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Seed Storage Protein Profile of Grain Legumes Grown in Iran, Using SDS-PAGE

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ABSTRACT

Seed protein profiles of 47 accessions belonging to eleven species and four tribes of grain legumes were studied, by extracting the total proteins from ten single seeds in each accession and performing SDS-Polyacrylamide gel electrophoresis. All eleven species were clearly recognizable from their protein banding patterns, but only *Phaseolus vulgaris* expressed high intraspecific variations, followed by *Lathyrus sativus.* Variation among accessions of other species was very limited. Cluster analysis, after quantifying the protein bands, using UPGMA procedure, showed phylogenetic relationships which were in a good concordance with species classification based on morphological characters. Accessions of tribe Vicieae formed one cluster *(Vicia faba, Lens culinaris, Pisum sativum, Lathyrus sativus* and *Vicia ervilia)* having nearly equal amounts of three categories of polypeptide: high, moderate and low molecular weight. The second cluster was a small tribe of Cicereae *(Cicer arietinum* accessions) having moderate and low molecular weight polypeptides. Accessions of Phaseoleae tribe formed the third cluster *(Phaseolus vulgaris, Vigna unguiculata* and *Vigna radiata),* having predominantly high molecular weight polypeptides. Finally, the more distinct tribe, Aeschynomeneae *(Arachis hypogaea* accessions), formed a separate cluster exhibiting a special banding pattern. A unique discrepancy was observed about *Glycine max,* which belongs to Phaseoleae but was clustered with Cicereae.

Keywords: Grain legumes, SDS-PAGE, Seed storage protein.

INTRODUCTION

The classification of Leguminosae, containing more than 21,000 species (Christou, 1994) is not well defined (Kupicha, 1977). The grain legumes, belonging to a "subfamily" of *Papilionidae* or *Faboidae,* were recently grouped into the five following tribes (Summerfield and Roberts, 1985): Phaseoleae, Vicieae, Cicereae, Aeschynomenae, and Gemistae. Most of the grain legumes belong to the first three tribes.

In recent years, grain legumes have played a primary role in the search for vegetable sources of proteins owing to the high protein content of the seed, ranging from 20% in pea to 40% in lupin (Cereletti, 1979). They can, therefore, be considered a good substitution to animal proteins in human diet, especially in the third world. However, the seed storage proteins of these legumes contain a low concentration of sulfur-containing aminoacids and plant breeders have to consider this problem in any improvement programes (Summerfield and Roberts, 1985).

The productive features, isozymes and protein polymorphisms of most grain legume crops are well documented (De Falco *et al*., 1991; Salmanowicz and Przybylska 1992; Labdi *et al*., 1996; Singh *et al*., 1994; Erskine and Muehlbauer, 1991; Koenig *et al.*, 1990). However, the comparative study of protein variation in these species is not well demonstrated. Hence, it is desirable to increase our knowledge of the genetic resemblance among the most important grain legumes by employing variations in seed storage proteins, which are their main common characteristics.

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Using protein polymorphism, a comparative study was undertaken to test the reliability of this method for estimating the genetic resemblance amoung eleven species of grain legumes.

MATERIAL AND METHODS

Plant Material

Forty-seven accessions of eleven species and four tribes of grain legumes were used in this study (Table 1). Ten single seeds were analysed in each accession.

Table 1. List and characteristics of grain legumes studied (after Sammerfield and Roberts, 1985).

no	Species	Tribe	Number of accessions	Common name	Chromosome number	Origin or site of domestication
1	Phaseolus vulgaris	phaseoleae	4	common bean	22	Mexic, Peru
2	Vigna unguiculata	"	2	cowpea	22, 24	" "
3	Vigna radiata	"	2	mung bean	22	Ethiopia, Africa
4	Glycine max	"	4	soybean	40(4x)	China
5	Vicia faba	Vicieae	2	faba bean	12, 14	Middle East
6	Lens culinaris	"	7	lentil	14	"
7	Pisum sativum	"	1	pea	14	Near East
8	Lathyrus sativus	"	8	chickling vetch	14	Middle East
9	Vicia ervilia	"	4	bitter vetch	14	Turkey, Europe
10	Cicer arietinum	Cicereae	11	chickpea	16	Middle East, Iran
11	Arachis hypogaea	Aeschynomeneae	2	groundnut	40(4x)	Bolivia, Argentina

Protein Extraction

Total salt soluble proteins were extracted by adding 30mg of ground seeds in lml of 50mM tris-HCI (pH 7.5) and 0.5 M NaCI at 4°C for 60 minutes. This was then frozen at –20°C and thawed 3 times during 24h to disrupt the tissue and release the proteins (Miller *et al*., 1972) and centrifugations were at 10000 g for 15 min.

SDS–PAGE Electrophoresis

One dimensional Sodium dodecylsulfate-polyacrylamide gel electrophoresis was carried out according to Laemmli (1970). Ten percent of resolving slab gels were used (l6×16×0.2 cm). Samples were prepared for electrophoresis by mixing 10 µl of extracted protein, 2.5 µl of 2-mercaptoethanol, and 7.5 µl of 0.002% bromophenol blue in 0.0625M tris-HC1 (pH 6.8), containing 10% glycerol and 2% SDS. All protein stainings were performed using Comassie Blue according to Hames and Rickwood, (1990).

Analysis of Data.

Protein band patterns with unambiguous resolutions were coded 0 or 1 depending on their absence or presence in each species. The resemblance matrices were calculated directly from data matrices, using the ratio of the number of 1-0 matches to the total number of bands as an index of genetic distance, this corresponds to a "simple matching coefficient" in the form of dissimilarity (Romesburg, 1990). In this method, "absence" contributed equally to "presence" in the calculation of dissimilarity. Finally, the NTSYS (Rohlf, 1993) computer program and the UPGMA method of clustering were used for converting resemblance matrices to the dendograms. The same procedures were performed for quantified banding patterns (0 to 9), but Euclidian distance (Romesburg, 1990) was used for calculating the genetic resemblance.

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RESULTS AND DISCUSSION

Within-Accession Homology

Electrophoretic single seed protein profiles of two accessions representing *Phaseolus vulgaris* (Red common bean), and *Arachis hypogaea* (groundnut) are presented in Figure 1. All individuals within each accession showed an identical number of bands with similar mobility and thereby intravarietal genetic homology. This was true for accessions of the other species studied. This indicates, therefore, that the SDS-PAGE procedure, using total protein samples, is not suitable to detect the seed storage protein polymorphism within varieties or within populations of grain legumes. This is in contradication with suggestions of Cooke (1992) and Kapse and Nerkar (1985). To find polymorphisms within accessions of grain legumes which are predominantely autogamous plants, the researchers may use some specific types of protein extracts (glutellins, albumins, isozymes) and analyse them using more than ten single seed samples.

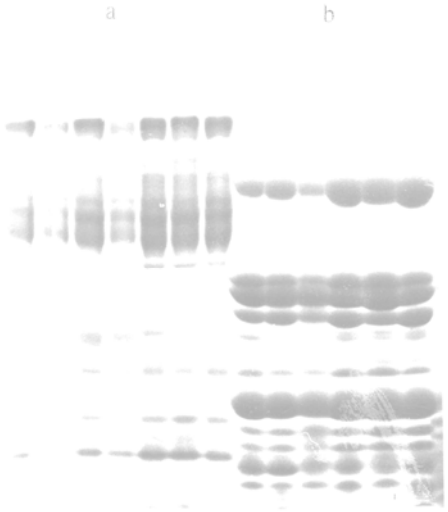

Figure 1. Single seed protein homology in two accessions representing *Phaseolus vulgaris* (a) and *Arachis hypogaea*.

Between-Accession Variation

Total protein homology was observed equally among accessions belonging to each species, except for *Phaseolus vulgaris* and *Lathyrus sativus.* Intraspecific variation for seed storage proteins in *Phaseolus vulgaris* has also been reported by Limongelli *et al*. (1996). In the case of *Lathyrus sativus* accessions (local populations collected from different regions of Iran), substantial protein polymorphisms were found for "Tabriz population of chickling vetch" which showed at least three different major bands (Figure 2). Other accessions studied presented the same banding pattern. The complete divergence of "Tabriz pouplation of chickling vetch" has previously been reported on both morphological characters and electrophoretic analysis (Mohamadi-Nassab. *et al.*, 1998). The protocol used here can, therefore, be useful for cultivar identification in two of the above-mentioned species.

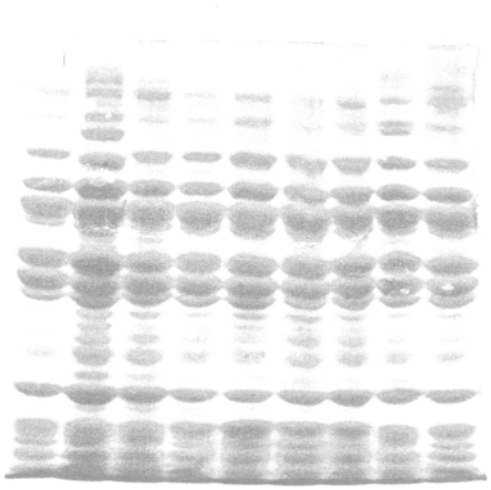

Figure 2. Different banding pattern observed for the "Tabriz population of *Lathyrus sativus*" (second sample from the left).

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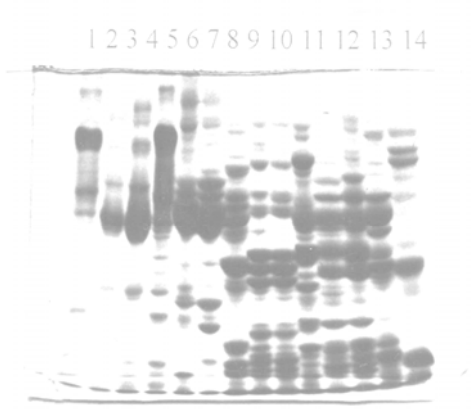

Figure 3. SDS-PAGE of seed storage proteins in 9 grain legume species. 1= *Phaseolus vulgaris* (Red c.v. bean), 2= *Phaseolus vulgaris* (Chiti cv.), 3= *Phaseolus Vulgaris* (Ablage cv.), 4= *Phaseolus vulgaris* (white), 5= *Vigna unguiculata*, 6= *Vigna radiata*, 7= *Vicia faba*, 8= *Cicer arietinum* (Jam cv.), 9= *C. arietinum* (Pirouz cv), 10= *Pisum sativum*, 11= *Lathyrus sativus* (Tabriz pop.), 12= *L. sativus* (Varamin pop.), 13= *Lens culinaris*, 14= *Glycine max*.

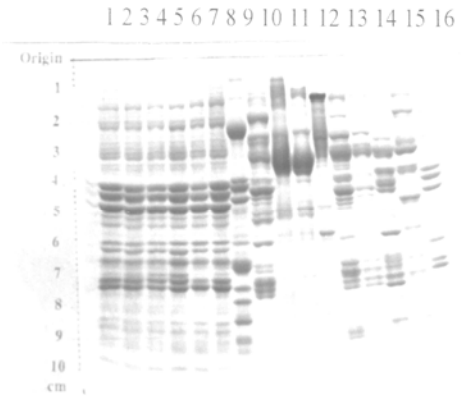

Figure 4. SDS-PAGE of seed storage proteins in seven grain legume species. 1, 2, 3, 4, 5, 6 and 16= Accessions of *C.arietinum*, 7= *Arachis hypogaea*, 8= *Vicia ervilia*, 9, 10 and 11= *Phaseolus vulgaris*, 12 and 13= *Lens culinaris*, 14= *Lathyrus sativus*, 15= *Pisum sativum.*

Interspecific Polymorphism

Examples of total seed protein profiles among species are presented in Figures 3 and 4. The differences between species are evident. All eleven species are clearly identifiable from the protein banding pattern. SDS-PAGE of total seed protein porfiles is, therefore, an efficient procedure for differentiating grain legume species. Several researchers have confirmed the usefulness of different SDS-PAGE proedures in plant taxonomic, evolutionary and genetic relationship studies (Ladizinisky and Hymowitz, 1979, Ladizininky and Van Oss, 1984 and Virinhos and Murry, 1983). The best concordance with the classification of grain legume species on the basis of morphological characteristics was obtained when the bands were quantified and an UPGMA procedure, using Euclidian distances, was applied. For example, Figure 5 presents just such a cluster analysis for one gel containing eleven species. Four groups representing the four tribes studied are recognizable. Accessions from the Vicieae tribe formed one cluster *(Vicia faba, Lens culinaris, Pisum sativum Lathyrus sativus* and *Vicia ervilia).* These species present three categories of polypeptides of high, moderate and low molecular weight, in nearly equal amounts (Figures 3 and 4). The second cluster included the small tribe of Cicereae *(Cicer arietinum* accessions) with moderate and low molecular weight polypeptides. Accessions of the Phaseoleae tribe formed the third cluster *(Phaseolus vulgaris*, *Vigna unguiculata* and *Vigna radiata),* Finally, the more distinct tribe Aeschynomeneae *(Arachis hypogaea* accessions*)* formed a separate group, showing a special banding pattern. *Glycine max* was an exception since it belongs to Phaseoleae but was culstered with Cicereae.

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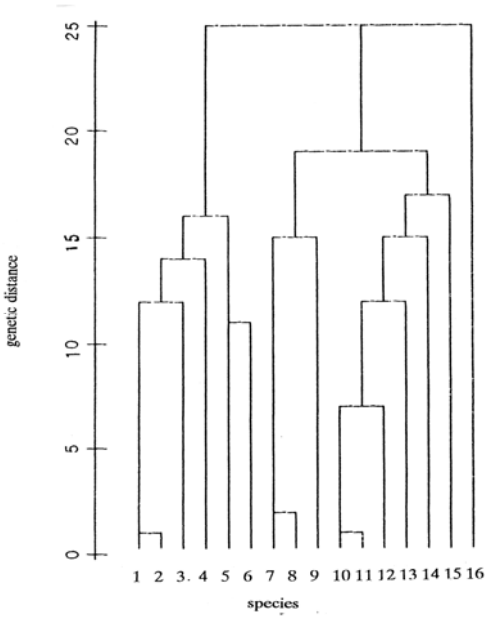

Figure 5. Dendrogram of 11 grain legume species based on seed storage protein profiles. 1 and 2= *Lathyrus sativus*, 3= *Pisum sativum*, 4= *Vicia ervilia*, 5= *Lens culinaris*, 6= *Vicia faba*, 7 and 8=*Cicer arietinum*, 9=*Glycine max* , 10, 11, 12 and 13= *Phseolus vulgaris*, 14= *Vigna unguiculata*, 15= *Vigna radiata*, 16= *Arachis hypogaea*

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بررسی پروتئین‌های ذخیره‌ای دانه در حبوبات زراعی ایران از طریق الکتروفورز SDS-PAGE

م. ولیزاده

چکیده

الگوی پروتئینی دانه در 47 نمونه (رقم یا جمعیت) متعلق به 11 گونه و 4 طایفه از حبوبات زراعی از طریق الکتروفورز در ژل سدیم دو دسیل سولفات- پلی‌اکریلامید، با استفاده از پروتئین‌های کل استخراج شده در 10 تک‌دانه از هر نمونه مورد مطالعه قرار گرفت. همه 11 گونه به طور روشن از روی الگوی نواربندی پروتئین‌ها قابل شناسایی بودند؛ ولی تغییرات درون گونه‌ای فقط در Phaseolus vulgaris و تا حدودی در Lathyrus sativus مشاهده گردید. این نوع تغییرات بین نمونه‌ای (درون گونه‌ای) در گونه‌های دیگر بسیار محدود و قابل صرفنظر بود. تجزیه خوشه‌ای پس از کمی کردن نوارهای پروتئینی و با استفاده از روش UPGMA نشان داد که مطابقت خوبی بین طبقه‌بندی حبوبات زراعی انجام شده براساس صفات مورفولوژیک و متداول وجود دارد. نمونه‌های طایفه ویسیه (Vicia ervilia, Lathyrus-sativus, Pisum sativum, Lens culinaris, Vicia faba) در یک گروه قرار گرفتند. در این گروه سه دسته پلی‌پپتید با وزن مولکولی سبک، متوسط و سنگین در مقدار تقریبا" یکسان وجود دارد. نمونه‌های طایفه سیسره (مخصوصا"Cicer arietinum) گروه دوم را تشکیل دادند که دودسته پلی‌پپتید با وزن مولکولی سبک ومتوسط دارند.گونه‌های طایفه فازئوله (Vigna radiata, Vigna unguiculata, Phaseolus vulgaris)گروه سوم را به وجود آوردند و عمدتا" پلی‌پپتیدهای با وزن مولکولی سنگین دارند. بالاخره، متمایزترین گروه را نمونه‌های طایفه اسکینومنه (Arachis hypogaea) به خود اختصاص داد که ازالگوی نواربندی خاصی برخورداراست. تنها ناهماهنگی موجود درمورد Glycine max دیده شد که به طایفه فازئوله تعلق داردولی درگروه طایفه سیسره قرارگرفت.

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