GENETIC STUDIES IN *Vicia* spp.

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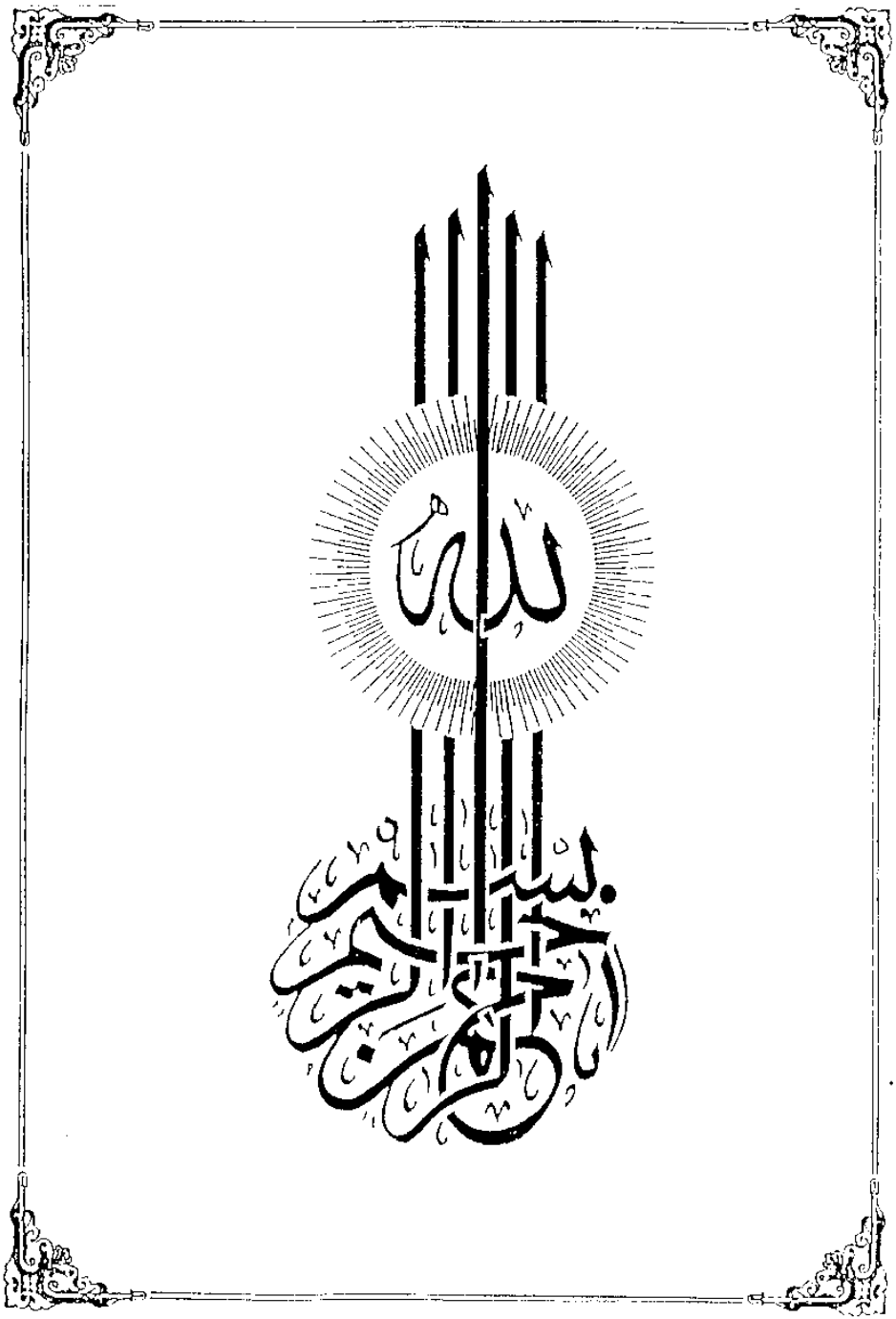

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CONTENTS

INTRODUCTION

The genus Vicia (120 - 150 species) is one of the most important genera belonging to the family Leguminosae, with a wide distribution over the temperate zones of both hemispheres but centered mainly in the Mediterranean region, Western Asia and Western South America. According to the Flora Europea, it was split into four sections: Cracca, Ervum, Euvicia and Faba (Ball 1968), although contradicting with other taxonomic systems (Fedchenko 1948, Stankevich 1982 and Cubero 1984).

Vicia faba L. known as broad beans, field beans or faba beans is an important legume grain in much of the north temperate zone and at higher altitudes in the cool season of some sub-tropical regions (Bond 1976). Being assigned to section Faba, it was assumed that V. narbonensis, V. galilaea, V. johannis and V. hyaeniscyamus which belong to the same section, are the wild relatives of V. faba L. (Zohary and Hopf 1973). This assumption was disputed by others, who indicated the remoteness of the V. narbonensis (VN) species group from V. faba L., as this species and the mentioned complex represent terminal points of two independent and divergent phylogenetic branches which may be traced back to a common evolutionary line (Hanelt et al. 1972, Schäfer 1973, Ladizinsky 1975, Cubero 1984 and Birch et al. 1985).

The subspecific classification of V. faba L., based mainly on seed size, defines the species into two subspecies : paucijuga and eu-faba (var. major, equina and minor), (Muratova 1931). This classification has been widely used since then. However, another classification

assumed paucijuga to be a geographical race of subspecies minor; (Hanelt 1972a), whereas Cubero (1974) and (1984) and Morens (1979) considered the varieties faba, equina, minor and paucijuga as botanical groups of one subspecies.

No wild progenitor of Vicia faba L. has yet been conclusively identified (Hanelt et al. 1972, Zohary and Hopf 1973, Zohary 1977 and Smartt 1984), and perhaps, the primitive wild form must have been closely related to the paucijuga type, being small seeded and autogamous ; (Cubero 1974, Ladizinsky 1975, Abdalla 1979, Cubero and Suso (1981) and Cubero (1984) or claimed to be allogamous and dehiscent (Moreno 1979).

Archaeological data, evolutionary trends and barriers between subspecies and types suggested that V. faba L. has originated in the Fertile Crescent, where it spread from there to different regions ; Smartt (1984) and Cubero (1984). Its domestication occurred in South Western Asia between Afghanistan and the marginal regions of the Eastern Mediterranean with an estimated period of 4000-7000 B.C. (Hanelt 1972, Hanelt et al. 1972, Zohary and Hopf 1973, Ladizinsky 1975, Zohary 1977 and Cubero 1984), which contradicts with (Abdalla 1979) who postulated that Egypt is the country of origin of V. faba L.

Faba beans (Vicia faba L.) is one of the major field crops grown in Egypt as it provides a substantial part of the protein in human diet. Its acreage reached 105,000 hectares, yielding 2,495 Kg/ha with a total production of 262,000 metric tonnes (FAO, 1981).

Cultivar identification by biochemical fingerprinting is becoming an important task in order to verify its identity and purity. However, such cultivar identification in faba beans has been executed only on a very limited scale on quite a few number of cultivars (Stegemann et al. 1980). The development of a network of biochemical genetical markers to identify and characterize each and every cultivar would certainly provide valuable tools for the fast checking of cultivar purity.

One of the major problems facing Vicia faba L. breeders in Egypt is flower and pod abscission which amounts to 50-90% drop of flowers and/or young pods carried by a plant. This in turn, results in a drastic reduction in seed yield. The rate of outcrossing varies from 5 to 50%, being a partially autogamous species, which might also affect yield production and stability.

The objectives of this investigation were to study :

1. The phylogenetic relationships between V. faba and other Vicia spp. representing the main sections of the genus through biochemical genetic markers such as seed protein electrophoresis, isozyme polymorphism and immunochemical analyses. Besides, the prediction of their interspecific crossability.
2. To assess the biochemical genetic identification and characterization of faba bean cultivars of different geographical origin and different botanical groups.
3. To study the genetic and biochemical basis of the shedding phenomenon among a group of cultivars and their hybrids.

SECTION 1

PHYLOGENETIC RELATIONSHIPS

REVIEW OF LITERATURE

Biochemcial Genetic Affinities

a. Seed Protein Electrophoresis

Danielsson (1949) examined the seed globulins from a number of different species of the Leguminosae and showed that, except in a few instances, each consisted of two major components ; vicilin and legumin. The molecular weight of both components were determined in pea and were found to be 186,000 and 331,000, respectively. Polyacrylamide gel electrophoresis (PAGE) was employed by Fox et al. (1964) to study the albumin patterns of seeds of 17 species of the Leguminosae. Protein patterns obtained from different species within a genus resembled one another more closely than those of species belonging to different genera. The potential usefuleness of this technique as a tool in taxonomic studies was also discussed. Globulins extracted from a number of differnt species of legume seeds were analyzed by Boulter et al. (1967) using disc electrophoresis. Genera of the tribe Vicieae were characterized by distinctive protein banding patterns. They usually exhibited a major protein band and other less prominent bands which occurred above the major band. The later ones varied in number according to the amount of protein being electrophorized and the distance over which the bands moved.

Ladizinsky (1975) studied the albumin profiles of seeds of V. faba and its taxonomically related species V. narbonensis, V. galilaea and V. hyaeniscyamus and found that its profile differed greatly from those of the other species, as only seven bands of the V. faba profile were shared with those of the wild species from a total of 14 bands. V. narbonensis exhibited eight bands in all the examined accessions regardless of their

morphological variation, of which five were in common with V. faba profile. He concluded that the albumin profile of the seed protein was a diagnostic trait of the species in section Faba, and that the examined species could not be considered as the wild progenitors of V. faba. Similarity of the profiles of V. galilaea and V. hyaeniscyamus was consistent with the morphological resemblance noted between these two species. Seed protein of 20 lines representing 14 vetch species were examined by Perrino et al. (1977) using the method of polyacrylamide gel disc electrophoresis. They reported differences between and within the species. Ladizinsky and Hymowitz (1979) summarized the main features of the seed protein profiles ; stability, uniformity, its additive nature and their significance in resolving specific taxonomic and evolutionary problems. They reported that stability of the seed protein profile is species - specific, highly stable and is slightly affected by environmental conditions or seasonal fluctuations and also chromosomal rearrangements. Variations in banding patterns between gels could be expressed by three methods - the R_f values, optical density values and the similarity index. The additive properties of the seed protein profile have been successfully utilized in studies aiming at elucidating the origin and evolution of polyploid plants. In a study involving different V. faba stocks and V. narbonensis, presumably its wild relative, Abdalla and Günzel (1979) concluded that V. narbonensis cannot be considered as an immediate ancestor of the cultivated species V. faba, where the protein profile of the former consisted of seven bands while the latter exhibited 21 bands.

Volodin et al. (1979) reported the predominance of intermediate inheritance of the albumin components in interspecific hybrids of peas and of the vetches, V. sativa and V. narbonensis, by means of polyacrylamide gel electrophoresis. Seed albumins of several members of the Papilionaceae including Pisum, Vicia and other species were investigated by Wolff (1980) to study the phylogenetic relationships among members of this family and to evaluate the usefuleness of this method in solving evolutionary problems. The albumin patterns of six systematically distinct species of Vicia showed very few similarities, which indicated that the albumin patterns were not suitable for studying the distantly related taxa. The albumin electrophoretic profiles of six species of section Faba along with V. bithynica revealed that V. bithynica was the closest to V. faba, (Perrino and Pignone 1981). Bhatty (1982) presented the albumin patterns and amino acid compositions for Vicia faba, Vicia sativa and other edible grain legume species.

The significance of seed protein electrophoresis in clarifying the origin of legume crops has been reviewed by Ladizinsky (1983) with special reference to soybean, chick pea, lentil, pea, common vetch (V. sativa), pigeon pea and broad beans. Its importance as a tool in tracing cereal evolution and the evolution of polyploid cereals due to its additive nature has also been reported.

Maplestone et al. (1985) reported qualitative variations in legumin subunit patterns on gel electrophoresis between five Vicia species and concluded that the heterogeneities of legumin genes in those examined species were comparable. Evolutionary relationships among 16 accessions

representing five species of Vicia were studied by Hussein and Salam (1985) using different electrophoretic techniques. Interspecific variations in the different protein fractions as revealed by SDS-PAGE under reduced and non-reduced conditions showed a close relationship between V. sativa and V. angustifolia and between V. dasycarpa and V. villosa. V. faba, V. sativa and V. angustifolia exhibited more distinct and sharp bands, especially of legumin - like fraction, than the other species. Abdel Tawab et al. (1987) reported variable relationships between 12 cultivars of Trifolium representing 11 species by using different electrophoretic methods. The albumin fraction proved to be useful in classification when dealing with closely related forms, whereas the globulin and glutelin fractions proved to be unreliable tools in chematoxonomic studies. The analysis of seed protein by polyacrylamide and SDS-gel electrophoresis were also reported.

b. Isozyme Polymorphism

Shannon (1968) reviewed literature concerning isozymes, their definition, morphology, distribution, detection in different organisms and the different techniques employed in isolating and distinguishing them. Brewbaker et al. (1968) described gel electrophoretic methods which were devised or modified for rapid and economic application to angiosperm tissues. Staining procedures that proved effective on a wide variety of tissues for isozyme activities such as esterase, catalase and others have also been reported with illustrative applications.

Amylase isozyme patterns in the hybrid progenies between V. pilosa and V. macrocarpa were examined by Yamamoto (1975) using polyacrylamide

disc electrophoresis in order to estimate the recombination of parental genes and the genetic homogeneity in the interspecific hybrid progenies. V. pilosa and V. macrocarpa exhibited two and three bands, respectively. However, the F_1 hybrids possessed the five bands of both parents which was in contrast with the appearance of fewer band - phenotypes possessing those parental bands in F_3 rather than F_4. This suggested the increment of homogeneity among the various genetic groups specifying the isozyme patterns in the F_4 generation. In (1979) he used the same method above to assess the relationships between the hybrid progenies of V. amphicarpa true and V. macrocarpa. Three amylase bands were observed for each parental species, including two α-amylase and one β-amylase band. The F_1 and F_2 hybrids showed the same bands derived from both parents, whereas the zymograms of the F_3 and F_4 were assembled in ten groups according to a pattern characterized by the presence or absence of both parental bands which was in agreement with the results obtained previously. Leaf and cotelydon extracts from 39 strains of 24 Vicia species representing the main sections of the genus were analyzed by Yamamoto (1980) using disc electrophoresis to detect isozyme polymorphism of the enzymes esterase, amylase, glutamate oxaloacetate transaminase (GOT) and indophenol -oxidase (IPO). Many bands were common to species or to series of sections ; however, some bands could be used to differentiate between certain taxonomic categories. Such characterizing bands did not indicate any clear phylogenetic trend or systematic correspondence, nor did the enzymatic similarities necessarily agree with taxonomic relationships. GOT showed less polymorphism than amylase or esterase and species that were polymorphic for other characters also showed enzyme polymorphism. Yamamoto and

Plitmann (1980), using the same materials and methodology employed above, observed a total of 17 bands for esterase, with 4-7 bands being displayed in each strain. In V. narbonensis and V. lutea only four bands were observed, as compared to four or five bands detected in all strains of series Sativae and species of section Ervum. In section Cracca, however, more bands were displayed and differences in banding pattern were observed among the two morphological groups representing the Villosae series. A total of six and four α-and β-amylase bands were detected, respectively, in Vicia strains, in contrast to a total of seven bands in the GOT pattern and nine bands in IPO. In general, there were more common versus differentiating specific bands between certain taxonomic categories, but such characterizing bands and patterns did not feature any clear general phylogenetic trend or systematic correlation. Esterase showed higher isozyme polymorphism which might be helpful in defining intraspecific entities. On the other hand, polymorphic species revealed also pronounced enzymatic polymorphism.

On the basis of karyotypic variations and isozyme polymorphism, Yamamoto et al. (1982) studied the genetic relationships between the V. marbonensis (VN) species group as well as V. faba and V. bithynica. Considerable differences were observed between the karyotypes of this group of species and those of V. faba and V. bithynica, in regard to the relative length of satellite chromosomes and the ratio of short to long arms of each chromosome. Similar variations were also deduced among the species, regarding their isozyme patterns and morphological characters which seemed to manifest clearer genetical relationships within the examined species. It was postulated that V. faba, V. bithynica and the VN species group were