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SEED PROTEIN PROFILING OF *PONGAMIA PINNATA* (L.) PIERRE FOR INVESTIGATING INTER AND INTRA-SPECIFIC POPULATION GENETIC DIVERSITY

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ABSTRACT

Genetic diversity study of intra and inter-specific population accession of *Pongamia pinnata* was undertaken in southern dry districts of Karnataka, India. Seed protein profile of twenty candidate plus trees was analyzed using Sodium Dodecyl Sulpate Poly Acrylamide Gel Electrophoresis (SDS-PAGE). Based on the banding pattern 13 and 21 subunits were observed for Chitradurga and Kolar accessions, respectively. Very low polymorphism of 33% was observed among the accessions of Kolar district whereas high monomorphism was observed within the accessions of Chitradurga. The molecular weight of protein subunits ranged between 9.37 kDa to 48.86 kDa, 21.78 kDa to 53.49 kDa for Chitradurga and Kolar district, respectively. Zone specific banding pattern was exhibited in both the Kolar and Chitradurga accessions. Specific intense bands at molecular weight ranging between 29 kDa and 43 kDa were fingerprinted monomorphically for Kolar accessions. The analysis of the seed proteins based on distance matrix exhibited six clusters. The accessions of Kolar could be grouped into a separate cluster (V) indicating that these genotypes originated from common stock. The accessions of Chitradurga showed scattering clusters indicating that these accessions could have originated from different parentage. The specific seed protein bands could be used as markers for identification of Pongamia genotypes from different dry zones.

KEY WORDS*:* *Pongamia pinnata*, Genetic Diversity, Protein profiling, Polymorphism

INTRODUCTION

Pongamia pinnata is an important source of biofuel, a sustainable substitute for fossil fuel in India. It is a well adapted tree to semi-arid and dry zones grown upto 1000m altitude. It is indigenous to Indian subcontinent and successfully grown in other regions of the world like Australia, China, United States (Anonymous, 1969). It is a multipurpose tree, the extracts from different parts of the tree are traditionally used as a source of medicine such as antidiarrheal (Brijesh *et al.,* 2006), antihyperammonenic (Musthafa *et al*., 2005), antihelmintic activity (Sunil *et al*., 2007). Presence of bioflavanoids like pongaflavanol, funicatachalcone from stem bark, furanoflavanol, chromenoflavones has been reported (Hao *et al.,* 2006). Detoxified cake of pongamia (Prabhu *et al.,* 2002) has been incorporated with cattle feed. In Karnataka state, a large population of Pongamia has been noted in dry arid agro-climatic zones that included the central and eastern dry region (Singh 1988).

The presence of high morphometric variation among these trees provides a reasonable ground to undertake a study of the genetic diversity across intra and inter-specific population of pongamia using molecular markers. An array of marker systems including protein markers and DNA markers are being used to study the genetic diversities in different plant systems. Seed protein is a very conservative trait among the oilseed species. Seed protein analysis by SDS-PAGE is a tool to understand the genetic diversity at protein level among the genotypes. Proteins exhibit distinct banding pattern in various plant species. Seed protein patterns can be used as a reliable tool for distinguishing the cultivars. Researchers have used seed protein profiling as genetic marker system since many years. Seed protein classification, structure, properties, evolutionary origin, modes of synthesis and deposition has been reviewed (Peter *et al*., 1995). Seed protein profiling by SDS-PAGE is less expensive, reproducible, reliable and efficient method. Seed protein markers are widely used for the identification of varieties (Cooke 1984) of agricultural and horticultural crops. The validity and simplicity of seed protein profiling has been well documented (Cooke 1988). High stable and reliable seed protein profiling make it a powerful tool in elucidating the taxonomic and evolutionary problems of cultivars (Ladizinsky *et al.,* 1979). The use of genetic and seed protein marker can be used to select elite accessions collected from different agro-climatic regions for tree improvement programs. Comparitive studies on the proteomic data in leguminous species has been reported (Yasmin *et al.,* 2010). Seed protein and enzyme polymorphism has been used for identification of cultivars of cotton (Nerkar and Rao 1993). Evolutionary affinities have been assessed in *Gossypium* species by seed protein electrophoresis (Lennart and Maung 1970). Comparitive electrophoretic studies of seed proteins in *Luffa* species has been documented (Singh and Roy 1990). Biochemical fingerprinting of genotypes has gained importance in the crop improvement and identification of wild and cultivated plant species. Genetic relationship has been analysed in the genus *Cicer* using seed proteins (Ahmad *et al*., 1992).

Inter and intra specific variation using SDS-PAGE seed protein analyses in chickpea germplasm have been reported (Asghar *et al.,* 2003). Genetic diversity in *Brassica* species using SDS-PAGE analysis has been studied (Rahman *et al.,* 2004). Genetic variation studies regarding interspecific genotypes of *Brassica* cultivars have been reported (Munazz *et al.,* 2009). 2D-PAGE and SDS-PAGE have also been associated with polymorphic protein isoforms and peanut subspecies Spanish type and runner type (Liang *et al.,* 2006).

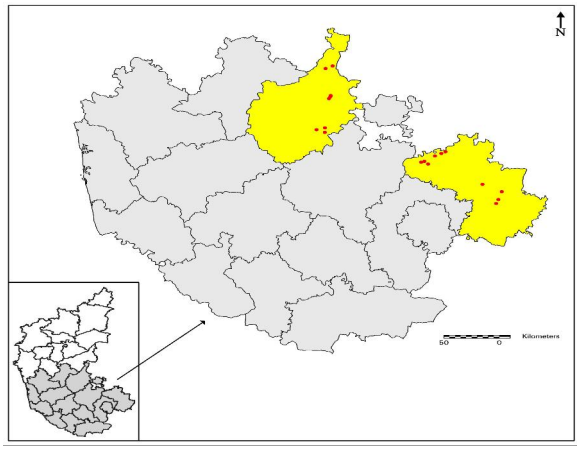
The present study was initiated to study the genetic diversity based on seed protein profiling across the selected accessions from two dry arid regions of Karnataka, India covered by high population of *Pongamia*.

MATERIALS AND METHODS

Sample Collection

Survey was conducted to study the morphometric diversity existing among the pongamia accessions in the southern dry arid regions of Karnataka, India. Chitradurga and Kolar districts were brought under the study (Figure1). Kolar and Chitradurga receive an average annual rainfall 600 mm to 700 mm and less than 400 mm, respectively. The area of sample collection for seed protein profiling lies between the altitudes of 510 m to 970 m and geographical position of N 13º 12'51.0" to N 14º 37'53.5" and E 76º37'38.03" to E 78º12'08.08". The candidate trees were marked randomly along the bunds, road side, canal side, farm bund. Forty and twenty seven accessions from Kolar and Chitradurga were marked respectively as candidate plus trees (CPTs) with emphasis given to the pod and seed characters. Ten CPTs selected from each district were randomly short listed for seed protein analysis (Table1). The dried seeds taken in triplicates were extracted with petroleum ether in a Soxtherm apparatus to determine the oil content.

FIG. 1: Karnataka state map (Southern districts-Chitradurga and Kolar) showing the sampling sites (shaded region).



Preparation of protein samples

Seeds were defatted using a mixture of chloroform: methanol: acetone (2:1:1) for extraction of soluble proteins. Defatted seeds were ground in 500 µl Tris HCl (0.12M pH-6.8) buffer and incubated overnight at 4ºC. Samples were centrifuged at 15,000 rpm for 15 min at 4ºC. The supernatant was separated and the steps were repeated for the complete extraction. 100 µl supernatant was taken and 500 µl chilled acetone was added to pellet the protein, which was then dissolved in 100 µl distilled water. Protein content of the extracts was determined by Bradford method (Bradford, 1976). Loading buffer (50 µl) was added to the sample and denatured in boiling water bath for 15 min.

Protein Profiling using SDS-PAGE

SDS-PAGE was used to prepare protein profiles as described by Laemmli (1970) and Davis (1964). 250µg of protein sample was loaded into wells in 10% gel slab along with protein molecular weight marker. Silver Staining of the gels was carried out as described by Merril (1979). The Relative Migration (RM) (Nerkar and Rao 1993) of each band was calculated as follows.

$$RM = \frac{\text{Distance migrated by the protein band from origin (cm)}}{\text{Distance migrated by tracking dye (cm)}}$$

or absence of the bands was recorded in a binary data matrix for pooling data of both districts. Based on the banding electrophoregrams, distance matrix was calculated and dendrogram was constructed using neighbor joining algorithm using the software PHYLIP (Phylogeny Inference Package-3.65) (Felsenstein J., 1993). Per cent polymorphism was calculated as follows.

$$\text{Polymorphism (\%)} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 10$$

RESULTS

Seed protein profiling was resolved into 13 and 21 subunits on electrophoregram for Chitradurga and Kolar, respectively. These banding patterns were credited to the variability in intensity of protein peptide accumulation at a particular molecular weight. The molecular weights of the peptides ranged from 9.37 kDa to 48.86 kDa, 21.78 kDa to 53.49 kDa for Chitradurga and Kolar districts, respectively. The relative migration value (Table 2) for Chitradurga samples ranged between 0.39-0.83 and that for Kolar ranged from 0.26 to 0.44. Low per cent polymorphism of 33% was observed among the accessions of Kolar whereas intra population of Chitradurga district exhibited high monomorphism. Zone specific intense bands (Figure 2) were observed among the inter population accessions. Specific peptide band at molecular weights between 29 kDa and 43 kDa was detected within the intra population of candidate plus trees of Kolar district. The cluster analysis based on the electrophoretic data, grouped the accessions into six clusters (Figure 3). CPT1 to CPT10 were seen in scattering clusters of I, II, III, IV and VI for Chitradurga accessions. The distance matrix ranged from 0.05 to 1.25(Table 3) for the accessions. The candidate plus trees ranging from 11 to 20 of Kolar were clustered into a separate group V. The clustering of genotypes showed independent relation with the pod characters recorded for the genotypes

TABLE 1: Accessions of *Pongamia pinnata* used for seed protein profiling

CPTs	Latitude	Longitude	Altitude(m)	Soil Type	Pod Characteristics				Oil Content (%)
					Texture	Tip	Size	Shape	
CPT 1	N 14 36 07.1	E 76 40 39.2	577	Red rocky	rough	beaked	medium	bulged	33.37
CPT 2	N 14 36 07.2	E 76 40 39.3	577	Red rocky	smooth	beaked	small	flat	36.31
CPT 3	N 14 36 07.3	E 76 40 39.4	577	Red rocky	smooth	beaked	medium	bulged	28.07
CPT 4	N 14 37 53.5	E 76 44 12.3	570	Red	rough	beaked	small	bulged	35.03
CPT 5	N 14 16 59.4	E 76 43 08.01	510	Red	rough	mucronate	small	bulged	33.34
CPT 6	N 14 18 16.1	E 76 43 48.02	600	Red rocky	rough	mucronate	medium	flat	39.42
CPT 7	N 14 19 00.0	E 76 43 55.04	600	Red	smooth	mucronate	small	bulged	35.05
CPT 8	N 13 56 57.06	E 76 37 38.03	610	Red	smooth	mucronate	medium	flat	40.03
CPT 9	N 13 55 25.06	E 76 41 53.2	600	Red	smooth	mucronate	medium	bulged	32.45
CPT 10	N 13 58 18.0	E 76 41 49.7	600	Red	smooth	mucronate	medium	bulged	33.61
CPT 11	N 13 12 51.0	E 78 09 32.5	900	Red	rough	mucronate	medium	bulged	31.15
CPT 12	N 13 15 24.3	E 78 10 33.8	910	Red	rough	mucronate	small	flat	34.01
CPT 13	N 13 20 26.7	E 78 12 08.08	920	Red	smooth	mucronate	small	flat	31.76
CPT 14	N 13 24 51.5	E 78 02 19.4	970	Red	rough	mucronate	medium	bulged	39.07
CPT 15	N 13 45 05.5	E 77 43 05.7	820	Red	smooth	mucronate	medium	bulged	31.17
CPT 16	N 13 43 55.7	E 77 41 01.8	800	Red	smooth	mucronate	medium	flat	36.63
CPT 17	N 13 42 14.6	E 77 37 52.9	770	Red	smooth	beaked	small	flat	33.29
CPT 18	N 13 38 37.6	E 77 32 40.5	880	Red	rough	beaked	medium	bulged	33.44
CPT 19	N 13 38 00.9	E 77 31 01.7	760	Red	rough	beaked	medium	flat	35.93
CPT 20	N 13 36 58.4	E 77 34 34.7	805	Red	rough	beaked	large	bulged	35.9

FIG. 2 Seed protein profiling of *Pongamia pinnata* accessions collected from (a) Chitradurga and (b) Kolar

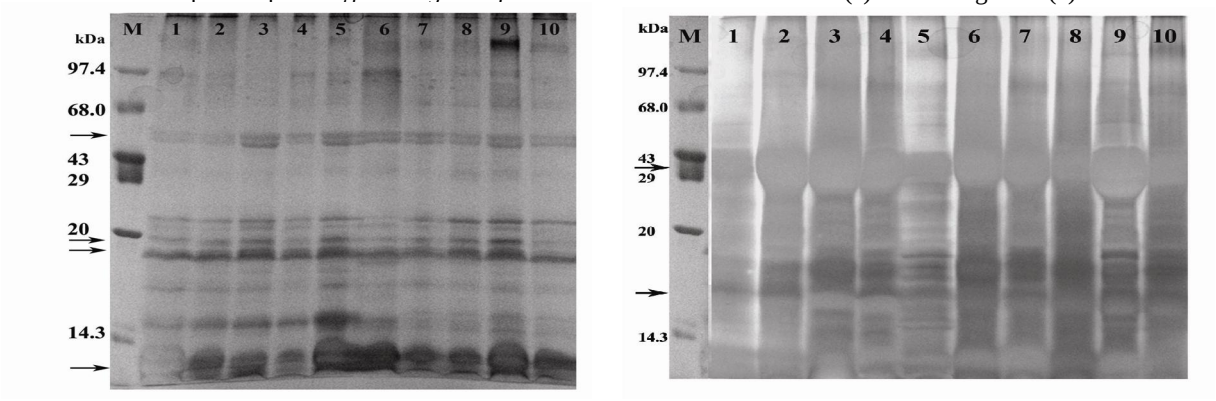
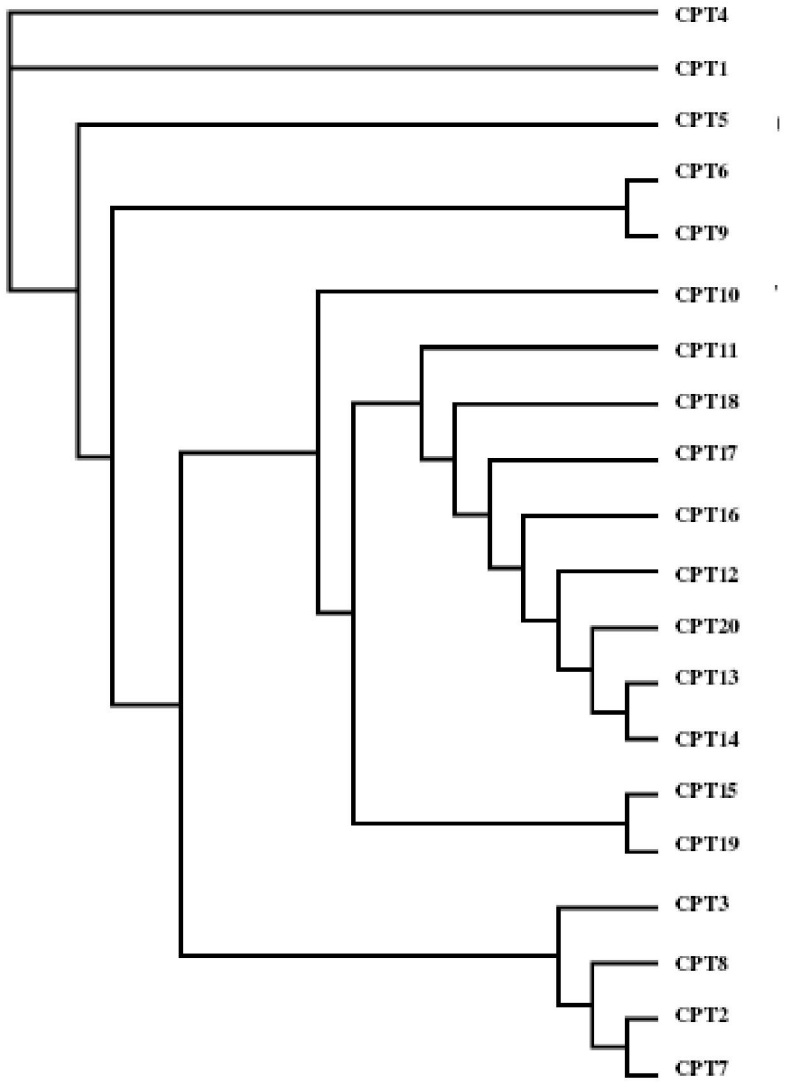


TABLE 2: Molecular weight and relative migration value of distinctly resolved protein subunits

Sl no	Molecular Weight (kDa)	Relative Migraton Value
	Chitradurga District	
1	48.86	0.39
2	21.40	0.61
3	12.50	0.76
4	9.37	0.83
	Kolar District	
5	53.49	0.26
6	48.87	0.28
7	29.33	0.38
8	27.85	0.39
9	25.94	0.41
10	21.78	0.44

FIG. 3: Dendrogram constructed based on the cluster analysis for twenty candidate plus trees (CPT) using seed protein markers in *Pongamia pinnata* (CPT 1 to 10- Chitradurga samples, CPT 11 to 20-Kolar samples)



DISCUSSION

Genetic diversity analysis is an efficient method that facilitate the identification of superior germplasm, acceralates the collection, conservation, improvement of germplasm for breeding programmes and tree improvement. Information regarding genetic similarity measures can be used for selection of superior quality planting material for improvement or for use in tree hybridization program. Biochemical fingerprints are used widely for its simple and effective method of genetic diversity analysis among the selected tree germplasm. Seed protein analysis has been used in genome relationship studies and genetic diversity studies within and between genotypes, which help in sorting of genetic population for genome mapping.

SDS-PAGE analysis is used as a selection tool for genetically diverse group of plant accessions for a better selection among the germplasm collections. A fair knowledge of genetic and biochemical marker data is essential to eliminate the duplicate genetic divergents and produce a collection of elite pongamia germplasm. In order to have a wider collection of germplasm for maintenance, then efficiently and effectively characterization of such diverse collection is necessary. The accessions from Chitradurga show heterogenous clustering in the dendrogram indicating that the population can be migrants or that the genetic material has been introduced from different areas or the possibility of exchange of germplasm by breeders or forest department personnel might have taken place. Similar results were reported in blackgram, where agronomic traits and SDS-PAGE markers have been analysed and no correlation was found between geographic location, agronomic traits and seed profiling (Ghafoor *et al*., 2005).

Based on the clustering analysis it is evident that latitudinal and altitudinal parameters that is geographic positions of trees could be used for selection of pongamia germplasm for further studies. The findings of the present

study revealed that low intra-specific variation was available in the selected candidate plus tree which is in accordance with the findings of Ghafoor *et al*., (2003) for seed protein in chickpea and contradicting with the findings of Mohammad *et al.,* (2007) who showed considerable variation in intra-specific chickpea germplasm. Homogenous and heterogenous groups were found among the accessions of Kolar and Chitradurga. The results of present study was further strengthened by the findings of Tayyaba *et al.,* (2006) in cultivated lentil germplasm collected from different agro-ecological zones of Pakistan showing low intraspecific variation. Groundnut species collected from different continents showed low interspecific genetic diversity as revealed by Asif *et al.,* (2004). Soyabean (*Glycine max*) lines evaluated for genetic diversity using seed protein electrophoresis revealed difficulty in establishing any relationship between the origin and clustering pattern (Faisal *et al.,* 2009). Odeigah and Osanyinpeju (1998) have reported that no correlation was found between seed morphology and seed protein profiles in bambara groundnut accessions from Nigeria which is in concurrence with our findings that suggest no correlation between pod characters and seed protein profile among the Kolar and Chitradurga accessions. SDS-PAGE analysis to differentiate at species level have been studied by Ghafoor *et al*., (2002) in *Vigna mungo* and *Vigna radiata* which revealed low level inter-specific genetic diversity and no correlation was observed between agronomic traits and geographical origin which is at par with our findings. Comparative seed protein profiling of Kabuli Chickpea genotypes was studied by Amjad *et al.,* (2009) who recorded no clear differentiation for the origin of genotypes. Comparison between morphological and seed protein profiling have been well documented by Ghafoor and Arshad (2008) in *Pisum sativum*. They also showed no correlation between cluster patterns with origin.

CONCLUSION

It could be concluded that the candidate plus trees from different geographical origins can be selected for germplasm maintenance by conventional vegetative propagation method or micropropogation. Inter- specific population genetic variation provides information on various aspects of divergent genotypes. Geographic zone specific seed protein markers could be used for the identification of candidate trees among different dry zones rich in pongamia population. Further, it is suggested that genotypes with similar intra-specific banding pattern should be characterized by multi-variant two dimensional seed protein analysis for further fine separation of protein molecules that occurred at same molecular weight lane. Further, DNA molecular markers could be employed for screening different pongamia population growing in different agro-ecological regions.

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Table 3: Distance matrix measure between the candidate plus trees of *Pongamia pinnata* by seed proteins (CPT 1 to 10-Chitradurga samples, CPT 11 to 20-Kolar**.**

CPT1	CPT1	CPT2	CPT3	CPT4	CPT5	CPT6	CPT7	CPT8	CPT9	CPT10	CPT11	CPT12	CPT13	CPT14	CPT15	CPT16	CPT17	CPT18	CPT19	CPT20
CPT1	0.00																			
CPT2	0.10	0.00																		
CPT3	0.15	0.05	0.00																	
CPT4	0.05	0.05	0.10	0.00																
CPT5	0.05	0.05	0.10	0.00	0.00															
CPT6	0.10	0.10	0.15	0.05	0.05	0.00														
CPT7	0.10	0.00	0.05	0.05	0.05	0.10	0.00													
CPT8	0.15	0.05	0.10	0.10	0.10	0.15	0.05	0.00												
CPT9	0.27	0.27	0.21	0.21	0.21	0.15	0.27	0.21	0.00											
CPT10	0.15	0.05	0.10	0.10	0.10	0.15	0.05	0.10	0.21	0.00										
CPT11	0.85	0.65	0.74	0.74	0.74	0.85	0.65	0.74	0.97	0.56	0.00									
CPT12	0.97	0.74	0.85	0.85	0.85	0.97	0.74	0.85	1.10	0.65	0.05	0.00								
CPT13	1.10	0.85	0.97	0.97	0.97	1.10	0.85	0.97	1.25	0.74	0.10	0.05	0.00							
CPT14	1.10	0.85	0.97	0.97	0.97	1.10	0.85	0.97	1.25	0.74	0.10	0.05	0.00	0.00						
CPT15	1.25	0.97	1.10	1.10	1.10	0.97	0.97	0.85	0.85	0.85	0.74	0.65	0.56	0.56	0.00	0.00				
CPT16	0.97	0.74	0.85	0.85	0.85	0.97	0.74	0.85	1.10	0.65	0.05	0.00	0.05	0.05	0.65	0.00				
CPT17	0.97	0.74	0.85	0.85	0.85	0.97	0.74	0.85	1.10	0.65	0.05	0.00	0.05	0.05	0.65	0.00	0.00			
CPT18	0.97	0.74	0.85	0.85	0.85	0.97	0.74	0.85	1.10	0.65	0.05	0.00	0.05	0.05	0.65	0.56	0.00	0.00		
CPT19	1.10	1.10	0.97	0.97	0.97	0.85	1.10	1.25	0.74	0.97	0.48	0.56	0.65	0.65	0.56	0.15	0.56	0.56	0.00	
CPT20	1.10	1.10	1.25	1.25	1.25	1.10	1.10	1.25	1.25	0.97	0.21	0.15	0.21	0.21	0.56	0.00	0.15	0.15	0.48	0.00

REFERENCES

Anonymous (1969) The Wealth of India raw materials. Vol III. C.S.I.R. New Delhi.

Ahmad, F., Slinkard, A.E., (1992) Genetic relationships in the genus Cicer L., as revealed by polyacrylamide gel electrophoresis of seed proteins. Theoritical and Applied Genetics 84: 688-692.

Asghar, R., Siddique, T., Afzal, M., (2003). Inter and intra-specific variation in SDS-PAGE electrophoregrams of total seed protein in chickpea (*Cicer arietinum* L.) germplasm. Pakistan Journal of Biological Science 6(24):1991-1995.

Asif, J., Abdul, G., Rashid, A., (2004) Seed protein electrophoresis in groundnut for evaluating genetic diversity. Pakistan Journal of Botany, 36(1): 25-29.

Amjad, H., Tariq, M. S., Manzoor, B. A., Nayyer, I., Ahsanul, M. H., Hina, A., (2009)Comparitive seed protein profiling of Kabuli Chickpea genotypes. Pakistan Journal of Botany 41(2): 703-710.

Bradford, M. M.,(1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Annals of Biochemistry 72:248-254.

Brijesh, S., Daswani, P.G., Tetali, P., Rojatkar, S.R., Antia, N.H., Birdi, T.J., (2006) Studies on P*ongamia pinnata* (L.) Pierre leaves: understanding the mechanism(s) of action in infectious diarrhea. Journal of Zhejiang University Science 7(8):665-674.

Cooke, R. J., (1984) The characterization and identification of crop cultivars by electrophoresis. Electrophoresis 5: 59-72.

Cooke, R. J.,(1988) Electrophoresis in plant testing and breeding. Advances in Electrophoresis 2 171-261.

Davis, B J (1964) Dise electrophoresis II, Method and application to human serum proteins Annals of New York Academic Science 121: 407-427.

Felsenstein, J., (1993) PHYLIP(Phylogeny Inference Package) ver. 3.56. Department of Genetics, University of Washington, Seattle.

Faisal, M. A. M., Afsari, S. Q., Muhammad, A., Muhammad, R. K., Asif, J., (2009) Evaluation of genetic diversity in soybean (*Glycine max*) lines using seed protein Electrophoresis. Australian Journal of Crop Science 3(2):107-112.

Ghafoor, A., Zahoor, A., Afsari, S. Q., Muhammad, B., (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, F.N., Afzal, M., Ashraf, M., Arshal, M., (2003) Inter relationship between SDS-PAGE markers and agronomic traits in chickpea (*Cicer arietinum* L.) Pakistan Journal of Botany 35 (4): 613-624.

Ghafoor, A., Ahmad, Z., (2005) Diversity of agronomic traits and total seed protein in black gram V*igna mungo* (L.) Hepper . Acta Biologica Cracoviensia Series Botanica 47(2): 69–75.

Ghafoor, A. and Arshad, M., (2008) Seed Protein Profiling Of Pisum Sativum L., Germplasm Using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis(SDS-PAGE) For Investigation Of Biodiversity, Pakistan Journal of Botany 40(6): 2315-2321.

Hao, Y., Zhang, S., Wu, J., Haihan, N., Lijuan, L., Yang, J., Qingxin, L., (2006); Pongaflavanol: a Prenylated Flavonoid from *Pongamia pinnata* with a Modified Ring A. Molecules 11: 786-791.

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

Lennart, B. J., Maung, M. T., (1970) Assessment of Evolutionary affinities in Gossypium by Protein Electrophoresis. American Journal of Botany 57(9): 1081-1092.

Ladizinsky, G., Hymowitz, T. (1979) Seed protein electrophoresis in taxonomic and evolutionary studies. Theoritical and Applied Genetics 54: 145-151.

Liang, X.Q., Luo, M., Holbrook, C.C., Guo, B.Z., (2006) protein profiles in Spanish and runner market type peanuts and potential markers. Plant Biology 6:24.

Merril, C.R., Shifrin, S., (1979). A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. Anals Biochemistry 98 (1): 231–237.

Musthafa, M. E., Perumal, S., Ganapathy, S., Tamilarasan, M., Kadiyala, B. D., (2005) Protective influence of *Pongamia pinnata* (Karanja) on blood ammonia and urea levels in ammonium chloride-induced hyperammonemia: antihyperammonemic effect of the leaf extract. Journal of Applied Biomedicine 3:1-6.

Mohammad, N., Ghafoor, A., Rashid, M. K., Habib, A., Afsari, S. Q., Haidar, A., (2007) Genetic diversity and geographic relationship among local and exotic chickpea germplasm. Pakistan Journal of Botany 39(5): 1575-1581.

Munazza, S., Salman, A. M., Malik, A. R., (2009) Electrophoretic Characterization and the Relationship between Some Brassica Species. Journal of Biology, 5(1): 1-4.

Nerkar, Y.S., Rao, T. N., (1993) Use of seed protein and enzyme polymorphism in the identification of cultivars of cotton. Seed Research, 375-394.

Odeigah, P.G.C., Osanyinpeju, A.O., (1998) Genetic Evaluating the genetic biodiversity of Bambara groundnut accessions from Nigeria using SDS-polyacrylamide gel electrophoresis. Resources and Crop Evolution (45): 451–458.

Peter, R. S., Johnathan, A. N., Arthur, S. T., (1995) Seed Proteins: Structures 'and Biosynthesis. The Plant Cell (7), 945-956

Prabhu, T.M., Devakumar, C., Sastry, V.R.B., Agerwal, D.K., (2002) Quantification of karanjin using High Performance Liquid Chromatography in raw and detoxified karanj (Pongamia glabra vent) seed cake. Asian-Australasian Journal of Animal Science 15(3) 416-420.

Rahman, M.M. and Hirata, Y. (2004) Genetic diversity in Brassica species using SDS-PAGE analysis. Journal of Biological Science, 4(2): 234-238.

Singh, N.P., (1988) The flora of Eastern Karnataka Vol I

233-234

Singh, B.P. and Roy, R.P., (1990) Comparitive electrophoretic studies of seed proteins in Luffa species. Journal of Indian Botanical Society 69:219-221.

Sunil, A. N., Malwadkar, G., Laware, R.B., (2007)Anthelmintic activity of *Pongamia glabra*. Songklanakarin Journal Science Technology 29(3): 755-757.

Tayyaba, S., Abdul, G., Muhammad, A., (2006) Geographic patterns of diversity of cultivated lentil germplasm collected from Pakistan as assessed by seed protein assays. Acta Biologica Cracoviensia Series Botanica 48(1): 77–84.

Yasmin, B., Habib, A., Sazia, S., Sajid, G., Imtiaz, A.K., (2010) Comparative Proteomic Studies in Leguminous Species. Asian Journal of Agricultural Sciences 2(3): 89-93.

ABBREVATIONS

SDS-PAGE-Sodium Dodecyl Sulpate
DNA-Deoxyribose Nucleic Acid
kDa- Kilo Dalton.
2D-PAGE-Two Dimensional Poly Acrylamide Gel Electrophoresis.
CPT- Candidate Plus Tree
Tris-HCL – Tris HydroChloric Acid
RM- Relative Migration
PHYLIP-Phylogeny Inference Package