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The Electrophoretic Contribution to the Solutions of Taxonomical Problems of Some *Lathyrus* L. Taxa Growing in Turkey

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ABSTRACT

Background: The seed proteins are used as molecular markers to clarify the systematic problems. Also, electrophoretic techniques are safe tools to identify the seed proteins. In present study, it was used the SDS-PAGE technique to solve the taxonomical problems of eight taxa of genus *Lathyrus* belong to three sections *Orobus, Lathyrostylis* and *Pratensis* according to the globulin B and glutelin.

Methods: The seed materials were collected from natural habitats and 0.5 g seed were homogenized and centrifuged based on extraction procedure. In addition, the amounts of seed storage protein subfractions were determined by using protein assay. The electrophoretic analysis were performed according to the 12% SDS-PAGE. The gel documentation system (Bio-Rad, USA) was used to analyse the electrophoretic data and UPGAMA was used to construct the dendogram to show the relationships among the species under focus.

Results: Current study showed that the species of section *Pratensis* were different from species of sections *Orobus* and *Lathyrostylis.* The differences among the studied taxa shown clearly and all studied taxa were choosen from the protein patterns.. Furthermore, present study demonstrated that *L. nivalis* has the highest globulin B and glutelin. Results of the present study generally supported the morphological data.

Key words: Cluster analysis, Electrophoresis, *Lathyrus*, Seed storage proteins.

INTRODUCTION

Legumes are important protein resources for human consumption in the developing countries (Kafadar *et al.,* 2019). *Lathyrus* L., which is a legume, has more than 200 taxa and the main center of variety is the eastern Mediterrenean and Irano-Turanian regions (Allkin *et al.,* 1986; Genc and Sahin, 2011; Marghali *et al.,* 2016). The genus has 79 taxa in Turkey, 25 of whose are endemic (Davis *et al.,* 1988; Guner and Ozhatay 2000; Ozbek-Yazici *et al.,* 2019). Kupicha (1983) classified 153 *Lathyrus* into 12 or 13 sections by using morphological data. The studies in the literature generally accepted Kupicha's classification as a reference and molecular studies confirmed this classification (Marghali *et al.,* 2014; Cildir *et al.,* 2017).

Different techniques including isozyme analysis (Sammour *et al*., 2019), fatty acid analysis (Bagci *et al.,* 2001; Arslan, 2017), electrophoresis (El-Shanshoury, 1997; Przybylska *et al.,* 2000; Ben Brahim *et al.,* 2002; Radwan *et al.,* 2013), RFLP, AFLP (Badr *et al.,* 2002; Belaid *et al.,* 2006) and RAPD techniques (Chtourou-Ghorbel *et al.,* 2001, 2002; Akter *et al.,* 2015) are used to classify the *Lathyrus* taxa as well as morphological characters. Seed storage proteins are significant biochemical markers because they are less affected by the environment and they are used in genetic variation studies and to characterize cultivars (Sofailan *et al.,* 2015). SDS-PAGE is also a powerful tool for the distinguishing seed proteins to solve the taxonomical problems (Ladizinsky and Hymowitz, 1979; Nikolic *et al.,* 2012). The reason for this is electrophoresis is a practical and reliable process for the genetic studies and it provides some superiorities compared to morphological techniques for the identifications of species (Sinha *et al.,* 2012; Sammour *et al.,* 2019). Present study demonstrated the relationships among the *Lathyrus* taxa comprising *L. aureus, L. brachypterus* var. *brachypterus, L. nivalis, L. digitatus, L. spathulatus, L. boissieri, L. pratensis, L. laxiflorus* subsp. *laxiflorus* from three sections of *Orobus, Lathyrostylis* and *Pratensis* in Turkey by using globulin B and glutelins patterns.

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MATERIALS AND METHODS

Dry seeds of eight *Lathyrus* species belong to three sections namely *Orobus, Lathyrostylis* and *Pratensis* (Kupicha 1983)were gathered from different localities of Turkey (Table 1) in 2004 and 2005. The laboratory studies were done in Firat University, Faculty of Science and Arts, Department of Biology Molecular Biology laboratory.

Seed proteins were obtained based on Vaz *et al.* (2004)' method. 0.5 g seed materials were grounded and homogenized with water, 5% (w/v) NaCl, 7% ethanol and 0.25% (w/v) NaOH, respectively. At first, the seed materials were centrifuged at 7800 g after the material was extracted with distilled water for 2 hours. The pellet was used to globulin B and glutelins in the next steps. Secondly, the original pellet was treated with 5% (w/v) NaCl for 2 hours and centrifugated at 7800 g for 15 minutes to take globulin B fractions in the supernatants. Later, the pellet was extracted with 75% ethanol (v/v) for 2 hours and centrifuged at 7800 g for 15 minutes to take the prolamins in the supernant. Lastly, the pellet was extracted with 0.25% (w/v) NaOH for 2 hours and suspension was centrifuged at 7800 g for 15 minutes to take glutelins in the supernatant. The samples of protein subfraction were deposited at -20°C until the electrophoretic analysis. The amounts of protein subfraction were measured by using Bio-Rad DC protein assay (Bio-Rad Laboratories, UK) and Fermentas [SMO431; (116.0 kDa (kilodalton), 66.2 kDa, 45 kDa, 35 kDa, 25 kDa, 18.4 kDa)] was used as a marker because the prolamin amounts were quantified lowest and the electrophoretic analysis of prolamins was not performed. The equal amount of protein subfractions was loaded on 12% SDS-PAGE (Laemmli, 1970). Electrophoretic analysis was conducted at 20 mA by using Protean II Electrophoresis Cell (Bio-Rad Laboratories, UK). The gels were stained using Coomassie Brilliant Blue (Sigma Aldrich Chemie, Germany) for 30 min. at 67°C and destained in destaining solution for 3-4 h at 67°C to view the protein bands.

Statistical analysis

A gel documentation system (Bio-Rad, USA) and Quantity 1-D analysis software were used to evaluate the electrophoretic results. The dendogram was formed with 4.0 % tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean).

RESULTS AND DISCUSSION

The electrophoretic results of globulin B and glutelin are given in Fig 1 and 2, respectively. The differences among the studied taxa were clearly observed and the present study demonstrated that they have distinct relationships (Fig 3 and 4). A relationship was observed between *L. digitatus* and *L. brachypterus* var. *brachypterus* (61.0 % similarity) and also *L. nivalis* demonstrated a high relationship with *L. boissieri* (50.0 % similarity) in first cluster according to the globulin B band profiles (Fig 3). In addition, *L. spathulatus* has less homology within studied five species of section *Lathyrostylis* and it is placed outer part of cluster I. *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus* (59.6 % similarity) also, are located in the second cluster. But *L. aureus,* which is a member of section *Orobus*, remained out of the clusters (Fig 3).

On the other hand, results of glutelin electrophoretic bands showed that studied taxa have been clustered in two clusters (Fig 4). In the cluster I, *L. brachypterus* var. *brachypterus* demonstrated a close similarity with *L. nivalis* (54.9% similarity) and also *L. boissieri* was associated with these two taxa (42.2% similarity for *L. brachypterus* var. *brachypterus* and 53.2% similarity for *L. nivalis*). *L. digitatus* and *L. spathulatus* also exhibited close similarity (49.5% similarity) in cluster I. Additionally, *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus* were placed in cluster II. However, *L. aureus* was located out of clusters (Fig 4). This study showed at the same time that globulin B amounts are higher than prolamin and glutelin amounts. *L. nivalis* has the highest globulin B amount (5.640 µg/ml) and glutelin amounts (4.219 µg/ml) while *L. boissieri* has the lowest globulin B amount (3.625 µg/ml) and glutelin amounts (2.227 µg/ml). The present study also found that the prolamin amounts of *Lathyrus* taxa were the lowest (Table 2).

Section *Orobus*

Kupicha (1983) suggests that a few species which are included in section *Orobus* (here represented by *L. aureus*) are rather similar to the members of section *Lathyrostylis.* But results of present study showed the globulin B and glutelin band patterns of *L. aureus* (member of section

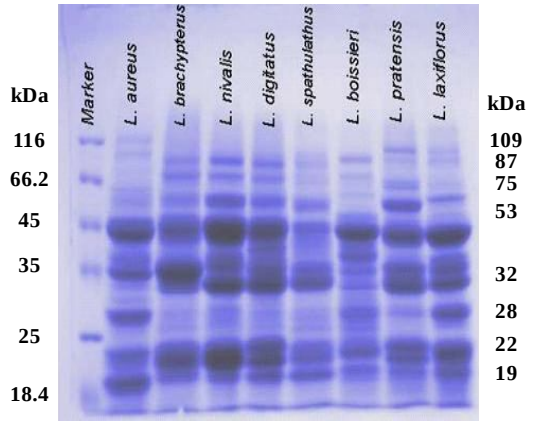


Fig 1: Electrophoretic band profiles of globulin B proteins of the studied taxa

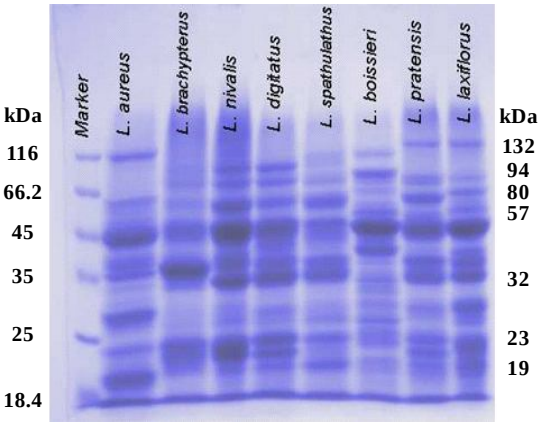


Fig 2: Electrophoretic band profiles of glutelin proteins of the studied taxa.

Table 1: Localities of the investigated *Lathyrus* taxa.

Section	Taxa	Locality	Herbarium no
Orobus	*Lathyrus aureus* (Stev.) Brandza	C3, Isparta Yukarıgökdere village, 1020 m	1100
Lathyrostylis	*L. brachypterus* Cel. var. *brachypterus* Cel.	B5, Kayseri Erciyes mountain edge, 1900 m	1110
”	*L. nivalis* Hand. Mazz. subsp.*sahinii* H. Genc	C4, Karaman Karadağ mountain edge, 1700 m	1120
”	*L. digitatus* (Bieb). Fiori	C3, Isparta, Eğirdir Kovada lake near, 900 m	1130
”	*L. spathulatus* Cel	C3, Konya Akşehir Cankurtaran village, open Pinus Forest, 1550 m	1140
”	*L. boissieri* Sirj	B7, Elazığ, Harput İnceler village 1380 m.	1150
Pratensis	*L. pratensis* L.	C3, Konya Beyþehir Gölyaka, Kubad-ı Abad Palace around, 1230 m	1160
”	*L. laxiflorus* (Desf.) O. Kuntze. subsp. *laxiflorus* Desf.	C3, Isparta, Pınargözü road, 1800 m	1170

Table 2: Protein amounts of the investigated *Lathyrus* taxa (µg/ml).

Taxa	Globulin B	Prolamin	Glutelin
L. aureus (Stev.) Brandza	4.476	0.632	2.643
L. brachypterus Cel. var. *brachypterus* Cel.	3.971	0.270	2.922
L. nivalis Hand.-Mazz. subsp.*sahinii* H. Genc	5.640	0.208	4.291
L. digitatus (Bieb). Fiori	5.159	0.365	3.124
L. spathulatus Cel	3.609	0.295	2.774
L. boissieri Sirj	3.625	0.607	2.227
L. pratensis L.	3.679	0.533	2.499
L. laxiflorus (Desf.) O. Kuntze. subsp. *laxiflorus* Desf.	4.180	0.389	3.235

Orobus) to be distinct from the storage band patterns of the members in section *Lathyrostylis* (five taxa) and *Pratensis* (two taxa). *L. aureus* has 19, 22, 28, 35, 45 and 28 kDa globulin B bandings and the globulin band profile of *L. aureus* with 28 kDa is common with *L. laxiflorus*. The 32 kDa glutelin band profiles of *L. aureus* is also,less dense than other taxa (Fig 1 and 2). Thus, *L. aureus* is placed outer part of the clusters I (including five members of section *Lathyrostylis* studied herein) and II (consisting the studied two members of section *Pratensis*) according to the globulin B and glutelin (Fig 3 and 4). The phylogenetic of hypothesis which is derived from molecular characters presented in the study of Asmussen and Liston (1998) suggested that Kupicha's section *Orobus* is monophyletic. However, Abou-El-Enain *et al.* (2007) claimed that the members of section *Orobus* in the study (comprising *L. aureus*) are gathered with members of section *Pratensis* (including *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus*) (Abou-El-Enain *et al.,* 2007). Furthermore, the results of the present study disagree with those of Kenicer *et al.* (2005) for the reason that the authors place all the members of section *Orobus* (comprise *L. aureus*) in a clade with *L. aphaca* and *L. laxiflorus* (Kenicer *et al.,* 2005).

Section *Lathyrostylis*

Members of *Lathyrostylis* share many characteristics with some species in section *Orobus*. Several researchers treat them as a part of a wider section *Orobus* (Czefranova, 1971; Kenicer *et al.,* 2005). However, Bassler (1966) suggested the two sections to be different (Abou-El-Enain *et al.,* 2007). Most of the following works concur with this delimitation (Davis *et al.,* 1970; Dogan *et al.,* 1992; Kenicer *et al.,* 2005; Abou-El-Enain, 2007). This study showed that the members of section *Lathyrostylis* have common globulin B band profiles. But, 45 kDa globulin B banding of *L. spathulatus* and 32 and 35 kDa globulin B banding of *L. boissieri* are less dense than other taxa (Fig 1 and 2). The findings of the present study also indicate that the two sections are distinct and the members of section *Lathyrostylis* (*L. brachypterus* var. *brachypterus*, *L. nivalis, L. digitatus, L. spathulatus* and *L. boissieri*) are grouped together (Fig 3 and 4). Similarly, Oskoueiyan *et al.* (2014) found that *L. aureus* is placed in different clusters from species of *Lathyrostylis*. In addition, Oskoueiyan *et al.* (2014) suggested that *L. digitatus, L. boissieri* and *L. brachypterus* are clustered in the same group. On the other hand, Davis (1970) indicated that *L. nivalis* is superficially close to *L. spathulatus*. Electrophoretic data presented here suggested that glutelin (49.3% similarity) band patterns of *L. nivalis* are similar to *L. spathulatus* but similarity of globulin B (24.1% similarity) band patterns of afore--mentioned species are low (Fig 3 and 4). However, Gunes (2012) indicated that *L. nivalis* and *L. spathulatus* have different seed morphologies.

Section *Pratensis*

Kupicha (1983) indicated that the members of section *Pratensis* (here represented by *L. pratensis* and *L. laxiflorus*

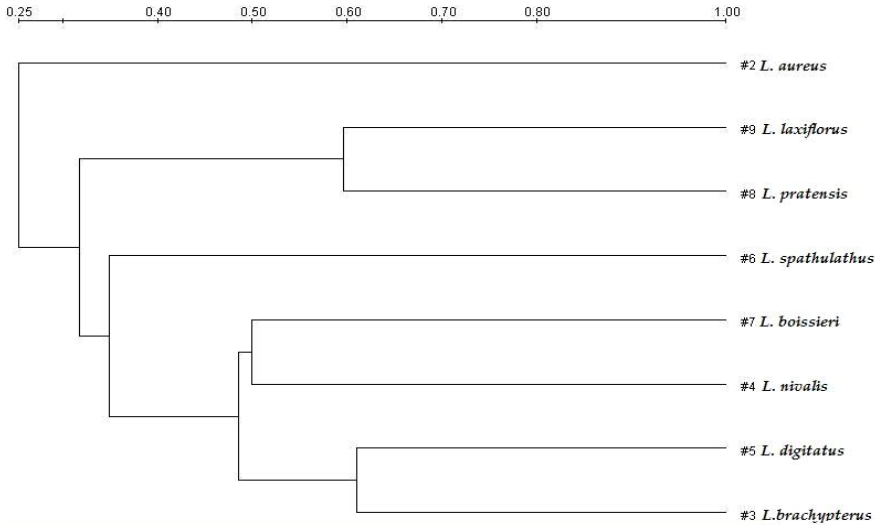


Fig 3: Dendogram of *Lathyrus* taxa based on seed globulin B profiles.

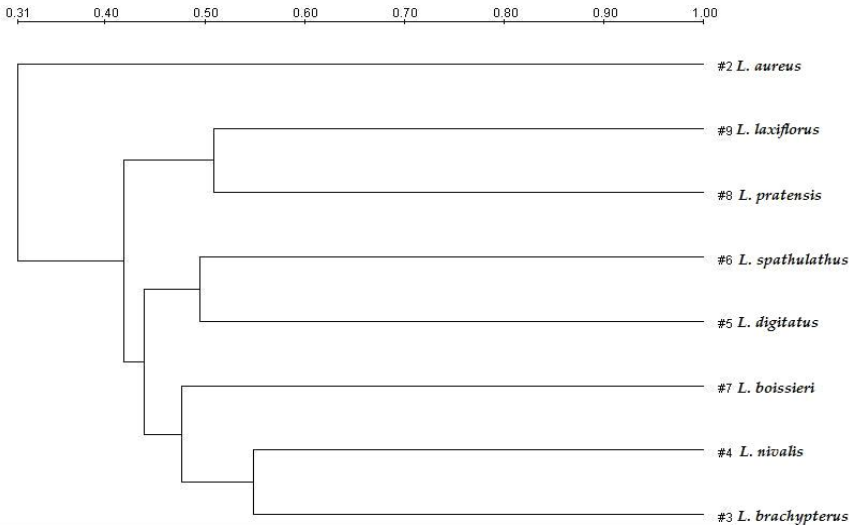


Fig 4: Dendogram of *Lathyrus* taxa based on seed glutelin profiles.

subsp. *laxiflorus*) form a well-defined group, sharing several distinctive characteristics. Current study demonstrated that the 28 kDa band pattern of *L. pratensis* is less dense than *L. laxiflorus.* This study also showed that *L. pratensis* and *L. laxiflorus* have similar globulin B and glutelin band patterns (Fig 1 and 2). Findings of the present study showed that the members of section *Pratensis are* clearly clustered distinctly from both species of sections *Orobus* and *Lathyrostylis* as the electrophoretic band patterns reveal (Fig 3 and 4). These results well agreed with the studies rely on chloroplast DNA characters (Asmussen and Liston, 1998), seed surface characters (Abou-El-Enain *et al.,* 2007) and petal micromorphology (Cildir *et al.,* 2012). Similarly, Emre *et al.* (2006) suggested that *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus* have a close affinity based on electrophoretic analysis of total seed proteins. Nevertheless, the findings of present study contrast with Dogan *et al.* (1992)'s morphological data; Dogan *et al.* (1992) indicated that *L. pratensis* and *L. laxiflorus* subsp. *laxiflorus* are located in different subgroups in cluster analysis.

CONCLUSION

Results of present study showed that the seed storage band patterns of *L. aureus,* which is a member of section *Orobus,* are distinct from the storage band patterns of the members in section *Lathyrostylis.* Thus, findings of present study have suggested that the two sections (section *Orobus* and *Lathyrostylis*) are distinct and the members of the section *Lathyrostylis* (*L. brachypterus* var. *brachypterus*, *L. nivalis, L. digitatus, L. spathulatus* and *L. boissieri*) are grouped together. Furthermore, the findings of the present study revealed that the members of the section *Pratensis* have *a* clearly distinct cluster from both species of sections *Orobus*

and *Lathyrostylis* based on all electrophoretic band patterns. On the other hand, this study showed that *L. nivalis* has the highest globulin B and glutelin amounts and it has been found that the prolamin amounts of the studied taxa are the lowest.

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