Received: 06 Jan 2024 | Revised: 16 Jun 2024 | Accepted: 19 Jun 2024

Electronic Journal of Plant Breeding

Research Note

Unravelling the biochemical traits of traditional rice landraces of Kerala

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Abstract
Biochemical characterisatics of 70 traditional landraces of rice (*Oryza sativa* L.) of Kerala including various micronutrients, rice bran oil and oryzanol content were analyzed. Traditional varieties have demonstrated their potential as valuable reservoirs of nutrients, indicating their suitability for incorporation into future breeding programmes aimed to develop nutritionally enriched rice varieties. Among the genotypes studied Oorkayama and Thekkan were identified with highest rice bran oil content and γ oryzanol content respectively. Fe, Zn, Na, K, carotene and anthocyanin content showed a wide range of variation among the genotypes. The GCV estimates were high for rice bran oil content and oryzanol content indicating higher magnitude of variability due to genotypes for these characters. The investigation suggests preponderance of additive gene effects for all the biochemical constituents under study as indicated by the high heritability coupled with high genetic advance.

Keywords: Traditional rice landraces, diversity, rice bran oil, oryzanol content

Kerala stands as a significant repository of rice biodiversity in the country. While scientific documentation regarding the precise count of traditional varieties in the state is lacking, reports suggest that the region predominantly cultivates around 2000 traditional varieties, as documented by Leenakumari (2012). In 1976, Kerala Agricultural University gathered close to 1000 traditional rice varieties from various regions within the state. This collection comprised aromatic, medicinal, and organic rice varieties, all well-suited to diverse soil conditions. Historically, these local varieties have met the nutritional, cultural, and ritualistic requirements of numerous tribal communities in the state.

In the realm of rice research in the country, the primary emphasis has been on the development of varieties with enhanced yield and resistance to pests or diseases. The majority of studies on traditional varieties have concentrated on their varietal characteristics. However, there has been limited attention paid to the nutritional aspects of these varieties, with only a few specialty rice types like Navara receiving consideration for their use in the traditional Indian medicinal system, Ayurveda, to address various ailments (Deepa *et al*., 2008). The objective of this study was to conduct a comparative analysis of the biochemical composition of traditional rice varieties cultivated in the state of Kerala.

Seventy traditional rice cultivars of Kerala were collected from various sources and raised during September 2022 - January 2023 at the uplands of the farm of Onattukara Regional Agricultural Research Station, Kayamkulam. The details regarding the cultivars studied are mentioned in **table 1.**

Estimation of Rice bran oil content: A quantity of 6 grams of crushed sample was placed in a packed bed within a thimble, and this assembly was then positioned in a distillation flask within a Soxhlet apparatus, with 10 ml of hexane. The extraction procedure was carried out to

Table 1. List of rice landraces used for the phytochemical profiling

S. No.	Name of landraces	S. No.	Name of landraces	S. No.	Name of landraces
1.	*Chenthadi*	25.	*Valiya Champan*	49.	*Oorpandi*
2.	*Chettadi*	26.	*Vellathondi*	50.	*Kodukanni*
3.	*Cheruvellari*	27.	*Anakodan*	51.	*Ittikandan*
4.	*Chuvanna Vellikannan*	28.	*Chellaam Chettivirippu*	52.	*Thavalakannan*
5.	*Karutha Chitteni*	29.	*Chettivirippu*	53.	*Thekkan Cheera*
6.	*Kokkan*	30.	*Cheruvirippu*	54.	*Kanali*
7.	*Kuttadan*	31.	*Ezhupunna Pokkali*	55.	*Vadakkan Vellarikayama*
8.	*Mullanchanna*	32.	*Kadamkudy Kuruka*	56.	*Marathoni*
9.	*Mundakakutty*	33.	*Kadamkudy Pokkali*	57.	*Chuvanna Chitteni*
10.	*Punchakayama*	34.	*Krishnakaomodha*	58.	*Thekkan Chitteni*
11.	*Athikkirai*	35.	*Kuthiru*	59.	*Kochuvittu*
12.	*Kariyadukkan*	36.	*Nayarambalam Pokkali*	60.	*Mullanchayama*
13.	*Kavugin Poothoola*	37.	*Oorkayama*	61.	*Karinellu*
14.	*Kalyanikutty*	38.	*Ponkuruka*	62.	*Karutha Kuruka*
15.	*Karutha Njavara*	39.	*Pokkali*	63.	*Eravapandy*
16.	*Kothambarikayama*	40.	*Rakthashali*	64.	*Thekkan*
17.	*Mattachembavu*	41.	*Traditional Virippu*	65.	*Gandhakashala*
18.	*Neicheera*	42.	*Varapuzha Pokkali*	66.	*Jeerakashala*
19.	*Njavara Manja*	43.	*Vyttila Kuruka*	67.	*Orthadian*
20.	*Nooranvella*	44.	*Kunju Kunju*	68.	*Karuthacheera*
21.	*Parambuvattan*	45.	*Valichoori*	69.	*Vellamundakan*
22.	*Kavunginpoothala*	46.	*Kannan Kulamban*	70.	*Veluthakattamodan*
23.	*Elappapoochampan*	47.	*Paalthondi*		
24.	*Arikkirai*	48.	*Vellari*		

completion, and the hexane was subsequently separated from the oil through evaporation. The weight of the rice bran oil extracted was then measured and documented.

Percentage of Yield = (RBO (g) / Initial weight of RB (g)) x 100

where, RBO is the rice bran oil and RB is the rice bran

Estimation of Oryzanol content: γ Oryzanol content of the rice bran oil sample was determined by spectrophotometric method according to the method described in CODEX (2015) by dissolving 0.2 g of rice bran oil sample in 100 ml of n-hexane and reading the absorbance at 314 nm in 1-cm quartz cuvette using a spectrophotometer.

γ Oryzanol content (%) = 100 x (1/W) x A x (1/359)

where, A is the Absorbance of the sample; W is γ-oryzanol weight (g) in 100 ml of the sample solution used for measurement and 359 is the specific extinction for γ oryzanol E (1%, 1cm)

Estimation of Na & K content of unpolished rice: Sodium & potassium in the seed was estimated by acid digestion and flame photometry (Jackson and Saunders, 1973). Oven dried seed samples were ground and 0.25 g of the ground sample was mixed with 10 ml HNO_3 in Teflon tubes. The tubes were kept for digestion in a microwave digester. The digested plant material was diluted to 100 ml with ultrapure (Type 1) water and read in a flame photometer, with necessary calibrations.

Estimation of Fe & Zn content of unpolished rice:Iron and zinc content of grain samples were estimated by using Atomic Absorption Spectrophotometer (Lindsay and Novell, 1978). 1 gm of seed was taken and powdered in the grinder. Powdered seed sample was digested in tri-acids (HNO_3+$HClO_4$+H_2SO_4) mixture (10:4:10) in sand bath. The digested sample was cooled for 30 minutes and made up to 50 ml with double distilled water. Then a known quantity of solution was used for subsequent analysis. A suitable blank was run simultaneously to account for the contamination from the reagents.

Estimation of Carotene content of unpolished rice:10 g of powdered rice sample was dispersed in 50 ml water saturated n-butanol to make a homogenous suspension. The suspension was shaken gently and allowed to stand

overnight (16 hours) at room temperature at dark. After that these were filtered through Whatman filter paper No.14 and volume of filtrate was made up to 100 ml. The absorbance (A) of the clear filtrate was measured at 440 nm in spectrophotometer against water saturated n-butanol as blank (Joy *et al*., 2015).

Beta carotene content (ppm) = 0.0105 + 23.5366*A

Estimation of Anthocyanin content of unpolished rice: Estimation of anthocyanin was done according to the method suggested by Ranganna (1977). One gm sample was collected from the grain. The sample was extracted using ethanolic HCl, filtered through Whatman No.1 filter paper. The filtrate was then diluted with ethanolic HCl to 50 ml. Absorbance was recorded at 535 nm. The anthocyanin content was calculated using the formula and was expressed as milligram per gram of the sample. Total OD (optical density) per 100 gram of sample (X) = [(absorbance at 535 nm) x (volume made up for the extract used for colour development) x (total volume) + 100] **+** [Volume (ml of extract) used x weight of sample taken].

Absorbance of the solution containing 1 mg per ml is equal to 98.2 (content).
Total anthocyanin in mg per 100 g of sample = X / 98.2

Statistical analysis: The data were subjected to analysis of variance to test for significant differences among the accessions using the statistical package GRAPES.

Variability studies: Analysis of variation revealed significant variation among the genotypes for all the biochemical traits (**Table 2).** The mean values of the various biochemical constituents analysed are presented in the **table 3.**

Rice bran oil content was highest for the variety *Oorkayama* (16.45 %). Lowest percentage is for *Thavalakannan* (0.663%) which was on par with Nooranvella (0.829%). This parameter showed a wide range from 0.6 to 16.5%. γ Oryzanol content of the rice bran oil sample was determined by spectrophotometric method which exhibited a wide range from 0.06 to 33.70%. Highest value was for *Thekkan* (33.703%), while lowest for *Oorkayama* (0.061%). Assessment of sodium in grain samples by acid digestion and flame photometry was maximum at 1.17 % for *Karutha Kuruka* and minimum at 0.346 % for *Eravapandy.* Phytochemical assessment of the potassium using flame photometry showed a widest range from 0.8 to 4.9 %. *Vellamundakan* with 4.899 % has the highest, while *Kadamkudy Kuruka* (0.753%) and *Vadakkan Vellarikayama* (0.76%) showed the least value. The obtained values were similar to the potassium content observed in other traditional rice varieties of Kerala as reported by Deepa *et al.* (2008).

Iron content of seed samples ranged from 25.55-288.85 mg/g. *Thekkan Chitteni* exhibited highest iron content and *Vadakkan Vellarikayama* had the lowest iron content. Seed samples evaluated for zinc content displayed an expansive range from 4.68- 46.07 mg/g. Significantly higher iron and zinc content of traditional genotypes compared to improved cultivars from Tamil Nadu was declared by Anandan *et al.* (2011). Jahan and Hasan (2014) and Anuradha *et al.* (2012) reported wide range of iron and zinc content in rice cultivars respectively. High variation for grain iron content (8.6 -62.9µg/g) was recorded with a mean of 22.78µg/g (Prasannakumari *et al.,* 2020).

Carotene content ranged from 0.165 -0.575 % among the 70 cultivars. Renuka *et al.* (2016) documented that the carotene content in unpolished aromatic indica rice varieties fell within the range of 0.12 to 0.99 mg/100g. The values obtained were comparable to another study on 84 land races of West Bengal where a much wider range of 0.025 to 3.48 mg/ 100g for iron and 0.085 to 19.53 mg/100g for zinc content was reported (Roy and Sharma, 2014). Anthocyanin content of the various rice cultivars were ranged from 1.216- 0.100%. Bhuvaneswari *et al*. (2020) and Sridevi *et al*. (2021) also discovered a wide range of anthocyanin content among traditional rice cultivars. A wide range for the biochemical constituents suggests that even though traditional landraces belong

Table 2. ANOVA of biochemical parameters estimated in the traditional rice landraces

S. No.	Characters	MSS of source	MSS of error	F
1.	Rice bran oil content	29.706	0.008	3763.997
2.	Oryzanol content	100.729	0.003	39926.988
3.	Na content	0.041	0.000	420.900
4.	K content	1.534	0.000	40024.79
5.	Fe content	3522.387	0.089	39720.609
6.	Zn content	98.340	0.002	40122.879
7.	Carotene content	0.014	0.000	53.789
8.	Anthocyanin content	0.196	0.000	39707.291

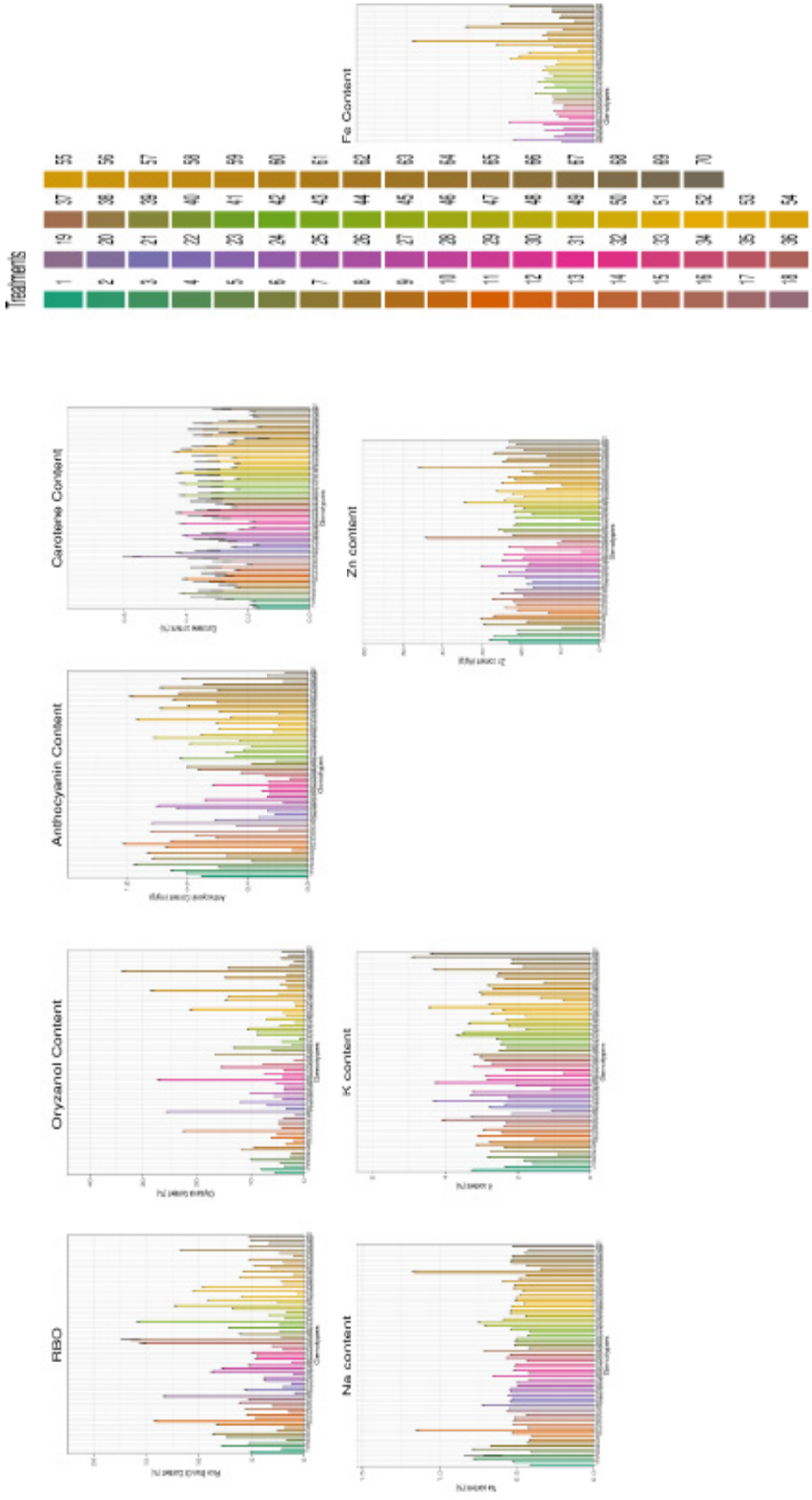

Fig. 1. Characterization of rice landraces based on biochemical parameters

Table 3. Mean biochemical composition of rice land races

S. No.	Genotype	Bran oil content (%)	Oryzanol (%)	Anthocyanin (mg/g)	Na (%)	Fe (%)	K (%)	Zn (%)	Carotene (%)
1	*Chenthadi*	5.141	5.497	0.698	0.412	47.05	3.261	23.185	0.175
2	*Chettadi*	2.156	8.116	0.800	0.522	101.10	2.358	27.960	0.185
3	*Cheruvellari*	7.794	3.630	0.904	0.778	73.75	1.585	26.965	0.185
4	*Chuvanna Vellikannan*	5.307	4.366	0.589	0.722	62.10	1.835	20.895	0.275
5	*Karutha Chitteni*	1.658	9.950	1.144	0.408	70.35	2.834	20.995	0.335
6	*Kokkan*	7.297	2.520	0.370	0.786	61.50	0.903	9.650	0.415
7	*Kuttadan*	8.623	2.298	1.025	0.671	82.20	2.760	29.450	0.320
8	*Mullanchanna*	2.487	11.668	0.535	0.430	98.90	2.371	18.605	0.235
9	*Mundakakutty*	1.824	9.363	1.056	0.416	74.55	3.139	30.250	0.265
10	*Punchakayama*	8.292	3.330	0.100	0.404	71.85	2.788	27.065	0.325
11	*Athikkirai*	14.096	1.975	0.938	0.528	31.35	1.534	12.935	0.405
12	*Kariyadukkan*	4.643	6.066	1.216	1.148	61.80	3.096	20.895	0.275
13	*Kavugin Poothoola*	5.472	5.041	0.908	0.426	59.10	2.450	24.280	0.310
14	*Kalyanikutty*	1.493	22.539	0.606	0.522	62.20	2.948	20.995	0.235
15	*Karutha Njavara*	5.638	4.093	0.738	0.518	69.95	2.391	21.990	0.335
16	*Kothambarikayama*	2.985	4.647	1.034	0.512	117.80	2.330	27.265	0.195
17	*Mattachembavu*	6.136	4.668	0.192	0.436	55.20	4.050	19.405	0.285
18	*Neicheera*	5.307	3.749	0.471	0.565	87.55	3.268	25.175	0.345
19	*Njavara Manja*	13.267	1.667	1.029	0.546	166.65	2.163	21.590	0.575
20	*Nooranvella*	0.829	25.615	0.610	0.722	34.85	1.067	16.815	0.425
21	*Parambuvattan*	5.610	3.269	0.325	0.540	105.85	2.792	18.605	0.330
22	*Kavunginpoothala*	1.980	7.047	0.217	0.530	92.35	2.362	17.015	0.245
23	*Elappapoochampan*	1.155	11.906	0.264	0.559	50.95	4.290	18.905	0.175
24	*Arikkirai*	3.795	3.833	0.867	0.536	127.55	2.272	25.870	0.265
25	*Valiya Champan*	3.795	5.605	0.995	0.542	64.10	3.298	23.085	0.325
26	*Vellathondi*	0.990	10.125	0.158	0.492	46.55	3.234	18.805	0.405
27	*Anakodan*	8.745	3.682	0.675	0.496	78.30	1.083	30.150	0.310
28	*Chellaam Chettivirippu*	7.755	3.565	0.269	0.422	47.45	2.036	18.010	0.225
29	*Chettivirippu*	5.280	5.288	0.251	0.657	80.90	4.240	24.875	0.185
30	*Cheruvirippu*	1.155	27.086	0.306	0.422	134.60	2.851	19.500	0.415
31	*Ezhupunna Pokkali*	4.620	3.883	0.257	0.516	40.80	2.891	24.475	0.185
32	*Kadamkudy Kuruka*	4.455	7.583	0.624	0.512	55.10	0.753	4.675	0.285
33	*Kadamkudy Pokkali*	4.950	3.707	0.258	0.506	60.60	2.340	19.105	0.345
34	*Krishnakaomodha*	1.980	15.447	0.115	0.432	64.75	3.214	23.085	0.425
35	*Kuthiru*	2.970	7.884	0.284	0.565	49.45	2.732	10.845	0.330
36	*Nayarambalam Pokkali*	15.345	1.795	0.441	0.540	47.35	2.917	9.550	0.245
37	*Oorkayama*	16.450	0.061	0.719	0.714	66.65	3.035	44.375	0.275
38	*Ponkuruka*	2.145	16.650	0.792	0.418	63.60	3.203	21.990	0.335
39	*Pokkali*	6.105	5.983	0.372	0.512	67.05	2.518	24.475	0.320
40	*Rakthashali*	2.310	13.044	0.210	0.508	93.95	2.296	25.770	0.415
41	*Traditional Virippu*	7.131	2.333	0.845	0.502	42.90	2.471	21.890	0.320
42	*Varapuzha Pokkali*	2.322	4.060	0.482	0.428	79.00	2.462	21.295	0.235
43	*Vyttila Kuruka*	15.754	0.902	0.540	0.414	63.60	2.583	4.675	0.320
44	*Kunju Kunju*	1.824	8.809	0.422	0.536	89.25	3.660	21.395	0.415

Table 3. Continued...

S. No.	Genotype	Bran oil content (%)	Oryzanol (%)	Anthocyanin (mg/g)	Na (%)	Fe (%)	K (%)	Zn (%)	Carotene (%)
59	*Kochuvittu*	5.804	3.046	0.787	0.597	73.25	2.692	16.615	0.245
60	*Mullanchayama*	3.151	4.383	0.599	0.482	82.50	2.831	19.800	0.170
61	*Karinellu*	4.809	3.178	0.888	0.436	75.00	1.277	46.070	0.345
62	*Karutha Kuruka*	1.990	14.752	1.170	1.170	49.65	2.369	12.735	0.265
63	*Eravapandy*	5.307	3.203	0.857	0.346	203.70	2.568	24.775	0.345
64	*Thekkan*	0.995	33.703	0.593	0.436	149.15	2.527	23.780	0.165
65	*Gandhakashala*	2.322	14.165	0.973	0.536	31.05	4.293	13.335	0.345
66	*Jeerakashala*	11.774	2.742	0.687	0.532	54.90	1.854	27.065	0.265
67	*Orthadian*	5.307	1.756	0.157	0.524	52.15	2.166	19.205	0.175
68	*Karuthacheera*	3.317	4.181	0.832	0.446	66.85	2.155	23.880	0.185
69	*Vellamundakan*	5.141	2.890	0.267	0.426	66.95	4.899	21.195	0.185
70	*Veluthakattamodan*	5.307	4.047	0.145	0.522	133.85	4.364	22.985	0.285
	Mean	5.256	7.603	0.599	0.539	80.37	2.631	21.562	0.298
	SE(d)	0.089	0.05	0.002	0.01	0.298	0.006	0.05	0.016
	CD	0.177	0.1	0.004	0.02	0.594	0.012	0.099	0.032
	CV	1.69	0.661	0.371	1.826	0.371	0.235	0.23	5.361

to the same geographical location of Kerala state, they retain their uniqueness.

GENETIC PARAMETERS: A sound knowledge on the extent of variability present in the available germplasm is a prerequisite to any breeding programme of a particular crop species. Since the observed variability is the sum of variation arising due to genotypic and environmental effects, knowledge on the nature and magnitude of genetic variation contributing to gain under selection is essential. The phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance of the various characters were estimated and presented in the table 4. All the characters exhibited a very high coefficient of variation.

The phenotypic coefficient of variation (PCV) for all biochemical characters ranged from 26.565 to 93.348 %. The genotypic coefficient of variation (GCV) for the various biochemical characters also followed a similar trend. The oryzanol content (93.348%) and rice bran oil content (73.335) exhibit the highest PCV, indicating substantial variation.

Information on heritability and estimates of genetic advance that could be obtained in the next cycle of selection are of vital importance to the breeder in deciding appropriate method of breeding. Highest heritability was observed for all the biochemical parameters namely rice bran oil content, oryzanol content, Na content, K content, Fe content, Zn content, carotene content and anthocyanin content indicating negligible influence of the environment on these characters. These findings align with previous studies by Pratap *et al.* (2018), Gupta *et al.* (2021), Krishnan *et al.* (2023), Basu *et al.* (2022) and Borah *et al.* (2023). In the present investigation genetic

Table 4. Estimates of genetic parameters with respect to various biochemical parameters in traditional rice landraces

Characters	PCV	GCV	Heritability (%)	Genetic advance (%)
Rice bran oil content	73.34	73.32	99.99	150.99
Oryzanol content	93.35	93.35	100	192.29
Na content	26.57	26.57	100	54.72
K content	33.29	33.29	100	68.58
Fe content	52.22	52.22	100	107.56
Zn content	32.52	32.52	100	66.99
Carotene content	28.06	28.06	100	57.81
Anthocyanin content	52.28	52.28	100	107.69

advance as percent of mean was high for oryzanol content, rice bran oil content, anthocyanin content and Fe content. All others traits exhibited moderate genetic advance as percent of mean. Moderate genetic advance with high heritability was also noted, indicating the potential for individual plant selection in further improving these traits. Study of coefficient of variation indicated that estimates of GCV for all characters studied are slightly less than PCV estimates thus indicating slight influence of environment on the performance of genotypes. This is in agreement with the findings of Sourav *et al.* (2024). High heritability and high genetic advance of characters is indicative of additive gene action suggesting the possibility of genetic improvement of those characters through section**.** Similar findings were also documented by Rajesh *et al.* (2016), Osman *et al.* (2012), Nithya *et al.* (2020) and Bhargavi *et al.* (2021*)*. Selection for these traits can effectively be carried out based on their phenotypic performance.

The present study clearly indicated that greater variability existed for all the characters studied among the 70 landraces of rice from Kerala. Prospective future breeding programmes for improving the nutritional constituents can utilise the traditional rice landraces as revealed by the high content of various constituents. The landrace *Oorkayama* and *Thekkan* were identified as having the highest rice bran oil content and γ oryzanol content respectively. The different varieties also displayed a wide range of Fe, Zn, Na and K content along with carotene and anthocyanin contents. The GCV estimates were high for rice bran oil content and oryzanol content indicating higher magnitude of variability for these characters. The investigation suggests preponderance of additive gene effects for all the biochemical constituents under study as indicated by the high heritability coupled with high genetic advance.

ACKNOWLEDGEMENT

The first author is grateful to Kerala Agricultural University for granting the Junior Research Fellowship for PG programme

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