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ASSESSMENT OF GENETIC DIVERSITY IN INDIGENOUS RICE ACCESSIONS OF NORTHERN PAKISTAN USING BIOCHEMICAL MARKERS

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Abstract

Indigenous rice germplasm was evaluated for total seed storage proteins diversity using 116 accessions. We obtained 18 visible polypeptide bands out of which eight were polymorphic (44.4%) and ten bands were found monomorphic (55.6%). Entire banding profile was divided into three groups (I, II and III) based on their molecular weight. Each of three groups had six bands. Glutelin and Prolamin fragments had high polymorphism percentage, while Albumin and Globulin subunits had low polymorphism percentage. Based upon the study using UPGMA all 116 accessions were categorized into seven clusters in which accession 7202 and 7205 were present in cluster II showing marked dissimilarity with other accessions. Cluster III had 74 accessions which showed that on the basis of storage protein profile low genetic diversity was present in these rice accessions.

Key words: Rice (*Oryza sativa*); Genetic diversity; SDS-PAGE analysis; Cluster analysis.

Introduction

Rice belonging to the family poaceae is the most important food item in terms of calorie intake. It is a source of nutrition for three billion people globally (Krishnamurthy *et al*., 2016). Rice crop can successfully grow in different agro-climatic conditions of about 114 countries of the world. Asia is the biggest rice producer and consumer of the world (Mallimar *et al*., 2015). Rice proteins, though smaller in quantity, are of high nutritional value and range from 7 to 10% of grain dry mass. Rice is an excellent crop because its endosperm stores and synthesizes both major classes of proteins, i.e., Glutelins and Prolamins. Glutelins are alkali soluble proteins which constitute 80-90% of total storage proteins of endosperm of rice and are simply consumable. Second class of proteins is prolamin an alcohol soluble protein which is five to ten percent of rice storage proteins is hard to digest (Salgotra *et al*., 2015). Increased protein content of rice can improve nutritional quality of rice. Most recent analyses indicate that human population will increase from 7.20 billion to 9.60 billion in 2050. Current yield trends are not enough to keep up with increasing demand. We will need more food, feed, and fiber (Fedoroff, 2015). The demand of rice as a protein food is going to be doubled by 2030. Short grain rice is cultivated in northern areas of Pakistan but its production and area under cultivation is decreasing (Bibi *et al.,* 2015). Demand for high yielding accessions had led to the erosion of landrace diversity and improvement of small group of standard, short duration, fertilizer responsive varieties of modern rice. Consequently landraces are moving out of cultivation through genetic erosion (Aravind *et al*., 2015; Reddy *et al*., 2016). Knowledge about the extent of genetic diversity is of utmost importance for designing an effective rice breeding and conservation program. Genetically diverse parents have the potential of higher yield per unit area (Roy *et al.,* 2015). Phenotypic diversity is not reliable because many yield attributing traits had low heritability, complex in nature, affected by environmental conditions, expensive and time consuming (Bibi *et al.*, 2009). Biochemical diversity particularly based on proteins which are primary gene products, easy to handle and are available throughout the year (Shinwari *et al*., 2014). To identify and characterize various storage proteins in rice cultivars and other plants germplasm various studies have been conducted to use SDS-PAGE technique (Akbar *et al* 2012; Meena & Shukla, 2013; Thanh & Hirata, 2014; Tripathy *et al*., 2015). In present study indigenous rice germplasm from northern Pakistan was exploited on the basis of storage protein to find genetically diverse rice accessions.

Material and Methods

One hundred and thirteen rice accessions and three checks IR6 (Coarse), JP5 (Japonica) and Super Basmati (Aromatic) were collected from the National Gene Bank of Plant Genetic Resources Institute (PGRI), National Agriculture Research Center (NARC) Islamabad. All rice accessions were belonging to cold areas of Northern Pakistan. Passport data of accessions used in this research is given in Table 1.

Proteins extraction: Four grains from each accession were dehusked and ground to make fine powder with mortar and pestle. Very fine powder (100mg) was taken into a 1.5ml eppendorf tube and mixed with 400µl of protein extraction buffer (2% SDS, 0.5M Tris-base pH 8, 5M Urea, 10% glycerol, and 1% 2-mercaptoethanol). Tracking dye Bromophenol blue (0.05%) was used. All samples were vortexed and left overnight at 25°C. Centrifugation at 20°C and 13,000rpm for 15 minutes was done for all samples. Supernatant was collected in another 1.5ml eppendorf tube and stored at –4°C till further use.

Table 1. List of rice germplasm from northern Pakistan used in present study.

Acc.	Prov.	Dist.	Alt.	Acc.	Prov.	Dist.	Alt.	Acc.	Prov.	Dist.	Alt.	Acc.	Prov.	Dist.	Alt.
7201	AJK	Muzf	1600	7645	KPK	Swat	650	7620	KPK	Chit	1620	7812	KPK	Chit	1940
7202	AJK	Muzf	1050	7646	KPK	Swat	650	7621	KPK	Chit	1670	7813	KPK	Chit	1910
7205	AJK	Muzf	850	7647	KPK	Swat	650	7622	KPK	Chit	1670	7814	KPK	Chit	1910
7210	AJK	Muzf	1000	7754	KPK	Swat	1550	7623	KPK	Chit	1850	7815	KPK	Chit	1910
7213	AJK	Muzf	1070	7826	KPK	Swat	870	7624	KPK	Chit	1850	7816	KPK	Chit	1930
7232	AJK	Muzf	980	7830	KPK	Swat	940	7625	KPK	Chit	1950	7818	KPK	Chit	1870
7592	AJK	Muzf	1100	7831	KPK	Swat	940	7626	KPK	Chit	2100	8083	KPK	Chit	1820
7664	AJK	Muzf	860	7832	KPK	Swat	940	7627	KPK	Chit	1450	8085	KPK	Chit	1995
7665	AJK	Muzf	900	7400	KPK	Dir	1190	7628	KPK	Chit	1450	7593	KPK	Malk	700
7668	AJK	Muzf	1400	7601	KPK	Dir	730	7629	KPK	Chit	1450	7594	KPK	Malk	700
7669	AJK	Muzf	1400	7603	KPK	Dir	800	7630	KPK	Chit	1520	7596	KPK	Malk	700
7670	AJK	Muzf	1400	7604	KPK	Dir	810	7783	KPK	Chit	240	7597	KPK	Malk	700
7675	AJK	Muzf	300	7605	KPK	Dir	810	7786	KPK	Chit	1470	7598	KPK	Malk	730
7676	AJK	Muzf	980	7606	KPK	Dir	1100	7787	KPK	Chit	1400	7599	KPK	Malk	730
7729	AJK	Muzf	220	7607	KPK	Dir	1100	7791	KPK	Chit	1416	7600	KPK	Malk	730
7660	AJK	Muzf	790	7608	KPK	Dir	1100	7792	KPK	Chit	1750	7839	KPK	Batg	1650
7736	AJK	Rawl	1080	7609	KPK	Dir	1200	7793	KPK	Chit	1750	7840	KPK	Batg	630
7397	KPK	Swat	960	7610	KPK	Dir	1200	7795	KPK	Chit	2000	7649	KPK	Mans	810
7632	KPK	Swat	2000	7402	KPK	Chit	1780	7796	KPK	Chit	1910	7650	KPK	Mans	940
7633	KPK	Swat	1050	7408	KPK	Chit	1850	7797	KPK	Chit	1490	7651	KPK	Mans	940
7634	KPK	Swat	1050	7611	KPK	Chit	1250	7798	KPK	Chit	1490	7652	KPK	Mans	1120
7635	KPK	Swat	1100	7612	KPK	Chit	1300	7802	KPK	Chit	1830	7654	KPK	Mans	1010
7636	KPK	Swat	1100	7613	KPK	Chit	1300	7803	KPK	Chit	1800	7656	KPK	Mans	920
7638	KPK	Swat	1200	7616	KPK	Chit	1500	7804	KPK	Chit	1860	7657	KPK	Mans	920
7642	KPK	Swat	800	7617	KPK	Chit	1500	7805	KPK	Chit	1800		IR 6		
7643	KPK	Swat	650	7618	KPK	Chit	1500	7806	KPK	Chit	1800		JP 5		
7644	KPK	Swat	650	7619	KPK	Chit	1560	7807	KPK	Chit	2035	Super Basmati			

Note: Acc. = Accession; Prov. = Province; Dist. = District; Alt. = Altitude

Electrophoretic profiles: Biochemical characterization of storage proteins was done on a vertical slab gel using discontinuous buffer system (12.0 x 13.8 cm^2). SDS-PAGE Model: AE-6530M, Japan was used for SDS-PAGE electrophoresis. Acrylamide (4.5%) stacking and 14% separation gel was used for separation of rice storage proteins (Laemmli, 1970). From each rice sample, ten microliters supernatant with the help of micropipette was loaded into the wells of the gel. Constant electric supply of 95 volts was supplied to electrophoresis apparatus having electrode buffer solution (0.025M Tris base, 0.129M glycine and 0.125% sodium dodecyl sulphate). Bromophenol Blue (BPB) was used as tracking dye and electrophoresis was stopped when BPB nearly reaches the ends of gel. Gel was shaken at 18rpm in a staining solution (water:methanol:acetic acid (50:44:6) and 2.25g coomassie brilliant blue (CBB R-250) for three hours on a shaker with 18rpm and then destained overnight in water: methanol:acetic acid (15:4:1: v/v). Each sample was scored twice for banding pattern of electrophoregrams. PAGE ruler (Thermo Scientific) was used for determination of molecular weight of proteins.

Statistical analysis: In protein banding profiles presence and absence of bands was coded as 1 or 0, respectively with clear resolutions. Genetic dissimilarity was calculated between pairs of accessions by measuring similarity index and by Dice coefficient. Only high frequency bands were utilized for scoring. Unweighted pair group method with arithmetic averages (UPGMA) using sequential agglomerative hierarchical and non-overlapping (SAHN) clustering method based on the genetic distance matrix was used for construction of dendrogram. NTSYS-pc (2.10e) (Exeter Genetic Software, Setauket, NY, USA) was used for analysis of genetic distance table and construction of dendrogram.

Table 2. Detailed profile of protein banding pattern of rice landraces from northern Pakistan.

Group	No. of bands	Weight in kDa	Major/ Minor	Protein type	Polymorphic/ Monomorphic	Polymorphism (%)	Present	Absent
I	A	180	Minor	High molecular weight	Monomorphic	0	116	0
	B	170	Minor	High molecular weight	Monomorphic	0	116	0
	C	130	Minor	High molecular weight	Monomorphic	0	116	0
	D	100	Minor	High molecular weight	Monomorphic	0	116	0
	E	70	Minor	High molecular weight	Monomorphic	0	116	0
	F	60	Minor	Waxy protein	Polymorphic	1.72	2	114
II	G	57	Minor	Precursor polypeptide of glutelin	Polymorphic	86.2	100	16
	H	52	Minor	Rapidly moving peptide subunit of glutelin	Polymorphic	96.5	112	4
	I	42	Minor	Albumin polypeptide sub units	Monomorphic	0	116	0
	J	39-38	Major	Alpha 1,2 (acidic) polypeptides of glutelin	Monomorphic	0	116	0
	K	37	Major	Alpha 3 (acidic) polypeptides of glutelin	Polymorphic	88.79	103	13
	L	34	Minor	Alpha 4 (acidic) polypeptides of glutelin	Polymorphic	65.51	76	40
III	M	26	Major	Globulin	Monomorphic	0	116	0
	N	20	Major	B 1 basic polypeptide subunit of glutelin	Polymorphic	93.96	109	7
	O	18-19	Major	B 2, 3 (basic) polypeptides subunit of glutelin	Monomorphic	0	116	0
	P	16	Major	Prolamin	Polymorphic	18.1	21	95
	Q	13	Major	Prolamin	Polymorphic	89.65	104	12
	R	10	Minor	Prolamin	Polymorphic	0	116	0

Results and Discussion

Storage proteins profile of northern areas rice accessions revealed a total of 18 clear and detectable bands by one-dimensional denaturing gel electrophoresis among 116 rice accessions (Fig. 1 and 2). Accessions 7419 and 7796 had 11 bands. Out of 18 bands eight bands (44.4%) were polymorphic among 116 accessions which were labeled as F, G, H, K, L, N, P, Q and R and ten bands (55.6%) were found monomorphic. Three groups were formed based on major storage proteins bands. Group I comprised of five high molecular weight (HMG) polypeptides (180-60kDa) that were well-conserved polypeptides and were not variable in any accession. Waxy protein (60kDa) of endosperm showed 1.72% polymorphism (Table 2). Group II comprised of six polypeptide protein bands (57-34kDa) out of which five protein bands were polymorphic. Polymorphism of 86.2% was exhibited by precursor polypeptide of glutelin which has low molecular weight of 57kDa. Rapidly moving polypeptide subunit of glutelin (52kDa) presented 96.5% polymorphism. Albumin (42kDa) polypeptide subunit was monomorphic. Single major monomorphic band of Alpha acidic subunits 1, 2 (39-41kDa) was also present in all accessions. Glutelin polypeptide subunit alpha-3 showed 88.79% variability. Glutelin polypeptide subunit (Alpha-4) represented 65.51% polymorphism. Six polypeptide subunits consisted of Group III, out of which 4 bands were polymorphic. β1 subunit of glutelin (20kDa) which is basic protein in nature presented 93.96% polymorphism. Prolamin polypeptide subunit of 10kDa, 13kDa and 16kDa exhibited, 0%, 89.65% and 18.1% polymorphism, respectively. In current study almost all rice accessions from northern Pakistan varied in their minor bands (Fig. 1A). Maximum 18 bands were scored for analysis in this study, while accessions 7664, 7656, 7618, 7402, 7608 and Super Basmati had 17 bands and in accessions 7419 and 7796 minimum 11 bands were recorded (Fig. 1B). Protein profile of all accessions was used to construct dendrogram based on Nei's unbiased genetic similarity coefficients. Genetic similarity coefficients varied from 0.74 to 1.00 in northern areas rice germplasm as shown in Fig. 2. At 0.91% similarity coefficient seven major clusters were formed (Table 3). Cluster I composed of eleven accessions, all having minor and major glutelin bands. Glutelins had more lysine (first

essential amino acid) than prolamin. Accessions belonging to cluster I can be used for high quality glutelin. Cluster II represents only two accessions (7202, 7205) both of them lacking prolamin subunit of 13kDa (Table 3). Accession 7205 was also lacking alpha-4 acidic subunit. Cluster III composed of 74 accessions including three checks varieties: IR6, JP5 and Super Basmati. Cluster IV included eighteen accessions. Cluster V composed of three accessions (7213, 7599 and 7636). Cluster VI grouped four accessions (7408, 7412, 7592 and 7654). Cluster VII had only three accessions (7797, 7796 and 7419). Value of similarity coefficients ranged from 0.74 for 7592-7202, 7418-7412, 7202-7418 and 7202-7408 pairs of accessions to 1.00 in accessions 7214-7407, 7408-7412 and 7232-7409. Accessions belonging to cluster V, VI and VII were found more diverse.

The SDS-PAGE technique is regularly used tool for the assessment of genetic diversity as quality of storage proteins is not affected by environmental fluctuations (Niazi, 2008; Sadia *et al.,* 2012; Amjad *et al*., 2014; Devraj *et al*., 2015; Haque *et al*., 2015). Narrow intra-specific genetic diversity was present in storage protein banding profile when 141 rice accessions were evaluated from northern Pakistan. Although there were 18 different polypeptides scored for cluster analysis but banding profile of 85% accessions was similar. These findings are similar with other research reports (Kusama *et al*., 2004; Swapan, 2015). Findings of this study are not consistent with the results of Rabbani *et al*., (2010) who verified a high inter-specific genetic diversity in Pakistani rice cultivars. Disagreement to the above studies may be contributed to the fact that rice germplasm utilized in these studies was from diverse geographic origin. Accessions 7205, 7419, 7593, 7617, 7636 and 7814 showed diversity in major polypeptide bands, while most of the northern area rice accessions had diversity in minor bands only. Mirali *et al*., (2007) reported that occurrence of major polypeptide protein bands in most of the germplasm proves that genes which code these polypeptide bands are mostly conserved. Germplasm from northern Pakistan was assessed by SDS-PAGE technique for seed storage proteins. Although variability in relative quantity of proteins was recorded but closely same banding pattern was reported for composition of storage proteins containing GBSS (granule-bound starch synthase) 60kDa band also known as waxy protein (Horibata *et al.,* 2004). Quantity of waxy protein is high in *indica* varieties, while *japonica* varieties lack waxy protein or present in small quantity (Ramli & Zin, 2015). Our research also confirms these findings as northern areas rice is short grain japonica type so waxy band (60kDa) was absent in all accessions. For exploitation of high hybrid vigor and identification of broader gene pool of rice provided by cluster analysis can be very helpful (Yamanaka *et al*., 2011). According to genetic distance, accessions from cluster V, VI, VII were most diverse. These accessions should be used for designing effective breeding programs for high quality genetically variable breeding lines. In current study, maximum eighteen bands were scored in northern areas germplasm, while accessions 7664, 7656, 7618, 7608, 7402 and Super-Basmati had seventeen bands each. Minimum eleven bands were recorded in accessions 7796 and 7419. Asghar *et al.,* (2004) observed twenty polypeptide bands in 35 rice accessions. In a different study 26 total bands were recorded (Sharief *et al.,* 2005). Twenty three scorable bands were present in 32 upland rice varieties by Tripathy *et al.,* (2015). In a different study on Iraqi germplasm, a total of 12 polypeptide bands were detected (Thanh & Hirata, 2014). Jugran *et al.,* (2010) observed seven polypeptide bands in 48 rice accessions of India. Difference in number of polypeptide bands may be attributed to gel consistency, size of the gel and germplasm used for evaluation. Another important observation recorded during this study was that four accessions (7814, 7636, 7593 and 7205) have high grain yield plant^{-1} and also showed absence of 16kDa and 13kDa prolamin subunit. This gives an indication to a correlation between grain yield per plant and prolamin marker in rice. Further research is needed to link protein markers with agronomically important traits.

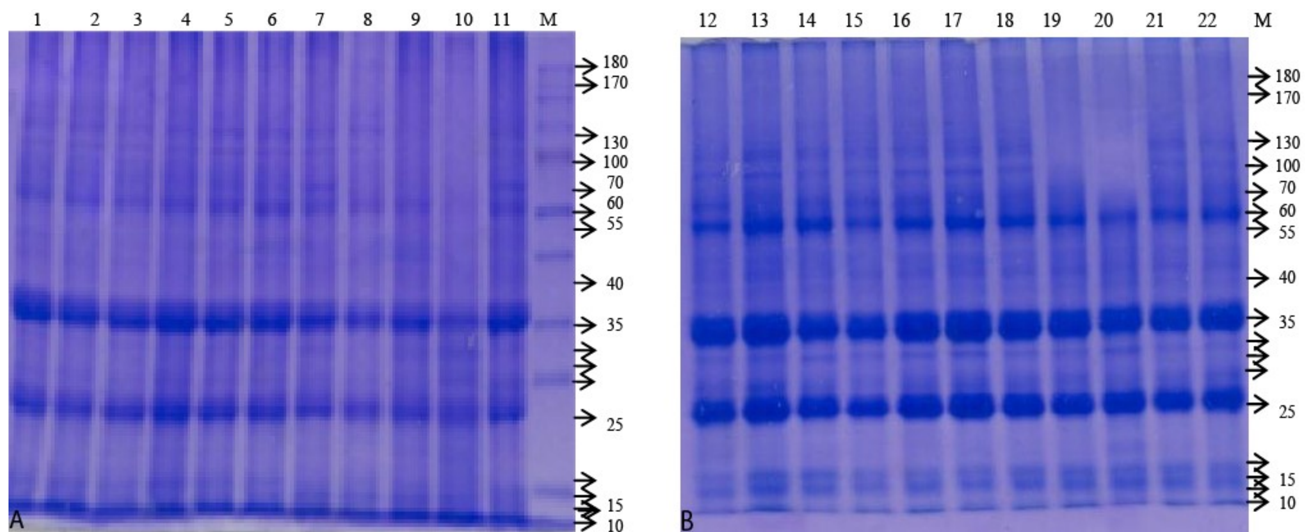

Fig. 1 (A and B). Electrophorograms showing types of protein banding pattern separated by 14% separation gel in different rice accessions from northern areas of Pakistan.

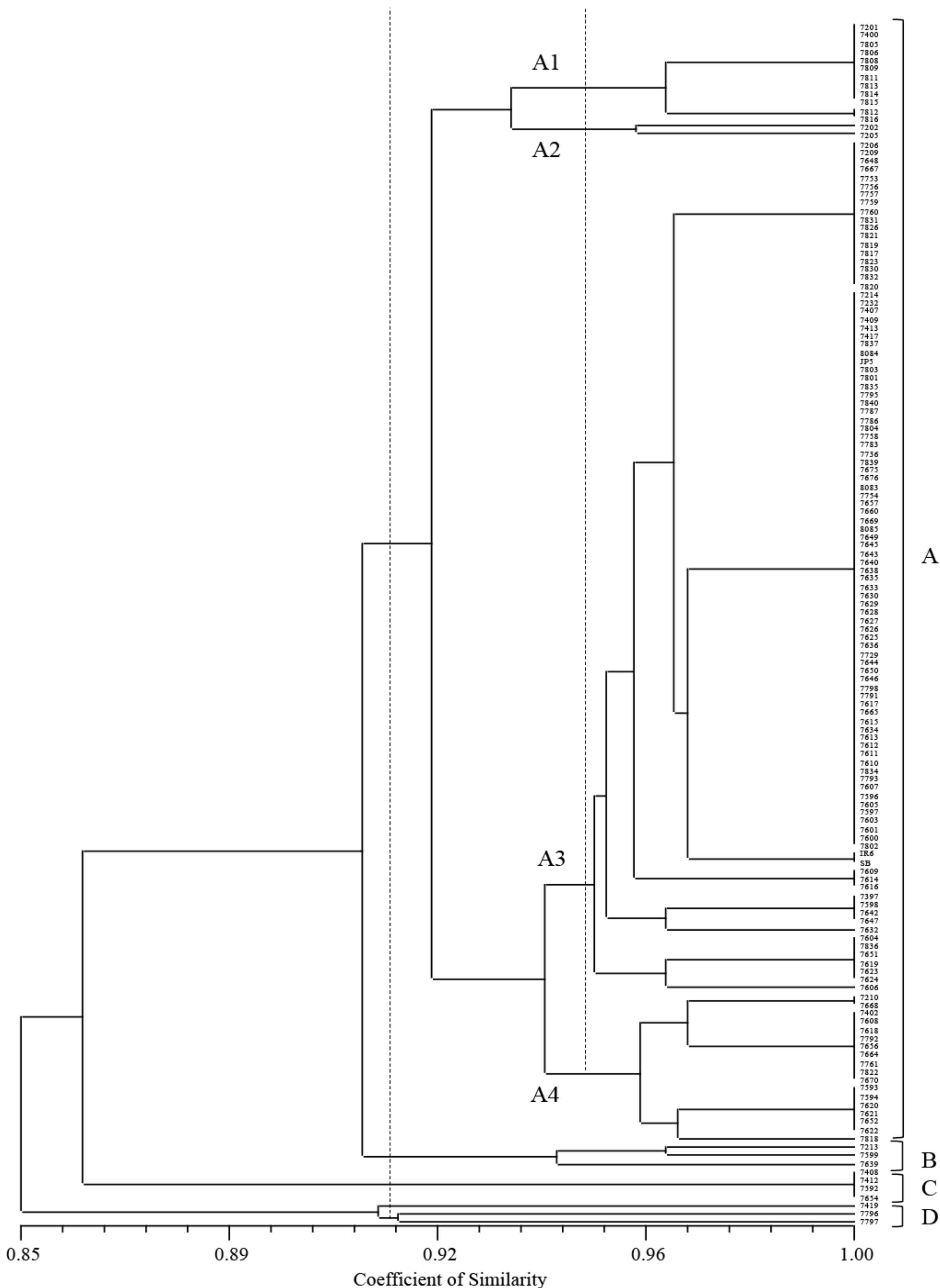

Fig. 2. Dendrogram showing relationship of 116 rice accessions from northern Pakistan on the basis of presence of common bands profile.

Table 3. Clustering of rice accessions based on seed storage proteins using SDS-PAGE analysis.

Cluster	No. of accessions	Accessions falling in each cluster
I	12	7201, 7400, 7806, 7809, 7805, 7811, 7813, 7814, 7815, 7812, 7816.
II	2	7205, 7202.
III	74	7803, 7835, 7840, 7787, 7786, 7804, 7758, 7795, 7783, 7736, 7839, 7675, 7676, 8083, JP5, 7754, 7657, 7660, 7669, 8085, 7649, 7645, 7643, 7640, 7638, 7635, 7633. 7630. 7629, 7628, 7627, 7626, 7625, 7636, 7729, 7644, 7650, 7648, 7798, 7791, 7617, 7665, 7615, 7634, 7613, 7612, 7611, 7610, 7834, 7793, 7607, 7596, 7605, 7597, 7603, 7601, 7600, 7802, Super-Basmati, 7609, 7614, 7616, 7397, 7598, 7642, 7647, 7632, 7604, 7836, 7651, 7619, 7623, 7624, 7606.
IV	18	7210, 7668, 7402, 7608, 7618, 7792, 7656, 7664, 7761, 7822, 7670, 7593, 7594, 7620, 7621, 7652, 7622, 7818.
V	3	7213, 7599, 7639.
VI	4	7408, 7412, 7592, 7654.
VII	3	7797, 7796, 7419.

Conclusion

Genetically diverse accessions on biochemical basis using SDS-PAGE could be of important consideration for designing effective breeding program for maintaining purity and to develop desirable recombinant breeding lines and cultivars for future breeding programs. SDS-PAGE in combination with two dimensional gel electrophoresis and molecular marker analysis is recommended for developing variations of isoforms of good quality protein subunits.

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References

Akbar, F., N. Yousaf, M.A. Rabbani, Z.K. Shinwari and M.S. Masood. 2012. Study of total seed proteins pattern of sesame (*Sesamum indicum* L.) landraces via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). *Pak. J. Bot*., 44: 2009-2014.

Amjad, H., A. Gul and T. Gulzar. 2014. Characterization of tomato germplasm through seed storage protein profiling by SDS-PAGE. *Pak. J. Bot*., 46: 827-832.

Aravind, J., F. Kahani and S. Hittalmani. 2015. Inter-specific *indica-japonica* drought tolerant rice genotypes for aerobic condition. *J. Rice. Res*., 3: 4

Asghar, R., R. Saddique, M. Afzal and S. Akhtar. 2004. Inter- and intra-specific variation in SDS-PAGE of total seed protein in rice (*Oryza sativa* L.) germplasm. *Pak. J. Biol. Sci*., 7: 139-143.

Bibi, A., F.M. Abbasi, M.A. Rabbani and K. Bibi. 2015. Genetic diversity assessment of indigenous rice germplasm from Northern Pakistan using agro-morphometric traits. *Pak. J. Bot*., 47: 1061-1067.

Bibi, S., I.A. Khan, H. Bughio, I.A. Odhano, M.A. Asad and A. Khatri. 2009. Genetics differentiation of rice mutants based on morphological traits and molecular marker (RAPD). *Pak. J. Bot.,* 41: 737-743.

Devraj, L., S.K. Tripathy, R. Kumar, M. Behera and R. Ranjan. 2015. Assessment of genetic diversity in quality protein maize (QPM) inbreds using ISSR markers. *J. Environ. Biol.*, 36: 985–992.

Fedoroff, N.V. 2015. Food in a future of 10 billion. *Agric. Food Security,* 4: 11.

Haque, S., P. Roy, A. Anandan, S. Samantaray, S.K. Pradhan and O.N. Singh. 2015. Genetic diversity study of seed proteins in rice drought tolerant donor accessions. *Rice Genom. Genet.,* 6: 1-5.

Horibata, T., M. Nakamoto, H. Fuwa and N. Inouchi. 2004. Structure and physicochemical characteristics of endosperm starches of rice cultivars recently bred in Japan. *J. Appl. Glycosci*., 51: 303-313.

Jugran, A., I.D. Bhatt and R.S. Rawal. 2010. Characterization of agro-diversity by seed storage protein electrophoresis: Focus on rice germplasm from Uttarakhand Himalaya, India. *Rice Sci.*, 17: 122-128.

Krishnamurthy, S.L., P.C. Sharma1, S.K. Sharma1, V. Batra1, V. Kumar and L.V.S. Rao. 2016. Effect of salinity and use of stress indices of morphological and physiological traits at the seedling stage in rice. *Ind. J. Exp. Biol.*, 54: 843-850.

Kusama, T., S.O. Yangi and M. Iwamoto. 2004. Comparison of rice (*Oryza sativa* L.) seed proteins from several varieties. *Agric. Biol. Chem.*, 48: 1649-1651.

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

Mallimar, M., P. Surendra, N.G. Hanamaratti, M. Jogi, T.N. Sathisha and R. Hundekar. 2015. Genetic variability for yield and yield attributing traits in F3 generation of rice (*Oryza sativa* L.). *Res. Environ. Life Sci*., 9: 24-28.

Meena, R.K. and P. Shukla. 2013. Genetic diversity of seed storage proteins in popular rice (*Oryza sativa* L.) varieties in Chhattisgarh and its application. *Res. J. Agric. Sci.*, 4: 329-332.

Mirali, N., S. El-Khouri and F Rizq. 2007. Genetic diversity and relationships in some *Vicia* species as determined by SDS-PAGE of seed proteins. *Biol. Plant*, 51: 660-666.

Niazi, A.R. 2008. Assessment of genetic diversity among wheat varieties in Sulaimanyah using random amplified polymorphic DNA (RAPD) analysis. *Jordan J. Biol. Sci.*, 1: 159-164.

Rabbani, M.A., Z.H. Pervaiz, Z.K. Shinwari, M.S. Masood and S.A. Malik. 2010. Assessment of genetic variability in rice (*Oryza sativa* L.) germplasm from Pakistan using RAPD markers. *Pak. J. Bot*., 42: 3369-3376.

Ramli, N.S. and N.H.M. Zin. 2015 Proteomic analysis of rice seed storage proteins in relation to nutrient quality of three different commercial rice types. *Jurnal Teknologi*, 77: 7-11.

Reddy, M.T., S.R. Pandravada, N. Sunil and N. Sivaraj. 2016. Genetic diversity and variability of indigenous landrace germplasm collection of rice from traditional agro-ecosystems in tribal areas of Telangana*, Indian J. Global Agric. Ecol.,* 6: 90-107.

Roy, P., M. Hasan, M.G. Rasul and M.M. Hossain. 2015. Seed culture of aromatic rice varieties under salt stress. *Amer. J. Bio. Life Sci*., 3: 260-264.

Sadia, T., Z.H. Pervaiz, M.Y. Mirza, M.A Rabbani and M.S. Masood. 2012. Assessment of phenotypic variability in rice (*Oryza sativa* L.) cultivars using multivariate analysis. *Pak. J. Bot*., 44: 999-1006.

Salgotra, R.K., B.B. Gupta, J.A. Bhat and S. Sharma. 2015. Genetic diversity and population structure of basmati rice (*Oryza sativa* L.) germplasm collected from north western Himalayas using trait linked SSR markers. *PLoS ONE,* 10: 1310-1319.

Sharief, A.E., S.A. Moursy, A.M. Salama, M.I. EL-Emery and F.E. Youssef. 2005. Morphological, molecular and biochemical identification of some rice (*Oryza sativa* L.) cultivars. *Pak. J. Biol. Sci.*, 5: 1275-1279.

Shinwari, Z.K., H. Rehman and M.A. Rabbani. 2014. SDS-PAGE based genetic divergence in safflower (*Carthamus tinctorius* l.). *Pak. J. Bot.*, 46: 811-815.

Swapan, T.K. 2015. Genotyping of scented rice landraces of Odisha using SDS-PAGE of total seed storage protein. *Res. J. Biotech.*, 10: 5-17.

Thanh, V.O.C and Y. Hirata. 2014. Seed storage protein diversity of three rice species in the Mekong Delta. *Biosph. Conserv*., 4: 59-67.

Tripathy, S.K., B.R. Mohapatra, P.K. Nayak, S. Pal, N. Senapati, G.B. Dash, D. Lenka, D. Swain and R. Ranjan. 2015. Revealing genetic variation in upland rice using seed storage protein profiling. *Res. Crops*, 16: 320-331.

Yamanaka, S., S.A. Jatoi, S. San-Yi, S.L. Kothari, T. Htut and K.N. Watanabe. 2011. Genetic diversity of Myanmar rice and their implementation on management methods. *Afr. J. Biotech.*, 10: 1290-1298.

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