BIOLOGIA PLANTARUM 39 (4): 553-559, 1997

The use of seed proteins revealed by SDS PAGE in taxonomy and phylogeny of some *Lathyrus* species

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Abstract

Electrophoretic seed protein patterns of 18 samples belonging to 14 species and 4 sections of *Lathyrus* are treated by principle component analysis (PCA). The morphological ground and karyological structure data of these samples are also discussed in the light of sectional and groups delimitation. The species under study of section *Cicercula* are separated into 3 groups and *L. hirsutus* referred to the most primitive species within *Lathyrus* species. This agrees with their previous grouping delimitation based on morphological characters and with chromosomal features such as karyotype structure. *L. aphaca* referred to the most advanced species in this genus, which agrees with the modification of this morphological characteristics and reduction in chromosome criteria. Section *Nissolia* has an intermediate position between section *Cicercula* and section *Aphaca*.

Additional key words: chromosomes, environmental factors, morphology, principle component analysis.

Introduction

The genus *Lathyrus* comprises about 170 species (Plitmann 1979), with centres in the Mediterranean sea countries. This genus has morphological characters related to the genus *Vicia*. Many species with unijugate leaves, stem often winged (*cf. Vicia*), leaves never imparipinnate, staminal tube usually truncate at apex, occasionally oblique, filaments never dilated style sometimes contorte. There are many species cultivated for forage and human food: *L. sativus* (grass pea), *L. hirsutus* (rough pea), *L. cicera* (flatpodded vetchling), *L. odoratus* (sweet pea), *L. ochrus* (ochrus), *L. sylvestris* (flat pea).

Many studies were carried out by Datta (1955) and Chaudhuri (1966) dealing with the genetic relationships within species of the genus *Lathyrus*. In addition to this numerical chromosome constancy species display a uniformity in chromosome morphology (all chromosomes are metacentric or submetacentric). Evolution,

Received 21 March 1996, *accepted* 19 June 1996.
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nevertheless, has resulted in a large increase in chromosome size (Rees and Hazarika 1969). In general, the total length of somatic chromosome set was longer in perennial species than in annual ones as reported by (Rees and Hazarika 1969). Species of the sections *Clymenus*, *Nissolia* and *Aphaca* have chromosome complements larger than those in the section *Cicercula*. The main karyotypic difference between species of the genus *Lathyrus* involves the shape of satellite chromosome (Yamamoto *et al.* 1984). The divergence and evolution within this genus is accompanied by a 3-fold increase in chromosome size which is directly correlated with 4-fold increase in their nuclear DNA amounts. Comparisons of the total DNA amounts showed discontinuity in the variation among species of this genus (Narayan 1982). Rees and Narayan (1977) clarified that divergence and evolution of *Lathyrus* accompanied by qualitative change within base sequences. Few reports were published on interspecific hybridization between *Lathyrus* species as it is difficult to obtain hybrids except for a few cross combinations.

Electrophoretic patterns of total seed proteins as revealed by polyacrylamide gel electrophoresis (PAGE) with sodium dodecyl sulphate (SDS) under reducing conditions have provided valide evidence for addressing taxonomic and evolutionary problems (Ladzinsky and Hymowitz 1979). This work was done to provide more information about taxonomical relationships between *Lathyrus* species.

Materials and methods

Seeds were obtained from the International Centre for Agriculture Research in Dry Areas, Aleppo, Syria (Table 1). The seeds were milled under cooling at 4 °C by using electric mil for 1 min and the total proteins were extracted for 2 h at 4 °C in 0.2 M Tris/HCl buffer, pH 8.0, and 1 mM phenylmethylsulphonyl fluoride (PMSF). The extract was centrifuged at 5000 *g* for 10 min and the supernatant was taken as total protein extract.

Characterization and molecular mass determination was carried out using one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were prepared for electrophoresis by precipitating protein solutions with 5 volumes of cold acetone at -20 °C for 2 h. Pellets obtained after centrifugation at 7500 *g* for 20 min were dissolved in 25 - 50 mm^3 of sample buffer [0.125 M Tris/HCl, pH 6.8, 2 % (m/v) SDS, 10 % (m/v) saccharose, 1 % (v/v) β-mercapto-ethanol, 0.1 % (m/v) bromophenol blue] and denatured by heating at 80 °C for 3 - 5 min. 17 % polyacrylamide slab gels were prepared as described by Laemmli (1970) and equal amount of proteins were loaded. Electrophoresis was carried out in Tris/glycine-SDS running buffer (0.25 M Tris, 1.88 M glycine, 0.1 % SDS). Gels were stained overnight in 20 cm^3 of 0.25 % kenacid blue R, 50 % (v/v) methanol, 7 % (v/v) glacial acetic acid and destained by shaking in 50 % methanol and 7 % glacial acetic acid. The destained gels were photographed on black and white film.

The bands produced by each sample, under reducing condition, were counted and their relative mobilities compared with those of standard marker protein. The presence or absence of each band was treated as a binary character in a data matrix

for computation using the program of Foucart (1982). The bands scored from the electrophoregrams were used for component analysis to produce a classification and grouping.

Table 1. The source and origin of different studied *Lathyrus* species.

No.	*Lathyrus*	Section	Origin	Strain
1	*sativus* L.	*Cicercula Medik*	TUR	1
2	*aphaca* L.	*Aphaca (Adans)*	URY	2
3	*inconspicuus* L.	*Cicercula*	IRN	5
4	*chloranthus* L.	*Cicercula*	IRN	6
5	*clymenum* L.	*Clymenum (Adans)*	AUS	14
6	*cicera* L.	*Cicercula*	AUS	15
7	*ochrus* DC.	*Clymenum*	AUS	18
8	*nissolia* L.	*Nissolia*	DDR	31
9	*hirsutus* L.	*Cicercula*	TUN	45
10	*inconspicuus* L.	*Cicercula*	IRO	260
11	*pseudocicera* Pamp.	*Cicercula*	SYR	343
12	*gorgoni* Parl.	*Cicercula*	JOR	496
13	*hierosolmitanus* Bioss.	*Cicercula*	SYR	555
14	*marmoratus* Bioss.	*Cicercula*	SYR	564
15	*gorgoni* Parl.	*Cicercula*	SYR	567
16	*annuus* L.	*Cicercula*	SYR	568
17	*blepharicarpus* Bioss.	*Cicercula*	SYR	684
18	*blepharicarpus* Bioss.	*Cicercula*	SYR	746

Results

Species under study of the genus *Lathyrus* from the sections *Cicercula*, *Clymenum*, *Nissolia* and *Aphaca* are annuals. The classification of these sections was based on morphological observation by Davis (1970), Meikle (1977) and Zohary and Feinbrun-Dothan (1966).

The analysis of seed proteins by SDS-PAGE revealed that seeds of *Lathyrus* are very rich in storage proteins with a large number of stable bands in the electrophoregram produced under reducing conditions. This reflects a number of genetic and phylogenic relationships which could be used as a criteria for the classification of species in this genus (Fig. 1).

The phenetic classification produced by the analysis of the electrophoregram revealed ordination species. The species belonging to a circle are represented as a group. These groups included species from the sections *Cicercula*, *Clymenum*, *Nissolia* and *Aphaca*. On the other hand, species from the section *Cicercula* were classified into three groups (I, II and III; Fig. 2).

Electrophoregram corresponding to different accessions of the same species *L. blepharicarpus* (17 and 18) showed a high level of similarity. These results confirm that seed protein profiles are stable within species, little difference induced by environmental factors (Harborn and Turner 1984).

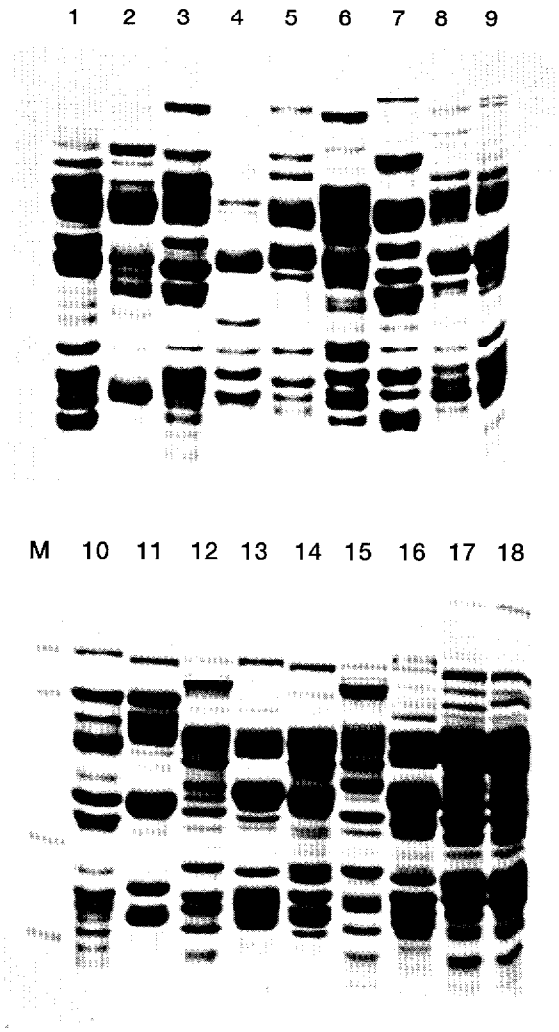

Fig. 1. Electrophoregrams produced by SDS-PAGE analysis of seed proteins of some *Lathyrus* species, numbered as in Table 1.

Most of species under investigation from section *Cicercula* are classified into three groups by principle component analysis (PCA). Group I included the following species: *L. sativus* (1), *L. chloranthus* (4), *L. cicera* (6), *L. inconspicuus* (3) and *L. hirsutus* (9). Group II included the species *L. pseudocicera* (11), *L. gorgoni* (12), *L. hiersolymitanus* (13) and *L. marmoratus* (14). Group III included the species *L. inconspicuus* (10), *L. gorgoni* (15), *L. annuus* (16), *L. blepharicarpus* (17 and 18).

Only two species from section *Clymenum*, *L. clymenum* (5) and *L. ochrus* (7), have a degree of similarity, so they are separated in two different groups (IV and V).

Group VI included only one species, *L. nissolia* from the section *Nissolia*. *L. aphaca* from the section *Aphaca* was isolated in a separate group (group VII).

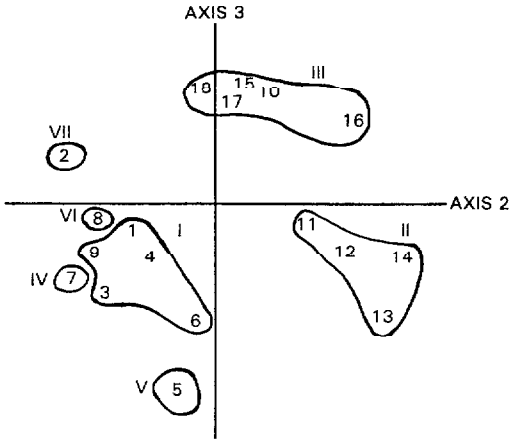

Fig. 2. Factorial analysis of correspondences applied to the matrix of produced proteins by SDS-PAGE characters of some *Lathyrus* species, numbered as Table 1.

Discussion

The application of principle component analysis techniques to variation of the seed protein patterns as revealed by polyacrylamide gel electrophoresis (PAGE) with sodium dodecyl sulphate (SDS) seems to be efficient in the nature of the correlation between these species. Separation into a group and separate species of *Lathyrus* by protein confirm the view of Vaughan (1983), that protein electrophoresis analysis provides a taxonomic evidence for the taxa at the species level, as has been shown for *Lotus* (Haider 1987) and *Vicia* (Sammour 1989) in *Fabaceae*, and for *Brassica* L. *Dipotaxis* DC. and *Erucastrum* (DC) C. Presl in *Brassiceae* (Sánchez-Yelamo *et al.* 1992).

Our analysis of seed protein electrophoresis data supports the sectional delimitation of *Lathyrus* section *Aphaca*, section *Nissolia* and section *Clymenum* as proposed by previous classification.

The species of *Lathyrus* section *Cicercula* do not cluster together on the base of seed protein similarities as assigned by previous morphological classification.

Yamamoto *et al.* (1984) were the first investigators who proposed a three groups of karyotype structure within this section depending on some variation in the shape of satellite chromosome as confirmed by seed protein electrophoresis.

Our results revealed that the first group includes the species *L. hirsutus* (9), *L. sativus* (1), *L. inconspicuus* (3), *L. chloranthus* (4) and *L. cicera* (6). Belonging to this group *L. hirsutus* (9) had a hairy stem, veins of leaflet were pinnate, corolla

pubescent, legume lanceolate and seed surface were tuberculate hairy, while the remaining species under the same group, stem were glabrous, veins of leaflet parallel, corolla and calyx were glabrous and legume was completely smooth. On morphological grounds evolution in this section may have been developed from *L. hirsutus* (9) which is the most primitive species, Bassler (1966) and Simola (1968). Seed protein components appear to run parallel to morphological development.

Despite the group II species *L. pseudocicera* (11), *L. gorgoni* (12), *L. momoratus* (14) and *L. hiersolymitanus* (13) and the group III species *L. blepharicarpus* (17, 18), *L. gorgoni* (15), *L. inconspicuus* (10) and *L. annuus* (16), all of these species have a glabrous winged stem, veins of leaflet parallel, calyx and seeds were glabrous, within these groups only *L. hiersolymitanus* (13) and *L. annuus* (16) differ in having tuberculate seed surface. Seed protein electrophoresis similarities separated these species into two groups. This may be because seed protein is often controlled by quantitative gene system (Ladzinski and Hymowitz 1979), and difference in karyotype structure.

L. clymenum (5) and *L. ochrus* (7) included under the section *Clymenium*, these two species as appeared in Fig. 1 have low degree of similarity by PAGE. The lower degree of similarity may be attributed to a considerable difference in karyotype, including differences in chromosome length between individual chromosomes of both species and also difference in some morphological variations such as pod shape and structure.

In the section *Nissolia*, only one species of *L. nissolia* (8) have a close position with a group I of section *Cicercula*, this species have many similar morphological characters such as pubescent pods, wingless seeds with tuberculate surface, parallel leaflet venation and similar karyological structure, but differ in having angled stem.

L. aphaca section *Aphaca* delimited at side position in Fig. 2 nearly the groups I and VI of section *Cicercula* and section *Nissolia*. This separate position may be as result of advancement of this species from the other by having an angled glabrous stem, parallel venation, glabrous pod and arm ratio found to be smaller than other sections.

As mentioned above our opinion depending on gene product supports the view of Bassler (1966) and Simola (1986) depending on morphological characters. So our results clarified that the species belonging to section *Cicercula* can be classified into three groups depending on PAGE and karyotype structure (Yamamoto *et al.* 1984) and *L. hirsutus* represented the primitive annuals *Lathyrus* species and *L. aphaca* was the most advanced species. *L. nissolia* is the linkage species between section *Cicercula* and section *Aphaca*.

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