Pak. J. Bot., 36(1): 25-29, 2004.

SEED STORAGE PROTEIN ELECTROPHORESIS IN GROUNDNUT FOR EVALUATING GENETIC DIVERSITY

ASIF JAVAID, ABDUL GHAFOOR AND RASHID ANWAR

Plant Genetic Resources Programme,
Institute of Agri Biotechnology and Genetic Resources,
National Agricultural Research Center, Islamabad, Pakistan.

Abstract

One hundred and fifty one accessions of groundnut representing five continents were evaluated for total seed protein by SDS-PAGE using slab type gel electrophoresis with 11.25 % polyacrylamide gel. Five major bands were recorded and most of the accessions were similar and only 8 differed for one band. Due to low genetic diversity for SDS-PAGE, 2D-electrophoresis was suggested to separate various proteins observed on gel. SDS-PAGE might be used for interspecific diversity and phylogenetic or evolutionary relationships among various species rather than intraspecific variation. Low genetic diversity may be attributed towards narrow genetic base of the crop or *A. hypogaea* spread all over the world from same origin. Further collection and acquisition of cultivated as well as wild groundnut should be strengthened to find a broad based germplasm for future use. Hybridization between accessions from two groups i.e., one with all 5 bands and other missing band 4 is suggested to investigate inheritance and linkage of this band. This might be linked with other agronomic characters that would help in planning experiments for marker assisted breeding in groundnut.

Introduction

Groundnut (*Arachis hypogaea*) is an annual warm-season plant of the legume family that originated in South America and groundnut has been cultivated since ancient times. The seeds contain 40-50% oil, 20-30% proteins and are an excellent source of B vitamins. Large-seeded varieties are used for roasting and confections and small-seeded types are used for oil. The groundnut meal is rich in protein and can be used as food or feed (Pardee, 2002). In Pakistan, the peanuts are consumed as roasted kernels. Because of the greater availability of suitable area for its cultivation, more than 90% of the total area under groundnut is now in Punjab of which nearly 87% is located in Rawalpindi, Jhelum and Attock districts (Hatam & Abbasi, 1994).

Availability of genetic variation is important for genetic improvement of the crop. Local and exotic germplasm can be used as source of genetic variation. Protein markers can act effectively to study the genetic variation of germplasm for its utilization in crop breeding programs. Many workers used seed protein electrophoresis for characterization of cultivated and wild species of groundnut (Ory & Cherry, 1972; Dawson & McIntosh, 1973; Cherry, 1975; Savoy *et al.*, 1978; Klozova *et al.*, 1983; Chiou *et al.*, 1988; Krishna & Mitra, 1988; Singh *et al*., 1993). The study showed that diversity exists for protein profiles and seed storage proteins have potential for aiding species classification and for serving as markers for interspecific hybridization studies. Low intraspecific diversity has been reported (Lanham *et al.*, 1994). Major variations between the accessions were confined to the region of arachin. There was no correlation between seed protein profiles and geographic distribution (Bertozo & Valls, 2001).

Table 1. Accessions of two clusters based on SDS-PAGE electrophoresis.

Cluster	Freq.	Accessions
Cluster I	8	PAK0090329 (CHINA), PAK0090251, PAK0090252, PAK0090257, PAK0090330, PAK0090332 (USA), PAK0090460 (SUDAN), PAK0090490 (INDONESIA)
Cluster II	143	PAK0090328, PAK0090348, PAK0090318, PAK0090325 (ARGENTINA), PAK0090327 (AUSTRALIA), PAK0090447, PAK0090452, PAK0090448 (BANGLADESH), PAK0090442 (BHUTAN), PAK0090300, PAK0090310 (BRAZIL), PAK0090456 (CANADA) PAK0090161, PAK0090443 (CHINA), PAK0090422 (EGYPT), PAK0090351 (HONDURAS), PAK0090291, PAK0090294, PAK0090296, PAK0090298, PAK0090303, PAK0090304, PAK0090306, PAK0090308, PAK0090311, PAK0090320, PAK0090321, PAK0090292, PAK0090295, PAK0090305, PAK0090307, PAK0090309, PAK0090354, PAK0090355, PAK0090357, PAK0090358, PAK0090375, PAK0090376, PAK0090444, PAK0090445, PAK0090446 (INDIA), PAK0090394, PAK0090395, PAK0090483, PAK0090484, PAK0090485, PAK0090487, PAK0090488, PAK0090489, PAK0090486 (INDONESIA), PAK0090334, PAK0090335 (IVORY COAST), PAK0090387, PAK0090388 (JAPAN), PAK0090302 (MALI), PAK0090449, PAK0090450, PAK0090451 (NEPAL), PAK0090319, PAK0090356, PAK0090362, PAK0090363, PAK0090379 (NIGERIA), PAK0090151, PAK0090152, PAK0090158, PAK0090171, PAK0090173, PAK0090423, PAK0090455, PAK0090458, PAK0090459, PAK0090517, PAK0090518, PAK0090653, PAK0090654, PAK0090655, PAK0090656, PAK0090657, PAK0090658, PAK0090736, PAK0090737, PAK0090170, PAK0090172 (PAKISTAN), PAK0090345, PAK0090350 (PERU) PAK0090333, PAK0090389, PAK0090390, PAK0090391, PAK0090392, PAK0090393, PAK0090453, PAK0090384 (SENEGAL), PAK0090364, PAK0090366, PAK0090373, PAK0090377, PAK0090378 (SUDAN), PAK0090322, PAK0090382 (TANZANIA), PAK0090383, PAK0090347 (UGANDA), PAK0090336, PAK0090337, PAK0090338 (UPPER VOLTA), PAK0090153, PAK0090154, PAK0090155, PAK0090156, PAK0090157, PAK0090159, PAK0090160, PAK0090162, PAK0090164, PAK0090169, PAK0090206, PAK0090254, PAK0090255, PAK0090256, PAK0090258, PAK0090259, PAK0090260, PAK0090261, PAK0090262, PAK0090301, PAK0090324, PAK0090331, PAK0090339, PAK0090340, PAK0090341, PAK0090342, PAK0090367, PAK0090370, PAK0090371, PAK0090374, PAK0090381, PAK0090386, PAK0090253 (USA), PAK0090365, PAK0090380 (ZAIRE), PAK0090349, PAK0090385 (ZIMBABWE).

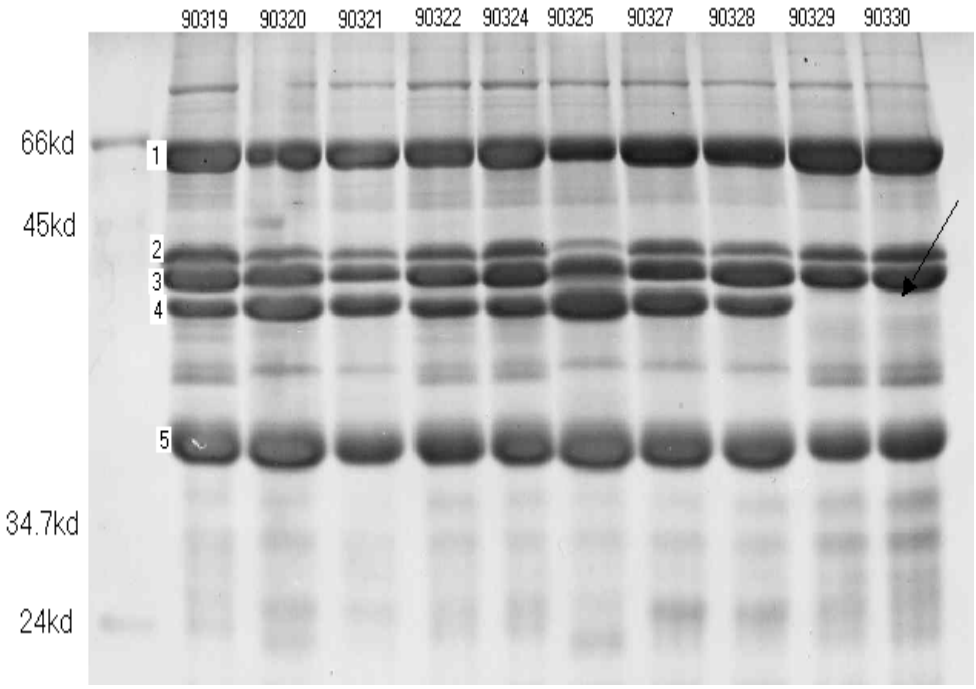

Fig. 1. Variation in protein bands on polyacrylamide gel of groundnut. The marker used was SDS-70 from Sigma Chemical Corporation. The arrow represents the absence of band 4.

The Genebank of Plant Genetic Resources Institute, NARC, Islamabad maintains a diverse collection of local and exotic groundnut germplasm (Bhatti *et. al.*, 1997). The objective of this study was to estimate the variation of seed storage proteins in groundnut germplasm.

Materials and Methods

One hundred and fifty one accessions of groundnut germplasm from the genebank of PGRI were used in the experiment (Table 1). The material included local germplasm from Pakistan (21) and exotic germplasm from Argentina (4), Australia (1), Bangladesh (3), Bhutan (1), Brazil (2), Canada (1), China (3), Egypt (1), Honduras (1), India (25), Indonesia (10), Ivory Coast (2), Nigeria (5), Peru (2), Senegal (8), Sudan (6), Tanzania (2), Uganda (2), USA (28) and Zimbabwe (2).

Total seed proteins were extracted from single grain. Seed was ground to fine powder with mortar and pestle. Sample buffer (400 µl) was added to 0.01 g of seed flour as extraction liquid and mixed thoroughly in eppendorf tube. The extraction buffer contained 0.5M Tris-HCl (pH6.8), 2.5%SDS, 10% glycerol and 5%2-mercaptoethanol. Bromophenol Blue (BPB) was added to the sample buffer as tracking dye to watch the movement of protein in the gel. To purify extraction, the homogenate samples were

mixed thoroughly by vortexing and centrifuged at 15000rpm for 5 minutes. The extracted proteins were recovered as supernatant and stored in the refrigerator until electrophoresis. Proteins along with a set of protein markers SDS-70 were analyzed through the slab type SDS-PAGE following discontinuous method of electrophoresis (Laemmli, 1970). The gel concentration was 11.25%. The electrophoresis was performed for 4 hours at 100V constant. The gel was stained with coomassie brilliant blue for about 20-30 minutes and then destained in 5% methanol 20% acetic acid until the color of background disappeared and the electrophoretic bands were clearly visible. After destaining the gels were dried at room temperature.

To avoid any ambiguity in the data, only major protein bands between 66 kd and 24 kd were considered for data recording. The data was recorded as presence or absence of protein markers and entered in a binary matrix. Data were analyzed using the computer software STATISTICA to construct dendrogram for the study of germplasm heterogeneity.

Results and Discussion

Five major bands were recorded and it was observed that protein profiles of most of the germplasm accessions were same for these bands (Fig. 1). The bands 1, 2, 3 and 5 were present in all the accessions whereas band 4 was missing in 8 accessions. Cluster analysis for germplasm accessions sorted the germplasm into two groups and the accessions in these two clusters along with origin are given in Table 1. All the accessions in which band 4 was missing were of exotic origin *i.e.*, one (PAK0090329) from China, five (PAK0090251, PAK0090252, PAK0090257, PAK0090330, PAK0090332) from USA, one (PAK0090460) from Sudan and one (PAK0090490) from Indonesia. All the other accessions (143) were in one group despite of the diverse origin as germplasm presents 5 continents *i.e.*, Asia, Africa, North America, South America and Australia. It was concluded that the material preserved in the genebank of PGRI exhibited low genetic diversity for SDS-PAGE (single dimension), therefore 2D-electrophoresis is needed to separate various portions of the gel and this technique has already been used by Li *et. al.*, (1998), who used single dimension and 2D-electrophoresis in 46 accessions of groundnut and reported the usefulness of later technique. Further DNA markers are also suggested to investigate intraspecific genetic diversity of *A. hypogaea*.

Low genetic diversity in *Arachis hypogaea* has been reported by Singh *et al*., (1993) and no geographical pattern for variation was found (Bertozo & Valls, 2001). Although the material included in the present study originated from various sources and differed from the material used by other researchers but even then low genetic diversity revealed insignificance of SDS-PAGE for studying intraspecific diversity in cultivated groundnut. Since many workers such as Cherry (1975), Klozova *et al.*, (1983), Krishna & Mitra (1988), Singh *et al.*, (1993), Lanham *et al.*, (1994) and Bertozo & Valls (2001) reported significant variation among different species of groundnut for seed storage proteins, therefore it is suggested to use different species of groundnut to increase the genetic diversity of the germplasm. This technique could better be used for interspecific diversity and phylogenetic or evolutionary relationships among various species. The accessions lacking band 4 were of exotic origin but there did not exist any relationship because these 8 accessions represent 3 continents. This did not suggest any relationship on the basis of geographic origin of cultivated groundnut that might be because of narrow genetic base of the crop or a limited portion of genepool represents cultivated species. Further, this

species originated from South America from where it spread allover the world (Larik, 1994). Diverse germplasm based on SDS-PAGE, as various workers reported diversity for different loci is suggested to assemble to have a large collection especially from centers of diversity.

It may be concluded that hybridization between accessions from two groups (one with all 5 bands and other missing band 4 is suggested to be conducted with the expectation that band 4 might be linked with agronomic characters. This would help in planning experiments for marker assisted breeding in groundnut.

References

Bertozo, M.R. and J.F.M. Valls. 2001. Seed storage protein electrophoresis in *Arachis pintoi* and *A. repens* (*Leguminosae*) for evaluating genetic diversity. *Genetic Resources and Crop Evolution*, 48(2): 121-130.

Bhatti, M.S., A. Qayyum and N. Kazmi. 1997. *Plant germplasm catalog*. Plant Genetic Resources Institute, National Agricultural Research Center, Islamabad.

Cherry, J.P. 1975. Comparative studies of seed proteins and enzymes of species and collections of *Arachis* by gel electrophoresis. *Peanut Science*, 2(2): 57-65.

Chiou, Y.Y., C.S. Hsu, F.Z. Tyan and T.T. Tsai. 1988. Physical and chemical study of proteins in various groundnut cultivars grown in Taiwan. *Journal of the Chinese Agricultural Chemical Society*, 26(2): 189-196.

Dawson, R and A.D. McIntosh. 1973. Varietal and environmental differences in the proteins of the groundnut (*Arachis hypogaea*). *Journal of the Science of Food and Agriculture*, 24(5): 597-609.

Hatam, M. and G. Q. Abbasi. 1994. Oilseed crops. In: *Crop production.* (Eds.): S. Nazir, E. Bashir and R. Bantel. National Book Foundation, Islamabad, p. 329-389.

Klozova, E., J. Svachulova, J. Smartt, E. Hadac, V. Turkova and V. Hadacova. 1983. The comparison of seed protein patterns within the genus *Arachis* by polyacrylamide gel electrophoresis. *Biologia Plantarum*, 25(4): 266-273.

Krishna, T.G. and R. Mitra. 1988. The probable genome donors to *Arachis hypogaea* L. based on arachin seed storage protein. *Euphytica*, 37(1): 47-52.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680–685.

Lanham, P.G., B.P. Forster, P. McNicol, J.P. Moss and W. Powell. 1994. Seed storage protein variation in *Arachis* species. *Genome*, 37(3): 487-496.

Larik, A.S. 1994. Genetic resources and their conservation in crop plants. In: *Plant breeding*. (Eds.): M.A. Khan, E. Bashir and R. Bantel. National Book Foundation, Islamabad, pp. 235-254.

Li, Y., S.Z. Huang and J.R. Fu. 1998. One and two-dimensional polyacrylamide gel electrophoretic analysis of seed polypeptide composition of peanut cultivars. *Acta Botanica Sinica*, 40(6): 534-541.

Ory, R.L. and J.P. Cherry. 1972. Protein from peanut cultivars (*Arachis hypogaea*) grown in different areas. V. Biochemical observations on electrophoretic patterns of proteins and enzymes. *Journal of American Peanut Research and Education Association*, 4(1): 32-40.

Pardee, W.D. 2002. *Peanut*. Encarta Encyclopedia, Microsoft Corporation, USA.

Savoy, C.F., D. Stoker and S.K. Pancholy. 1978. Relatedness of "Jenkins", "Hawthorne" and "Storers Jumbo" peanuts. *Proceedings of American Peanut Research and Education Association, Inc.*, 10(1): 76.

Singh, A. K., S. Gurtu and R. Jambunathan. 1993. Phylogenetic relationships in the genus *Arachis* based on seed protein profiles. *Euphytica*, 74(3): 219-225.

(Received for publication 26 June 2002)