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Genetics and mapping of new isozyme loci in *Vicia faba* L. using trisomics

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Abstract Polymorphism in ten enzyme systems (ACO, ACP, AAT, EST, FK, ME, NAG, PRX, 6PGD, and SOD) in *Vicia faba* L. was analyzed, revealing 13 loci, six of which have not been reported before. Inheritance, genetics, possible location, and linkage analysis were studied in 13 different F_2 populations trisomic for four of the six chromosomes (nos. 3, 4, 5, and 6) of the species. Each of these loci exhibited typical Mendelian inheritance except for those involved in the trisomic chromosome. Five loci have been assigned to a specific chromosome: *Est-2* to chromosome 3, *Fk-2* to chromosome 4, *Prx-1* to chromosome 5, and *Sod-1* and *Pgd-p* to chromosome 6. *Nag-1* and *Pgd-c* displayed a linkage of 22.8 cM indicating a clear homology with chromosome 5 of garden pea on which both markers are syntenic.

Key words *Vicia faba* · Isozyme · Trisomic · Mapping

Introduction

It is widely recognized that the development of a linkage map of any species provides a valuable tool in many areas of plant genetics and breeding. Genes coding for enzymes have made important contributions to the construction of such maps in different crops. Such has not been the case in *Vicia faba* because, in comparison with other pulse crops, faba bean has been the focus of little effort in this area and few isozyme loci have contributed to the preliminary map of the species. Most of the isoenzymatic polymorphisms in this species have been used in evolutionary and taxonomic studies (Ladizinsky 1975; Yamamoto 1975, 1979; Yamamoto and Plitmann 1980; Polignano and Sonnante 1992), inbred-line recognition (Gates and Boulter 1979, 1980), cultivar identification (Bassiri and Rouhani 1977; Kaser and Steiner 1983), and outcrossing-rate estimation (Peat and Adham 1984; Carre et al. 1991; Suso and Moreno 1995). Although most of these authors described genetic variation, no information on the inheritance of the observed isozymes was reported. Prior to 1990 details of the inheritance of only seven isozyme loci was available (Gates and Boulter 1979; Suso and Moreno 1982; 1986; Peat and Adham 1984; Mancini et al. 1989). Recently, new isozyme loci have been identified (Torres et al. 1993a; Suso et al. 1993) and some linkage groups including isozyme markers have also been established. However, up to now only three isozyme loci have been clearly associated with a specific chromosome (Van de Ven et al. 1991; Torres et al. 1993a).

The cytological tools available in faba bean for assigning genes and linkage groups to their respective chromosomes is limited to translocation stocks (Sjödin 1971) and primary trisomics (Cabrera and Martín 1989; Cabrera et al. 1989). To-date, five of a possible six primary trisomics have been characterized by our group (Martín and Barceló 1984), and these offer useful tools to enhance the map in *V. faba*. In the present study we report the inheritance, genetics, and linkage of 13 isozyme loci in faba bean, six of them described for the first time: aconitate hydratase (*Aco-1*), fructokinase (*Fk-2*), malic enzyme (*Me-4*), beta-N-acetyl-glucosaminidase (*Nag-1*), peroxidase (*Prx-3*), and 6-phosphogluconate dehydrogenase (*Pgd-c*). Primary trisomics have been used to establish the chromosomal location of some of the loci. Both positive and negative results on linkage and chromosomal location will provide new information useful for the construction of a more complete map of the species.

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

Female parent	Male parent	F_1 trisomic chromosome	F_2 population size	F_2 no. of trisomic plants	Segregating isozyme loci
Vf 6	2	V	45	10	*Aat-1, Aco-2, Prx-1, Sod-1*
	2	VI	38	3	*Aat-2, Aco-1, Prx-1, Sod-1*
	33	III	19	4	*Est-1, Est-2, Nag-1, Pgd-p, Pgd-c, Prx-3*
	33	IV	48	11	*Est-1, Est-2, Nag-1, Pgd-p, Pgd-c, Prx-3*
	76	V	50	8	*Me-4, Est-2, Pgd-p, sod-1*
	108	V	40	3	*Acp-1, Pgd-p*
	108	VI	40	9	*Pgd-p*
	159	IV	35	6	*Acp-2, Fk-2, Pgd-p, Prx-1, Prx-3, Sod-1*
	159	V	44	5	*Acp-2, Fk-2, Pgd-p, Prx-1, Prx-3, Sod-1*
	159	VI	54	15	*Acp-2, Fk-2, Pgd-p, Prx-1, Prx-3, Sod-1*
	166	IV	52	18	*Pgd-c, Sod-1*
	166	V	38	6	*Pgd-c, Sod-1*
	166	VI	36	7	*Pgd-c, Sod-1*

Materials and methods

Thirteen F_2 populations, derived from plants trisomic for chromosomes III, IV, V, and VI (T3, T4, T5, and T6), were scored for allozyme phenotypes (Table 1). Primary trisomics where 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 and characterized as outlined by Martín and Barceló (1984). Crosses were made, and progenies were grown, in the greenhouse to avoid interference by insects.

Most of the isozyme analyses were performed on extracts from young leaves. Nevertheless, for several systems other organs, such as roots or seeds, were tested in order to improve band resolution and to optimize banding patterns. Thus for FK, NAG and PRX, healthy root tips were used in the F_2 screening.

Horizontal starch-gel electrophoresis was performed on 12% starch gels according to Gottlieb (1973). Samples of leaves or root tissue were extracted in 2–3 drops of tris-maleate buffer, pH 8. In the case of peroxidase isozymes, the buffer did not contain 2-mercaptoethanol. Three different gel systems were used. System I consisted of tris-citrate/lithium borate buffer, pH 8 (Selander et al. 1971), System II of histidine buffer, pH 6.5 (Cardy et al. 1980), and System III of citrate/N-(3 aminopropyl- morpholine) buffer, pH 6.1 (Clayton and Tretiak 1972). Slices from system-I gels were stained for: aspartate aminotransferase (AAT; E.C. 2.6.1.1), beta-N-acetyl-glucosaminidase (NAG; E.C. 3.2.1.30), malic enzyme (ME; E.C. 1.1.1.40), peroxidase (PRX; E.C. 1.11.1.7), and superoxide dismutase (SOD; E.C. 1.15.1.1). System-II gels were used for: aconitate hydratase (ACO; E.C. 4.2.1.3), fructokinase (FK; E.C. 2.7.1.4), and 6-phosphogluconate dehydrogenase (PGD; E.C. 1.1.1.44). Finally, acid phosphatase (ACP; E.C. 3.1.3.2) and esterase (EST; E.C. 3.1.1.-) were stained in slices from System-III gels. Most of the assay solutions were prepared according to standard recipes (Wendel and Weeden 1990) except for NAG (Weeden and Marx 1987) and FK (Muelbauer et al. 1989). Enzymes were named using the nomenclature proposed by the International Union of Biochemists (1984) and different isozymes (products of different genes) were designated as indicated by Weeden (1988).

When the marker under study was located on a trisomic chromosome the normal co-dominant ratio was modified due to the presence of the extra chromosome. Thus, in order to locate a specific isozyme locus on its respective chromosome, the goodness-of-fit to the 1:2:1 segregation ratio or to the "critical ratio" were tested by chi-square analysis (see Discussion).

The chi-square test for homogeneity of data from different F_2 families was carried out according to the procedure described by Mather (1957). Data were pooled when homogeneous and used for the final linkage and inheritance test. Linkage between loci was examined using a contingency chi-square test. Recombination fractions were estimated using maximum-likelihood formulae as applied by the LINKAGE-1 program (Suiter et al. 1983), and justified using the threshold LOD>3 achieved by the MAPMAKER program (Lander et al. 1987).

Results

Polymorphism was present for 13 of the 28 isozymes resolved in the ten enzyme systems. The results obtained for each enzyme system are summarized in Table 2 and explained below:

ACP. As reported previously (Torres et al. 1993 a), this enzyme system exhibited two anodal zones of activity. In the present study only the most slowly migrating form (*Acp-2*) was polymorphic in crosses 6×108 and 6×159. The ACP activity was so intense that in some families (e.g. 6×108 T6) the activity bands merged together, and the three possible variants could not be clearly distinguished. Thus, these data were omitted from the analysis. Segregation ratios in 6×159 T4 and 6×159 T6 did not fit either the normal or the critical ratios. Some of the F_2 individuals could have been misclassified for the reason described above. Nevertheless, progenies 6×108 T5 and 6×159 T5 were clearly resolved and the segregation pattern did fit the 1:2:1 ratio expected for a single nuclear gene. As the data from both families were homogeneous, and the pooled data fit the ratio expected for diallelic inheritance, these results were included in the linkage analysis.

AAT. AAT activity was tested in both leaf and root tissues. Leaf extracts yielded better resolution than roots although the banding pattern was similar. Three isozyme loci were resolved as outlined in a previous study (Torres et al. 1993 a), but only *Aat-2* showed variation in cross 6×2 (Fig. 1) whereas 6×2 T5 segregation did not fit either the co-dominant or the trisomic ratio. In contrast, individuals from 6×2

Table 2 Goodness-of-fit to disomic and trisomic ratios of the isozyme loci studied

Isozyme loci[a]	Trisomic chromosome	Cross	Population size	Chi^2 for heterogeneity	Chi^2 for disomic ratio	Chi^2 for trisomic ratio			Odds ratio (2n + 1/2 n)
						2n	2n + 1 (RCA)[b]	2n + 1 (RCCA)[c]	
Acp-2	IV	6 × 159	35 (4)[g]		8.49*	7.31*		7.36*	
Acp-2	V	6 × 108	38 (1)[d]		0.11				
Acp-2	V	6 × 159	44 (1)		1.64				
Acp-2	**V**		82	0.85 (*df* 2)	0.93				
Acp-2	VI	6 × 159	52 (4)		6.58*	12.51*		73.15*	
Aat-2	V	6 × 2	45 (4)		6.60*	9.70*		17.00*	
Aat-2	VI	6 × 2	38 (1)		0.89				
Aco-2	V	6 × 2	45 (1)		1.09				
Aco-2	VI	6 × 2	38 (1)		0.11				
Fk-2	IV	6 × 159	33 (2)[e]		10.21*	0.75	2.68		
Fk-2	V	6 × 159	43 (1)		0.21				
Fk-2	VI	6 × 159	54 (1)		1.26				
Me-4	V	6 × 76	49 (1)		4.92				
Est-1	III	6 × 33	19 (1)		2.26				
Est-1	IV	6 × 33	48 (1)		1.79				
Est-2	III	6 × 33	19 (3)[f]		5.21	0.60	1.79		12.59
Est-2	IV	6 × 33	48 (1)		0.79				
Est-2	V	6 × 76	48 (1)		5.13				
Nag-1	III	6 × 33	19 (1)		3.00				
Nag-1	IV	6 × 33	48 (1)		1.50				
Pgd-p	III	6 × 33	19 (1)		4.37				
Pgd-p	IV	6 × 159	35 (4)		11.23*	3.97		27.75*	
Pgd-p	IV	6 × 33	48 (1)		3.67				
Pgd-p	**IV**		83	11.60 (*df* 2)*	3.87				
Pgd-p	V	6 × 108	40 (1)		0.95				
Pgd-p	V	6 × 159	44 (1)		0.50				
Pgd-p	V	6 × 76	49 (1)		0.02				
Pgd-p	**V**		133	0.85 (*df* 4)	0.61				
Pgd-p	VI	6 × 108	40 (2)		8.55*	0.21	0.64		
Pgd-p	VI	6 × 159	54 (3)		5.41	1.85	0.04		430.54
Pgd-p	**VI**		94	0.79 (*df* 2)	13.49*	0.78	0.11		
Pgd-c	III	6 × 33	19 (1)		0.47				
Pgd-c	IV	6 × 166	52 (1)		0.12				
Pgd-c	IV	6 × 33	48 (1)		0.33				
Pgd-c	**IV**		100	0.39 (*df* 2)	0.06				
Pgd-c	V	6 × 166	38 (1)		1.16				
Pgd-c	VI	6 × 166	26 (1)		3.56				
Prx-1	IV	6 × 159	34 (1)		1.94				
Prx-1	V	6 × 159	44 (3)		5.59	0.98	0.91		16.95
Prx-1	V	6 × 2	45 (2)		7.71*	1.82	3.43		
Prx-1	**V**		89	0.59 (*df* 2)	12.96*	1.81	3.57		
Prx-1	VI	6 × 159	54 (4)		6.15*	9.46*		22.38*	
Prx-1	VI	6 × 2	38 (1)		1.16				
Prx-1	**VI**		92	5.89 (*df* 2)	1.57				
Prx-3	III	6 × 33	19 (1)		0.89				
Prx-3	IV	6 × 159	34 (4)		6.71*	17.55*	0.01		
Prx-3	*IV*	*6 × 33*	48 (1)		4.50				
Prx-3	**IV**		82	8.97 (*df* 2)*	2.49				
Prx-3	V	6 × 159	43 (1)		1.70				
Prx-3	VI	6 × 159	41 (1)		1.00				
Sod-1	IV	6 × 159	35 (1)		4.89				
Sod-1	IV	6 × 166	52 (1)		2.77				
Sod-1	**IV**		87	4.52 (*df* 2)	2.79				
Sod-1	V	6 × 159	44 (1)		2.32				
Sod-1	V	6 × 166	38 (1)		0.95				
Sod-1	V	6 × 2	45 (1)		0.60				
Sod-1	V	6 × 76	49 (1)		1.65				
Sod-1	V		176	4.68 (*df* 6)	0.81				
Sod-1	VI	6 × 159	41 (5)[h]		4.32	5.67		2.44	
Sod-1	VI	6 × 166	36 (3)		2.44	1.72	0.26		9.28
Sod-1	VI	6 × 2	38 (2)		14.42*	1.01	1.83		
Sod-1	VI		115	8.54 (*df* 4)	11.07*	2.71		0.38	

[a] Isozymes in bold, pooled data from the different families trisomic for a specific locus
[b] RCA, random chromosome association
[c] RCCA, random complete chromatid association
[d] (1), F_2 families that fit the typical Mendelian segregation ratio
[e] (2), F_2 families that fit the trisomic ratio
[f] (3), F_2 families that fit both the normal and the trisomic ratio
[g] (4), F_2 families that did not fit neither the normal nor the trisomic ratio
[h] (5), F_2 families that fit both the normal and the trisomic ratio considering random chromatid association due to the existance of a trisomic recessive (aaa) individual

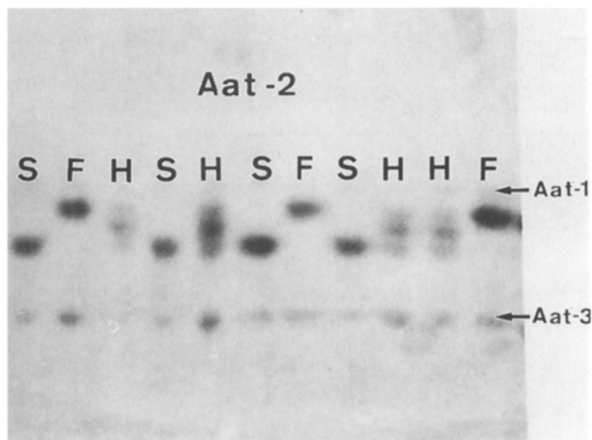

Fig. 1 Faba-bean aspartate aminotransferase patterns demonstrating variation in the isozyme *Aat-2*. Phenotype designations are: *H* heterozygous; *S* slow; *F* fast. Heterozygous individuals have three bands, indicating that the isozyme is dimeric. The anode is at the top of the figure

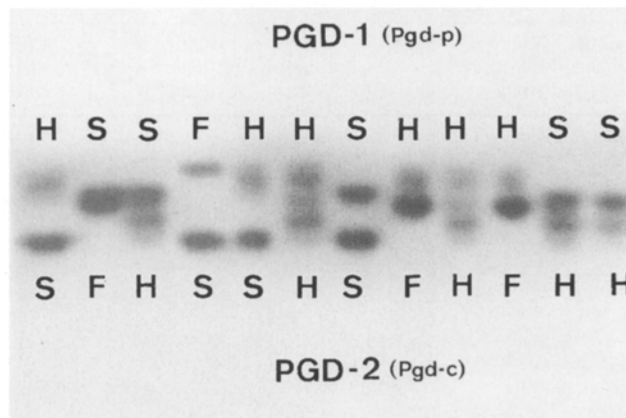

Fig. 2 6-Phosphogluconate dehydrogenase phenotypes obtained from an F_2 population demonstrating variation in both the plastidial (*Pgd-p*) and the cytosolic (*Pgd-c*) isozymes. Phenotype designations are the same as in Fig. 1. Heterozygous individuals have three bands indicating the dimeric structure of both loci. The anode is at the top of the figure

T6 showed typical Mendelian segregation. The faba-bean soaked-pollen extract (Weeden and Gottlieb 1980) contained only the less-anodal AAT (*Aat-3*) indicating the cytoplasmic nature of this locus.

ACO. Isozyme analysis for ACO was done on extracts from leaves, roots and seeds. Similar banding patterns were resolved with these different organs, but leaf zymograms gave the more intense bands. Two anodal zones of activity were observed in leaf extracts of *V. faba*. The less-anodal isozyme was designated *Aco-2* and scored in cross 6×2. The active form of this locus behaved as a monomer and gave a good fit to the expected 1:2:1 ratio.

FK. FK was analyzed in both roots and seeds, but root extracts yielded the best resolution. Five zones of activity were observed. Only the second anodal zone, designated as *Fk-2*, was polymorphic in cross 6×159. The two-banded zymotype of the heterozygous individuals suggested that *Fk-2* is functionally monomeric. A normal Mendelian segregation was observed at this locus in 6×159 T5 and T6. Nevertheless, a good fit to the critical ratio was observed in the family 6×159 T4 indicating that the locus is located on chromosome 4.

ME. Four zones of bands were observed in anodal slices. The three most-anodal bands were monomorphic and stained very poorly. The less-mobile zone, designated as *Me-4*, produced the darkest bands on the gel and was polymorphic in 6×76. The segregation ratio in the family 6×76 T5 did not differ significantly from the expected disomic ratio.

EST. Several zones of EST were detected in both anodal and cathodal slices. The two most-anodal esterases (*Est-1* and *Est-2*) were polymorphic in cross 6×33, while 6×76 showed variation only for *Est-2*. The monomeric nature of both isozymes was confirmed as reported earlier (Torres et al. 1993a). Segregation patterns for *Est-1* approximated a 1:2:1 ratio in 6×33 T3 and T4, as did *Est-2* in 6×33 T4 and 6×76 T5. *Est-2* could be located on chromosome 3 on the basis of data from 6×33 F_2 progeny exhibiting a better fit to the trisomic, than to the normal ratio.

NAG. From analyzing root extracts, one anodal zone of NAG activity was resolved with variation in cross 6×33. The enzyme (*Nag-1*) was monomeric because the heterozygous individuals were two-banded. In the two families analyzed, T3 and T4, the χ^2 values did not deviate from the expected co-dominant ratio.

PGD. Variation was found in the two zones of anodal activity resolved for this enzyme system. *Pgd-1* was analyzed in crosses 6×33, 6×76, 6×108 and 6×159, while *Pgd-2* showed variation in 6×33 and 6×166. Both loci produced intense and well-resolved bands allowing the unambiguous score of the three allozymic variants (Fig. 2). Heterozygous individuals exhibited a three-banded pattern indicating that both loci are functionally dimeric. Subcellular location studies from soaked-pollen extracts (Weeden and Gottlieb 1980) identified *Pgd-2*as the cytosolic specific form. Thus, loci *Pgd-1* and *Pgd-2* have been named as *Pgd-p* and *Pgd-c* respectively. *Pgd-c* in 6×108 and 6×159 T6 segregated as expected for a trait located on the trisomic chromosome, as clearly shown by the pooled data from both families. Data from 6×159 T4 did not fit either a normal or a trisomic segregation. Nevertheless, in the rest of the F_2 generations studied both loci fit a 1:2:1 segregation ratio, confirming the single-gene inheritance of the isozyme.

PRX. Peroxidase activity was present in both anodal and cathodal slices. Genetic analysis was performed on the most-anodal isozyme, *Prx-1*, which showed variation in

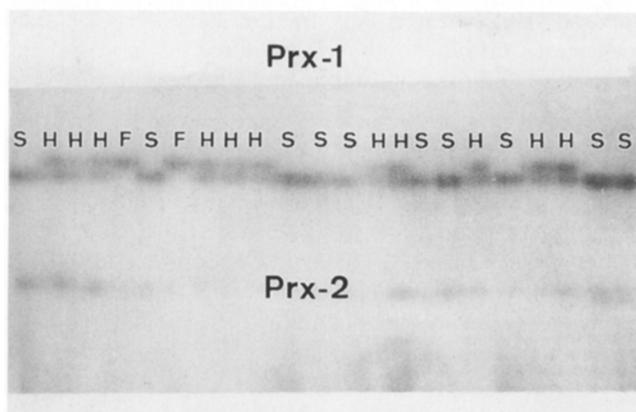

Fig. 3 Phenotypes of the anodal peroxidases in root extracts of faba bean segregating at *Prx-1*. Phenotype designations are the same as in Fig. 1. The anode is at the top of the figure

6×159 and 6×2. *Prx-2* was monomorphic in all the families analyzed (Fig. 3). A third locus, designated as *Prx-3*, was scored on cathodal slices. The inheritance of *Prx-3* was studied in the families 6×159 and 6×33. The heterozygous phenotypes in *Prx-1* and *Prx-3* exhibited only the two parental bands, suggesting that both loci are monomeric. *Prx-1* and *Prx-3* segregated in the expected Mendelian ratio in most of the F_2 families. Only two exceptions were detected: *Prx-3* showed disturbed segregation that did not fit either the normal or the critical ratio in 6×159 T4. On the other hand, *Prx-1* was unambiguously located on chromosome 5 because the homogeneous pooled data from 6×2 T5 and 6×159 T5 clearly exhibited a trisomic segregation ratio.

SOD. Three zones of white bands were observed on a blue background when the gel was viewed under white light. The most-anodal zone, *Sod-1*, was the only polymorphic one in crosses 6×2, 6×76, 6×159 and 6×166. The enzyme was a dimer as evidenced by the three-banded zymotype of *Sod-1* heterozygotes. This isozyme gave a good fit to the co-dominant ratio for the families derived from trisomics for chromosomes 4 and 5 (families 6×2, 6×76, 6×159 and 6×166). By contrast, the segregations of 6×2 T6, 6×159 T6 and 6×166 T6 clearly showed the trisomic segregation ratio expected for a locus located on chromosome 6.

Using the Mapmaker program a linkage between *Nag-1* and *Pgd-c* was detected in cross 6×33. In 6×33 T3 the estimated recombination fraction (r) was 0.29 with a LOD=0.96, and in 6×33 T4 r=0.18 with a LOD=5.88. When the data were pooled a recombination fraction of 0.21 with a LOD=6.50 was obtained. The homogeneity of the recombination fractions from these families was tested as proposed by Morton (1956). Since the obtained value of χ^2 (1 df)=1.57 is clearly non-significant, the recombination fraction of 0.21 (or 22.79 cM using Kosambi's mapping function) from pooled data is a good estimation of the linkage intensity.

Discussion

F_2 trisomic plants in *V. faba* can be readily distinguished from disomics by cytological techniques. Thus, the disomic and trisomic groups were tested separately (Table 2). As reported by Hermsen (1970), the advantage of this procedure is that the ratios are tested twice and the rate of chromatid association can be estimated. Finally, the frequency of trisomics gives an insight into the transmission rate of the extra chromosome in each trisomic. Table 3 shows the critical ratios expected in a F_2 population derived from a trisomic plant (*AAa*) considering either random chromosome association or random complete chromatid association according to Hermsen (1970). The segregation in disomic plants is independent of the segregation-type considered, which is not the case in the trisomic group. The random chromosome association does not permit the formation of homozygous-recessive trisomic individuals (*aaa*). Thus, the group of trisomic plants was tested against the expected ratio taking into account only two genotype/phenotype classes (*AAA* and *AAa*/*Aaa*). When the *aaa* genotype was detected, as in the *Sod-1* cross 6 159 T6, the 2n+1 individuals were tested against the expected ratio assuming random complete chromatid association. In this case all three genotypes/phenotypes are possible (Table 3).

From the 51 segregations considered in this study, 36 [noted as (1) in Table 2] fit the typical Mendelian ratio, indicating that these loci were not located on their respective trisomic chromosome.

Four isozymes [see (2) in Table 2] matched the trisomic ratio. Thus, *Fk-2* was unambigously assigned to chromosome 4, while results from the crosses 6×108 T6 for *Pgd-p*, 6×2 T5 for *Prx-1* and 6×2 T6 for *Sod-1*, supported the location of these isozyme loci on their respective trisomic chromosomes.

Table 3 Critical ratios in the F_2 assuming random chromosome association and random complete chromatid association. F_1 Genotype: *AAa*. Male-transmission rate = 0

Item	Genotype/Phenotype					
	2n			2n + 1		
Segregation type	*AA*	*Aa*	*aa*	*AAA*	*AAa*/ *Aaa*	*aaa*
Random chromosome association	4	4	1	2	7	0
Random complete chromatid association	4	4	1	12	32	1

In four cases, and due to the relatively small size of some families (6×33 T3 for *Est-2*, 6×159 T6 for *Pgd-p*, 6×159 T5 for *Prx-1* and 6×166 T6 for *Sod-1*), the segregation of isozyme loci fits both the normal and the trisomic ratio [see (3) in Table 2]. In these cases the odds ratio, comparing the probability that the locus is located on the trisomic chromosome with the probability that is not located on the trisomic chromosome, was calculated. Odds ratios indicate how many times one hypothesis is more probable than the other. A similar method, known as LOD scoring (Lander and Botstein 1989), was used in the linkage analysis. Our results showed that the odds ratio in the four families mentioned above clearly supported the hypothesis of trisomy (Table 2), confirming the location of *Pgd-p*, *Prx-1*, and *Sod-1* on chromosomes 6, 5, and 6, respectively, as mentioned above, and indicating the location of *Est-2* on chromosome 3. In the case of *Est-2* (in 6×33 T3), the results did not directly assign this locus to chromosome 3; this hypothesis was however supported by the fact that the homozygous recessive trisomic (*aaa*) was not observed and the odds ratio was more than 12 in favour of trisomy (Table 2). Nevertheless, further analyses with a larger population size are necessary to confirm this hypothesis.

In the case of *Sod-1* (6×159 T6) the data fit both the normal and the trisomic ratio. In addition to this, a homozygous-recessive trisomic individual (*aaa*) was detected in this cross, and the odds ratio considering random complete chromatid association was in favour of disomy. Nevertheless, results from 6×2 and the odds ratio in 6×166 strongly supported the hypothesis of trisomy (Table 2). Since the data from the three previously mentioned families were homogeneous, and the pooled data perfectly fit the critical ratio, the final conclusion was that *Sod-1* was located on chromosome 6.

As shown by Torres et al. (1993 a) the isozyme locus *Tpi-1* shares the same linkage group with the 45*s* ribosomal gene array (*Rrn*) and can be associated with the large metacentric chromosome (Chromosome 1) possessing the nucleolar-organizer region. As *Pgd-2* (=*Pgd-c*) proved to be tightly linked to *Tpi-1* (Torres et al. 1993b) it could be concluded that *Pgd-c* and *Nag-1* are located on chromosome 1. The same linkage was reported by Weeden and Wolko (1990) in pea where *Nag-1* and *Pgd-c* where tightly linked. The subcellular compartmentation analysis performed in our study, and the highly conserved subcellular distribution among taxa (Weeden 1988), demonstrate the homology of both loci indicating that the linkage has also been conserved in *Vicia*.

Another example of a linkage conservation between the two species (*Prx-1* and *Acp-1*) has been recently reported (Torres et al. 1993a). In faba bean these two linkage groups have been located on different chromosomes (the present study has shown that *Prx-1* lies on chromosome 5) while in pea, the four loci share the same linkage group on chromosome 5. This could be interpreted as evidence for a chromosome rearrangement between faba bean and pea which occurred during evolution from a common ancestor. However, further analysis of a family segregating for all the isozymes involved is needed to comfirm this hypothesis.

Altogether, five new isozyme loci have been assigned to a specific chromosome in *V. faba* and one new linkage group has been clearly established. The fact that most of the markers analyzed so far seem to segregate independently (except for *Nag-1* and *Pgd-c*), even after having been located on the same chromosome (*Pgd-p* and *Sod-1*), is not at all surprising. Considering the enormous size of the *V. faba* genome, linkage between such a small number of characters is unlikely. The study of additional allozymic variants in wider faba-bean crosses, and the inclusion of RAPD and RFLP markers in our analysis, will allow us to identify new linkage groups that have so far escaped statistical detection.

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