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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (17): 2890-2894, 2007
ISSN 1028-8880

Electrophoretic Analysis of Total Protein Profiles of Some *Lathyrus* L. (Sect. Cicercula) Grown in Turkey

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Abstract: In this study, total 8 species (Section Cicercula) belong to 14 different geographic distributions collected from 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. Hierarchical cluster analysis and Euclidean distance were used for comparison between species and calculating the genetic resemblance respectively. Dendogram was formed using average linkage. Electrophoretic protein profiles of seed cotyledons were showed that all species formed two clusters. The first one consisted of the *L. stenophyllus*, *L. hirsutus*, *L. chloranthus*, *L. cicera* and *L. sativus* second one by three species (*L. annuus*, *L. cassius* and *L. phaselitanus*). In addition, very little differences were observed in total protein profiles of the examined sample species (*L. cicera*, *L. hirsutus* and *L. chloranthus*) from nine geographical regions belong to section Cicercula. Protein amount was found to be highest in *L. hirsutus* and lowest in *L. cicera*.

Key words: Taxonomy, SDS-PAGE, cicercula, *Lathyrus*, total seed protein, cluster analysis

INTRODUCTION

Fabaceae is third largest family of flowering plants comprises about 269 genera and 5100 species in the world (Mabberly, 1997; Ellison *et al*., 2006). The genus *Lathyrus* is placed in the tribes of *Vicieae* of Fabaceae family and divided into 13 sections (Kupicha, 1983). It has 152 species and shows a broad diffusion throughout the world (Alkin *et al*., 1986). This 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 been described from Turkey in recently years (Ertekin and Saya, 1990; Ertekin, 1994; Davis *et al*., 1988; Maxted and Goyder, 1988; Guner and Ozhatay, 2000).

Legume seeds have a high level of protein content which contain average of 20-25% protein, 2-3 times more than cereals (Cai *et al*., 2001; Monsoor and Yusuf, 2002; Gepts *et al*., 2005; Duranti, 2006). Therefore, grain legumes have played a primary role in the search for vegetable sources of proteins and investigations on economically viable legumes as alternative foods broaden the protein sources for human nutrition (Valizadeh, 2001; Sena *et al*., 2005). Seed proteins are highly stable, being unaffected by environmental conditions and easily to handle. Thus, electrophoretic banding patterns of total seed proteins and seed storage protein subfractions (albumins, globulins, prolamins, glutelins) as revealed by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis) have provided valid evidence for addressing taxonomic and evolutionary problems (Ladizinsky and Hymowitz, 1979; Kamel *et al*., 2003; Freitas *et al*., 2004; Ribeiro *et al*., 2004; Fukuda *et al*., 2005).

The aim of the present study was to investigate both interspecific and intraspecific variations in Section Cicercula by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis) technique. In this study, total 8 species belong to 14 different geographic distributions were collected and studied for the analysis of seed storage protein profiles to examine their relationship. Protein amounts of samples were also determined.

MATERIALS AND METHODS

Dry seeds of *Lathyrus* species were collected from various areas of Turkey. Outcross was not observed among studied species. Details about the seed materials are given in Table 1. The laboratory study was carried out

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Table 1: Localities of investigated *Lathyrus* species

Species	Section	Province	Locality
L. annuus L.	Cicercula	Mugla	Osmaniye village
L. cassius Boiss.	Cicercula	Sanlıurfa	Sanlıurfa-Viransehir road
L. cicera L.	Cicercula	Burdur	Burdur Education Faculty campus
L. cicera L.	Cicercula	Elazig	University campus
L. cicera L.	Cicercula	Burdur	Burdur Denizli road
L. sativus L.	Cicercula	Antalya	Cobanishak village
L. stenophyllus Boiss. and Heldr.	Cicercula	Antalya	Phaselis
L. phaselitanus Hub.-Mor and Davis	Cicercula	Antalya	Phaselis
L. hirsutus L.	Cicercula	Konya	Near to Iliirmak bridge
L. hirsutus L.	Cicercula	Isparta	Egirdir-Aksu road
L. hirsutus L.	Cicercula	Isparta	Egirdir Balkırı village
L. hirsutus L.	Cicercula	Elazig	University campus
L. chloranthus Boiss.	Cicercula	Isparta	Sarkikaraagac-Yenisarbademli road
L. chloranthus Boiss.	Cicercula	Mus	Sutluce village

in the Molecular Identification Laboratory, Department of Biology, Faculty of Sciences and Art, Firat University, Elazig, Turkey in 2006.

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 the ratio 1:10 (w/v), using 0.1 M Tris-HCl buffer (pH: 7.5). Total protein was extracted after centrifugation at 17.600 g for 20 min at 4°C and supernatants were used for analysis. Proteins in the supernatants were quantified using Bio-Rad DC protein assay Kit (Bio-Rad Laboratories, UK). The samples were boiled for 5 min prior to loading, then average equal 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, 1, respectively, in statistical analysis. On the gel, five distinct regions were determined and called as A, B, C, D and E being equivalent to decreasing MWs of 97, 66, 45, 30 and 20.1 kDa (kilodalton), respectively; (Amersham Biosciences, UK) (Fig. 1). We analyzed the gels in a numerical classification for all protein bands. 3 bands in region A (a_1 to a_3), 3 bands in region B (b_1 to b_3), 6 bands in region C (c_1 to c_6), 4 bands in region D (d_1 to d_4) 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 total proteins by SDS-PAGE revealed that seeds of *Lathyrus* are very rich in storage proteins with a large number of stable bands on the gel (Fig. 1). Many studies based on the electrophoretic analysis of seed proteins have been used to examine genetic variability and systematic problems in several legumes such as genus *Astragalus* (Acık *et al*., 2004), genus *Lupin* (Vaz *et al*., 2004), genus *Pisum* (Jha and Ohri, 2002), genus *Phaseolus* (Przybylska and Przybylska, 1993), genus *Lathyrus* (Przybylska *et al*., 1999, 2000; Emre *et al*., 2006), genus *Onobrychis* (Emre *et al*., 2007).

The electrophoregrams were evaluated on the basis of band mobility and relative intensity. A dendogram of studied species based on the total seed protein profile is given in Fig. 2 regions of electropherogram was the most informative part of the gel that revealed the largest number of different protein banding profile in the electropherogram of species, belong to Section Cicercula in genus *Lathyrus* (Fig. 1). Protein profiles of each species showed its own electrophoresis pattern with subunits of varied molecular weights. Differences were observed in both presence and absence of a particular band. A strong dark band was present in d_3 band (between 30 and 20.1 kDa major protein band) in all of the species studied except for *L. cicera* which has dark band. Protein amounts in all species were also quite similar as shown in Table 2. Protein amount was found to be highest in *L. hirsutus* (225.393 μg mL^{-1}) from Isparta (Egirdir-Aksu road) and lowest in *L.cicera* (206.677 μg mL^{-1}) from Burdur (Burdur Education Faculty campus).

Electrophoretic protein banding profiles of seed cotyledons were showed that all species formed two clusters. The first one consisted of the *L. stenophyllus*,

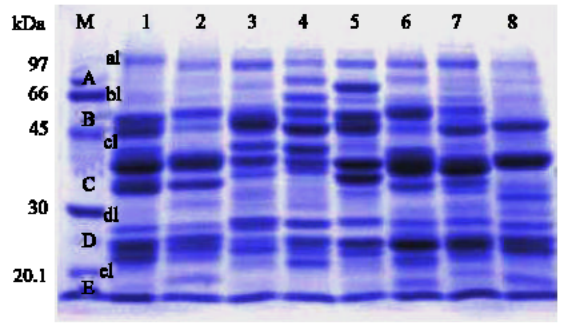

Fig. 1: SDS-PAGE of total seed proteins in eight species. *M*: *Marker*; 1: *L. annuus* (*Mugla*); 2: *L. cassius* (*Sanlyurfa*); 3: *L. cicera* (*Burdur*); 4: *L. sativus* (*Antalya*); 5: *L. stenophyllus* (*Antalya*); 6: *L. phaselitanus* (*Antalya*); 7: *L. hirsutus* (*Konya*) 8: *L. chloranthus* (*Isparta*)

Table 2: Protein amounts of investigated *Lathyrus* species

Species	Protein amounts (μg mL^{-1})
L. annuus L.	214.540
L. cassius Boiss.	208.006
L. cicera L.	206.677
L. cicera L.	216.201
L. cicera L.	219.080
L. sativus L.	209.677
L. stenophyllus Boiss. and Heldr.	217.087
L. phaselitanus Hub.-Mor. and Davis	222.846
L. hirsutus L.	224.839
L. hirsutus L.	225.393
L. hirsutus L.	213.654
L. hirsutus L.	219.413
L. chloranthus Boiss.	213.986
L. chloranthus Boiss.	218.748

L. hirsutus, *L. chloranthus*, *L. cicera* and *L. sativus* second one by three species (*L. annuus*, *L. cassius* and *L. phaselitanus*).

Protein profiles of *L. cicera* and *L. sativus* show a high level of similarity to each other. Both species had almost the same protein bands but some differences were observed in protein profiles. For example, while *L. cicera* has strong dark band profile, *L. sativus* has faint band in b_2 band. Also in c_3 band, *L. sativus* has strong dark band but *L. cicera* has dark band. Furthermore, in cluster I, *L. stenophyllus* also shows similar band patterns with *L. hirsutus*. In addition *L. chloranthus* is similar with these two species (*L. stenophyllus* and *L. hirsutus*). On the other hand according to band profiles of *L. annuus*, *L. cassius* and *L. phaselitanus* all three species formed Cluster II. *L. annuus* and *L. cassius* higher degree of similarity than *L. phaselitanus* which endemic for Turkey.

These results are agreement with an earlier study done by El-Shanshoury (1997) who found that *L. sativus*, *L. chloranthus*, *L. cicera* and *L. hirsutus* were in the same

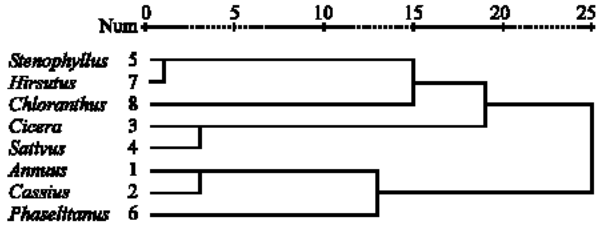

Fig. 2: Dendogram of *Lathyrus* species based on total seed protein profiles

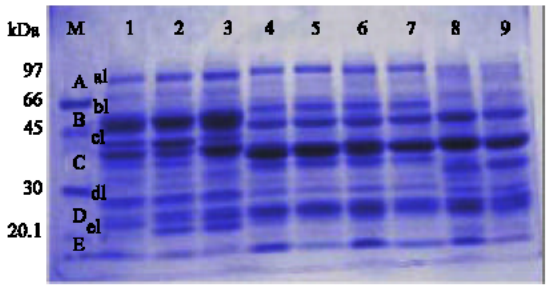

Fig. 3: SDS-PAGE of total seed protein bands among species according to the geographic distribution. *M: Marker*; 1, 2, 3: *L. cicera (Burdur*, *Elazig*, *Burdur-Denizli road*; respectively); 4, 5, 6, 7: *L. hirsutus* (*Konya*, *Isparta* (*Egirdir-Aksu* road), *Isparta* (*Egirdir Balkyry* village), *Elazig*); 8, 9: *L. chloranthus* (*Isparta*, *Mus*)

cluster but *L. annuus* was member of the other cluster. In another study, isozymic results are partly consistent with our results (Brahim *et al*., 2002). In this study (done by Brahim *et al*., 2002), *L. sativus* and *L. cicera* formed the same cluster and had great similarity but *L. hirsutus* was in another cluster. Also in another study (results from RAPD analyses) done by Chtourou-Ghorbel *et al*. (2002) showed that *L. sativus* more closely related to *L. cicera*. Similarly, Present results show that these two species (*L. sativus* and *L. cicera*) have great homology each other and also *L. hirsutus* was in different position in same cluster. In addition, *L. annuus* has different electrophoretic band patterns and was another cluster. Furthermore *L. sativus* and *L. cicera* were reported to be similar with respect to some properties, such as morphological traits (Jackson and Yunus, 1984), karyotype (Yamamoto *et al*., 1984). On the contrary, Przybylska *et al*. (2000) indicated that *L. sativus* and *L. cicera* are definitely different in the composition of seed albumins and globulins.

Band profiles of the examined taxa from nine geographical regions belong to section Cicercula are shown in Fig. 3. Very little differences were observed in the examples of total seed protein bands among taxa according to the geographic distribution.

According to present results, it seems justified to recommend a wider use of electrophoretic analysis of seed storage proteins in taxonomic investigations of leguminous plants. The other prominent result of our study is similarity of electrophoretic band patterns of *L. sativus* to band patterns of *L. cicera*. As a result, further investigation of phylogenetic relationships between these species needed by analysis of genetic variations between the species, such as analysis of subprotein fractions (albumins, globulins etc.) and isozymic studies.

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