Pak. J. Bot., 43(3): 1429-1432, 2011.

DETERMINATION OF GENETIC DIVERSITY IN THE *VICIA* L. (SECTION *VICIA*) BY USING SDS-PAGE

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Abstract

The interspecific and intraspecific variations in some taxa of *Vicia* is explained by SDS-PAGE method. In this study, nine *Vicia* L. Taxa, collected from different regions of Turkey have been studied for the analysis of seed storage protein profiles to examine their relationship. The differences among species were observed and all nine taxa were clearly identifiable from the protein patterns. Electrophoretic data were documented by using a gel documentation system (Bio-Rad, USA) and analysed by using Quantity 1-D analysis software and a dendogram has constructed with 4.0 % tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean). The dendogram from SDS-PAGE analysis showed that all studied taxa constituted two clusters. The first one consisted of *V. noeana* var. *noeana, V. noeana* var. *megalodonta, V. truncatula, V. peregrina, V. michauxii* and *V. grandiflora* second one by *V. mollis, V. hybrida*, and *V. assyriaca.* Present results showed that all studied species have similar total protein content. But *V. truncatula* (86.837 µg/ml) and *V. hybrida* (83.209 µg/ml) have highest total protein content. Whereas *V. grandiflora* (65.860 µg/ml) has low total protein content.

Introduction

Vicia L. comprises about 210 species, is recognised 22 sections in two subgenera (*Vicia* and *Vicilla*), widely distributed along regions of Europe, Asia and the American regions (Kupicha, 1976; Weber & Schifino-Wittmann, 1999). The Mediterranean area is its principal centre of diversification (Naranjo *et al.,* 1998). In Turkey, 64 species, 22 subspecies and 18 varieties of this genus have been recorded (Davis, 1970; Davis *et al.,* 1988; Vural, 2000; Inceer & Ayaz, 2005).

Vicia species are morphologically diversified and the morphological approach is rather difficult to account for the entire genetic variation existing in the *Vicia* (Haider & El-Shanshoury, 2000). Therefore, electrophoretic analysis of seed storage proteins were used in investigating genetic diversity and evaluation of taxonomic and genetic associations in the *Vicia* and numerous other plants at generic, specific and intra-specific levels, in addition to morphological characters (Ladizinsky & Hymowitz, 1979; Mirali *et al*., 2007; Çelebi *et al.,* 2009; Hameed *et al.,* 2009; Emre *et al.,* 2010). The objective of the present study was to investigate interspecific variations in section *Vicia* by sodium dodecyl sulphate polyacrilamyde gel electrophoresis (SDS-PAGE) technique.

Materials and Methods

Dry seeds of *Vicia* species (*Vicia truncatula, Vicia assyriaca, Vicia noeana* var. *noeana, Vicia noeana* var. *megalodonta, Vicia peregrina, Vicia michauxii, Vicia mollis, Vicia hybrida, Vicia grandiflora* var. *grandiflora*) were collected from various areas of Turkey (Fig. 1). 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 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) were used as marker. The samples were boiled for 5 minutes prior to loading. 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 dendogram was constructed with 4.0 % tolerance in UPGAMA (Unweighed Pair-Group Arithmetic Mean).

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 *Astragalus, Lathyrus, Vicia, Onobrychis* (Ayaz *et al.,* 1999; Przybylska *et al.,* 2000; Acık *et al.,* 2004; Beyazbenli, 2006; Emre *et al.,* 2007). The differences among species were observed and all nine taxa were clearly identifiable from the protein patterns. The total seed protein banding patterns of nine taxa were illustrated in Fig. 2 and protein amounts of *Vicia* species studied were given Table 2.

The all studied taxa of section *Vicia* 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 taxa constituted two clusters (Fig. 3). The first one consisted of *V. noeana* var. *noeana, V. noeana* var. *megalodonta, V. truncatula, V. peregrina, V. michauxii* and *V. grandiflora* second one by *V. mollis, V. hybrida*, and *V. assyriaca.* In cluster I, *V. peregrina* and *V. michauxii* found to have higher similarity to each other (53%) than *V. grandiflora* when the results of cluster I were compared. It was reported that *V. peregrina* and *V. michauxii* have sometimes been confused (Davis,1970). But Şahin & Babaç (1990) demonstrated that *V. peregrina* different from that of *V. michauxii* in terms of karyological data. In previous studies, the isozyme cladogram and morphological characters showed that *V. peregrina* linked *V. michauxii* (Jaaska, 1997; Leht & Jaaska, 2002; Jaaska & Leht, 2007). On the contrary, a previous study revealed that *V. michauxii* and *V. peregrina* differed by seeed albumin patterns and total protein band profiles (El-Shanshoury & Soliman, 1996; Przybylska & Zimniak-Przybylska, 1997). On the other hand, it was reported that *V. hybrida* showed low similarity with *V. peregrina* and *V. michauxii* and it was placed in another cluster based on isozyme and total protein band patterns (El-Shanshoury & Soliman, 1996; Jaaska, 1997). In addition, Ayaz *et al*. (1999)

found that total protein profiles of *V. hybrida* were different from protein profiles of *V. peregrina* especially 45 kDa, 29 kDa, 20 kDa, 14 kDa protein bands. Also, *V. noeana* var. *noeana* and *V. noeana* var. *megablandato* closer to each other (66%) rather than the other member of cluster I (*V. truncatula)*. Isozyme and morphological results obtained from Jaaska & Leht (2007) revealed that *V. noeana* was clustered together with *V. michauxii* and *V. peregrina.* On the other hand, Davis (1970) indicated that *V. assyriaca* close to *V. noeana*, and connected to it by transitional forms. Whereas present results showed that electrophoretic band patterns of *V. assyriaca* different from that of *V. noeana.* In cluster II, *V. mollis* and *V. hybrida* closer to each other (51%) than *V. assyriaca* which is partly differ from these two species of cluster II. Jaaska & Leht (2007) demonstrated that *V. mollis* and *V. hybrida* are placed in one cluster based on isozyme characters. Whereas the morphology tree show them apart (Jaaska & Leht 2007). Also, it was determined quantities of total seed proteins in the present study (table 2). Present results showed studied species have similar total protein content. But *V. truncatula* (86.837 µg/ml) and *V. hybrida* (83.209 µg/ml) have highest total protein content. Whereas *V. grandiflora* (65.860 µg/ml) has low total protein content.

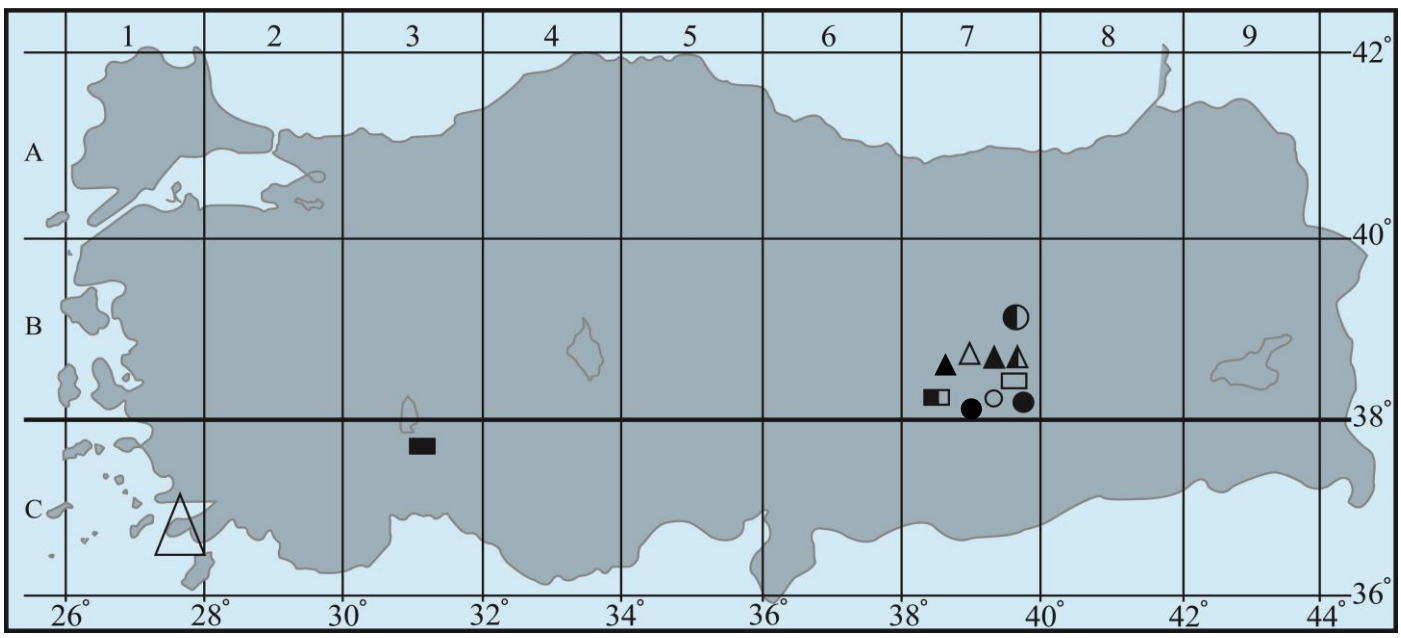

Fig. 1. Geographic locations of investigated *Vicia. Vicia truncatula* (■); *Vicia assyriaca* (●); *Vicia noena* var. *noeana* (▲); *Vicia noena* var. *megalodonta* (○); *Vicia peregrina* (△); *Vicia michauxii* var. *Stenophylla* (▭); *Vicia mollis* (▲); *Vicia hybrida* (◧); *Vicia grandiflora* var. *grandiflora* (◧).

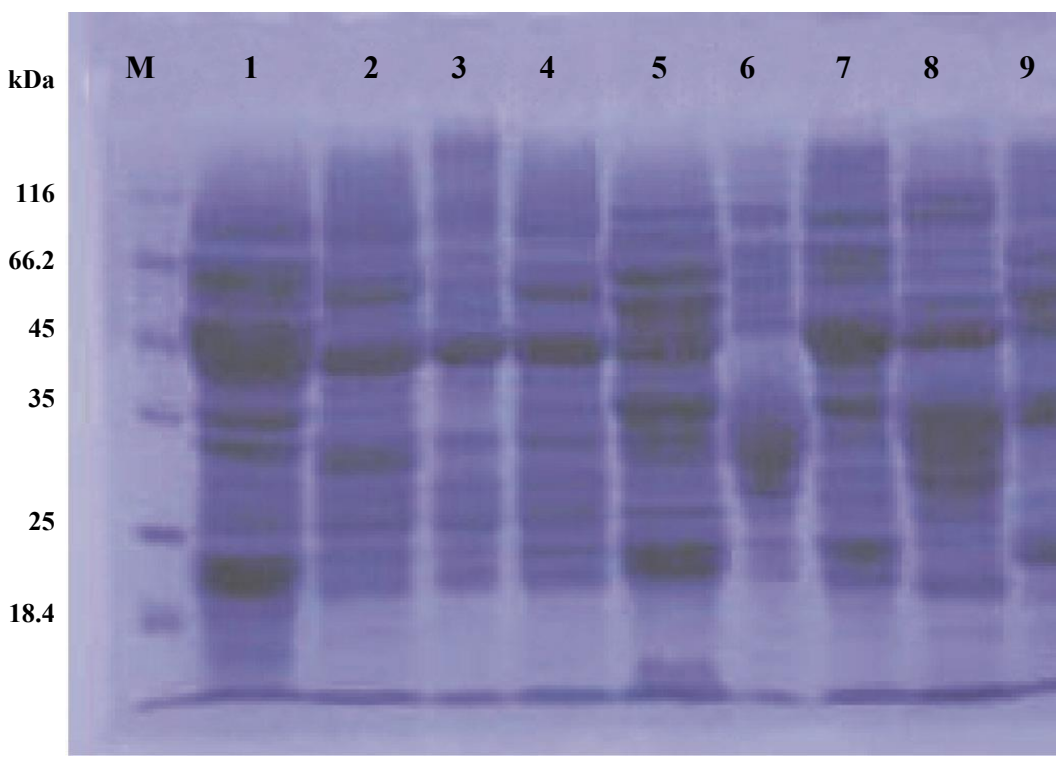

Fig. 2. SDS-PAGE of total seed proteins in nine taxa. M: *Marker;* 1: *Vicia truncatula;* 2: *Vicia assyriaca;* 3: *Vicia noeana* var. *noeana;* 4: *Vicia noeana* var. *megalodonta;* 5: *Vicia peregrina;* 6: *Vicia michauxii;* 7: *Vicia mollis;* 8: *Vicia hybrid;* 9: *Vicia grandiflora* var. *Grandiflora.*

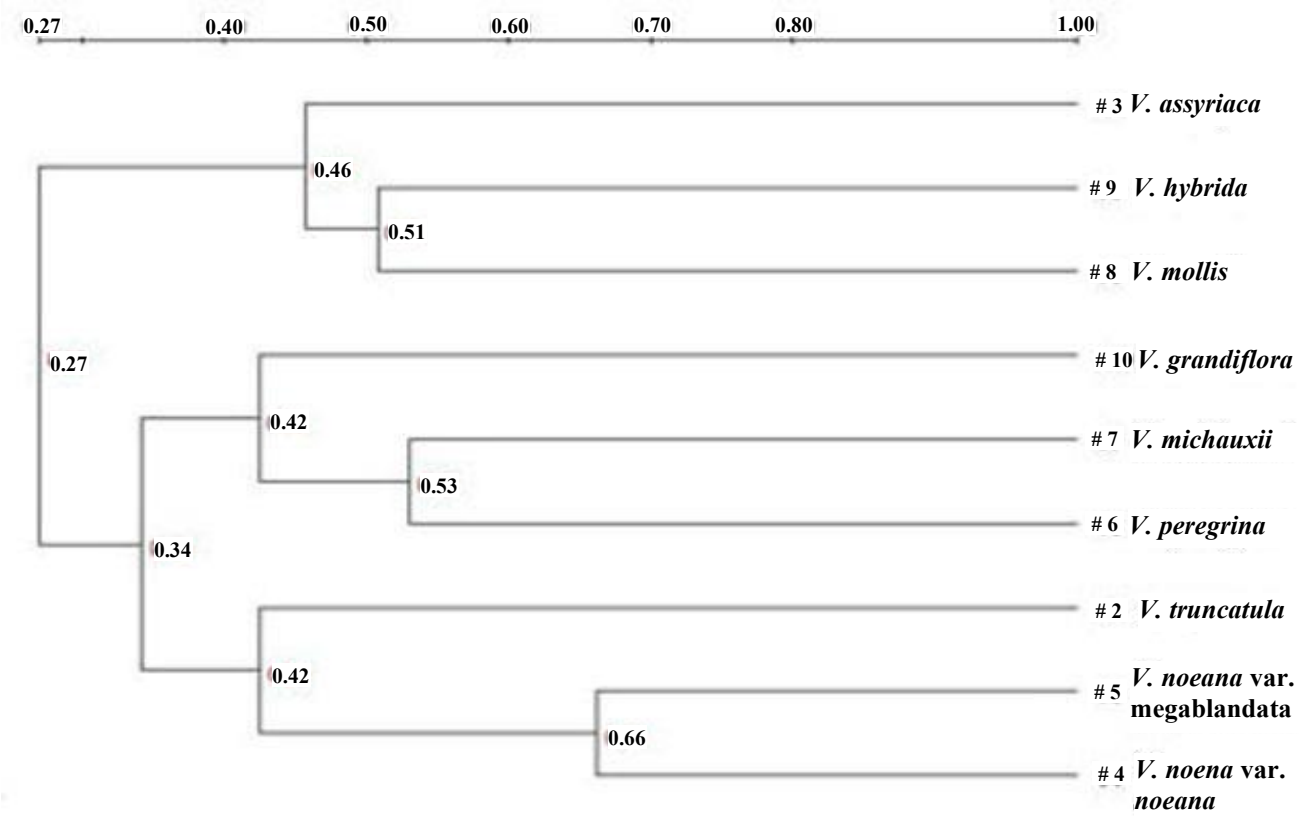

Fig. 3. Dendogram of *Vicia* taxa based on total seed protein profiles.

Table 1. Localities of investigated *Vicia* species.

Taxa	Coordınates	Locality
Vicia truncatula Fischer ex Bieb. (Sect. *Vicia*)	37º39'59.00"N; 31º17'15.00" E	C3, Isparta, Pınargözü, 1800 m
Vicia assyriaca Boiss. (Sect. *Vicia*)	38º15'58.41"N; 39º45'26.78" E	B7, Diyarbakır Ergani road 45 th km, 800 m
Vicia noeana Reuter ex Boiss. var. *noenoa* (Sect. *Vicia*)	38º40'20.97"N; 39º04'04.40"E	B7, Elazığ, around Cip dam,1100 m
Vicia noeona Reuter ex Boiss. var. *megalodonta* Rech. (Sect. *Vicia*)	38º11'46.64" N; 39º50'00.75" E	B7, Diyarbakır, Ergani-Diyarbakır road 15th. km, 800 m
Vicia peregrina L. (Sect. *Vicia*)	38º40'35.31"N; 39º11'43.29"E	B7, Elazığ, Fırat University campus, 1060 m
Vicia michauxii Sprengel var. *stenophylla* Boiss. (Sect. *Vicia*)	38º26'31.65"N; 39º19'19.42"E	B7, Elazığ, Sivrice, around University camp, 1100 m
Vicia mollis Boiss. &Hausskn.(Sect. *Vicia*)	38º34'22.36"N; 39º03'33.51" E	B7, Elazığ, Hankendi, Tilek hill, 1050 m
Vicia hybrida L. (Sect. *Vicia*)	38º 11'48.00"N; 38º52'07.92" E	B7, Malatya, Pötürge, Gündüzköy, 1250m
Vicia grandiflora Scop. var. *grandiflora*	38º52'41.97" N; 39º41'10.24" E	B7, Tunceli, Tunceli-Elazığ road, querceus forst, 1100 m

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

Taxa	Total protein amounts (µg/ml)
Vicia truncatula	86.837
Vicia assyriaca	74.790
Vicia noeana var. *noenoa*	71.061
Vicia naeona var. *megalodonta*	80.139
Vicia peregrina L.	82.697
Vicia michauxii var. *stenophylla*	81.627
Vicia mollis	79.116
Vicia hybrida L.	83.209
Vicia grandiflora var. *grandiflora*	65.860

Acknowledgement

This study submitted as a poster in International Symposium 7th. Plant Life of South west Asia (7th PLoSWA), 25-29 June 2007, Eskişehir/TURKEY.

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(Received for publicaiton 10 February 2010)