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Total Electrophoretic Band Patterns of Some Onobrychis Species Growing in Turkey

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Abstract: The aim of this study was to investigate interspecific variations in Sections Lophobrychis, Onobrychis, Hymenobrychis of genus Onobrychis by sodium dodecyl sulphate polyacrilamyde gel electrophoresis (SDS-PAGE) technique. In this study total eight species collected from different regions of Turkey have been studied for the analysis of seed storage protein profiles to examine their relationship. Electrophoretic data were documented by using a gel documentation system and analysed by using Quantity 1-D analysis software and also the dendogram were formed in UPGAMA. Studied species of genus Onobrychis sections Lophobrychis, Onobrychis, Hymenobrychis cluster together on the basis of seed protein similarities as designed by previous morphological classification. The formed dendogram from SDS-PAGE analysis showed that all studied species constituted two clusters. The first one consisted of *O. caput-galli, O. aequidendata, O. fallax, O. armena, O. viciifolia* and second one by *O. hypargyrea, O. galegifolia* and *O. cappadocia*. Protein amounts of all species were also found to be between 49.864-56.966 μg ml^{-1}.

Key words: *Onobrychis* · SDS-PAGE · total protein · legume seeds · similarity matrix · UPGAMA

INTRODUCTION

The genus *Onobrychis* Miller extends from the mediterranean region to Central Asia. The majority of the species are restiricted to North-west Asia, especially Iran and Anatolia, making this area the main centre of genetic diversity of the genus [1]. Many species are exploited as high protein fodder plants for several animals and they play an important role in enrichment of soil, increasing the nutritive value of drought-resistant pasture [2].

Onobrychis an extremely difficult genus with many of the worst problems in Anatolia-one of the main centres of the genus [3]. The taxonomy of the genus continues to be a subject of much confusion, mainly because of the different approaches to species delimitation, resulting in varying numbers of recognized species [4]. Recently Yildiz *et al.* [1] suggested based on fruit morphology genus *Onobrychis* consist of 170 species, eight sections and two subgenera. In Flora of Turkey (volume 3) many of the species dealth with cannot be defined or keyed out satisfactory and numerous taxonomic problems throughtout the genus await solution and genus is represented with 54 species and divided into five sections [3-5]. Grain legumes are widely recognized as important sources of food and feed proteins. In many regions of the world, legume seeds are the unique supply of protein in the diet [6]. Legume seeds have a high level of protein content, ranging from approximately 200 to 400 g kg^{-1} as compared to other sources of plant proteins [7]. Legume seeds are highly stable, unaffected by environmental conditions. Therefore electrophoretic techniques for total seed protein analysis have been recognized as a valid source of taxonomic evidences and were used to adress taxonomic relationships at the generic and specific levels [8]. In literatures, most limited studies have been performed on the genus *Onobrychis* and as far as the literature search show electrophoretic analysis and protein amounts of species (exclude from *O. viciifolia)* from genus *Onobrychis* examined in the present study has not yet been investigated.

The objective of the present study was to investigate interspecific variations in Sections Lophobrychis, Onobrychis, Hymenobrychis by sodium dodecyl sulphate polyacrilamyde gel electrophoresis (SDS-PAGE)

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Table 1: Localities of investigated *Onobrychis* species

Species	Section	Province	Locality
O. caput-galli (L.) Lam.	Lophobrychis	Manisa	Near to Kula dam lake
O. aequidentata (Sibth. and Sm.) d'Urv	"	Manisa	Salihli
O. fallax Freyn and Sint.	Onobrychis	Elazig	University campus
O. armena Boiss. and Huet	"	Usak	Usak-Akarca road
O. viciifolia Scop.	"	Usak	Banaz
O. hypargyrea Boiss.	Hymenobrychis	Gediz	Gediz-Simav road
O. galegifolia Boiss.	"	Elazig	Harput
O. cappadocia Boiss.	"	Elazig	University campus

technique. In this study total eight species 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.

MATERIALS AND METHODS

Dry seeds of *Onobrychis* species 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 and Ohri [9]. 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 equal amount of each sample was loaded on to the 12% SDS-PAGE [10]. 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 dendogram were formed with 4.0% tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean). Also, similarity matrix was constructed by Dice coefficient using Quantity 1-D (Bio-Rad) and expressed as percentages.

RESULTS AND DISCUSSION

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* [11], genus *Lupin* [12], genus *Pisum* [9], genus *Phaseolus* [13], genus *Lathyrus* [14-16]. The differences among species were observed and all eight species were clearly identifiable from the protein patterns. The total seed protein banding patterns of eight species were illustrated in Fig. 1. Additionally, protein amounts of studied species were given in Table 2 and also similarity matrix were given Table 3. Protein amounts of all species were also found to be between 49.864-56.966 μg ml^{-1}.

The all studied species of genus Onobrychis sections Lophobrychis, Onobrychis, Hymenobrychis cluster together on the basis of seed protein similarities as designed by previous morphological classification. The formed dendogram from SDS-PAGE analysis showed that all studied species constituted two clusters (Fig. 2). The first one consisted of *O. caput-galli*, *O. aequidendata*, *O. fallax*, *O. armena*, *O. viciifolia*

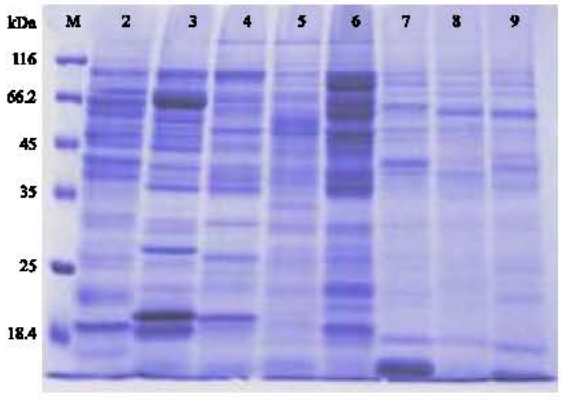

Fig. 1: SDS-PAGE of total seed proteins in eight taxa. M: Marker; 1: *O. caput-galli*; 2: *O. aequidentata*; 3: *O. fallax*; 4:*O. armena*; 5: *O. viciifolia*; 6: *O. hypargyrea*; 7: *O. galegifolia* 8: *O. cappadocia*

Table 2: Protein amounts of investigated *Onobrychis* species

Species	Protein amounts (μg ml^{-1})
O. caput-galli (L.) Lam.	56.966
O. aequidentata (Sibth. and Sm.) d'Urv	54.294
O. fallax Freyn and Sint.	51.251
O. armena Boiss. and Huet	54.531
O. viciifolia Scop.	54.971
O. hypargyrea Boiss.	53.178
O. galegifolia Boiss.	49.864
O. cappadocia Boiss.	51.724

Table 3: Similarity matrix of investigated *Onobrychis* species

	2	3	4	5	6	7	8	9
2	100.0	48.2	37.2	37.8	45.8	34.7	35.7	34.2
3	48.2	100.0	29.7	47.5	47.3	33.9	22.8	25.0
4	37.2	29.7	100.0	44.2	49.4	47.0	42.3	46.4
5	37.8	47.5	44.2	100.0	58.3	39.9	32.5	27.1
6	45.8	47.3	49.4	58.3	100.0	43.6	36.8	33.8
7	34.7	33.9	47.0	39.9	43.6	100.0	54.8	55.0
8	35.7	22.8	42.3	32.5	36.8	54.8	100.0	62.9
9	34.2	25.0	46.4	27.1	33.8	55.0	62.9	100.0

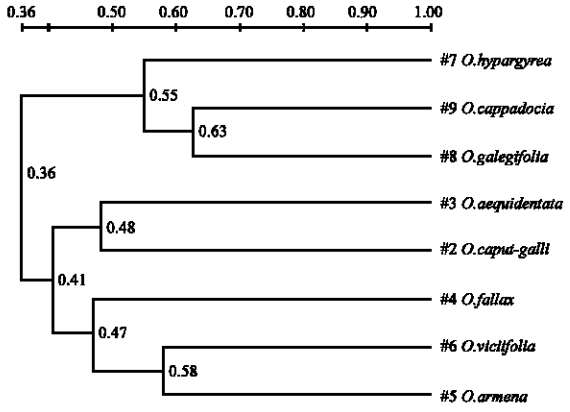

Fig. 2: Dendogram of *Onobrychis* species based on total seed protein profiles

second one by *O. hypargyrea*, *O. galegifolia and O. cappadocia*. *O. armena* and *O. viciifolia* found to have higher similarity to each other than *O. fallax* when the results of cluster I were compared. Also *O. caputgalli* and *O. aequidendata* which are the members of section Lophobrycis closer to each other rather than the other members of cluster I. Similarly, in cluster II, *O. galegifolia* and *O.cappadocia* closer to each other than *O. hypargyrea* which is partly differ from these two species of cluster II.

In a study chromosomal criteria and phylogenetic implications in the genus *Onobrychis* done by Abou-El-Enain [2] revealed that two species (*O. caput-galli* and *O. aequidendata*) from section Lophobrychis, it can be concluded that the recorded variation in chromosome numbers in each of *O. caput-galli* and *O. aequidendata*, can be referred to the differences in their taxonomic delimitation, at least at the subspecies level. Abou-El-Enain [2] also suggested that section Lophobrychis has a comparatively highly derived organization and can be considered as a heterogenous unit in the genus *Onobrychis*. But results from our present study showed that *O. caput-galli* and *O. aequidendata* have similar total band profiles especially between 116 and 35 kDa. Therefore, 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 RAPD studies.

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