ACTA BIOLOGICA CRACOVIENSIA Series Botanica 48/1: 77–84, 2006

GEOGRAPHIC PATTERNS OF DIVERSITY OF CULTIVATED LENTIL GERMPLASM COLLECTED FROM PAKISTAN, AS ASSESSED BY SEED PROTEIN ASSAYS

TAYYABA SULTANA[1], ABDUL GHAFOOR[2*], AND MUHAMMAD ASHRAF[1]

[1]*Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[2]*Plant Genetic Resources Programme, National Agricultural Research Centre, Islamabad, Pakistan*

Received July 15, 2005; revision accepted February 15, 2006

Seed protein profiles were studied in 144 lentil accessions intensively collected from all over Pakistan. Heterogeneous populations were isolated on the basis of SDS-PAGE, and 13 polymorphic protein peptides were found, representing almost all the variation reported so far in lentil. The low diversity of accessions from the Northern Area and North Western Frontier Province, the most geographically diverse areas, suggested the need for more exploration so that the maximum genetic diversity of the areas can be truly represented. Clusters based on agro-ecological zones did not prove adequate for evaluation of lentil resources, whereas 63 of 108 accessions (58.3%) were grouped together by altitude and provincial distribution. The study confirmed the wealth of phenotypic divergence in the local lentil. A small sample of accessions from a particular region might not reflect the actual diversity within that region. Samples representing total diversity in particular countries or regions should be evaluated, so that a representative rather than a random set of accessions can be included in investigations of diversity on regional or continental scales. As Pakistan is in the vicinity of the centre of diversity of lentil, high variation of various parameters is expected, and that can be found only if a complete set of germplasm is studied.

Key words: *Lens culinaris*, cluster analysis, genetic diversity, landraces, SDS-Polyacrylamide Gel Electrophoresis.

INTRODUCTION

One approach to assembling a gene pool is to collect material from diverse geographic origins, with a concentration of accessions from the presumed centres of diversity in individual samples (Laghetti et al., 1998; Ghafoor et al., 2003b). Representative samples from the complete geographic range of the crop species can help to ensure conservation of co-adapted gene complexes (Frankel, 1984; Frankel et al., 1995; Brown, 1978; Beuselinck and Steiner, 1992). The multilocus nature of most characters provides information useful to breeders, but the complexity of inheritance makes predication of breeding outcomes difficult. The use of gene products (proteins) or low-molecular components (terpenes, flavonoids) has solved this problem in part.

Among biochemical techniques, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used, due to its reliability and simplicity in describing the genetic structure of crop germplasm (Gepts, 1989; Murphy et al., 1990). Over the last two decades, considerable interest has been focused on the use of electrophoretic methods for reliable discrimination and identification of plant varieties (Sammour, 1985; Sammour et al., 1994; Przybylska and Zimniak-Przybylska, 1995). Electrophoresis adds information to taxonomy and should not be dissociated from morphological, anatomical and cytological observations (Boulter et al., 1966; Ghafoor et al., 2002). Seed protein profiles and molecular markers obtained by electrophoresis have been successfully used to study taxonomical and evolutionary relationships of several crop plants (Gepts and Bliss, 1988; Gepts et al., 1988; Sammour, 1989; Rao et al., 1992; Das and Mukarjee, 1995).

Cultivated lentil (*Lens culinaris* Medik) is thought to have originated in the Fertile Crescent of the Mediterranean region in Western Asia, dating back to the beginnings of agriculture, from where it spread to the rest of world (Ladizinsky, 1979; Duke,

*e-mail: ghafoor59pk@yahoo.com

PL ISSN 0001-5296

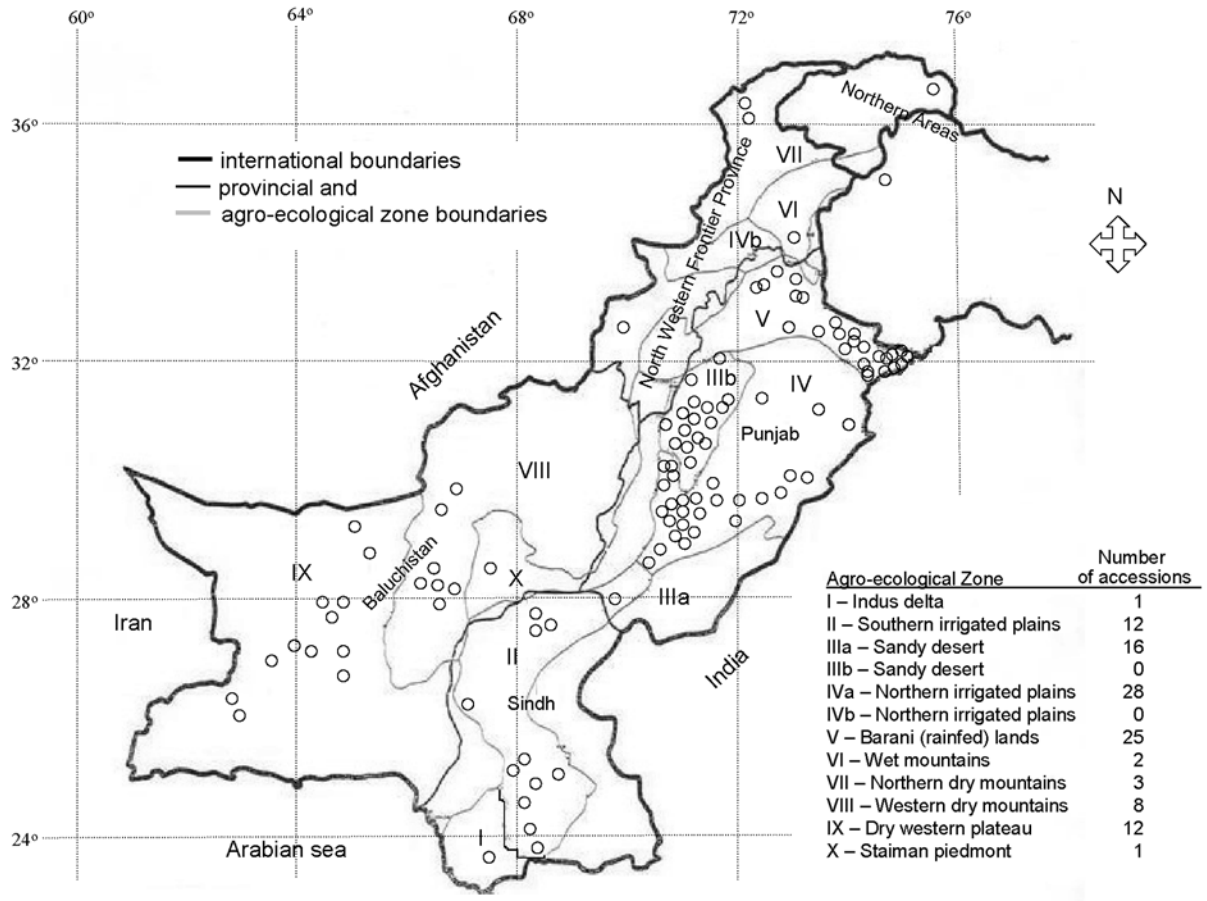

Fig. 1. Lentil germplasm collecting sites (o) in Pakistan. One circle indicates twenty samples.

1981). Lentil is unique because it can be grown in marginal environments in which other crops cannot be cultivated (Cubero, 1981). Moreover, its high protein content from 19% to 36%, 55% starch, low levels of toxic and antinutrient factors, and its ability to grow under water-deficit stress conditions, are the main attributes that make this an important crop (Savage, 1988; Bhatty, 1998). In view of the importance of the crop, and the need to enhance the scope and usefulness of its genetic resources, in this study we undertook to access and evaluate genetic diversity in lentil collected from geographic regions of Pakistan, on the basis of seed storage protein profiles.

MATERIALS AND METHODS

Accessions representing the complete geographic distribution of lentil in Pakistan (N = 144) were selected for SDS-PAGE analysis (Sultana, 2004). These accessions, mainly landraces, were collected from farmers' fields in lentil-growing areas of Pakistan (Fig. 1). Seed protein profiles were determined for 20 plants sampled at random within each accession to investigate inter- and intra-accession variation. For extraction of proteins, single seeds were ground to fine powder with mortar and pestle. Then 400 µl protein extraction buffer [Tris-HCl (pH 6.8), 2.5% SDS, 10% Glycerol, 5% 2- mercaptoethanol and Bromophenol Blue (BPB)] was added to 0.01 g seed flour and mixed thoroughly in an Eppendorf tube with a small glass rod. Seed protein extracts were analyzed through vertical slab gel in a discontinuous buffer system following the method of Laemmli (1970) by loading 10 µl protein extract solution in 9.5% polyacrylamide gel. Different researchers have used gel concentrations ranging from 7.5% to 17.0% (Masood et al., 2003; Piergiovanni and Taranto, 2005). The proteins in the gels were stained with Coomassie Brilliant Blue (CBB R 250). To check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions. The molecular weights of the dissociated polypeptides were determined using molecular weight protein standards in the SDS–70 Kit (Sigma Chemical Company, U.S.A.).

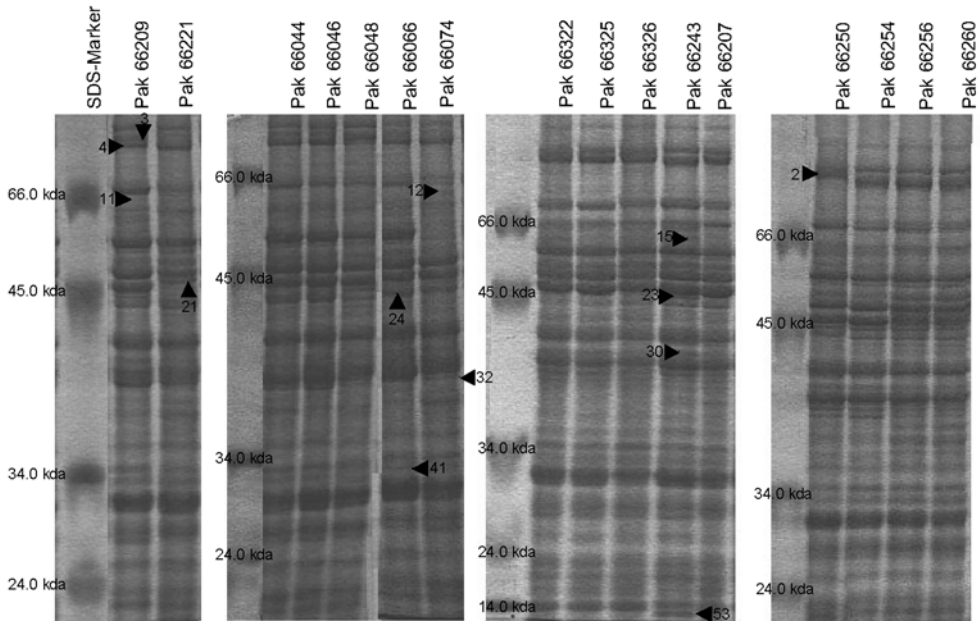

Fig. 2. Variation of seed proteins of lentil germplasm. Arrows idicate 13 polymorphic peptides. Gel was devided into six regions of bands: I – >66 kda; II – 45–66 kda; III – 34–45 kda; IV – 24–34 kda; V – 18–24 kda; VI – <14 kda.

After staining and destaining of the gels, genetic diversity was estimated based on the presence or absence of peptides. The presence/absence of the peptide was entered in a binary data matrix. The scores were 1 for the presence and 0 for the absence of a band. To avoid taxonomic weighting, the intensity of bands was not taken into consideration. The SDS-PAGE data were analyzed for simple statistics and cluster analysis with STATISTICA 6.0 and SPSS 7.5.1 for Windows (Statsoft, 2001; SPSS, 1996). The data were analyzed on the basis of provinces, agro-ecological zones, and altitude at intervals of 500 m. The binary matrix was used to calculate the coordinates for graphic presentation of accessions based on provincial and clustering patterns in scatter diagrams with the use of SPSS 7.5.1. The agro-ecological zones followed *Agro-Ecological Regions of Pakistan*, which divides the country into 10 zones on the basis of geology, climate, agricultural land use and water availability (PARC, 1980). A similarity index was used to construct a dendrogram by the UPGMA method (Sneath and Sokal, 1973).

RESULTS

SDS-PAGE was run in various combinations, and it turned out that a 9.5% acrylamide gel concentration and 10 µl sample gave the best resolution (Fig. 2). The seed protein profile data were split into two categories of accessions, homogeneous and heterogeneous, on the basis of within-accession variation. Of 144 accessions, 108 were homogeneous and the others were heterogeneous. In total, 55 protein bands were recorded, ranging in molecular weight from 14 KDa to 66 KDa, and 13 of these were polymorphic. Occasionally the density or sharpness of a few peptides varied, but this variation was not considered. On the basis of banding pattern, the gel was divided into six regions with intervals of molecular markers. Region I had 9 bands of more than 66 KDa, of which 3 were polymorphic. Region II ranged from 45 KDa to 66 KDa, with 14 protein peptides, of which 5 were polymorphic. Region III ranged from 34 KDa to 45 KDa, with 19 protein subunits, of which 4 were polymorphic. Regions IV and V, ranging from 18 KDa to 34 KDa, had 9 monomorphic bands. Region VI, ranging from 14 KDa to 18 KDa, had 4 bands, one of which was polymorphic.

As shown in Table 1, band B_2 exhibited variation in the accessions collected from Baluchistan and Punjab. The material collected from Punjab Province was polymorphic for all 13 protein peptides; the next most polymorphic germplasm was that collected from Baluchistan. When these data were analyzed on the basis of agro-ecological zone and altitude, it indicated that the germplasm collected from zones 4 and 5 was diverse, with more than half of the loci heterogenous. The first range of altitude (0–500 m) revealed diversity for all loci. The accessions of cluster I were distributed more in zones 4 and 9 (Tab. 1, Fig. 3). Cluster II consisted of accessions collected from zones 2 and 4. Cluster III consisted of zones 4 (6.7% of accessions), 5 (6.7%),

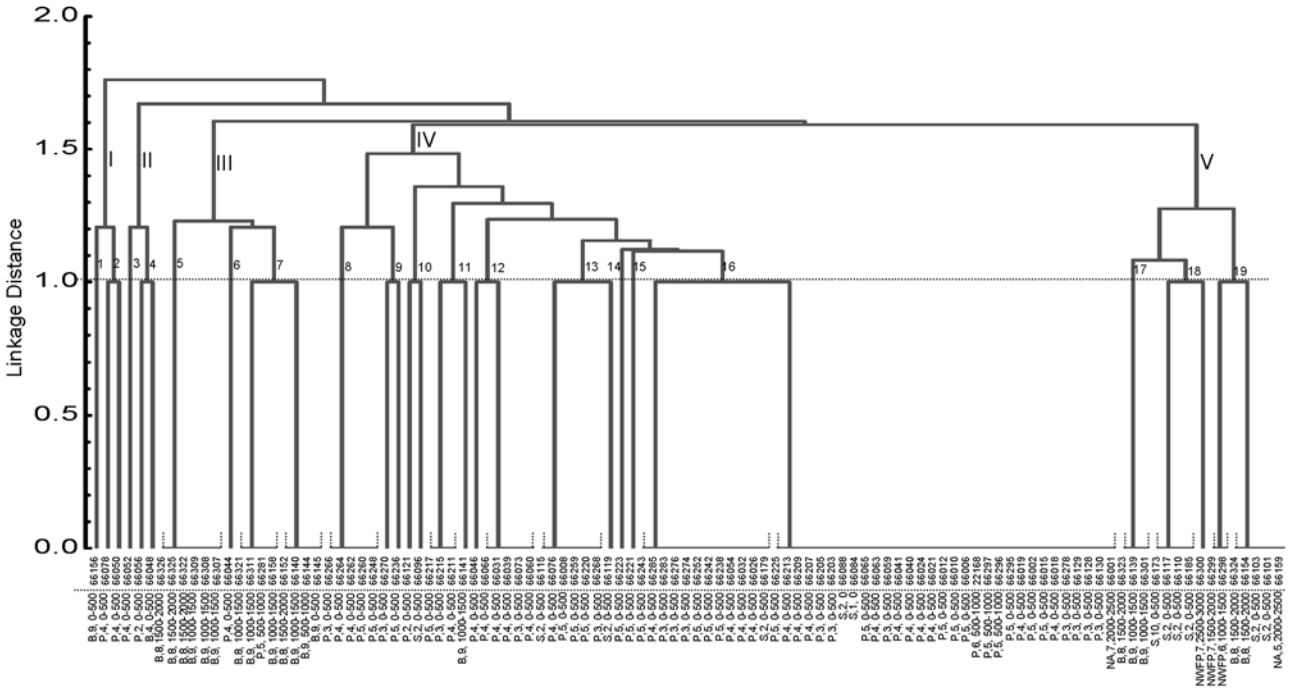

Fig. 3. Cluster diagram of 108 homozygous accessions of *Lens culinaris* based on SDS-PAGE markers. Collection sites were Punjab (P), Baluchistan (B), Sindh (S), Northern Areas (NA) and North Western Frontier Province (NWFP). Numbers 1 to 10 are agro-ecological zones given in Figure 1. The last segment presents the altitude range of the collection site.

8 (33.3%) and 9 (53.3%). The accessions of cluster IV were distributed in all zones except 8 (Western Dry Mountains) and 10 (Staiman Piedmont). The cluster V accessions were distributed in all zones except 1 and 4. The accessions of zones 4 and 9 had the maximum representation in the clusters, appearing in four of the five. The high diversity of the accessions collected from Punjab Province may be attributable to sampling, as this area has been explored the most in the last two decades. The lowest degree of polymorphism was found in the accessions from the Northern Area and North Western Frontier Province, where variation was observed in two protein peptides. These areas are geographically diverse and one should expect more genetic diversity, but their accessions and thus the actual diversity of their germplasm was less well represented. These areas should be sampled more intensively so that the genetic diversity of lentil in the area can be accurately portrayed.

The heterogeneous accessions were not considered for analysis and presentation due to low intra-accession variation. All the polymorphic peptides observed within heterogeneous accessions were also recorded in the material evaluated, hence all the variation for SDS-PAGE was included in the analysis and presentation. The cluster diagram using UPGMA revealed 5 clusters, and these were subdivided into 19 sub-clusters (Fig. 3). Three accessions were in cluster I, 3 in II, 15 in III, 71 in IV, and 16 in cluster V. The level of association for grouping on the basis of provinces and altitude was low; 13 of 15 accessions of cluster III were collected from Baluchistan, mostly from high altitudes, whereas 61 of 71 were from Punjab and were mostly from low altitudes. In terms of the three geographic parameters, province and altitude grouped the accession sub-clusters, whereas agro-ecological zone was independent of the clustering pattern. The provincial distribution data indicated some relation with protein bands, and thus were plotted in a scatter diagram on the basis of the first two components, which contributed to 26.23% of the total variation (Fig. 4). Separation on the basis of PC_1 and PC_2 revealed that the germplasm collected from Punjab was scattered, and many of these accessions overlapped due to similarity. The scatter diagram revealed that 21.43% of the accessions from Punjab, 20% of those from Sindh, 30% of those from Baluchistan, 50% of those from the North Western Frontier Province and 100% from the Northern Area were represented on the basis of dissimilarity.

The scatter diagram for PC_1 and PC_2 revealed separation on the basis of classification, and 26.7% of the accessions of cluster III, 19.7% of IV and 31.2% of cluster V were represented on the basis of similarity, whereas all the accessions of cluster I were scattered.

TABLE 1. Diversity of 13 polymorphic peptides for three geographic parameters in lentil germplasm collected from Pakistan

		Polymorphic protein band												
	f	B_2	B_3	B_4	B_{11}	B_{12}	B_{15}	B_{21}	B_{23}	B_{24}	B_{30}	B_{32}	B_{41}	B_{53}
Province														
Baluchistan	20	1/0	1	1/0	1	1	1/0	0	1/0	1/0	0	1/0	1	1
Northern Areas	2	1	1	1/0	1	1	1	0	1	1/0	0	0	1	1
NWFP	4	1	1	0	1	1	1	0	1	1/0	0	1/0	1	1
Punjab	70	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0
Sindh	12	1	1	1/0	1	1	1/0	0	1	1/0	0	1/0	1	1
Agro-ecological zone														
Zone 1	1	1	1	1	1	1	1	0	1	1	0	0	1	1
Zone 2	12	1	1	1/0	1	1	1/0	0	1/0	1/0	1/0	1/0	1	1
Zone 3a	16	1/0	1	1/0	1	1	1	0	1/0	1/0	0	1/0	1/0	1
Zone 4a	28	1/0	1/0	1	1/0	1/0	1	0	1/0	1/0	1/0	1/0	1/0	1
Zone 5	25	1/0	1	1/0	1	1	1	1/0	1/0	1/0	0	1/0	1/0	1/0
Zone 6	2	1	1	1/0	1	1	1	0	1	1	0	0	1	1
Zone 7	3	1	1	1/0	1	1	1	0	1	1	0	1/0	1	1
Zone 8	8	1/0	1	1/0	1	1	1	0	1/0	0	0	1/0	1	1
Zone 9	12	1/0	1	1/0	1	1	1/0	0	1/0	1/0	0	1/0	1	1
Zone 10	1	1	1	0	1	1	1	0	1	0	0	1	1	1
Altitude (m)														
0–500	81	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0
50–1000	5	1	1	1/0	1	1	1	0	1/0	1/0	0	0	1	1
100–1500	11	1/0	1	1/0	1	1	1/0	0	1/0	1/0	0	1/0	1	1
1501–2000	8	1/0	1	1/0	1	1	1	0	1/0	1/0	0	1/0	1	1
2001–2500	2	1	1	1/0	1	1	1	0	1	1/0	0	0	1	1
2500–3000	1	1	1	0	1	1	1	0	1	1	0	1	1	1

1 – indicates the presence of the protein band; 0 – indicates absence of the protein band; 1/0 – indicates polymorphism of the protein band in the germplasm; *f* – number of accessions; NWFP – North Western Frontier Province.

DISCUSSION

Sampling and assays have been successfully used to study the geographic variation of seed storage protein (Bogyo et al., 1980; Erskine and Muehlbauer, 1991; Piergiovanni and Taranto, 2003; Ghafoor et al., 2003a). In the present investigation, four zones of the electrophoretic profile exhibited variation, with major differences in region II, where 5 of 14 protein peptides were polymorphic. Similarity of banding patterns between accessions may be due to duplications in the germplasm, but this should be confirmed with two-dimensional electrophoresis (e.g., Celis and Bravo, 1984; Beckstrom-Sternberg, 1989; Higginbotham et al., 1991; Picard et al., 1999; Thiellement et al., 2005). In our study, SDS-PAGE showed low intraspecific variation. SDS-PAGE should be used to select diverse accessions from various sources, preferably from the centre of diversity, so that a broad-based gene pool can be acquired. For better management of the gene bank, precise and comprehensive knowledge of agricultural and biochemical data (proteins and DNA) is essential so that duplicates can be eliminated; this will help in compiling a core collection of lentil germplasm.

According to Perry and McIntosh (1991), differentiation by geographic region of origin is useful in substantiating postulated regions of diversity or gene centres. Rare alleles occurring in only one or two apparently random populations can be considered mutants, migrants or the result of other coincidental events (van Hintum and Elings, 1991). Alleles common in restricted areas occur mostly in high mountainous areas. This could indicate that genetic material has been introduced from the foothills of

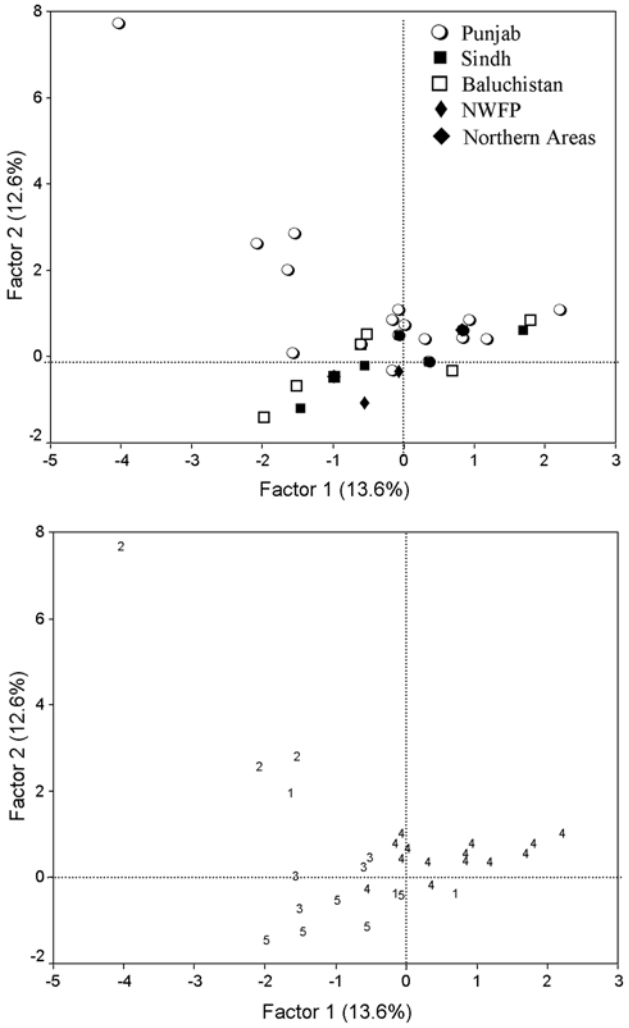

Fig. 4. Scatter diagram of 108 accessions of lentil based on SDS-PAGE for distribution against provinces (above) and clusters (below).

the North Western Frontier Province to the high mountains of the North Western Frontier Province and Northern Area. Migration of landraces into new regions, followed by some degree of contamination by mixing with other landraces, can be expected in a country like Pakistan, where movement of germplasm from one area to another is not restricted. Areas with a high levels of environmental stress will present mixtures with interesting types of tolerance to environmental stresses but which are homogeneous; these areas require less extensive sampling for the purpose of conserving genetic resources.

Clusters based on agro-ecological zones did not prove useful for the studied lentil germplasm, whereas altitude and provincial distribution grouped 63 accessions out of 108 (58.3%). Cluster analysis also grouped many same-source accessions separately, perhaps due to exchange of germplasm by breeders or transport of pulses to different markets from where seed of various origins is disseminated throughout the country. According to Smith et al. (1995), linkage clustering and PCA are useful for preservation and utilization of germplasm.

On the whole, the multivariate approach proved to be a very useful tool, in that it produced five clusters based on SDS-PAGE, much more differentiated by provincial distribution than by subdivision of clusters solely according to the altitude and agro-ecological zone of the collecting site. The study confirmed the wealth of phenotypic diversity in the local lentil germplasm. The variation within Pakistan seems largely attributable to province. Further collection missions to the main lentil-growing areas should concentrate efforts on sampling as many geographically and ecologically distinct areas as possible, rather than collecting extensively from fields close to motorable roads in individual provinces, as has already been suggested by Pecetti et al. (1996) for tetraploid wheat. For cowpea, Laghetti et al. (1998) suggested sending collection expeditions to areas where genetic erosion is taking place, along with the areas where the existing genetic diversity has not yet been sampled (Padulosi, 1993).

Brown (1978), Frankel (1984) and Huh and Huh (2001) considered geographic origin the best criterion for examining variation, whereas Kumar and Arora (1992) reported no definite relationship between genetic diversity and geographic distribution. Previous studies on germplasm from Pakistan as reported by Piergiovanni and Taranto (2003) indicated low diversity of seed proteins in lentil, but in the present study the large collection we examined showed diversity for all three regions, and almost all the polymorphic bands reported earlier in cultivated lentil were observed in the present material. A small number of germplasm collections from a particular region might not accurately represent the actual diversity within that region. Samples representing the total diversity in a particular country or region should be evaluated, so that a representative rather than random set of accessions can be used for investigations of diversity on the regional or continental level. As Pakistan is in the vicinity of the centre of diversity of lentil, high variation of various parameters is expected, and that can be found only if a complete set of germplasm is studied. Although germplasm collected from high altitudes of the Northern Areas exhibited low genetic diversity, the high geographic diversity of the area means that some classic diversity should be expected, and further collection missions to the area are suggested.

SDS-PAGE on 9.5% acrylamide in a large gel can be employed to study intraspecific diversity. It enabled us to isolate landraces, which require critical evaluation to isolate pure lines. There is a wealth

of inter- and intra-accession variation in local lentil germplasm that is yet to be collected. Further collections are needed to expand the gene pool. Except for Punjab Province, all areas in Pakistan should be explored before their local germplasm becomes extinct. SDS-PAGE showed a low level of diversity in our accessions, but higher than in other legumes, and it can be increased by the addition of more extensively sourced germplasm.

REFERENCES

Beckstrom-Sternberg SM. 1989. Two-dimensional gel electrophoresis as a taxonomic tool: evidence from the Centrspermae. *Biochemical and Systematic Ecology* 17: 573–582.

Beuselinck PR, and Steiner JJ. 1992. A proposed framework for identifying and utilizing plant germplasm resources. *Field Crop Research* 29: 261–272.

Bhatty RS. 1998. Composition and quality of lentil (*Lens culinaris* Medik): A review. *Canadian Institute of Food Science and Technology* 21: 144–160.

Bogyo TP, Porceddu E, and Perrino P. 1980. Analyses of sampling strategies for collecting genetic material. *Economic Botany* 34: 160–174.

Boutler D, Thurman DA, and Turner BL. 1966. The use of disc electrophoresis of plant proteins in systematics. *Taxon* 15: 135–143.

Brown AHD. 1978. Isozymes, plant population genetic structure and genetic conservation. *Theoretical and Applied Genetics* 52: 145–157.

Celis JE, and Bravo R. 1984. *Two dimensional gel electrophoresis of proteins: methods and applications*. Academic Press, New York.

Cubero JI. 1981. Origin, taxonomy and domestication. In: Webb C, Hawtin G [eds.], *Lentil*, 15–38. C.A.B., Landon, UK.

Das S, and Mukarjee KK. 1995. Comparative study on seed proteins of *Ipomoea*. *Seed Science and Technology* 23: 501–509.

Duke JA. 1981. *Handbook of legumes of world economic importance*, 52–57. Plenum Press, New York.

Erskine W, and Muehlbauer FJ. 1991. Allozyme and morphological variability: outcrossing rate and core collection formation in lentil germplasm. *Theoretical and Applied Genetics* 83: 119–125.

Frankel OH. 1984. Genetic perspectives of germplasm conservation. In: Arber W, Limensee K, Peacock WJ, Starlinger P [eds.], *Genetic manipulation: impact on man and society*, 161–170. Cambridge Univ. Press, Cambridge.

Frankel OH, Brown AHD, and Burdon JJ. 1995. *The conservation of plant biodiversity*. Cambridge University Press, Cambridge.

Gepts P, and Bliss FA. 1988. Dissemination pathways of common bean (*Phaseolus vulgaris, Fabaceae*) deduced from phaseolin electrophoretic variability. II. Europe and Africa. *Economic Botany* 42: 86–104.

Gepts P, Kmiecik K, Pereira P, and Bliss FA. 1988. Dissemination pathways of common been (*Phaseolus vulgaris, Fabaceae*) deduced from phaseolin electrophoretic variability. 1. The Americas. *Economic Botany* 42: 73–85.

Gepts P. 1989. Genetic diversity of seed storage proteins in plants. In: Brown AHD, Clegg MT, Kahler AL, and Weir BS [eds.], *Plant population genetics, breeding and genetic resources*, 64–82. Sinauer Associates Inc., Sunderland, Massachusetts.

Ghafoor A, Ahmad Z, Qureshi AS, and Bashir M. 2002. Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE. *Euphytica* 123: 367–378.

Ghafoor A, Gulbaaz FN, Afzal M, Ashraf M, and Arshad M. 2003a. Inter-relationship between SDS-PAGE markers and agronomic characters in chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany* 35: 613–624.

Ghafoor A, Ahmad Z, Hashmi NI, and Bashir M. 2003b. Genetic diversity based on agronomic traits and SDS-PAGE markers in relation to geographic pattern of blackgram [*Vigna mungo* (L.) Hepper]. *Journal of Genetics and Breeding* 57: 5–11.

Higginbotham JW, Smith JSC, and Smith OS. 1991. Quantitative analysis of two-dimensional protein profiles in inbred lines of maize (*Zea mays* L.). *Electrophoresis* 12: 425–431.

Huh MK, and Huh HW. 2001. Genetic diversity and population structure of wild lentil Tare. *Crop Science* 41: 1940–1946.

Kumar L, and Arora PP. 1992. Multivariate analyses in chickpea. *Indian Journal Pulses Research* 5: 1–5.

Ladizinsky G. 1979. The origin of lentil and wild gene pool. *Euphytica* 28: 179–187.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* 227: 680–685.

Laghetti G, Pienaar BL, Pasdulosi S, and Perrino P. 1998. Ecogeographical distribution of *Vigna savi* in southern Africa and some areas of the Mediterranean basin. *Plant Genetic Resources Newsletter* 115: 6–12.

Masood S, Ghafoor A, and Javaid A. 2003. *Laboratory manual; Characterization and evaluation of plant genetic resources*. Plant Genetic Resources Institute, NARC, Islamabad, Pakistan.

Murphy RW, Sites JW, Buth DG, and Haufler CH. 1990. Protein 1: isozyme electrophoresis. In: Hillis DH, Moritz C [eds.], *Molecular systematics*, 45–126. Sunderland, MA.

PARC 1980. *Agro-ecological Regions of Pakistan*. Social Sciences Divisions, Plant Sciences Division, Pakistan Agricultural Research Division (PARC), Islamabad, Pakistan.

Pecetti L, Annicchiario P, and Damania AB. 1996. Geographic variation in tetraploid wheat (*Triticum turgidum* spp. *Turgidum convar*. Durum) landraces from two provinces in Ethiopia. *Euphytica* 43: 395–407.

Perry MC, and McIntosh MS. 1991. Geographical patterns of variation in the USDA soybean germplasm collections. I. Morphological traits. *Crop Science* 31: 1350–1355.

Picard P, Greneche MB and Zivy M. 2005. Two-Dimensional Electrophoresis, potential of two-dimensional electrophoresis in routine identification of closely related durum wheat lines. *Electrophoresis* 18: 174–181.

Piergiovanni AR, and Taranto G. 2003. Geographic distribution of genetic variation in a lentil collection as revealed by SDS-PAGE fractionation of seed storage proteins. *Journal of Genetics and Breeding* 57: 39–46.

Piergiovanni AR, and Taranto G. 2005. Assessment of the genetic variation in Italian lentil populations by electrophoresis (SDS-PAGE) of seed storage proteins. *Plant Genetic Resources Newsletter* 141: 33–38.

Przybylska J, and Zimniak-Przybylska Z. 1995. Electrophoretic pattern of seed globulins in the Old World *Lupinus* species. *Genetic Resources and Crop Evolution* 42: 69–75.

Rao R, del Vaglio M, Paino MDU, and Monti L. 1992. Identification of *Vigna* ssp. Through specific seed storage polypeptides. *Euphytica* 62: 39–43.

Sammour RH. 1985. *The use of protein characters in the taxonomy of pea and beans*. Ph.D. Dissertation, Tanta University, Tanta, Eygpt.

Sammour RH. 1989. Electrophoresis of the seed proteins of *Vicia faba* L. and its immediate progenitors: A reappraisal. *Plant Breeding* 104: 196–201.

Sammour RH. 1994. Species relationships in Genus *Lens* as indicated by electrophoresis – A reappraisal. *Lens Newsletter* 21: 1–4.

Savage GP. 1988. The composition and nutritive value of lentils. *Nature Abstract Review* 58: 319–343.

Smith SE, Guarino L, Doss ALA, and Conta DM. 1995. Morphological and agronomic affinities among Middle Eastern alfalfas accessions from Oman and Yemen. *Crop Science* 35: 1118–1194.

Sneath PHA, and Sokal RR. 1973. *Numerical taxonomy: The principles and practice of numerical classification*. W.F. Freeman & Co, San Francisco.

SPSS, Inc, 1996. SPSS for windows, release 7.5.1. Standard version. http://www.spssscience.com

STATSOFT, INC. 2001. STATISTICA (data analysis software system), Version 6. www.statsoft.com

Sultana T. 2004. *Geographical distribution of lentils in Pakistan as indicated by data on genetic markers*. Ph.D. Dissertation, Quaid-i-Azam University, Islamabad, Pakistan.

Thiellement H, Bahrman N, Damerval C, Plomion C, Rossignol M, Santoni V, de Vienne D, and Zivy M. 1999. Proteomics for genetic and physiological studies in plants. *Electrophoresis* 20: 2013–2026.

van Hintum TH, and Elings JL. 1991. Assessment of glutenin and phenotypic diversity of Syrian durum wheat landraces in relation to their geographical origin. *Euphytica* 55: 209–215.