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Comparative Study of Proteins in Seeds of some Species of *Trigonella* from Iran

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Abstract

The present study is the first comparative study of some *Trigonella* species from Iran, reporting the seed protein content and possible use of SDS-PAGE profile of seed proteins in the *Trigonella* taxonomy. Quantitative analysis of seed proteins of the species and putative populations of *Trigonella* showed that content of proteins varied from 23.52 to 46.6% D.W. where the lowest protein content in *T. monantha* and the highest protein content in *T. aphanoneora* . The proteins content of seeds in most of the wild species of *Trigonella* was higher than that of *T. foenum-graecum* L. (29.93% D.W.). The electrophoretic patterns of seed proteins were compared for correct identification of the species. The electrophoretic profile varied among some putative populations of a single species. Overall, this study showed the quantitative and qualitative differences among the studied species and putaive populations. The values of seed protein electrophoretic profiles in the taxonomy of *Trigonella* species are discussed.

Keywords: *Leguminosae (=Fabaceae), Seed protein; SDS-PAGE, Trigonella*

Introduction

Plant proteins provide nearly 65% of the world supply of proteins for humans with 45%-50% and 10%-15% from cereals and legumes or vegetables, respectively (Mahe et al., 1994). Importance of plant proteins in the average diet varies from the least developed regions (where animal proteins are scarce and poverty precludes the consumption of meat) to the highly developed regions (where animal production is particularly abundant). Nevertheless, there is now an expanding consumption of protein food of legume and vegetable origin throughout the world. Among the plant species, legumes are considered as the major source of dietary proteins (Bressani and Elias, 1980; Norton et al., 1985).

Characteristically, grain legume seeds have large protein content, ranging from 20% to 40% of their dry matter, according to species, genotypes within species, and environment (Otoul,1976; Norton et al.,1985; Baudoin,1991). Storage proteins in legume seeds are mainly located in the cotyledonary tissues. Embryonic axis and testas contribute represent small proportions of the seed mass. The major storage proteins in legume seeds are the globulins which usually account for about 70% of the total protein. Glutelins (10-20%) and albumins (10-20%) make up the remainder. The principal storage globulins in most legumes are legumin and vicilin, the latter predominating in common bean (Jansman, 1996).

Fenugreek (*Trigonella foenum-graecum* L.), one of the condiments known to mankind, has been cultivated for a very long time. It has been popularly used as a food and as a medicine in the Mediterranean region, western Asia, northern India, and Africa. Fenugreek seeds contain 26% protein, 5.8% fat and 44.1% carbohydrate (Patil et al., 1997). Because of the high protein content (up to 36%) and its favorable amino acid composition, fenugreek seed is equal in value with soy (Makai and Balatinez, 1998). *Trigonella* contains about 58 wild species in Iran and many chemical properties of them remain to be elucidated.

Electrophoretic techniques for proteins and isoenzymes have been applied successfully in taxonomic classification in various families, such as *Poaceae* (Johnson et al., 1967; Duvall and Biesbor, 1989),

Cucurbitaceae (Pasha and Sen, 1910) and *Fabaceae* (Misset and Fontenelle, 1992). They are also a usefull tool in identifying varieties and cultivars, and establishing genetic diversity in cultivated plants such as *Vitis vinefra* (Altube et al., 1992) *Lolium rigidum* (Bravi et al., 1994), *Hevea brasiliensis* (Leconte et al, 1994), *Glycine max* (Pascale et al., 1994) and *Bromus* (Aiassa et al., 1995).

The purpose of this investigation is to provide seed protein data of some *Trigonella* species from Iran, and apply them to assess the relationships of 18 samples belonging to 6 species and four sections of *Trigonella*.

Materials and Methods

Plant material: The seeds of *Trigonella* species and putative populations were obtained from the Alborz gene bank, Karaj, Iran. The locations of the *Trigonella* species and putative populations were presented in Fig. 1. The data on the studied taxa are given in Table 1. For the study of proteins changes during subsequent generations, seeds of some *Trigonella* species were grown in Alborz experimental field. Seeds were cultivated in March at green house and after two months the plants were transferred to the field in May. In the subsequent year flowering was occurred in June and legumes were harvested at various days after anthesis in July.

Extraction and measurement of proteins: Seeds of *Trigonella* species and putative populations were powdered and directly homogenized in a mortar cooled on ice using a buffer containing:1M Tris-HCl, pH 6.8. After centrifugation at 27200 g for 1 hour at 4˚C, the supernatant was filtered and then transferred to eppen tubes and the samples keeped on ice at 4˚C. Total protein concentrations were measured by modified spectrophotometric method of Lowry at 660 nm using BSA as the standard (Laemmeli, 1970).

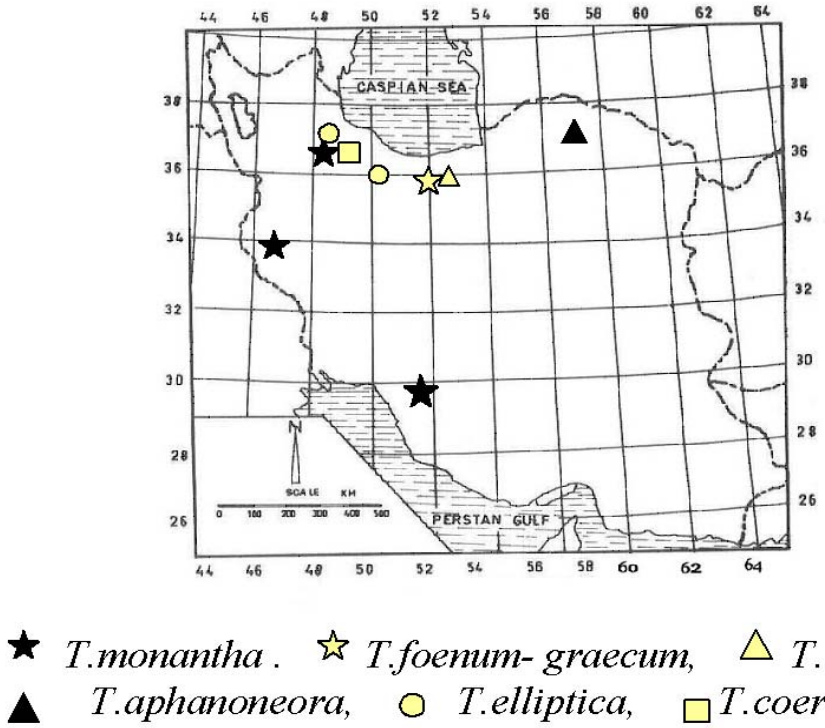

Figure 1. Locations of the *Trigonella* species and putative populations studied.

Qualitative assay: Sodium dodecyl sulphate polyacrylamid gel electrophoresis (SDS-PAGE) was used to resolve the protein pattern of the seeds extracts: 12.5% resolving gel, 4.5% stacking gel, tris-HCl buffer pH 6.8, and loading approximately 100 µg of protein extracts to each well. After the electrophoresis was run, the bands in the gels were stained by coommassie brilliant blue G250 for 24 hr and destained by methanol-acetic acid-water (1:1:8) for 24 hr. SDS-PAGE was performed according to Laemmeli (1970). Molecular weight of the protein bands was determined by simultaneous running of standard molecular weight proteins. Standard molecular weights of proteins were purchased from Sigma Co. The qualitative differences between the species were estimated by the number and Rm (relative mobility) values of protein bands.

Apparatus used: Beckman high-speed centrifuge (J2-21M) and Shimadzu UV-visible recording spectrophotometer (UV-160) with 10 mm matched quartz cells were used for centrifugation of the extracts

and determination of the absorbance, respectively. SDS-PAGE gel electrophoresis was done using a LKB (Sweden) apparatus.

Table 1. Taxon, geographical origin, voucher number and protein content. Values are the means of three samples ± SE.

Species	*Section*	Voucher number	Locality and year of harvest	Protein content (%D.W.)
T. coerulescens	*Biebersteiniana e*	2816	Zanjan-1998	35.32±1.47 (34.15±2.02)*
T. foenum-graecum		-	Tehran-2000	29.93±3.33
T.aphanoneora	*Gladiatae*	-	Bojnourd-2000	46.60±0.95
T.elliptica	*Ellipticae*	770	Qazvin-1996	44.17±1.37
T.elliptica	*Ellipticae*	460	Zanjan-1995	36.12±0.81
T.tehranica	*Ellipticae*	76	Tehran (Damavand)-1994	33.46±2.28
T.tehranica	*Ellipticae*	120	Tehran (Karaj)-1994	38.78±0.15
T.monantha	*Ellipticae*	151	Kohkiloie&Boierahmad-1993	25.03±1.10 (29.50±0.98)*
T.monantha	*Bucerates*	1960	Kermanshah-1993	26.23±0.84 (26.04±0.99)*
T.monantha	*Bucerates*	2024	Kermanshah-1993	30.24±0.18
T.monantha	*Bucerates*	1835	Kermanshah-1993	30.84±1.41 (26.60±0.27)*
T.monantha	*Bucerates*	1809	Kermanshah-1993	32.78±1.27
T.monantha	*Bucerates*	1855	Kermanshah-1993	34.67±1.55 (24.66±0.28)*
T.monantha	*Bucerates*	1942	Kermanshah-1994	32.57±1.11
T.monantha	*Bucerates*	2111	Kermanshah-1994	23.52±0.53
T.monantha	*Bucerates*	1771	Kermanshah-1995	33.08±1.26
T.monantha	*Bucerates*	1773	Kermanshah-1996	34.34±0.40
T.monantha	*Bucerates*	463	Zanjan-1995	27.70±1.79 (24.78±0.70)*

**Each of the parenthetical values represents the protein content in the seeds obtained at the second year in the experimental field.*

Interpretation and cluster analyses: Cluster analyses were done using SPSS 9.05 for Windows. To estimate species/putative populations similarity as indicated by protein electrophoresis patterns, Jaccard's index were determined. Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence). The resulting data matrix was used for cluster analysis using average linkage method.

Results and Discussion

Protein content of seeds of the species and putative populations of *Trigonella* collected from different regions of Iran were determined (Table 1). The results showed that content of protein varied from

23.52 to 46.6% dry weight where *T. monantha* (voucher no. 2111) had the lowest protein content and *T. aphanoneora* (Section *Ellipticae*) had the highest (Fig. 2). Moreover, the protein content of the different putative populations of *T. monantha* showed a considerable variation and ranged from 23.52 to 34.67 % dry weight. Protein content of different putative populations of *T.monantha* collected at the same year from different regions of Kermanshah province also varied and ranged from 30.24 to 34.67% dry weight. The protein contents of seeds whitin most wild species of *Trigonella* was higher than that of the *T. foenum-graecum* L. (29.93% D.W.). Thus other wild species of *Trigonella* can also be considered as the new natural protein sources.

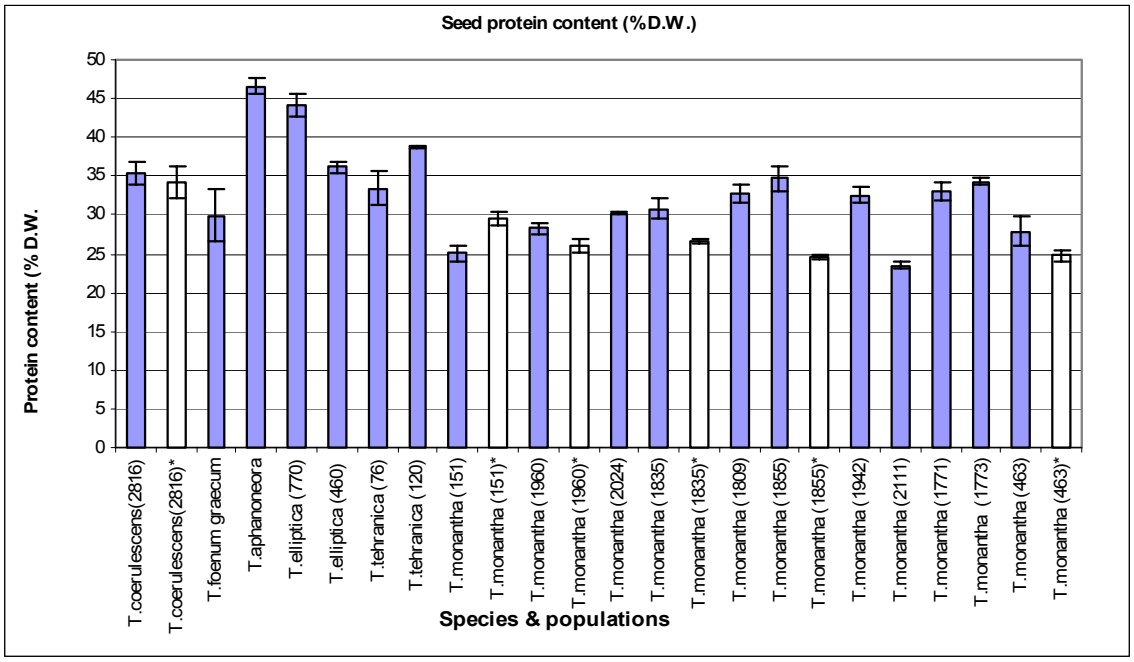

Figure 2. Protein content (% D.W.) in seeds of different species and putative populations of *Trigonella*. Asterisked samples represent the seeds obtained at the second year in the experimental field.

The content of proteins in the new seeds of some species produced in experimental field reduced but in *T.monantha* (voucher no.151) the content increased.

In this study, seeds were chosen as plant materials for protein analysis since they represent the most stable and comparable plant stages (Boulter and Thurman, 1968). According to the comparative analysis of the protein patterns in the SDS-PAGE system (Fig. 3)

sixteen to twenty three prominent protein bands with Rm values from 0.21 to 0.80 (about 70,000 to 6,500 Da) were detected in the seed proteins extracts of *Trigonella foenum graecum* and *T. monantha* (voucher no.1809 and 1855), respectively. No variation was found between replications. Some of the bands were common to all the species and the putative populations while some others were specific to the species or the putative populations. For example, two bands (about 18,000 and 45,000Da) are exclusive of *T. T. foenum-graecum* L. (Fig. 3**A**). The seeds of *T. monantha* (voucher no. 1960*) which was obtained from voucher no. 1960 in the experimental field has relatively the same profile with that of the putative population no.1960. Thus the new condition did not affect the protein profile of the seeds. This result is in accordance with the results of Carreras et al., (1997). The protein electrophoretic profiles are almost stable within species, being little affected by environmental factors (Harborne and Turner, 1984). According the view of Vaughan (1983) the protein electrophoretic analyses provide taxonomic separation of taxa at the species level. According to the results obtained from the analysis of different putative populations of three species (*T. monantha*, *T.elliptica* and *T.tehranica*) somewhat protein variation was observed for some putative populations. According to the protein electrophoretic profiles of different putative populations of *T. monantha* the profiles of different putative populations are not same (Figs. 3A and B). The protein electrophoretic pattern of some putative populations of *T. monantha* (e.g., Figs. 3A, 4,6; 3B, 1,2 and 4,5,6) are same.

The similarity matrix and the dendrogram obtained from cluster analysis are presented in Table 2 and Fig. 4, respectively. According to the stability of the seeds protein electrophoretic profile of the species under different conditions (e.g. Harborne and Turner, 1984) and based on the obtained dendrogram (Fig. 4) some of the different putative populations of a single species can be belong to the different species and the current nomenclature of the *Trigonella* should be reassessed according to their other characteristics and with other proper methods.

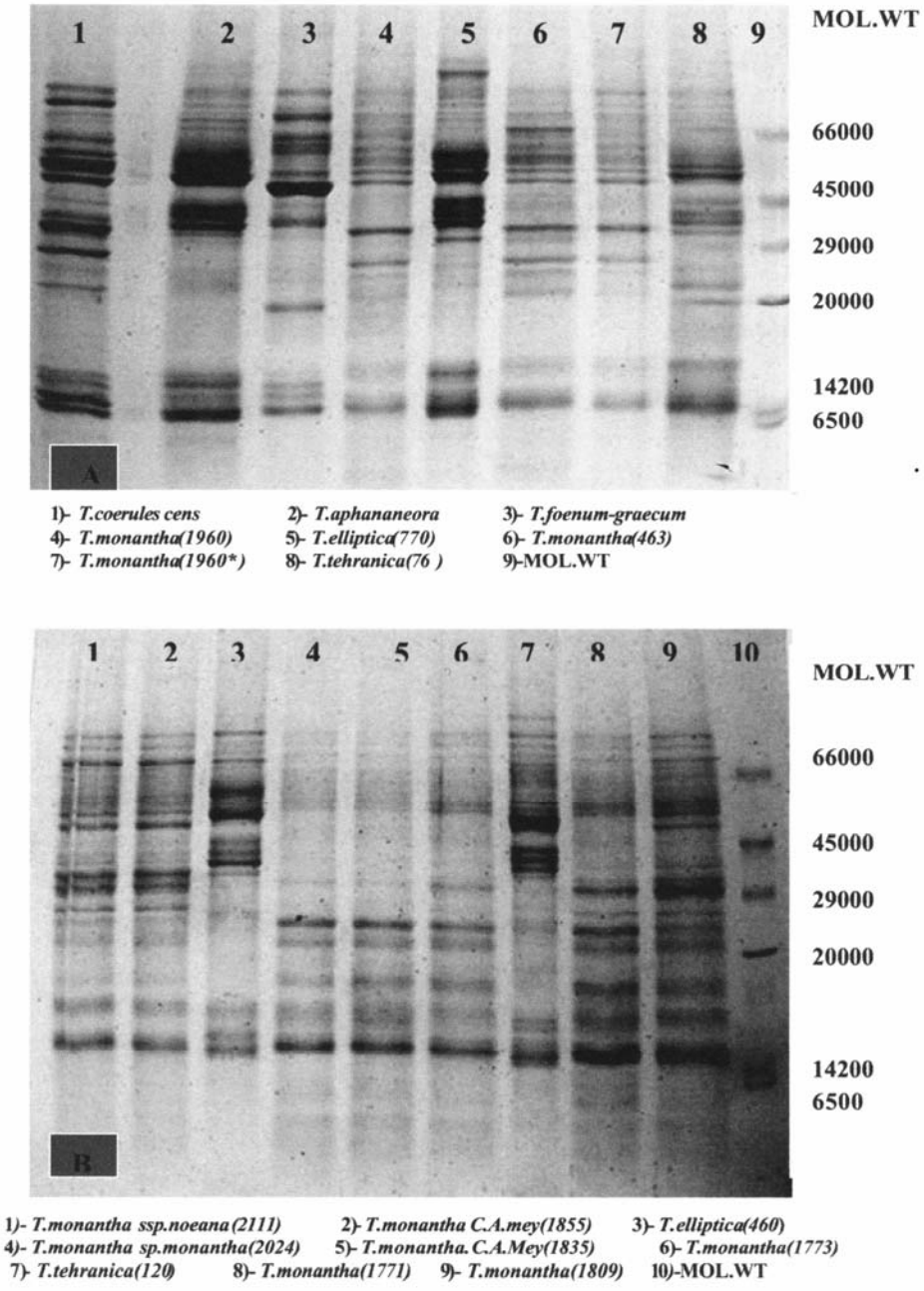

Figure 3. SDS-PAGE analysis (12.5% acrylamid, coommassie brilliant blueG250 staining) of seed proteins at different *Trigonella* species and putative populations from Iran. The parenthetical values represent voucher numbers.

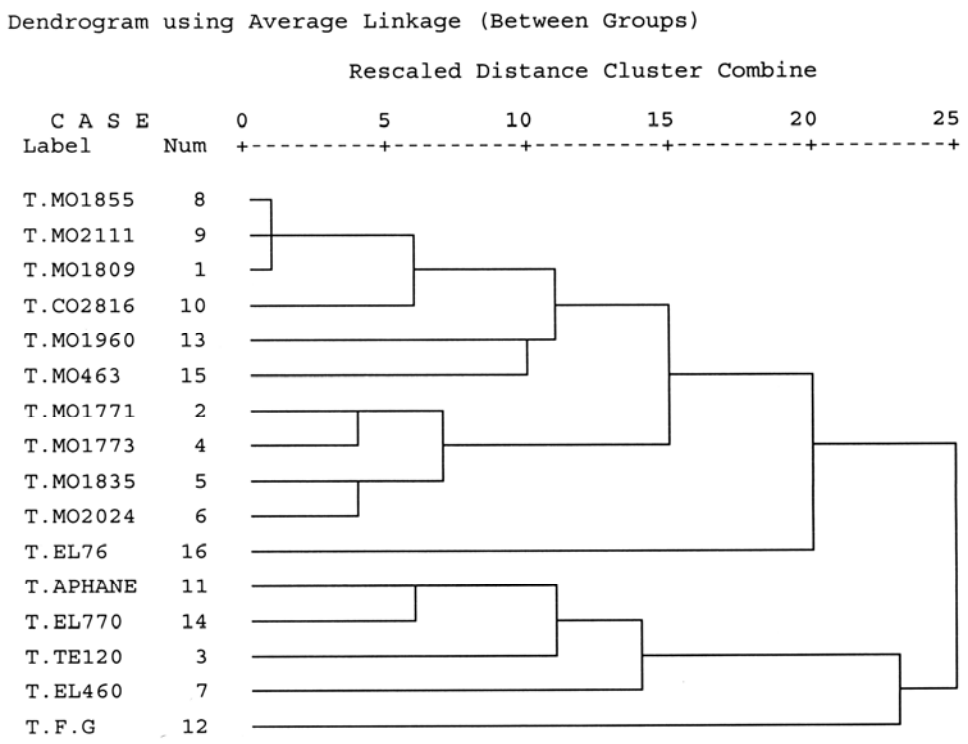

Figure 4. Cluster analysis of the *Trigonella* species and putative populations according to the seed protein data.

In summary, as the seeds proteins patterns can be considered stable for each species and being little affected by environmental factors (Carreras et al., 1997), the present nomenclature of *Trigonella* species specially *T. monantha* and may be other species should be reassessed.

Acknowledgments

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