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The Use of Seed Proteins Revealed by SDS-PAGE in Taxonomy of Some *Lathyrus* L. Species Grown in Turkey

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Abstract: In this study total 9 taxa belong to four different sections (Pratensis, Orobon, Orobus and Platystylis) collected from different regions of Turkey have been studied for the analysis of seed storage protein profiles to examine their relationship by sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) technique. Data obtained from electrophoresis of seed proteins were analysed using hierarchical clustering analysis and Euclidean distance was used for calculating the genetic resemblance. Dendogram was formed using average linkage. Electrophoretic protein profiles of seed cotyledons were showed that all taxa except for *L. brachypterus* var. *brachypterus* formed two clusters. The first one consisted of *L. pratensis*, *L. laxiflorus* subsp. *laxiflorus*, *L. roseus* and the second one *L. digitatus*, *L. spathulatus*, *L. boissieri L. nivalis* and *L. aureus*. In addition, total protein profile differences were observed when four taxa originated from 9 geographical regions belong to three sections (Sections Platystylis, Pratensis and Orobon) were analysed. Protein amount was found to be highest in *L. spathulatus* and lowest in *L. brachypterus* var. *brachypterus*.

Key words: Cluster analysis, *Lathyrus*, seed protein, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), taxonomy, total protein

INTRODUCTION

Fabaceae is one of the largest plant families of flowering plants comprises about 269 genera and 5100 species in the world (Mabberly, 1997). The genus Lathyrus is placed in the tribes of *Vicieae* of Fabaceae family and divided into 13 sections (Kupicha, 1983). *Lathyrus* has 152 species and shows a broad diffusion throughout the world (Alkin *et al*., 1986). The *Lathyrus* L. genus is represented with 70 taxa in the level of species, subspecies and variety and is divided into 10 sections in Turkey (Davis, 1970). Additionally, six taxa have later been described from Turkey (Ertekin and Saya, 1990; Ertekin, 1994; Davis *et al*., 1988; Maxted and Goyder, 1988; Gunes and Ozhatay, 2000).

The systematic methodology mainly based on morphology has been improved by the incorporation of various characters such as physiology, ecology or biochemical characters. Several biochemical analysis, especially of proteins, make it possible to establish differences at various taxonomic levels (Vaughan, 1983). Leguminous plants are characterized by a high protein content in the seeds. Therefore they are of remarkable agricultural interest (Przybylska *et al*., 1977). The protein contents of legumes are about double those of most cereals in that on average the percentage of protein is 21-25% (Monsoor and Yusuf, 2002; Shahidi *et al*., 2001). The electrophoretic analysis of proteins found in seed and storage organs has been recognized as a powerful tool of experimental taxonomy in detecting intraspecific variations and evaluating interspecific relationships (Przybylska, 1995; Przybylska *et al*., 2000). Although many studies have been performed on the genus *Lathyrus*, the electrophoretic analysis and protein amounts of taxa examined in the present study has not yet been investigated.

The aim of the present study was to investigate both interspecific and intraspecific variations in genus *Lathyrus* by sodium dodecyl sulphate polyacrilamyde gel electrophoresis (SDS-PAGE) technique. In this study total 9 taxa and 14 different geographic distributions of these taxa belong to four different sections (Pratensis, Orobon, Orobus and Platystylis) collected from different regions of Turkey have been studied for the analysis of seed storage protein profiles to examine their relationship. Protein amounts of samples were also determined.

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MATERIALS AND METHODS

Dry seeds of *Lathyrus* species were collected from various areas of Turkey. Details about the seed materials were given in Table 1.

Seed proteins were extracted as described by Jha and Ohri (2002). Seed coats were removed prior to extraction and cotyledons were obtained. These were homogenized in 0.1M Tris-HCl buffer (pH: 7.5). Total protein was extracted after centrifugation at 17.600 g for 20 min at and supernatants were used for analysis. Proteins in the supernatants were quantified using Bio-Rad DC protein assay (Bio-Rad Laboratories, UK). The samples were boiled for 5 min prior to loading, then average 200 μg protein of each sample was loaded on to the 12% SDS-PAGE (Laemmli, 1970). Electrophoresis was performed in the Protean II electrophoresis cell (Bio-Rad Laboratories, UK) at 20 mA until the bromophenol dye (BDH Laboratory Supplies Poole, England) front had reached the bottom of the gel. The gels were stained in Coomassie Brilliant Blue (Sigma Aldrich Chemie, Germany) solution for 30 min at 67°C and destained in destaining solution for 3-4 h at 67°C to visualise the proteins.

Statistical analysis: Evaluation of protein profiles was done visually with an emphasis on qualitative banding differences. The bands were divided into four distinct groups of strong dark, dark, faint and no band and four groups represented by 4, 3, 2 and 1, respectively, in statistical analysis. On the gel, five distinct regions can be distinguished designed as A, B, C, D and E being equivalent to decreasing MWs (Molecular weight; 97 kDa (kilodalton), 66, 45, 30 and 20.1 kDa, respectively; Amersham Biosciences, UK) (Fig. 1). We propose a numerical classification for all protein bands. Seven bands in region A (a_0 to a_6), 4 bands in region B (b_1 to b_4), 4 bands in region C (c_1 to c_4), 3 bands in region D (d_1 to d_3) and, 2 bands in region E (e_1 to e_2) were distinguished.

Data obtained from electrophoresis of seed proteins were analysed using the SPSS (version 10.0) program (SPSS, Chicago, IL, USA). Hierarchical cluster analysis was used for comparison between species and Euclidean distance was used for calculating the genetic resemblance. Dendogram was formed using average linkage.

RESULTS AND DISCUSSION

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 under reducing conditions (Fig. 1). This reflects a number of genetic and phylogenic relationships which could be used as a criteria for the classification of species in this genus (El-Shanshoury, 1997).

The electrophoregrams were evaluated on the basis of band mobility and relative intensity and a dendogram

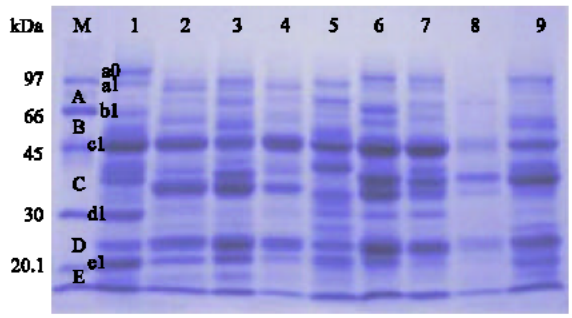

Fig. 1: SDS-PAGE of seed total proteins in nine taxa. M: Marker; 1: *L. aureus* (*Isparta*); 2: *L. digitatus* (*Isparta: Golcuk*); 3: *L. spathulatus* (*Konya*); 4: *L. nivalis* (*Karaman*); 5: *L. boissieri* (*Elazig*); 6: *L. pratensis* (*Konya*); 7: *L. laxiflorus* subsp. *laxiflorus* (*Isparta: Pinargozu*) 8: *L. brachypterus* var. *brachypterus* (*Karaman*); 9: *L. roseus* (*Konya*)

Table 1: Localities of examined taxa

Taxa	Section	Province	Locality
L. aureus (Stev.) Brandza	Orobus	Isparta	Egirdir,Yukarı Gokdere village
L. brachypterus Cel. var. *brachypterus*	Platystylis	Kayseri	Erciyes Mountain
L. nivalis Hand.-Mazz	Platystylis	Konya	Karaman, Karadag
L. digitatus (Bieb.) Fiori	Platystylis	Isparta	Golcuk
L. digitatus (Bieb.) Fiori	Platystylis	Isparta	Kovada lake
L. sphathulatus Cel	Platystylis	Konya	Aksehir, Cankurtaran village
L. boissieri Sirj	Platystylis	Elazığ	Harput Inceler village
L. pratensis L.	Pratensis	Konya	Beysehir, Golyaka
L. pratensis L.	Pratensis	Isparta	Yenisarbademli Pinargozu road
L. laxiflorus (Desf.) O.Kuntze subsp. *laxiflorus*.	Pratensis	Isparta	Pinargozu
L. laxiflorus (Desf.) O.Kuntze subsp. *laxiflorus*	Pratensis	Trabzon	Dernek pazari
L. laxiflorus (Desf.) O.Kuntze subsp. *laxiflorus*	Pratensis	Isparta	Egirdir-Aksu road
L. roseus Stev	Orobon	Konya	Hadim
L. roseus Stev	Orobon	Isparta	Aksu

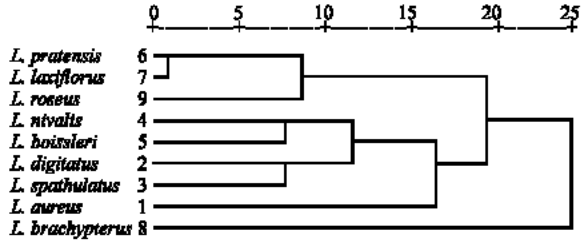

Fig. 2: Dendogram of *Lathyrus* taxa based on total seed protein profiles

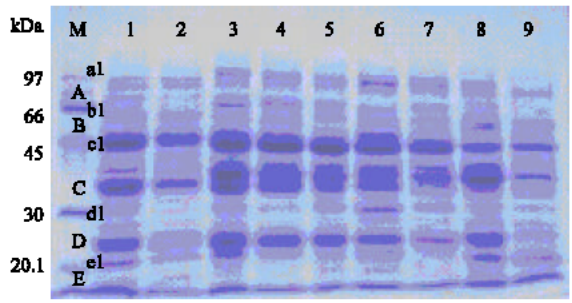

Fig. 3: SDS-PAGE of total seed protein bands among taxa according to the geographic distribution. M: Marker; 1, 2: *L. digitatus* (*Isparta: Golcuk and Egirdir*, respectively); 3, 4: *L. pratensis* (*Konya and Isparta*); 5, 6, 7: *L. laxiflorus* subsp. *laxiflorus* (*Isparta: Pinargozu, Trabzon, Isparta: Egirdir*); 8, 9: *L. roseus* (*Konya, Isparta*)

Table 2: Protein amounts of examined taxa

Taxa	Protein amounts ($\mu g\ mL^{-1}$)
L. aureus (Stev.) Brandza	218.305
L. brachypterus Cel *var. brachypterus*	157.397
L. nivalis Hand.-Mazz.	195.825
L. digitatus (Bieb.) Fiori	203.355
L. digitatus (Bieb.) Fiori	212.325
L. spathulatus Cel.	226.832
L. boissieri Sirj.	211.218
L. pratensis L.	223.842
L. pratensis L.	212.436
L. laxiflorus (Desf.) O.Kuntze subsp. *laxiflorus*	216.533
L. laxiflorus (Desf.) O.Kuntze subsp. *laxiflorus*	207.231
L. laxiflorus (Desf.) O.Kuntze subsp. *laxiflorus*	211.107
L. roseus Stev.	216.423
L. roseus Stev.	204.462

of *Lathyrus* taxa based on the total seed protein profile is given in Fig. 2. B and C region of the electropherogram was the most informative part of the gel that revealed the largest number of different protein profile in the electropherogram of *Lathyrus* taxa shown in Fig. 1. Each taxa protein showed its own electrophoresis pattern with subunits of varied molecular weights. Differences were observed in both presence and absence of a particular band. The most distinctive sample that analyzed was *brachypterus* with some faint protein bands and 1 strong dark band. Between 30 and 20 kDa major protein band (d_3 band) was present in all of the species studied, except *L. brachypterus* var. *brachypterus* that has a dark band instead of strong dark band in the same molecular weight. Protein amounts in all taxa were also quite similar except for *L. brachypterus* var. *brachypterus* that it's protein amount was lowest (Table 2).

Electrophoretic protein profiles of seed cotyledons were showed that all taxa except for *L. brachypterus* var. *brachypterus* formed two clusters. The first one consisted of the *L. pratensis*, *L laxiflorus* subsp. *laxiflorus*, *L. roseus*, second one by five taxa (*L. digitatus*, *L. spathulatus*, *L. boissieri L. nivalis* and *L. aureus).* Total protein profile of *L. brachypterus var. brachypterus* were distinguished from the other members of the Platystylis. This may be being this taxon endemic for Turkey (Davis, 1970).

In section pratensis, protein profiles of *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus* show a high level of similarity to each other. Both species had the same major protein bands but some minor bands were different from each other *L. roseus*, belonging to Section Orobon, was also found to be these two taxa (*pratensis* and *laxiflorus).* *L. roseus*, very distinctive species, standing somewhat between Sections *Lathyrus* and *Orobus* (Davis, 1970). Although basic protein bands of *L. roseus* similar to species of Section Orobon it can be distinguished in other bands from *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus*. In e_2 band, for example, *L. roseus* was found to be different from two species. In b_3 band also diversity observed so that two species have faint band but *L. roseus* has dark band. In d_1 band, while two species have dark band *L. roseus* has faint band profile. According to band profiles of *L. roseus*, *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus* all three taxa formed Cluster I. *L. digitatus*, *L. spathulatus*, *L. nivalis*, *L. boissieri* and *L. aureus* taxa formed cluster II. *L. nivalis* shows similarity with *L. boissieri* and *L. digitatus* with *L. spathulatus*. *L. aureus*, belong to Sect. Orobus, also show similarity with taxa of Section Orobon (*digitatus*, *spathulatus*, *nivalis* and *boissieri*). In a_1 band *L. nivalis* differ from others. While *L. nivalis* has faint band other four members of cluster II, have dark band. In a_4 band, *L. spathulatus* has dark band, other taxa have faint band profile. Beside *L. digitatus* superficially similar to *L. spathulatus,* protein band patterns of two species also close to one another. In a_0, b_1, d_1 bands, *L. aureus* different from other taxa.

Band profiles of the examined taxa from nine geographical regions belong to three sections (Platystylis, Pratensis and Orobon) are shown in Fig. 3. Little differences were observed in the examples of total seed

protein bands among taxa according to the geographic distribution. For example, in b_2 and d_1 bands of *L. pratensis,* differences were observed in Konya and Isparta samples. Similarly, while the protein profiles of two samples of *L. laxiflorus* subsp. *laxiflorus* (Isparta: Pinargozu and Egirdir) have almost the same protein bands, other sample of *L. laxiflorus* subsp. *laxiflorus* (Trabzon: Dernekpazari) shows little differences in c_4, e_1 and e_3 bands.

Electrophoretic protein profiles and amounts of proteins of sections Pratensis, Orobon, Orobus and Platystylis were examined by SDS-PAGE. Total seed protein data reported in the present study are essentially consistent with Flora of Turkey except for *L. brachypterus* var. *brachypterus* (Davis, 1970) and also little differences were observed in the examples of total seed protein bands among taxa according to the geographic distribution. Protein amounts of all species were also found to be between 157.397-226.832 μg mL^{-1}. Further analysis of genetic variations between the species, such as analysis of subprotein fractions (albumins, globulins etc.), RAPD studies, which have been used as a taxonomic tool in many species, may able to further resolve the phylogenetic relationships between these species.

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