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RESEARCH ARTICLE

Evaluation of glycemic index, glycemic load and biochemical traits of rice associated with anti-diabetic properties

BN Rithesh[1], S Ramchander[1,4], S Manonmani[1], D Uma[3], S Rajeswari[1,5] & P Jeyaprakash[1,2]*

[1]Department of Rice, Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University (TNAU), Coimbatore 641 003, Tamil Nadu, India
[2]Department of Genetics and Plant Breeding, Anbil Dharmalingam Agricultural College and Research Institute (TNAU), Trichy 620 027, Tamil Nadu, India
[3]Department of Biochemistry, Centre for Plant Molecular Biology & Biotechnology (CPMB&B), TNAU, Coimbatore 641 003, Tamil Nadu, India
[4]School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore 641 114, Tamil Nadu, India
[5]Department of Cotton, CPBG, TNAU, Coimbatore-641003, Tamil Nadu, India

*Email: jeyaprakash.p@tnau.ac.in

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Abstract

Diabetes mellitus, a modern lifestyle disease and metabolic disorder, is closely associated with an increased risk of cardiovascular disease. Research on carbohydrates, particularly white rice with a high glycemic index, has been linked to an increased risk of type II diabetes, heart disease, and cancer. In this study, we aimed to understand the nutritional composition, estimated glycemic index, and glycemic load of twenty-eight rice accessions, particularly focusing on those with low starch digestibility associated with low GI levels. The proximate composition analysis revealed that tested rice accessions exhibited higher levels of genetic variation for amylose (18.45 - 25.97%), phenolic content (5.00-34.08%), protein (5.52-14.54%), and crude fibre (1.64-3.91%) content in brown rice. Huge variability for estimated glycemic index, ranging from low to high GI was observed among all the varieties (49.37 - 78.58%). Traditional varieties *viz.,* Thavalakannan and Kavuni depicted low estimated glycemic index (49.37 % and 54.55 %) and moderate glycemic load (14.60 and 15.80), respectively. The estimated glycemic index exhibited significant and negative association with amylose (r = -0.57**), phenolic (r = -0.67**), and crude fibre (r = -0.52**) content. In contrast, glycemic load showed a significant positive correlation with the amount of carbohydrate content. Principal component analysis revealed considerable variability among rice accessions' biochemical traits with the first two principal components accounting for 68.57% of the total variance. The hierarchical clustering based on Darwin software identified two major clusters. Cluster I comprise popular varieties and Cluster II contains traditional varieties with low to moderate glycemic index. Moreover, identifying rice varieties with lower glycemic index can facilitate the development and enhancement of breeding lines for the diabetic population.

Keywords

rice; carbohydrate; glycemic index; glycemic load; type II diabetes

Introduction

India is one of seven countries in Southeast Asia (SEA) with a high prevalence of diabetes, with approximately 74.2 million cases as of 2021(1). It harbors the highest number of adult diabetes cases, accounting for 1 in 7 adults living in India with diabetes worldwide. However, estimates for the number of persons with diabetes in the SEA region are 90 million, and by 2045 this number is expected to rise substantially to 152 million, representing a 68% increase (1). The increased rate of type II diabetes in most developing countries is mainly attributed to active mechanization towards lifestyle transitions and higher rates of urbanization (2). Variations in blood sugar levels after eating carbohydrate-rich foods have gained much attention among health-conscious customers. Glycemic load (GL) and Glycemic index (GI) are concepts that can be used to indicate both the quantity and quality of carbohydrates (3). GI is an important property of the starch fraction, which characterizes the carbohydrate in a different type of food-based blood glucose level (4), whereas GL is defined as the product of a food's GI value and its carbohydrate content; in contrast, it represents the combination of the quantity and quality of carbohydrates consumed (5). GI classifies food as low (≤ 55), intermediate (> 55–69) or high GI (≥ 70) (6). The *in vitro* method of digestion has gained wide attention with a high correlation between a glycemic response that closely mimics the *in vivo* situation (7, 8).

Rice is the primary source of food for people across Asia and Africa. The removal of many essential nutritional components during polishing leads to micronutrient deficiencies among the rice-consuming population (9). Consuming more polished rice was thought to be a risk factor for type II diabetes due to its high glycemic response (10). The physical and chemical structure of rice also reflects the degree of variation in glycemic response among the varieties. Starch is one of the major components of rice and plays an important role in defining its quality. The starch digestibility rate plays a crucial role in determining how much and how quickly glucose is absorbed into the bloodstream after consuming starchy foods. Additionally, these digestibility rates of starch can be classified into three types based on the length of time rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (11). For rice varieties, amylose content, resistant starch, and gelatinization properties are key factors that influence the glycemic index (GI) and serve as predictors of the starch digestion rate (12). The ratio of amylose - amylopectin significantly affects the variation in glycemic response due to the impact on starch content (13). The glycemic response may be linked to amylose's straight chain linear structure as rice cultivars with high amylose content (AC) have low Gl values (14). However, the rate of starch digestion cannot be accurately predicted by the amylose concentration alone (15). Other factors, such as degree of processing, cooking methods, varieties, and chemical components like resistant starch content, phytonutrients, dietary fibre, protein, and fat content, also play a significant role in starch digestibility or glycemic response (16, 17).

The traditional landraces are highly adaptable to various environmental conditions and have also been employed in the indigenous systems of Indian medicine such as Ayurveda, Siddha, and Unani (18, 19). These landraces contribute significantly to local food security and have been a great source of genes for novel alleles with high genetic variability as well as a rich supply of diverse agronomic and biochemical features (20). Traditional pigmented rice (black, purple, red, and brown) is thought to be nutritionally dense due to its high concentration of bioactive phytochemicals such as phenolic compounds, which exhibit a broad range of anti-cancer properties (21, 22). Moreover, the current popularity of traditional rice varieties has gained significant relevance due to the reduced incidence of cardiovascular disease. Several studies also found that the estimated glycemic index (EGI) may be an important variable in rice breeding to screen for traditional varieties associated with low GI. Therefore, this preliminary study was conducted to investigate the glycemic response of rice landraces and adaptable varieties as well as their therapeutic values that help to identify elite donors for use in breeding programs aimed at developing low glycemic index (GI) rice varieties for the diabetic population.

Materials and Methods

Genetic material and Sample Preparation

The experiment was carried out at the Department of Rice (Paddy Breeding Station (PBS)), Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India (11^0 N latitude and 77^0 E longitude with an elevation of 426.72 m above the mean sea level). The twenty-eight diverse rice accessions, including traditional landraces, used in the present study are depicted in Table 1 and Fig. 1. The rice grains of different accessions collected from

Table 1. List of traditional and popular varieties used in the study

S.No.	Accessions	Grain classification
1	GEB 24	Fine grain
2	TKM 9	Short bold red grain
3	BPT 5204	Medium slender
4	CO 43	Medium slender
5	CO (R) 50	Medium slender
6	CO 51	Medium slender
7	ASD 16	Short bold
8	ADT 37	Short bold
9	ADT 43	Medium slender
10	ADT (R) 45	Medium slender
11	IR 20	Medium slender
12	IR 36	Long slender
13	IR 50	Long slender
14	CO RH 3	Medium slender
15	Improved White Ponni	Medium slender
16	Pusa Basmati 1	Extra long slender
17	CR 1009 *Sub 1*	Short bold
18	Bhavani	Long slender
19	Swarna	Short bold
20	Improved Kavuni	Black rice
21	Kavuni	Black rice
22	Norungan	Red rice
23	Purple puttu	Purple rice
24	Thavala Kannan	Bold red rice
25	Jeeraga Samba	Very small and fine grain
26	Rasacadam	Fine quality and white rice
27	Sivapuchithiraikar	Bold grain
28	Mappillai samba	Medium slender and Red rice

Fig. 1. Phenotypic variation in grain color of traditional rice varieties used in the present investigation.

PBS were de-husked to make brown and polished (white) rice using a laboratory rice mill (Pearlest grain polisher-Kett, Japan) and then each was ground into flour using a small-volume powder mixer. Finely powdered samples were collected, sieved using 100 mesh sieves for nutritional analysis, and kept at defroster storage (-20° C) until analysis.

Determination of proximate composition

The amylose content was determined following Juliano's simplified procedure (23) with minor modifications. The Anthrone method (24) was used to calculate the total percentage of rice's Carbohydrate content (CHO). By utilizing the Folin-Ciocalteau reagent and the method described by (25), the total phenolic content of the flour sample was determined and expressed as µg of Gallic acid (GAE) g^{-1}. The protein content was determined using Lowry's method (26). The Association of Official Analytical Chemists' standard method (962.09) of analysis was used to assess the crude fibre content (27).

The *in-vitro* starch digestibility was analyzed by the method of Goni (28) with slight modification. Brown rice flour (50 mg) was cooked in 5 ml of water for 30 mins, and 10 ml of HCl-KCl buffer (pH = 1.5) was added to the sample. For each sample, 0.2 ml of a solution containing 1 g of pepsin in 10 ml of HCl-KCl buffer was added. The samples were then incubated at 40°C for 1 hour in a shaking water bath and the volume was adjusted to 25 ml with Tris-maleate buffer (pH= 6.9). After incubation at 37°C, the aliquots were collected at 30-minute intervals over 3 hours (30, 60, 90, 120 and 180 mins). The enzyme activity in the aliquots was inactivated by heating at 100°C for 5 mins and refrigerated. Then, 3 ml of 0.4M Sodium acetate buffer (pH 4.75), and 60 µl amyloglucosidase was added to hydrolyze the digested starch to glucose. Finally, the samples were incubated at 60°C for 45mins and glucose content in each aliquot was estimated using a glucose oxidase peroxidase kit. Glucose was converted into starch by multiplying with 0.9.

The kinetics of starch hydrolysis and estimated GI were determined by a non-linear first-order equation:

$$C = C\infty\,(1-e^{-kt})$$

Where, C = Concentration of starch hydrolyzed at time (t min), C∞ = equilibrium percentage of starch hydrolyzed after 180 min, and k = kinetic constant. The variable, C∞ and k were estimated for each treatment based on the data obtained from the in vitro starch digestion. The area under the hydrolysis curve (AUC) was calculated using the equation:

$$AUC = C\infty_{(tf-t0)} - C\infty/K(1 - e^{-k(tf-t0)}$$

where, C∞ corresponds to the equilibrium percentage of starch hydrolyzed after 180 min, tf is the final time (180 min), t_0 is the initial time (0 min) and k is the kinetic constant. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (White bread). The estimated glycemic index (EGI) was calculated from the hydrolysis index using the corresponding equation given by Goni (28).

$$EGI = 39.71 + 0.549HI$$

Glycemic load is obtained by multiplying the estimated glycemic index by the net available carbohydrates contained in a nominal serving (50 g) of the food divided by 100 (29).

Mathematically, $GL = GI \text{ x available carbohydrate} \frac{(g)}{100}$

The available carbohydrate was calculated by subtracting fibre from the total carbohydrates of the samples.

Statistical analysis

The replicated data was analyzed statistically by a Completely Randomized Design and the significance between the tested varieties for various traits was presented at $p<0.05$. The results were presented as mean ± standard error (d) of three replicates. The Pearson correlation analysis was performed to find out the associations among each trait with R software (https://CRAN.R project.org/). Principal Component Analysis (PCA) was performed using the packages 'variability', 'Agricolae', 'FactoMineR', and 'facto extra' of R studio 4.2.3. The tree-based hierarchical clustering of mean biochemical data was derived through Darwin software 6.0.

Results

Genetic variation for glycemic index and glycemic load

The data were analyzed statistically and revealed a significant difference among all the accessions irrespective of the traits (Table 2a and 2b). The wide variation of glycemic index ranging from low (49.37 ± 0.38 %) to high (78.58 ± 0.14 %) with a mean value of 66.92 ± 0.40 % was observed among the accessions, whereas the glycemic load ranged from medium (14.60 ± 0.41%) to high (26.24 ± 0.57%) with the mean value of 21.32 ± 0.38%. Traditional variety Thavalakannan with red colour rice exhibited low EGI and GL of 49.37 ± 0.38 % and 14.60 ± 0.41 followed by black rice variety Kavuni with 54.55 ± 0.12% and 15.80 ± 0.40, respectively. The highest value was recorded for short bold rice ADT 37 (78.58 ± 0.14 %) for GI and long slender grain IR 50 (26.24 ± 0.57) for GL (Table 2a). The frequency distribution graph (Fig. 2) concludes that most accessions come under moderate to high glycemic index and glycemic load.

Proximate composition of rice accessions

Amylose is a major determinant of rice cooking and eating qualities. In this study, the amylose content of brown rice ranged from 18.45 to 25.97% with a mean value of 22.39% (Table 2a). The traditional variety Rasacadam had the highest amylose content (25.97 ± 0.09), followed by black rice Kavuni (25.66 ± 0.47), and the lower value of amylose content was recorded for CO 43 (18.45 ± 0.43%). Brown rice with high to intermediate amylose content had varied glycemic index. The carbohydrate content of polished rice was higher than that of corresponding brown rice, as seen in Table 2b. A wide range of carbohydrate content (58.99 ± 1.38%-74.78 ± 0.75%) was observed among the 28 accessions of brown rice with a mean value of 65.82 ± 1.00%, whereas polished rice ranged accordingly from 64.32 ± 0.89% (TKM 9) to 83.54 ± 0.98 % (Pusa Basmati1) with a high mean value of 76.18 ± 0.76%. The pigmented variety, Purple Puttu (58.99 ± 1.38%), had a very low carbohydrate percentage, and CO (R) 50 had the highest value with 74.78 ± 0.75% in brown rice. The results also showed that the polishing process removed the outer bran layers of brown rice, which contain essential nutritional components, and turned it into a simple carbohydrate food.

Rice is rich in antioxidants, especially pigmented varieties, which are often associated with the outer bran layer. The value of total phenolic content for brown rice ranged from 5.00 ± 0.06 µGAE g^{-1} to 34.08 ± 0.39 µGAE g^{-1} with the mean value of 11.48 ± 0.27 µGAE g^{-1} and in the case of polished rice, it varied from 2.21 ± 0.10 µGAE g^{-1} to 20.41 ± 0.60 µGAE g^{-1} with the mean value of 6.12 ± 0.29 µGAE g^{-1} (Table 2b). The pigmented variety Purple Puttu had the highest value of phenolic content (34.08 ± 0.39 µGAE g^{-1} and 20.41 ± 0.60 µGAE g^{-1}), followed by black rice Kavuni. This indicates removing outer layers during polishing decreased the phenolic content in polished white rice.

The protein content of rice determines its nutritional quality, as it is the second most important component of grains after starch. The result indicated that the difference between brown and white rice is due to the presence of the bran portion, which significantly increases the protein level of brown rice. Our findings also revealed that the protein content varied, significantly among all rice accessions. The traditional varieties such as Kavuni (14.54 ± 0.02 mg/g), Mapillai Samba (13.98 ± 0.23 mg/g), and Sivapuchithiraikar (13.16 ± 0.04 mg/g) had the highest protein content. The brown rice ADT 43 (5.52 ± 0.06 mg/g) had the lowest protein content with a mean of 9.26 ± 0.12 mg/g. The protein content of polished rice varied from 3.14 ± 0.04 mg/g (GEB 24) to 8.32 ± 0.03 mg/g (Mapillai Samba), with a mean value of 5.51 ± 0.15 mg/g (Table 2b). The crude fibre content of brown rice varied from 1.64 ± 0.06% (Jeeraga Samba) to 3.91 ± 0.11% (Mapillai Samba) with a mean value of 2.15 ± 0.09%. In the case of polished rice, it varied from 1.03 ± 0.05 (BPT 5204) to 2.45 ± 0.07% (Mapillai Samba) with a mean of 1.60 ± 0.04% (Table 2b). This showed that traditional rice had greater crude fibre content than brown and polished rice.

Table 2a. Variation in Amylose content and Estimated glycemic index in brown rice

S.No	Accessions	Amylose (%)	Hydrolysis Index (%)	Estimated Glycemic index (%)	GI Categorization	Glycemic Load
1	GEB 24	22.09 ± 0.80	50.93 ± 0.51	67.61 ± 0.28	Medium	21.29 ± 0.54
2	TKM 9	19.69 ± 0.72	69.06 ± 1.11	77.54 ± 0.61	High	23.69 ± 0.06
3	BPT 5204	19.75 ± 0.05	49.49 ± 0.65	66.82 ± 0.36	Medium	20.93 ± 0.52
4	CO 43	18.45 ± 0.43	60.99 ± 0.57	73.12 ± 0.31	High	24.55 ± 0.36
5	CO (R) 50	21.49 ± 0.56	51.28 ± 0.74	67.80 ± 0.71	Medium	24.76 ± 0.42
6	CO 51	21.84 ± 0.53	49.61 ± 0.57	66.89 ± 0.31	Medium	21.02 ± 0.40
7	ASD 16	22.14 ± 0.88	57.82 ± 0.75	71.39 ± 0.41	High	21.48 ± 0.49
8	ADT 43	21.62 ± 0.28	48.01 ± 0.75	66.01 ± 0.41	Medium	23.12 ± 0.52
9	ADT (R) 45	23.49 ± 0.50	41.12 ± 0.66	62.23 ± 0.36	Medium	20.95 ± 0.26
10	ADT 37	20.36 ± 0.14	70.96 ± 0.25	78.58 ± 0.14	High	25.02 ± 0.28
11	IR 20	23.45 ± 0.70	58.08 ± 0.55	71.53 ± 0.30	High	22.57 ± 0.28
12	IR 36	20.14 ± 0.82	43.18 ± 1.15	63.36 ± 0.63	Medium	22.79 ± 0.21
13	IR 50	23.27 ± 0.81	61.03 ± 1.17	73.15 ± 0.64	High	26.24 ± 0.57
14	CO RH 3	20.80 ± 0.62	60.15 ± 0.72	72.66 ± 0.39	High	20.99 ± 0.28
15	Improved white ponni	23.88 ± 0.05	47.26 ± 1.25	65.60 ± 0.68	Medium	21.23 ± 0.35
16	Pusa basmati 1	22.62 ± 0.31	59.12 ± 0.58	72.10 ± 0.32	High	23.52 ± 0.58
17	CR 1009 *Sub* 1	23.32 ± 0.33	63.94 ± 0.57	74.74 ± 0.31	High	23.68 ± 0.28
18	Bhavani	21.06 ± 0.35	66.57 ± 0.22	76.18 ± 0.12	High	22.49 ± 0.36
19	Swarna	22.27 ± 0.26	51.90 ± 0.59	68.14 ± 0.32	Medium	20.62 ± 0.08
20	Improved Kavuni	23.71 ± 0.75	29.52 ± 0.52	55.88 ± 0.29	Medium	19.56 ± 0.49
21	kavuni	25.66 ± 0.47	27.11 ± 0.22	54.55 ± 0.12	Low	15.80 ± 0.40
22	Norungan	24.75 ± 0.09	50.44 ± 0.05	67.34 ± 0.03	Medium	22.08 ± 0.26
23	Purple puttu	23.10 ± 0.53	35.50 ± 1.10	59.15 ± 0.60	Medium	16.77 ± 0.60
24	Thavalakannan	23.88 ± 0.85	17.64 ± 0.69	49.37 ± 0.38	Low	14.60 ± 0.41
25	Jeeraga samba	19.97 ± 0.16	50.83 ± 1.12	67.56 ± 0.62	Medium	22.58 ± 0.74
26	Rasacadam	25.97 ± 0.09	39.71 ± 1.07	61.46 ± 0.59	Medium	18.52 ± 0.36
27	Sivapuchitiraikar	23.66 ± 0.53	45.62 ± 0.63	64.70 ± 0.35	Medium	19.04 ± 0.22
28	Mapillai samba	24.43 ± 0.39	33.88 ± 1.19	58.27 ± 0.65	Medium	17.17 ± 0.24
	Mean ± SE(d)	22.39 ± 0.46	49.67 ± 0.71	66.92 ± 0.40	Medium	21.32 ± 0.38

*Data are based on the average of three replicates and the observations for each replicate with their standard error (d).

Table 2b. Mean performance of biochemical traits in traditional, brown and polished rice.

S.No	Accessions	Brown rice				Polished rice			
		CHO (%)	Phenolic (µg GAE/g)	Protein (mg \ g)	Crude Fibre (%)	CHO (%)	Phenolic (µg GAE/g)	Protein (mg \ g)	Crude Fibre (%)
1	GEB 24	65.02±1.41	8.71±0.25	8.73±0.07	2.05±0.02	79.63±0.42	2.58±0.15	3.14±0.04	1.47±0.03
2	TKM 9	63.46±0.67	13.84±0.06	6.07±0.16	2.34±0.10	64.32±0.89	8.81±0.02	4.23±0.07	1.53±0.06
3	BPT 5204	64.59±1.58	8.47±0.17	6.38±0.31	1.95±0.08	78.46±0.33	4.18±0.41	3.38±0.08	1.03±0.05
4	CO 43	69.05±0.80	9.69±0.37	8.28±0.16	1.89±0.05	81.81±0.75	3.09±0.33	3.25±0.10	1.45±0.01
5	CO (R) 50	74.78±0.75	8.33±0.18	10.21±0.04	1.74±0.06	76.24±1.10	4.18±0.34	3.93±0.10	1.36±0.06
6	CO 51	64.65±0.99	5.00±0.06	7.57±0.28	1.81±0.09	68.31±0.73	2.78±0.17	3.53±0.05	1.72±0.01
7	ASD 16	61.85±1.50	9.01±0.08	8.74±0.16	1.66±0.13	79.63±1.44	3.23±0.18	5.74±0.15	1.14±0.01
8	ADT 37	72.13±1.97	5.88±0.29	9.51±0.15	2.06±0.06	80.12±0.36	4.42±0.15	5.23±0.14	1.61±0.05
9	ADT 43	69.33±1.18	7.41±0.29	5.52±0.06	1.98±0.05	78.38±1.03	6.06±0.28	3.57±0.09	1.40±0.10
10	ADT (R) 45	65.49±0.68	7.14±0.24	10.94±0.12	1.80±0.03	75.05±0.56	5.00±0.33	7.13±0.14	1.47±0.07

11	IR 20	64.79±0.96	7.65±0.25	8.91±0.16	1.69±0.08	71.10±1.17	5.54±0.42	5.62±0.20	1.28±0.03
12	IR 36	73.65±0.17	8.33±0.45	9.26±0.06	1.72±0.10	77.39±0.49	4.92±0.30	6.00±0.35	1.30±0.11
13	IR 50	73.44±1.11	7.21±0.08	10.25±0.11	1.72±0.07	74.12±1.14	4.99±0.06	7.18±1.02	1.38±0.02
14	CO RH 3	59.75±0.61	7.07±0.23	9.04±0.01	1.97±0.08	68.67±0.40	2.21±0.10	6.24±0.12	1.47±0.01
15	Improved white ponni	66.68±1.02	9.45±0.31	8.56±0.08	1.94±0.22	75.01±0.52	3.89±0.44	5.82±0.13	1.39±0.01
16	Pusa basmati 1	67.11±1.84	6.33±0.44	9.17±0.12	1.86±0.09	83.54±0.98	3.24±0.22	6.51±0.12	1.72±0.05
17	CR 1009 *Sub* 1	65.32±0.43	6.53±0.08	9.18±0.40	1.95±0.15	68.24±0.47	2.95±0.14	7.58±0.23	1.34±0.01
18	Bhavani	61.13±0.84	10.64±0.58	8.83±0.11	2.09±0.06	81.50±0.36	3.02±0.50	4.23±0.08	1.33±0.04
19	Swarna	62.69±0.50	8.91±0.24	7.76±0.05	2.17±0.04	80.72±0.21	2.52±0.24	5.99±0.33	1.67±0.03
20	Improved kavuni	72.95±1.44	22.72±0.27	8.85±0.14	2.95±0.08	80.90±1.55	8.26±0.34	6.70±0.10	2.14±0.05
21	Kavuni	60.97±1.33	24.32±0.45	14.54±0.02	3.06±0.15	76.26±0.90	13.06±0.44	7.38±0.08	2.02±0.06
22	Norungan	68.84±0.80	11.53±0.21	8.29±0.05	3.28±0.09	82.28±1.00	8.11±0.23	3.74±0.09	2.21±0.04
23	Purple puttu	58.99±1.38	34.08±0.39	7.64±0.03	2.33±0.15	73.15±1.33	20.41±0.60	3.59±0.11	1.93±0.09
24	Thavalakannan	61.52±1.17	22.01±0.50	9.30±0.15	2.40±0.07	79.32±0.51	15.34±0.42	5.73±0.11	2.09±0.02
25	Jeeraga samba	68.47±1.60	7.82±0.66	11.19±0.06	1.64±0.06	73.57±1.09	4.97±0.31	7.38±0.07	1.28±0.04
26	Rasacadam	62.37±0.56	17.45±0.14	9.43±0.10	2.12±0.11	74.24±0.57	5.00±0.39	6.60±0.08	2.01±0.01
27	Sivapuchitiraikar	60.99±0.43	9.90±0.06	13.16±0.04	2.12±0.07	77.41±0.43	7.21±0.31	6.42±0.11	1.59±0.02
28	Mapillai samba	62.84±0.24	16.09±0.26	13.98±0.23	3.91±0.11	73.69±0.45	11.36±0.28	8.32±0.03	2.45±0.07
	Mean ± SE(d)	**65.82±1.00**	**11.48±0.27**	**9.26±0.12**	**2.15±0.09**	**76.18±0.76**	**6.12±0.29**	**5.51±0.15**	**1.60±0.04**

*Data are based on the average of three replicates and the observations for each replicate with their standard error (d).

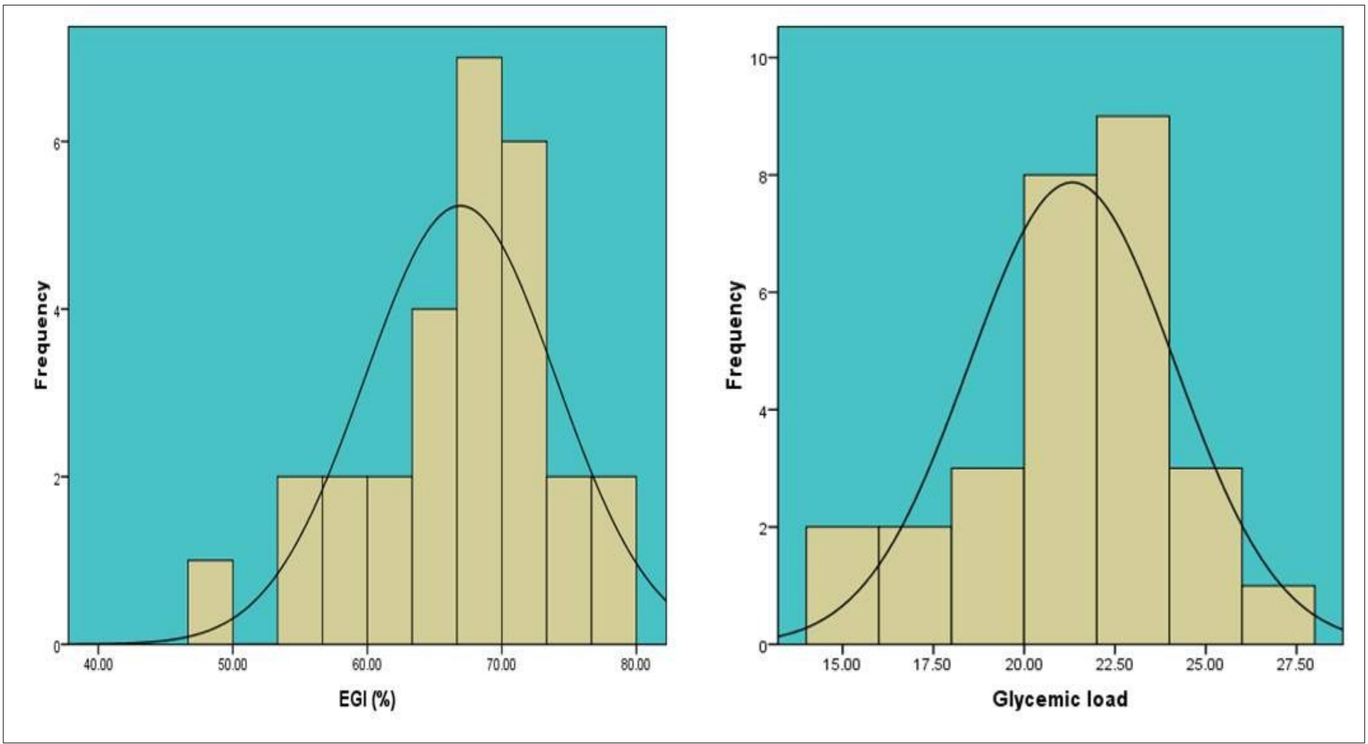

Fig. 2. Frequency distribution of EGI and GL among traditional and popular rice varieties.

Correlation analysis

Association among the various biochemical traits was observed in brown rice (Fig. 3). GL and EGI showed a significant positive correlation (r = 0.84**). The important trait amylose content showed a significant negative association with EGI and GL (r = -0.57**) whereas it associated positively with crude fibre (r = 0.51**), total phenolic content (r = 0.43**) and total protein (r = 0.35*). A significant positive correlation was observed between CHO and GL (r = 0.59**). Total phenolic content was negatively correlated with GI (r = -0.67**), GL (r = -0.74**), and CHO (r = -0.36*) was positively associated with crude fibre (r = 0.56**). Crude fibre content also showed a significant negative association with GI (r = -0.52**), GL (r = -0.57**), and a positive association with total protein (r = 0.36*), respectively.

Principal Component Analysis

PCA is a widely used multivariate statistical technique for identifying the maximum variability among components. The PCA for the biochemical traits under study revealed

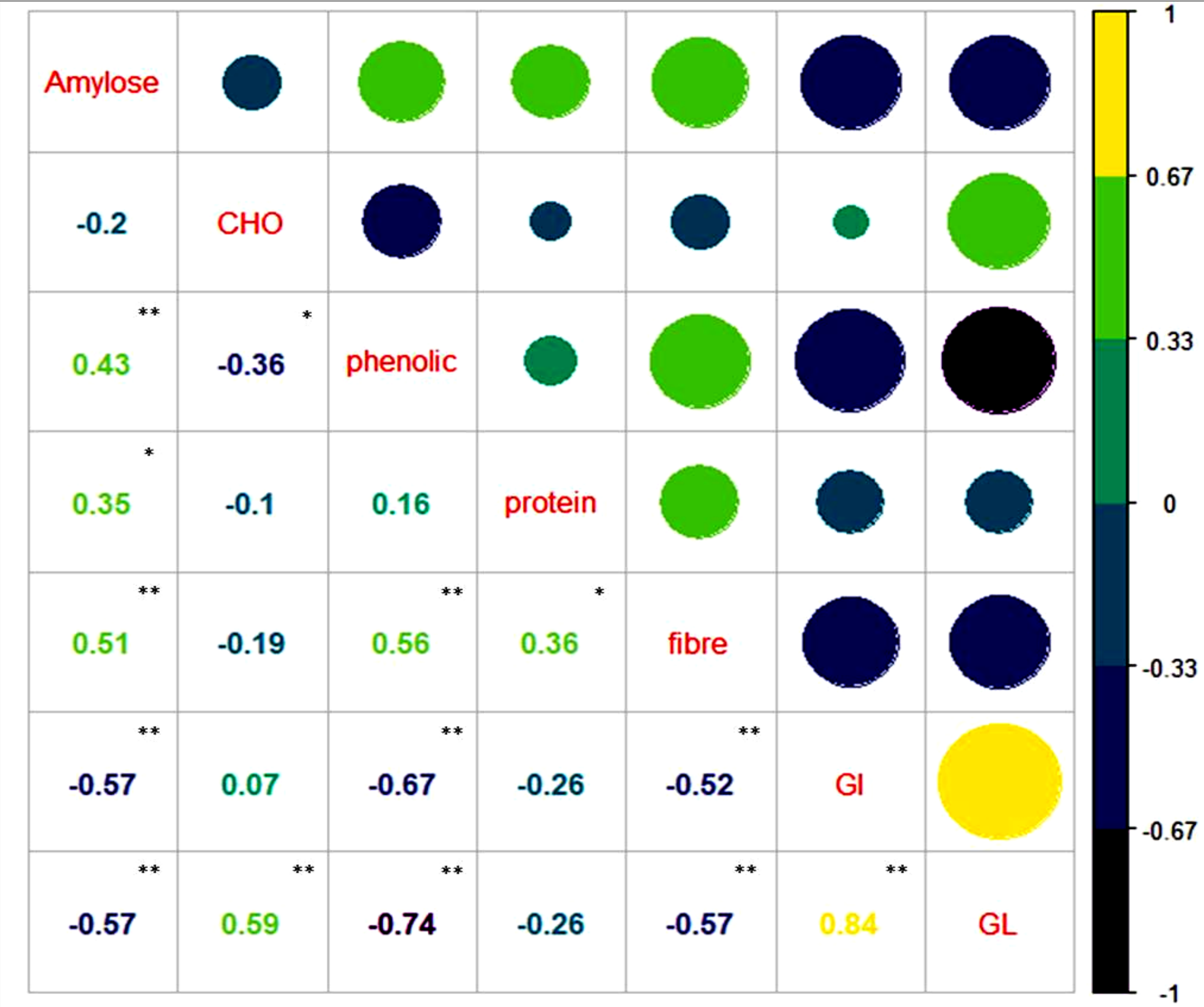

Fig. 3. Correlation coefficients among various biochemical traits in brown rice. *Significance at 95% level (P value <0.05), ** Significance at 99% level (P value <0.01)

the presence of variability among all the rice accessions. The factor loadings of each variable, eigenvalues, percentage of variance, and cumulative percentage of variance for all seven principal components are depicted in Table 3 and Fig. 4. The first two PCs had eigenvalues greater than 1 and accounted for a cumulative variance of 68.57%. The first principal component (PC1) with an eigenvalue of 3.715 accounted for 53.069% of the total variability. Traits such as amylose (0.379), phenolics (0.422), protein (0.222), and fibre (0.389) contributed positively to PC1, whereas traits like the estimated glycemic index (-0.435) and glycemic load (-0.483) exhibited negative contributions. PC2 with an eigenvalue of 1.086 and accounting for 15.510% of the total variation showed negative loadings for carbohydrate content (-0.701) and protein (-0.526), respectively.

A biplot was constructed between PC1 and PC2, which illustrates the relationships and relative contributions of various biochemical traits across the first two

Table 3. The factor loadings, eigen values, percent of variance and cumulative percent of variance for all principal components.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Amylose	0.379	-0.252	-0.035	-0.741	-0.412	-0.271	-0.006
CHO	-0.235	-0.701	0.536	0.085	0.071	-0.076	-0.384
Phenolic	0.422	0.193	0.251	0.365	0.093	-0.762	-0.013
Protein	0.222	-0.526	-0.694	0.149	0.396	-0.119	-0.016
Fibre	0.389	-0.219	-0.026	0.520	-0.652	0.319	0.057
EGI	-0.435	0.153	-0.405	0.104	-0.423	-0.328	-0.571
GL	-0.483	-0.244	-0.044	0.089	-0.238	-0.343	0.724
Eigenvalue	3.715	1.086	0.878	0.530	0.500	0.291	0.001
% Variance	53.069	15.510	12.537	7.564	7.142	4.162	0.016
Cumulative % of variance	53.069	68.579	81.115	88.679	95.822	99.984	100.000

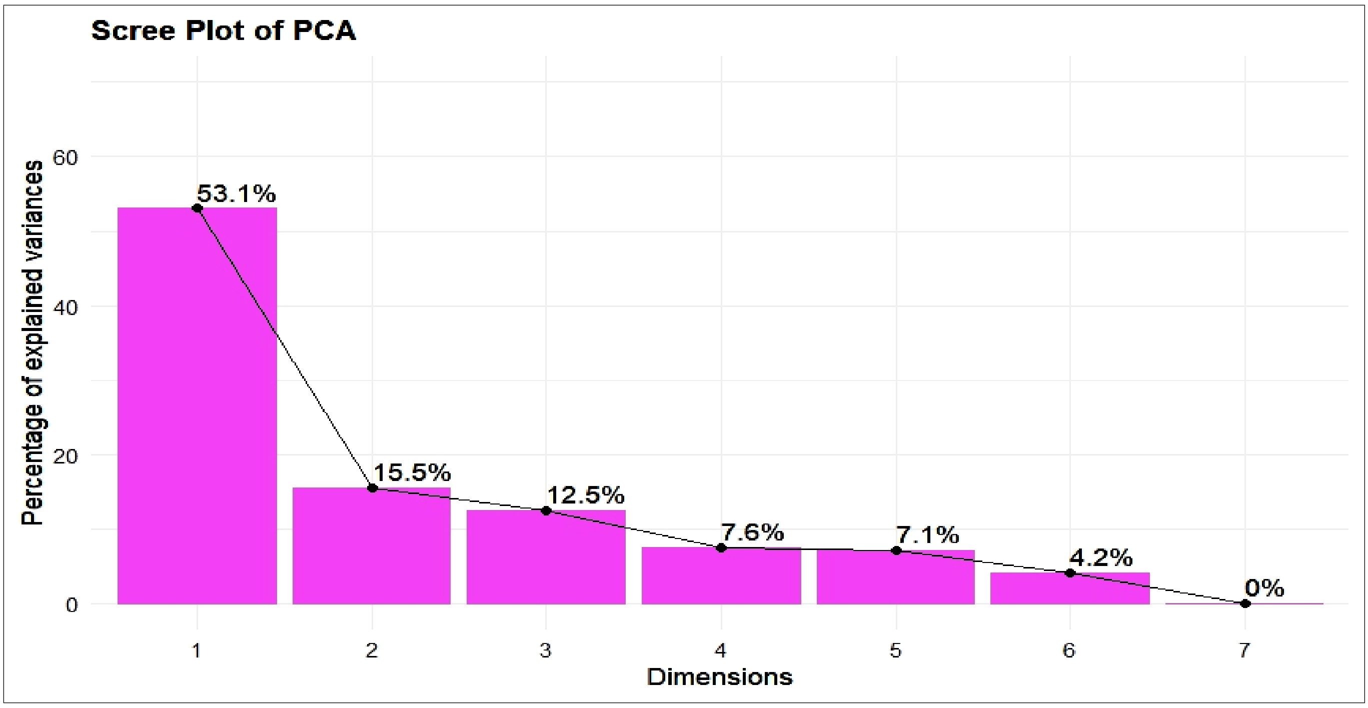

Fig. 4. Scree plot depicting the relationship between all the principal components and their contribution to the percentage of variation

principal components (Fig. 5). The length of a loading vector reflects the extent to which that trait contributes to the total variation. The GI and GL vectors have a relatively small angle between them, indicating a positive correlation between these two traits. Similarly, GI and CHO are also positively correlated as the vectors are with an aligned acute angle (< 90^0). The result also showed total phenolics, Crude fibre, and Amylose content had a strong negative correction with Gl forming a wide obtuse angle (< 90^0). The interaction between accessions and the variables is presented in the PCA biplot (Fig. 5). The varieties positioned in the same quadrant as the variable vectors are expected to have similar characteristics. The traditional varieties, namely, Thavalakannan, Purple puttu, Kavuni, Maapillai samba, Rasacadam, Improved kavuni Norungan, and Sivapuchithiraikar are grouped based on high biochemical and low to moderate glycemic index values.

Diversity analysis

The tree-based hierarchical clustering of mean biochemical data was derived through Darwin software 6.0, which divided the rice accessions into two major clusters with dissimilarity values of 2.40 to 33.96 (Fig. 6). The diversity analysis revealed that the rice accessions were in Cluster I with two sub-clusters, *viz.,* sub-cluster I (14 accessions), and sub-cluster II (8 accessions), which possess intermediate amylose content with moderate to high GI. The accessions of Cluster II (6 accessions) hold traditional varieties with low to moderate GI values.

Discussion

Genetic variation for glycemic index and glycemic load

Cereal grains with lower digestibility are essential in the dietary management of cardiovascular diseases (30). Rice is often regarded as a high-glycemic food, having a wide range of GI values from low 54 to as high as 121 (13, 31).

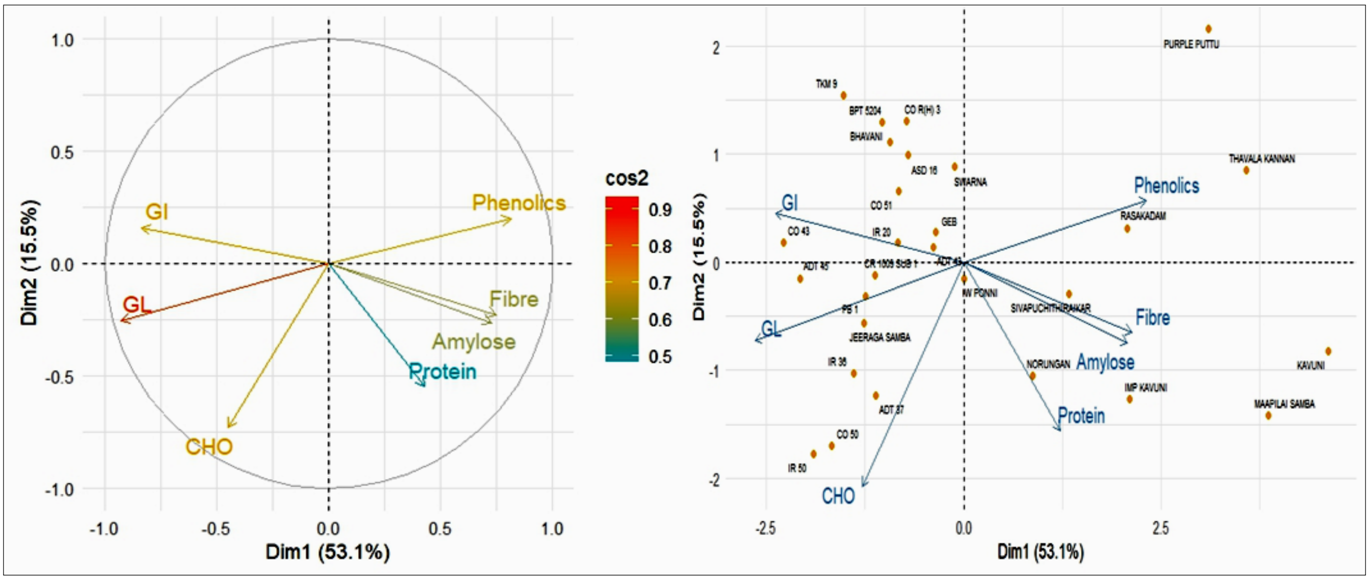

Fig. 5. PCA biplot for the variables and accessions along the first 2 principal components [Dim: dimension or Principal component i.e. PC1 and PC2].

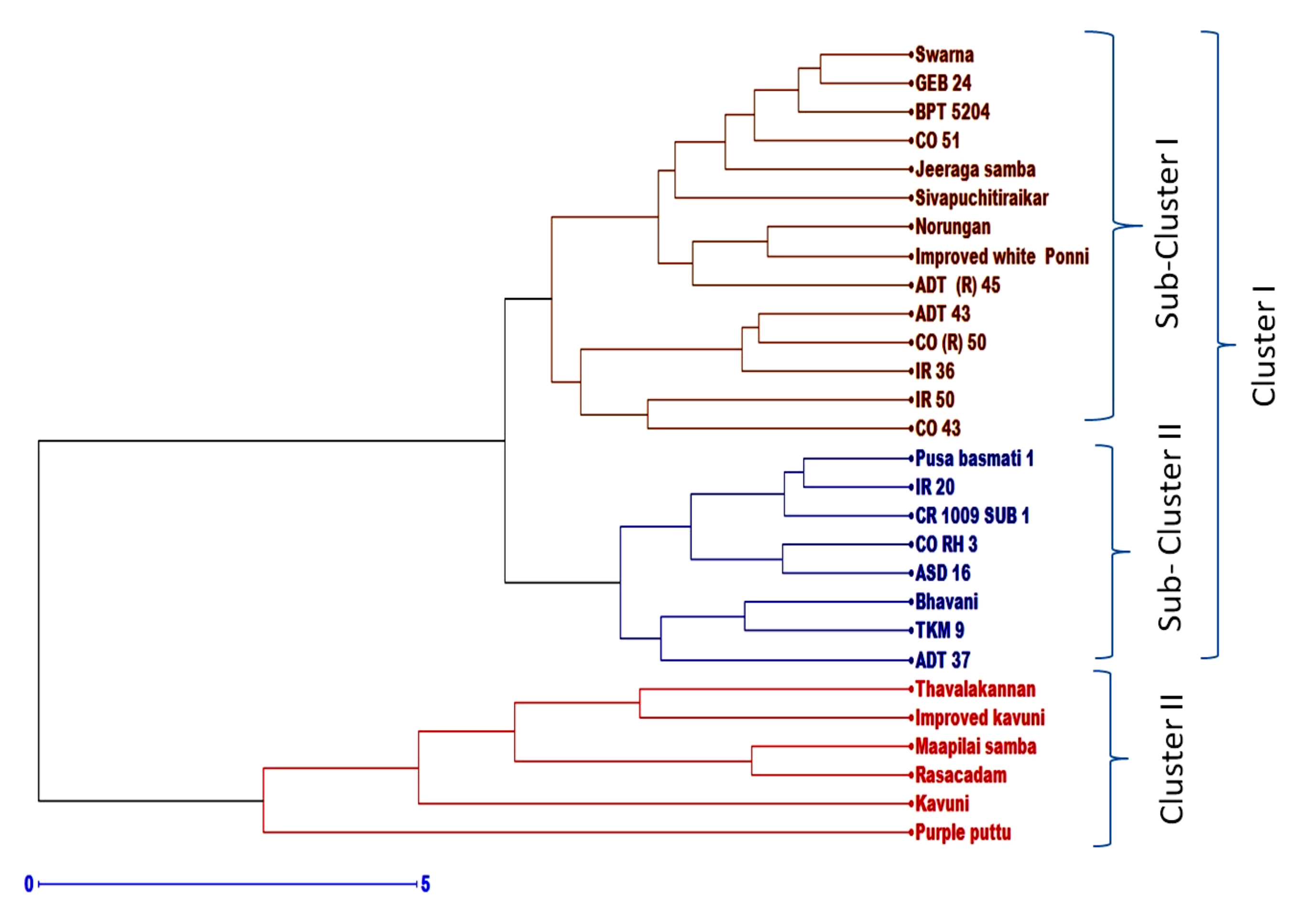

Fig. 6. Tree based on hierarchical clustering of biometrical traits in traditional and popular rice varieties

Among the different methods of GI estimation, the *in-vitro* approach of GI measurement is simple, rapid, and does not require a human participant (7, 11, 28). The analysis showed that 26 rice accessions had moderate to high levels of estimated glycemic index value and were by the system of classification of GI (6). The GI of two pigmented rice varieties, Thavalaikannan and Kavuni, exhibited lower glycemic index values, specifically 49.37% and 54.55%, accompanied by moderate glycemic load. Similarly, studies also showed that the pigmented rice with high amylose content had a low GI and the non-pigmented white rice had a high GI (32). Despite having equal starch contents, the current study revealed that colored rice had a lower GI level and a higher nutritional content. Furthermore, variations in rice's nutritional makeup, processing techniques, and other food-related factors may also contribute to variations in its GI value (16). The study also found that GL negatively correlated with amylose content and positively with EGI aligning with an earlier report (33). Therefore, the lower rate of starch digestibility in pigmented rice had been linked to lower GI and GL, respectively.

Proximate composition of rice accessions

Amylose content is the main component of rice starch that influences the glycemic index, with rice higher in amylose having a lower GI value (31). The study showed that the amylose content of brown rice varied greatly in terms of glycemic responses, ranging from intermediate (18.45 %) to high (25.97 %). The rice varieties with equal amylose concentration may also differ in GI due to differences in chemical composition and cooking technique. This is following the report by (18), which indicates that the pigmented rice varieties have different rates of starch digestion but similar amylose content. Our study also showed a complicated relationship between different nutrient compositions that influence rice's glycemic responses. Cereals are mostly composed of carbohydrates, especially starch, which is the body's main source of energy. Polished white rice is obtained by removing the outer bran layer of brown rice during the milling process. As a result, excessive consumption of polished white rice can raise carbohydrate intake increasing blood glucose levels. The present investigation showed a wide range of carbohydrate content that varied from 58.99% to 74.78% in brown rice and 64.32% to 83.54% in polished rice varieties, which aligns with the findings of the previous study by (19) with no significant difference in carbohydrate content between traditional and high-yielding varieties. A strong positive correlation was found between GL and carbohydrate content which is per (34) who observed a significant relationship between GL and total carbohydrate intake.

Phenols play a significant role in determining the antioxidant properties of cereal grains (32). The present study revealed that the traditional pigmented rice has a higher phenolic level than the non-pigmented kind. A strong negative correlation was also exhibited between phenolic content and GI, GL, and carbohydrate content.

This was in accordance with the result observed by (35). This suggests that the carbohydrate content was influenced by thicker bran layers with high antioxidant activity, which may help to manage cardiovascular diseases and delay the glycemic index or response. However, studies also showed that rice grains with darker pericarp colour such as red and black, contain higher amounts of polyphenols (36, 37). A significant positive association between grain phenolic content and antioxidant activity has also demonstrated possible health advantages in terms of preventing cardiovascular issues like diabetes, high blood pressure, cancer, and heart disease (38).

Protein is a significant non-starchy component that influences the digestibility of starch in rice (39). Rice protein content and composition vary between brown rice and white rice according to the milling process. Brown rice has more protein than polished white rice. Additionally, pigmented rice has a higher protein content compared to non-pigmented rice (40). As reported by (41) native medicinal rice cultivars, their total crude protein content ranged from 6.67% to 10% of milled rice. The study showed a considerable difference in protein content amongst all the kinds, ranging from 5.52 to 14.54 mg/g for brown rice and 3.14 to 8.32 mg/g for polished rice. Similarly, the highest protein content in brown rice revealed that protein plays an important role in regulating eating quality and might be a reason for their protein-starch interaction resulting in lower GI.

Unmilled rice contains significantly more dietary fibre and nutrients than milled or polished white rice (42). Similarly, a study reported that the total dietary fibre level in the medicinal rice variety Njavara was significantly higher than that in the non-medicinal rice variety (18). The present study showed that the crude fibre content of brown rice was significantly higher than that of polished rice. Similarly, pigmented rice had higher crude fibre than non-pigmented rice. Crude fibre content also exhibited a significant negative association with GI and GL which might be due to its thick bran portion. The dietary fibre content in different rice varieties played a key role in determining their glycemic response, with higher fibre content being associated with a lower glycemic index (43). Moreover, the fibrous texture of rice grains contributes significantly to slower glucose absorption resulting in a lower glycemic index in the diet.

Principal component analysis

PCA simplifies data by identifying the key variables that account for the majority of variation in genotypes (44). In this study, the first two principal components explain a cumulative variance of 68.57% with an eigenvalue > 1 and effectively capture a substantial portion of the overall variation. Traits like amylose, total phenolics, protein, and fibre exhibited strong positive loadings on PC1, highlighting their significant role in distinguishing the rice varieties. Conversely, the glycemic index (GI) and glycemic load (GL) had negative loadings, suggesting that varieties with low GI and GL values tend to have higher levels of amylose, total phenolics, and crude fiber content, or vice versa. PC2 accounts for about 15.510% of the variation and is primarily distinguished based on total carbohydrate and protein content showing negative loadings. However, these Eigenvalues are also used to select important principal components (PCs) that significantly contribute to the total variability (45).

The result showed that the biplot created between PC1 and PC2 effectively demonstrates the relationships among different biochemical traits in the rice varieties. Traits that have vectors closely aligned with each other, such as GI and GL indicate a positive correlation, suggesting that varieties with a higher GI tend to also have a higher GL. Similarly, the acute angle between the glycemic index (GI), and carbohydrate content (CHO) suggests a positive relationship between these traits aligning with established metabolic responses to foods high in carbohydrates. The negative correlation between estimated glycemic index and total phenolics, crude fibre, and amylose content also suggested that rice varieties with higher levels of these biochemical compounds tend to have lower glycemic responses. Furthermore, studies also reported correlations among variables based on the angles observed in the biplot (44). This study also showed that traditional varieties such as Thavalakannan, Purple Puttu, Kavuni, Maapillai Samba, Rasacadam, and Improved Kavuni cluster together in the same quadrants and are characterized by high biochemical content and low to moderate GI values. Therefore, by identifying and selecting specific traits associated with lower glycemic responses it is possible to create rice varieties that are both high-yielding and nutritionally superior.

Diversity analysis

Based on the biochemical traits, a dendrogram was created using the UPGMA clustering method to study the genetic variability among the varieties with Darwin 6.0 software (46). The diversity analysis showed that Cluster I comprises popular rice varieties with good quality and appearance. This group also covers a wide characteristic of grain features, such as acceptable size and shape, indicating marketability and commercial value. The presence of the traditional variety, sivapuchithiraikar, and norungan in this cluster I might be attributed to certain traits associated with modern varieties. The accessions of Cluster II hold traditional varieties, namely Thavalakannan, Maapilai samba, Improved Kavuni, Kavuni, Rasacadam, and Purple puttu, with low to moderate glycemic index values. Most of these traditional varieties are high in nutritional value compared to commercial varieties and genetically more distant from those in Cluster I. However, the cluster analysis indicates a high degree of heterogeneity in attributes among the varieties and also suggests some possible parents for use in future breeding efforts aimed at improving the nutritional value of the variety (47). As a result, this study provides critical insights into the nutritional and glycemic properties of various rice varieties, particularly focusing on the benefits of pigmented traditional rice for cardiovascular disease management.

Conclusion

The present investigation revealed a considerable variance in the starch composition and nutritional contents of the rice varieties based on their chemical structure. The starch digestibility was shown to be influenced by amylose, crude fibre, total protein, and total phenolics content, leading to a reduced glycemic index value and supporting the use of an indirect method for EGI analysis in food and products. Among the varieties, Thavalakannan, Kavuni, Improved Kavuni, Maapillai Samba, Rasacadam, and Sivapuchithiraikar exhibited superior nutritional profiles contributing to a lower glycemic response. Our study also showed that polishing rice significantly removes the outer bran layers of the grains turning the rice into a simpler carbohydrate that raises blood sugar levels more quickly. Therefore, consuming polished rice could increase the risk of cardiovascular diseases by promoting higher glycemic responses, potentially leading to health issues such as type II diabetes. Additionally, the dietetic benefit of traditional landraces can be utilized as potential donors for understanding the genetic basis of the glycemic index. Identifying important genomic regions or potential genes associated with these quality traits is crucial for enhancing rice varieties' nutritional security with a low glycemic index. Furthermore, these findings could serve as a nutritional alert for managing cardiovascular diseases and diabetes mellitus, contributing to better overall health.

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Authors' contributions

All authors contributed to the study and designed the experiment. Material preparation, biochemical, and quality-related analysis were performed by BNR. Data analysis and results interpretation were done by BNR, SR, and PJ. The manuscript was written by BNR and SR. All authors read and commented on the manuscript. The final draft of the manuscript was revised and finalized by SR and PJ.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest in the content of the manuscript and study undertaken.

Ethical issues: This article does not contain any studies with human participants and animals by any authors.

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