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Variability of storage proteins and esterase isozymes in *Vicia sativa* subspecies

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Abstract

The relationships among 20 samples belonging to 6 subspecies of *Vicia sativa* based on the variability of seed storage proteins and esterase isozyme electrophoretic patterns was discussed in relation to variation in their morphology and chromosome characters. Electrophoretic protein profiles of different accessions of the same subspecies showed identical (*e.g. macrocarpa* and *cordata*) or similar (*e.g. amphicarpa*) patterns, confirming the stablity of seed storage proteins within these subspecies. However, considerable variation of protein patterns were observed within accessions of both *nigra* and *sativa* subspecies, which could be correlated to different geographical origins. Esterase pattern revealed a sharp distinction for each subspecies according to the number and loci of allelic bands. The dendrogram delimited the subspecies *incisa* and *sativa* as two separate groups, while the other subspecies were grouped together in another group.

Additional key words: common vetch, chromosomes, esterase, isozymes, numerical analysis, seed storage proteins.

Introduction

The species in the genus *Vicia* are morphologically diversified because leaflets are variable from large to oblong, calyx from truncate to dentate; teeth from shorter than the tube, equal to the tube to longer than the tube and pods from narrow, wide to torulose (Zohary and Plitmann 1979). Moreover, there is astonishing variation in basic chromosome number (x = 5, 6 and 7) and chromosomal criteria (Holling and Stace 1974).

Vicia sativa complex is a highly variable group with sympatric occurrence (Zohary and Plitmann 1979). Classification of this complex has been a matter of controversy. On the basis of morphological characters, Mettin and Hanelt (1964) and Hanelt and Mettin (1966) considered each type as a separate species, while Ball (1968) and Davis and Plitmann (1970) classified all types in one or two species.

Vicia sativa complex displays a wide variation in chromosome morphology with different karyotypes that differ considerably from one another in relative length of specific chromosomes, arm ratios and size of satellites (Watanabe and Yamada 1958, Yamamoto 1966). The group contains a series of karyotypes of decreasing chromosome numbers starting with 2n=14 (the basic chromosome number of the genus *Vicia*) to 2n=12 and 10. The reduction of the basic chromosome number in this species may be restricted to a complete chromosomal fusion-fission cycle (Schubert *et al.* 1995).

Self pollination in this group was examined and various hybrid generations were produced when crossing was carried out within and between types of the same chromosome number (Mettin and Hanelt 1973, Yamamoto 1967, 1971, 1974, 1977).

Seed protein polymorphism studied by Ladizinsky and Waines (1982) on 42 accessions of *Vicia sativa* representing five different karyotypes with 2n=10, 12 and 14 revealed that all accessions, except those with 2n=14, have highly variable protein profiles and the variation within and between karyotypes was of the same magnitude and no protein bands could be correlated to a particular karyotype.

The difficulty in the identification of *Vicia sativa* subspecies by morphological characters could be overcomed by applying electrophoretic procedure of seed storage proteins and isozymes that has rapidly become a very convenient tool for subspecies identification (Robinson and Megarrity 1975, Dalling *et al.*

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1979, Wrigley *et al.* 1982, Ferguson and Grabe 1986, Mejia and McDaniel 1986, Hamrick and Godt 1989, 1996, Holger and Joachim 1995).

The present work describes the use of variability of seed storage proteins and esterase isozyme electrophoretic patterns to distinguish between different samples representing 6 subspecies of *Vicia sativa* complex.

Materials and methods

Seeds representing 20 samples belonging to 6 subspecies of *Vicia sativa* L. of different origins were obtained from the International Center for Agriculture Research in Dry Areas (ICARDA), Aleppo, Syria (Table 1).

Characterization and molecular mass determination of seed storage proteins was carried out using one-dimensional SDS-polyacrylamide gel electrophoresis. Samples were prepared for electrophoresis by extracting proteins from 0.5 g seed powder in 1 cm^3 of 0.2 M Tris/HCl, pH 8, and 1 mM phenylmethylsulphonyl fluoride (PMSF) for 2 h in a refrigerator. The extract was centrifuged at 5 000 *g* for 20 min and proteins in the supernatant were precipitated with 5 volumes of cold acetone at -20 °C for 2 h. Pellets obtained after centrifugation at 7 500 *g* for 20 min were dissolved in 0.02 cm^3 of sample buffer (0.125 M Tris/HCl, pH 6.8, 2 % m/v SDS, 10 % m/v sucrose, 1% v/v β-mercaptoethanol, 0.1% m/v bromophenol blue) and denaturated by heating at 80 °C for 3 - 5 min.

Table 1. Origin, accession number and serial number of the studied *Vicia sativa* subspecies.

Subspecies	Origin	Accession number	Coded number
sativa	Italy	371, 372	1
	Japan	462, 464, 465	2
nigra	United Kingdom	1380, 1428, 11458	3
	Afghanistan	592	4
	Egypt	-	5
macrocarpa	United Kingdom	1942, 1943, 2050	6
	United Kingdom	2069, 2217	7
amphicarpa	Syria	2613	8
	Syria	2614	9
cordata	Italy	888	10
	United Kingdom	1361	11
incisa	United Kingdom	1091	12

17 % SDS-polyacrylamide gel slabs were prepared as described by Laemmli (1970). Equal amounts of proteins were loaded per track. Electrophoresis was carried out in Tris/glycine-SDS running buffer (0.25 M Tris, 1.88 M glycine, 0.1 % SDS) using vertical gel electrophoresis unit (Mini-Protein Cell, *BioRad*, USA) at 140 V for the first 15 min followed by 150 V until the indicator dye reached the bottom of the gel. Gels were stained overnight in 20 cm^3 of 0.25 % kenacid blue, 50 % (v/v) methanol, 7 % (v/v) glacial acetic acid and destained by shaking overnight in 50 % methanol and 7 % glacial acetic acid. The relative mobilities of the band were caùlculated and the presence or absence of each band was treated as a binary character.

For extraction of crude isoesterase enzymes, 0.5 g of seed powder was extracted with 1 cm^3 of the Tris/HCl buffer without SDS. Separation of enzymatic bands was carried out in 7.5 % non-denaturing polyacrylamide gel slabs prepared as described by (Laemmli, 1970). Aliquots (0.05 cm^3) of the crude extract were loaded onto the gel and electrophoresis was carried out at a constant current of 25 mA per gel under temperatute 4 °C. The gels were stained according to the procedures of (Soltis *et al.* 1983). Gels were incubated in 0.1 M Na-phosphate buffer, pH 6.0 for 10 min, then stained for 2 h in a solution containing 1 % α-naphthyl acetate and 1 % Fast blue RR. The presence or absence of each enzymatic band was also treated as a binary character.

Data from protein and isoesterase were processed for cluster analysis performed using the program NTSYS PC (numerical taxonomy and multivate analysis system) as described by Rohlf (1993).

Results

The SDS-PAGE patterns of seed proteins (Fig. 1) show that the total number of electrophoretic bands ranged from 34 in subspecies *incisa* to 41 for both subspecies *nigra* and *cordata*. A great homology of protein patterns within the range of 97 - 65 kD can be recognized amongst all studied subspecies except the subspecies *macrocarpa* and *incisa* which showed the absence of two bands and one band, respectively, at this range. These two subspecies included two extra bands at the range of 40 - 38 kD. Another area of great similarity in protein profiles was observed in the range of 32 - 29 kD with the absence of only two bands of 32 - 31 kD in subspecies *incisa*.

Electrophoretic protein profiles corresponding to different accessions of the same subspecies (*e.g. macrocarpa* and *cordata*) showed identical patterns. Moreover, different accessions of *amphicarpa* subspecies showed highly similar protein patterns. However, considerable variations of the electrophoretic protein profiles were observed within accessions of the same subspecies *e.g. nigra* and *sativa* (Fig. 1).

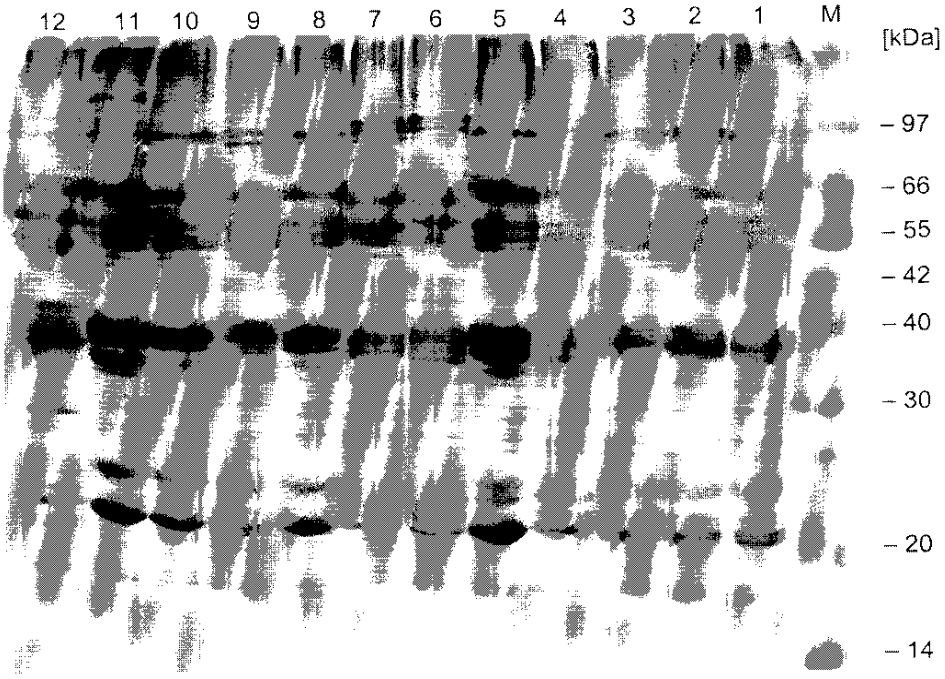

Fig. 1. Electrophoretic profile produced by SDS-PAGE of seed storage proteins of the studied *Vicia sativa* subspecies as numbered in Table 1.

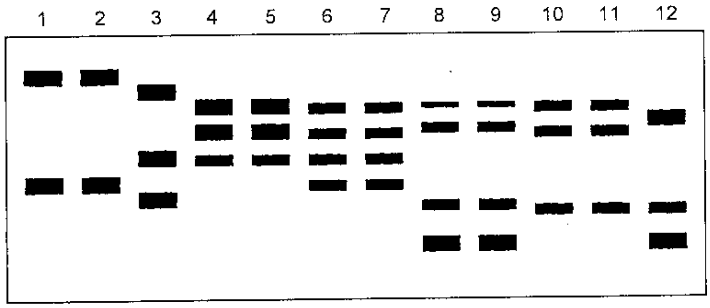

Fig. 2. Zymogram of esterase (EST) patterns of the studied *Vicia sativa* subspecies as numbered in Table 1.

Analysis of esterase (EST) isozyme patterns revealed a variability among the six examined subspecies. The number of EST bands ranged from 2 to 4 bands (Fig. 2). Different accessions belonging to the same subspecies showed identical EST paterns. However, some different patterns could be observed between accessions belonging

to subspecies *nigra*.

In the subspecies *sativa* this enzyme appeared as dimer, two homozygous loci, one allele for each locus. In subspecies *nigra* it appeared as monomer, one locus in which EST pattern showed polymorphic alleles except in the samples from the United Kingdom which showed a dimer, in three heterozygous loci. In both subspecies *amphicarpa* and *macrocarpa* the enzyme appeared dimer in four heterozygous loci with two alleles. EST patterns of subspecies *cordata* is dimer in three heterozygous loci. The subspecies *incisa* appeared as dimer in two heterozygous.

The relationships between the examined samples, as inferred from the analysis of combined data of seed storage proteins electrophorogram produced under SDS-PAGE and esterase zymogram are illustrated in the dendrogram (Fig. 3) which delimited the subspecies *incisa* and *sativa* as two separate groups, while the other subspecies were grouped together in another group.

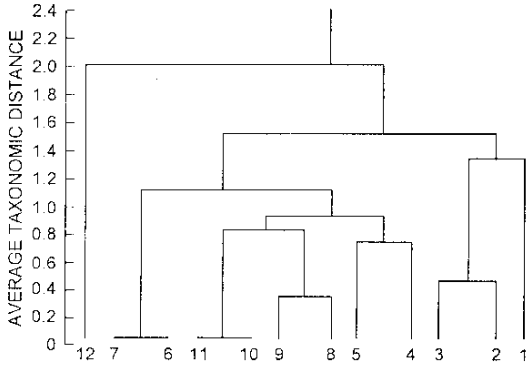

Fig. 3. Dendrogram illustrating the relationships of the studied *Vicia sativa* subspecies as numbered in Table 1.

Discussion

The present study based on gene expression products (seed storage proteins and esterase isozyme) are considered for the identification of these subspecies. The phenetic classification produced by the analysis of storage proteins and esterase electrophoregrams indicated complete identity between different accessions of each *macrocarpa* and *cordata* subspecies, as well as high level of similarity between different accessions of *amphicarba* subspecies. This confirms that seed storage proteins are stable within these subspecies (Ladizinsky and Hymowitz 1979).

While, on the other hand, little variations of storage proteins and esterase electrophoregrams were observed within accessions belonging to *nigra* and *sativa* subspecies, which could be correlated to different geographical origins. The dendrogram delimited subspecies *incisa* and *sativa* in separate groups which may be correlated to the presence of some specific morphological characters, *i.e.* large hairy dentate leaflets, narrow calyx teeth and broad pods, compared with the other subspecies. EST pattern of subspecies *incisa* also confirms this separation. Yamamoto (1977) reported a failure of hybrid formation between the taxa belonging to subspecies *amphicarpa* and *incisa* (both having 2n=14). He correlated this with some differences in the protein patterns and the presence of extra alleles at cathode locus for *amphicarpa*, beside some main morphological differences (short plants, subterranean fruiting branches and calyx teeth shorter than the tube) in *amphicarpa*, not in *incisa*.

Although subspecies *sativa* and *nigra* have the same chromosome number (2n=12), with similar karyotypes (Hollings and Stace 1974). Dendrogram based on the data from seed storage proteins and esterase isozyme delimited each subspecies with its representative accessions in a separate group, except *nigra* samples collected from United Kingdom which were clustered in the group of subspecies *sativa*. This indicated that no protein bands could be correlated to a particular karyotype (Ladizinisky and Waines 1982).

The dendrogram also clustered the accessions of subspecies *amphicarpa* into a separate group. The distinct separation of subspecies *amphicarpa* is supported by the results of karyotype data described by Yamamoto (1977). These results revealed that this subspecies has a specific centromere position, chromosome length, and hetero-chromatic band pattern. This is confirmed also by morphological characteristics as proposed by Zohary and Plitmann (1979): large leaflet, calyx teeth tapering from middle, coarsely dentate stipules, long flower, large pods and big seeds.

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