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Genetic Diversity of Seed Storage Proteins in Pigmented and Non-pigmented Indigenous Paddy (*Oryza sativa* L.) Landraces of Assam, India

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ABSTRACT

Background: Pigmented rice particularly, red and black rice have come into prominence due to their promising antioxidant property. In Assam, all major ecotypes of paddy *viz*., Ahu, Sali, Bao, Boro have large number of indigenous landraces with red kernel. Although Assam is recognised as a secondary centre of origin and diversity yet gene erosion is assuming serious proportion. It is acknowledged that many indigenous cultivars are facing extinction and there is apprehension that many may be lost even before they are evaluated and characterised. Among different methods, seed protein profile is recognised as fingerprint for a race by International Seed Testing Association (ISTA) as it can resolve interspecific and intraspecific variation without ambiguity.

Methods: The present study involves, molecular characterisation and genetic diversity analysis of pigmented and non-pigmented landraces. Seed protein profiles for the paddy landraces were worked out by SDS-PAGE. SDS-PAGE was carried out with two phase gradient buffer system with upper stacking gel and lower separating gel to ensure denaturation of protein while migrating through it.

Result: Among the twenty landraces a total of 15 proteins bands were observed in the size range of 12.1 Kd to >122.0 Kd. Highest protein bands were observed in cultivar *Ranjit Amon, Bismuthi* and *Boro* (ecotype) all with 15 protein bands. On the other hand, lowest protein bands were recorded in *Amona* with only 9 bands. Among the protein bands six with molecular weight >122.0 Kd; 112.0 Kd; 97.4 Kd, 90.0 Kd and 15.0 Kd were consistently found in all the landraces. The dendrogram revealed a total of four clusters. The landrace *Amona* (red) did not belong to the any cluster and most distantly related to others. On the other hand, some are genetically identical. *Agnisali*, *Suhagmani* and *Tengeri* (all white) had no genetic distance between them and hence identical at molecular level. Within the same cluster both red and white rice landraces were observed. Our study suggested that several proteins bands were detected only on specific rice and it could be as biochemical markers for further research.

Key words: Cultivars, Indigenous, Non-pigmented, Pigmented, SDS-PAGE.

INTRODUCTION

Indigenous landraces of major crops are the product of natural evolution and selection pressure and hence gift of nature, which sustained the food security of mankind for thousands of years. Red rice is a group of paddy where the kernel colour has characteristics of red to purple red colour of varied intensity. Red rice is relatively unfamiliar to general public since it is seldom marketed but it has attracted the attention of researchers because anthocyanin is known to be a powerful antioxidant. Pigmented (red, brown, purple and black) rice in particular has gained a lot of attention as raw materials for the production of commercial health food supplements due to its high phenolic, anthocyanin and antioxidant contents. Based on cultivation practice and season in Assam paddy cultivars are classified into four major groups, *viz*., Ahu (autumn paddy), Sali (winter paddy), Boro (summer paddy) and Bao (deep water paddy). Red rice landraces are found among all the four groups, particularly in Bao group which are rich in nutritive and nutraceutical values. Loying *et al*. (2010) studied such lesser-known landraces, particularly their genetic diversity is very limited. Protein polymorphism serves as genetic marker as they are direct products of active genes and are quite polymorphic and generally heritable (Gepts, 1986). Both DNA based and seed protein profile-based markers are used for molecular characterization by a number of workers. However, DNA based markers generate variable designate results since different primers generate different profiles, which are often not reproducible and consistent. By contrast seed protein profile is consistent, stable and reproducible since seed proteins are storage protein and only variation is due to genetic factors. The high stability of seed protein profile and its additive nature make it a promising tool for

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distinguishing genotypes of particular plant species. Therefore, in the present study SDS-PAGE technique was employed for analysis of seed protein diversity in different red and white indigenous landraces collected from different districts of Assam. That apart even far and many landraces of conventional white rice are also endangered to various degrees of continuous negligence. This loss of agrobiodiversity is a matter of serious concern and threat to food security (Swaminanthan, 2011). The present study was undertaken in the backdrop of such scenario. The study involves molecular characterization and diversity analysis of pigmented (red) and non-pigmented (white) by seed protein profile with SDS-PAGE technique. Seed protein electrophoretic profile is gaining acceptance and International Seed Testing Association (ISTA, 1996) has recognised it as a tool for identification of cultivar species and to study intraspecific variability.

MATERIALS AND METHODS

Altogether 20 indigenous landraces of paddy were collected for evaluation. Among them eleven were pigmented red rice and other nine landraces were non pigmented. All these landraces were collected from the different districts of Assam, *viz*., Nagaon, Sonitpur, Darrang, *etc*. The pigmented red paddy landraces used in this study were *Amona* (AM1), *Biroi* (BI2), *Kabra* (KA3), *Kablam* (KA4), *Kabrabadam* (KA5), *Bil Bao* (BB6), *Godasali* (GS7), *Lalkartisali* (LK8), *Ronga Kurmi* (RK9), *Kura Binni* (KB10), *Kokowa* (KK11) (Fig 1) and the non pigmented white paddy landraces were *Agnisali* (AS12), *Lotasali* (LS13), *Suhagmani* (SM14), *Kalizira* (KZ15), *Laki* (LK16), *Tengeri* (TG17), *Ranjit Amon* (RA18), *Boro* (BR19), *Bismuthi* (BS20) (Fig 1). Seed protein profiles were worked out by SDS-PAGE method outlined by Laemmli (1970). Manually dehusked grains (300 mg) were extracted with ice-cold Tris-buffer 0.2 M (PH: 7.5). Seed proteins were resolved in two phase acrylamide gel, stacking gel 4%, separating gel 13.5%. Protein bands were visualized by staining with Coomassie Brilliant Blue R-250 stains. Protein molecular weight marker (PMW-M, Bangalore Gennie) was co-electrophoresed to determine the molecular weight of the individual protein. After gel scoring the data were analysed to prepare similarity index (SI) matrix using Nei and Lis coefficient. From this, the dendrogram was prepared by unweighted pair group method with arithmetic average (UPGMA) using NTSYS PC V2, 02j Software.

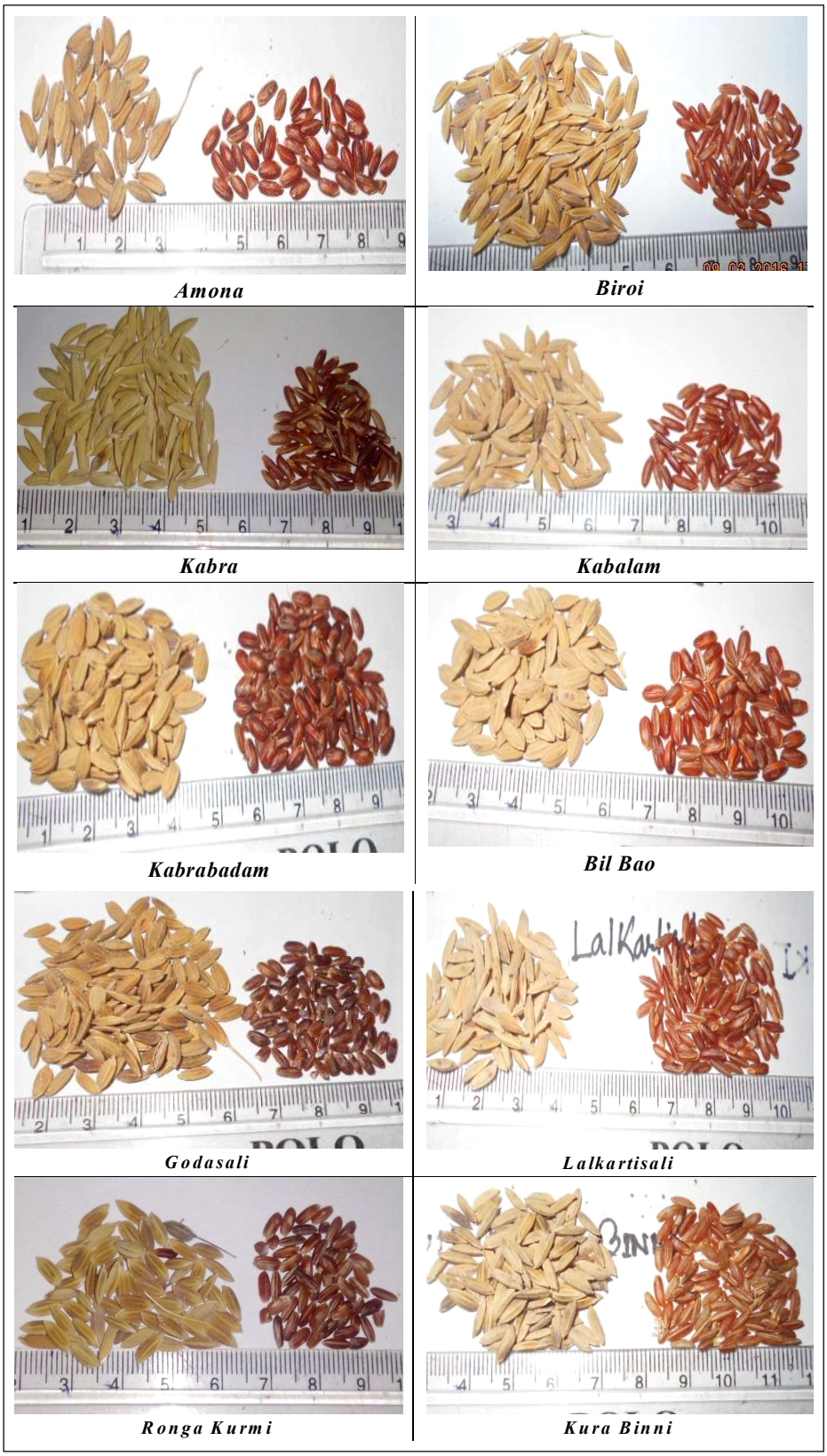
RESULTS AND DISSCUSSION

A number of workers working with different paddy groups show that genetic diversity in paddy can be effectively analysed and documented based on seed protein profile. Analysis of seed protein profile resolved by SDS-PAGE revealed a total of 15 protein bands among the landraces in the present study (Table 1). Considerable variation has been observed, in protein profile which is evident from the fact that seed protein varied from 12.1 Kd to > 122 Kd (Table 1). Among the landraces, the highest number of 15 protein

Table 1: Frequency distribution of seed protein in red and white paddy landraces of Assam.

Em (cm)	Mol.wt (Kd)	*Godasali*	*Lalkartisali*	*Ronga Kurmi*	*Kura Binni*	*Kokowa*	*Agnisali*	*Suhagmani*	*Laki*	*Tengeri*	*Ranjit Amon*	*Boro*	*Bismuthi*	*Amona*	*Biroi*	*Kabra*	*Kablam*	*Kabrabadam*	*Bil Bao*	*Lotasali*	*Kalizira*	T2
0.2	>122.0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	12
0.8	>122.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20
0.9	>122.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20
1.1	~122.0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	13
1.4	112.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20
1.6	104.0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	12
1.9	97.4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20
2.2	90.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20
2.7	74.0	1	1	0	1	0	0	0	0	0	1	1	1	0	1	1	1	1	0	0	0	11
3.3	61.4	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18
3.7	52.1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	17
5.2	32.2	0	1	0	1	0	0	0	1	0	1	1	1	0	1	0	1	1	1	1	1	12
6.2	22.2	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	19
7.5	15.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20
8.5	12.1	1	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	16
T 1		13	14	11	14	12	13	13	14	13	15	15	15	09	12	11	12	11	11	11	11	

1: Presence of protein band; 0: Absent; T1: Total protein band for a landrace; T2: Total landrace for a particular protein band.



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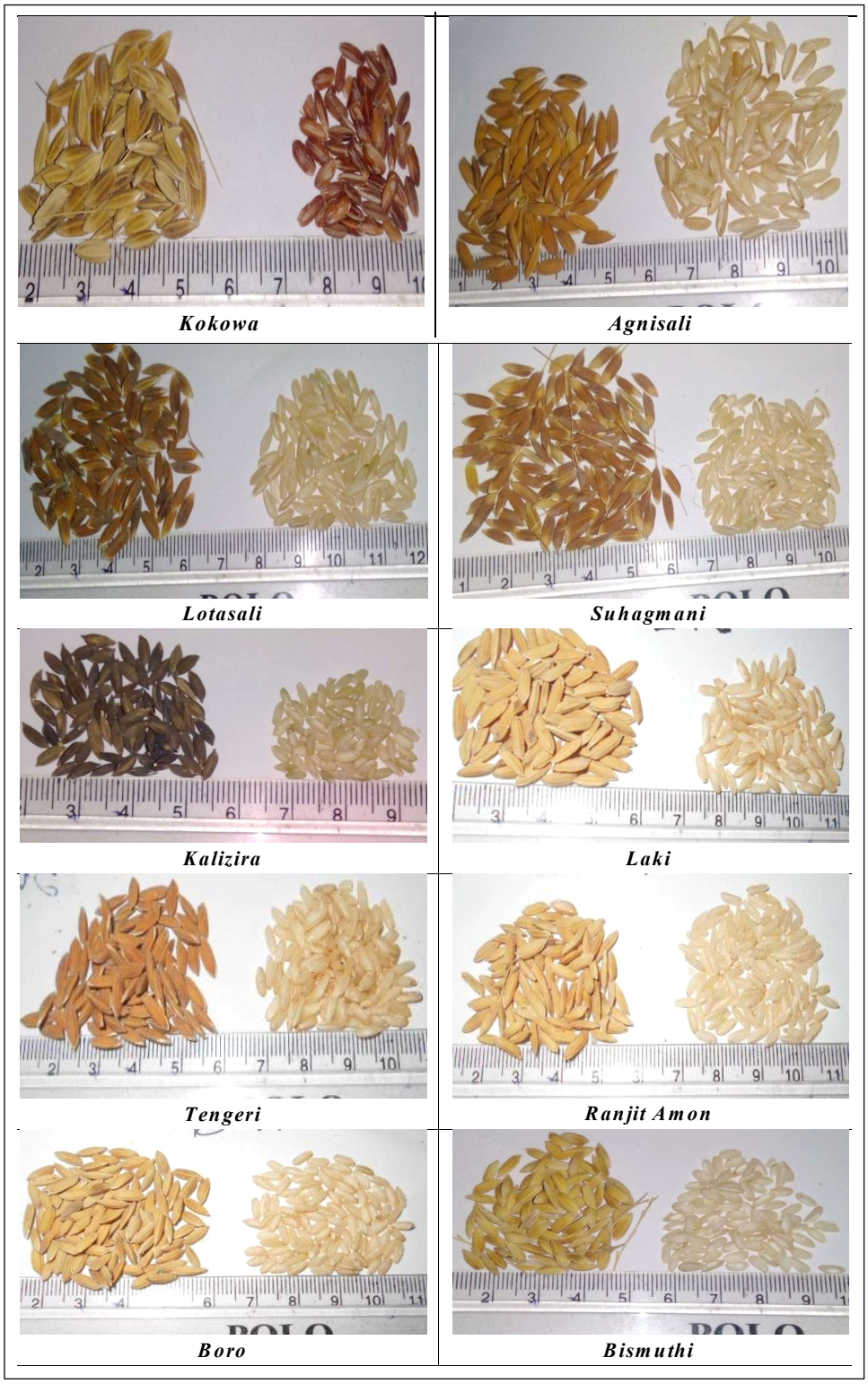

Fig 1: Indigenous paddy landraces with grain and dehusked grain collected from different parts of the state of Assam.

Table 2: Similarity index matrix based on seed protein profile of indigenous landraces of Assam.

	Godasali	*Lalkartisali*	*Ronga Kurmi*	*Kura Binni*	*Kokowa*	*Agnisali*	*Suhagmani*	*Laki*	*Tengeri*	*Ranjit Amon*	*Boro*	*Bismuthi*	*Amona*	*Biroi*	*Kabra*	*Kabalam*	*Kabrabadam*	*Bil Bao*	*Lotasali*	*Kalizira*
Godasali	1.00																			
Lalkartisali	0.80	1.00																		
Ronga Kurmi	0.87	0.80	1.00																	
Kura Binni	0.80	1.00	0.80	1.00																
Kokowa	0.80	0.73	0.80	0.73	1.00															
Agnisali	0.87	0.80	0.87	0.80	0.93	1.00														
Suhagmani	0.87	0.80	0.87	0.80	0.93	1.00	1.00													
Laki	0.80	0.87	0.80	0.87	0.87	0.93	0.93	1.00												
Tengeri	0.87	0.80	0.87	0.80	0.93	1.00	1.00	0.93	1.00											
Ranjit Amon	0.87	0.93	0.73	0.93	0.80	0.87	0.87	0.93	0.87	1.00										
Boro	0.87	0.93	0.73	0.93	0.80	0.87	0.87	0.93	0.87	1.00	1.00									
Bismuthi	0.87	0.93	0.73	0.93	0.80	0.87	0.87	0.93	0.87	1.00	1.00	1.00								
Amona	0.60	0.67	0.73	0.67	0.67	0.73	0.73	0.67	0.73	0.60	0.60	0.60	1.00							
Biroi	0.67	0.73	0.53	0.73	0.60	0.67	0.67	0.73	0.67	0.80	0.80	0.80	0.67	1.00						
Kabra	0.73	0.67	0.60	0.67	0.67	0.73	0.73	0.67	0.73	0.73	0.73	0.73	0.73	0.93	1.00					
Kabalam	0.67	0.73	0.53	0.73	0.60	0.67	0.67	0.73	0.67	0.80	0.80	0.80	0.67	1.00	0.93	1.00				
Kabrabadam	0.60	0.67	0.47	0.67	0.53	0.60	0.60	0.67	0.60	0.73	0.73	0.73	0.73	0.93	0.87	0.93	1.00			
Bil Bao	0.60	0.67	0.47	0.67	0.53	0.60	0.60	0.67	0.60	0.73	0.73	0.73	0.73	0.93	0.87	0.93	1.00	1.00		
Lotasali	0.60	0.67	0.60	0.67	0.67	0.73	0.73	0.80	0.73	0.73	0.73	0.73	0.73	0.93	0.87	0.93	0.87	0.87	1.00	

bands was observed in *Ranjit Amon*, *Boro* and *Bismuthi*. On the other hand, lowest number of protein bands was observed in *Amona* with 9 protein bands (Table 1). Three other cultivar is *Lalkartisali*, *Kura Binni* and *Laki* exhibited second highest number of protein bands with 14 protein bands each (Fig 4, Table 1). However, their profile was different. Among the protein bands, some were consistently found to be present in all the landraces. Among them two have molecular weight >122 Kd, while another had molecular weight 112 Kd (Fig 4). Among low molecular weight protein only the one with molecular weight 15.0 Kd was found in all the 20 landraces (Table 1). On the other hand, the protein with molecular weight 74 Kd occurred in lowest frequency since it was found in only 11 landraces (Table 1). Loying *et al*. (2010) worked with 10 deep water paddy (Bao) cultivars of Assam and reported a 22 protein bands ranging in size from 97.40 Kd to-~13.2 Kd. The authors also reported three protein bands of 26.7, 17.0 and 15 .7 Kd as molecular marker for deep water paddy. Tiwari (2010) worked with 12 indigenous landraces of Boro rice or spring rice reported a total 14 protein bands in the size range of 16.2 to 9.0 Kd. Like Joha paddy, Boro landraces also exhibited considerable genetic diversity and three proteins with molecular weight 16.2, 24.75 and 31.4 Kd were found to be marker for Boro group landraces, since they were consistently found in all the landraces (Dutta Roy *et al*., 2010).

Analysis of similarity index (SI) matrix revealed considerable variability among the landraces as revealed by SI values. Among the landraces, SI values varied from 47% to 100% (Table 2). On the higher size 100% SI value was observed between a number of landraces *e.g*., between *Agnisali* and *Tengeri* as well as between *Tengeri* and *Suhagmani* (Table 2). On the other hand, *Amona* exhibited the least similarity with any other landrace which is reveal from the fact that the SI value of *Amona* with other landraces varied from 60 to only 73 (Table 2). The variability in SI matrix was reflected in the dendrogram which reveal the phylogenetic relationship among the landraces. Dendrogram analysis reveals a total 4 cluster (Fig 2). The first and biggest cluster contains a total of 7 landraces with similarity coefficient among them ranging from 85 to 100 (Fig 2). The second cluster had 5 landraces *viz*., *Lalkartisali*, *Kura Binni*, *Ranjit Amon*, *Boro* and *Bismuthi*. The fourth cluster was the smallest with two landraces, *viz*., *Godasali* and *Ronga Kurmi* with similarity coefficient of 85 between them (Fig 2). The most notable aspect of the dendrogram is that red rice and white rice did not form separate clusters; rather both red and white rice cultivars were found to belong to same cluster. The first cluster had seven landraces and out of them, five were red rice while the remaining two were white. Likewise, in cluster 2 there were five landraces and out of them, three were white while the rest two were red (Fig 2). However, those landraces which exhibited 100% similarity were either red or white *e.g*. *Agnisali*, *Suhagmani* and *Tengeri* exhibited 100% SI value and all three were white (Fig 2). Similarly, *Biroi*, *Kabalam* exhibited 100% similarity and both are red (Fig 2). Apart from qualitative difference, there was also some quantitative difference also as revealed by pixel intensity analysis (Fig 3). Pixel intensity spectra reveal that most of the high molecular weight proteins have low concentration of protein and in the gel, they appeared as very thin band and corresponding pixel intensity are also lowest. By contrast most of the low molecular weight protein had relatively high concentration of protein. In the gel they appeared as thick and deeply stain bands. Among them the protein with molecular weight 15 Kd was found to be thickest and most prominent in the gel and its corresponding pixel intensity was also found to be highest. Another low molecular weight

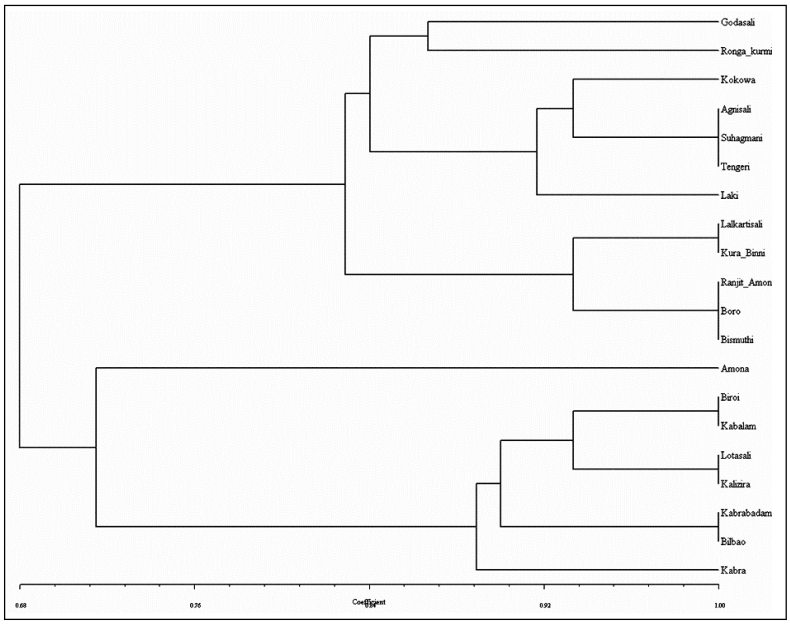

Fig 2: Clustering of indigenous rice cultivars based on seed protein electrophoretic profile using Jaccard's similarity coefficient.

protein with size 22.2 Kd was found to exhibit second highest pixel intensity implying that protein concentration for this band is second highest (Fig 3).

Among the landraces in the present study, two namely *Amona* and *Ronga kurmi* appears to be unique and very distantly related to the remaining landraces. Because they did not belong to any cluster in the dendrogram (Fig 2). The landrace *Amona* exhibited genetic distance of about 0.84 and SI value in the range of 57.14 to 69.23 implying that genetically it is most distantly related to other landraces in the present study. The other landrace *Ronga kurmi* also exhibited similar genetic dissimilarity with others. By contrast, some landraces are morphologically different with aspect to seed morphology, but at molecular level they were found

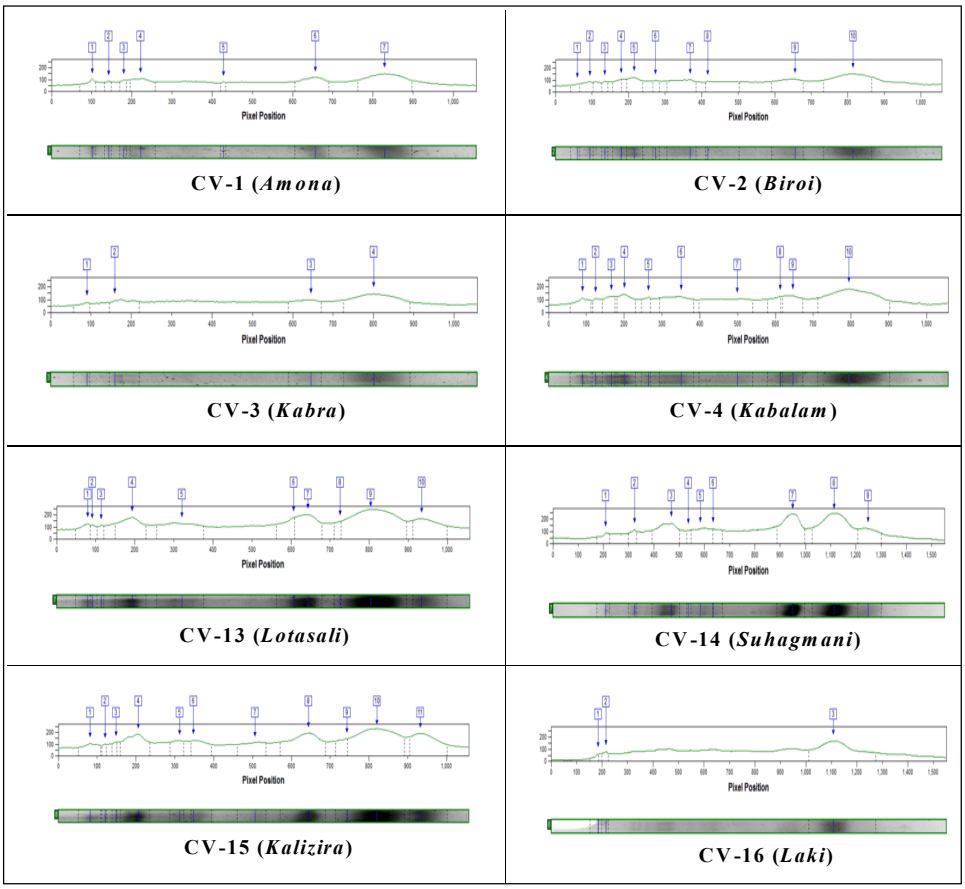

Fig 3: Pixel intensity analysis showing the relative proportion of individual proteins of few indigenous rice landraces.

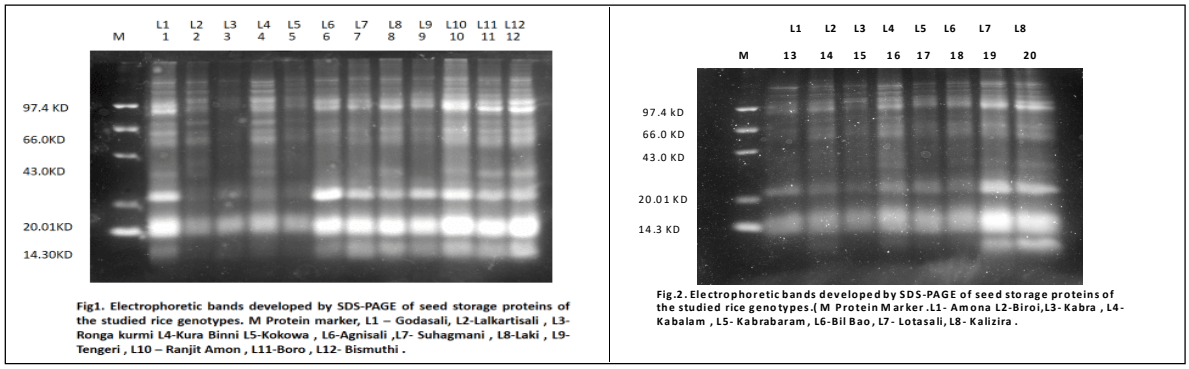

Fig 4: Seed protein profile of indigenous rice landraces resolved in 13.5% polyacrylamide gel. M: Protein molecular weight marker; Lane 1 to 20: Individual landraces of red and white cultivars.

to be identical. For instance, *Tengeri, Suhagmani* and *Agnisali* were morphologically distinct but at molecular level they were, found to be identical with genetic distance 0.0 between them (Fig 2).

Dendrogram analysis showed that within the same clusters there is both red rice as well as white rice landraces in random and there was no separate cluster for red or white rice. Quantitative study by pixel intensity analysis showed that mostly low molecular weight proteins particularly the one with 15 Kd have relatively high concentration of protein while the high molecular weight proteins occur in relatively low concentration. However, in the present study two high molecular weight protein (97.4 and 90.0 Kd) occurred in relatively high amount. Santos *et al*. (2013) predicted three proteins in wild rice *Oryza glumaepatula* as glutelin (34-36 Kd), albumin (15-25 Kd) and prolamin (15-18 Kd).

CONCLUSION

Several rice protein bands have been detected only on specific rice both of SDS PAGE. This study suggested the specific protein bands can be used as biochemical markers for rice and further more study to predict their functional protein. No crop has as much genetic variability as paddy and paddy represents the largest gene pool with a recorded number of 75,0000 to 10,0000(IRRI). Many workers, corroborates the views that molecular analysis is a powerful and proven tool to find out genetic similarity and dissimilarity among closely related landraces and it can be used to identify a particular landrace or cultivar as recognized by International Seed Tenting Association (ISTA).

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Conflict of interest: None.

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