Bio-physicochemical study for glutinous and nonglutinous rice with genetic diversity of Bangladeshi rice germplasm

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Abstract

Glutinous rice is popularly known as sticky rice in Japan, Korea, China, Thailand, Philippine, Malaysia, Indonesia and some other countries. People of those countries preferably take it as cooked rice and various kind of processing food. Information on physicochemical properties of this glutinous rice is available in those countries, while in Bangladesh, there is no information about physicochemical properties and polymorphisms of the glutinous rice. Therefore, the study was conducted to characterize the physicochemical properties and polymorphisms of glutinous rice varieties. A set of 36 rice germplasms were used in this study. All parameters of physicochemical properties were analyzed as per laboratory manual of Grain and Nutritional Quality division of BRRI as well as molecular trait was analyzed as per laboratory manual of Biochemistry and Molecular Biology Department under university of Dhaka. All rice germplasm were analyzed by the cleaved amplified polymorphic sequence marker RM190F-GBSSW2R/*AccI* for determination of G/T single nucleotide polymorphism (SNP) of the *Waxy* gene. In this study, 7 glutinous rice samples have shown T polymorphism (AGTTATA sequence) whereas 29 nonglutinous rice germplasms have shown G polymorphism (AGGTATA sequence). The range of amylose content of all glutinous rice (T polymorphism) varied from 5% to 8.4%, whereas nonglutinous rice (G polymorphism) varied from 11% to 29%.

KEY WORDS

glutinous rice, nonglutinous rice, polymorphism and amylose content

1 | INTRODUCTION

Rice is the staple food and around 3.5 billion people are consuming the rice (*Sreepada and Vijayalaxmi*, 2013). In Asia, it is the predominant staple food for 17 countries including Bangladesh. Highest amount of carbohydrate among the nutrients accumulates in the rice endosperm. Starch is one of the most common carbohydrate for human diet. It consists of amylose and amylopectin. Different rice varieties with different grain size and shape as well as diverse characters are available throughout the world. There are many rice varieties including glutinous rice (or *Waxy* rice) and nonglutinous rice with variation in the apparent amylose content. Amylose content is a key determinant of the cooking and processing quality of rice (*Oryza sativa* L). Low amylose levels are usually associated with tender, sticky, cohesive, glossy cooked rice, while higher amylose levels tend to cook dry, fluffy and separated grains. Glutinous rice differ from nonglutinous rice principally in having no amylose in its starch, which is essentially entirely amylopectine (*Vidal and Juliano*, 1967). Similarly, glutinous rice differs from other types of rice as because the grain starch contains essentially no amylose (0–2% dry basis) and high amount of amylopectin (*Juliano*, 1979), which is responsible for the sticky quality of cooked glutinous rice. Generally, amylose content of *Waxy* rice starches ranged from 0.1% to 3.25% (*Zuo et al*., 2009; *Chang et al*., 2007). Glutinous rice, however differs from the non-glutinous rice mainly in having low (<5%) or almost no amylose in its starch but basically high in amylopectin; Glutinous showed the lowest amylose content (4.2%) reported by *Hyun et al*., 2011. In despite of the very low-amylose and high-amylopectin contents, each *Waxy* rice variety gives different eating quality characteristics to the processed rice products (*Kim et al*., 1996; *Keeratipibul et al*., 2008). In Bangladesh, people of very limited region (ethnic group) traditionally consume this type of rice. Furthermore, a little bit information on physicochemical properties of glutinous rice germplasm obtained from Bangladesh. On the other hand, there is no specific information on the molecular traits (single nucleotide polymorphisms) of glutinous rice germplasm. Therefore, we proposed this research to identify the molecular traits of glutinous rice germplasm available in Bangladsh.

2 | MATERIALS AND METHODS

A set of 36 rice germplasm used in this study were composed of 33 local cultivars and 3 BRRI developed HYVs, which were collected from Genetic Resource and Seed division (GRS) of Bangladesh Rice Research Institute (BRRI), Gazipur.

Length and breadth of milled rice was measured in millimeter using slide calipers. Micro Kjeldahl procedure of *AOAC* (2005) is used for the determination of nitrogen and crude protein is calculated by multiplying the nitrogen content by a factor 5.95. An estimation of the gelatinization temperature (GT) is indexed by the alkali digestion test (*Little et al.,* 1958). GT is measured by the alkali spreading value. Cooking time is that time when starch granules are fully disappear in the boiling water with increasing time (Ranghino method). Volume expansion of cooked and uncooked milled rice was measured by water displacement method. Amylose in rice is discharged by treatment with dilute alkali. By the addition of Tri-iodide ion, amylose produces blue color. The absorbance of blue color produced in aqueous solution is measured by UV-spectrophotometer at 620 nm as described by *Williams et al.,* (1958) as modified by *Juliano*, (1971).

Restriction endonuclease is called the molecular scissor. AGGTATA/AGTTATA polymorphism was detected by restriction endonuclease cleavage. It can cut DNA by recognizing a specific sequence. The G/T (AGGTATA/AGTTATA sequence) polymorphism in intron 1 of the *Waxy* gene (In1G and In1T alleles) was determined by restriction enzyme digest of a polymerase chain reaction (PCR) fragment generated from this genomic region. Specifically, the DNA fragment containing the polymorphic sequence of In1 SNP was amplified from genomic DNA using forward primer RM190F and reverse primer GBSSW2R with restriction enzyme (*AccI*) in the PCR reaction. The product size was 252 bp, which generated two fragments of 124 bp and 128 bp after digestion with the restriction enzyme (*AccI*).

The degree of chalkiness was determined using milled rice upon visual observation. An international standard scale was used for classifying endosperm chalkiness of milled rice (*Khush et al*., 1979). The percent of head rice was calculated by weight of head rice divided by weight of milled rice and multiplied by hundred.

3 | RESULTS

3.1 | Glutinous rice

Out of 36 rice germplasm 7 had glutinous rice including Athabinni, Binni-1, Binni-2, Cylindrical Tapi-629, Gohatibinni, Kalabinni-1 and Rangabinni (Table-1). Picture shows glutinous rice and glutinous cooked rice (Figure-1 and Figure-2). The range of amylose content of all glutinous rice varied from 5% to 8.4%. Among these cultivars 2 had long slender (LS), 4 had long bold (LB) and 1 had medium bold (MB) type grain. Range of protein content varied from 7.8% to 8.5%. Range of alkali spreading value varied from 5.3 to 7.0. All glutinous rice have shown low gelatinization temperature except Binni-2 which have shown intermediate gelatinization temperature, highest cooking time (18 min) and highest volume expansion (3.6). Lowest volume expansion was shown in Athabinni, Gohatibinni, Kalabinni-1 and Rangabinni. All glutinous rice (Figure-3) have shown T polymorphism (AGTTATA sequence) with opaque grain (0% chalkiness). Glutinous rice had better head rice recovery than the nonglutinous rice. The range of head rice recovery of glutinous rice varied from 58.3% to 64.6% (Table-1).

3.2 | Nonglutinous rice

Out of 36 rice germplasm 29 had nonglutinous rice including Baoijhaki, Boradudhkalam, Boylam, BR 11, BRRI dhan 28, BRRI dhan 29, Depa, Dudhkalam, Dudsail, Harilaxmi, Hatishail, Joalbogh, Jol, Kalimanik, Karailadhan, Kharmao, Khorma, Kanaibansi, Lathamona, Ledabinni. Magoibalam, Maloti, Molladigha, Motichak, Neda, Pakhisail, Patnai-231, Poushmoricha and Topaboro (Table-1). Picture shows nonglutinous rice and nonglutinous cooked rice (Figure-1 and Figure-2). The range of amylose content of all nonglutinous rice varied from 11% to 29%. Among these cultivars 8 had long slender (LS), 9 had long bold (LB), 1 had medium slender, 8 had medium bold (MB) and 3 had short round type grain. Range of protein content varied from 7.0 to 10.7%. Range of alkali spreading value varied from 3.2 to 6.8. Among nonglutinous rice, 20 have shown intermediate gelatinization temperature and 9 have shown low gelatinization temperature. Range of cooking time varied from 15 to 23 minutes. Among them 13 cultivars had more than 18 minutes cooking time. Range of volume expansion varied from 3.4 to 4.4. Among them 19 cultivars had more than 3.6 volume expansion. All nonglutinous rice (Figure-3) have shown G polymorphism (AGGTATA sequence) with different types of chalkiness like opaque, white belly, white center and translucent. Range of head rice recovery of these cultivars varied from 17.7% to 69% (Table-1).

3.3 | Correlations of different traits

Pearson correlations of glutinous and nonglutinous rice based on protein content, alkali spreading value, cooking time, volume expansion ratio and amylose content has shown in Table-2. In the case of glutinous rice cooking time was significant and negatively correlated with alkali spreading value whereas volume expansion ratio was significant and positively correlated with cooking time. Amylose content was positively correlated with alkali spreading value but negatively correlated with protein content, cooking time and volume expansion. In the case of nonglutinous rice cooking time was highly significant and positively correlated with protein content whereas cooking time was highly significant and negatively correlated with alkali spreading value. Amylose content was negatively correlated with alkali spreading value but positively correlated with protein content, cooking time and volume expansion. Head rice recovery was significant and positively correlated with alkali spreading value but highly significant and negatively correlated with cooking time.

3.4 | Cluster analysis of different traits

Four properties such as amylose content, volume expansion ratio, polymorphisms and chalkiness based clustering provided seven clusters at level below 5 for rice germplasm (Figure-4). Cluster-1 consists of Ledabinni which had 11% amylose, G nucleotide and opaque grain. Cluster-2 consists of seven rice cultivars; included Binni-1, Rangabinni, Cylindrical Tapi-629, Kalabinni-1, Binni-2, Athabinni and Gohatibinni having the range of amylose content of 5% to 8.4%. These cultivars have shown T nucleotide and opaque grain. Cluster-3 consists of two rice cultivars; included Khorma (17%) and Kanaibansi (18%) having G nucleotide and opaque grain. Cluster-4 consists of five rice cultivars; included Baoijhaki, Dudsail, BR 11, Harilaxmi and Neda. Volume expansion and amylose content of these cultivars varied from 3.7 to 3.9 and 21% to 25% as well as they have shown the G nucleotide and translucent grain. Cluster-5 consists of five rice cultivars; included Kharmao, Topaboro, Hatishail, Maloti and Kalimanik. These rice cultivars had white centre (Wc) type grain. Cluster-6 consists of BRRI dhan29 which had the highest amylose (29%) and highest volume expansion (4.4) as well as it has shown the G nucleotide with white centre (Wc) type grain. Cluster-7 consists of fifteen rice cultivars; included Lathamona, Pakhisail, BRRI dhan28, Depa, Karailadh, Patnai-231, Joalbogh, Magoibalam, Dudhkalam, Motichak, Boradudhkalam, Boylam, Jol, Molladigha and Poushmoricha. Theses rice cultivars had the range of amylose content of 25.2 to 29% as well as they have shown the G nucleotide with white belly (Wb) type rice grain.

3.5 | Statistical analysis

All data were arranged for computation in Microsoft Excel softwares. Correlation coefficient and dendrogram were done through SPSS v17.0 software for Pearson correlation and hierarchical cluster (Gomez and Gomez, 1984).

4 | DISCUSSION

Prathepha et al, (2005) reported that 50 glutinous rice and 40 nonglutinous rice present in 90 accession of rice. The range of amylose content varied from 5.9% to 9.4% for glutinous rice and 12.9% to 30.5% for nonglutinous rice of these accessions. Similarly, glutinous rice and nonglutinous rice shows wide variation in amylose content reported by *Ayres et al*., (1997). All most similar result has been shown in this present study. The range of amylose content of all glutinous rice varied from 5% to 8.4% whereas the range of amylose content of all nonglutinous rice varied from 11 to 29% among 36 rice germplasm.

In this study range of alkali spreading value varied from 5.3 to 7.0 in glutinous rice, whereas range of alkali spreading value varied from 3.2 to 6.8 in nonglutinous rice. Higher level of disintegration has been shown in glutinous rice compare to nonglutinous rice. *Prathepha et al*., (2005) reported similar statement, starch granules of glutinous rice varieties showed high levels of disintegration in alkali (KOH) solution, while starch granules of nonglutinous rice cultivars were significantly more resistant to alkali digestibility. Similar result reported by *Bao et al*., (2002), almost all of nonglutinous rice cultivars had low digestibility of starch granules in alkali solution. In glutinous rice, it has been reported that a high level of alkali digestion was found in starch granules of rice from China and Japan.

Glutinous rice contains a G to T mutation at the 5' splice site of Wx intron 1 that leads to incomplete post-transcriptional processing of Wx pre-mRNA reported by (*Wang et al*., 1995; *Isshiki et al*., 1998; *Bligh et al*., 1998; *Cai et al*., 1998 and *Hirano et al*., 1998). *Isshiki et al*., (1998) demonstrated that the allele of rice *Waxy* gene that controls the production of lower amounts of Wx protein is Wxb (base T at the 5' splice site of Wx intron 1), whereas the Wxa allele (base G at the 5' splice site of Wx intron 1) produces a higher level of Wx protein than Wxb. In addition, there is a little variation in the intensity of Wx protein within cultivars with Wxa or Wxb alleles. Based on these results, rice scientists have concluded that the G to T mutation at the 5' splice junction of the first intron is the major reason for variation in the expression of the rice *Waxy* gene.

For Thai indica rice, *Prathepha*, (2003) reported that intermediate- and high-amylose cultivars contained G at the 5' splice site of Wx intron 1, whereas low-amylose cultivars and glutinous rice had T at the at the 5' splice junction of the first intron. The G to T mutation at the 5' splice junction has assessed by PCR-RFLP method (Prathepha, 2003 and *Ayres et al*., 1997). All most similar result were found in this study. All glutinous rice (Figure-3) have shown T polymorphism (AGTTATA sequence) with opaque grain (0% chalkiness). All nonglutinous rice (Figure-3) have shown G polymorphism (AGGTATA sequence) with different types of chalkiness like opaque, white belly, white center and translucent.

The starchiness in sticky rice gives it a distinct opaque whiteness different from the more translucent appearance of regular rice grains (*Wittenberg*, 2007). Similar result has been shown in this study; all glutinous rice had opaque grain. Whereas, most of the nonglutinous rice had white belly, white center and translucent grain but few grains had opaque.

The quality of rice products from *Waxy* rice are correlated with other physicochemical characteristics such as alkali digestibility of the rice starch reported by *Kim et al*., (1996). Similar result has been shown in this study; amylose content was positively correlated with alkali spreading value but negative correlated with protein content, cooking time and volume expansion for glutinous rice. *Yadav et al*., (2007) reported that cooking time had highly significant and positive correlation with amylose content. Similar result has been shown in this study; amylose content was positively correlated with cooking time for nonglutinous rice.

5 | CONCLUSION

Information of physicochemical and polymorphism for glutinous rice will be helpful to familiar with Bangladeshi people. Since amylose level is one of the most important breeding traits in the development of new rice cultivars. A set of 36 rice germplasam, 7 glutinous rice have shown T polymorphism (AGTTATA sequence). The range of amylose content of all glutinous rice varied from 5% to 8.4%. Amylose content was positively correlated with alkali spreading value but negative correlated with cooking time and volume expansion for glutinous rice. Dissimilar result has shown in nonglutinous rice.

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CONFLICT OF INTEREST

The authors declare that they do not have any clash of interest.

ETHICAL APPROVAL

The research of this study any human or animal was not involved for testing.

Table 1 Glutinous and nonglutinous rice germplasm based on different traits

SL. No.	Cultivars	Size & Shape	Protein content (%)	Alkali spreading value	Gelatinization temperature	Cooking time (min)	Volume expansion ratio	Amylose content (%)	Polymorphism	Chalkiness	Head rice recovery (%)
1	Athabinni	LB	8.2±0.78	6.6±0.24	Low	16±0.65	3.1±0.09	8±0.78	T	Opaque	64.6±0.26
2	Binni-1	LB	8.4±0.90	6.1±0.16	Low	16±0.44	3.3±0.21	5±0.91	T	Opaque	64.4±0.89
3	Binni-2	LB	8.4±0.65	5.3±0.37	Intermediate	18±0.83	3.6±0.18	6±1.06	T	Opaque	63.7±0.61
4	Cylindrical Tapi-629	LB	7.8±0.50	7.0±0.06	Low	16±0.72	3.4±0.06	6.1±0.20	T	Opaque	63.4±0.59
5	Gohatibinni	LS	8.3±0.25	6.7±0.16	Low	15±0.70	3.1±0.17	8.4±1.1	T	Opaque	63.5±0.45
6	Kalabinni-1	LS	8.2±0.10	6.5±0.25	Low	16±0.81	3.1±0.08	6±0.96	T	Opaque	58.3±1.48
7	Rangabinni	MB	8.5±0.39	7.0±0.06	Low	16±0.70	3.1±0.06	5.2±0.15	T	Opaque	60.3±0.70
8	Ledabinni	MB	8.4±0.39	6.8±0.20	Low	16±0.51	3.4±0.11	11±0.55	G	Opaque	50.3±1.86
9	Khorma	LS	7.6±0.38	4.0±0.18	Intermediate	17±0.32	3.7±0.13	17±1.53	G	Opaque	60.3±0.62
10	Kanaibansi	LS	7.5±0.39	3.2±0.14	Intermediate	17±0.84	3.7±0.33	18±2.08	G	Opaque	58.5±0.74
11	Boradudhkalam	LB	7.5±0.38	5.0±0.26	Intermediate	21±0.90	3.7±0.14	26±1.53	G	Wb5	44.2±0.58
12	Boylam	MB	10.2±0.15	3.8±0.20	Intermediate	22±0.84	3.6±0.14	25.2±0.7	G	Wb5	29.0±1.44
13	Dudhkalam	LB	7.0±0.26	4.9±0.19	Intermediate	19±0.63	3.4±0.27	26±1.22	G	Wb5	34.2±1.48
14	Jol	LB	9.6±0.25	3.5±0.35	Intermediate	23±0.52	3.9±0.25	28±1.00	G	Wb1	49.5±1.58
15	Molladigha	LB	10.3±0.15	3.5±0.14	Intermediate	23±0.42	4.0±0.16	27±0.57	G	Wb5	28.7±1.39
16	Motichak	MB	10.5±0.06	4.0±0.25	Intermediate	22±1.00	3.4±0.07	26±0.06	G	Wb5	17.7±1.35
17	Poushmoricha	MB	7.7±0.66	4.8±0.13	Intermediate	18±1.24	3.6±0.17	29±0.52	G	Wb5	54.5±1.48
18	BRRI dhan 29	MB	7.2±0.39	6.0±0.71	Low	19±0.52	4.4±0.22	29±0.52	G	Wc5	65.4±0.50
19	Hatishail	LB	8.5±0.45	6.0±0.74	Low	18±0.79	3.6±0.05	24±1.10	G	Wc1	61.0±1.53
20	Kalimanik	LB	8.0±0.10	5.6±0.10	Low	18±0.58	3.7±0.21	25±1.31	G	Wc5	62.0±0.79
21	Kharmao	LS	7.2±0.58	3.7±0.22	Intermediate	18±0.60	3.9±0.08	21±1.07	G	Wc9	53.0±0.77
22	Maloti	MB	8.2±0.34	5.7±0.27	Low	15±0.00	3.6±0.08	23±0.74	G	Wc1	63.0±0.65
23	Topaboro	SR	9.6±0.24	4.4±0.26	Intermediate	21±0.91	3.7±0.10	22±0.78	G	Wc5	66.0±0.95
24	Baoijhaki	SR	8.4±0.33	4.8±0.13	Intermediate	16±1.16	3.9±0.32	21±1.00	G	Tr	64.0±0.77

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SL. No.	Cultivars	Size & Shape	Protein content (%)	Alkali spreading value	Gelatinization temperature	Cooking time (min)	Volume expansion ratio	Amylose content (%)	Polymorphism	Chalkiness	Head rice recovery (%)
25	BR 11	MB	7.5±0.33	5.6±0.23	Low	17±0.42	3.7±0.05	25±0.25	G	Tr	64.0±0.53
26	BRRI dhan 28	MS	8.6±0.32	6.1±0.19	Low	18±0.36	4.3±0.24	28±0.83	G	Tr	68.0±0.56
27	Depa	LB	7.6±0.15	5.1±0.11	Intermediate	19±0.66	3.6±0.20	29±0.25	G	Tr	38.0±1.39
28	Dudsail	SR	9.0±0.30	4.9±0.41.	Intermediate	16±0.88	3.7±0.06	21±0.74	G	Tr	69.0±0.46
29	Harilaxmi	LB	9.2±0.06	4.7±0.20	Intermediate	20±0.51	3.7±0.06	24±1.66	G	Tr	66.0±0.55
30	Joalbogh	LB	9.8±0.13	4.9±0.23	Intermediate	19±1.00	3.9±0.19	26±0.81	G	Tr	62.0±0.52
31	Karailadhan	LS	7.9±0.46	4.5±0.24	Intermediate	15±1.38	3.7±0.08	29±0.58	G	Tr	54.0±1.22
32	Lathamona	LS	8.4±0.33	6.3±0.26	Low	16±0.96	3.7±0.13	28±1.00	G	Tr	61.0±0.45
33	Magoibalam	MB	10.7±0.06	3.8±0.26	Intermediate	21±0.84	3.4±0.20	27±0.35	G	Tr	61.0±1.23
34	Neda	LS	10.5±0.25	4.6±0.08	Intermediate	19±0.54	3.9±0.32	23±0.82	G	Tr	49.0±1.80
35	Pakhisail	LS	7.7±0.24	4.3±0.18	Intermediate	18±0.58	3.7±0.10	28±0.67	G	Tr	62.0±0.55
36	Patnai-231	LS	7.5±0.15	5.5±0.30	Low	17±0.58	3.4±0.21	29±0.58	G	Tr	61.0±1.01

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Table 2 Pearson correlations of glutinous and nonglutinous rice germplasm

Correlations					
Parameter	**Protein content (%)**	**Alkali Spreading Value**	**Cooking Time (mm)**	**Volume Expansion ratio**	**Amylose content (%)**
Polymorphism (Glutinous)	**T**	**T**	**T**	**T**	**T**
Alkali Spreading Value	-.404				
Cooking Time (mm)	.196	-.794*			
Volume Expansion ratio	-.135	-.684	.799*		
Amylose Content (%)	-.217	.180	-.393	-.354	
Head Rice Recovery (%)	-.104	-.284	.102	.407	.336
Polymorphism (Nonglutinous)	**G**	**G**	**G**	**G**	**G**
Alkali Spreading Value	-.341				
Cooking Time (mm)	.578**	-.517**			
Volume Expansion ratio	-.024	.043	.076		
Amylose Content (%)	.028	-.018	.310	.192	
Head Rice Recovery (%)	-.282	.371*	-.564**	.290	-.128
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

Figure 1 Glutinous (left) and non-glutinous (right) rice endosperms stained with KI-I_2 solution

Figure 2 Glutinous (left) and non-glutinous (right) cooked rice endosperms

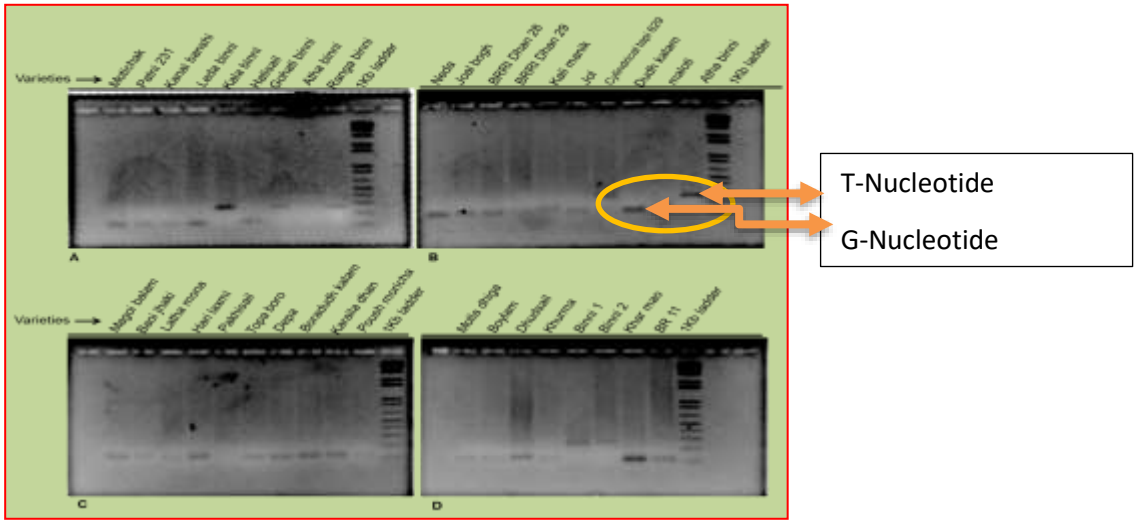

Figure 3 Agarose gel electrophoresis of PCR products showing the single nucleotide substitution (G/T polymorphisms)

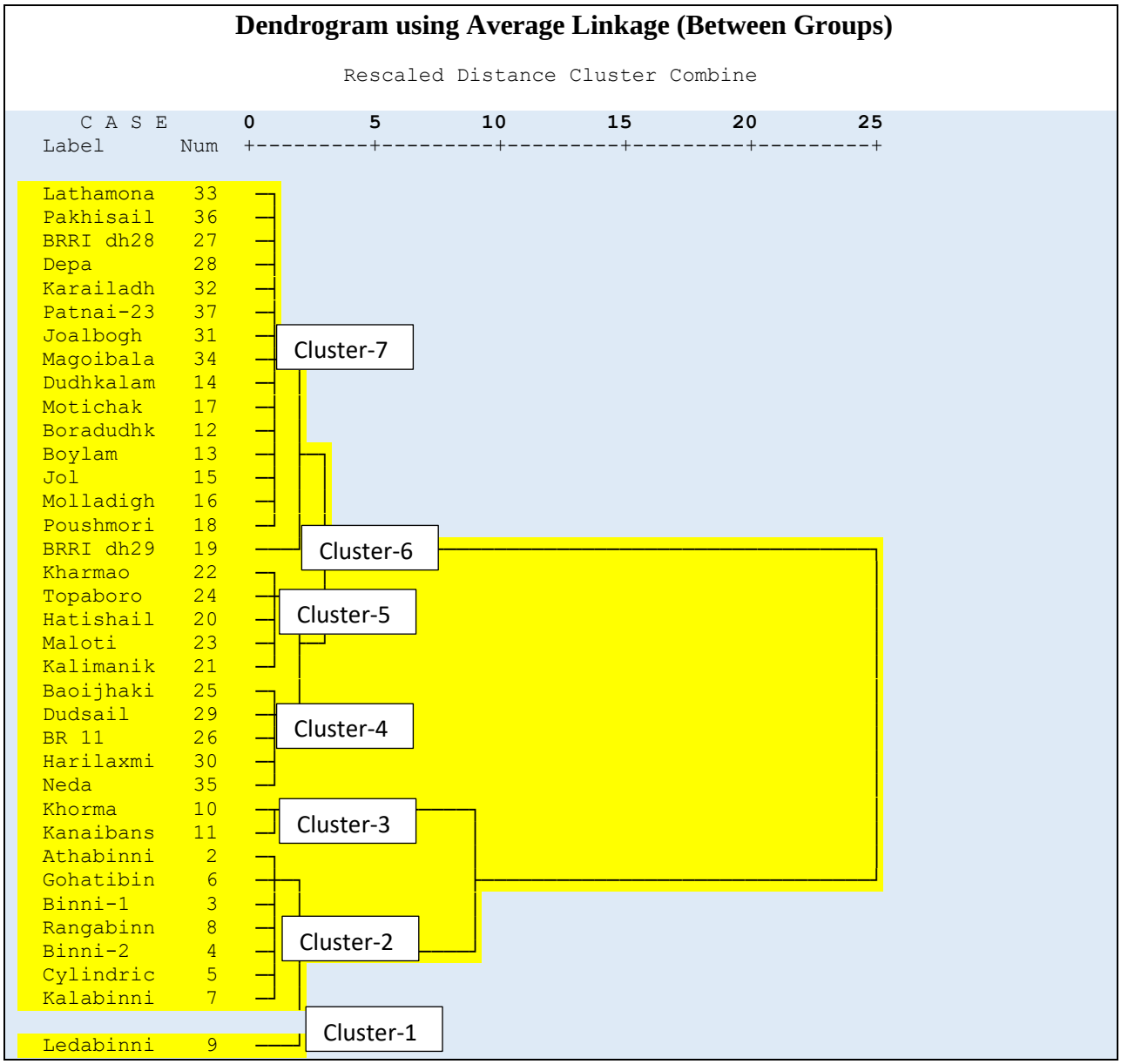

Figure 4 Dendrogram of rice germplasm based on different traits

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