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An Electrophoretic Analysis of the Seed Proteins of some *Vicia* L. Species from Northeast Anatolia (Turkey)

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Abstract

Soluble (non-reducing) and non-soluble (reduced) protein profiles of nine taxa of *Vicia* species are *V. sepium L*., *V. peregrine L*., *V. hybrida L*. and *V. sativa L*. subsp. nigra (L.) Ehrh, from Sect. *Vicia*, *V. cassubica L*. and *V. cracca L*. subsp. cracca from Sect. Cracca, *V. tetrasperma* (L.) Schreb. and *V. hirsute* (L.) S. F. Gray. from Sect. Ervum and *V. bithynica L*. from Sect. Faba were studied. . The non-reducing protein profile from the seeds exhibited a major set of proteins with molecular masses in the range of 66-34.7 kDa and the reduced protein profile showed major proteins with molecular masses in the range of 66-45 kDa. Spectrophotometric determinations of non-reduced protein amount was found significantly different ($p = 0.05$) in the seeds of *V. hirsute*, *V. sativa* subsp, nigra and *V. bithynica* while the remaining species were not determined significantly different. As for reduced protein amount, the amount of reduced protein of *V. hirsute* was found significantly different among the species ($p = 0.05$). The highest level of non-reduced and reduced proteins were determined for the seed of *V. hirsute*.

Introduction

The systematic methodology mainly based on morphology has been improved by the incorporation of physiology, ecology or biochemical characters. Severel biochemical analysis, especielly of proteins, meke it possible to establish differences at various taxonomic levels (Vaughan, 1983). One of the biochemical methods more extensively used for taxonomic purposes has been the electrophoretic analysis of the proteins found in seed and storage orgaras. These proteins are physilogically stable and easly to handle (Ladizinsky and Hymowitz, 1979). Seed proteins have received extensive attention in literature (Derbyshire *et al*., 1976; O'Kenedy *et al*., 1979; Lalonde *et al*., 1984; Aliaga-Morell *et al*., 1987; Gifford, 1988; Collada *et al*., 1988). Considerable insight has been dained as to their structure and synthesis during seed development and to their role as storage proteins following seed inhibition (Higgins, 1984). Seed proteins have previously been classified according to their exraction by a series of solvents (Osborne, 1924; Shewry and Miflin, 1985). The storage proteins in the majority of plant species studied are of the globin type. Two classes have been described: 11 S or legumin type' globulins and 7 S or vicilin type' (Derbyshire *et al*., 1976; Casey *et al*., 1986). Legumins, i.e. 11/12 S storage proteins, are the major seed proteins the legumens *Vicia faba* L. (Bailey and Boulter, 1970), *Pisum sativum* L. and in other *Vicia* species (Derbyshire *et al*., 1976). Similar proteins with the significant legumin characters (acidic subunits of Mr = c. 30-40 KD, basic subunits of Mr = c. 20 KD, linked together by S-S bridges and forming hexametric molecules in the storage tisues) have also been recognized as major storage proteins in many dicot and monocot species (De Klerk and Engelen, 1985; Fischer and Schopfer, 1988; Hasegawa *et al*., 1978; Jensen, 1984; Jensen and Buttner, 1981; Konishi *et al*., 1985; Simon *et al*., 1985). In this investigation we analysed the occurence of the soluble and insoluble proteins in some *Vicia* species collected from Northeast Anatolia (Turkey) were surveyed. No studies have previously been reported on electrophorotic separation of the storage proteins of given 9 *Vicia* taxa from Turkey.

Materials and Methods

Seed materials: The seeds used in this study for protein extraction were collected from Northeast Anatolia (Turkey) are *V. sepium* L., *V. peregrine* L., *V. hybrida* L. and *V. sativa* L. subsp. nigra (L.) Ehrh, from Sect, *Vicia*, *V. cassubica L*. and *V. cracca L*. subsp. cracca from Sect. Cracca, *V. tetrasperma* (L.) Schreb. and *V. hirsute* (L.) S. F. Gray. from Sect. Ervum and *V. bithynica* L. from Sect. Faba.

Protein extraction: A modification of Gifford (1988) method (Gifford *et al*., 1982) was used for the protein exraction from the seeds. Seed coats were removed prior exraction. Endosperm plus embryo were ground in Tris-glycine-buffer (0.01 M Tris; 0.08 M glycine), pH (.2 + 2 percent NaCl (Jensen and Berthold, 1989) in a ratio of 1 g seed material to 10 ml buffer. After 30 min stirring the slurry was centrifugated at 16.000 g for 20 min at 10°C. The supernatants, containing the soluble proteins, were removed. The pelled was thoroughly reexracted twice with a duble volume of buffer to exract all soluble proteins. The pelled containing the reduced storage proteins was suspented in TGP buffer (0.01 M Tris, 0.08 M glycine) pH 8.2 + 2 percent NaCl and an equal volume of 62 mM Tris-HCl (pH 6.8) buffer with 3.05 percent (w/v) SDS and 10.7 percent (w/v) glycerol. The suspension was boiled for 8 min. After centrifugating the supernatant was applied for SDS-PAGE.

Electrophoresis: Proteins in the supernatant were quantified by the medhot of Laemmli (1970) and electrophoretically separated according to Jensen and Lixue (1991) in SDS-PAGE. Fifty microgram of protein were applied to each slot and at 4°C under a constant of intensity of a mA per slot for 3.5 h. Reaching a final voltage of 300 V. Gels were stained with silver nitrate (Sambrook and Russell,1989). The standard proteins (MW-SDS-702, Sigma Chemical Co.)

were employed are trypsin inhibitor (20.1 kDa) trypsinogen (24 kDa), carbonik anhydrase (29 kDa), glyceraldhyde-3-phosphate dehydrogenase (34,7 kDa), egg albumin (45 kDa) and bovine albumin (66 kDa).

Results and Discussion

Seeds of some *Vicia* L. species were extracted for SDS-soluble seed proteins and their electrophoretic behaviour was determined by SOS-PAGE. The saver stained gray protein profiles obtained after separating these proteins in their reduced (non-soluble) (+ME) and nonreduced (soluble) (-ME) states are shown in Fig. 1. The total soluble and non-soluble protein amount of the seeds were given in Table 1. The non-reducing gel (Fig. 1a) shows that the soluble protein profile from the seeds are different and has a major set of proteins with molecular masses in the range of 66-34.7 kDa. Whereas the nonsoluble protein profile has major proteins with molecular masses in the range of 66-45 kDa (Fig. 1b).

Table 2 shows presence or absence of non-reducing and reducing seed proteins in *Vicia* species separated by SDS-PAGE. There were dramatic changes in the protein composition of the seeds were classified according to extraction procedures (see Materials and Methods). The non-reduced protein amount was found significantly different ($p = 0.05$) in the seeds of *V. hirsute*, *V. sativa* and *V. bithynice* while the remaining species were not determined significantly different. As for reduced protein amount, the amount of reduced protein of *V. hirsute* was found significantly different among the species ($p = 0.05$k The highest level of non-reduced and reduced protein were determined for the seed of *V. hirsute*.

When the proteins from seeds of *Vicia* of nine taxa was electrophoresed, the non reducing protein profiles (Fig. 1a) of the various species had a common protein band with an estimated molecular weight of 34.7 kDa and 24 kDa. The reducing protein profiles (Fig. 1b) of the various species had a common protein band with an estimated molecular weight of 34.7 kDa . The 66 kDa protein band as in non reducing protein profiles were identified for the species *V. cracca* subsp. cracca, *V. hybrida*, *V. sativa* subsp. ngra, *V. bithynica* and *V. peregrine*. The non reducing protein profiles of some species except *V. sepium*, *V. tetresperrna*, *V. hirsute* and *V. hybrida* and *V. sepium*, *V. tetrasperma* and *V. peregrine* were found to be specific for 45 kDa and for 29 kDa protein bands, respectively while *V cassubica*, *V. bithynica* and *V. peregnine* for 20 kDa and *V. cracca* subsp. cracca, *V. bithynice* and *V. peregrine* for 14 kDa protein bands were found specific (Table 2).

The reducing protein profiles exhibited most of the protein bands at 34.7 kDa in all *Vicia* species except that *V. sepium* and *V. hirsute*, The 66 kDa protein band as in reducing protein profiles was determined in *V. sepium*, 45 kDa in *V. tetrasperma*, *V. bithynice* and *V. peregrine*, 29 kDa in *V. sepium*, *V. tetrasperma* and *V. bithynica* and 20 kDa in *V. terrasperma* and *V. sativa* subsp. nigra (Fig. 1b). In many seeds, storage proteins are localized within an amorphous matrix contained in single membrane bound organelles called protein bodies (Lott, 1980), Commonly, these proteins are globulins (Derbyshire *et al*., 1976). However, in some seeds inclusions of crystalloid storage ,proteins are also found embeded in the matrix.

Table 1: Protein content of non-reducing and reducing fractions of *Vicia* L. seeds. Results are the means of three replicates of 5 seeds of each. For comparisons among means the analysis of variance was used

Species	Protein (µg/g dry wt)	
	Soluble (non-reduced)	non-soluble (non-reduced)
V. tetrasperma (L.) Schreb	100.4c	61.4b
V. hirsute (L.) S. F. Gray	160.1e	75.6d
V. cracca L. subsp. cracca	100.8c	63.1b
V. hybrida L.	120.3d	70.7c
V. sativa L. subsp. nigra (L.) Ehrh	85.6b	52.2a
V. bithynica L.	84.8a	51.8a
V. peregrine L.	120.1d	70.1c
V. sepium L.	100.6c	63.4b
V. cassubica L.	101.3c	62.5h

Means in columns followed by different letters are different at the $p = 0.05$ level

Table 2: Presence (+) or absence (-) of non-reducing and reducing seed proteins in some *Vicia* L. species collected from Northeast Anatolia (Turkey)

Species	Non reducing protein bands (kDa)							Reducing protein bands (kDa)						
	66	45	34.7	29	24	20	14	66	45	34.7	29	24	20	14
V. sepium L.	-	-	+	-	+	-	-	+	-	-	+	-	-	-
V. cassubica L.	-	+	+	+	+	+	-	-	-	+	-	-	-	-
V. tetrasperma (L.) Schreb	-	-	+	-	+	-	-	-	+	+	+	-	+	-
V. hitsuta (L.) S. F. Gray	-	-	+	+	+	-	-	-	-	-	-	-	-	-
V. cracca L. subsp. cracce	+	+	+	+	+	-	+	-	-	+	-	-	-	-
V. hybrida L.	+	-	+	+	+	-	-	-	-	+	-	-	-	-
V. sativa L. subup. nigra (L.) Ehrh	+	+	+	+	+	-	-	-	-	-	-	-	+	-
V. bithynica L.	+	+	+	+	+	+	+	-	+	+	+	-	-	-
V. peregrina L.	+	+	+	-	+	+	+	-	+	+	-	-	-	-

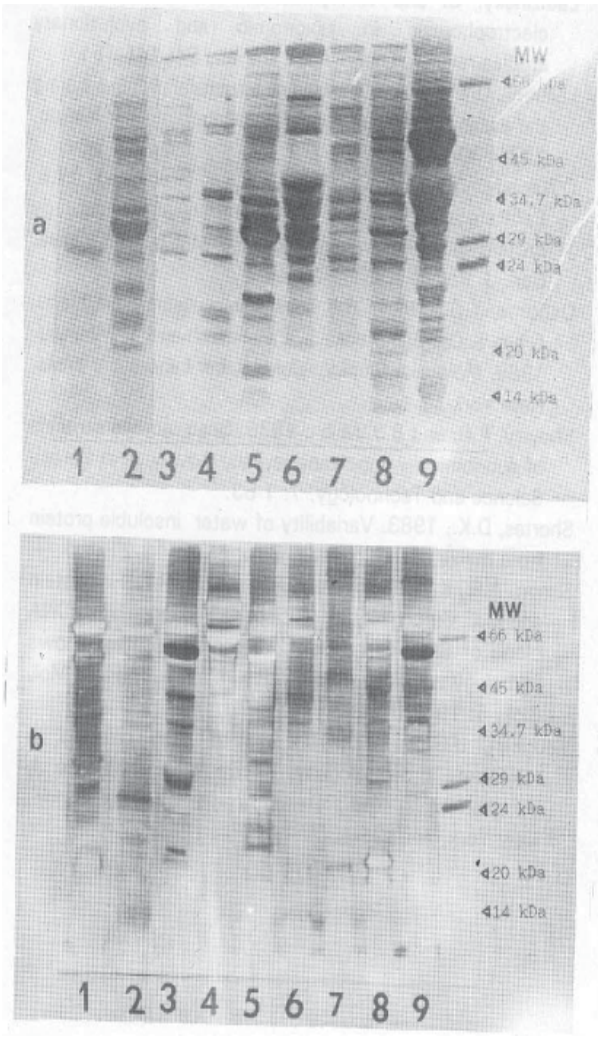

Fig. 1: SDS-PAGE protein profiles from seeds of *Vicia* L.; a) non-reducing protein profile (Tris-glycine-buffer extracts + 2 percent NaCl), b) reducing protein profiles (Tis-glycine-buffer extracts + 2 percent NaCl and Tris-SDS-glycerol). 1; *V. sepium* L., 2; *V. cassubica* L., 3; *V. tetrasperma* (L.) Schreb, 4; *V. hirsuta* (L.) S. F. Gray, 5; *V. cracca* L. subsp. cracca., 6; *V. hybrida* L., 7; *V. sativa* L. subsp. nigra (L.) Ehrh., 8; *V. bithynica* L. 9; *V. peregrina* L. (45, 34.7, 20 and 14 kDa molecular weights in standard couldn't clearly be seen because of the low density in staining)

Ultrastructural studies have shown that protein bodies localized in the storage parenchyma of seeds of several Pinus species contain crystalloid protein inclusions.

Although legumins have been reported to be the predominant storage protein in seed plants, exceptions are known (Danielsson, 1949). In gymnosperms, legumins has also been detected in the seeds of Ginkgo biloba (Jensen and Berthold, 1989), but not in Macrozamia commur (Blagrove *et al*., 1984). In Abies species, all major legumin proteins were characterised at 55 kDa (Jensen and Lixue, 1991). Most of the species of Poaceae family store predominantly prolamine. The oat accumulates 11S globulins and rice store glutelins (Kasarda *et al*., 1976; Shewry and Miflin, 1985). In some species of Leguminasae, such as *Vicia faba* L. and *Pisum sativum* L., the 11 and 12S proteins were described as the major seed proteins (Derbyshire *et al*., 1976). 30-40 and 20 kDa proteins were recognized in many dicot and monocot species (De Klerk and Engelen, 1985; Fischer and Schopfer, 1988; Hasegawa *et al*., 1978; Jensen, 1984; Jensen and Buttner, 1981; Konishi *et al*., 1985; Simon *et al*., 1985). Similarly, we determined most of the soluble proteins between the ranges at 30-40 and 20 kDa. This paper does not include the classification of protein types in the given species, but more detailed analyses will be required to detect protein types and classes bay increasing the number of species in the genus upon this work.

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