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Research Article

Variability among Rice (*Oryza sativa* L.) Genotypes for Physicochemical, Cooking and Antioxidative properties for exploitation in quality breeding

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Abstract
In the present investigation, 67 advance genotypes of rice were analyzed for their physicochemical, cooking, pasting and antioxidant properties. Wide variations were observed in most of the traits under study such as amylose (9.96–22.43%), amylopectin (77.57–90.04%), starch (20.65–52.11%), volume expansion ratio (1.71–4.67 ml), length of blue gel (59.00–116.00 mm) and cooking time (19–26 minutes). Peak viscosity and final viscosity were found to be highest in genotype VL-7852 (292.5 RVA) and VL-30425 (496.08 RVA), respectively. Antioxidative activities were found correlated with total polyphenols. DPPH radical inhibition was recorded in the range of 9.23-17.34 µM Trolox equivalents/g DW, while ferric reducing antioxidant power (FRAP) was in the range of 6.76-30.19 µM Trolox equivalents/g DW. Principal component analysis (PCA) was done to find out relationships among groups of variables in a data set and between genotypes. Two major clusters were formed using agglomerative hierarchical clustering (AHC) that explained good variation in the traits. The existence of wide variability for various parameters in the present study suggests that the selected genotypes might be useful for rice breeders working on the development of new varieties with value-added traits.

Key words
Variability; *Oryza sativa*; Cooking quality; Antioxidative properties

Introduction

Rice (*Oryza sativa*, L.) is the major food grain crop and has been considered to be the best staple food among all other cereal grains owing to its superior nutritional quality. India is well known as a rice producing country with 95.32 million tonnes during the 2011-12 and area under paddy cultivation was increased by six per cent to about 44.50 million hectares (GOI annual report, 2011-2012). The characterization of rice in relation to consumers and export is attributed to physicochemical, cooking and eating qualities. The cooking and eating qualities of rice are basically determined by the properties of the starch (Wani *et al.,* 2012). Rice starch is usually digested quite rapidly as compared to other starchy foods. The gelatinization temperature, volume expansion ratio, water uptake, gel consistency, amylose, amylopectin and starch content are the significant properties that influence the cooking and eating characteristics of rice grains (Wani *et al.,* 2012). Gelatinization temperature determines the time required for cooking rice. It directly affects the physical properties of the starch granule, which in turn influences the quality ratings of cooked rice. Harder gel consistency is associated with harder cooked rice and this feature is particularly evident in high-amylose rice. If gel consistency is soft, the cooked rice has a higher degree of tenderness. An increase in gelatinization temperature has been observed with the decrease in alkali spreading value of rice starch. Many of the cooking and eating characteristics of milled rice are influenced by the ratio of two kinds of starches, amylose and amylopectin. If amylose content is high, the rice grains will show high volume expansion ratio and high degree of flakiness. The rice grains cook dry, are less tender and become hard upon cooling. If amylose content is low, the rice grains will cook moist and sticky (Wani *et al.,* 2012). In rice varieties, not only the physicochemical, cooking and eating qualities but also the pasting characteristics play important role in quality determination (Rachmat *et al.,* 2006).

Polyphenolic compounds, recognized to have protective functions against oxidative damage, are well known natural antioxidants and are associated with reduced risk of chronic diseases. The phenolic content in rice grain is greatly affected by genotype and environment (Goffman and Bergman, 2004). The interest in health benefit of whole grain rice may drive breeding programs further to enhance the nutritional potential of rice. Surveying rice genotypes from a wide collection of germplasm could identify promising rice lines with better physicochemical properties and a high antioxidant capacity (Shen *et al.,* 2009). Therefore, the present study was undertaken to evaluate different rice genotypes grown under North-western Indian Himalayan region for their physicochemical, cooking, pasting and

antioxidative properties to be utilized for future rice breeding programmes .

Materials and methods

Plant Material: The experimental materials consisting of 67 advanced breeding genotypes of rice (*Oryza sativa* L.) that were grown at the experimental farm, Hawalbagh, Almora (29°56′ N, 79°40' E and 1250 m above MSL) under North-western Indian Himalayan conditions during *kharif* season 2011-12, in randomized block design with three replications. Grains with translucent pericarp were harvested and sun-dried to bring the moisture content up to 12-14% for the analysis. Details of genotypes were presented in Table 1. Preparation of rice flour for analysis was done shortly before analysis by using Newport scientific super mill grinder with a 0.25 mm sieve. The whole grain and rice flour samples of each genotype were kept at 4^0C in screw-capped plastic containers for further use. All the experiments were done in triplicate and results were expressed on dry weight basis.

Determination of Physicochemical Properties:

Volume expansion ratio (VER) was determined as volume of cooked rice to volume of raw rice by following the procedure of Juliano (Juliano, 1972). Alkaline spreading value (ASV) was estimated by following the standard method of Juliano and Villareal (1993) and the spreading of each grain was rated visually by 7-point numerical scale. A low ASV corresponds to a high gelatinization temperature (GT) and *vice-versa*. Gel consistency (GC) was determined based on the consistency of milled rice paste gelatinized by boiling in dilute alkali and cooled to room temperature (Little *et al.*, 1958). The degree of disintegration and the transparency of paste dissolved out of the kernels were evaluated using a 7 point scale (Bhattacharya, 1979). Starch and amylose content were estimated by anthrone and iodine reagent respectively (Juan *et al.*, 2006). Amylopectin was calculated by using formula:

$amylopectin(\%) = 100\% - amylose\%$ (Jane et al., 1999). Protein content was estimated by dye-binding method (Bradford, 1976).

*Determination of Cooking Quality:*Water uptake (WU) is considered as an important economic attribute of rice as it gives an indirect measure of volume increase on cooking. For water uptake measurement, standard method (Anonymous, 2004) was followed. Cooking time and solids released by rice into cooking water have also been considered as a cooking quality attributes (Juliano, 1972). Cooking time (CT) and solids in cooking water (SCW) were determined by following the methods as reported by Oko *et al.* (2012).

Determination of Pasting Properties:

The pasting behaviour was studied with Rapid Visco-analyser (RVA) (Newport Scientific, Australia) using rice flour slurries (12% concentration on dry matter basis). The temperature–time conditions included a heating step from 50 to 95^0C at a rate of 7.5^0C /min, a holding period of 5 min at 95^0C followed by a cooling step to 50^0C at the same rate and a holding period of 1 min at 50^0C. The bowl speed was 160 rpm. Peak time, peak viscosity, trough viscosity, final viscosity, breakdown viscosity and setback viscosity were recorded and analyzed with the Thermocline Windows Data Collection (Newport Scientific, Australia) software.

Determination of Antioxidative properties:

Rice water extract of all rice genotypes were prepared. Milled rice flour (10 g) and water (250 mL) were mixed, and heated at 100^0C for 15 min. Water extract was filtered and followed by centrifuged at 1500×g for 10 min. Rice extracts were used to determine total polyphenols content and antioxidative properties as per standard methodologies. The total polyphenolic (TPP) compounds were determined by Folin Ciocalteu reagent (Singleton and Rossi, 1965) and calculated from a standard calibration curve based on tannic acid (0–0.1 mg/mL) and the results were expressed as tannic acid equivalents in mg per g dry weight (mg TAE/g DW). Scavenging effects on DPPH and 2,2-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) free radicals by rice aqueous extract was measured following standard methods (Brand-Williams *et al*., 1995; Arnao *et al*., 2001) respectively. Trolox was employed as a reference and the DPPH radical scavenging activity was expressed in µM Trolox equivalents (TE)/ g dry weight while ABTS radical scavenging activity was expressed in percent inhibition. The ferric reducing antioxidant power (FRAP) assay was followed as suggested by Benzie and Strain (1996).

*Statistical analysis:*Data represent the mean of three samples of one replication for each rice genotype. The genotypic mean value of each parameter was used for statistical analysis using SPSS programme (SPSS Inc., Version 10, Chicago, Illinois, IL, USA). For multifactorial comparison, the classification and discrimination of rice genotypes using physicochemical, cooking and antioxidative properties were attained by PCA and AHC using JMP 9.2 and SAS 9.3 softwares. Varimax method was performed to produce orthogonal transformations to the reduced factors to identify the high and low correlations.

Results and Discussion

Variations in Physicochemical and Cooking Quality Properties: The rice genotypes under study showed wide variation for most of the

physicochemical and cooking attributes (Table 2). This is due to wide genetic basis of the tested rice genotypes. Most of the rice genotypes exhibited less than 20 percent amylose (low amylose rice) with a wide variation between 9.96 (VL-31441) and 22.43% (VL-7620) suggesting that there are considerable levels of genetic diversity. Most consumers prefer rice with intermediate or lower amylose content (Rachmat *et al.,* 2006). Similarly, considerable variations were observed in amylopectin and starch content. Amylopectin and starch content ranged from 77.57 (VL-7620) to 90.04% (VL-31441) and from 20.65 (VL-31334) to 52.11% (VL-40063) respectively. Volume expansion ratio varied between 1.71 and 4.67 ml and wide variations were also found in length of blue gel [59.00 (VL-40004) − 116.00 mm (VL-31441)]. Rice with similar amylose content can be differentiated according to the tenderness. Cooked rice with softer gel consistency was moretender. Most of the rice genotypes exhibited soft gel consistency with intermediate GT (70-74^{0}C). Results of present study were in agreement with earlier finding (Juliano, 1979), where they found that the volume expansion ratio was positively correlated with amylose content and the gel type. The protein content was appreciably high (>6.2%) for all tested genotypes and varied between 6.29 (VL-31072) and 8.67% (VL-30425) (Table 1). The present study was in agreement with earlier findings (Villareal *et al.,* 1990; Umadevi *et al.,* 2010), which showed that the protein content in 9 genotypes of rice ranged between 6.0–9.0%. Solids in cooking water and water uptake content ranged between 0.03 – 0.11% and 60.00 – 490.00%, respectively. Cooking time ranged between 19 (VL-7742) and 26 min (VL-8116).

*Variations in Pasting Properties :*Wide variation was observed in pasting properties among the tested rice genotypes. The peak and final viscosity of all genotypes ranged between 33.58 (VL-31072) and 292.50 (VL-7852) and 49.58 (VL-31072) – 496.08RVU (VL-30425), respectively (Table 3). It might be due to the nutritional composition of the considered genotypes, affecting the cooking property of rice (Wani *et al.,* 2012). Most of the rice genotypes exhibited less than 200 RVU setback viscosity with a considerable variation between 16.00 (VL-31072) and 232.83RVU (VL-31290) . The setback viscosity is related to the gel network formation and is correlated to the amount of the amylose present in the rice starch (Jane *et al.,* 1999). This is consistent with present findings because most of the rice genotypes, which exhibited low amylose content, exhibited low setback viscosities. Most of the rice genotypes under study exhibited less than 250 Rapid Visco Units through viscosity with a considerable variation between 33.83 (VL-31072) – 250.50 RVU (VL-31290) . Peak time was found low for all low amylose genotypes and exceeded 7 min in high amylose genotypes, while breakdown was found higher for all low amylose genotypes. In low amylose varieties, amylopectin is predominating which causes granules to be swollen more easily (Li *et al.,* 2008).

Variations in Antioxidative Properties :

Total polyphenols content in rice genotypes ranged between 0.55 (VL-40067) and 2.56 mg TAE/gram DW (VL-30569) (Table 4). DPPH radical inhibition activity of water extract from rice genotypes recorded in the range of 9.23 (VL-40067) to17.34 µM TE/gram DW (VL-8094) while ABTS radical inhibition activity in water extract from rice genotypes found in the range of 23.00 (VL-40095) to 58.18% (VL-30927). FRAP value in rice genotypes aqueous extract found in the range of 6.76 (VL-40119) to 30.19 µM TE/g DW (VL-30953). The results of present study are in agreement with the report of Goffman and Bergman (2004), in which rice genotypes with different pericarp color were evaluated and antioxidant activity (FRAP value) ranged between 10.0 and 13.1 µM TE/g DW. Rice possesses different compounds with antioxidant activity including polyphenols, but variations are observed in the concentration of these compounds in the grains, mainly due to genotype, pericarp color and processing (Melissa and Enio, 2011).

Principal Component Analysis (PCA):

The principal component analysis and their correlation are shown in Fig. 1a. The principal component PC1 represented 23.00% of variability, while the principal component PC2 represented 17.41% of variability. The interpretation of the PCA results can be explained by positioning of loading plot over score plot (Parihar *et al.,* 2013). Superimposition showed the respective rice genotypes for their variables. All parameters were occupied on the right side of the biplot and among the parameters the water uptake, solids in cooking water, ABTS, length of blue gel, starch and setback were observed on the right upper side of the biplot with positive loading for both PC 1 and 2, while protein, cooking time, break down, amylopectin were grouped together with slightly lesser positive loadings on the right lower side of the biplot. This suggests that protein, cooking time, breakdown, and amylopectin exhibit positive correlationbetween them. The amylose and peak time were observed on the left upper side of the biplot and TPP and FRAP were found in middle portion of biplot. Based on this mathematical rule, uncorrelated variables occur at right angles to one another because the cosine of the angle between them is cosine $90^o = 0$, or not correlated. Similarly, the cosine of 0 is 1, which denotes a positive correlation between the variables (Lopez *et al.,* 2006). The amylose showed negative correlation with trough, breakdown, final viscosity, cooking time and setback viscosity from which it can be suggested that high level of amylose restricts the

swelling of granules, thereby reducing the peak viscosity. Amylose content might be responsible for high water uptake ratio, as Frei *et al.* (2003) had reported that rice with high amylose content tends to absorb more water upon cooking. Solids in cooking water affect the stability of the cooked rice (Normita and Cruz, 2002). The variation in values may be as a result of the variation in rice consistency, seen in the bursting of the grains during and after cooking, as they are of different genotypes (Normita and Cruz, 2002). The protein content showed positive correlation with trough, breakdown, final viscosity, and setback. Starch content also showed positive correlation with cooking time. The results are in agreement of previous study by Noosuk *et al.*, (2003). However, positive correlation between amylose content and viscosity was reported (Charoenthaikij *et al.*, 2009). In present finding, setback, breakdown and trough viscosity were found negatively correlated with amylose content. Mishra and Rai (2006) also suggested that setback may largely be determined by degree of amylose polymerization. Similarly, breakdown of the different rice genotypes revealed that they were significantly different with the same pattern as viscosity and were negatively correlated with amylose content. Radical scavenging activities of rice genotypes were found well correlated with total polyphenols while *in vitro* assessment of FRAP showed negative correlation with other antioxidant properties. Previous studies reported (Goffman and Bergman, 2004) that antioxidant activity have positive correlation with the total phenol content and is especially associated with the content of tannic acid and catechins.

Agglomerative hierarchical clustering (AHC):

In order to see patterns of clustering between the rice genotypes, AHC analysis was used. Using a similarity level, 67 rice accessions were classified into mainly two clusters (Fig. 2). From AHC, an important conclusion can be drawn that based on composition, the differences between the accessions studied are still great enough to classify them correctly, on the basis of the variables introduced in the present analysis. Cluster 1 consisted of 23 genotypes (19, 63, 21, 17, 30, 66, 28, 22, 14, 6, 15, 62, 60, 7, 8, 5, 54, 9, 11, 16, 12, 4, 3) grouped in to two groups viz., group 1 with 16 accessions and group 2 with 7 accessions. Accessions in cluster I showed higher amylose, starch, volume expansion ratio, water uptake, FRAP and lower peak viscosity, trough viscosity, breakdown, final viscosity and setback. The higher amylose, setback, volume expansion ratio, water uptake, and peak time in cluster I is mainly contributed by group 1 of cluster I. Cluster 2 comprised of a mixture of 44 accessions (50, 49, 48, 44, 64, 53, 40, 38, 46, 36, 42, 41, 35, 52, 34, 45, 43, 33, 32, 26, 24, 25, 61, 57, 56, 51, 47, 67, 58, 59, 55, 20, 37, 13, 39, 65, 27, 23, 29, 31, 58, 21)) grouped in to two groups viz., group 1 with 21 accessions and group 2 with 23 accessions. Accessions in cluster II showed lower amylose, starch, volume expansion ratio, water uptake, FRAP, peak time and higher peak viscosity, trough, breakdown, final viscosity and setback viscosity. The lower amylose, starch, volume expansion ratio, water uptake, and peak time in cluster II is mainly contributed by group 2 of cluster II. The major characteristics shared by both cluster I and cluster II were solids in cooked water, cooking time, length of blue gel and total polyphenols.

Conclusion

In conclusion, this study has shown that sufficient variability was found for the physicochemical, cooking and antioxidative properties in rice genotypes studied. All the important traits analyzed may be used in the breeding programmes to increase variability for different physicochemical, cooking and antioxidative characteristics and to make suitable selections that are acceptable to consumers. From a breeding point of view, the high variability suggests that it could be possible to obtain appreciable responses to selection for these traits. The results of present study could help rice breeders to identify interrelationship between important physicochemical, cooking quality, pasting and antioxidative properties to find out the better rice genotypes and breed these characteristics into improved varieties.

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Table 1. Details of rice (non-glutinous, translucent color) genotypes along with Alkali spreading value (ASV), Gel consistency (GC), gelatinization temperature (GT) and 1000 seed weight

Sample No.	Genotype Name	ASV	GC	GT	*1000 Seed weight	Sample No.	Genotype Name	ASV	GC	GT	*1000 Seed weight
1	VL-30924	1	S	74.5-80	21.05	35	VL-31290	2	S	74.5-80	22.88
2	VL-30925	4	S	70-74	21.06	36	VL-31329	2	S	74.5-80	26.83
3	VL-30926	6	S	<70	23.46	37	VL-31335	2	S	74.5-80	27.71
4	VL-30927	2	S	74.5-80	27.57	38	VL-31450	2	S	74.5-80	26.63
5	VL-30928	6	S	<70	17.37	39	VL-31451	2	S	74.5-80	18.98
6	VL-30929	6	H	<70	23.33	40	VL-31452	1	S	74.5-80	22.24
7	VL-30934	7	S	<70	17.02	41	VL-7620	2	S	74.5-80	18.00
8	VL-30938	6	H	<70	19.38	42	VL-30560	2	S	74.5-80	23.69
9	VL-30960	6	S	<70	24.21	43	VL-7742	1	S	74.5-80	23.96
10	VL-31000	1	S	74.5-80	24.82	44	VL-30569	2	S	74.5-80	26.88
11	VL-31072	6	M	<70	22.03	45	VL-7820	1	S	74.5-80	16.91
12	VL-31076	5	S	70-74	20.99	46	VL-7702	2	S	74.5-80	27.41
13	VL-30930	2	M	74.5-80	20.69	47	VL-7954	2	S	74.5-80	26.09
14	VL-30935	7	H	<70	24.81	48	VL-7852	2	S	74.5-80	26.13
15	VL-30937	7	S	<70	24.48	49	VL-30424	5	S	74.5-80	29.55
16	VL-30948	5	S	70-74	20.44	50	VL-30425	6	S	<70	24.26
17	VL-30953	6	S	<70	20.44	51	VL-31484	2	M	74.5-80	24.57
18	VL-30956	7	H	<70	25.57	52	VL-31486	1	S	74.5-80	24.38
19	VL-31003	6	S	<70	27.17	53	VL-31487	2	S	74.5-80	34.22
20	VL-31077	2	S	74.5-80	21.18	54	VL-40102	7	S	<70	23.89
21	Pusa bas-1	5	S	70-74	19.05	55	VL-40063	5	S	70-74	26.43
22	Pusa sugandha-2	7	S	<70	25.09	56	VL-40004	2	H	74.5-80	26.24
23	Bas-370	2	S	74.5-80	18.31	57	VL-40003	3	M		24.99
24	VL-31407	2	S	74.5-80	22.16	58	VL-40002	5	M	70-74	24.03
25	VL-31441	2	S	74.5-80	23.72	59	VL-40119	5	S	70-74	22.97
26	VL-31348	1	S	74.5-80	24.07	60	VL-40056	7	S	<70	21.18
27	VL-8116	2	M	74.5-80	23.12	61	VL-40058	3	M		24.13
28	VL-8158	5	S	70-74	20.33	62	VL-40061	6	S	<70	19.39
29	VL-8051	1	S	74.5-80	25.11	63	VL-40062	3	M		27.38
30	VL-8094	2	S	74.5-80	26.64	64	VL-40067	1	S	74.5-80	25.7
31	VL-31334	2	S	74.5-80	23.51	65	VL-40068	2	S	74.5-80	27.28
32	VL-31339	2	S	74.5-80	28.15	66	VL-40095	5	M	70-74	25.58
33	VL-31449	4	S	70-74	23.33	67	VL-40107	6	M	<70	24.69
34	VL-31284	2	M	74.5-80	27.36						

S= Soft, M= Medium H= Hard, *Seed weight in gram

Table 2. Basic statistics for physicochemical properties of 67 tested rice genotypes

	Trait	Unit	Mean	Median	Mode	SD	Min	Max
Group attribute 1	Amylose	%	18.01	18.24	18.24	2.51	9.96	22.43
(Physicochemical properties)	Amylopectin	%	81.99	81.76	81.76	2.51	77.57	90.04
	Starch	%	31.91	30.80	33.15	7.21	20.65	52.11
	Protein	%	7.29	7.12	6.98	0.53	6.29	8.68
	VER	ml	2.68	2.43	2.05	0.76	1.71	4.67
	LBG	mm	90.84	94.00	98.00	11.32	59.00	116.00
Group attribute 2	CT	Min	22.86	23.00	24.00	2.29	19.00	26.00
(Cooking quality)	SCW	g	0.07	0.07	0.06	0.02	0.03	0.11
	WU	ml	248.73	240.00	80.00	112.24	60.00	490.00

VER- Volume expansion ratio, LBG- Length of blue gel, CT -Cooking time, SCW- Solids in cooked water, WU - Water uptake

Table 3. Basic statistics for pasting properties of 67 tested rice genotypes

	Trait	Unit	Mean	Median	Mode	SD	Min	Max
Group attribute 3	Peak viscosity	RVU	142.28	140.92	171.25	53.62	33.58	292.50
(Pasting properties)	Trough viscosity	RVU	134.54	134.75	N/A	47.70	33.83	250.50
	Breakdown viscosity	RVU	6.62	0.08	0.58	13.16	2.83	53.58
	Final viscosity	RVU	262.80	262.33	N/A	96.19	49.58	496.08
	Setback viscosity	RVU	121.85	112.50	72.92	49.74	16.00	232.83
	Peak Time	Min	6.64	6.93	7.00	0.44	5.78	7.03

Table 4. Basic statistics for antioxidative properties of 67 tested rice genotypes

	Trait	Unit	Mean	Median	Mode	SD	Min	Max
Group attribute 4	TPP	mg TAE/gram DW	1.034	0.887	1.227	0.429	0.55	2.56
(Antioxidative properties)	ABTS	% inhibition	44.623	44.818	44.364	6.248	23.00	58.18
	FRAP	µM TE/gram DW	17.768	17.968	10.493	5.846	6.76	30.19
	DPPH	µM TE/gram DW	13.308	13.159	16.459	2.213	9.23	17.34

TPP: Total polyphenols, ABTS: Scavenging effects on ABTS free radicals, DPPH: Scavenging effects on DPPH free radicals, FRAP: ferric reducing antioxidant power

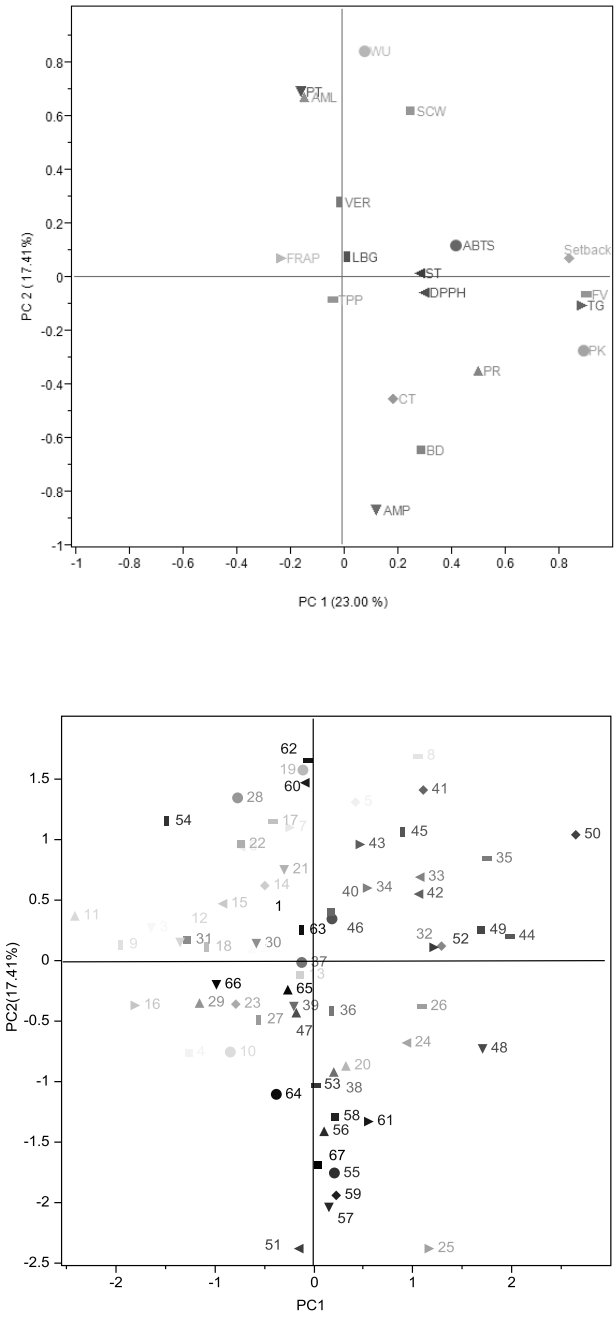

Fig 1. (A) Loading plot of PC1-PC2 for rice genotypes. Loading variables are Amylose (AML), Amylopectin (AMP), Starch (ST), Protein (PR), Peak viscosity (PK), Trough (TG), Breakdown (BD), Final viscosity (FV), Setback, Peak time (PT), Volume expansion ratio (VER), Length of blue gel (LBG), Cooking time (CT), Solids in cooked water (SCW), Water uptake (WU) and antioxidative parameters (TPP, ABTS, FRAP, DPPH).
(B) Score plot for rice genotypes

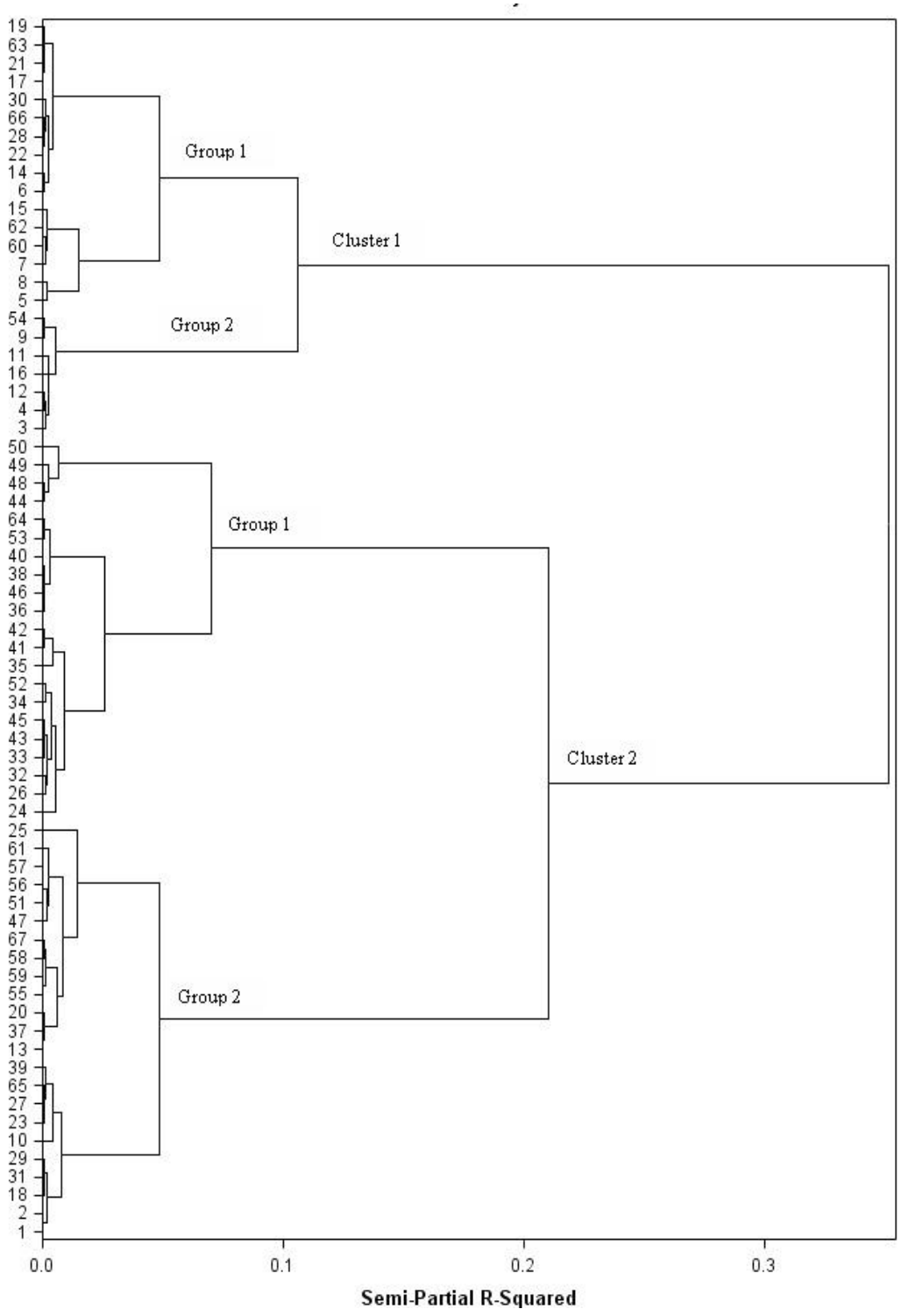

Fig 2. Cluster analysis of 67 rice genotypes by Agglomerative hierarchical clustering (AHC) method.