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Seed protein profiling of rice genotypes

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Abstract

Biochemical characterization and quantification of seed protein of a diversified group of 117 rice genotypes consist of land races, wild rice, advanced breeding lines and popular varieties of Chhattisgarh India was done in the present study. Total fifteen bands were observed in which eight were polymorphic. Total seed protein was quantified by Bradford methods and on the basis of protein percent the group was divided in three categories less medium and high three cultivars i.e. Badagadakhurta (8.27%) followed by Kalokuchi (8.05%) and Nunki Gurmatia (7.20%) among 117 showed highest seed protein percent. Similarity coefficients varies from 65 to 100% based on jaccard's the genotypes were grouped into six clusters and shows Cluster I and II (d) had 5 genotypes present, cluster I had two subgroup Kalamkati and G-21 similar and Rajeshwari, IR 87728-367-B-B and Uri are some differences between genotypes similarity coefficient of this cluster 55% while cluster II (a) 15 genotypes and three subgroup cluster II (a) Moroberekan genetically different to the other. clusters II (c) two, cluster II (e) divide into two subgroup and present 29 genotypes, and cluster II (b), the largest one with 114 genotypes this cluster three genotypes Poornima, Basmati, and E 2526 are different diversity than other.

Overall on the cluster Rajeshwari, IR-87728-267 and Uri genotypes was found to be different. Thus, the It is also most likely that the protein marker would then be able to estimate the amount of genetic diversity in the genus and perhaps obtain an indication as to the origin of the rice genotypes. On the basis of cluster analysis the genotypes, Rajeshwari, IR-87728-267 and Uri for protein marker and Badagadakhurta and ARC 10376 for protein content seems to be different and further would be used to enhancement of nutritional quality of rice.

Keywords: Seed storage protein, bradford assay, SDS-PAGE, genetic diversity, polymorphism, varietal identification

Introduction

Rice is major crop of India and Chhattisgarh is one of the biggest contributor in rice production with an average of 3.6 million ha land with the productivity of 1.2 to 1.6 t/ha depending upon the rainfall (http://agricoop.nic.in 2016-2017) ^[4] Chhattisgarh has diverse collection with rice germplasm that need to be characterize. Compared with other cereals, rice has a relatively low protein contents ranging from 7% to 10% of the grain dry weight, (Khush GS 1997). Storage proteins are coded by multi gene families, accumulate in large quantities during seed development in special organelles called protein bodies. Rice storage proteins are divided into four fractions: albumins, globulins, prolamin, and glutelin. Glutelin fraction is the predominant protein in rice endosperm. However the number of major protein is almost similar in all genotypes, the number of bands and their position is genotype specific because of variation in protein subunits and amino acid content. storage proteins can be used as biochemical marker as these proteins are polymorphic and generally little affected by environmental conditions during the seed development and maturation (Nerker and Rao, 1993; Sadia et al., 2009; Mehetre and Dahat, 2001) ^[15,9] Genetic diversity of storage proteins can be utilized for pedigree analysis as well as nutritional quality improvement by identifying lines with more or variably expressed storage protein.

Material and Methods

In the present study 170 cultivars were used for identification on the basis of seed storage protein analysis since it represents a valid alternative and improved approach to identify relatedness and characterization among cultivars (Mennella *et al.*, 1999; Iqbal *et al.*, 2005) ^[10, 5]. Seeds of 170 rice genotypes (Table 1) (The experimental materials consist of total 170 genotypeps which include 9 wild rice, 69 landraces, 14 germplasm rice collection from undivided Madhya Pradesh and Chhattisgarh, popular donors rice varieties and 78 advanced breeding lines for different yield and yield contributing genotypes) of diverse agronomic characters were obtained from IGKV, Raipur. C. G. India. Seed protein was isolated from fine powder of dehulled rice grains by keeping rice flour in Tris urea buffer extraction buffer (0.125M Tris-HCl pH 6.8, 4% SDS, 4M Urea, 5% β merceptoetanol) for about 5 minutes and

centrifuged at 4 °C at 15000 rpm for 10 minutes. The protein was quantified by Bradford assay (1976) and SDS-PAGE was performed using a 5% stacking gel and 12% separating gel. 50 µg of protein was loaded and Thermofisher Scientific tricolor pre strain Protein Mol. Wt. Marker10 to 170 kDa was loaded for size determination. The samples were run on SDS-PAGE gels at 25 °C for Stacking gel to the power supply at 90 volt and resolving gel at 70 volts using Tris-glycine buffer pH 8.3. The gels were stained in staining solution (9% methanol, 2% acetic acid and 0.05% coomassie brilliant R-250) for 6 hours, and de-stained in de-staining solution (9% methanol, 2% acetic acid) for about 8 to 10 hours until the protein bands appeared clearly. Staining, de-staining was conducted on shaker at the speed of 1200 r/Hour. After washed with distilled water, the gels were scanned by gel documentation system Bands were scored on the basis of presence (1) and absence (0). Relationship among rice genotypes was estimated using Jaccard's similarity coefficient and a

UPGMA (Unweighted pair group method) tree. Cluster analysis is an aims to classify a sample of subjects (or objects) on the basis of a set of measured variables into a number of different groups such that similar subjects are placed in the same group. Cluster analysis has no mechanism for differentiating between relevant and irrelevant variables. Therefore, the choice of variables included in a cluster analysis must be underpinned by conceptual considerations. Bands were scored for their presence (1) and absence (0) and a scoring matrix was generated. The pairwise genetic distance 1973) calculated (Nei. was by Popgene v1.32 (http://www.ualberta.ca/fyeh). The dendrogram was constructed based on similarity co-efficient, using the unweighted pair group method of arithmetic averages (UPGMA) employing sequential, agglomerative hierarchic and nonoverlapping clustering (SAHN) (Sneath and Sokal, 1979) in NTSYSpc, version 2.1 (Applied Biostatistics Inc., USA).

Table 1: List of genotypes and their details u	used for SDS-PAGE analysis
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S. N.	Rice genotype	Characteristics feature	S.N.	Rice genotype	Characteristics feature
1	Annada	Realese variety for drought tolerant, early maturing, realease from silk board	86	M-2260	Local accession from C.G.
2	ARB 8	Projeny derived from IR-64 and Buddha, drought tolerant, semi tall genotype	87	M-2298	Local accession from C.G.
3	Abhya	Released high yielding, resistant to gall midge	88	M-2463	Local accession from C.G.
4	Azucina	drought tolerant donor, tall (japonica group)	89	MAHESHWARI	Release variety of IGKV, Raipur C.G.
5	Arb6	Projeny derived from IR-64 and Buddha, drought tolerant, semi tall genotype	90	AVT-1-IME3	Advance Breeding Line
6	Bamleshwari	This is the only bacterial blight resistant variety released for M.P. and Chhattisgarh.	91	DURGESHWARI	Release variety of IGKV, Raipur C.G.
7	Buddha	Landrace from Karnataka	92	SHYAMLA	Release variety of IGKV, Raipur C.G.
8	Bakal	Landrace, deep root system	93	AVT2IME 10	Advance Breeding Line
9	Bas-370 -	Release variety	94	RAJESHWARI	Release variety of IGKV, Raipur C.G.
10	Bhataphool	Landrace, drought tolerant	95	Chandrahasini	Release variety
11	Batroo	Landrace, drought tolerant	96	JALDUBI	Release variety of IGKV, Raipur C.G.
12	Bhatajhooli	Landrace, drought tolerant	97	AVT2-A-6	Advance Breeding Line
13	Badshabhog	bhog Small grain, aromatic rice	98	AVT2-IMAE-12	Advance Breeding Line
14	CT9993	Japonica, drought tolerant	99	Indira Sugandhit Dhan1	Release variety of IGKV, Raipur C.G.
15	Cross 116	Drought tolerant	100	AVT2E-TP-6	Advance Breeding Line
16	Chaptigurmutiya	Landrace, high yield. Bold seeded, tall, purple base	101	AVT-1-ASG	Advance Breeding Line
17	Deshi lal dhan	Semi dwarf drought tolerant early maturing landrace	102	AVT2E-TP-5	Advance Breeding Line
18	Deshi no17	Semi dwarf drought tolerant early maturing landrace	103	SUVT3/7	Advance Breeding Line
19	Dagaddeshi	Drought tolerant landrace of CG	104	DT-10	Advance Breeding Line
20	IR62266	Drought tolerant line (mainly because of osmatic adjustment)	105	M-1433	Local accession from C.G.
21	IC267982	Drought Tolerant line	106	RP-Bio-226	Resistant to BLB, improved versio of BPT 5204
22	IR64	Release variety	107	IR-84984-83-15-Bo	Advance breeding line
23	IR55419-04	High yielding breading material	108	IC-267982	Drought Tolerant line
24	Kranti	Derived from cross 116	109	IBD-1	Release variety
25	Lalmati	Landrace from UP., small grain with red kernel and aromatic.	110	BPT-5204	-
26	Laloo-14	Landrace, semi tall, drought resistance.	111	M-144	Local accession from C.G.
27	Mahamaya	Release variety 112 MTU-1010		Local Accession from CG	
28	MTU1010	High yielding	113	SUVR-3/7	-
29	Samleshwari	Release variety	114	Dubraj	Aromatic rice high yielding tall, medium slender grain
30	Swarna	Resistant to BLB and Tolerant to many diseases	115	M-1051	Local accession from C.G.
31	Safri 17	High Yielding	116	Saltu Gurmatia (2788)	Germplasm line
32	Shennong89366	Drought tolerant donor	117	Tulsi Gurmatia (3239)	Germplasm line

33	Swarna sub1	Swarna-Sub1 is the flood-tolerant version of the popular mega-variety Swarna (MTU 7029) in eastern India.	118	Bangla Gurmatia (2711)	Germplasm line
34	Vandana	Early maturing drought tolerant semi tall released from bihar.	119	DJOGOLONDJOG OL	Drought tolerance
35	IBD 1	Drought tolerant, resistance to BLB, Blast and stem borer. Derived from cross of Swarna x IR42253 Maturity110 days,	120	Maheshwari	Release variety of IGKV, Raipur C.G.
36	Danteshwari	Release variety of IGKV, Raipur C.G.	121	Botki Gurmatia (2728)	Germplasm line
37	Poornima	It has a good escape as well as tolerance capacity to moisture stress.	122	Chapti Gurmatia	Germplasm line
38	IR87728-367-b-b	Donor for QTL1.1	123	Chapti Gurmatia (3011)	Germplasm line
39	Swarna sub1	Swarna-Sub1 is the flood-tolerant version of the popular mega-variety Swarna (MTU 7029) in eastern India.	124	G-42	Advance breeding Line
40	IR84984-83-15- 110-b	Advance breeding line	125	Badagadkhuta	-
41	RRF78	-	126	Karmamasuri	-
42	Shabhagi dhan	High yield, drought tolerant, released variety, bold grain type	127	RPF-78	-
43	MTU1010	High yielding	128	Jabar Sail	
44	Punjab bas 3	Resistance to BLB	129	Kalamkati	-
45	RYT 3275	Resistance to BLB	130	Dhalshaita	-
46	Aganni	Land race resistance to gall midge	131	Dhangar	-
47	Karma masuri	It possesses resistance to Gm biotype 1, 4 & 5 and tolerant to leaf blast and brown spot	132	Bhinuhangin	-
48	Safri 17	High yielding	133	Gulmurli	-
49	RP bio 226	Resistant to BLB, improved versio of BPT 5204	134	Srikamal	-
50	Dubraj	Aromatic rice high yielding tall, medium slender grain	135	Elaychi	Aromatic rice high yielding tall, medium slender grain
51	BPT 5204	Resistant to BLB (xa5,Xa21), short slender, aromatic popular rice variety	136	Bisni-1	Germplasm
52	Jitpiti	Resistant to gall midge	137	RR-152	Advance breeding Line
53	PR122	Resistance to blast (Pi2)	138	RR-137	Advance breeding Line
54	IR64	Multiple resistant, high yielding, semi dwarf, medium grain stable genotype	139	RR-149	Advance breeding Line
55	SLO16	Drought donor	140	IR-8	Advance breeding Line
56	Kalokuchi223	Drought donor	141	Reg -1035	Landraces
57	Kaliya	Drought donor	142	Reg-1038	Landraces
58	Pratao	Drought donor	143	G-106	Advance breeding Line
59	Chuvadau130	Drought tolerance	144	G-114	Advance breeding Line
60	Chengri-2	Drought tolerance	145	G-121	Advance breeding Line
61					
	CR5272	Drought tolerance	146	G-134	Advance breeding Line
62	Epagri 2	Drought tolerance	147	G-194	Advance breeding Line
62 63	Epagri 2 Djoglon-djoglo	Drought tolerance Drought tolerance	147 148	G-194 G-196	Advance breeding Line Advance breeding Line
62 63 64	Epagri 2 Djoglon-djoglo WR1	Drought tolerance Drought tolerance Wild rice Oryza nivara	147 148 149	G-194 G-196 G-198	Advance breeding Line Advance breeding Line Advance breeding Line
62 63 64 65	Epagri 2 Djoglon-djoglo WR1 WR2	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150	G-194 G-196 G-198 G-200	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line
62 63 64 65 66	Epagri 2 Djoglon-djoglo WR1 WR2 WR3	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151	G-194 G-196 G-198 G-200 G-203	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line
62 63 64 65 66 67	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151 152	G-194 G-196 G-198 G-200 G-203 ARC-10376	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line
62 63 64 65 66 67 68	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara Wild rice Oryza nivara Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151 152 153	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces
62 63 64 65 66 67 68 69	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR41	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara	147148149150151152153154	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces
62 63 64 65 66 67 68 69 70	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR41 WR73	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151 152 153 154 155	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces
62 63 64 65 66 67 68 69 70 71	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR41 WR73 WR99	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151 152 153 154 155 156	G-194 G-196 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces
62 63 64 65 66 67 68 69 70 71 72	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR41 WR73 WR99 WR132	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151 152 153 154 155 156 157	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces - Landraces Advance breeding Line
62 63 64 65 66 67 68 69 70 71 72 73	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR41 WR73 WR99 WR132 E1701	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151 152 153 154 155 156 157 158	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces - - Landraces Advance breeding Line - Landraces
62 63 64 65 66 67 68 69 70 71 72 73 74 74	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara Germplasm line Germplasm line	147 148 149 150 151 152 153 154 155 156 157 158 159	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces - - Landraces Advance breeding Line - Landraces Landraces Landraces
62 63 64 65 66 67 68 69 70 71 72 73	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR41 WR73 WR99 WR132 E1701	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara	147 148 149 150 151 152 153 154 155 156 157 158	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces - - Landraces Advance breeding Line - Landraces
62 63 64 65 66 67 68 69 70 71 72 73 74 75	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1703	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza nivara Germplasm line Germplasm line Germplasm line	147 148 149 150 151 152 153 154 155 156 157 158 159 160	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926 Nagina-22	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces - - Landraces Advance breeding Line - Landraces Landraces Landraces Release variety
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 78	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1702 E1703 E1827	Drought tolerance Drought tolerance Wild rice Oryza nivara	147 148 149 150 151 152 153 154 155 156 157 158 159 160 161	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926 Nagina-22 BAM-5997	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces - - Landraces Advance breeding Line - Landraces Landraces Landraces Landraces Landraces Landraces Landraces
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1703 E1827 E2010	Drought tolerance Drought tolerance Wild rice Oryza nivara Germplasm line	147 148 149 150 151 152 153 154 155 156 157 158 159 160 161	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926 Nagina-22 BAM-5997 G-100	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces - - Landraces Advance breeding Line - Landraces Landraces Release variety Landraces Advance breeding Line
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 78	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1701 E1702 E1703 E1827 E2010 E2312	Drought tolerance Drought tolerance Wild rice Oryza nivara Wild rice Oryza latifolia Germplasm line Germplasm line Germplasm line Germplasm line Germplasm line Germplasm line	147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926 Nagina-22 BAM-5997 G-100 G-47	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces Landraces Advance breeding Line - Landraces Landraces Landraces Release variety Landraces Advance breeding Line Advance breeding Line
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1701 E1702 E1703 E1827 E2010 E2312 E2367	Drought tolerance Drought tolerance Wild rice Oryza nivara Germplasm line	$\begin{array}{c} 147\\ 148\\ 149\\ 150\\ 151\\ 152\\ 153\\ 154\\ 155\\ 156\\ 157\\ 158\\ 159\\ 160\\ 161\\ 162\\ 163\\ 164\\ 165\\ 166\\ \end{array}$	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926 Nagina-22 BAM-5997 G-100 G-47 G-1	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces Landraces Advance breeding Line - Landraces Landraces Landraces Release variety Landraces Advance breeding Line Advance breeding Line Advance breeding Line
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 82	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1701 E1702 E1703 E1827 E2010 E2312 E2367 M4628	Drought tolerance Drought tolerance Wild rice Oryza nivara Germplasm line Germplasm line	$\begin{array}{c} 147\\ 148\\ 149\\ 150\\ 151\\ 152\\ 153\\ 154\\ 155\\ 156\\ 157\\ 158\\ 159\\ 160\\ 161\\ 162\\ 163\\ 164\\ 165\\ \end{array}$	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926 Nagina-22 BAM-5997 G-100 G-47 G-1 G-8 G-102 G-5	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1703 E1827 E2010 E2312 E2367 M4628 E1857 E2526 M-114	Drought tolerance Drought tolerance Wild rice Oryza nivara Germplasm line	$\begin{array}{r} 147\\ 148\\ 149\\ 150\\ 151\\ 152\\ 153\\ 154\\ 155\\ 156\\ 157\\ 158\\ 159\\ 160\\ 161\\ 162\\ 163\\ 164\\ 165\\ 166\\ 167\\ 168\\ \end{array}$	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-555 BAM-5446 BAM-5926 Nagina-22 BAM-5997 G-100 G-47 G-1 G-1 G-8 G-102 G-5 G-21	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces Landraces Advance breeding Line - Landraces Landraces Landraces Release variety Landraces Advance breeding Line Advance breeding Line
62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 82	Epagri 2 Djoglon-djoglo WR1 WR2 WR3 WR32 WR36 WR 41 WR73 WR99 WR132 E1701 E1702 E1703 E1827 E2010 E2312 E2367 M4628 E1857 E2526	Drought tolerance Drought tolerance Wild rice Oryza nivara Germplasm line Metal accession from C.G. Germplasm line Germplasm line	$\begin{array}{c} 147\\ 148\\ 149\\ 150\\ 151\\ 152\\ 153\\ 154\\ 155\\ 156\\ 157\\ 158\\ 159\\ 160\\ 161\\ 162\\ 163\\ 164\\ 165\\ 166\\ 167\\ \end{array}$	G-194 G-196 G-198 G-200 G-203 ARC-10376 Juma Koi Murali ARC 10955 BPT-1004 RYT-3275 BAM-5446 BAM-5926 Nagina-22 BAM-5997 G-100 G-47 G-1 G-8 G-102 G-5	Advance breeding Line Advance breeding Line Advance breeding Line Advance breeding Line Landraces

Result

The obtained result revealed variation in storage protein content (Table 2) it ranged from 1.01 per cent to 8.05 percent. Highest protein content was recorded in Badagadakhurta (8.27%) followed by Kalokuchi (8.05%) and Nunki Gurmatia (7.20%). The genotype ARC 10376 had reported lowest protein content with value of 1.01%.

A total of fifteen bands were present (PMW-B size ranges between 17 -130 kDa) (Figure 1.). The protein banding pattern was characterized by no. of polymorphic and monomorphic in the increasing order. The genotypes shows differed in number of bands by their mobility (position) and intensity. The result on the band map of the protein profiles suggested that the specific genotypes could be differentiated based on the position of bands but not on numbers, as some of the genotypes expressed similar number of bands. In present study, the wide range of protein peptides (high to low molecular weights) showed the potential for discriminating rice genotypes and can create additional variability to supplement existing germplasm. Prolamine ranges of 13 to 17 kDa, while 40 kDa was acidic subunit of glutelin 20 kDa basic subunit and 26 kDa was the globulin. Seed storage proteins polymorphism can be used as a potential molecular varietal identification marker for and economic characterization of rice germplasm as reported earlier by Netra and Prasad (2007)^[12]. Chauhan and Nanda (1984) and Sengupta and Chattopadhyay (2001) their result on the banding pattern of the protein profiles suggested that the specific genotype could be differentiated either based on the position or intensity of bands but not on number, as some of the varieties expressed similar number of bands. Presence of low intensity of bands can also be used as reflection of quantity of particular polypeptide chain of those proteins.

A cluster analysis based on the Jaccard's similarity coefficient was performed on the 117 genotypes and resulted in bifurcations covering a large range (65 to 100%) of similarity coefficient (Fig. 2). The similarity value (1.3) was quite high for genotypes included in clusters I, and II b. Bhanaspanchi, Bangla gurmatia, were diverse type grouped in cluster I and Rajeshwari, IR-87728-267 and Uri in cluster I respectively. Rajeshwari, IR-87728-267 and Uri genotypes are different. Thus, the It is also most likely that the protein marker would then be able to estimate the amount of genetic diversity in the genus and perhaps obtain an indication as to the origin of the rice genotypes. These days' protein markers are in routine use and widely accepted as a reliable tool for the deten11 ination of genetic identity of crop varieties (Weir, 1990)^[18].

Table 2: Genotypes	divided int	o different	group
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Wild rice Variet	ies Landrace	s Advance breeding	Germplasm
Annada, ARB ARB6, Bamleshv IR36, M TU1010 IR64, Kranti, I Samleshwa ri, Sv WR3, uBD1,Danteshwa WR99, Badshahbhog, A masuri, Safri 17, Durgeshwari, Rajeshwari, Ch Indira sugndf Elayachi, Jeeradh Tarunbhog, CHII Basmati 370, Bas Swarna,	 vari, CT9993, Punjab Bas3, Mahamaya, varna, Swarna dana, ri,Poorn ima, ganni, Karma Dubraj, Jitpiti, Shymala, andrahasini, it dhan-1, an, Nagina22, R-8, CGZR-1, mati 1, IR64, 	 Batro, Ial Dhan, adDeshi, Botki 28), Dau 130, IR 62266, IC267982, IR42253, OGOL, 84984- 17-83-48-1-BSahabhagi D panchi, IR84984-83-15-862- B, IR 90019 akhuta, 159-B, IR 90019-22-28-2-B, B-6, I 45-40, 78, IR 55419-04, IR 86931-B-400 Y -211, 86918-B305, IR 87728-75-B-B, 2, BAM 87728-367-B-B,IR 84984- 83-15- 926, B, CR 5272, EPAGRI-2, PINKA RYT 3275, PR 122, SLO-16, Ka 45-37, SL AVT-1-IME-3, R1570, AVT-2 AS CHAU BPT 204(Improved), BPT5204(proved), AVT-2-IME-10, AVT-2 AVT-1- ASG, R-RHZ-LI- ar, Dhala RRHZ-IB-13, R-RHZ-SM-14, R-F MI-30, R-56, RR100, A-GM-AS- GG9, G93,G100,G102,Cross116 GMATN47,IR55419,KALO KUCHI,G132,G134,Kalamk ati,G136,G158,G173,G186,G19 G196, G198, G200, G203, R-RF- ARC10955,R-RF-75, RR152, RR RR-149, RR-8 M011, G104, abri, 35, Reg- 0-1-1,IR 	han, -17- RRF- D, IR IR 110- EO, Gurmatia(2676),Gurmatia (3053), Bangla Gurmatia (3053), Bangla Gurmatia (2711), Sultu Gurmatia (2788) Bisni-I, CHAPTI GURMATIA Chepti Gurmatia (3011), Jhunl Gurmatia (2739), Kalam Nunk Gurmatia (2788), Srikamal, 47, 47, 48, 49, 49, 40, 469,

Table 3: Mean values for see	d protein content (%)	in rice genotypes

S. No.	Variety Name	Protein content (%)	Sr. No.	Variety Name	Protein content (%)
1	Annada	2.99	86	Shenong	1.33
2	ARB8	2.56	87	IR 87728-367-B-B	1.55
3 4	Abhaya	2.51	88 89	WR-132	4.71 2.99
4 5	Azucina ARB6	5.97 1.71	89 90	CHAU-DAU-130 Karma Masuri	4.03
6	Bamleshwari	1.71	90 91	E-1702	1.55
7	Buddha	1.44	91	M-1433	4.72
8	Bakal	2.56	93	AGANNI	1.44
9	Bhataphool	1.97	94	IR 84984-83-15-110-B	1.55
10	Batroo	1.71	95	DT-10	5.07
11	Bhatajhooli	2.29	96	Gul murali	5.6
12	CT 9993	1.49	97	Bhinuhangin	1.6
13	Cross 116	1.87	98	Dhangar	2.67
14	Deshi lal Dhan	1.81	99	Srikamal	1.97
15	Deshi No. 17	1.97	100	Elayachi	5.76
16	Dagad Deshi	1.23	101	Jeeradhan	1.17
17	Kranti	2.19	102	Bisni-I	1.65
18 19	Lalmati Laloo-14	3.15 1.49	103 104	RR-152 RR-137	6.67 6.83
20	Mahamaya	1.49	104	RR-149	6.93
20	Samleshwari	1.49	105	RR-8 M011	5.65
21	Swarna	4.87	100	Reg-1035	1.23
23	Shennong-89366	2.08	107	Reg-1038	1.17
24	Durgeshwari	1.17	109	CHIR-8	6.67
25	Shyamala	1.97	110	CGZR-1	5.55
26	Chandrahasini	1.07	111	IET-23829	5.01
27	Rajeshwari	1.33	112	Basmati-370	3.15
28	AVT-2-A-6	2.45	113	Basmati-1	2.56
29	AVT-2-IME-12	1.6	114	R-RHZ-MI-30	4.21
30	Indira sugndhit dhan-1	1.44	115	Azucena	1.49
31 32	AVT-2-E-TP-6 AVT-2-E-TP-5	1.71 1.97	116 117	IR-64 RR-100	6.98
32	Swarna sub 1	2.45	117	BAM-1292	1.17 2.56
33	Vandana	1.07	118	BAM-1292 BAM-5446	2.08
35	IBD-1	1.76	110	BAM-5926	2.56
36	Poornima	1.39	120	Nagina-22	1.81
37	Shubhangi Dhan	4	122	BAM-5997	1.92
38	Aganni	1.49	123	Karigilas	2.08
39	BPT 5204 [Improved]	2.08	124	WR-1	5.08
40	Jitpiti	1.97	124	WR-2	4.97
41	SLO 16	1.12	126	WR-32	4.49
42	Kalia	1.65	127	E-1857	1.92
43	PRATAO	2.03	128	E-2520	2.13
44	Chuva Dau 130	3.09	129	E-1702	2.56
45	DJOGOLON DJOGOL	2.45	130	E-1827	3.09
46 47	Maheshwari Botki Gurmatia (2728)	2.24 6.03	131 132	E-2367 E-1703	1.81 1.76
47	CHAPTI GURMATIA	6.13	132	E-1703 E-1701	1.70
49	Chepti Gurmatia (3011)	6.88	133	E-2010	2.08
50	AVT-2-IME-10	1.28	135	E-2312	1.07
51	Jaldubi	6.36	136	M-4628	1.28
52	Danteshwari	6.88	137	M-114	4.98
53	Safri 17	1.07	138	M-1051	1.92
54	Dubraj	2.61	139	G-1	1.44
55	Dhalashita	1.33	140	G-5	2.24
56	Gurmatia (2676)	6.67	141	G-8	1.07
57	Jhunki Gurmatia (2739)	7.2	142	G-21	2.03
58	Kalam Gurmatia (3053)	7.03	143	G-42	2.13
59 60	Nunki Gurmatia (2784)	7.20	144	G-47	1.39
60 61	Sultu Gurmatia (2788) Tulsi Gurmatia	6.29 5.6	145 146	G-69 G-93	1.92 2.35
62	M-144	1.49	146	G-100	2.35
63	Jabar Sail	1.49	147	G-100 G-102	1.12
64	PRF-78	5.6	140	G-102 G-104	2.45
65	RYT-3275	3.04	150	G-108	1.07
66	SUVT3/7	2.35	151	G-127	2.03

67	MTU-1010	2.67	152	G-134	2.13
68	RP-BIO-226	2.45	153	G-114	1.39
69	BPT-5204	1.44	154	G-196	1.92
70	Kalamkati	1.07	155	G-198	2.35
71	Kalanamak	1.55	156	G-194	2.08
72	R-56	3.89	157	G-200	1.12
73	Kadamphool	2.03	158	G-203	2.45
74	WR-3	3.77	159	Mikhudeb	2.93
75	Jhilli	1.39	160	Koi murali	1.07
76	Swarna	1.6	161	WR 132	3.65
77	Bhansapanchi	6.27	162	PR 122	1.39
78	Banda	1.71	163	Juma	1.21
79	Bada gada khurta	8.27	164	ARC 10376	1.01
80	Chau Dau	1.87	165	Uri	1.6
81	kali aus	2.45	166	EPAGRI-2	1.55
82	GP-145-40	5.31	167	ARC 10955	2.93
83	Dular	6.05	168	BPT-5204	1.65
84	Moroberekan	1.06	169	WR 73	4.99
85	Kalokuchi	8.05	170	IRM-3	4.69

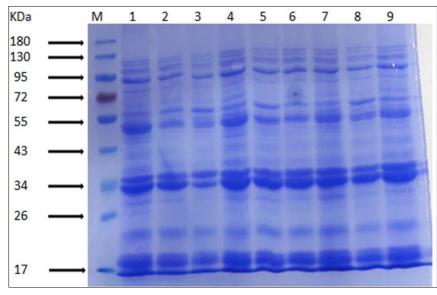


Fig 1: Types of protein banding pattern separated by 12% separating gel in different rice varieties.

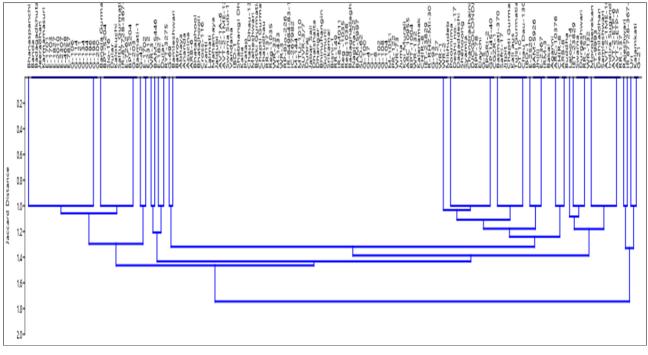


Fig 2: Clustering pattern of 170 genotypes of rice for seed protein profile as revealed by dendogram.

Discussion

It is concluded the genotype with highest protein content was found in Bada gada khurta (8.27%) followed by Kalokuchi (8.05%) and Nunki Gurmatia (7.20%). at seed storage protein level is present rice varieties / genotypes which can be utilized in breeding programs aimed at increasing level of genetic diversity which ultimately will be useful for the development of new improved genotypes of rice. Also to increase chances of developing more diverse germplasm of rice, the genotypes showing maximum genetic distance (Rajeshwari, IR-87728-267 and Uri) will be utilized in further breeding programs.

References

- Bhowmik A, Omura T, Kumamaru T. Screening of rice verities for endosperm storage proteins. Plant Breeding. 1990; 105:101-105.
- Ghafoor A, Ahmad Qureshi AS, Bashir M. Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiate* (L.) R. Wilczek based on morphological traits and SDSPAGE. Euphytica. 2002; 123:367-378.
- 3. Hammer Q, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica. 2001; 4(1):9.
- 4. http://agricoop.nic.in 2016-2017
- 5. Iqbal SH, Ghafoor A, Ayub N. Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. Pakistan Journal of Botany. 2005; 37:87-96.
- Jugran A, Bhatt ID, Rawal RS. Characterization of agrodiversity by seed storage protein electrophoresis: Focus on rice germplasm from Uttarakhand Himalaya, India. Rice Science. 2010; 17(2):122-128.
- 7. Kagawa H, Hirano H, Kikuchi F. Variation of glutelin seed storage protein in rice (*Oryza sativa* L.). Japanese Breed. 1988; 38(3):327-332.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227:680-685.
- 9. Mehtre SS, Dahat DV. Cultivar purity subcommittee report. Newsletter of the Association of Official Seed analysts. 2001; 59(1):40-57.
- Mennella G, Onofaro SV, Tonini A, Magnifico V. Seed storage protein characterization of Solanum species and of cultivars and androgenic lines of *S. melongena* by SDS-Page and AE-hplc. Seed Science and Technology, 1999; 27:23-35.
- 11. Naz F, Farooq S, Arshad R, Afzaal M, Akram M. Discriminating upland and lowland rice genotypes through proteomic approach. Pakistan Journal of Botany. 2006; 38:1731-1738.
- 12. Nethra N, Prasad SR, Vishwanath K, Dhanraj KN, Gowda R. Identification of rice hybrids and their parental lines based on seed, seedling characters, chemical tests and gel electrophoresis of total soluble seed proteins. Seed Sci. & Technol. 2007; 35:176-186.
- 13. Osborne TB. The proteins of the wheat kernel. Carnegie Inst. Washington, Washington, D.C, 1907.
- Patra N, Chawla HS. Biochemical and RAPD molecular markers for establishing distintiveness of basmati rice (*Oryza sativa* L.) varieties as additional descriptors for plant variety protection. Indian J. Biotechnology. 2010; 9:371-377.
- 15. Sadia M, Malik SA, Rabbani MA, Peaece SR. Electrphoretic characterization and the relationship

between some brassica species. Electron. J Biol. 2009; 5:1-4.

- 16. Subbaiah SV, Singh S, Kumar RM, Viraktamath BC, Ahmad MI. Agrotechniques for hybrid cultivation and seed production. Directorate of Rice Research, Hyderabad, India, 2005, 1-29.
- 17. Yamagata H, Sugimoto T, Tanaka K, Kasai Z. Biosynthesis of storage proteins in developing rice seeds. Plant physiology. 1982; 70(4):1094-1100.
- Weir BS. Genetic Data Analysis Methods for Discrete Population Genetics Data Sinaver Assoc, Suderland, Massachuetts, 1990.