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Effect of pigmentation on physical, phytochemical and antioxidant properties of traditional rice landraces from Odisha region (India)

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A study was conducted in the Odisha region of India to assess genetic parameters, heritability, and trait associations in twenty traditional pigmented and non-pigmented rice landraces. The evaluation focused on twenty-five physical, phytochemical, and antioxidant properties. The results showed potential for enhancing desired traits in pigmented rice due to a wide range of genotypic variation, high heritability, and substantial genetic advances. These improvements were particularly observed in characteristics such as porosity, total soluble sugar, phytochemicals, and antioxidant properties in pigmented rice. Additionally, properties like the length-breadth ratio of grains, thickness, diameter, thousand grain weight, and bulk density of grains can be used for trait improvement in non-pigmented rice. Physical attributes like thickness, grain weight, and porosity, as well as phytochemical traits like total soluble sugar and protein content, were notably higher in pigmented rice. Correlations between traits indicated that Pigmented rice was associated with phytochemical and antioxidant properties, while Non-pigmented rice was linked to amylose content, density, and the length-breadth ratio of grains. Two promising pigmented genotypes, Bodikaberi and Mahipaljeera, were identified and could be valuable for future rice breeding programs. Moreover, these genotypes have potential applications in the food industry for creating value-added products to enhance nutritional quality and could also be relevant to the cosmetic industry due to their superior antioxidant properties.

Keywords: Antioxidant properties, Physical properties, Phytochemicals, Pigmented rice, Variability

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Rice (non-pigmented) is the primary source of nutrition, to meet daily calorie and protein intake of more than two billion people around the world[1]. Poor nutritional quality of the non-pigmented rice (NP) as compared to pigmented rice (P) is a major concern for the nutritional and health security of the rice consuming population[2]. To address the food and nutritional security of the world populace, inclusion of P rice that are enriched with several bioactive compounds with antioxidative properties such as anthocyanin, phenolic acids, flavonoids, pro-anthocyanidins, tocopherols, tocotrienols, oryzanol[2] in the breeding programme could be an efficient choice.

Changes in food habits, driven by better health consciousness need inclusion of quality rice in the diet. This has led to an increased demand of P rice varieties in the food industry. The market value, consumer preference and popularity of rice is not only judged by its nutritional quality but is also dependent on its physical attributes[3]. Though the value of each trait varies according to local cuisine and culture[4], knowledge on physical traits is essential to classify rice grain which will aid in designing and optimization of suitable equipments required for post-harvest processing[5].

The study of variability in physical and nutritional quality traits of P rice is required for its

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Abbreviations

P rice: Pigmented Rice; NP rice: Non-pigmented rice; MC: moisture content; L: length; B:breadth; L/B: length breadth ratio; Th: thickness; ED: equivalent diameter; GD: geometric mean diameter; GA: grain area; GV: grain volume; DS: degree of sphericity; TSW: thousand seed weight; BD: bulk density; PD: particle density; GP: grain porosity; TSS: total soluble sugar; TAC: total anthocyanin content; GO: gamma oryzanol; TPC: total phenolic content; TFC: total flavonoid content; ABTS: 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; CUPRAC: cupric-reducing antioxidant capacity successful utilization in plant breeding programme. Understanding the genetic architecture of these traits and finding the promising genotypes is crucial to initiate the breeding programme. Key parameters such as heritability, variance component, genotypic and environmental variance are essential to unravel the gene action governing the desired trait. Further, heritability coupled with genetic advance offers the most effective condition for selection of a specific character[6]. Knowledge about the relationship amongst the traits is a prerequisite for executing any breeding programme[7] as it is essential to determine the main target traits for improving nutritional quality.

The Jeypore tract of Odisha, being the secondary centre of origin of rice, preserves many traditional landraces. These local landraces enriched with nutritive and nutraceutical values[8] remain mostly underutilized and unexplored. There is no report on the physical, phytochemical and antioxidant properties of P rice of Odisha region and a systematic effort on documentation of nutritional quality of P rice of this region needs to be initiated. Due to the lack of sufficient reports, the beneficial properties of these traditional landraces still remain unknown to consumers and to the food industry. So to capitalize the health benefits of these valuable resources, extensive research by the stakeholders needs to be undertaken so that they can be made available to consumers for inclusion in their diet as specialty functional food. Hence this study was designed to (1) characterize P and NP rice genotypes with respect to variation in physical, phytochemical and antioxidant properties, (2) calculate broad sense heritability (BSH) and the expected genetic advance (GA) in order to get information on achievable improvement of respective traits by breeding, (3) study the correlation between the traits to know the association type and magnitude among the traits, and (4) identify the most promising genotypes.

Materials and Methods

Twenty P and NP rice genotype (P rice- Baranga, Bodikaberi, Gondiachampeisiali, Kaniar, Kantakaamala, Kathidhan, Kundadhan, Landi, Latachaunri and Mahipaljeera; NP rice- Chinamal, Dudhamani, Kaberi, Kalikaranji, Kusumal, Lakdimachi, Latamahu, Laxmibilash, Magura and Radhabati) grown in Randomized Block Design (RBD) during Kharif (2017) at the experimental plot of ICAR-National Rice Research Institute (20.45_N, 85.93_E, Cuttack, Odisha) were used in this study. Recommended agronomic package of practices and plant protection measures were followed during the growing season. The grains were harvested at physiological maturity, sundried for 3-4 days to maintain the final moisture content of 12-13%. The grains were then stored at 4°C in vacuum sealed plastic containers till further use.

Physical traits

100 g of rice grains were de-hulled by Satake rice huller (Japan) and 14 physical traits were determined. Grain moisture content (MC:%) was estimated by Indosaw digital moisture meter with an accuracy of ±0.2% on dry weight basis. Rice grain analyzer (Satake, Japan) with an accuracy of 0.02 mm was used to measure grain length (L:mm), width (W:mm) and thickness (Th:mm). Length–breadth ratio (L/B) was estimated by dividing length by breadth of grain. Thousand seed weight (TSW:g) was determined by counting and weighing 1000 grain on a digital electronic balance with 0.001 g sensitivity. The equivalent diameter (ED:mm) of the grain is the diameter of a spherical particle estimated by using the equation ($ED=\{[L\ (W+T)^2]/4^{1/3}\}$) by Mohsenin[9]. The geometric mean diameter (GD:mm) depicts the central tendency of length, width and thickness by using the product of their values was calculated according to formula ($Dg=\{LWT\}^{1/3}$) of Mohsenin[9]. The grain area (GA:mm^2) was estimated according to the formula ($GA=\pi\ BL^2/2L\text{-}B$) of Jain & Bal[10]. Grain volume (GV:mm^3) was determined according to the formula ($GV=\pi B^2\ L^2/6\{2L\text{-}B\}$, where $B=[(WT)^{0.5}]$ given by Jain & Bal[10]. Degree of sphericity (DS:%) was calculated according to formula ($DS=[\{LWT\}^{1/3}]/L$), the particle density (PD:g mL^{-1}), bulk density (BD:g mL^{-1}) and grain porosity (GP:%) was calculated as per the formula of Mohsenin[9].

Proximate composition

For the analysis of proximate composition, phytochemical and antioxidant activities, the dehulled samples were ground by the grinding machine (Glen mini grinder) and sieved through 100 mesh size. Proximate composition of the grain such as amylose content was estimated as per Juliano *et al.*[11] and expressed in percentage. Crude protein content of the grain was estimated as per AOAC methodology[12] using the formula N X 5.95 and expressed in percentage. Total soluble sugar (TSS) was estimated following anthrone method of Hedge & Hofreiter[13] and expressed in percentage.

Phytochemical and antioxidant properties

Phytochemical properties such as total anthocyanin content (TAC) in the samples was determined using method described by Fuleki & Francis[14] and expressed as mg 100 g^{-1}. Gamma-oryzanol (GO) was estimated according to Bucci *et al.*[15] and expressed as mg 100 g^{-1}. Total phenolic content (TPC) was determined by modified protocol of Zilic *et al.*[16] where catechol was used as standard instead of catechin and expressed as mg Catechol equivalent (CE) 100 g^{-1}. Total flavonoid content (TFC) was determined according to Eberhardt *et al.*[17] and expressed as mg Catechine equivalent (CEt) 100 g^{-1}. Antioxidant activities such as ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging was assayed by modified protocol of Serpen *et al.*[18] and expressed as % inhibition. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay was estimated according to the method of Zhu *et al.*[19] and expressed as % inhibition. Ferric reducing antioxidant power (FRAP) activity was measured as per Mau *et al.*[20] using different concentration of ascorbic acid for standard curve and result was expressed as µg ascorbic acid equivalent (AAE) g^{-1}. Cupric ion reducing antioxidant capacity (CUPRAC) was determined according to the method of Apak *et al.*[21] using different concentrations of trolox for standard curve preparation and was expressed as µg trolox equivalent (TE) g^{-1}.

Statistical analysis

The design of experiment followed was RBD. The estimation of mean, range, and coefficient of variation (CV%), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), environmental coefficient of variation (ECV), broad sense heritability (BSH) and genetic advance (GA) was done by using Cropstat software (Version 7.0). DMRT test was done to differentiate the mean for all the traits at 5% significance level by using SAS software (version 9.2). Correlation and PCA analysis was done with the help of paleontological statistics (PAST) software version 4.02.

Results and Discussion

Estimation of genetic parameters of variation

Physical, phytochemical and antioxidant properties of the 20 rice samples were estimated and the result is presented in graphical form (Fig. 1a and Fig. 1b). Significant variation with respect to range, mean, GCV, PCV, BSH and GA were observed for the above parameters and are presented in Table 1a and Table 1b. Among the studied traits, highest CV was observed for TAC (72.33%) followed by CUPRAC (58.06%) in P rice. However, in NP rice, TPC (54.33%) followed by TAC (47.71%) had shown higher variability. Existence of such variation is essential and ideal to facilitate the breeding programme. Such variation in P rice was also observed previously by other researchers[8,22]. However, variability observed in P rice was comparatively lower to NP rice except for B, GP, protein, TAC, TFC and CUPRAC.

In P rice, highest PCV and GCV was observed for TAC (72.43, 72.23%, respectively) followed by CUPRAC (58.06, 58.05%, respectively) which was almost the same, while in NP rice, highest PCV and GCV was observed for TPC (54.49, 54.18%, respectively) followed by TAC (50.16, 45.12% respectively). This signifies a higher contribution of genetic constitution of plants to phenotypic expression as compared to effect of environment on these traits. Similar findings have also been reported earlier[23]. Though presence of high genetic variability for a trait signifies its amenability to selection but it is the heritability that separates heritable variation from total phenotypic variation observed for the traits and decides which traits are to be selected. In this study, higher heritability (h^2) was observed for all the traits in both P and NP rice which indicates the possibility of selection of these traits. Lowest values of BSH ($h^2 \leq 30\%$) were observed for L, GrA, GrV in P rice whereas B was observed with low heritability in NP rice genotypes. High heritability along with genetic advance together decides the possible improvement of traits after selection. Genetic advance was observed to be higher (>30.0%) in GP, TSS, TAC, GO, TPC, TFC, ABTS, FRAP and CUPRAC in P rice (highest in CUPRAC: 99.97% and TAC: 99.72%). The traits (L/B, Th, GD, TSW, BD) had higher phenotypic variation in NP rice compared to P rice for which higher GA was observed (highest in TPC: 99.78%). The higher GA correlates with the possibility of desirable improvement in grain physical and nutritional quality.

Comparison of mean of genotypes

Physical properties

Significant differences among the genotypes were observed for physical traits of P and NP rice (Fig. 1a

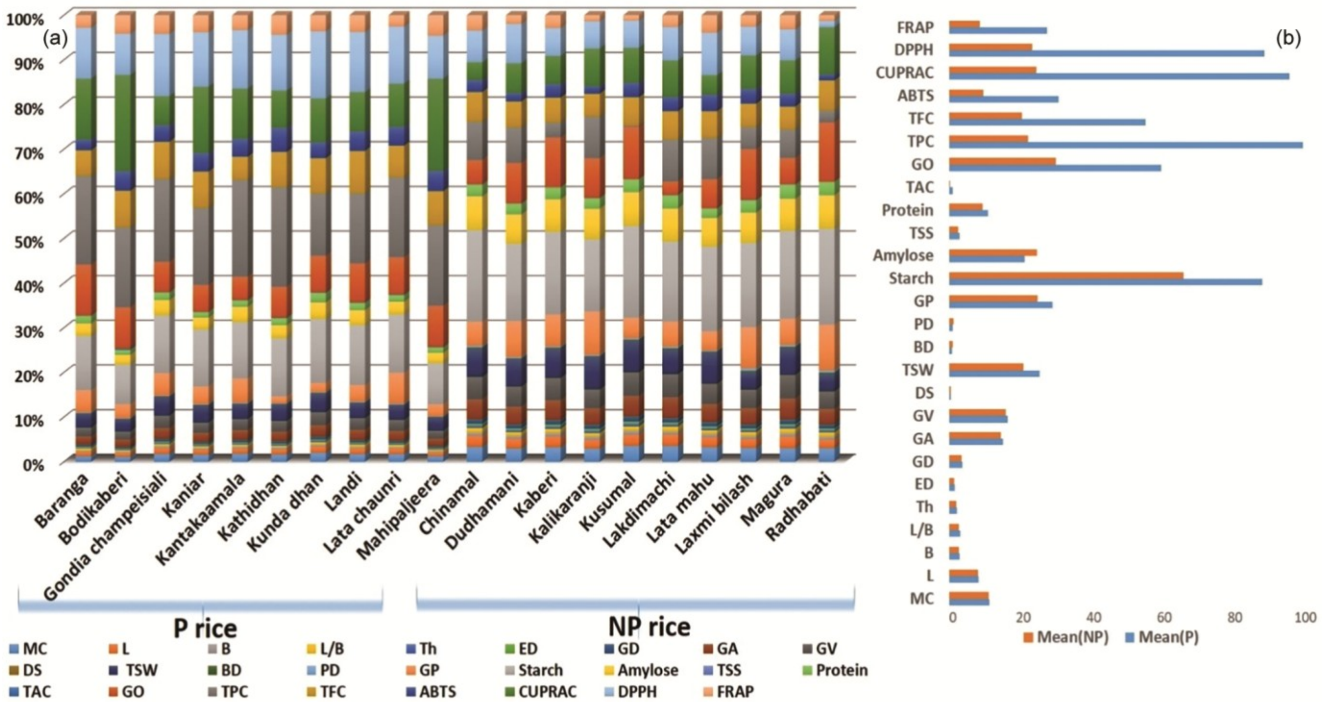


Fig. 1 — (a) Physical properties, proximate composition, phytochemical, antioxidant properties of Pigmented and Non-pigmented rice, (b) comparison of mean of Pigmented and Non-pigmented rice.

MC: grain moisture content (%); L: grain length (mm); B: grain breadth (mm); L/B: length breadth ratio; Th: thickness of grain (mm); ED: equivalent diameter (mm); GD: geometric mean diameter (mm); GrA: grain area (mm^2); GrV: grain volume (mm^3); DS: degree of sphericity (%); TSW: thousand seed weight (g); BD: bulk density (g mL^{-1}); PD: particle density (g mL^{-1}); GP: grain porosity (%); total soluble sugar (%); TAC: total anthocyanin content (mg 100 g^{-1}); GO: gammaoryzanol (mg 100 g^{-1}); TPC: total phenolic content (mg CE 100 g^{-1}); TFC: total flavonoid content (mg CEt 100 g^{-1}); ABTS: 2,2'-azino-bis 3-ethyl benzo thiazoline- 6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; CUPRAC: cupric-reducing antioxidant capacity; NP: Non-pigmented rice; P: Pigmented rice ; CV: coefficient of variation (%); PCV: phenotypic coefficient of variation (%) ; GCV: genotypic coefficient of variation (%); ECV: environmental coefficient of variation (%); h^2: broad sense heritability (%); GA: genetic advance over mean at 5% selection intensity

and Fig. 1b) though mean values did not differ for MC, L, B, L/B, ED, GD, GrA, GrV and DS. Variation in physical traits of rice grain among P and NP rice were also previously observed[8,22]. The MC of both P and NP rice lies within the acceptable range as observed earlier[8]. MC was found to have significant positive correlation with grain L, B, ED, GD, GrA and GrV (Fig. 2). Moisture-dependent physical properties of rice grains are important to design post-harvest equipment for handling and storage of the product. Swelling of grains with increase in moisture content may influence the physical traits[24].

Longest and shortest grain was observed in NP genotype Lakdimachi (9.12 mm) and Radhabati (6.18 mm). Highest L/B ratio of 3.66 was observed in Lakdimachi. Both P and NP rice genotypes were observed to have long bold grain with L more than 6.0 mm and L/B ratio less than 3.0. However, Devraj *et al*.[8] observed longer grain of NP rice as compared to P rice. Highest grain Th of 2.18 mm was observed in NP rice genotype Kalikarnji and lowest Th (1.88 mm) was observed in P rice genotypes Landi and Latachaunari. Grain dimensions such as L, B and Th are important in determining aperture size in designing of grain handling machinery equipment required for processing of rice[25]. Mean Th, found higher in P rice (2.04 mm) compared to NP rice (1.86 mm), signifies that P rice would be more resistant to breakage during the milling process as compared to NP rice. It also determines the market value of the rice grain, consumer preference and is a deciding factor for production of value added products. Such long bold grain P rice may be utilized in making suitable nutrient rich value added products like popped rice, puffed rice and flaked rice.

Gondiachampeisiali was found to have highest ED (1.57 mm) and GD (3.91 mm) whereas lowest value was observed in NP rice genotypes Radhabati

Table 1a — Estimation of genetic variables for physical properties of pigmented and non-pigmented rice genotypes

	Range		Mean		CV		PCV		GCV		ECV		h^2		GA	
Parameter	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP
MC	9.18-12.19	9.86 -12.39	11.26	11.20	8.36	7.08	8.4	7.09	8.31	7.03	1.23	0.53	97.83	99.43	16.79	14.46
L	7.36-8.73	6.18-9.12	8.25	8.03	4.95	11.4	6.24	11.75	3.17	11.03	5.37	4.03	25.79	88.22	3.34	21.3
B	2.27-3.24	2.39-2.93	2.88	2.71	10.18	6.98	10.91	8.73	9.38	4.61	5.56	7.41	73.98	27.93	16.63	5.04
L/B	2.56-3.41	2.66-3.66	2.95	2.71	10	12.1	11.25	37.71	8.56	36.17	7.3	10.65	57.95	92.03	13.44	71.46
Th	1.88-2.14	1.66-2.18	2.04	1.68	4.99	9.3	5.03	36.22	4.96	36.21	0.84	1.09	97.18	99.91	10.06	73.87
ED	1.28-1.57	1.17-1.49	1.46	1.37	5.8	6.93	6.45	7.39	5.07	6.43	3.99	3.64	61.71	75.69	8.24	11.57
GD	3.20-3.90	2.90-3.72	3.63	3.08	5.58	8.26	6.21	36.09	4.86	35.89	3.87	3.78	61.25	98.9	7.81	72.86
GrA	13.43-16.10	11.37-16.49	15.18	14.7	4.68	10.8	6.19	11.17	2.34	10.48	5.74	3.86	14.23	88.07	1.79	3.22
GrV	14.48-17.72	12.18-17.99	16.58	15.9	5.03	11.1	6.52	11.46	2.82	10.73	5.88	4.01	18.66	87.76	2.55	20.77
DS	0.41-0.48	0.38-0.48	0.44	0.43	4.71	7.58	5.81	8.04	3.26	7.1	4.81	3.77	31.5	78.02	3.71	12.91
TSW	22.78-28.86	12.56-27.26	25.54	21	8.84	22.6	9.02	22.77	8.65	22.44	2.56	3.87	91.92	97.11	16.91	45.5
BD	0.58-0.78	0.62-1.17	0.69	0.86	9.17	22	9.32	22.3	9.02	21.71	2.34	5.14	93.71	94.69	18.04	46.35
PD	0.84-1.19	0.79-1.76	0.99	1.16	13.07	26	13.23	26.32	12.89	25.7	3.0	5.68	94.85	95.34	25.89	51.52
GP	11.11-50.00	14.29-36.36	29.19	25	40.05	33.7	40.41	34.22	39.68	33.14	7.66	8.51	96.41	93.82	79.92	67.67

MC: grain moisture content (%); L: grain length (mm); B: grain breadth (mm); L/B: length breadth ratio; Th: thickness of grain (mm); ED: equivalent diameter (mm); GD: geometric mean diameter (mm); GrA: grain area (mm^2); GrV: grain volume (mm^3); DS: degree of sphericity (%); TSW: thousand seed weight (g); BD: bulk density (g mL^{-1}); PD: particle density (g mL^{-1}); GP: grain porosity (%); NP: Non-pigmented rice; P: Pigmented rice; CV: coefficient of variation (%); PCV: phenotypic coefficient of variation (%); GCV: genotypic coefficient of variation (%); ECV: environmental coefficient of variation (%); h^2: broad sense heritability (%); GA: genetic advance over mean at 5% selection intensity

Table 1b — Estimation of genetic variables for proximate composition, phytochemical and antioxidative properties of pigmented and non-pigmented rice genotypes

	Range		Mean		CV		PCV		GCV		ECV		h^2		GA	
	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP
Amylose	19.61-23.34	22.99-27.01	21.38	24.76	5.29	5.1	5.35	5.15	5.23	5.05	1.13	0.99	95.55	96.26	10.59	10.19
Protein	9.13-12.67	7.59-10.64	10.9	9.44	10.38	9.94	10.38	10.06	10.37	9.81	0.51	2.27	99.76	94.92	21.18	19.69
TSS	2.04-3.55	1.73-3.61	2.76	2.45	15.61	25.40	15.61	78.74	15.60	78.74	0.66	0.54	99.81	99.99	15.61	78.74
TAC	0.45-2.84	0.05-0.35	0.96	0.19	72.33	47.71	72.43	50.16	72.23	45.12	5.4	21.93	99.94	80.89	99.72	83.7
GO	35.75-87.88	10.56-43.19	59.99	30.17	31.86	37.16	31.86	37.22	31.86	37.1	0.96	3.02	99.91	99.34	64.99	75.91
TPC	82.95-169.09	0.91-34.77	135.88	22.33	20.49	54.33	20.5	54.49	20.48	54.18	0.83	5.82	99.84	98.86	41.81	99.78
TFC	35.22-76.89	17.11-23.78	55.57	20.47	33.83	10.25	36.39	10.48	31.05	10.02	18.98	3.05	72.79	91.5	54.73	19.85
ABTS	18.45-42.17	4.54-13.59	30.86	9.57	26.78	28.15	26.78	28.19	26.76	28.11	0.74	2.15	99.92	99.42	54.61	57.49
DPPH	85.34-91.69	4.56-36.15	89.23	23.49	2.23	36.7	2.26	36.73	2.2	36.67	0.5	2.01	95.17	99.7	4.42	74.9
FRAP	15.07-42.13	3.77-16.60	27.77	8.59	36.26	45.6	28.66	45.63	28.6	45.57	1.86	2.3	99.58	99.74	58.45	93.06
CUPRAC	42.25-202.25	13.29-34.12	96.39	24.68	58.06	26.48	58.06	26.66	58.05	26.29	1.34	4.45	99.94	97.21	99.97	53.28

TSS: total soluble sugar (%); TAC: total anthocyanin content (mg 100 g^{-1}); GO: gammaoryzanol (mg 100 g^{-1}); TPC: total phenolic content (mg CE 100 g^{-1}); TFC: total flavonoid content (mg CEt 100 g^{-1}); ABTS: 2,2'-azino-bis 3-ethyl benzo thiazoline- 6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; CUPRAC: cupric-reducing antioxidant capacity; NP: Non-pigmented rice; P: Pigmented rice; PCV: phenotypic coefficient of variation (%); GCV: genotypic coefficient of variation (%); ECV: environmental coefficient of variation (%); h^2: broad sense heritability (%); GA: genetic advance over mean at 5% selection intensity

(1.69; 1.17 mm, respectively). ED and GD values of P and NP rice lies between 1.35 to 1.50 mm and has been reported to have an important role in determining the diameter of pores in a sieve used in a grading machine[26]. Lakdimachi and Dudhamani showed highest GrA for ED and GD (16.50, 16.43 mm, respectively). Highest GrV was observed in Dudhamani (17.99 mm^3) followed by Lakdimachi (17.74 mm^3) and Gondiachampeisiali (17.72 mm^3). Knowledge of grain surface is important during modelling of grain drying, aeration, heating and cooling[27]. For P and NP rice GrA was 15.18 and 14.66 mm^2, respectively and GrV was found to be 16.58 and 15.89 mm^3, respectively. Though P and NP rice did not significantly differ for GrA, P rice may take more time for cooking due to higher thickness of the grain as thickness and surface area of grain influences the diffusion of water during cooking process[28] and is correlated with optimal cooking time[29]. Highest DS was observed in Kalikarnji (0.49%) whereas the lowest value was recorded in Lakdimachi (0.38%). Sphericity, an important criteria for designing the grain hoppers for milling process[27] decides the packaging size[5]. Similar value of DS observed in both P and NP rice (~0.4%) suggest that both require similar package size.

	MC	L	B	L/B	Th	ED	GD	SA	SV	DS	TSW	BD	PD	GP	Starch	Amylose	Protein	Tss	TAC	GO	TPC	TFC	ABTS	DPPH	FRAP
L	0.58																								
B	0.45	0.36																							
L/B	-0.05	0.41	-0.70																						
Th	0.10	0.20	0.67	-0.44																					
ED	0.52	0.67	0.90	-0.35	0.76																				
GD	0.51	0.67	0.89	-0.33	0.77	1.00																			
SA	0.60	0.99	0.45	0.31	0.30	0.75	0.75																		
SV	0.60	0.98	0.52	0.24	0.37	0.80	0.80	1.00																	
DS	-0.28	-0.65	0.39	-0.84	0.54	0.11	0.11	-0.56	-0.49																
TSW	0.27	0.44	0.65	-0.26	0.77	0.78	0.79	0.52	0.57	0.21															
BD	-0.22	-0.30	-0.56	0.31	-0.71	-0.65	-0.66	-0.37	-0.42	-0.30	-0.86														
PD	-0.27	-0.42	-0.66	0.30	-0.64	-0.73	-0.73	-0.49	-0.54	-0.20	-0.85	0.80													
GP	-0.11	-0.16	-0.22	0.08	0.08	-0.16	-0.15	-0.17	-0.17	0.08	-0.04	-0.23	0.39												
Starch	0.12	0.14	0.24	-0.08	0.46	0.33	0.34	0.18	0.20	0.19	0.39	-0.44	-0.23	0.27											
Amylose	0.09	0.09	-0.20	0.22	-0.40	-0.20	-0.21	0.04	0.01	-0.33	-0.31	0.41	0.21	-0.23	-0.83										
Protein	-0.16	0.14	-0.18	0.35	0.23	0.04	0.06	0.15	0.14	-0.10	0.19	-0.05	0.08	0.23	0.55	-0.37									
Tss	-0.26	-0.31	0.14	-0.32	0.35	0.05	0.06	-0.26	-0.23	0.52	0.13	-0.30	-0.20	0.11	0.21	-0.39	0.10								
TAC	-0.17	0.14	0.21	-0.03	0.35	0.28	0.28	0.17	0.19	0.13	0.37	-0.36	-0.23	0.17	0.52	-0.57	0.27	0.45							
GO	-0.40	-0.23	0.03	-0.14	0.41	0.06	0.07	-0.17	-0.14	0.41	0.31	-0.42	-0.13	0.41	0.64	-0.69	0.48	0.59	0.73						
TPC	0.02	0.15	0.37	-0.16	0.65	0.47	0.48	0.22	0.26	0.31	0.56	-0.52	-0.39	0.14	0.89	-0.79	0.49	0.29	0.71	0.73					
TFC	0.12	0.20	0.44	-0.24	0.52	0.48	0.49	0.27	0.31	0.28	0.37	-0.46	-0.35	0.14	0.71	-0.67	0.19	0.41	0.76	0.64	0.80				
ABTS	0.16	0.22	0.46	-0.22	0.56	0.51	0.52	0.29	0.33	0.26	0.50	-0.47	-0.40	0.03	0.82	-0.80	0.37	0.38	0.77	0.69	0.92	0.91			
DPPH	0.17	0.28	0.40	-0.12	0.61	0.53	0.54	0.34	0.38	0.19	0.62	-0.59	-0.43	0.19	0.94	-0.79	0.56	0.19	0.62	0.65	0.94	0.74	0.88		
FRAP	0.00	0.17	0.40	-0.18	0.56	0.46	0.47	0.24	0.28	0.25	0.50	-0.36	-0.35	-0.03	0.70	-0.74	0.37	0.30	0.74	0.67	0.87	0.78	0.91	0.77	
CUPRAC	-0.21	-0.05	0.26	-0.21	0.53	0.29	0.30	0.02	0.06	0.40	0.38	-0.36	-0.22	0.20	0.58	-0.67	0.29	0.53	0.82	0.83	0.79	0.76	0.80	0.65	0.88

Fig. 2 — Correlation heat map of physical, phytochemical and antioxidant activity of Pigmented and Non-pigmented rice. Significant correlations are colored either in red (negative) or green (positive). Shades of green indicate increasing positive correlation coefficient; shades of red indicate increasing negative correlation coefficient.

MC: grain moisture content (%); L: grain length (mm); B: grain breadth (mm); L/B: length breadth ratio; Th: thickness of grain (mm); ED: equivalent diameter (mm); GD: geometric mean diameter (mm); GrA: grain area (mm^2); GrV: grain volume (mm^3); DS: degree of sphericity (%); TSW: thousand seed weight (g); BD: bulk density (g mL^{-1}); PD: particle density (g mL^{-1}); GP: grain porosity (%); total soluble sugar (%); TAC: total anthocyanin content (mg 100 g^{-1}); GO: gammaoryzanol (mg 100 g^{-1}); TPC: total phenolic content (mg CE 100 g^{-1}); TFC: total flavonoid content (mg CEt 100 g^{-1}); ABTS: 2,2'-azino-bis 3-ethyl benzo thiazoline- 6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; CUPRAC: cupric-reducing antioxidant capacity; NP: Non-pigmented rice; P: Pigmented rice; CV: coefficient of variation (%); PCV: phenotypic coefficient of variation (%); GCV: genotypic coefficient of variation (%); ECV: environmental coefficient of variation (%); h^2: broad sense heritability (%); GA: genetic advance over mean at 5% selection intensity

Highest TSW was observed in P genotype, Kaniar (28.86 g) and lowest value in Laxmibilash (14.06 g). Laxmibilash was found to possess highest BD (1.17 g mL^{-1}) and PD (1.76 g mL^{-1}) whereas lowest BD was noted in Gondiachampeisiali (0.58 g mL^{-1}). Grain weight determines the ease of cleaning grains using aerodynamic force[29]. It is also a useful index to "milling outturn" in determining the relative amount of foreign matter in a given lot of paddy[5]. So milling outturn ratio in P rice could be higher as compared to NP rice. Higher TSW of P rice compared to NP rice may occur due to the greater thickness of the grain. TSW has also been reported to be associated with yield[30]. So, higher TSW is an added advantage in P rice along with enriched antioxidant compounds.

Understanding BD of grains is essential for designing of silos and storage bins[31], whereas PD finds application in separation of impurities present in the cereal grains using pneumatic separators, as seeds of various impurities and rice grain differ greatly in true density among each other[32]. Both BD and PD of P rice (0.69, 0.99 g mL^{-1}, respectively) was lower than NP rice (0.86, 1.16 g mL^{-1}, respectively) which was due to higher porosity observed in P rice (29.19%), compared to NP rice (24.95%). The lower density in P rice may be due to the presence of empty space within husk. Our finding contradicts with that of Devraj *et al.*[8] as they reported higher density of P rice compared to NP rice. According to Varnamkhasti *et al.*[33], low percentage of porosity as observed in NP rice could cause difficulties in active drying process. This may result in slower drying of rice as low porosity offers less resistance to air combustion product in convective drying with forced draft[5].

Proximate composition

Genotypes were found to have significant difference in proximate composition such as amylose, TSS and protein content (Fig. 1a). Cooked rice with intermediate amylose content (20-25%) is soft and fluffy which is mainly preferred in Indian subcontinent. Amylose content was highest in NP rice, Dudhamani (27.01%) and lowest in P rice Kantakamala (19.61%).

Amylose content of studied rice germplasm lies in intermediate range with the exception of P rice Kantakamala (19.61%) and NP rice Dudhamani (27.01%). Highest protein content of 12.67% and 12.43% was observed in P rice Kaniar and Baranga, respectively and lowest value was observed in NP rice Chinamal (8.79%). TSS was found to be highest in Kusumal (3.6%) and lowest in Latamahu (1.7%). P rice was observed to have higher proximate composition except amylose content (Fig. 1b). TSS in rice grain may have an effect on the flavour and color reactions that take place during cooking or processing[34]. Similarly, high protein content may enhance the sensory properties of cooked rice which signifies that P rice may attract consumer acceptability not only due to enriched nutritional composition but also due to improved flavour accompanied by high sugar and protein content. Similar findings were also reported by other researchers[24,25].

Phytochemical and antioxidant properties

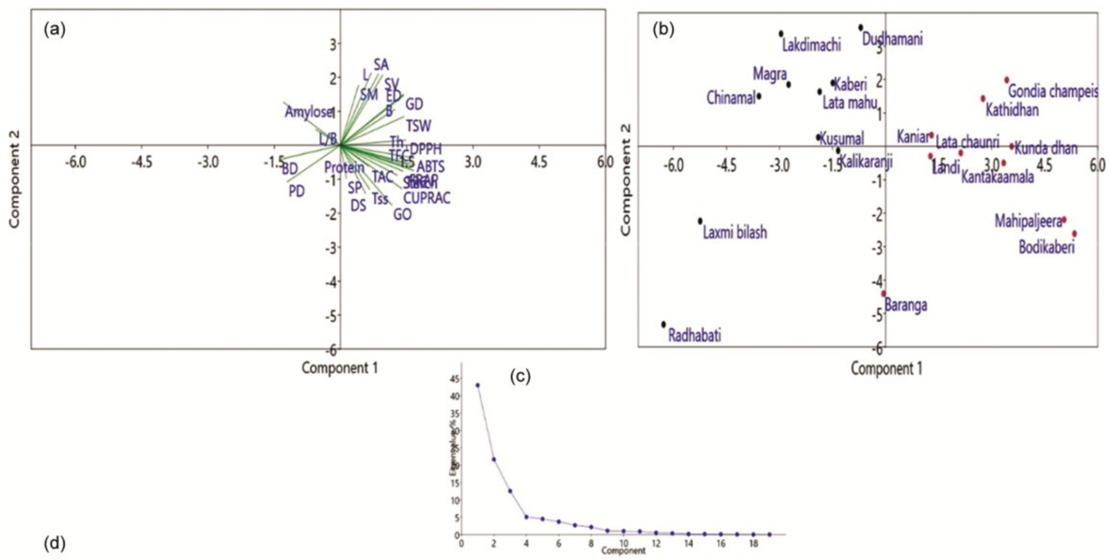
Phytochemical properties such as TAC, GO, TPC and TFC of P and NP rice genotypes are presented in (Fig. 1a and Fig. 1b). Genotypes were found to be significantly different for all the phytochemical traits. In P rice genotypes mean TAC was 0.96 mg 100 g^{-1} and in NP rice it was 0.19 mg 100 g^{-1}. Highest TAC was observed in Bodikaberi (2.85 mg 100 g^{-1}). GO ranged from 35.75 (Kathidhan) to 87.88 mg 100 g^{-1} (Bodikaberi) in P rice genotypes whereas it ranged from 10.56 (Lakdimachi) to 40.81 mg 100 g^{-1} (Laxmibilash) in NP rice genotypes. Highest TPC was observed in Bodikaberi (169.09 mg 100 g^{-1}) and lowest in Kusumal (0.91 mg 100 g^{-1}). In P rice, TPC ranged from 82.95 to 169.09 mg 100 g^{-1} and in NP rice genotypes, it ranged from 0.91 to 33.30 mg 100 g^{-1}. TFC was found to be highest in Bodikaberi (76.89 mg 100 g^{-1}) and lowest in Magura (17.11 mg 100 g^{-1}) and Laxmibilash (18.67 mg 100 g^{-1}). TFC ranged from 35.22 to 76.89 mg 100 g^{-1} in P rice and from 17.11 to 23.78 mg 100 g^{-1} in NP rice.

ABTS, CUPRAC, DPPH and FRAP antioxidant activities estimated in the P and NP rice are presented in Fig. 1a and Fig. 1b. Genotypes were found to be significantly different for all the antioxidant activities. Highest ABTS activity (42.17% inhibition) was observed in Mahipaljeera and lowest value in Radhabati (4.54% inhibition). In P rice, the value ranged from 18.45 to 42.17% inhibition, whereas in NP rice it ranged from 4.54 to 12.14% inhibition. Mean ABTS activity in P rice was 30.86% inhibition whereas it was 9.57% inhibition in NP rice. CUPRAC activity ranged from 13.29 in NP rice (Chinamal) to 202.25 µg TE g^{-1} in P rice (Bodikaberi). Mean CUPRAC activity in P rice was 96.39 µg TE g^{-1} and in NP rice it was 24.69 µg TE g^{-1}. Highest DPPH activity was observed in Latachaunri (91.53% inhibition) and lowest activity in Radhabati (4.56% inhibition). In P rice, DPPH inhibition percentage was more than 85% while in NP rice it varied from 4.56 to 36.16% inhibition. In P rice, FRAP activity ranged from 20.86 (Baranga) to 42.13 µg AAE g^{-1} (Mahipaljeera) and NP rice it ranged from 4.18 (Radhabati) to 14.10 µg AAE g^{-1} (Latamahu). Mean FRAP activity was 25.77 µg AAE g^{-1} in P rice and for NP rice it was 9.19 µg AAE g^{-1}. Both phytochemical traits and antioxidant activity were observed to be higher in P rice compared to NP rice (Fig. 1b). The higher content of antioxidant activity in P rice was due to the presence of more amount of phenolics, anthocyanin and proanthocyanidin in the aleurone layer of P rice compared to NP rice[8,21,35].

Association analysis

Knowledge of trait association is essential to change and improve the nutritional pattern of rice. The trait association relationship can be used to select rice varieties for preparation of specific type of value added products. Significant positive correlation ($r \geq 0.7$) of L with GrA, GrV; B and Th with ED, GD; ED and GD with GrA, GrV; ED with GD; GrA with GrV; TSW with Th, ED, GD; BD with PD; TAC with GO, TPC, TFC, ABTS, FRAP, CUPRAC; GO with TPC, CUPRAC; TPC with TFC, ABTS, DPPH, FRAP, CUPRAC; TFC with ABTS, DPPH, FRAP, CUPRAC, ABTS with DPPH, FRAP,CUPRAC; DPPH with FRAP; FRAP with CUPRAC was observed (Fig. 2). Significant negative correlation ($r \geq 0.7$) of L/B with DS; Th with BD; ED and GD with PD; BD, PD with TSW; Amylose with TPC, ABTS, DPPH, FRAP was also observed.

Association of physical traits with each other were also previously observed[23,31]. Interestingly physical traits such as Th, ED, GD, TSW; proximate



(d)

	MC	L	B	L/B	Th	ED	GD	SA	SV	DS	TSW	BD	PD	GP	Starch	Amylose	Protein	Tss	TAC	GO	TPC	TFC	ABTS	DPPH	FRAP	CUPRAC
PC 1	0.065	0.112	0.198	-0.090	0.238	0.228	0.231	0.138	0.154	0.091	0.232	-0.220	-0.194	0.021	0.227	-0.208	0.101	0.107	0.207	0.187	0.267	0.246	0.270	0.267	0.251	0.223
PC 2	0.284	0.342	0.170	0.075	0.024	0.241	0.237	0.337	0.334	-0.227	0.136	-0.067	-0.174	-0.157	-0.122	0.204	-0.098	-0.209	-0.142	-0.283	-0.120	-0.092	-0.093	-0.061	-0.106	-0.204

Fig. 3 — The principal component analysis (PCA) of Pigmented and Non-pigmented rice genotypes. (a) scatter plot showing the genotypic relationship, (b) scatter plot showing trait variables, (c) screeplot, (d) loadings of principal components 1 and 2

composition such as protein and TSS content were found to have positive association with phytochemical and antioxidant activity. However negative association of BD and amylose was observed with the phytochemical and antioxidant activity. The association of physical traits with antioxidant activities has been established for the first time by this study. Kaur *et al*.[22]; Devraj *et al*.[8] have also reported the association of phytochemical traits with antioxidant activity. This study is an initial attempt to distinguish pigmented and non pigmented landraces of Odisha region based on physical, phytochemical and antioxidant properties and establishing association among these traits. However, it is essential to extend the scope of the study to include a broader range of diverse genotypes in order to strengthen the reliability of the results. Findings of Bastia *et al*.[36]; Sanghamitra *et al*.[37], that recorded similar trait association of phytochemicals with antioxidant traits in an association mapping population of about 120 genotypes proves the rigidity of trait association findings of this study.

Principal component analysis and identification of suitable genotypes

All the physical, phytochemical and antioxidant activity traits studied in P and NP rice genotypes were subjected to PCA (Fig. 3) to reveal the pattern of genotypic variation on the studied traits while removing data redundancy[38]. The first two axis of principal component having eigenvalue more than one captured 65.55% of the total variation revealing that ABTS, DPPH, FRAP, TPC, TFC, L, GA and, GV are the key determinants of phenotypic variation. The cluster analysis clearly distinguished P rice and NP rice on the basis of phytochemical and antioxidant activity except amylose content, L/B ratio, BD and PD. Bodikaberi (P1) and Mahipaljeera (P2) formed a separate group within the P rice due to higher phytochemicals and antioxidant properties. Bodikaberi (Fig. 1a and Fig. 1b) was identified with long bold grain with Th of 2.05 mm, and TSW of 26.44 g. Further it was found to possess TAC of 2.85 mg 100 g^{-1}, GO of 87.88 mg 100 g^{-1}, TPC of 169.09 mg CE 100 g^{-1} , TFC of 76.89 mg CEt 100 g^{-1}, ABTS of 41.95% inhibition, CUPRAC of 202.25 µg TE g^{-1}, DPPH of 86.97% inhibition and FRAP of 39.37 µg AAE g^{-1}. Mahipaljeera was also observed with long bold grain with Th of 2.14 mm, TSW of 27.40 g with TAC of 1.04 mg 100 g^{-1}, GO of 84.50 mg 100 g^{-1}, TPC of 165.23 mg CE 100 g^{-1}, TFC of 69.33 mg CEt 100 g^{-1}, ABTS of 42.17% inhibition, CUPRAC of 187.87 µg trolox g^{-1}, DPPH of 87.95% inhibition, FRAP of 42.13 µg AAE g^{-1} making them the most promising ones amongst all studied genotype.

Conclusion

The study revealed a notable increase in both variation and heritability, as well as a significant genetic advance, for the examined grain physical and nutritional quality traits in P rice genotypes. This suggests a potential for enhancing these traits in P rice varieties. The insights gained into the physical characteristics through this research will be instrumental in devising processing and handling machinery to mitigate post-harvest losses. The higher thickness, grain weight, and porosity observed in P rice, in comparison to NP rice, underscores the need for distinct processing machinery tailored to these specific attributes. The identified associations among traits will facilitate the screening of germplasm for the purpose of breeding superior varieties with improved grain and nutritional quality. Notably, this study marks a pioneering achievement by establishing a correlation between physical traits and antioxidant compounds as well as antioxidant activity. The promising genotypes singled out for their quality traits hold substantial promise for incorporation into future plant breeding initiatives, aiming to develop advanced lines and value-added products. Given the prevalence of lifestyle-related diseases, the endorsement and conservation of these nutritionally enriched P rice varieties from the Odisha region assumes paramount importance in promoting a well-balanced diet and overall health.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

NB: Biochemical analysis, Drafting and Reviewing the Manuscript; GK: Physical trait analysis; Manuscript writing and Editing PS: Conceptualization, drafting and editing; SS: field experiment Resources; SSN: Literature survey, data analysis; and SKP: Editing the manuscript.

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