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Determination of Genetic Variations in *Lathyrus* L. Species Based on SDS-PAGE Analyses of Seed Storage Proteins (Albumin, Globulin A, Glutelin)

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Abstract

The present study reports on variations for banding pattern of subfractions (albumins, globulin A, glutelins) of seed storage proteins in some *Lathyrus* species (*L. clymenum, L. ochrus, L. nissolia, L. aphaca* L. var. *affinis, L. aphaca* L. var. *biflorus, L. aphaca* L. var. *pseudoaphaca, L. aphaca* L. var. *modestus*) collected from their natural habitats in Turkey. Electrophoretic data were documented by using a gel documentation system and analysed using Quantity 1-D analysis software; dendrograms were produced with 4.0 % tolerance in UPGAMA (Unweighted Pair-Group Arithmetic Mean). The differences between taxa were observed and all seven species were clearly identifiable from their protein banding patterns in view of both strength and positioning of the bands. The dendrograms of seed storage proteins using UPGAMA showed that all the studied taxa formed two clusters in this study. The present results demonstrated that the four studied subspecies of *L. aphaca* (members of section *Aphaca*) are much more closely related to each other rather than *L. ochrus, L. clymenum* and *L. nissolia.* The results of the present study generally support morphological classifications of Davis (1970), Kupicha (1983) and Dogan *et al.* (1992).

Keywords: Albumin; Genetic Diversity; Globulin A; Glutelin; *Lathyrus*; SDS-PAGE; UPGAMA.

Tohum Depo Proteinlerinin (Albumin, Globulin A, Glutelin) SDS-PAGE Analizlerine göre *Lathyrus* L. Türlerinde Genetik Varyasyonun Belirlenmesi

Özet

Bu çalışma, Türkiye'deki doğal habitatlarından toplanan bazı *Lathyrus* (*L. clymenum, L. ochrus, L. nissolia, L. aphaca* L. var. *affinis, L. aphaca* L. var. *biflorus, L. aphaca* L. var. *pseudoaphaca, L. aphaca* L. var. *modestus*) türlerindeki tohum depo proteinlerinin alt fraksiyonlarının (albumin, globulin A, glutelin) band paternlerindeki varyasyonu rapor etmektedir. Bu çalışmada, elektroforetik veriler, jel dökümantasyon sistemi ile görüntülendi ve Quatity 1-D analiz yazılımı kullanılarak analiz edildi ve dendogramlar%4 tolerans ile UPGAMA (Unweighted Pair-Group Arithmetic Mean) ile oluşturuldu. Taksonlar arasındaki farklılıklar gözlendi ve yedi tür açık bir biçimde bantların durumundan ve koyuluğundan dolayı protein band paternlerine gore ayırt edildi. Bu çalışmada, UPGAMA kullanılarak yapılan tohum depo proteinlerinin dendogramları göstermiştir ki çalışılan bütün yedi takson iki küme oluşturmuştur. Mevcut çalışmalar göstermiştir ki *L. aphaca*'nın (*Aphaca* seksiyonunun üyeleri) çalışılan alt türleri, *L. ochrus, L. clymenum* ve *L. nissolia* türlerine gore birbirlerine çok daha yakın kümelenmişlerdir. Genel olarak bu çalışmanın sonuçları Davis (1970), Kupicha (1983) ve Doğan vd. (1992)'nin morfolojik çalışmalarını desteklemektedir.

Anahtar kelimeler: Albumin, genetik çeşitlilik, globulin A, glutelin, *Lathyrus*, SDS-PAGE, UPGAMA

1. Introduction

With more than 650 genera and 18,000 species, legumes are the third largest family of higher plants [1]. *Lathyrus* L., which is a member of the *Viciae* tribe (Leguminosae), consists of approximately 160 annual and perennial species, many of which are economically important, used as forage, human food or ornamental plants, and have a long history as cultivated plants [2-4]. The species within the *Lathyrus* are divided into 13 sections, based on the morphological characteristics throughout world [5]. The *Lathyrus* L. is represented by 75 taxa at the level of species, subspecies and variety, and is divided into 10 sections in Turkey [6-13].

Besides morphological data, several techniques have been used to examine the infrageneric classification within the genus *Lathyrus*, including karyotype analyses [14,15]. RFLP and numerous genetic marker assays based on PCR such as RAPD, inter simple sequence repeats (ISSR), and AFLP [16,17], nuclear and chloroplast DNA restriction site analysis [18-20], as well as isozyme and seed storage proteins electrophoresis [21-24]. Since seed storage proteins are physiologically stable, electrophoretic band profiles of seed proteins have provided important evidence for addressing taxonomic problems [25-28]. Seed storage proteins are classified into four major groups, based on extraction properties [29]; albumins (extract in water), globulins (extract in diluted salt; the great storage proteins of legume seeds), glutelins (extract in weak basic or acidic solutions) and prolamins (extract in alcohol/water mixtures) [30,31].

The objective of present study was to determine the genetic diversity among seven *Lathyrus* taxa (*L. clymenum, L. ochrus, L. nissolia, L. aphaca* L. var. *affinis, L. aphaca* L. var. *biflorus, L. aphaca* L. var. *pseudoaphaca, L. aphaca* L. var. *modestus*) based on seed storage protein subfractions (albumin, globulin A and glutelin) by using SDS-PAGE to contribute the solution of taxonomical problems in *Lathyrus*. No studies have previously been reported on electrophoretic separation of the storage protein subfractions of *Lathyrus* taxa in this study.

2. Materials and Methods

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

Table 1. Localities of investigated *Lathyrus* taxa

Taxa	Herb. no	Section	Province	Locality	Altitude
L. clymenum L.	1270	*Clymenum* (Adans.) DC.	Mugla	Knidos	70 m
L. ochrus (L.) DC.	1280	*Clymenum* (Adans.) DC.	Mugla	Marmaris	400 m
L. nissolia L.	1290	*Nissolia* (Adans.) Reichb.	Burdur	Bagsaray	890 m
L. aphaca L. *var. affinis* (Guss.) Arc.	1301	*Aphaca* (Adans.) Reichb.	Mugla	Marmaris	500 m
L. aphaca L. *var. biflorus* Post.	1302	*Aphaca* (Adans.) Reichb.	Burdur	Bagsaray	870 m
*L. aphaca*L. *var. pseudoaphaca* (Boiss.) Davis	1303	*Aphaca* (Adans.) Reichb.	Isparta	Near to Kovada lake	900 m
L. aphaca L. *var. modestus* P.H.Davis	1304	*Aphaca* (Adans.) Reichb.	Isparta	Egirdir Balkiri village	910 m

Seed protein subfractions from *Lathyrus* seeds were extracted as described by Vaz et al. [32]. Seed coats were removed prior to extraction and cotyledons were obtained. These were homogenised with water, 75 % ethanol and 0.25 % (w/v) NaOH, respectively to obtain protein subfractions. Firstly, to obtain albumins and globulins A seed meal extracted with distilled water for 2 hours and then centrifugated at 7800 g for 15 minutes. Supernatant that contain albumin and globulin A dialyzed 1/100 against distilled water overnight at 4 °C and then centrifuged for 15 minutes at 7800 g. Supernatant was containing albumins and pellet obtained after the dialysis (extracted in 1 ml distilled water) was contained globulin.

The procedure was sequentially repeated to obtain prolamins and glutelins from the pellet containing the insoluble material. The pellet was resuspended in 75 % ethanol (v/v) for 2 hours and centrifuged at 7800 g for 15 minute to obtain prolamins. Lastly, the glutelin fraction was obtained by treating 0.25 % (w/v) NaOH for 2 hours after the suspension was centrifuged at 7800 g for 15 minutes. The pellet fractions were dried overnight in the air at 4 °C. All samples were kept at -20 °C until used. Marker was used from Fermentas [SMO431; (116.0 kDa (kilodalton), 66.2 kDa, 45 kDa, 35 kDa, 25 kDa, 18.4 kDa)]. The samples were boiled for 5 minutes prior to loading, then equal amount of protein from each sample was loaded in to the 12 % gel [33]. 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 to 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 protein bands.

2.1. Statistical analysis

Electrophoretic data were documented by using a gel documentation system (Bio-Rad, USA) and analysed by using Quantity 1-D analysis software. Dendograms were formed with 4.0 % tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean). Similarity matrix was constructed by Dice coefficient using Quantity 1-D analysis software (Bio-Rad) and expressed as percentages.

3. Results and Discussion

This study presents electrophoretic data concerning the diversity of seed storage proteins by using SDS-PAGE in seven species of *Lathyrus*. The differences between species were observed and all seven taxa were clearly identifiable from the protein banding patterns. The seed storage protein banding patterns of seven taxa are illustrated in Figure 1 (albumin), Figure 2 (globulin A), and Figure 3 (glutelin), except for prolamins, as their quantities were quite low.

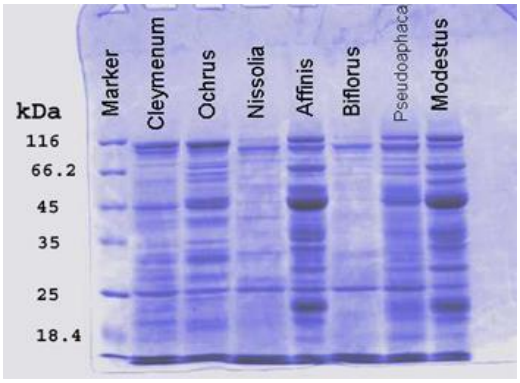

Figure 1. Electrophoretic band profiles of albumin of studid taxa

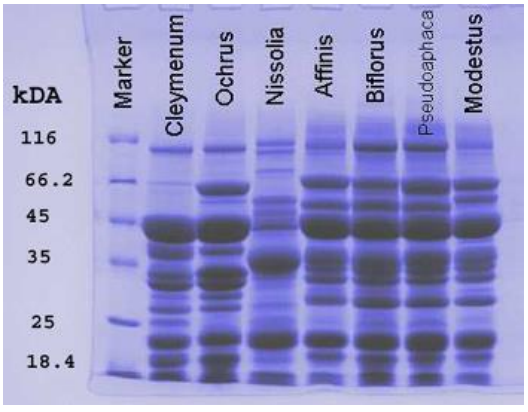

Figure 2. Electrophoretic band profiles of globulin A of studid taxa

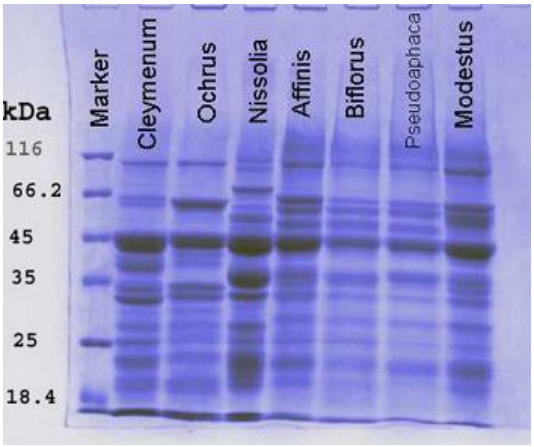

Figure 3. Electrophoretic band profiles of glutelin of studid taxa

Additionally, the protein amounts of the studied taxa are given in Table 2 and dendrograms were produced according to SDS-PAGE analysis of albumin (Figure 4), globulin A (Figure 5) and glutelins (Figure 6).

Table 2. Protein amounts of investigated *Lathyrus* taxa

	Seed storage protein subfractions (µg/ml)			
Taxa	**Albumin**	**Globulin A**	**Prolamin**	**Glutelin**
L. cleymenum	2.681	7.833	0.354	2.096
L. ochrus	2.354	8.557	0.160	2.206
L. nissolia	2.741	6.824	0.405	3.368
L. aphaca var. affinis	3.653	7.511	0.183	2.547
L. aphaca L. *var. biflorus*	2.197	8.036	0.414	1.515
*L. aphaca*L. *var. pseudoaphaca*	2.897	7.704	0.363	1.331
L. aphaca L. *var.modestus*	4.441	7.119	0.271	2.294

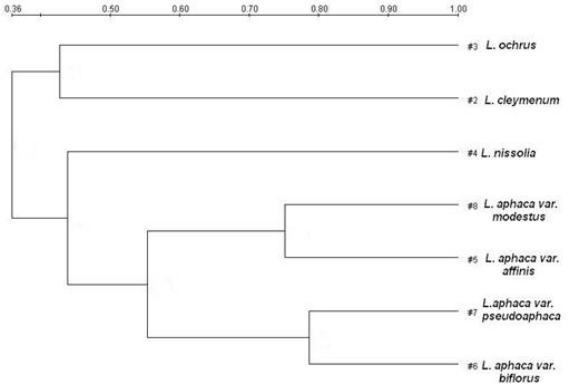

Figure 4. Dendogram of *Lathyrus* taxa based on seed albumin profiles

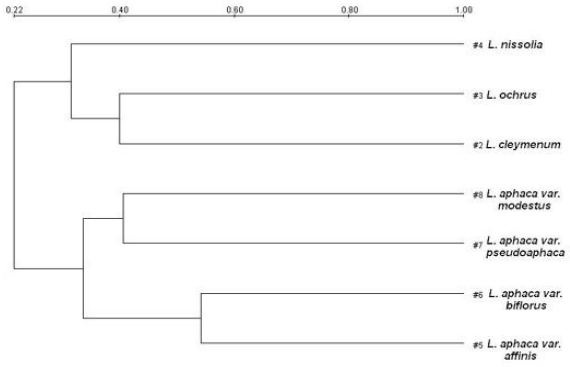

Figure 5. Dendogram of *Lathytrus* taxa based on seed globulin A profiles

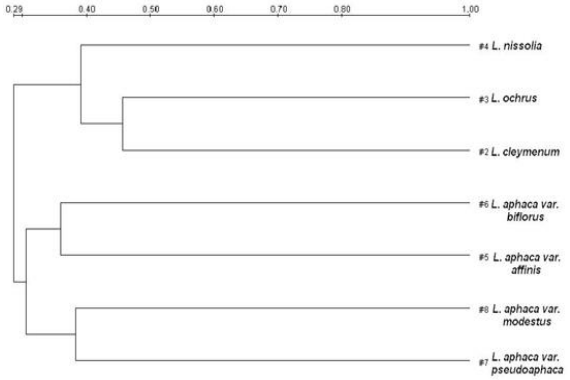

Figure 6. Dendogram of *Lathyrus* taxa based on seed glutelin profiles

Present results showed that *L. aphaca* var. *affinis* and *L. aphaca* var. *modestus* have the highest albumin content (3,653 µg/ml and 4,441 µg/ml, respectively).

L. ochrus (8.557 µg/ml) and *L. aphaca* var. *biflorus* (8.036 µg/ml) had higher globulin content than the other studied species. Furthermore, it was determined that *L. nissolia* has the highest glutelin content (3.368 µg/ml) and *L.aphaca* var. *biflorus* has the highest prolamin amounts (0.414 µg/ml) among the species studied. However, prolamin amounts of all studied taxa were quite low. Therefore electrophoretic banding pattern was not determined for prolamines. The results of the present study generally support morphological classifications of Davis [6], Kupicha [5] and Dogan et al. [34]. *L. nissolia* was placed in monotypic *Nissolia; L. ochrus* and *L. clymenum* was placed under section *Clymenum;* and *L. aphaca* was placed under section *Aphaca* [5,6, 34].

The dendrograms of seed storage proteins using UPGAMA showed that all the studied taxa formed two clusters in this study. The present results demonstrated that the four studied subspecies of *L. aphaca* (members of section *Aphaca*) are much more closely related to each other rather than the other species. Based on albumin and glutelin data (Figures 4 and 6, respectively), *L. aphaca* var. *pseudoaphaca* and *L. aphaca* var. *modestus* exhibited a close relationship with each other and close affinity was determined between *L. aphaca* var. *affinis* and *L. aphaca* var. *biflorus*. On the other hand, *L. aphaca* var. *biflorus* has close similarity with *L. aphaca* var. *pseudoaphaca*, while *L. aphaca* var. *modestus* has close similarity with *L. aphaca* var. *affinis*, being founded on globulin A data (Figure 5). In addition, strong similarities were shown between *L. ochrus* and *L. clymenum*, which are members of section *Clymenum*, based on the seed albumins, globulins and glutelins by the present study. Morphologically, it was reported that *L. ochrus* and *L. clymenum* have common properties, such as wide petiole wings, hollow finger-like pouches on the standard and spatulate style [5, 17]. The present results support those of several previous studies including RAPD data [16], AFLP data [17] and isozyme similarity results [35]. Mohammed Ali et al. [19] also reported that the two species of section *Clymenum* (*L. clymenum, L. ochrus*) have two 5S rDNA loci on the long arm of chromosome 2. In addition, Mohammed Ali et al. [19] indicated that *L. aphaca* takes an intermediate position between species of the sections *Clymenum* and *Lathyrus.* However, a SDS-PAGE analysis study by El-Shanshoury [21] indicated that two species from section *Clymenum, L. clymenum* and *L. ochrus*, have a low degree of similarity, so they are separated into two different groups and *L. nissolia* is clustered within another group. Also, in a comparative isozymic polymorphism study done by Brahim et al. [3] showed that the low level of allozyme variation between *L. ochrus* and *L. nissolia*. A study by Abou-El-Enain et al. [36] suggested that *L. nissolia* are nested in different subclusters from *L. clymenum* and *L. ochrus*.

Conversely, in the present study, *L. nissolia*, which is a member of monotypic *Nissolia* section, showed similarity with *L. ochrus* and *L. clymenum* based on albumin and glutelin sub fractions (Figure 4 and Figure 6), and *L. nissolia* showed affinity with subspecies of *L. aphaca*, based on globulin A (Figure 5). Chloroplast DNA data from a study by Asmussen & Liston [18] suggested that *L. nissolia* occurs as sister to *L. ochrus* and *L. clymenum.* Another study by Kenicer et al. [37] supported the results of the present study. In contrast, Abou-El-Enain et al. [36] reported that *L. nissolia* has differently clustered subgroups from *L. ochrus* and *L. clymenum.*

Based on SDS-PAGE results, it can be concluded that electrophoretic analysis of seed storage proteins can be used as powerful tools to solve taxonomical problems among species.

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