

TOTAL SEED STORAGE PROTEIN PATTERNS OF SOME *LATHYRUS* SPECIES GROWING IN TURKEY USING SDS-PAGE

İRFAN EMRE^{1*}, DILEK TURGUT-BALIK², HASAN GENÇ³ AND AHMET ŞAHİN⁴

¹Firat University, Faculty of Education, Department of Primary Education, Elazığ, Turkey

²Yildiz Technical University, Faculty of Chemical and Metallurgical Engineering,
Department of Bioengineering, Istanbul, Turkey

³Mehmet Akif University, Faculty of Education, Department of Primary Education, Burdur, Turkey

⁴Erciyes University, Faculty of Education, Kayseri, Turkey

*Corresponding Author: e-mail: iemre@firat.edu.tr; phone: +90 424 237 00 00/4843; fax: +90 424 236 50 64

Abstract

Present study determined the variation of total electrophoretic seed protein patterns and protein amounts in some *Lathyrus* species viz., *L. tukhtensis*, *L. cilicicus*, *L. saxatilis*, *L. annuus*, *L. hierosolymitanus*, *L. gorgoni* var. *gorgoni*, *L. Lycicus* and *L. odoratus* collected from their natural habitats of different localities in Turkey. Electrophoretic data were documented by using a gel documentation system (Bio-Rad, USA) and analysed by using Quantity 1-D analysis software and also the dendogram were formed with 4.0% tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean). The differences among species were observed and all 9 taxa were clearly identifiable from the protein patterns. The formed dendogram from SDS-PAGE analysis showed that all studied taxa constituted two clusters. The first one consisted of *L. saxatilis*, *L. gorgoni*, *L. annuus*, *L. hierosolymitanus*, *L. lycicus* and *L. phaselitanus* second one by *L. tukhtensis*, *L. cilicicus* and *L. odoratus*. In cluster I, *L. saxatilis* and *L. gorgoni* were found to have higher similarity to each other. Also, it was reported that quantities of total seed proteins in the present study. *L. lycicus* (79.906 µg/ml) has highest total protein content whereas *L. cilicicus* (65.860 µg/ml) has lowest total protein content.

Introduction

Genus *Lathyrus*, which is a member of the *Viciae* tribe (family *Leguminosae*), consists of about 160 annual and perennial species and many of them are economically important, used as forage, human food or ornamental plant and have a long history as cultivated plants (Chtourou-Ghorbel *et al.*, 2001, Brahim *et al.*, 2002, Lewis *et al.*, 2005). Classification of *Lathyrus* has varied in the history of the genus (Brahim *et al.*, 2002). Kupicha's (1983) morphology-based monograph represents only worldwide treatment of genus. She proposed infrageneric classification with 13 sections (Kupicha, 1983; Asmussen & Liston, 1998). The *Lathyrus* L. genus is represented with 75 taxa in the level of species, subspecies and variety and it is divided into 10 sections in Turkey (Davis, 1970; Ertekin & Saya, 1990, Ertekin, 1994; Davis *et al.*, 1988; Maxted & Goyder, 1988; Guner & Ozhatay, 2000; Genç & Şahin; 2008; Genç, 2009).

The systematic methodology especially based on morphology chiefly has been improved by the incorporation of physiology, ecology or biochemical traits (Ayaz *et al.*, 1999; Przybylska *et al.*, 2000). It was reported that biochemical and molecular analysis, particularly of electrophoretic analysis of seed proteins as revealed by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis) have provided valid evidence for detecting intraspecific variation and assessing interspecific relationships (Ladizinsky & Hymowitz, 1979; Kamel *et al.*, 2003; Javaid *et al.*, 2004; Çelebi *et al.*,

2009; Hameed *et al.*, 2009). 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 the genus *Astragalus* (Acik *et al.*, 2004), genus *Lupin* (Vaz *et al.*, 2004), genus *Pisum* (Jha & Ohri, 2002), genus *Lathyrus* (Przybylska *et al.*, 1999, 2000, Emre *et al.*, 2006; Emre *et al.*, 2007a, Emre, 2009), genus *Onobrychis* (Emre *et al.*, 2007b), genus *Phaseolus* (Przybylska & Przybylska, 1993) and genus *Vicia* (Przybylska & Zimniak-Przybylska, Z., 1997; Ayaz *et al.*, 1999; Mirali *et al.*, 2007; Emre, 2007; Emre *et al.*, 2007c). Present study reports the variation of total electrophoretic seed protein patterns and protein amounts in some *Lathyrus* species collected from their natural habitats of different localities in Turkey.

Materials and Methods

Dry seeds of *Lathyrus* species viz., *L. tukhtensis* Czecz., *L. cilicicus* Hayek&Siehe, *L. saxatilis* (Vent.) Vis., *L. annuus* L., *L. hierosolymitanus* Boiss., *L. gorgoni* Parl. var. *gorgoni*, *L. lycicus* Boiss., *L. phaselitanus* Hubb.-Mor&Davis, *L. odoratus* L., were collected from various areas of Turkey. Details about the seed materials are given in Table 1.

Seed proteins were extracted as described by Jha & Ohri (2002). Seed coats were removed prior to extraction and cotyledons were obtained. These were homogenised in 0.1M 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 (Bio-Rad Laboratories, UK) and on the gel, Fermentas (116.0 kDa (kilodalton), 66.2 kDa, 45 kDa, 35 kDa, 25 kDa, 18.4 kDa) used as marker. The samples were boiled for 5 minutes 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

Electrophoretic data were documented by using a gel documentation system (Bio-Rad, USA) and analysed by using Quantity 1-D analysis software and also the dendrogram were formed with 4.0% tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean).

Results and Discussion

Kupicha's (1983) classification based on morphological characters proposed 153 species of *Lathyrus* into four great sections viz., *Orobos* (54), *Lathyrus* (33) *Notolathyrus* (23) and *Lathyrstylis* (20); other four sections comprises two to seven species (*Aphaca*, *Clymenum*, *Linearicarpus*, *Pratensis*) and last five sections are monotypic (*Orobon*, *Nissolia*, *Viciopsis*, *Neurolobus*, *Orobastrum*) (Asmussen & Liston, 1998; Badr *et al.*, 2002). Furthermore, Doğan *et al.*, (1992) determined 54 *Lathyrus* species grown in Turkey to nine sections based on forty morphological characters. They suggested that there are basically nine sections (*Orobos*, *Lathyrstylis*, *Aphaca*, *Nissolia*, *Orobon*, *Gorgonia*,

Table 1. Localities of investigated *Lathyrus* species

Species	(Davis <i>et al.</i> , 1970)	Kupicha (1983)	Doğan <i>et al.</i> , (1992)	Locality
<i>L. tukhtensis</i> Czecz.	<i>Platystylis</i>	<i>Lathyrrosthyllis</i>	<i>Lathyrrosthyllis</i>	Karaman, Ermenek Gülnar road, 1340 m
<i>L. cilicicus</i> Hayek&Siehe	“	<i>Lathyrrosthyllis</i>	<i>Lathyrrosthyllis</i>	Trabzon, Araklı, 1950 m.
<i>L. saxatilis</i> (Vent.) Vis.	<i>Orobastrum</i>	<i>Viciopsis</i>	<i>Nissolia</i>	Isparta, Eğirdir, edge of Kovada lake, 900 m
<i>L. annuus</i> L.	<i>Cicerula</i>	<i>Lathyrus</i>	<i>Lathyrus</i>	Mugla Osmaniye village, 500 m
<i>L. hierosolymitanus</i> Boiss.	“	<i>Lathyrus</i>	<i>Lathyrus</i>	Muğla, Marmaris, İçmeler-Bozburun road 3rd km, 450 m
<i>L. gorgoni</i> Parl. var. <i>gorgoni</i>	“	<i>Lathyrus</i>	<i>Gorgonia</i>	Antalya, Ünsallar district, around Prison, 40 m
<i>L. lycicus</i> Boiss.	“	<i>Lathyrus</i>	<i>Cicerula</i>	
<i>L. phaseitanus</i> Hubb.-Mor & Davis	“	<i>Lathyrus</i>	Not examined	Antalya, Kemer, Phaselis, 20m,
<i>L. odoratus</i> L.	<i>Lathyrus</i>	<i>Lathyrus</i>	Not examined	Muğla,Dalyan, İztuzu road, 10m,

Clymenum, *Cicerula* and *Lathyrus*) which are grouped in two subgenera, viz., subgenus *Lathyrus* and subgenus *Orobus* (Doğan *et al.*, 1992). In addition, Davis (1970) recognized 58 *Lathyrus* species from Turkey under ten sections viz., *Orobus*, *Platystylis*, *Pratensis*, *Lathyrus*, *Orobastrum*, *Cicerula*, *Clymenum* and *Aphaca*, in addition to monotypic sections *Orobon* and *Nissolia* in flora of Turkey (vol. III) (Table 1). In this study, SDS-PAGE it was used the as a tool for assessing genetic variation and determining the relationships among distinct species belonging to genus *Lathyrus* including three sections *Lathyrosthyllis* (*L. tukhtensis* and *L. cilicicus*), *Viciopsis* (monotypic section; *L. saxatilis*), *Lathyrus* (*L. annuus*, *L. hierosolymitanus*, *L. gorgoni*, *L. lycicus*, *L. phaselitanus* and *L. odoratus*). *Linearicarpus* and *Orobastrum* (monotypic section) (Kupicha, 1983). The differences among species were observed and all 9 taxa were clearly identifiable from the protein patterns. The total seed protein banding patterns of 9 taxa are illustrated in Fig. 1 and protein amounts of *Lathyrus* species were given Table 2.

The all studied taxa of genus *Lathyrus* cluster together on the basis of seed protein similarities as designed by previous morphological classification. The formed dendrogram from SDS-PAGE analysis showed that all studied taxa constituted two clusters (Fig. 2). The first one consisted of *L. saxatilis*, *L. gorgoni*, *L. annuus*, *L. hierosolymitanus*, *L. lycicus* and *L. phaselitanus* second one by *L. tukhtensis*, *L. cilicicus* and *L. odoratus*. In cluster I, *L. saxatilis* and *L. gorgoni* were found to have higher similarity to each other. Present study demonstrated that *L. saxatilis*, which is member of monotypic section *Viciopsis*, was gathered together with members of section *Lathyrus*. Davis (1970) indicated that *L. saxatilis* is a species of doubtful systematic position. Kupicha (1983) stated that *L. saxatilis* resembles the fruit of some members of the section *Lathyrus*. But findings of Doğan conflict with current results. Doğan *et al.*, (1992) indicated that *L. saxatilis* placed different cluster from elements of section *Lathyrus* (*L. annuus*, *L. hierosolymitanus*) and it was nested together with members of section *Nissolia*. Furthermore, present findings determined *L. gorgoni* grouped together with elements of section *Lathyrus* and section *Viciopsis* (*L. saxatilis*). It was found that *L. gorgoni* has exhibited closer similarity with *L. saxatilis* than members of section *Lathyrus*. Doğan *et al.*, (1992) proposed a new section *Gorgonia* to accomodate *L. gorgoni* in addition to *L. rotundifolius* and *L. undulatus*. But *L. gorgoni* has been placed in section *Lathyrus* by Kupicha (1983) and also it has been placed in section *Cicerula* by Davis *et al.*, (1970). Results of present study was supported by several other studies such as chloroplast DNA restriction site data (Asmussen & Liston, 1998), electrophoresis of seed storage proteins (El Shanshoury, 1997), AFLP data (Badr *et al.*, 2002), seed surface characters (Abu El Enain *et al.*, 2007) which reported that *L. gorgoni* has been clustered together with elements of section *Lathyrus*.

Table 2. Protein amounts of investigated *Lathyrus* species.

Taxa	Total protein amounts (µg/ml)
<i>L. tukhtensis</i>	69.674
<i>L. cilicicus</i>	59.860
<i>L. saxatilis</i>	65.209
<i>L. annuus</i>	75.395
<i>L. hierosolymitanus</i>	65.627
<i>L. gorgoni</i> var. <i>gorgoni</i>	75.279
<i>L. lycicus</i>	79.906
<i>L. phaselitanus</i>	73.395
<i>L. odoratus</i>	65.441

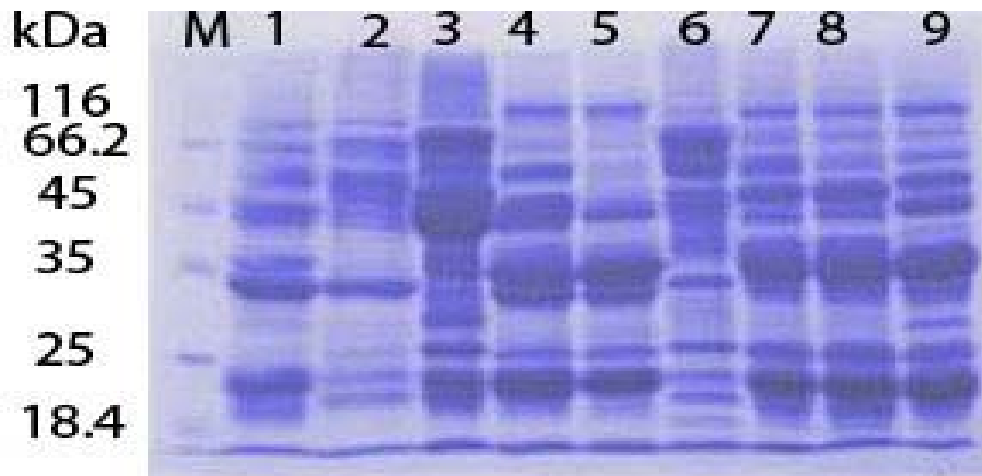


Fig. 1. SDS-PAGE of total seed proteins in studied *Lathyrus* taxa. M: Marker; 1: *L. tukhtensis*; 2: *L. cilicicus*; 3: *L. saxatilis*; 4: *L. annuus*; 5: *L. hierosolymitanus*; 6: *L. gorgoni*; 7: *L. lycicus*; 8: *L. phaselitanus*; 9: *L. odoratus*

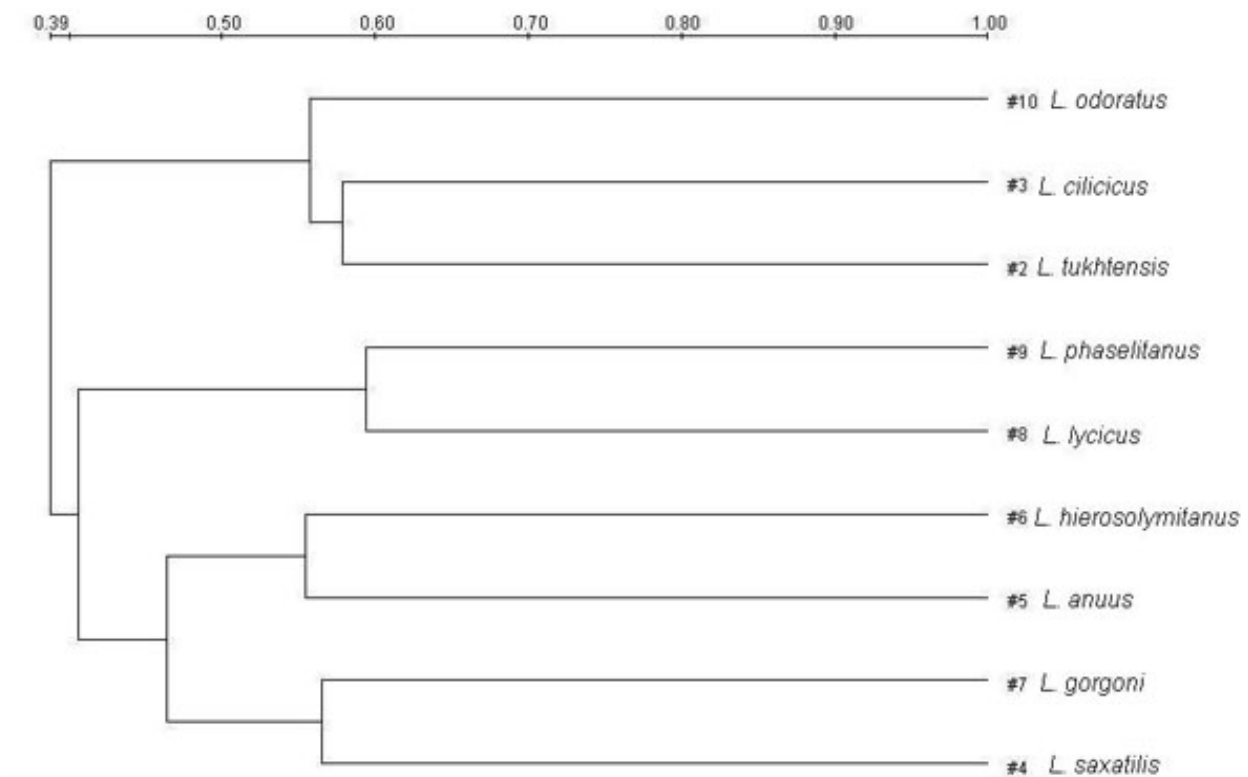


Fig. 2. Dendrogram of *Lathyrus* species based on total seed protein profiles.

Present study demonstrated that *L. annuus* and *L. hierosolymitanus* were closest each other. A study done by Asmussen & Liston (1998) demonstrated that *L. annuus* and *L. hierosolymitanus* exhibited close affinity each other based on chloroplast DNA. El-Shanshoury (1997) found that most of species from section *Cicerula* are separated into three groups according to findings obtained from electrophoresis of total seed proteins. Results of study done by El-Shanshoury (1997) showed that *L. annuus* and *L. hierosolymitanus* had close similarity. The affinity between some species of section *Lathyrus*, especially *L. annuus* and *L. hierosolymitanus*, is supported by both AFLP and isozyme data in addition to cpDNA restriction site data and cluster analysis of the seed

characters (Asmussen & Liston, 1998; Badr *et al.*, 2002; Abou-Ebu-El Enain *et al.*, 2007). Also, *L. lycicus* was clustered with *L. phaselitanus* when the results of cluster I were compared. Davis (1970) indicated that *L. phaselitanus* is allied to *L. Lycicus*. In cluster II, *L. tukhtensis* and *L. Cilicicus* were closer to each other. In addition, it was found that *L. odoratus*, placed in cluster II, was nested together with these two species. Also, it determined that quantities of total seed proteins in the present study (Table 2). *L. lycicus* (79.906 µg/ml) has highest total protein content whereas *L. cilicicus* (59.860 µg/ml) has lowest total protein content.

References

- Abu-El-Enain, M.M., M.H.A. Loufty and A.A. Shehta. 2007. Seed surface characters and their systematic significance in the genus *Lathyrus* (*Leguminosae*, *Papilionoideae*, *Vicieae*). *Feddes Reportorium*, 118(7-8): 269-285.
- Acık, L., M. Ekici and M. Celebi. 2004. Taxonomic relationships in *Astragalus* sections *Hololeuce* and *Synochreati* (*Fabaceae*): evidence from RAPD-PCR and SDS-PAGE of seed proteins. *Ann. Bot. Fennici*, 41: 305-317.
- Asmussen, C.B. and A. Liston. 1998. Chloroplast DNA characters, phylogeny, and classification of *Lathyrus* (*Fabaceae*). *American Journal of Botany*, 85(3): 387-401.
- Ayaz, F.A., H. Inceer and S. Hayirlioglu-Ayaz. 1999. An electrophoretic analysis of the seed proteins of some *Vicia* L. species from Northeast Anatolia (Turkey). *Pakistan Journal of Biological Sciences*, 2(4): 1139-1142.
- Badr, A., H. El Shazly, H. El Rabey and L.E. Watson 2002. Systematic relationships in *Lathyrus* sect. *Lathyrus* (*Fabaceae*) based on amplified fragment length polymorphism (AFLP) data. *Can. J. Bot.*, 80: 962-969.
- Brahim, N.B., A. Salhi, N. Chtourou, D. Combes and M. Marrakchi. 2002. Isozymic polymorphism and phylogeny of 10 *Lathyrus* species. *Genetic Resources and Crop Evolution*, 49: 427-436.
- Chtourou-Ghorbel, N., B. Lauga, D. Combes and M. Marrakchi. 2001. Comparative genetic diversity studies in the genus *Lathyrus* using RFLP and RAPD markers. *Lathyrus Lathyrism Newsletter*, 2: 62-68.
- Çelebi, A., L. Açık and Z. Aytaç. 2009. Biosystematics studies among *Ebenus* L. species based on morphological, RAPD-PCR and seed protein analyses in Turkey. *Pak. J. Bot.*, 41(5): 2477-2486.
- Davis, P.H. 1970. *Lathyrus* L. *Flora of Turkey and East Aegean Islands*, Vol.3. Edinburgh University Press, Edinburgh.
- Davis, P.H., R.R. Mill and T. Kit. 1988. *Flora of Turkey and East Aegean Islands*, Vol.10. Edinburgh University Press, Edinburgh.
- Doğan, M., A. Kence and C. Tıgın. 1992. Numerical taxonomic study on Turkish *Lathyrus* (*Leguminosae*). *Edinb J Bot.*, 49(3): 333-341.
- El-Shanshoury, A.R. 1997. The use of proteins revealed by SDS-PAGE in taxonomy and phylogeny of some *Lathyrus* species. *Biologia Plantarum*, 39(4): 553-559.
- Emre, İ., D. Turgut-Balık, H. Genç and A. Şahin. 2006. The use of seed proteins revealed by SDS-PAGE in taxonomy of some *Lathyrus* L., species grown in Turkey. *Pakistan Journal of Biological Sciences*, 9(12): 2358-2361.
- Emre, İ. 2007. Genetic diversity studies in the genus *Vicia* (Section *Vicia*) by using SDS-PAGE. International Symposium 7th. *Plant Life of South west Asia (7th PLoSWA)*, 25-29 June, Eskişehir/Turkey.
- Emre, İ., D. Turgut-Balık and A. Sahin. 2007a. Electrophoretic analysis of total protein profiles of some *Lathyrus* L. (Sect. *Cicerula*) Grown in Turkey. *Pakistan Journal of Biological Sciences*, 10(17): 2890-2894.
- Emre, İ., D. Turgut-Balık, A. Şahin and M. Kürşat. 2007b. Total electrophoretic band patterns of some *Onobrychis* species growing in Turkey. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 2(2): 123-126.

- Emre, İ., D. Balık-Turgut and A. Şahin. 2007c. Determination of total seed protein in patterns in some *Vicia* L., taxa by using sds-Page. *International Symposium 7th. Plant Life of South west Asia (7th PLoSWA)*, 25-29, June, Eskişehir/Turkey.
- Emre, İ. 2009. Electrophoretic analysis of some *Lathyrus* L. species based on seed storage proteins. *Genetic Resources and Crop Evolution*, 56: 31-38.
- Ertekin, S.A. 1994. A New Record for the Flora of Turkey. *Turk. J Bot.*, 18(1): 33-34.
- Ertekin, S.A. and O. Saya. 1990. A New Record for the Flora of Turkey. *Turk J Bot.*, 15(1): 75-77.
- Genç, H. and A. Şahin. 2008. A new species of *Lathyrus* L. (section *Cicercula*; Fabaceae) from Turkey. *Botanical Journal of Botany*, 158(2): 301-305.
- Genç, H. 2009. *Lathyrus nivalis* subsp. *sahinii* subsp. nov. (sect. *Platystylis*, Leguminosae) from Turkey. *Nordic Journal of Botany*, 27(5): 402-404.
- Guner, F. and N. Ozhatay. 2000. *Lathyrus* L., in Guner A, Ozhatay N, Ekim, T, Baser KHC. eds. *Flora of Turkey and the East Aegean Islands* vol. 11. Edinburgh University Press, Edinburgh.
- Hameed, A., T.M. Shah., B.M. Atta., N. Iqbal., M.A. Haq and H. Ali. 2009. Comparative seed storage protein profiling of Kabuli chickpea genotypes. *Pak. J. Bot.*, 41(2): 703-710.
- Javaid, A., A. Ghafoor and R. Anwar 2004. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pak. J. Bot.*, 36(1): 25-29.
- Jha, S.S. and D. Ohri. 2002. Comparative study of seed protein profiles in the genus *Pisum*. *Biologia Plantarum*, 45(4): 529-532.
- Kamel, E.A., H.Z. Hassan and S.M. Ahmed. 2003. Electrophoretic characterization and the relationship between some Egyptian Cruciferae. *Online Journal of Biological Sciences*, 3(9): 834-842.
- Ladizinsky, G. and T. Hymowitz. 1979. Seed protein electrophoresis in taxonomic and evolutionary studies:review. *Theor Appl Gene.*, 54: 145-151.
- Kupicha, F.K. 1983. The infrageneric structure of *Lathyrus*. *Notes RBG Edinburg*, 41(29): 209-244.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature*, 227: 680-685.
- Lewis, G., B. Schrire, B. Mackinder and M. Lock. 2005. Legumes of the world. *Royal Botanic Gardens*, Kew, UK.
- Maxted, N. and D.J. Goyder. 1988. A New Species of *Lathyrus* Sect. *Lathyrus* (Leguminosae, Papilionodeae) from Turkey. *Kew Bulletin*, 43(4): 711-714.
- Mirali, N., S. El-Khoury and F. Rizq. 2007. Genetic diversity and relationships in some *Vicia* species as determined by SDS-PAGE of seed proteins. *Biologia Plantarum*, 51(4): 660-666.
- Przybylska, J. and Z.Z. Przybylska. 1993. Electrophoretic seed albumin patterns in the cultivated Phaseolus species:albumin variants with trypsin inhibitory activity. *Genetica Polonica*, 34(1): 15-25.
- Przybylska, J. and Z.Z. Przybylska. 1997. Electrophoretic seed albumin patterns in *Vicia* species of sect. *Hypechusa* and *Peregrina* (Fabaceae). *Plt. Syst. Evol.*, 208: 239-248.
- Przybylska, J., Z.Z. Przybylska and P. Krajewski. 1999. Diversity of seed albumins in some *Lathyrus* species related to *L. sativus*:An electrophoretic study. *Genetic Resources and Crop Evolution*, 46(3): 261-266.
- Przybylska, J., Z.Z. Przybylska and P. Krajewski. 2000. Diversity of seed globulins in *Lathyrus sativus* L. and some related species. *Genetic Resources and Crop Evolution*, 47: 239-246.
- Vaz, C.A., C. Pinheiro, J.M.N. Martins and C.P.P. Ricardo. 2004. Cultivar discrimination of Portuguese *Lupinus albus* by seed protein electrophoresis:the importance of considering “glutelins” and glycoproteins. *Field Crops Research*, 87: 23-34.

(Received for publication 10 January 2008)