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ORIGINAL PAPER

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# Development of a composite map in *Vicia faba*, breeding applications and future prospects

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**Abstract** A composite map of the *Vicia faba* genome based on morphological markers, isozymes, RAPDs, seed protein genes and microsatellites was constructed. The map incorporates data from 11 $F_2$ families for a total of 654 individuals all sharing the common female parent Vf 6. The integrated map is arranged in 14 major linkage groups (five of which were located in specific chromosomes). These linkage groups include 192 loci and cover 1,559 cM with an overall average marker interval of 8 cM. By joining data of a new $F_2$ population segregating for resistance to ascochyta, broomrape and others traits of agronomic interest, have been saturated new areas of the genome. The combination of trisomic segregation, linkage analysis among loci from different families with a recurrent parent, and the analysis of new physically located markers, has allowed the establishment of the present status of the *V. faba* map with a wide coverage. This map provides an efficient tool in breeding applications such as disease-resistance mapping, QTL analyses and marker-assisted selection.

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## Introduction

The number of species with molecular maps developed is increasing fast, including not only the main cultivated annual crops (Loarce et al. 1996; Cho et al. 1998; Jan et al. 1998) but also fruit trees (Foolad et al. 1995; Sondur et al. 1996; Sharon et al. 1997) or forest species (Byrne et al. 1995; Remington et al. 1999). Molecular maps of legume crops are available in soybean (Shoemaker and Specht 1995), pea (Timmerman-Vaughan et al. 1996), common bean (Freyre et al. 1998), chickpea (Ratnaparkhe et al. 1998) and lentil (Ford et al. 1999). In *Vicia faba*, Sirks (1931) described the first linkage groups with 19 genetic factors. Subsequently, isoenzyme analyses were performed (Suso and Moreno 1982; Mancini et al. 1989) and linkage studies including morphological characters were considered (Cabrera and Martín 1989). With the advent of molecular techniques, Van de Ven et al. (1991) established the first linkage groups in *V. faba* with RFLP (Restriction Fragment Length Polymorphisms), RAPD (Random Amplified Polymorphic DNAs), isozymes and morphological markers. Since then, the faba bean map has been completed with new isozymes (Suso et al. 1993; Torres et al. 1995, 1998), and specific genome regions have been saturated with morphological markers, RFLPs, RAPDs or seed storage protein genes physically located (Torres et al. 1993; Satovic et al. 1996; Vaz Patto et al. 1999).

To obtain an informative map useful for scientific and breeding purposes, linkage and cytogenetic data should be combined. In *V. faba*, this is possible due to the availability of trisomics that can be obtained from polyploids (Poulsen and Martin 1977), translocation lines (Sjödin 1971; Schubert et al. 1983) and asynaptic mutants (Gonzalez and Martin 1983). Up to now, five from the six primary trisomics of the species have been identified and characterized (Martín and Barceló 1984). These trisomic

plants are very useful for mapping purposes considering their acceptable fertility and wide crossing possibilities (Cabrera et al. 1989). As a result, the use of a recurrent asynaptic parent in one or different crosses, followed by the analysis of their segregant progenies, allowed the assignment of linkage groups or markers to specific chromosomes (Torres et al. 1995; Satovic et al. 1996; Torres et al. 1998; Vaz Patto et al. 1999).

The grouping of information from several families to obtain more complete linkage maps has been described in different species, such as sorghum, rice, barley, maize or tomato (Causse et al. 1996; Qi et al. 1996, 1998; Van Wordragen et al. 1996; Dufour et al. 1997; Cho et al. 1998). The increased number of mapped loci and the higher statistical resolution to detect genetic linkage among them are some of the advantages of this approach. As the segregant population derived from different families is larger, the location of any marker and the internal order of each linkage group are also more accurate. Common markers can also be used as a control, checking discrepancies among linkage groups from different families. In faba bean, Vaz Patto et al. (1999) used this latter approach to assign previously independent linkage groups and/or markers of different studies, to specific chromosomes. Nevertheless, data from different populations were not aligned in order to develop and integrate the saturated linkage map of the crop.

The availability of the saturated linkage map facilitates marker-assisted breeding as well as quantitative trait loci (QTLs) mapping. A skeletal map with a uniform distribution of markers can be extracted from a high-density map, and can be applied to detect and map loci underlying quantitative traits. In *V. faba* , previously reported maps have allowed the detection of agronomically important genes, including QTLs associated with seed weight and broomrape/ascochyta resistance (Vaz Patto et al. 1999; Román et al. 2002, 2003).

The present study contributes to the development and saturation of the *V. faba* map by analysing a new $F_2$ population with isozymes, RAPDs, seed protein genes as well as microsatellites or SSRs (Simple Sequence Repeats) physically located on chromosome 1. The combined use of trisomic families and physically located markers allowed the assignment of linkage groups to specific chromosomes. In addition, we have established the correspondence and orientation among the different linkage groups of the *V. faba* maps published so far. Common markers present in the asynaptic line Vf 6, the female parent in all crosses, as well as co-dominant markers, have facilitated the combination of data to establish the present status of the map in this species.

## Materials and methods

### Plant material

The new molecular analysis was carried out in a $F_2$ disomic family (n=196) derived from the cross Vf 6×Vf 136. Three $F_2$ families derived from the cross Vf 6×Vf 27 [one disomic and two trisomic for chromosomes 4 (T4) and 6 (T6)] used by Vaz Patto et al. (1999) were also included in the microsatellite analysis since they displayed high variability for such markers (Table 1). DNA extraction was performed from young leaves of $F_2$ individuals, using the method described by Lassner et al. (1989) and modified by Torres et al. (1993).

To establish the correspondence and alignment of the markers in a consensus map, segregation data of the above described families were joined to seven $F_2$ populations used in a previous study (Satovic et al. 1996). These families were trisomic for chromosomes 3, 4, 5 and 6 (T3, T4, T5 and T6). In all cases the female parent was the asynaptic line, Vf 6, used as a source of trisomy (Table 1).

### Isoenzyme analysis

Variability among parental lines was tested for nine isoenzyme systems. Three of them (aconitase, ACO, E.C. 4.2.1.3; phosphogluconate dehydrogenase– PGD, E.C. 1.1.1.44; superoxide dismutase, SOD, E.C. 1.15.1.1) detected three polymorphic loci (*Aco*-2, *Pgd*-p and *Sod*-1) that were included in the linkage analysis. Description, genetic and chromosome location of each isoenzymatic locus is described in previous papers (Torres et al. 1998).

### RAPD and seed protein genes analysis

Twenty to forty nanograms of genomic DNA in a final volume of 25 µl per reaction was used in the analysis with RAPD primers. Reaction mixture composition and amplification conditions were those described by Williams et al. (1990) with slight modifications (Torres et al. 1993). From 419 RAPD primers surveyed on the parental lines, we selected 45 that provided 124 clear and consistent polymorphic bands in the $F_2$ population. Out of 45 primers, 42 were purchased from OPERON Technologies (Alameda, USA) and were named OP. The remaining three primers named MER were used

**Table 1** Families considered in the total linkage analysis

| Female parent | Male parent | Trisomic $F_1$ chromosome | No. of $F_2$ plants | Authors |
|---|---|---|---|---|
| Vf 6 | Vf 2 | 5 | 45 | Satovic et al. 1996 |
| | Vf 2 | 6 | 38 | |
| | Vf 33 | 3 | 19 | |
| | Vf 33 | 4 | 48 | |
| | Vf 159 | 4 | 35 | |
| | Vf 159 | 5 | 44 | |
| | Vf 159 | 6 | 54 | |
| | Vf 27 | 4 | 59 | Vaz Patto et al. 1999; |
| | Vf 27 | 6 | 60 | present study |
| | Vf 27 | None | 56 | |
| | Vf 136 | None | 196 | Present study |

since they amplified intense and consistent products in previous studies (Torres et al. 1993).

Five seed-protein genes (USP, vicilin, legumin A, legumin B3 and legumin B4) were also surveyed. Legumins B3 and B4 map together in chromosome 3 (Vaz Patto et al. 1999), and were previously physically located by PCR with sorted or micromanipulated chromosomes and by in situ hybridisation (Macas et al. 1993a, b; Fuchs and Schubert 1995). The sequences and characteristics of the primers used for the detection of length polymorphisms among these genes were previously reported (Macas et al. 1993a). Amplification conditions were similar to those used with RAPD primers with some modifications (Vaz Patto et al. 1999), in order to maximise amplification and resolution of the products. Reaction mixtures were covered with mineral oil and amplified products were electrophoresed on 1% Seakem agarose, 1% Nu-Sieve agarose, 1×TBE gels, and visualised by ethidium bromide staining.

### SSR analysis

A total of 12 SSR loci were surveyed in the parental lines involved in the crosses. Six of them (GATA-2, GA II-68, GATA-11, JF1-AG3, GAII-59 and GA-4) showed polymorphic patterns in the cross Vf 6×Vf 27, and consequently the three $F_2$ families (175 individuals) from this cross were analysed. In case of the cross Vf 6×Vf 136, only microsatellites JF1-AG3, GAII-59 and GA-4 generated polymorphic products, and were analysed in the 196 $F_2$ individuals. These microsatellites were physically located on chromosome 1 of the species by PCR with flow-sorted chromosomes (Pozarkova et al. 2002).

PCR reactions were performed in 20 $\mu$l of 50 ng of plant genomic DNA, 1×buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.001% gelatin], 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 200 nM of each primer and 0.6 U of *Taq* DNA polymerase (Promega) using the following conditions: initial denaturation at 94°C for 5 min, 35 or 40 cycles of 1 min at 94°C, 50 s at respective annealing temperature and 2 min at 72°C, and final extension for 10 min at 72°C (Pozarkova et al. 2002).

When the polymorphism between the parental lines was easily identifiable, the PCR products were analyzed on ethidium bromide-stained 2% agarose gels. Only when the alleles were not easily distinguishable, acrylamide gels were used. In these cases amplification products were mixed with a loading buffer [0.006 M EDTA pH 8.0, 7% Ficoll (400), 0.16% bromophenol blue and 0.16% xylene cyanol] in a 1:6 proportion. From the mixture, 15 $\mu$l were loaded in a vertical 10% non-denaturing polyacrylamide gel (1-mm thick and 20-cm long). Amplified products were electrophoresed for 6–8 h at 19 mA. Electrophoresis buffer was 1×TBE (0.09 mM TRIS-borate pH 8.0, 2 mM EDTA) and bands were visualised by ethidium bromide staining.

### Statistical analysis

Segregation data from nine $F_2$ families derived from trisomic plants and two $F_2$ disomic populations were joined to create the *V. faba* composite map. Goodness-of-fit to the normal co-dominant ratio 1:2:1 (isozymes and codominant SSRs or legumin genes) or the dominant 3:1 ratio (morphological traits and RAPD markers) was tested by chi-square analysis. Those markers that did not show disomic segregation were excluded from the analysis.

In order to create a composite map, we assumed that: (1) the genes encoding morphological and isozyme phenotypes were homologous 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 identified by 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 the products with the same MW detected in all the plants, even if they were obtained in different crosses. Missing values were assigned to all the individuals of a family when (a) the marker did not segregate, (b) the RAPD marker segregates but the amplification was very faint and phenotypes could not be distinguished unambiguously, and (c) the marker did not fit the expected Mendelian segregation.

Once the partial maps for each cross were available, the process of integration was possible by using common maternal markers that allowed the development of a skeletal map (the Vf 6 map). The order of the markers thus obtained was considered as fixed for further mapping. Once the Vf 6 map was set, all the linkage groups from different crosses containing common maternal markers and placed on the same linkage group in the Vf 6 map, were arranged. For this purpose, at least two common markers are needed in order to unambiguously integrate two linkage groups. In cases where this requirement was fulfilled markers from all the groups were ordered according to its distance to the skeletal markers thus obtaining a single linkage group. Since many adjacent markers in the new integrated linkage group were not shared by all the mapping populations considered, the recombination fraction between them can not be directly assessed. Therefore, the distance (cM) between a pair of co-segregating markers in the integrated map generally coincided with the distance between them as calculated by their '$F_2$ family map'.

Data from different families were pooled and multilocus linkage groups (LGs) were constructed using MAPMAKER V2.0 (Lander et al. 1987). Recombination fractions were justified by using a LOD score of 3 as the threshold, and genetic distances (cM) were inferred by Kosambi's mapping function (1944).

## Results and discussion

The linkage analysis of family Vf 6×Vf 136 included a total of 131 markers (three isozymes, two seed-protein genes, two microsatellites and 124 RAPDs). From these markers, 27 (23 RAPDs, three isozymes and one seed-protein gene) were common with previous studies (Satovic et al. 1996; Vaz Patto et al. 1999), allowing the construction of a consistent composite map. The joint segregation analysis consisted of 501 markers and 654 individuals**,** and revealed 14 major LGs with a number of markers ranging from 5 to 103 (chromosome 1). Five LGs were unambigously assigned to specific chromosomes and included 192 markers: two morphological traits, (*yf* y *Sc*), six isoenzymatic loci (*Aco*-2*, Acp*-2*, Lap*-1*, Adh*-2*, Prx*-1*, Sod*-1), three seed-proteins genes (USP, B3 and B4), 176 RAPDs and four microsatellites (GATA-2, GA II-68, GATA-11 and GAII-59). One hundred and forty nine markers were attached to unassigned LGs and for the rest 160 remained unlinked.

For brevity, only those LGs assigned to specific chromosomes are reported and displayed in Fig. 1. Apart from the chromosomal sequences, a set of morphological traits and isozymes, previously located but which could not be integrated in their corresponding chromosome in joint analysis, are listed on each figure. Similarly**,** putative locations of the genes and the QTLs coding disease or parasitic plant resistance in the species are shown on the right. An extended explanation on additional symbols is displayed in Tables 2 and 3 together with the original references. The descriptive systematic used in each group will be as follows: (1) description of markers in the group; (2) common markers that allowed the joint analysis of data; (3) new markers ascribed to the *V. faba* map that

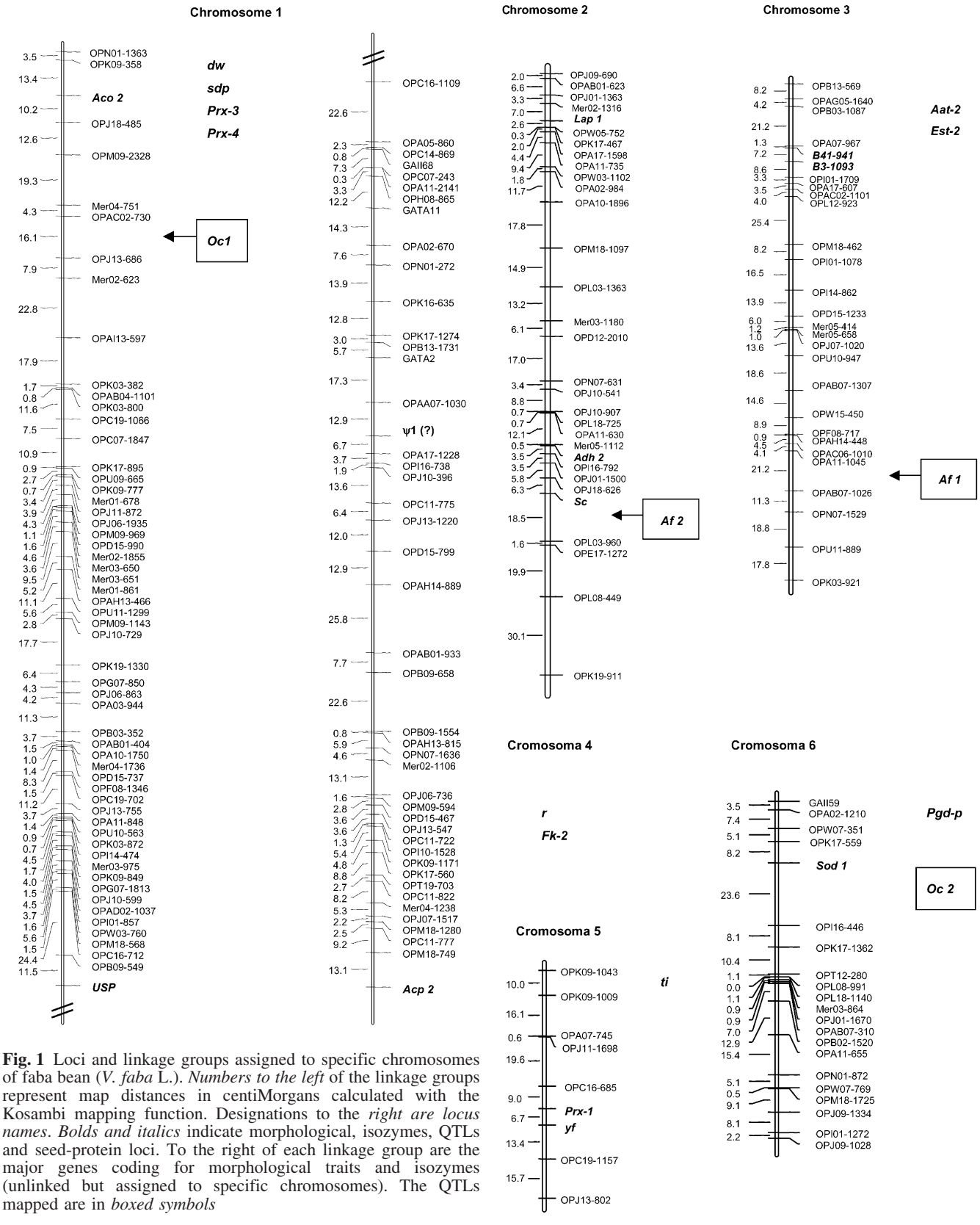


**Fig. 1** Loci and linkage groups assigned to specific chromosomes of faba bean (*V. faba* L.). *Numbers to the left* of the linkage groups represent map distances in centiMorgans calculated with the Kosambi mapping function. Designations to the *right are locus names*. *Bolds and italics* indicate morphological, isozymes, QTLs and seed-protein loci. To the right of each linkage group are the major genes coding for morphological traits and isozymes (unlinked but assigned to specific chromosomes). The QTLs mapped are in *boxed symbols*

have saturated new areas of the genome; (4) markers that allowed the location of the LG on a specific chromosome; and (5) mapped length of the chromosome. Nomenclature used for the chromosome, LG designation and the isozyme loci was as proposed by Satovic et al. (1995).

## Chromosome 1

The linkage group assigned to this chromosome included two morphological markers *sdp* (the solid distribution of pigment on the flower) and *dw* (short internodes), 96 RAPDs, two isozyme loci (*Aco*-2 and *Acp*-2), the seed-protein gene USP, the pseudogene of the legumin B4 ($\psi$1) and three microsatellites (GATA-2, GAII-68 and GATA-11). Ten markers were common with previous studies (Vaz Patto et al. 1999), and 32 new markers (31 RAPDs and the pseudogene $\psi$1) have been incorporated in the integrated map.

The location of $\psi$1 on this chromosome revealed a contradictory result in the study. Macas et al. (1993a) located the pseudogene of legumin B4 either on chromosome 4 or on the distal part of the long arm of chromosome 5. Nevertheless our linkage analysis clearly attached this marker with the LG assigned to chromosome 1 of the species. Once the homology of the fragment amplified (332 bp) with the one described by Heim et al. (1989) as pseudogen $\psi$1 was checked, PCR reactions with the flow-sorted chromosones of the parental lines involved in the crosses were performed. Results suggest that none of the lines contain the B4 pseudogene on chromosome 1 (data not shown). In parallel, segregation analyses of $\psi$1 in different $F_2$ populations derived from plants trisomic for chromosomes 4 and 5 were also carried out, revealing, in any case, a good fit to the 1:2:1 ratio. With no additional information available we do not know how to reconcile both hypotheses. Thus, the assignment of the pseudogene on this array should be questioned until the reasons for these contradictory results are elucidated.

On the other hand, the presence of the seed-protein gene USP and the three chromosome 1 specific microsatellites, allowed the precise assignment of this linkage group. Further confirmation is derived from previous studies carried out by González (1985), Cabrera et al. (1989) and Satovic et al. (1996), that discarded the position of the morphological genes *sdp* and *dw* on chromosomes 2, 3, 4, 5 and 6. Other trisomic segregation studies have also rejected the position of loci *Aco*-2, on chromosomes 3, 4, 5 and 6, and *Acp*-2, on chromosomes 3, 4, 5 and 6 (Table 2). Microsatellite JF1-AG3, and the isozyme loci *Prx*-3 and *Prx*-4, were also ascribed to this array by linkage analysis but in different mapping populations. The lack of common markers in the vicinity of the three loci did not allow the inference of their correct position on the chromosome (Fig. 1). As expected, for the large metacentric chromosome of the species, this LG was the longest described in the study and covered 800 cM.

## Chromosome 2

This chromosome included 28 RAPD markers, two isozyme loci (*Lap*-1 and *Adh*-2) and the morphological marker *Sc* (black seed colour). Five common markers made possible the grouping of data. *Sc* was previously located on chromosome 2 by Sjödin (1971) and allowed the assignment of the complete array to this chromosome. The isozyme loci ascribed to this LG do not deny this assignment since *Lap*-1 was discarded to be on chromosomes 4 and 6, and *Adh*-2 on chromosomes 4, 5 and 6 (Table 2). This group covered approximately 250 cM.

## Chromosome 3

Twenty six RAPDs and the legumins B3 and B4 were associated to this group. Vaz Patto et al. (1999), using trisomic segregation, unambiguously assigned both genes to this chromosome. Data from different progenies were pooled by means of two common markers and the analysis of the family Vf 6×Vf 136 allowed the integration of 15 new RAPDs. The isozyme loci *Est*-2 and *Aat*-2p, although remain unlinked in this study, were previously ascribed to this chromosome (Table 2). This linkage group covers 277 cM.

## Chromosome 4

Linkage analysis of markers previously assigned to this chromosome (Satovic et al. 1996) revealed surprising results. Ten RAPD markers and the isozyme locus *Fk*-2 exhibited typical trisomic deviation in families derived from a plant trisomic for chromosome 4. However, none of the pairwise combination of these markers tested in diploid families, nor the addition of new marker loci in following studies with different mapping populations, reveal linkage relationships among them. Up to now we do not know the reasons for such an outcome.

## Chromosome 5

Seven RAPD markers, the isozyme locus *Prx*-1 and the morphological marker *Yf/yf* (yellow flower) are included in this group. The chromosomal location was possible due to the presence of three common loci that exhibited trisomic segregation in different $F_2$ progenies derived from plant T5. Thus, in previous studies (Table 2), *Prx*-1 (Torres et al. 1995), *Yf/yf* and the RAPD marker OPC19 1157 (Satovic et al. 1996) were unequivocally assigned to this chromosome. Determinate growth (*Ti/ti*), assigned to this chromosome as well (Satovic et al. 1996), could not be integrated in the group after joint analysis. The total length covered by this array is 95.7 cM.

**Table 2** Chromosomal location of major genes coding for morphological traits and isozymes in faba bean

| Morphological trait | Gene | Chromosome | | | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| Unifoliata | *un* | | | No | | No | | Cabrera et al. 1989; Satovic et al. 1996 |
| Determinate growth | *ti* | | | | | Yes | | ICARDA 1986; Cabrera et al. 1989; Satovic et al. 1996 |
| Short internodes | *dw* | Yes[1] | No | No | No | No | No | González 1985; Cabrera et al. 1989; Satovic et al. 1996 |
| Solid distribution of pigment on flower | *sdp* | Yes[1] | No | No | No | No | No | González 1985; Cabrera et al. 1989; Satovic et al. 1996 |
| Yellow pigment on flower | *yf* | | | | | Yes | | González 1985; Cabrera et al. 1989; Satovic et al. 1996 |
| Hilum colour | *n* | | No | | No | No | No | Cabrera et al. 1989; Satovic et al. 1996 |
| Red seed-coat | *r* | | | | Yes | | | Cabrera et al. 1989; Satovic et al. 1996 |
| Black seed color | *Sc* | | Yes | | | | | Sjödin 1971; Vaz Patto et al. 1999 |
| **Isozymes** | **Gene** | **Chromosome** | | | | | | **References** |
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| Aspartate aminotransferase | *Aat*-2 | | | Yes | | | | Torres et al. 1995, 1998 |
| Aconitate hydratase | *Aco*-2 | Yes[2] | | | | | | Torres et al. 1995, 1998; Román 2000 |
| Acid phosphatase | *Acp*-2 | Yes[2] | | | | | | Torres et al. 1995, 1998 |
| Alcohol dehydrogenase | *Adh*-2 | | Yes[2] | | | | | Torres et al. 1998; Vaz Patto et al. 1999 |
| Esterase | *Est*-1 | | | No | No | | | Torres et al. 1995 |
| | *Est*-2 | | | Yes | | | | Torres et al. 1995 |
| Fructokinase | *Fk*-2 | | | | Yes | | | Torres et al. 1995 |
| Glucose-6-phosphate isomerase | *Gpi*-2 | | | | No | No | No | Torres et al. 1998 |
| Leucine amino-peptidase | *Lap*-1 | | Yes[2] | | | | | Torres et al. 1998; Vaz Patto et al. 1999 |
| Malic enzyme | *Me*-4 | | | | | No | | Torres et al. 1995 |
| Beta-N-acetyl-glucosaminidase | *Nag*-1 | | | No | No | | | Torres et al. 1995 |
| 6-Phosphogluconate dehydrogenase | *Pgd*-p | | | | | | Yes | Torres et al. 1995, 1998 |
| | *Pgd*-c | | | No | No | No | No | Torres et al. 1995, 1998 |
| Peroxidase | *Prx*-1 | | | | | Yes | | Torres et al. 1995 |
| | *Prx*-3 | Yes[2] | | | | | | Torres et al. 1998 |
| | *Prx*-4 | Yes[2] | | | | | | Torres et al. 1995 (referred as *Prx*-3) |
| Superoxide disumtase | *Sod*-1 | | | | | | Yes | Torres et al. 1995 |

[1] Indirect assignment of the loci after discarding its position on the rest of the chromosomes
[2] Loci assigned by linkage analysis

## Chromosome 6

This group joins 19 RAPDs, the isozyme locus *Sod*-1 and the microsatellite GAII-59. Data from different families were pooled by means of three common markers, and segregation data from the new Vf 6×Vf 136 $F_2$ population allowed the integration of additional markers (six RAPDs and microsatellite GAII-59). The trisomic segregation observed in families T6 analyzed in previous studies for five loci, *Sod*-1 (Torres et al. 1998), OPK17 1362 (Satovic et al. 1996), and OPL08 991, OPL18 1140 and Mer03 867 (Vaz Patto et al. 1999), facilitated the precise assignation of the LG to this chromosome. This fact was also supported by the trisomic segregation observed for microsatellite GAII-59 in the 6×27 T6 population. Although the microsatellite GAII-59 was originaly isolated as chromosome 1-specific (Pozarkova et al. 2002), additional experiments using flow-sorted chromosomes showed that except for a locus on chromosome 1 the primers also amplified a slightly longer fragment from chromosome 6 (data not shown). The product from chromosome 6 exhibits length polymorphism and corresponds to the marker mapped in this study. The length covered by this integrated group was of 135.6 cM. *Pgd*-p, also located in this chromosome according to trisomic segregation (Torres et al. 1995), remained unlinked in the present map.

Although the total map length obtained with this composite map has been 1,559 cM, it does not necessarily correspond to the real genetic distance. It is very likely that some linkage groups actually cover the same chromosomal regions but we were not able to check this because the markers segregate in different families. The use of dominant markers in the $F_2$ also raises the problem of phases between markers. We were not able to prove the existence of linkage between many pairs of actually

**Table 3** Major genes and QTLs coding disease / parasitic plant resistance in faba bean

| Trait | Gene / QTL | Chromosome | Linked / flanking markers | References |
|---|---|---|---|---|
| Rust resistance[1] | *Uvf*-1 | Unknown | $OPD13_{736}$; $OPL18_{1032}$; $OPI20_{900}$ | Avila et al. 2003 |
| Broomrape resistance[2] | *Oc1* | 1 | $OPJ13_{686}$/$OPAC02_{730}$ | Román et al. 2002 |
| | *Oc2* | 6 | $OPAC06_{342}$/$OPN07_{849}$ | Román et al. 2002 |
| | *Oc3* | Unknown | $OPW15_{533}$/$OPAA07_{807}$ | Román et al. 2002 |
| *A. fabae* resistance[2] | *Af1* | 3 | $OPA11_{1045}$/$OPAB07_{1026}$ | Román et al. 2003 |
| | *Af2* | 2 | $OPE17_{1272}$/$OPJ18_{626}$ | Román et al. 2003 |

[1] Monogenic trait
[2] Polygenic trait

linked markers because they were in repulsion phase and the number of individuals in some of our $F_2$ families was rather limited.

Maps generated using molecular markers often have greatly inflated lengths, compared with those predicted from chiasma counts (Nilsson et al. 1993; Sybenga 1996). Although the reason for this discrepancy is not fully understood, some explanations have been proposed. First, chiasma frequencies may be underscored because it is not possible to distinguish between a single chiasma or two closely adjacent ones (Sybenga 1975). A second hypothesis is that a different mechanism from chiasmata (gene conversion) is also responsible for recombination. Nevertheless, this effect can be ruled out since the frequency of recombination generated by such a mechanism is extremely low. Third, and perhaps more important, is that recombination frequencies may be biased upward because of the misclassification of phenotypes in genetic mapping. Nilsson et al. (1993) found that, even at a low frequency of misclassification, the estimated recombination values became strongly biased, and this bias was always upwards.

The number of chiasmata is dependent on the species and the length of the chromosome with the longer the chromosome, the more is the occurrence of chiasmata (Tease and Jones 1995). Considering that *V. faba* presents one of the largest genomes among legumes (about 13,000 bp; Bennet and Leitch 1995) and chromosome 1 is the largest of the species, it is feasible that a high number of chiasmata may occur. This statement, together with the fact that some linkage groups might cover the same chromosomal regions, is a possible explanation of the apparent expansion in the length of this map, in particular for chromosome 1.

Combining trisomic segregation, linkage analysis of loci detected in different populations, and the use of physically located markers, has facilitated the establishment of a more complete genetic linkage map with wide coverage of the chromosomes. The present map has allowed the detection of QTLs for important agronomic traits, such as seed weight or broomrape and *Ascochyta fabae* resistance, as well as to tag a gene controlling resistance to *Uromyces fabae* (Table 3).

The use of a recurrent asynaptic parent (Vf 6) in all our crosses allowed us to join data from different progenies by means of common markers present in the female parent. Moreover, the segregation analysis of molecular markers in trisomic families for chromosomes 3, 4, 5 and 6 directly assign their corresponding LGs to chromosomes. However, recovering trisomic plants for chromosome 1 (the largest of the *V. faba* genome) is difficult and, if obtained, they are infertile. In the case of a plant trisomic for chromosome 2, the number of individuals recovered is not large enough to be used in segregation and linkage studies. Consequently, the location of markers in chromosomes 1 or 2 is only possible: (1) by excluding their presence on other chromosomes considering trisomic family segregation, or (2) using physically located markers.

The development of the latter type of markers is of great interest in faba bean in order to assign LGs to chromosomes 1 or 2. Apart from legumins B3 and B4, the seed protein marker USP, physically localized on chromosome 1 (Macas et al. 1993a), has been successfully used to assign LGs to a specific chromosome (Vaz Patto at al. 1999). With the aim of integrating physically localized markers into existing LGs, new microsatellites from chromosome 1-specific DNA libraries of *V. faba* were developed (Pozarkova et al. 2002), and subsequently tested in this study. From the six microsatellites polymorphic in the cross 6×27, three of them (GATA-2, GATA-11 and GAII-68) map to the same LG as USP, further confirming its correct assignation to chromosome 1. The rest of the microsatellites from this chromosome that show Mendelian segregation remained unlinked. Future saturation of the *V. faba* genome should allow us to include them in their specific chromosome. In the case of cross Vf 6×Vf 136, JF1-AG3 maps on a LG possibly belonging to chromosome 1, but was difficult to infer its correct position for the reason explained above. GAII-59 was unexpectedly linked to chromosome 6 which, in turn, was explained by the presence of two independent loci; while GA-4 did not fit the expected 1:2:1 segregation and was omitted to the linkage analysis. After discarding an error in the scoring (the bands were clear and repeatable), and with no evidence of gametophytic selection favouring some of the alleles, we ignore the causes of this lack of fit.

It should be mentioned that different isozyme markers (*Prx*-3, *Prx*-4, *Est*-2; *Aat*-2; *Fk*-2 or *Pgd*-p) as well as the morphological markers (*dw*, *sdp* and *ti*), all of them were previously assigned to chromosomes considering trisomic segregation (Table 2) and could not be attached to

specific LGs. These functional genes could be located in other regions not so densely covered by RAPD markers, due to its tendency to group in specific areas close to the centromeres (Schmidt et al. 1995). A future approach should consider the use of new microsatellites as well as other types of markers, such as AFLPs or STSs, that might saturate new regions of the genome. This approach should also help to clarify the discrepancy betwen physical localization and the observed segregation of pseudogene $\psi 1$, which is reminiscent to the results obtained with legumin B3 in our previous studies (Macas et al. 1993a; Vaz Patto et al. 1999).

*V. faba* belongs to what is considered the basal genus in the *Viceae* and thus, information about the arrangement of genes on its genome should be very important for examining the evolution of the genome structure in the tribe. Several studies show a relatively high degree of gene order conservation in the legumes. A pioneering report established the common gene order across at least 40% of the lentil and pea genomes (Weeden et al. 1992); and further suggested, based on isozyme loci, the conservation of many chromosomal regions in *Vicia* (Torres et al. 1993, 1995; Vaz Patto et al. 1999). Subsequently, Menacio-Hautea et al. (1993) discovered a high degree of linkage conservation between the mung bean and cowpea. Extensive regions of similarity were also found among linkage segments of pea, lentil and chickpea maps (Simon and Muehlbauer 1997; Ellis and Poyser 2002).

At present, molecular studies of faba bean have been limited to morphological traits, isozymes, a few seed-protein genes and microsatellites, and a great number of RAPD markers. The latter enabled genetic maps to be constructed rapidly, but such maps cannot be aligned with those of other species. New DNA standard markers, useful for cross-referencing genetic information, are needed to reveal additional areas of homology and to detect possible rearrangements among these genera. To overcome this, we are transforming a set of RAPD markers into SCARs to correlate linkage maps across different progenies or legume species. PCR primers are being designed so that polymorphism between parents of different mapping populations (or even legume species) can be detected, either directly after restriction digestion. For breeding purposes, special interest is being focused on the RAPD markers tightly linked to genes or QTLs of agronomic interest (Table 3). Besides, a set of chromosome-specific RAPDs, detected by trisomic segregation and evenly spaced along the six chromosomes of the species, is also being transformed into standard markers. Thus, alignment between chromosome/linkage groups among the *Viciae* might be facilitated in the future. There is little information available to predict the transferability of SCAR/STS markers but their ease of assay enables them to be tested empirically. Even a relatively low level of transferability will facilitate the elaboration of meaningful comparative maps for trait mapping and marker-assisted breeding in all legumes species, including those with few marker resources available today.

A map useful for marker-assisted selection requires saturation, especially in the regions harbouring agronomically important traits. However, *V. faba* possesses one of the largest genomes among legumes (approximately 13,000 Mb), compared with other better-characterized crops as soybean, pea or *Medicago* (about 1,200 Mb, about 4,000 Mb and 450 Mb, respectively). The large genome sizes seriously complicate the development of saturated linkage maps, as well as the identification and location of important genes. Thus, our future aims also extend the genome coverage to attach all currently unassigned markers and reduce the number of linkage groups to six.

Considering all the limitations that the process of map integration involves, this map could be seen as the best approximation that could be obtained with the present data. Nevertheless, the reported map will be very useful for our future research since it would enable us to pre-select markers to saturate relevant genome regions (namely those where putative QTLs for specific traits are located). Moreover, we will continue the mapping of new major genes and QTLs of agronomic interest, and refining their position in the map to facilitate marker-assisted selection in the species.

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