THE PRACTICAL APPLICATION OF MOLECULAR MARKER TECHNIQUES IN PLANT BREEDING

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PRIMARY GENETIC MAP OF A WIDE FABA BEAN (*Vicia faba* L.) CROSS "GERMANY X MOROCCAN"

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ABSTRACT

This research was conducted at the Molecular Genetic Laboratory of Plant Breeding Department, Georg-August University, Germany, from 1999-2001. The main objective of this research is to develop a genetic linkage map in faba bean (*Vicia faba* L.). A Population of F7 recombinant inbred lines (RILs) derived from the cross 34Morocco x Kristall25 was used. The 34Morocco is a pure line bred from ICARDA, Syria. Kristall25, developed at Hohenheim, Germany, is an inbred line from German cv. Kristall. The RIL population consists of 253 lines. The number of lines analyzed with RAPD was 57. Those lines were chosen randomly from the whole population of RILS. Mapmaker /Exp. 3.0 was applied to the data. The Haldane function LOD > 3.0 and maximum likelihood distance of 40 cM was used. The 48 selected decamer primers amplified a total of 115 polymorphic bands with an average of 2.3 polymorphic bands per primer. Fragment sizes ranged from about 550 bp to 2400 bp. The present map comprises a total of 858.3 cM and classified into 14 linkage groups.

Key words: Faba bean, linkage map, RAPD, RIL

INTRODUCTION

Faba bean (*Vicia faba* L.), also referred to as broad bean, horse bean or field bean, occupies nearly 2.3 x 10^6 ha world-wide (FAO, 2000). The most common use of faba bean is as human food in developing countries (mainly Asia and North Africa), with China the largest producer with 1.1 x 10^6 ha planted annually (FAO, 2000).

The origin of *Vicia faba* L. is still debated (Duc, 1997). No wild progenitor has been found, and major differences exist between *V. faba* and the most related species that belong to the *Narbonensis* complex (*V. narbonensis, V. galilea, V. johannis, and V. hayeniscyamus).* Geographically, the origin of *Vicia faba* L. is considered to be the Near East. The subspecies *V. paucijuga,* presently found from Afghanistan to India, is a primitive form. Earliest findings of major faba beans (*Vicia faba major*) in Eastern Iraq date after 1000 A.D. The presence of large seeded types in

Central Europe is proven only as late the Middle Ages (Hanelt, 1972). These types expanded in the sixteenth century towards Mexico and southern America. Small seeded types (*Vicia faba minor*) are found in Ethiopian area and have been favored by North European agriculture (Duc, 1997). Medium seeded types (*Vicia faba equina*) have developed throughout Middle East and North Africa with major concentration in Egypt.

The development of a detailed linkage map for *V. faba* will greatly increase the efficiency of genetic and breeding studies in this important crop. In comparison to other legume species, the faba bean (*Vicia faba* L.) has been the focus of little research in this area. The 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) are worth mentioning. 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 Martin, 1977), translocation stocks (Schubert *et al.*, 1983) and asynaptic mutants (Gonzales and Martin, 1983). After successful identification and characterization of five of the six possible primary trisomics (Martin and Barcelo 1984), crosses between an asynaptic line and a normal diploid parent proved to be advantageous in localizing genes to their respective chromosomes (Cabrera *et al.*, 1989; Torres *et al.*, 1995). On the other hand, preliminary analysis of genetic linkage in faba bean includes the study of morphological traits (Cabrera and Martin, 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) reported, that when two F2 populations (20 F2 individuals derived from the cross Vf6 x Vf173 and 44 F2 plants from the cross Vf6 x Vf35) were analyzed using isozyme, RFLP and RAPD, 11 independently assorting linkage groups were identified in this population. Satovic *et al.* (1996) analysed 13 F2 families of faba bean descending from a trisomic plant. They have been analyzed for morphological, isozyme and RAPD markers. Data were pooled to genetically map partially overlapping sets of informative genetic markers. The study revealed a total of 10 linkage groups, six of which have been precisely assigned to specific chromosomes. The most recent map in faba bean has been reported by Vaz Patto *et al.* (1998). Different F2 families of faba bean descending from trisomic plants were analyzed for morphological, isozyme, RAPD markers and seed protein genes. Linkage analysis revealed 14 linkage groups. A composite map was also developed from these results and those previously reported (Torres *et al.*, 1993; Satovic *et al.*, 1996). The numbers of linkage

groups were reduced to 13 with total distance of approximately 1200 cM (Vaz Patto *et al.*, 1999).

The expected length of the *Vicia faba* genetic map is 700 - 1500 cM (Torres *et al.*, 1993). This 1500 cM number has not yet been reached until now. A complete map is one in which all regions of all chromosomes are covered by markers; and the number of linkage groups is the same as the haploid number of chromosome of the species (Peterson, 1996). *Vicia faba* has x = 6 chromosomes. So, a fully elaborated linkage map in *V. faba* consists of six linkage groups. The main objectives of this work is to develop a genetic linkage map.

MATERIALS AND METHODS

Plant material

For the construction of a linkage map, a population of recombinant inbred lines (RILs) derived from the cross 34Morocco x Kristall25 was used. The 34Morocco is a bean pure line BPL228/ILB141 from ICARDA, Syria. Kristall25, developed at Hohenheim, Germany, is an inbred line from the German cultivar Kristall. These parents were chosen because of their large genetic and phenotypic difference (Schill *et al.*, 1997). Further information about the differences of these two genotypes are presented below.

The F2 plants from the cross 34Morocco x Kristall25 and from the cross Kristall25 x 34Morocco were self-pollinated and advanced to the F7 generation using single-seed descent. The final population consists of 253 lines including reciprocal lines (114 lines derived from the cross 34Morocco x Kristall25, and 139 lines were from the cross Kristall25 x 34Morocco). All 253 lines were tested in the field. A random sample of 57 lines was characterized based on RAPDs. Seed multiplication was conducted in a cage throughout, that guaranteed that no bees entered the cage i.e., no cross pollination occurred.

Random amplified polymorphic DNA (RAPD)-analysis

DNA was isolated using a modified version of the protocol described in Lassner *et al.* (1992). The DNA concentration was determined using a DNA fluorometer TK0100 (Hoefner). The quality of DNA was checked by gel electrophoresis. DNA quality was judged by the quality of the visualized band under the ultra violet light. RAPD marker analysis was performed essentially as described by Williams *et al.* (1990). The amplification was performed in a Perkin Elmer Thermo Cycler Type 480 and programmed for 45 cycles. After denaturation at 94°C for 30 seconds, each cycle consisted of 1 minute at 92°C followed by 1 minute at 35°C and 2 minutes at 72°C. After 45 cycles the last step completed the amplification at 72°C for 5

minutes. The amplification products were separated by electrophoresis through 1.8% PeqGoldUniversal agarose gel (Table 6), at 80 volts for 4 hours. Agarose was dissolved in TAE-buffers. It was stained for 20 minutes with ethidium bromide (40 µl ethidium bromide in 500 ml distilled water), then washed with tap water for 10 minutes, photographed and scanned under ultra violet light.

Construction of the genetic map

To construct the genetic map, 57 RI F7 lines were chosen randomly from the whole population. Segregation data were analyzed with MAPMAKER/EXP 3.0 computer program (Lander *et al.*, 1987; Lincoln *et al.*, 1993).

RESULTS

Table 1. Distribution of the markers, average distance between markers in a linkage group and length of the genetic map of RILs in the faba bean population under study

Linkage group	Number of markers	Average distance (cM)	Length (cM)
1	6	13.96	69.8
2	2	19.00	19.0
3	16	18.58	278.7
4	6	15.02	75.1
5	5	15.60	62.4
6	2	6.30	6.3
7	12	17.52	192.7
8	9	8.53	68.2
9	4	13.57	40.7
10	5	17.35	69.4
11	2	14.00	14.0
12	3	18.60	37.2
13	3	13.00	26
14	2	13.7	13.7
Mean		14.66	
Total	77		973.2

The 48 selected primers amplified a total of 115 polymorphic bands (data not shown) with an average of 2.3 polymorphic bands per primer. Fragment sizes ranged from about 550 bp to 2400 bp. With linkage criteria LOD >3.0 and a distance of less than 40 cM, 77 RAPD marker loci were classified into 14 groups (Figure 1). There

were 38 unlinked markers. These markers were omitted from the analysis as far as the linkage map and QTL is concerned. The distribution of markers along the genetic map was visualized by the cumulative number of markers along the different linkage groups.

As shown in Table 1, the size of the 14 linkage groups ranged from 6.3 cM to 278.7 cM. The total length of map is 973.2 cM. The largest linkage group contains 16 loci (linkage group 3). The distance between markers ranges from 1.9 cM to 38.5 cM, with an average of 14.66 cM.

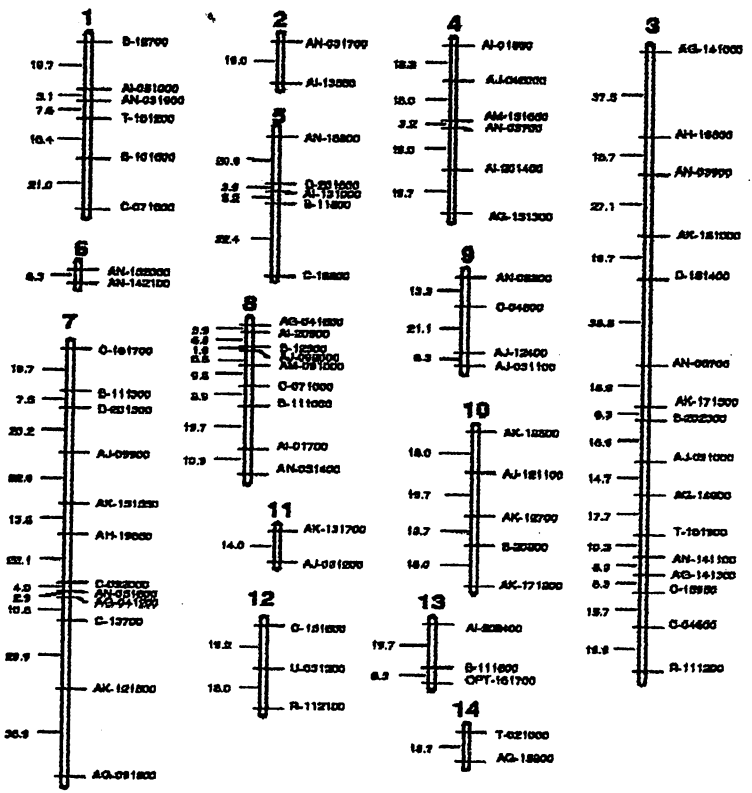
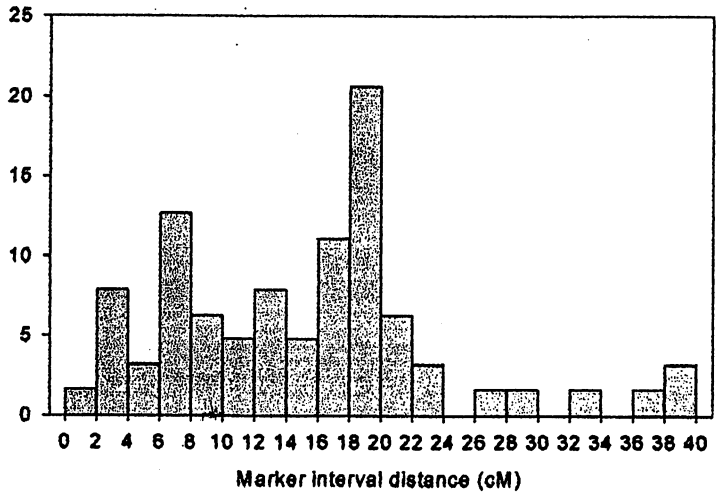


Figure 1: Primary linkage map of faba bean (*Vicia faba*) with 77 RAPD-markers. Number to the left of a linkage group represents map distance in cM calculated with the Haldane mapping function. All linkages shown have a LOD score of at least 3.0. Designations to the right are locus names

In the total number of markers observed, 1.6 % were nearly mpletely linked (distance less than 2 cM), and 30.1 % showed a stance less than 10 cM; 49.2 % of the markers were separated distance 10 to 20 cM, and 19.1 % by distance between 20 to 38 l (Figure 2).



igure 2: Frequency distribution distance (cM) between marker pairs

DISCUSSION

A total of 77 markers were found segregating in 57 RI F7- mple lines (Figure 2). The total map distance defined among ked markers was 973.2 centiMorgan. A comparison of the present netic map with the hitherto published maps shows that its total ngth resembles the map of Vaz Patto *et al.* (1998) with a map ngth of 984.5 cM. However these maps are still small when mpared to the expected size of the *Vicia faba* genome (1500 cM; z Patto *et al.*, 1998). Besides the total map length, a complete ap is obtained only when the number of linkage groups corresponds the haploid number of chromosomes. The haploid chromosome mber of the faba bean is six (x = 6), therefore, six linkage groups, stead of the 14 linkage groups detected here, were expected. ese results reflect problems associated with the use of relatively all populations (here: N = 57).

In view of the relatively large size of the linkage group 3, it ay putatively be assigned to chromosome number one of the faba

bean (Figure 3). In the standard karyotype, this chromosome is the longest one (Torres *et al.* 1993). It is a metacentric chromosome and about twice as long as each of the five subtelocentric chromosomes (Cabrera *et al.*, 1989).

The average distance between markers in the present map is 14.66 cM when all 77 RAPD markers were used. Although the density of the map was lower than in the faba bean map constructed by Vaz Patto *et al.* (1989) which has an average distance of 12.95 cM, the map presented here can be considered as sufficiently dense for a QTL analysis. In general, a marker distance of about 20 cM is still adequate to detect QTL (Tanskley *et al.*, 1993). In the present map, there are 12 gaps larger than 20 cM: one on the linkage group 1, three on the linkage group 3, two on the linkage group 5, five on the linkage group 7, and one on the linkage group 9.

Clusters of markers were found throughout the linkage groups. Clustering of markers throughout the linkage groups are in contrast to the nature of metacentric chromosomes, which have the centromere in the middle of the chromosome. It is expected that a reduction of recombination frequencies occurs in the vicinity of the centromere (Tanksley *et al.* 1992). Barcelo and Martin (1990) reported that faba bean has six pairs of chromosomes of which one pair is metacentric carrying the nucleolus organizer region and the remaining five pairs are essentially subtelocentric and of similar length. Since the connection of the detected linkage groups to chromosomes and chromosome arms is unclear, these present findings cannot be further interpreted.

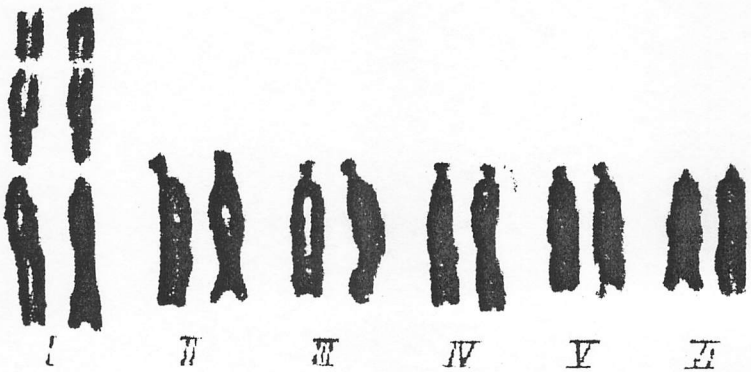


Figure 3: Standard karyoptype of chromosomes of *Vicia faba* (Schubert *et al.*, 1986)

CONCLUSION

As an additional tool for the genetic improvement of faba bean, an Old World grain legume, a preliminary linkage map of a wide cross was established.

Regarding the mean value, genotypic standard deviation and heritabilities (data was not shown), the 57 RI F7-sample lines chosen at random from the total set of 253 RI F7-lines were obviously well representative of this set. This implies that the 57 RI F7-sample lines are a priori acceptable as a basis of a primary linkage map for this cross.

To create a linkage map, RAPD markers were established for this cross and the 57 RI F7-sample lines. A total of 77 markers were assayed segregating in the 57 RI lines and grouped into 14 linkage groups. The total map distance defined among linked markers was 973.2 centiMorgan. The haploid chromosome number of faba bean is x = 6, therefore, six linkage groups, instead of the 14 linkage groups presented here, were ultimately expected. The actual result reflects problems associated mostly with the relatively small population (N = 57). This map nevertheless is significantly improving the present knowledge of the faba bean genetic situation.

The average distance between markers of the recent faba bean map is 14.66 cM Haldane. This map can be considered as sufficiently dense for a QTL analysis.

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