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

M. Valizadeh

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 “sub-family” of *Papilionidae* or *Faboidae*, were recently grouped into the five following tribes (Summerfield and Roberts, 1985): Phaseoleae, Vicieae, Cicereae, Aeschynomeneae, 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 programs (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.

1 Department of Agronomy, Faculty of Agriculture, Tabriz University, Tabriz, Islamic Republic of Iran.
Using protein polymorphism, a comparative study was undertaken to test the reliability of this method for estimating the genetic resemblance among 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>
<thead>
<tr>
<th>no</th>
<th>Species</th>
<th>Tribe</th>
<th>Number of accessions</th>
<th>Common name</th>
<th>Chromosome number</th>
<th>Origin or site of domestication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phaseolus vulgaris</td>
<td>phaseoleae</td>
<td>4</td>
<td>common bean</td>
<td>22</td>
<td>Mexic, Peru</td>
</tr>
<tr>
<td>2</td>
<td>Vigna unguiculata</td>
<td></td>
<td>2</td>
<td>cowpea</td>
<td>22, 24</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>Vigna radiata</td>
<td></td>
<td>2</td>
<td>mung bean</td>
<td>22</td>
<td>Ethiopia, Africa</td>
</tr>
<tr>
<td>4</td>
<td>Glycine max</td>
<td></td>
<td>4</td>
<td>soybean</td>
<td>40(4x)</td>
<td>China</td>
</tr>
<tr>
<td>5</td>
<td>Vicia faba</td>
<td>Vicieae</td>
<td>2</td>
<td>faba bean</td>
<td>12, 14</td>
<td>Middle East</td>
</tr>
<tr>
<td>6</td>
<td>Lens culinaris</td>
<td></td>
<td>7</td>
<td>lentil</td>
<td>14</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>Pism sativum</td>
<td></td>
<td>1</td>
<td>pea</td>
<td>14</td>
<td>Near East</td>
</tr>
<tr>
<td>8</td>
<td>Lathyrus sativus</td>
<td></td>
<td>8</td>
<td>chickling vetch</td>
<td>14</td>
<td>Middle East</td>
</tr>
<tr>
<td>9</td>
<td>Vicia ervilia</td>
<td></td>
<td>4</td>
<td>bitter vetch</td>
<td>14</td>
<td>Turkey, Europe</td>
</tr>
<tr>
<td>10</td>
<td>Cicer arietinum</td>
<td>Cicereae</td>
<td>11</td>
<td>chickpea</td>
<td>16</td>
<td>Middle East, Iran</td>
</tr>
<tr>
<td>11</td>
<td>Arachis hypogaea</td>
<td>Aeschnomeneae</td>
<td>2</td>
<td>groundnut</td>
<td>40(4x)</td>
<td>Bolivia, Argentina</td>
</tr>
</tbody>
</table>

**Protein Extraction**

Total salt soluble proteins were extracted by adding 30mg of ground seeds in 1ml of 50mM tris-HCl (pH 7.5) and 0.5 M NaCl 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 dodecyl sulfate-polyacrylamide gel electrophoresis was carried out according to Laemmli (1970). Ten percent of resolving slab gels were used (16×16×0.2 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-HCl (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.
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 contradiction with suggestions of Cooke (1992) and Kapse and Nerkar (1985). To find polymorphisms within accessions of grain legumes which are predominantly 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.

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

Figure 2. Different banding pattern observed for the “Tabriz population of *Lathyrus sativus*” (second sample from the left).
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 profiles is, therefore, an efficient procedure for differentiating grain legume species. Several researchers have confirmed the usefulness of different SDS-PAGE procedures in plant taxonomic, evolutionary and genetic relationship studies (Ladizinsky and Hymowitz, 1979, Ladizinsky and Van Oss, 1984 and Virihnos 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 clustered with Cicereae.

ACKNOWLEDGEMENTS

The author would like to thank the Gene Bank, at the Plant Breeding Institute, Karaj for providing the majority of plant materials and the Department of Research Affairs, Tabriz University for their financial support.
REFERENCES

بررسی پروتئین‌های ذخیره‌ای داه در حیوانات زراعی ایران از طریق الکتروفوروز SDS-PAGE

م. ویزاده

چکیده

الگوی پروتئینی داه در 47 نمونه (واقعی یا جمعیت) متعلق به 11 گونه و 4 طایفه از حیوانات زراعی از طریق الکتروفوروز در زل سدیم دو دسیل سولفات- پلی اکریلیمید، با استفاده از پروتئین‌های کل استخراج شده در 10 نک‌دانه از هر نمونه مورد مطالعه قرار گرفت. همه 11 گونه به طور روش‌های روزی الگوی توپرینی درون گونه‌ها، یا Phaseolus vulgaris به واسطه یک شناسایی می‌پردازند. ویلی نگیرنده درون گونه‌ای فقط در شناخته‌گر درده، با توجه به سایر محدودیت‌های گونه‌ای، این نوع نگیرنده صورت نمی‌گیرد. این نگیرنده بین نمونه‌ای (درون گونه‌ای) در گونه‌های مبتنی بر سیسی و قابل صرف‌نظر بود. تجربه خوشه‌ای پس از کمی کردن نوارهای پروتئین و یا UPGMA نشان داد که مطالعه خوبی بین طبقه‌بندی حیوانات زراعی انجام شده برحسب صفات مورفولوژیک و مداوم و وجود دارد. نمونه‌های طبقه ویژه (Vicia ervilia, Lathyrus-sativus, Pisum sativum, Lens culinaris, Vicia faba روی گروه Cicer arietinum, C. reticulatum) قرار گرفتند. در این گروه سه دسته پلی‌پتید با وزن مولکولی سبک، متوسط و سنگین در مقدار تقریباً یکسان وجود دارد. نمونه‌های طیفه سپر (مخصوصاً Cicer arietinum) گروه دوم را تشکیل دادند که دو دسته پلی‌پتید با وزن مولکولی سبک و متوسط دارند. نمونه‌های طیفه فازوله (Vigna radiata, Vigna unguiculata, Phaseolus vulgaris) پلی‌پتیدهای با وزن مولکولی سنگین دارند. با این حال، میزان توزین گروه را نشان می‌دهد. نمونه‌های طیفه اسکینومه (Arachis hypogaea) به کمیت اضافه‌داری و پاک‌داری خاصی برخوردارند. نهایتاً نشان می‌دهد که طیفه فازوله مودود در گروه سپر قرار گرفت.