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EVALUATION OF THE CHLROPLAST DNA AMONG VICIA FABA L. GERMPLASM USING RESTRICTION- SITE ANALYSIS*

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Abstract – A restriction-site analysis of chloroplast DNA (cpDNA) was carried out to evaluate the level of diversity in *Vicia faba* L. germplasm selected from different geographical regions using 11 restriction endonucleases. We analyzed 214 restriction sites in 18 accessions of *Vicia faba.* All of the accessions had identical cpDNAs, pointing out the ancestral character of all the accessions are of only one gene pool, and all of them must have evolved through the same maternal lineage. Molecular size of the chloroplast DNAs obtained was 123.25 kb, indicating that it had lost one of the inverted repeats. Due to lack of cpDNA diversity, it is concluded that the broad bean has passed through a genetic bottleneck during domestication and lost most of its cytoplasmic variability. The present study favors the monophyletic origin of its cytoplasm, and accordingly, of *Vicia faba.*

Keywords – Chloroplast DNA- restriction site analysis - vicia faba L. germplasm

1. INTRODUCTION

Faba bean (*Vicia faba* L.) is a grain legume grown for its high seed protein content (about 30%). *Vicia faba* is known to have been cultivated from early Neolithic times. It can be said that it has been known from the beginning of Agriculture. It is logical to suppose that the use of *Vicia faba* as a cultivated species began in the Near East, following the Neolithic culture as it spread across the inhabited world [1]. The plant was known in other regions, particularly in Mediterranean countries.

The faba bean has attracted the interest of taxonomists and evolutionists for a long time. Various approaches for intra- and inter-specific taxonomy have been reported based on geographic origin, morphology, karyotype, or isozymes [2-7]. These criteria are influenced by environmental factors. Genetic diversity, determined with molecular markers, has been explored in the *Vicia* family mainly at the interspecific level [8, 9]. Molecular based methods such as restriction site analysis of chloroplast DNA (cpDNA) have produced significant contributions to our understanding of crop evolution. Molecular approaches can provide numerous conservative markers that are easily interpreted and valuable in identifying progenitors of crop plants. Restriction site analysis of cpDNA, because of the many suitable features of the chloroplast genome such as small size, evolutionary conservatism, and predominant uniparental inheritance, has been increasingly used for genetic

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evolutionary, phylogenetic, and biosystematic studies at the inter- and intraspecific levels in a number of crop species [8, 10-14].

Information about genetic diversity within elite germplasm of the faba bean is far from being adequate for breeding purposes. Moreover, breeders can only capitalize on the genetic variation within the species because *Vicia faba* cannot successfully be crossed with any related species. Information on the geographic structure of genetic diversity within species is important to optimize the identification of useful genes. However, there has been no comprehensive study to determine the nature of chloroplast genetic diversity within *V. faba* germplasm that is of fundamental importance. I have, therefore, examined chloroplast genomes of 18 accessions of *Vicia faba* using restriction endonuclease analysis.

2. MATERIALS AND METHODS

a) Plant materials

A list of the accessions investigated is given in Table 1. The seed samples were obtained from the ICARDA, Alleppo, Syria.

Table 1. List of accessions of *Vicia faba* used in the present study

Number	Accession No.	Origin	Number	Accession No.	Origin
1	ILB 4171	Portugal	10	ILB 3509	Syria
2	ILB 3597	Syria	11	ILB 2856	India
3	ILB 350	Egypt	12	ILB 107	Afghanistan
4	ILB 996	Great Britain	13	ILB 4168	Portugal
5	ILB 4967	Madagascar	14	ILB 4342	Libya
6	ILB 500	Iran	15	ILB 4338	Libya
7	ILB 4204	Portugal	16	ILB 3664	Spain
8	ILB 80	Egypt	17	ILB 3558	Syria
9	ILB 363	Egypt	18	ILB 3809	Canada

b) Chloroplast DNA extraction and Restriction endonuclease analysis of pure cpDNA

cpDNA were extracted from young leaves according to Ogihara and Tsunewaki [15] with some modifications. In the greenhouse, three to five week old plants were kept in darkness for 24 hrs just before harvesting the fresh leaflets. From 20- 50 g of young leaflets, crude cpDNA was isolated from the pellet of low- speed centrifugation using a buffer specially prepared to preserve organellar structure intact. It was then purified by CsCl/ ethidium bromide centrifugation.

Pure cpDNA of 18 accessions were digested with 11 restriction enzymes (*Hin*dIII, *Xba*I, *Bam*HI, *Dra*I, *Nco*I, *Sal*I, *Xho*I, *Ava*I, *Pvu*II, *Pst*I, *Kpn*I) according to the manufacturer's instructions (Amersham Pharmacia Biotech) and fractionated overnight by 0.85% agarose gel electrophoresis in 1× TAE buffer. *Hin*dIII digested lambda DNA and 1 kb ladder served as molecular size markers. For better resolution of bands, the digested cpDNAs was electrophoresed on a bridge type electrophoresis unit and allowed to migrate about 12 cm on the gel. The molecular sizes of each fragment were calculated by making comparisons to molecular size markers after photographs had been taken.

3. RESULTS

An example of the cpDNA restriction fragment patterns of 18 accession of *Vicia faba* digested with *Ava*I, *Bam*HI, *Dra*I and *Pvu*II is shown in Fig 1. Consequently, upon single digestion with 12 six-cutter restriction endonuclease enzymes, the electrophoretic pattern of cpDNA fragments were clearly reproducible in all 18 accessions. The estimated genome size from the restriction fragments generated by six enzymes: *Hin*dIII, *Ava*I, *Xho*I, *Pvu*II, *Sal*I and *Pst*I were 121.2, 122.1, 123.8, 126.5, 123 and 122.9 kb, respectively. The average size of chloroplast genome was 123.25 kb. The size estimated in this study corresponds well with the estimates of *Vicia faba* and its close relatives [8]. An analysis of 214 restriction sites revealed that all the 18 accessions exhibited similar patterns in all endonuclease digests and no intraspecific variation was detected.

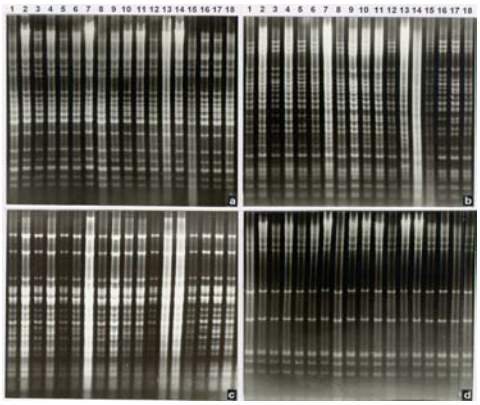

Fig. 1. Restriction fragment patterns of chloroplast DNAs from 18 *Vicia faba* Accessions (see table 1) digested with *Ava*I (a), *Bam*HI (b), *Dra*I (c), and *Pvu*II (d)

4. DISCUSSION

The present study revealed that the chloroplast genome is highly conserved in *Vicia faba*. The most common characteristic feature is the almost complete lack of intraspecific variation in the cpDNA among the accessions collected from the entire distribution area of broad bean. This result may indicate that the broad bean was domesticated from an ancestor that possessed little cpDNA diversity, or the broad bean experienced a cytoplasmic bottleneck during domestication and lost much of its variation. The latter syndrome has occurred in the history of many cultivated plants [16], and it has probably affected the broad bean as well. Such conservative nature of the chloroplast genome has been reported both at the intraspecific and interspecific levels in other plant taxa such as Sativa species complex [9]. Perhaps the best study with which to compare our findings is an analysis of *Lens* [10], which also lacks the inverted repeat [17]. Mayer & Soltis [10] surveyed 339 restriction sites and found only a single restriction site loss and a single insertion within 114 accessions of the cultivated lentil, and 112 accessions (of 114) of *Lens culinaris* ssp. *culinaris* exhibited identical cpDNA restriction-site patterns.

One of the characteristics of the broad bean chloroplast genome is the absence of the large inverted repeated sequences [8, 18, 19]. This event has reduced the genome size of these species to ~123 kb. The size estimated in this study for accessions of *Vicia faba* approximates to 123 kb. It appears, therefore, that all the accessions have lost the repeat structure. Palmer and his co-workers [17, 19, 20] have suggested that the inverted repeat confers stability to the chloroplast genome, and

that sequence rearrangements are more common when it is absent. In the present study we did not identify any rearrangements unique to the broad bean. It was evident, however, that the chloroplast genome of *Vicia faba* is extremely divergent from that of its closest wild relative, Narbonensis species complex and the presence of a startling difference in RFLP patterns between *V. faba* and wild species in the Narbonensis species complex revealed that none of the wild species in the complex can be considered the immediate wild progenitor of *V. faba* [8].

Our study has shown that there is no discriminant polymorphism between accessions with different geographical origins, pointing out that the ancestral character of all the accessions are of only one gene pool. The complete lack of intraspecific variation can only be explained by assuming that cytoplasm of *Vicia faba* had been differentiated at the original region before its introduction to other parts of the world. The present result favors the monophyletic origin of its cytoplasms, and accordingly, of *Vicia faba*. On the other hand, it can be suggested that *Vicia faba* chloroplast genome differentiated at the original region and has not changed appreciably after its introduction. In conclusion, archeaobotanical findings agree with our main results from cpDNA RFLP assays in that: there is a close relationship between broad beans from different geographical regions and all of them must have evolved through the same maternal lineage.

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