

# QTL Detection for Agronomic Traits in Faba Bean (*Vicia faba* L.)

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## SUMMARY

Complex agronomic traits are normally under polygenic control. The traits evaluated in faba bean in the present study may be grouped into three main categories: floral characters, yield distribution and yield characters, according to other authors. A F<sub>2</sub> population derived from the cross 29H x Vf 136 was used for QTLs analysis. A total of 15 QTLs were detected for 8 out of 10 traits evaluated. The analysis detected 2, 5 and 8 QTLs for floral characters, yield distribution traits and yield characters, respectively. QTLs for different traits co-localized in three regions of the map located in linkage groups 3, 4 and 13, which suggests the existence of common pleiotropic genes for the control of these traits. Alternatively, these regions might be hot-spots QTLs, i.e. chromosomal regions with several QTLs controlling different traits as reported for several organisms.

The present work constitutes a first step towards the identification of molecular markers for agronomic traits, some of them yield related, in faba bean. After validation, the putative QTLs detected in this work will provide valuable tools for pyramiding QTLs of all these traits in a faba bean breeding program.

## KEY WORDS

*Vicia faba*, agronomic traits, QTLs, breeding

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Received: June 26, 2005

## Acknowledgements

This study was supported by grants SC97-005-C2-1 and RTA01-30 from the Spanish Instituto Nacional de Investigaciones Agrarias (INIA). C.M. Avila was supported by a contract financed by the Juan de la Cierva Program from the Ministerio de Educación y Ciencia of Spain.

## INTRODUCTION

Since the dawn of civilization legumes, together with cereals, have been fundamental to the development of modern agriculture providing protein in human diet, edible oils, and fodder and forage for animals. Faba bean (*Vicia faba* L.) is one of the oldest legume crops mainly grown for human and animal dietary needs. Like other grain legumes, faba bean contributes to sustainable agriculture by fixing atmospheric nitrogen in symbiosis with soil bacteria. This unique ability reduces the dependence of farmers on extensive use of chemical fertilizers protecting soil and water quality. In addition, legumes play a critical role in crop rotation, improving soil physical conditions and decreasing the amount of diseases and weed populations which in turn leads to lower consumption of herbicides and fungicides.

The advantages of grain and forage legumes are largely under-exploited in Europe. Legumes represent only 5% of the agricultural area in the European Union compared with 20-30% in North and South America, Australia and Asia. Moreover, in the case of faba bean, the total area of cultivation has steadily decreased in many countries over the last century. The recent surge of the Bovine Spongiform Encephalopathy (BSE) attributed to the use of animal-based cattle feed has increased the demand for plant protein in the EU that has been covered with large imports of soybean from other countries. This dependence has produced a renovated interest for alternative sources of protein that might be covered by several traditional legumes crops.

Large-scale production of faba bean has been historically hindered by low and unstable yields as well as by susceptibility to several diseases (Duc, 1997; Knott, 1997). To turn faba bean into an attractive alternative crop for farmers breeding efforts should be directed to the identification of high yielding genotypes of faba beans well adapted to each farming condition. Agronomic traits in faba bean, as in other species, are usually complex characters determined by several interacting components, some of which are under polygenic control. This fact greatly hampers the selection process and the success of traditional breeding programs. Elucidation of the genetic basis of these yield-related characters is of outmost importance to achieve yield increases that might reinforce future faba bean cultivation in European farming systems.

The advent of molecular markers has greatly facilitated the detection of genomic regions controlling quantitative characters. Using linkage maps, it is possible to estimate the number of loci controlling genetic variation in a segregating population and to characterize these loci (Quantitative trait loci-QTLs). Furthermore, since QTL-linked molecular markers allow the indirect selection of interesting genotypes,

they constitute an essential tool for the development of Marker Assisted Selection (MAS) programs.

In *V. faba*, this strategy has been successfully used to identify QTLs conferring resistance against diseases such as *Orobanche crenata* Forsk. (Roman et al., 2002) and *Ascochyta fabae* Speg. (Roman et al., 2003, Avila et al., 2004) as well as molecular markers closely linked to a resistance gene against *Uromyces viciae-fabae* (Pers.) J. Shört (Avila et al., 2003b). However, few efforts have been performed so far to improve breeding for agronomic traits using molecular markers. To our knowledge, only Ramsay et al. (1995) tried this approach to map QTLs related to the agronomic traits affecting yield, although their results were limited due to the low density of the map used. The main objective of the present study was therefore, the development of a molecular genetic linkage map in faba bean suitable to identify QTLs for important agronomic traits in the species.

## MATERIAL AND METHODS

### Plant material

A population of 159 F<sub>2</sub> plants from the cross between the faba bean inbred lines 29H and Vf 136 was used for mapping and the corresponding F<sub>2:3</sub> families were tested for yield components in the field (Table 1). Parents were remarkably contrasted for the traits of interest.

### Field evaluation

One hundred and fifty nine F<sub>2:3</sub> families were evaluated in a field trial following a completely randomized design. Each entry was represented by ten plants in a single row. Distance between rows was 70 cm and plants were spaced 10 cm within the row. Both parents were included in the evaluation process. Field studies were conducted at the CIFA (Centro de Investigación y Formación Agraria, Córdoba, Spain) using standard agronomic practices for the region.

According to Nadal et al. (2003), the traits evaluated in this study can be grouped into three different categories: (1) floral characters as flowers per inflorescence (FPI) and nodes with flowers (NF), (2) yield distribution: height (cm) of the lowest node bearing flowers (HLF), number of lateral branches with pods (BRWP), height (cm) of the lowest node bearing pods (HLP), distance (cm) between the first node bearing flowers and the first node bearing pods (FNPF) and (3) yield characters as pod length (cm) (PL), fertilized ovules per pod (OPP) and the primary components of yield: seeds per mature pod (SPP) and pods per plant (PPP).

### Molecular analyses

Molecular analyses were carried out using plant tissue from young leaves or roots of the F<sub>2</sub> progeny. RAPD amplification was performed according to Williams et

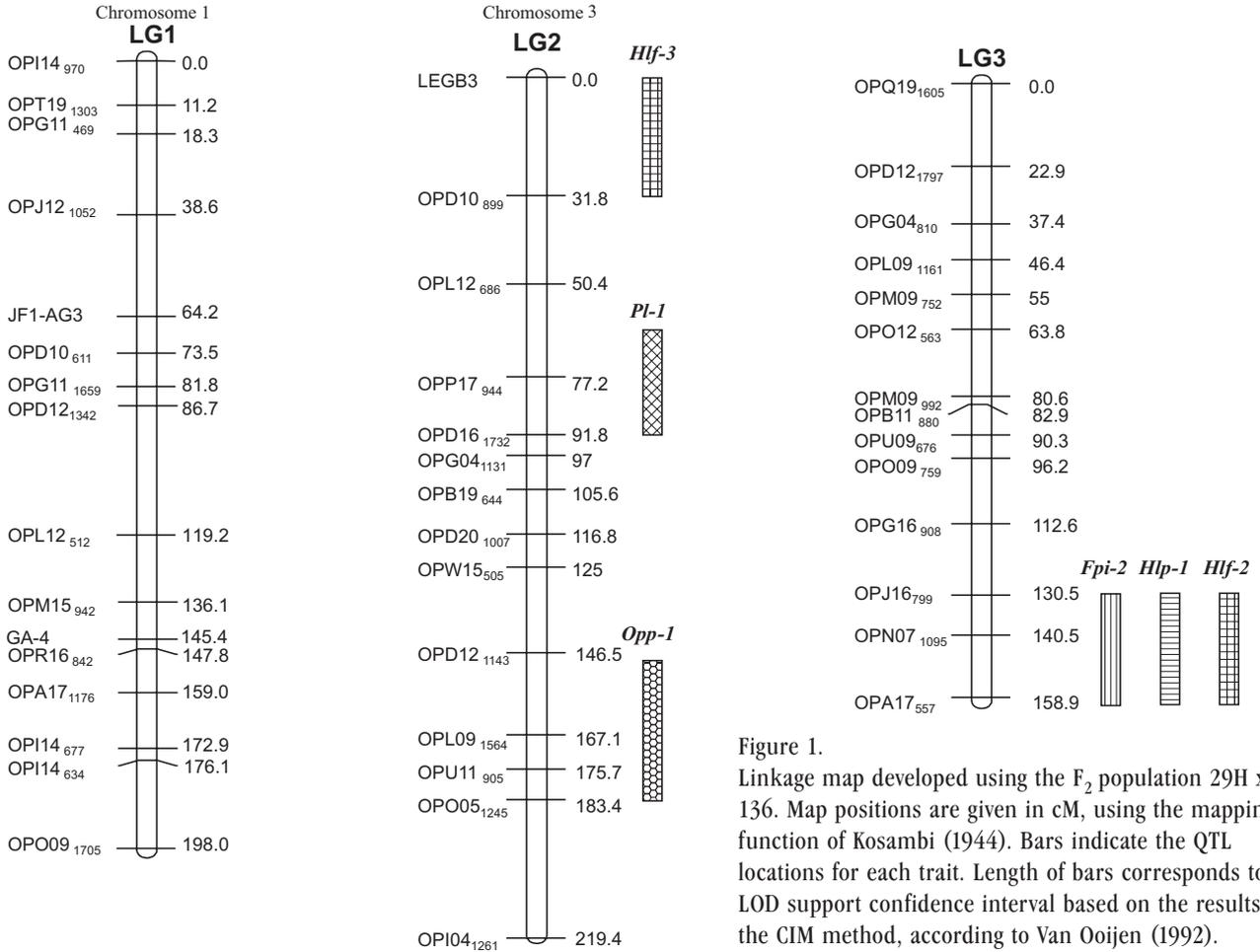
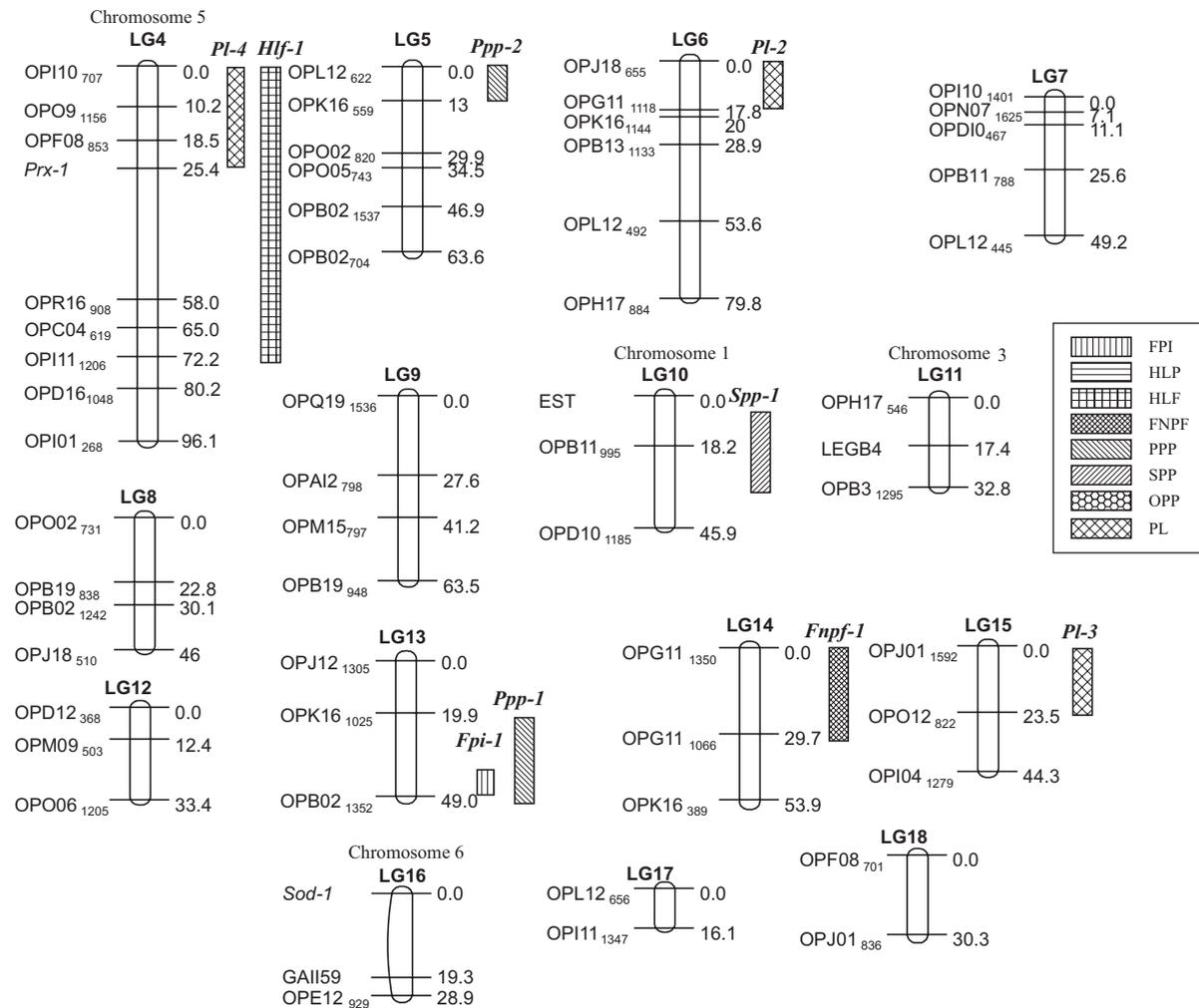


Figure 1. Linkage map developed using the F<sub>2</sub> population 29H x Vf 136. Map positions are given in cM, using the mapping function of Kosambi (1944). Bars indicate the QTL locations for each trait. Length of bars corresponds to 2 LOD support confidence interval based on the results of the CIM method, according to Van Ooijen (1992).



al. (1990) and Welsh and McClellan (1990), following the modifications of Torres et al. (1993). For isozyme markers, horizontal starch-gel electrophoresis was performed according to Gottlieb (1973) and all the assay solutions were adapted from those reported by Wendel and Weeden (1990). Amplification conditions for legumins B3 and B4 were similar to those of RAPDs with modifications described by Vaz Patto et al. (1999). Microsatellite markers were amplified according to Macas et al. (1993a) and Vaz Patto et al. (1999). Isozyme loci (Torres et al., 1998; Avila et al., 2003a), legumines (Macas et al., 1993a; Macas et al., 1993b; Fuchs and Schubert, 1995), and microsatellite markers (Pozarkova et al., 2002) used in this map were previously localized on specific chromosomes of the species.

### Map construction

Each marker was tested against the expected segregation ratio using a chi-square goodness of fit in the  $F_2$  population. The linkage map was constructed using MAPMAKER/EXP version 3.0 (Whitehead Institute, Cambridge, Mass., USA; Lincoln et al., 1993). A LOD threshold of 3 was established to consider significant linkage. Recombination fractions were converted to centiMorgans (cM) using the Kosambi's mapping function (Kosambi, 1944).

### QTL analysis

The linkage map and the quantitative data of agronomic traits were used for the QTL analyses. QTL location was performed using three different methods included in the QTL CARTOGRAPHER software (Basten et al., 2001): Linear regression (LR, in LRmapqtl), Simple Interval Mapping (SIM, Model 3 in Zmapqtl) and Composite Interval Mapping (CIM; Model 6 in Zmapqtl). Markers to be used as cofactors for CIM were selected by Forward-Backward

stepwise regression (FB method in SRmapqtl). A minimum LOD of 3 was established to consider a QTL as significant. Uncertainty of the map position for the QTLs was calculated using a 2-LOD-support interval (Van Ooijen, 1992) (Figure 1).

## RESULTS

### Map

A linkage map was developed using a  $F_2$  population from a cross between two inbred lines of faba bean contrasting for several agronomical traits affecting yield (Table 1). One hundred and three marker loci segregating in the  $F_2$  population were mapped into 18 linkage groups (LGs) ranging from 2 to 16 marker loci and covering 1308 cM of the faba bean genome. Out of these 103 markers, 95 were RAPD markers, 3 isozyme loci (*Est-1*, *Prx-1* and *Sod-1*), 2 seed protein genes (legumins B3 and B4) and 3 microsatellites (GA-4, GAII-59 and JF1-AG3).

### Traits

Simple correlation coefficients between the studied characters were computed (Table 2) revealing a positive significant correlation between HLP and HLF ( $r = +0.82^{***}$ ). HLP and HLF were positively correlated with FPI ( $r = +0.41^{***}$  and  $+0.34^{**}$ , respectively) and HLP was also positively correlated with FNFP ( $r = +0.45^{***}$ ). BRWP showed a positive relationship with FPI ( $r = +0.27^*$ ) and a negative significant correlation with HLF and NF ( $r = -0.22^*$ ). A positive relationship between BRWP and PPP ( $r = +0.66^{***}$ ) was recorded as well, indicating the importance of the number of branches with pods in determining the final yield of faba beans.

All the evaluated traits within the group named "yield characters" were positively correlated with

Table 1. Phenotypic data obtained after traits evaluation in the parental lines (29H and Vf 136), and the  $F_{2,3}$  progeny.

Vegetal material	Traits	29H	Vf 136	$F_{2,3}$		
		Average	Average	Average	Min	Max
Floral characters	FPI <sup>a</sup>	7.24	4.84	5.48	3.98	6.88
	NF <sup>b</sup>	6.21	17.63	17.35	7.00	21.67
Yield distribution characters	HLF <sup>c</sup>	30.20	12.56	16.75	9.60	31.88
	HLP <sup>d</sup>	30.20	18.33	20.68	11.86	34.25
	FNPF <sup>e</sup>	0.00	9.38	4.10	0.00	13.00
	BRWP <sup>f</sup>	6.80	3.56	4.58	3.00	10.00
Yield characters	PPP <sup>g</sup>	31.60	20.56	27.75	15.43	49.50
	PL <sup>h</sup>	4.26	5.43	5.90	4.66	7.31
	SPP <sup>i</sup>	3.14	2.58	2.93	1.89	4.03
	OPP <sup>j</sup>	3.44	2.77	3.36	2.47	4.35

\* Significant at  $P < 0.05$ , \*\* Significant at  $P < 0.005$ , \*\*\* Significant at  $P < 0.001$

(a)FPI: Flowers per inflorescence; (b)NF: Number of nodes with flowers; (c)HLF: Height of the first node bearing flowers (cm) (d)HLP: Height of the first node bearing pods (cm); (e)FNPF: Difference between the height of the first node bearing pods and the first one bearing flowers (cm); (f)BRWP: Number of branches with pods; (g)PPP: Pods per plant; (h)PL: Pod length (cm); (i)SPP: Seeds per pod; (j)OPP: Ovules per plant

Table 2. Correlation coefficients among the traits evaluated in the F<sub>2:3</sub> population derived from the cross between the *V. faba* genotypes 29H and Vf 136.

Traits	FPI <sup>a</sup>	NF <sup>b</sup>	HLF <sup>c</sup>	HLP <sup>d</sup>	FNFP <sup>e</sup>	BRWP <sup>f</sup>	PPP <sup>g</sup>	PL <sup>h</sup>	SPP <sup>i</sup>	OPP <sup>j</sup>
FPI <sup>a</sup>	1	+0.07	+0.34**	+0.42***	+0.09	+0.27*	+0.06	-0.07	-0.02	+0.02
NF <sup>b</sup>	-	1	-0.22*	-0.03	+0.28	-0.03	+0.14	-0.02	-0.01	-0.08
HLF <sup>c</sup>	-	-	1	+0.83***	-0.15	+0.09	+0.05	-0.07	-0.10	-0.00
HLP <sup>d</sup>	-	-	-	1	+0.45***	+0.04	+0.03	-0.03	-0.15	-0.02
FNFP <sup>e</sup>	-	-	-	-	1	+0.00	+0.04	+0.04	-0.12	-0.07
BRWP <sup>f</sup>	-	-	-	-	-	1	+0.66***	+0.03	-0.02	+0.01
PPP <sup>g</sup>	-	-	-	-	-	-	1	-0.01	-0.12	-0.06
PL <sup>h</sup>	-	-	-	-	-	-	-	1	+0.68***	+0.72***
SPP <sup>i</sup>	-	-	-	-	-	-	-	-	1	+0.87***
OPP <sup>j</sup>	-	-	-	-	-	-	-	-	-	1

\* Significant at P < 0.05, \*\* Significant at P < 0.005, \*\*\* Significant at P < 0.001

(a)FPI: Flowers per inflorescence; (b)NF: Number of nodes with flowers; (c)HLF: Height of the first node bearing flowers (cm); (d)HLP: Height of the first node bearing pods (cm); (e)FNFP: Difference between the height of the first node bearing pods and the first one bearing flowers (cm); (f)BRWP: Number of branches with pods; (g)PPP: Pods per plant; (h)PL: Pod length (cm); (i)SPP: Seeds per pod; (j)OPP: Ovules per plant

each other. Thus, a high correlation was detected between the following pairs of traits: PL-SPP, PL-OPP and SPP-OPP (r= +0.68\*\*\*, +0.72\*\*\* and +0.87\*\*\*, respectively). Similar results on positive correlations between pod length (PL) and seed number per pod (SPP) were reported by Musallam et al. (2004) in faba bean. Besides, SPP has been reported to be a stable parameter in *V. faba* (Suso et al., 1996; Thomson et al., 1997), suggesting that this trait might be critical to improve yield stability in the species. Since parental lines in the study differ for this trait, the QTL analyses may reveal genomic regions useful for breeding for stable yield in faba bean.

### QTL analysis

Linear regression, Simple Interval Mapping (SIM) and Composite Interval Mapping (CIM) methods were used to identify QTLs for the evaluated agronomical traits. Since the results obtained with the three methods were consistent, only the results derived from the CIM method are presented (Table 3). Phenotypic values of the parents and their progeny for all characters analysed are shown in Table 1.

Table 3 presents the 15 putative QTLs detected using CIM. Out of these, 2 were related to floral characters, 5 to yield distribution and 8 to yield characters.

### Floral characters

Two putative QTLs, with LOD values higher than 3, were detected for FPI in linkage groups 13 and 3 (*Fpi-1* and *Fpi-2*) (Table 3; Figure 1). The maximum LOD values were 4.2 and 3.5, respectively and accounted for 20.3% and 13.1 % of the observed phenotypic variation. No significant QTLs for NF were identified since the maximum LOD value was 2.8 in LG 4.

### Yield distribution characters

For 3 of the traits considered for yield distribution (HLF, HLP and FNFP), five QTLs were detected (Table 3; Figure 1). Three of them (*Hlf-1*, *Hlf-2* and *Hlf-3*), related to HLF, were located in LGs 4, 3 and 2 (LOD = 3.3, 3.2 and 3.1, respectively) and explained 9.1%, 11.7% and 17.2 % of the observed phenotypic variation (Table 3; Figure 1). A QTL related to HLP (*Hlp-1*) was located on LG 3 (LOD value 3.3) and explained 15.1% of the variation. Another QTL for FNFP (*Fnpf-1*) was mapped in LG 14 displayed a LOD value of 3.1 and explained a 41.2% of the phenotypic variation. No QTL for BRWP was detected.

### Yield characters

Eight QTLs were detected for the yield characters evaluated. Two of them, had an effect on PPP (*Ppp-1* and *Ppp-2*) and were mapped in LGs 13 and 5, respectively (Table 3; Fig 1). Their maximum LOD values were 5.5 and 3.6 and accounted for the 57.5% and 51.6% of the observed variation, respectively. Four QTLs for PL were identified (*Pl-1*, *Pl-2*, *Pl-3* and

Table 3. Putative QTLs detected for agronomic traits in faba bean (*Vicia faba* L) by composite interval mapping (CIM).

	Traits	QTL	LG <sup>a</sup>	Flanking Markers	LOD	Var % <sup>b</sup>	Gene Action <sup>c</sup>
Floral characters	FPI <sup>d</sup>	<i>Fpi-1</i>	LG 13	OPK16 <sub>1025</sub> -OPB02 <sub>1352</sub>	4.2	20.3	OD
		<i>Fpi-2</i>	LG 3	OPN07 <sub>1095</sub> -OPA17 <sub>557</sub>	3.5	13.1	PD
Yield distribution characters	HLF <sup>e</sup>	<i>Hlf-1</i>	LG 4	OPF08 <sub>853</sub> - <i>Prx-1</i>	3.3	9.1	PD
		<i>Hlf-2</i>	LG 3	OPN07 <sub>1095</sub> -OPA17 <sub>557</sub>	3.2	11.7	A
		<i>Hlf-3</i>	LG 2	LEGB3-OPD10 <sub>899</sub>	3.1	17.2	D
	HLP <sup>f</sup>	<i>Hlp-1</i>	LG 3	OPN07 <sub>1095</sub> -OPA17 <sub>557</sub>	3.3	15.1	PD
	FNPF <sup>g</sup>	<i>Fnpf-1</i>	LG 14	OPG11 <sub>1350</sub> -OPG11 <sub>1066</sub>	3.1	41.2	OD
Yield characters	PPP <sup>h</sup>	<i>Ppp-1</i>	LG 13	OPK16 <sub>1025</sub> -OPB02 <sub>1352</sub>	5.5	57.5	OD
		<i>Ppp-2</i>	LG 5	OPL12 <sub>622</sub> -OPK16 <sub>559</sub>	3.6	51.6	D
	PL <sup>i</sup>	<i>Pl-1</i>	LG 2	OPP17 <sub>944</sub> -OPD16 <sub>1732</sub>	6.6	19.2	A
		<i>Pl-2</i>	LG 6	OPJ18 <sub>655</sub> -OPG11 <sub>1118</sub>	4.2	12.7	PD
		<i>Pl-3</i>	LG 15	OPJ01 <sub>1592</sub> -OPO12 <sub>822</sub>	3.9	34.6	OD
		<i>Pl-4</i>	LG 4	OPO9 <sub>1156</sub> -OPF08 <sub>853</sub>	3.2	12.0	A
	SPP <sup>j</sup>	<i>Spp-1</i>	LG 10	OPB11 <sub>995</sub> -OPD10 <sub>1185</sub>	3.6	22.6	OD
	OPP <sup>k</sup>	<i>Opp-1</i>	LG 2	OPL09 <sub>1564</sub> -OPU11 <sub>905</sub>	3.3	13.2	OD

(a)LG: Linkage group; (b)Var%: Proportion of phenotypic variance explained

(c)Gene Action: A, additive effect D, dominance; PD, partial dominance and OD, over-dominance (Stuber et al. 1992)

(d)FPI: Flowers per inflorescence; (e)HLF: Height of the first node bearing flowers (cm); (f)HLP: Height of the first node bearing pods (cm)

(g)FNPF: Difference between the height of the first node bearing pods and the first one bearing flowers (cm); (h)PPP: Pods per plant

(i)PL: Pod length (cm); (j)SPP: Seeds per pod; (k)OPP: Ovules per plant

*Pl-4*) and associated with LGs 2, 6, 15 and 4 (Figure 1). LOD values were 6.6, 4.2, 3.9 and 3.2, respectively, and explained 19.2%, 12.7%, 44.6% and 12.0% of the observed phenotypic variation (Table 3). Analysis of the seeds per pod (SSP), resulted in the detection of one QTL (*Spp-1*) in LG 10. *Spp-1* had a LOD value of 3.6 and explained 22.6 % of the phenotypic variation (Table 3; Figure 1). The last QTL with significant effect (LOD= 3.3) on the number of ovules per plant (OPP) was located in LG 2 (Figure 1) and explained the 13.2% of the observed variation.

### QTLs Effects

The effects shown for each of the QTLs are summarized in Table 3. Gene action was determined using the classification described by Stuber et al. (1992). Six QTLs (*Fpi-1*, *Fnpf-1*, *Ppp-1*, *Pl-3*, *Spp-1* and *Opp-1*) presented overdominance, 2 QTLs (*Hlf-3* and *Ppp-2*) dominance, 4 (*Fpi-2*, *Hlf-1*, *Hlp-1* and *Pl-2*) showed partial dominance and the last 3 QTLs (*Hlf-2*, *Pl-1* and *Pl-4*) presented an additive gene action.

### Assignment of QTLs to chromosomes

Six of the QTLs reported were assigned to specific chromosomes of the species since they were located in linkage groups carrying physically localized markers. This is the case for LG 2 that was adscribed to chromosome 3 by the legumin B3 previously located on this chromosome (Vaz Patto et al., 1999; Macas et al., 1993a; 1993b). Therefore *Hlf-3*, *Pl-1* and *Opp-1* associated to this linkage group are located on chromosome 3 (Figure 1). In the same way, the isoenzymatic marker *Prx-1* was previously assigned to

chromosome 5 by Torres et al. (1998). Consequently, LG 4 carrying QTLs *Pl-4* and *Hlf-1* can be adscribed to this chromosome. The same authors located *Est-1* on chromosome 1, allowing the assignment of *Spp-1*, placed on LG 10 to the largest chromosome of the species.

### DISCUSSION

In this study, a F<sub>2:3</sub> population of the cross 29H×Vf136 was used for mapping and detection of QTLs related to agronomic traits in faba bean. The linkage map spanned 1308 cM and included 103 molecular markers arranged in 18 linkage groups. The analysis identified a total of 15 QTLs (2 for floral characters, 5 for yield distribution traits and 8 for yield components) distributed on 9 of the 18 LGs (Figure 1). Six of the QTLs reported could be assigned to specific chromosomes. Thus, *Spp-1* was located on chromosome 1, *Hlf-3*, *Pl-1* and *Opp-1* on chromosome 3 and *Pl-4* and *Hlf-1* on the chromosome 5 of the species.

To our knowledge, the only previous study on QTLs related to agronomic traits in faba bean was that of Ramsey et al. (1995). Some of the characters studied by these authors (pod length, flowers per node, number of stems and seeds per pod) were also considered in the present study. They used a genetic linkage map consisting of 23 molecular markers distributed on seven linkage groups spanning 300 cM of the *V. faba* genome. Despite the low density of the map, the number of QTLs detected was high. This may be due to different reasons. First, the LOD threshold to accept

the existence of a QTL (LOD=2) may have been too low leading to the detection of false positives. The second reason might be the exotic origin of one of the parents used in their cross. One of the lines (VF 172) was a *paucijuga* type, considered as the closest relative to the unknown wild ancestor of *V. faba*, while the other was a traditional European cultivar. Both parents strongly differed for the traits under study, increasing the probability of QTL detection. None of the molecular markers used by these authors were common with the present study preventing a detailed comparison of results.

Several QTLs for different traits were located in the same chromosomal regions. This is particularly the case for the region between OPJ16<sub>799</sub> and OPA17<sub>557</sub> on LG 3 where QTLs *Hlp-1*, *Hlf-2* and *Fpi-2* were mapped. The same situation was also found for *Pl-4* and *Hlf-1*, on LG 4 (Figure 1) and *Fpi-1* and *Ppp-1*, on LG 13 (Figure 1). The identical localization of QTLs *Hlp-1*, *Hlf-2* and *Fpi-2* controlling 3 traits highly correlated seems to indicate that the region harbours common pleiotropic genes coding for these traits. The same consideration may be extended to QTLs *Pl-4* and *Hlf-1*, and *Fpi-1* and *Ppp-1* although, in these cases, no significant correlation between the corresponding traits was found. Alternatively, it might be possible that a concentrated distribution of QTLs or hot-spot occurs. The existence of such chromosomal regions with several QTLs controlling different traits have been reported in several organisms (Li et al., 2000; Fridman et al., 2000; Schadt et al., 2003). A higher map density will be required to elucidate which of the hypotheses is correct. In any case, the regions holding the QTLs described above are of great interest for further studies of gene cloning and functional genomics. Apart from these QTLs, additional ones controlling the same characters were detected in independent regions of the genome. No QTLs for NF and BRWP were detected stressing the need of additional efforts to locate the QTLs responsible of these traits.

Most of the putative QTLs detected in this study (12 out of 15) showed dominant rather than additive effects (Table 3), which may be considered less appropriate for a faba bean breeding program. As widely known, MAS programs usually rely on QTLs with additive effects that can be readily applied for breeding purposes. Nevertheless, it has to be considered that commercial production of faba bean is mainly based on improved populations and synthetic cultivars comprising several breeding lines. Synthetic cultivars take advantage of heterosis and heterogeneity (usually found in faba bean) to

The use of an F<sub>2</sub> population and the corresponding F<sub>2,3</sub> families have proven a convenient approach to study yield-related traits in this species. QTL detection for yield components has been usually carried out in

permanent populations such as Recombinant Inbred Lines (RILs) and then, validated across multiple test locations before their application in MAS (Orf et al., 1999a; 1999b; Baum et al., 2003; Beattie et al., 2003; Tar'an et al., 2004; Zhang et al. 2004). However, inbreeding in faba bean has been reported to be the main responsible for yield depression (Nadal et al., 2003). In fact, selfing faba bean has been related to a decrease in the number of seeds per pod (Hebblethwaite et al., 1984), the number of seeds per plant (Rowland, 1958; Drayner, 1959; Free, 1966; Poulsen, 1975; Monti and Frusciante, 1982; Filippetti and de Pace 1986; Suso et al., 1996) and the mean seed weight (Free, 1996), among other traits. As a consequence, inbreeding depression is a factor that greatly hampers the use of RIL populations for QTL detection of yield components in this species.

The results of this work may be useful in future breeding programs of the species, throughout the inclusion of the best combination of alleles in the breeding lines. Molecular markers linked to the detected QTLs can be used to tag desirable alleles accelerating cultivar improvement. However, verification of putative QTLs concerning their effects and accurate chromosome map position is an essential step prior to the use in an applied breeding program. For this purpose, we plan to analyse different F<sub>2</sub> populations from the same faba bean cross as described by Romagosa et al. (1999). Basically these authors propose to analyse the QTL-flanking markers developed using a new population derived from the same parental genotypes. If the QTL are real, the flanking markers should co-segregate with the traits of interest in this new population.

This approach will also allow to test the stability of the QTLs in different environments as well as to confirm their effects in an independent sample of lines.

The present study constitutes the first step towards the identification of molecular markers suitable for agronomic traits selection in faba bean. Once putative QTLs for yield-related traits are validated, these results will be combined with our molecular markers developed for ascochyta blight, rust and broomrape resistances to develop highly yielding faba bean cultivars with combined resistance against these diseases.

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