more families, with the

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

Female parent	Male parent	F_1 trisomic chromosome	F_2 population size	F_2 no. of trisomic plants	Segregating morphological trait loci	Segregating isozyme loci
Vf6	2	5	45[a]	10	*un, ti, r*	*Aat-1, Aco-2, Prx-1, Sod-1*
	2	6	38[a]	3	*un, ti, r*	*Aat-2, Aco-1, Prx-1, Sod-1*
	33	3	19[a]	4	*sdp, yf, n*	*Est-1, Est-2, Nag, Pgd-p, Pgd-c, Prx-3*
	33	4	48[a]	11	*sdp, yf, n*	*Est-1, Est-2, Nag, Pgd-p, Pgd-c, Prx-3*
	76	5	50	8	*dw, sdp*	*Me-4, Est-2, Pgd-p, Sod-1*
	108	5	40	3	*dw, n*	*Acp-1, Pgd-p*
	108	6	40	9	*dw, n*	*Pgd-p*
	159	4	35[a]	6	*sdp, yf, n*	*Acp-2, Fk-2, Pgd-p, Prx-1, Prx-3, Sod-1*
	159	5	44[a]	5	*sdp, yf, n*	*Acp-2, Fk-2, Pgd-p, Prx-1, Prx-3, Sod-1*
	159	6	54[a]	15	*sdp, yf, n*	*Acp-2, Fk-2, Pgd-p, Prx-1, Prx-3, Sod-1*
	166	4	52	18	*dw, sdp*	*Pgd-c, Sod-1*
	166	5	38	6	*dw, sdp*	*Pgd-c, Sod-1*
	166	6	36	7	*dw, sdp*	*Pgd-c, Sod-1*

[a] F_2 families scored for RAPD markers

same origin, for only the two former markers (Table 1). Chromosome and linkage group designations, as well as morphological trait and isozyme loci nomenclature, were used as proposed by Satovic et al. (1995).

Methods

Morphological traits

Six morphological traits have been included in the study: determinate growth (*Ti/ti*), short internodes (*Dw/dw*), solid distribution of pigment on flower (*Sdp/sdp*), yellow pigment on flower (*Yf/yf*), hilum colour (*N/n*), and red seed-coat (*R/r*). All the traits were reported previously as being of monofactorial inheritance (Picard 1963; Moreno and Cubero 1971; Sjödin 1971 b; Moreno et al. 1981; ICARDA 1981, 1986; Cabrera 1988 a, b).

Isozymes

Ten 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; esterase, EST, E.C. 3.1.1.; fructokinase, FK, E.C. 2.7.1.4; malic enzyme, ME, E.C. 1.1.1.40; beta-N-acetyl-glucosaminidase, NAG, E.C. 3.2.1.30; peroxidase, PRX, E.C. 1.11.1.7; phosphogluconate dehydrogenase, PGD, E.C. 1.1.1.44; and superoxide dismutase, SOD, E.C. 1.15.1.1) revealing 13 polymorphic loci, were included in the analysis. Results obtained for each isozyme locus, concerning their genetics and chromosome location, have been previously reported (Torres et al. 1995).

RAPD analysis

Seven F_2 families (Table 1) were chosen for the RAPD marker analysis (6×2 T4 and T5; 6×33 T3 and T4; 6×159 T4, T5, and T6) since they showed increased levels of polymorphism in morphological and isozyme loci.

DNA was extracted from unexpanded, or partially expanded, leaflets and approximately 20 ng of genomic DNA was used as a template in a 25-µl polymerase chain reaction (PCR) mixture employing the procedure of Torres et al. (1993 a). The reaction conditions were as described by Williams et al. (1990) with slight modifications (Torres et al. 1993 a).

A total of 133 primers were surveyed in the parental lines involved in the crosses; 117 of them were purchased in commercially available kits from OPERON Technologies (Alameda, USA) and are named as OP. The rest, named P or CS, were chosen since they produced intense and consistent amplification products in a previously reported study (Torres et al. 1993 a).

Seventy five of the primers (56.4%) revealed intense and clearly scorable bands and were chosen for the analysis of the F_2 families.

The nomenclature used by Michelmore et al. (1991) was employed in describing RAPD loci adding the letters A, B, or C depending on the segregating cross (6×2, 6×33 and, 6×159, respectively).

Statistics

As we have followed a combined approach (joint segregation analysis in families derived from trisomic plants) to map the *V. faba* genome, we had to develop a suitable statistical procedure in order to create a composite map, bearing in mind the results of gene localization.

Goodness-of-fit to the normal co-dominant ratio of 1:2:1 for isozymes or the dominant 3:1 ratio for morphological and RAPD markers was tested by chi-square analysis. However, when the marker under study was located on a chromosome in a trisomic state the normal co-dominant 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) the type of marker segregation (co-dominant vs dominant), (2) the parent carrying the dominant allele, and (3) the 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 was demonstrated by González (1985). These critical ratios were distinguished from the normal ratios by means of chi-square tests.

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

Contingency tables (Mather 1957) were employed to check the homogeneity of the segregation ratios for the loci analyzed in more than one family, and the significance determined by the chi-square test. If segregations of a locus were proven to be homogeneous in different families they were pooled for the analysis of linkage. Missing values were assigned to all the individuals of a family when (1)

the marker did not segregate, (2) the RAPD marker did segregate but the amplification was so faint that it was impossible to screen it unambiguously, and (3) the marker failed to exhibit the normal Mendelian segregation.

The linkage relationships were determined by the software MAPMAKER V2.0 (Lander et al. 1987) using a LOD score of 3.0 as the threshold for considering significant linkage. Recombination fractions were converted to centiMorgans (cM) using the mapping function of Kosambi (1944).

When a linkage between a given pair of loci was significant (LOD>3.0) considering the data pooled over more than one family, the maximum-likelihood estimates of the recombination fraction were calculated from data of each family separately. Finally, these estimates were tested for homogeneity by applying the statistics proposed by Morton (1956; recently reviewed by Beavis and Grant 1991).

Results

Morphological markers

The segregation results obtained for each morphological trait locus are summarized in Table 2. Since all the morphological markers were dominant and the male parent was always recessive homozygous, all the trisomic F_1 plants were expected to have the genotype AAa for the locus located on the extra chromosome.

In most cases segregation ratios fit to normal Mendelian expectations. In two cases (*Ti/ti* in 6×2 T5 and *Dw/dw*

Table 2 Goodness-of-fit to disomic and trisomic ratios of the morphological-trait loci

Locus[a]	Trisomic chromosome	Cross	Population size	Chi² for heterogeneity	Chi² for disomic ratio	Chi² for trisomic ratio	
						2n	2n+1 (RCCA)[b]
Ti	5	6×2	43 (2)[d]		4.10*	0.08	–
Ti	6	6×2	48 (1)[c]		1.00		
Dw	4	6×166	52 (1)		1.64		
Dw	5	6×108	40 (4)[f]		13.30*	4.63	–
Dw	5	6×166	38 (1)		0.88		
Dw	5	6×76	50 (2)		6.00*	0.03	–
Dw	5		128	7.82 (2 gl)*			
Dw	6	6×108	40 (1)		0.13		
Dw	6	6×166	34 (1)		0.04		
Dw	6		74	1.05 (1 gl)	0.02		
Sdp	3	6×33	18 (1)		0.67		
Sdp	4	6×33	43 (1)		0.38		
Sdp	4	6×159	26 (3)[e]		1.28	0.75	–
Sdp	4	6×166	40 (1)		0.13		
Sdp	4		109	0.52 (2 gl)	1.35		
Sdp	5	6×76	49 (1)		0.55		
Sdp	5	6×108	40 (1)		0.13		
Sdp	5	6×159	31 (1)		0.10		
Sdp	5	6×166	38 (1)		0.88		
Sdp	5		158	0.26 (3 gl)	1.42		
Sdp	6	6×108	40 (1)		0.53		
Sdp	6	6×159	54 (1)		2.00		
Sdp	6	6×166	28 (1)		1.71		
Sdp	6		122	4.28 (2 gl)	0.01		
Yf	3	6×33	18 (3)		3.63	0.22	–
Yf	4	6×159	26 (1)		0.46		
Yf	4	6×33	43 (1)		0.07		
Yf	4		69	0.15 (1 gl)	0.39		
Yf	5	6×159	31 (3)		0.53	3.38	–
Yf	6	6×159	54 (1)		0.62		
N	3	6×33	13 (3)		0.64	0.24	–
N	4	6×159	25 (1)		0.01		
N	4	6×33	27 (1)		1.00		
N	4		52	0.55 (1 gl)	0.41		
N	5	6×108	34 (1)		0.35		
N	5	6×159	23 (4)		6.39*	28.34*	21.01*
N	5		57	1.99 (1 gl)	4.26*	36.75*	13.36*
N	6	6×159	45 (1)		1.67		
R	5	6×2	39 (1)		0.08		
R	6	6×2	34 (3)		3.18	0.75	

[a] Loci in bold, pooled data from the different families trisomic for a specific chromosome
[b] RCCA, random complete chromatid association
[c] (1), F_2 families that fit the typical Mendelian segregation ratio
[d] (2), F_2 families that fit the trisomic ratio
[e] (3), F_2 families that fit both the normal and the trisomic ratio
[f] (4), F_2 families that did not fit neither the normal nor the trisomic ratio

in 6×76 T5) a good fit to the critical trisomic ratio was observed. Thus, it was confirmed that *Ti/ti* is located on chromosome 5. Results from *Dw/dw* in families derived from plants trisomics for the same chromosome were equivocal (Table 2). Thus, a firm conclusion concerning its location could not be established. In five cases (*Sdp/sdp* in 6×159 T4, *Yf/yf* in 6×33 T3 and in 6×159 T5, *N/n* in 6×33 T3, and *R/r* in 6×2 T6) segregation fitted both the normal and the critical ratios probably due to the relatively small F_2 population size. Nevertheless, the location of *Yf/yf* on chromosome 5 was subsequently established by linkage analysis (see Discussion).

Considering the RCA model it is impossible to obtain an F_2 plant showing a recessive phenotype for the gene located on the extra chromosome. Thus, only diploid plants were tested for a trisomic ratio. In cases where trisomic inheritance was proven, as in *Ti/ti* in 6×2 T5, plants showing a recessive phenotype were not detected. Nevertheless, in the case where a recessive phenotype was detected (*N/n* in 6×159 T5) the ratios of trisomic plants were also tested against the trisomic ratio considering the RCCA model.

Isozyme markers

Results of the genetics and inheritance of isozyme markers obtained in the same study have been reported elsewhere (Torres et al. 1995).

RAPD markers

The 75 primers used in the analysis resolved 308 scorable polymorphic markers (4.11 RAPDs per primer on average) in at least one of the seven F_2 families. Unique markers were polymorphic only in families derived from one cross while common markers were polymorphic in more than one cross.

The segregation ratios obtained for the RAPD markers were classified into four groups (Table 3) based on segregation types; 30 RAPDs exhibited trisomic segregation, 25 of them displayed typical Mendelian segregation in other families trisomic for a different chromosome as shown in Table 4. As it was impossible to check the corresponding normal segregation in at least one other family, the five remaining markers were omitted from the analysis. These 25 markers were of particular interest since they allowed us to assign respective linkage groups to a specific chromosome. The results of segregation analysis for the rest of the RAPD markers have been omitted for brevity.

Table 3 Segregation patterns for RAPD

F_2 Family	Segregation type				Total
	Normal diploid	Trisomic	Neither	Not scored	
6×2 T5	61	7	9	3	80
6×2 T6	56	0	4	20	80
6×33 T3	71	3	2	24	100
6×33 T4	86	6	7	1	100
6×159 T4	41	7	6	43	97
6×159 T5	64	2	10	21	97
6×159 T6	51	0	6	40	97
Common	137	5	8	12	162[a]
Total	567	30	52	164	813

[a] 24 RAPDs screened in 6×2 T5 and T6, 27 RAPDs in 6×33 T3 and T4, and 20 RAPDs in 6×159 T4, T5 and T6

The trisomic segregation model used in testing goodness-of-fit of the trisomic (2n+1) F_2 plants depended on the parent carrying the dominant allele of the marker in question. When in a family derived from a dominant homozygous female parent (AA) a recessive trisomic plant was detected, the RCCA model was used for the same reason as for the morphological trait loci. This situation occurred in five cases (OPK-17A_{744}, OPC-19$_{883}$, P-6B_{386}, OPK-06C_{781} and OPA-02BC_{1210}) out of ten. On the other hand, when the male parent was dominant homozygous for a given marker, the RCA model was used. In two cases (OPC-19BC_{1157} and OPK-17BC_{1362}) the segregation fitted both normal and trisomic ratios. Nevertheless, the tight linkage with other markers previously localized clearly indicated that the loci were present on chromosomes 5 and 6, respectively.

A total of 282 RAPDs were considered in the linkage analysis. The rest (26 RAPDs) were excluded as they did not show normal diploid segregation in any of the families analyzed, or else because the segregations obtained from different F_2 populations were not homogeneous (only two cases).

Linkage analysis

The linkage analysis of data pooled from 13 F_2 families has revealed 48 linkage groups containing one morphological trait locus (*yf*), nine isozyme loci (*Aat-2*, *Aco-2*, *Est-2*, *Pgd-c*, *Pgd-p*, *Prx-1*, *Prx-3*, *Nag-1* and *Sod-1*) and 147 RAPDs. Six of them (I–VI) were clearly assigned to specific chromosomes (Fig. 1). Three other linkage groups included isozyme as well as RAPD markers (VII–IX) (Fig. 2). The rest of the linkage groups contained only RAPD loci.

Almost all linkage relationships reported were established on data pooled from more than one family. Due to the relatively limited size of the F_2 families (Table 1) only in a few cases were data from a unique population sufficient to justify a linkage with a threshold of LOD>3. Nevertheless, a conservation of linkage in different families was always detected. Moreover, the tests of homogeneity of the recombination fractions between a pair of loci estimated in different families proved to be a good approach to check the reliability of the linkages obtained. Thus, most of the tests were not significant, justifying the linkage relationships established independently. The only exception was found in linkage group V (Fig. 1) and is explained below.

Table 4 Goodness-of-fit to disomic and trisomic ratios of the RAPD markers located on specific chromosomes

RAPD marker	Size of PCR product (bp)	Trisomic family	Genotype of female parent	Population size	Chi^2 for heterogeneity	Chi^2 for disomic ratio	Chi^2 for trisomic ratio	
							2n	2n+1
OPC-11A	941	6×2 T5	AA	43 (2)[b]		5.65*	0.05	–
		6×2 T6		32 (1)[a]		0.67		
OPC-14A	1150	6×2 T5	aa	39 (2)		7.19*	0.02	0.89
		6×2 T6		31 (1)		1.30		
OPK-17A	1702	6×2 T5	aa	45 (2)		13.70*	0.17	3.43
		6×2 T6		31 (1)		0.01		
OPK-17A3	744	6×2 T5	AA	44 (2)		4.36*	0.05	0.07
		6×2 T6		30 (1)		2.18		
P-4A	505	6×2 T5	AA	40 (2)		4.80*	0.00	–
		6×2 T6		31 (1)		0.01		
OPA-02B	668	6×33 T3	AA	18 (1)		1.85		
		6×33 T4		43 (2)		7.45*	0.14	–
OPA-02B	614	6×33 T3	aa	18 (2)		8.96*	0.91	1.79
		6×33 T4		43 (1)		0.19		
OPA-07B	1297	6×33 T3	aa	18 (2)		6.00*	0.91	0.02
		6×33 T4		43 (1)		1.31		
OPC-11B	912	6×33 T3	AA	18 (1)		1.85		
		6×33 T4		44 (2)		4.36*	0.36	–
OPC-16B	1059	6×33 T3	AA	16 (1)		0.00		
		6×33 T4		44 (2)		4.36*	0.36	–
OPC-19B	883	6×33 T3	AA	18 (1)		1.85		
		6×33 T4		45 (2)		6.23*	0.23	2.78
OPK-17B	422	6×33 T3	aa	17 (2)		4.41*	0.47	0.02
		6×33 T4		45 (1)		0.01		
P-6B	360	6×33 T3	AA	18 (1)		0.67		
		6×33 T4		31 (2)		3.88*	0.19	3.43
OPD-15C	732	6×159 T4	AA	28 (2)		4.76*	0.09	–
		6×159 T5		44 (1)		0.12		
		6×159 T6		49 (1)		0.55		
		Pooled		93 (5)[e]	0.07 (*df* 1)	0.61		
OPH-08C	642	6×159 T4	aa	29 (2)		6.08*	0.13	0.02
		6×159 T5		44 (1)		0.12		
		6×159 T6		– (4)[d]				
		Pooled		– (6)[f]				
OPJ-06C	340	6×159 T4	aa	28 (2)		4.76*	0.28	0.11
		6×159 T5		44 (1)		3.03		
		6×159 T6		– (4)				
		Pooled		– (6)				
OPJ-13C	1169	6×159 T4	aa	31 (2)		4.74*	0.13	0.11
		6×159 T5		38 (1)		1.72		
		6×159 T6		51 (1)		2.36		
		Pooled		89 (7)[g]	4.14 (*df* 1)*	0.09		
OPK-06C	1803	6×159 T4	aa	– (4)				
		6×159 T5		21 (2)		5.73*	0.50	0.02
		6×159 T6		50 (1)		0.67		
		Pooled		– (6)				
OPK-06C	781	6×159 T4	AA	33 (2)		6.31*	0.38	0.14
		6×159 T5		41 (1)		0.40		
		6×159 T6		51 (1)		0.79		
		Pooled		92 (5)	1.17 (*df* 1)	0.06		
OPK-19C	562	6×159 T4	aa	30 (1)		0.04		
		6×159 T5		19 (2)		7.74*	0.23	3.50
		6×159 T6		40 (1)		0.53		
		Pooled		70 (5)	0.09 (*df* 1)	0.48		
OPC-02BC	1210	6×33 T3	AA	18 (1)		3.63		
		6×33 T4		43 (1)		0.63		
		6×159 T4		– (4)				
		6×159 T5		40 (1)		0.13		
		6×159 T6		49 (2)		4.25*	0.44	1.36
		Pooled		101 (5)	2.87 (*df* 2)	1.19		

Table 4 (Continued)

RAPD marker	Size of PCR product (bp)	Trisomic family	Genotype of female parent	Population size	Chi^2 for heterogeneity	Chi^2 for disomic ratio	Chi^2 for trisomic ratio	
							2n	2n+1
OPC-19BC	1157	6×33 T3	AA	18 (1)		0.67		
		6×33 T4		45 (1)		0.19		
		6×159 T4		34 (1)		0.35		
		6×159 T5		38 (3)[c]		0.88	3.09	–
		6×159 T6		50 (1)		0.24		
		Pooled		147 (5)	1.23 (*df* 3)	0.27		
OPC-19BC	758	6×33 T3	AA	18 (1)		0.07		
		6×33 T4		45 (1)		0.07		
		6×159 T4		34 (1)		0.35		
		6×159 T5		37 (2)		5.63*	0.14	
		6×159 T6		50 (1)		0.00		
		Pooled		147 (5)	1.09 (*df* 3)	1.19		
OPC-16ABC	446	6×2 T5	AA	44 (1)		0.48		
		6×2 T6		35 (2)		5.04*	0.00	–
		6×33 T3		18 (1)		0.07		
		6×33 T4		45 (1)		0.90		
		6×159 T4		30 (1)		2.18		
		6×159 T5		44 (1)		1.09		
		6×159 T6		– (4)				
		Pooled		181 (5)	4.66 (*df* 4)	0.02		
OPK-17BC	1362	6×33 T3	AA	17 (1)		0.02		
		6×33 T4		45 (1)		0.90		
		6×159 T4		28 (1)		1.71		
		6×159 T5		41 (1)		0.40		
		6×159 T6		40 (3)		2.13	2.13	–
		Pooled		131 (5)	0.79 (*df* 3)	2.14		

[a] (1), F_2 families that fit the typical Mendelian segregation ratio (3:1)
[b] (2), F_2 families that fit the trisomic ratio (see Discussion)
[c] (3), F_2 families that fit both the normal and the trisomic ratio but the marker in question was located on a specific chromosome since belong to a localized linkage group (see Figs. 1 and 2)
[d] (4), F_2 families where the PCR amplification products were too faint to score them unequivocally
[e] (5), RAPD markers that were included in linkage analysis pooling the data from more than one family
[f] (6), RAPD markers that were included in linkage analysis considering the data from one single family
[g] (7), RAPD markers not included in linkage analysis since the chi^2 for heterogeneity was found significant

Linkage group I included two isozyme markers (*Nag-1* and *Pgd-c*) and one RAPD marker. As was previously reported (Torres et al. 1993 a, 1995), this linkage group can be precisely assigned to the metacentric chromosome 1.

Est-2 and two more RAPD markers formed Linkage group II (Fig. 1). *Est-2* in 6×33 T3 fits both the normal and the trisomic ratios but the odds ratio observed was more than 12 times in favour of trisomy, indicating that the locus might be located on chromosome 3 (Torres et al. 1995). This hypothesis has been confirmed by the fact that one of the RAPD markers included in the sequence. OPK-$17B_{422}$, closely linked to *Est-2*, showed a clear trisomic segregation in the same family allowing us to assign linkage group II to chromosome 3 of the species.

Fk-2 and ten RAPD markers exhibited typical trisomic deviation in families derived from the plant trisomic for chromosome 4. However, none of the pairwise combinations of the above mentioned markers exceeded a threshold LOD>3.

Linkage group III included *Prx-1*, *Yf/yf* and one RAPD marker. *Prx-1* was precisely located on chromosome 5 of the species (Torres et al. 1995). Segregation of *Yf/yf* and OPC-$19BC_{1157}$ in the family 6×159 T5 fits both ratios, but this result could be explained by the dominant nature of those markers making them less informative. A sequence formed by P-$11B_{1013}$ and OPC-$19BC_{758}$ and seven other unlinked RAPD markers (Linkage group IV) was also assigned to chromosome 5 since it clearly exhibited trisomic deviation in families derived from plants with an extra copy of this chromosome.

An isozyme and four RAPD markers formed linkage group V. As previously reported (Torres et al. 1995) the isoenzymatic locus *Sod-1* is located on chromosome 6. RAPDs OPA-$02BC_{1210}$, OPI-$16ABC_{446}$ and OPK-$17BC_{1362}$, closely linked to this locus, when segregating in families derived from the plant trisomic for chromosome 6 also fitted to critical trisomic ratios. This linkage group was established on data from five trisomic families. Nevertheless, the test of homogeneity of recombination fractions between *Sod-1* and OPI-$16ABC_{446}$ was significant, as was that between the latter RAPD marker and OPK-$17BC_{1362}$. As mentioned before, this was the sole inconsistent result detected in our study. The recombination fraction between *Sod-1* and OPI-$16ABC_{446}$ varied from r=0.04

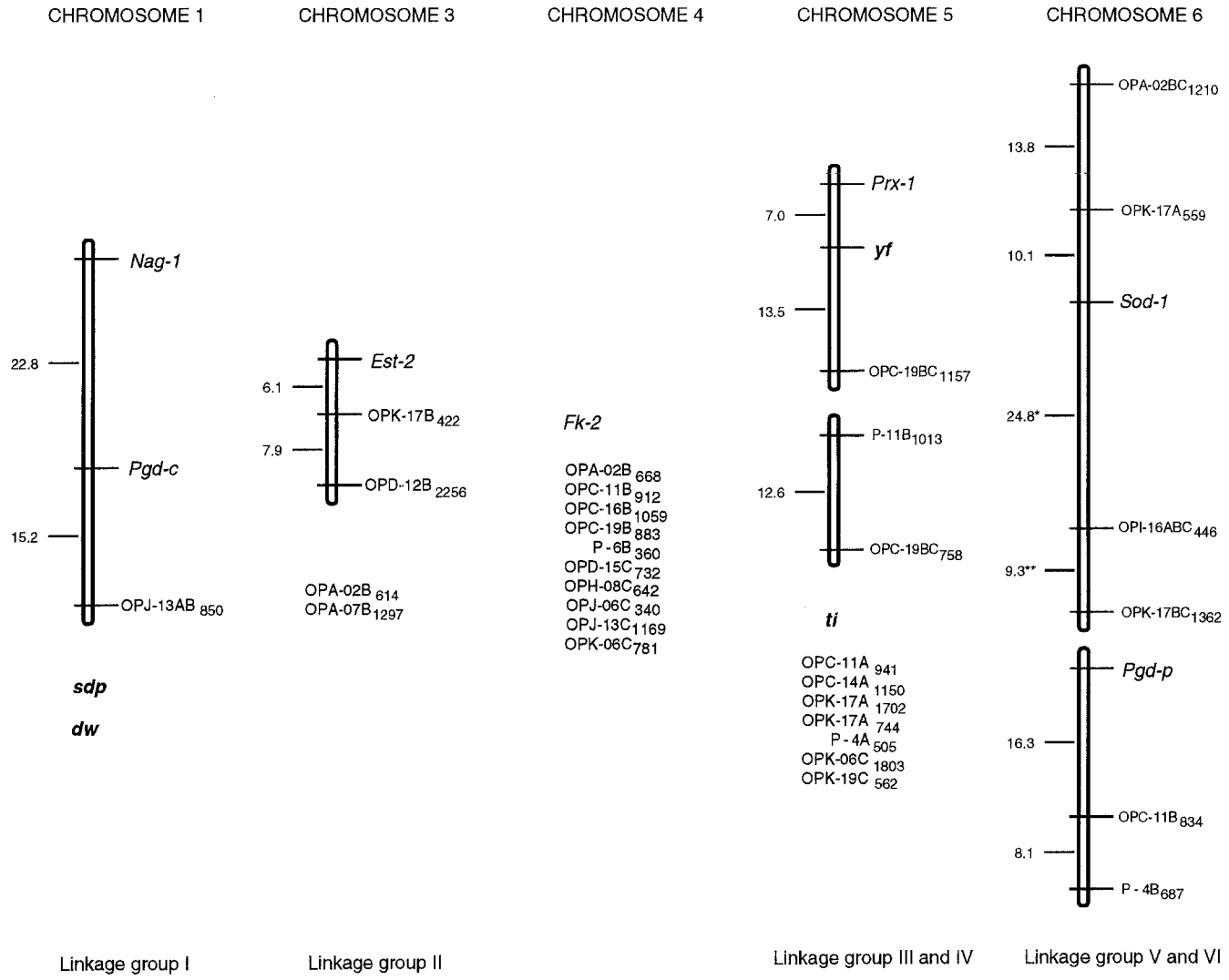

Fig. 1 Loci and linkage groups assigned to a specific chromosome of faba bean (*V. faba* L.). *Numbers* to the left of a linkage group represent map distances in cM calculated with the Kosambi mapping function. All linkages shown have a LOD score of at least 3.0 *Designations* to the right are locus names. *Italics* indicate isozyme loci. *Bold and italics* indicate morphological trait loci. Test of homogeneity of recombination fraction estimates significant at 5% (*), at 1% (**)

(with LOD=4.08) in the family 6 159 T4 to r=0.33 (with LOD=0.63).

Pgd-p and two more RAPD markers (OPC11B$_{834}$ and P-4B$_{687}$) were included in linkage group VI. *Pgd-p* has also been unambiguously assigned to chromosome 6 (Torres et al. 1995). This enabled us to locate the complete sequence on the same chromosome.

It was not possible to assign the rest of the linkage groups to specific chromosomes. Linkage groups VII, VIII and IX consisted of both isoenzymatic and RAPD markers whereas the rest included only RAPD markers. Figure 2 shows these three linkage groups as well as linkage groups from X to XIII that included more than four RAPDs. Moreover, we detected eight linkage groups formed by four RAPDs, three groups of three RAPDs, and 24 groups of two RAPDs (data not shown).

Discussion

Our result confirm previous studies carried out by Sjödin (ICARDA 1986) and Cabrera et al. (1989) concerning the localization of *Ti/ti* (determinate growth) on chromosome 5. It is known that *Dw/dw* (short internodes) does not map on chromosomes 2 (González 1985), 3 (Cabrera et al. 1989), 4 (this study), 5 (Cabrera et al. 1989), or 6 (González 1985; Cabrera et al. 1989; this study). It can thus be concluded that this gene must presumably be located on chromosome 1 of *V. faba* L.

A similar situation is found in the case of the gene encoding the solid distribution of pigment on the flower (*Sdp/sdp*). Previous studies, as well as our results, confirm that *Sdp/sdp* can not be situated on chromosomes 2 (González 1985), 3 (Cabrera et al. 1989; this study), 4 (Cabrera et al. 1989; this study), 5 (Cabrera et al. 1989; this

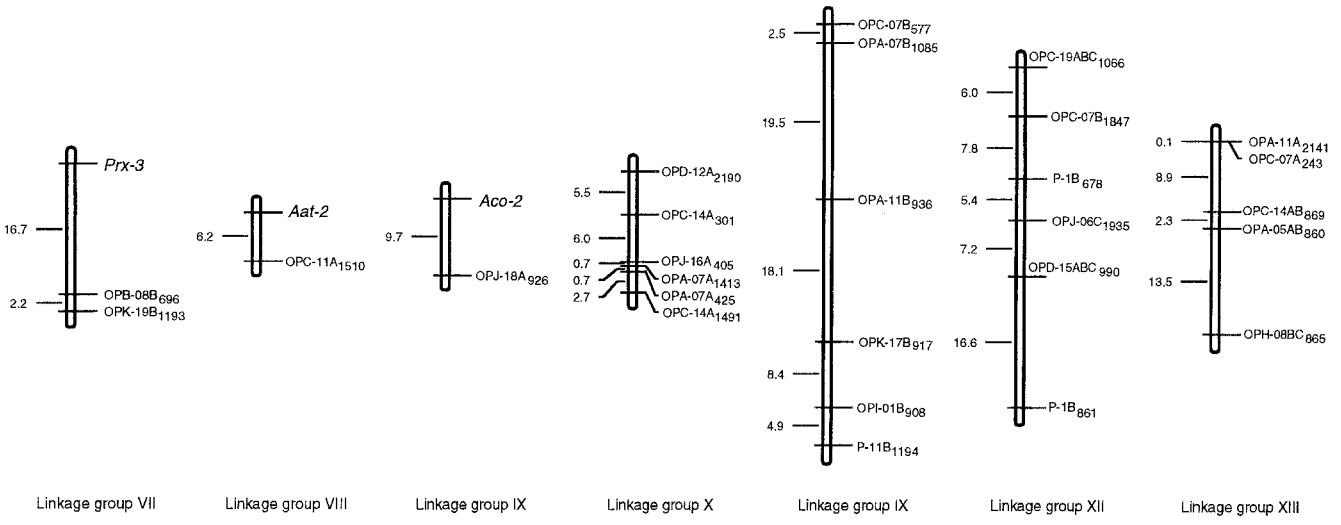

Fig. 2 Linkage groups not assigned to specific chromosome of faba bean (*V. faba* L.)

study), or 6 (González 1985; Cabrera et al. 1989; this study). Nevertheless, the localization of both loci (*Dw/dw* and *Sdp/sdp*) on chromosome 1 should be confirmed by further studies. The extreme difficulty in obtaining a trisomic for this chromosome explains why such direct confirmation is still lacking. Although co-segregating in the same families, neither of these two loci exhibited joint segregation with any of the markers of linkage group I located on chromosome 1. These results can be explained by the enormous size of the metacentric chromosome 1 of this species and the few markers so far located on it.

Concerning the gene encoding yellow pigment on the flower (*Yf/yf*), the literature revision, as well as our results, clearly discard the possibility of the localization of this gene on chromosomes 2 (González 1985), 3 (Cabrera et al. 1989), 4 (Cabrera et al. 1989; this study), or 6 (González 1985; Cabrera et al. 1989; this study). In our study, data from *Yf/yf* (6×33 T3 and 6×159 T5) fits both the normal and the trisomic ratio. Bearing in mind the results obtained by Cabrera et al. (1989), in the case of the 6×33 T3 family, this result could be explained by the small F_2 population size. On the other hand, joint segregation analysis revealed that the *Yf/yf* gene is clearly associated with linkage group III localized on chromosome 5. The unambiguous location of *Prx-1* on this chromosome proved this hypothesis.

Our data concerning *N/n* (hilum color) confirm the results previously reported by Cabrera et al. (1989). It could be concluded that the gene *N/n* is located either on chromosome 1 or on chromosome 3. In spite of having analyzed an F_2 family derived from a plant trisomic for chromosome 3, in which the gene *N/n* was segregating, we were not able to confirm either of the two hypotheses. Again, the low F_2 population size gave ambiguous results with segregation data fitting both the normal and the trisomic ratio. Finally, in the case of *R/r* (encoding red seed-coat), our data do not contradict its location on chromosome 4 as reported previously by Cabrera et al. (1989).

It is obvious that more linkage groups have been identified than there are chromosomes. Although we were able to locate a number of loci on a specific chromosome, the linkage analysis failed to detect the linkage relationships between them, as in the case of *Fk-2* and ten RAPD markers localized on chromosome 4. This is not an unexpected result and for several reasons. First, many markers did not segregate in the same family and consequently linkage relationships between them could not be established. Moreover, the dominant nature of morphological and RAPD markers, the presence of markers in the repulsion phase, and the relatively small population size in some cases, made it difficult to confirm the linkage considering as a threshold a LOD score greater than 3. Thus, it can be concluded that some linkage groups might mark the same chromosome.

As five out of the six possible faba bean trisomics can be obtained and identified relatively easily they constitute a very useful tool in assigning loci to their respective chromosomes. Combining this approach with the linkage analysis of the polymorphic loci detected in different crosses gives good and reliable results for the development of a map of the species. However, the lower fertility of trisomic plants may produce F_2 family sizes that are relatively small for segregation and linkage analysis studies. In these cases, the problem of justifying linkage can be solved by using the same asynaptic line as a common female parent involved in crosses followed by the establishment of a composite map. Considering this approach, it is very important to include in the linkage analysis only those markers that show clear Mendelian F_2 segregation ratios and to pool the data from different families only if they are homogeneous. Finally, the test of homogeneity of recombination fractions has proven to be very useful in further checking the reliability of data. In the present study, the test of homogeneity was significant only in cases where screening and/or assigning of RAPD markers was erroneous. Only in the case of linkage group V mentioned before were we not able to provide a simple explanation.

We expect that in further studies the use of an asynaptic line (Vf 6) as a common female parent in new crosses will enable us not only to map more markers but also to prove the association between the established linkage groups.

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