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ORIGINAL ARTICLE

Evaluation of variability and environmental stability of grain quality and agronomic parameters of pigmented rice (*O. sativa* L.)

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Abstract Eleven pigmented rice genotypes were evaluated to estimate genetic parameters, heritability and association. The results indicated that, genotypic variation was high among the lines. The distinct seasonal effect on plant performance for antioxidant capacity, anthocyanin, flavonoids, head rice recovery and test weights was also observed. Wet season favoured the crop performance in all genotypes as compared to drought conditions. The differential accumulation of different quality traits such as AOA, anthocyanin content, flavonoids content, etc showed high heritability, which would be transfer to high yeilding popular rice cultivars through conventional or geneticaly modification techniques. The line Mamihunger was chosen as donor of the high-quality rice grain and Annapurna for high yield. Further, Mamihunger are foreseen to be good in nutritional quality and industry use.

Keywords Pigmented rice · Variability · Antioxidant · Anthocyanin · Oryzanol · Nutraceutical

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Introduction

Growing of pigmented rice has a long history, and being utilised for food, medicine, cultural and religious activities from ancient India. Before the introduction of high yielding varieties. Which is a white grain used for food but not for medicinal use. Pigmented rice mostly distributed in rice-growing Asian countries such as India, Sri Lanka, Philippines, China, and Japan. In India, it is mostly distributed in East, South, and the hilly tracts of the West & Northeast. Some of these pigmented rice also reported for plains of Western Uttar Pradesh, Punjab, Gujarat etc. The acceptance of high-yielding rice varieties in the 1970s and the demand for white rice led to a drastic reduction of the area under pigmented rice in India. Presently, rice occupied an area of 44.11 mha, production of 105.48 MT and a productivity of 2.39 t ha^{-1} (2014–2015) (Agricultural statisticsat a glance 2016), which is 2.8 times production than in 1970–1971 (37.59 MT). But, now we are self-sufficient of white rice grain and moving towards quality attributes for value addition in rice. Pigmented rice has become increasingly interested for good source of bioactive compounds (Chitra et al. 2010). These bioactive compounds are higher in antioxidant, anthocyanin, phenolic acids, flavonoids, pro-anthocyanidins, tocopherols, tocotrienols, γ-oryzanol (Prabhu and Jaydeep 2015; Sanghamitra et al. 2017) and high rate of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity in comparison to white rice (Oki et al. 2005). It is not only the rice type that is richest bioactive compound but preventive or nutraceutical effects has even more impressive health benefits for reducing the chronic disease like cardiovascular disease, type-2 diabetes, obesity, cancer etc. Thus, it find favour for health conscious of consumers (Okarter and Liu 2010; Bett-Garber et al. 2013) and has been classified as a

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functional food or superfood (Abdel-Aal et al. 2006; Yawadio et al. 2007; Prabhu and Jaydeep 2015).

Botanically, pigmented rice is wild, weedy, or cultivated types, and caryopsis are red or purple or brown coloured covered with dark or light coloured husk. The pigmented cultivars are high tolerance to unfavourable environments such as low fertile soil, deep water, salinity and cold conditions but poor yielder. Thus considerable environmental impacts on grain quality and yield traits apparently expected.

Gradually, it is gaining demand and higher value per unit in market due to better nutritional composition of the grains. Thus, the improvement of pigmented rice should be accelerated to meet the higher grain quality standards for the food industry and good yield for farmers. Today, breeding in white rice has a deep research platform but breeding in pigmented rice has very few reports. Earlier report says, genotypic variability for grain quality and yield traits exists in the pigmented rice. But, understanding the genetic architecture of quality traits and searching the valuable genotypes is essential to starts the breeding programme. Grain quality and yield traits are most likely quantitative in nature genetically and expected to be influenced by genetic constitution of the plants, environment fluctuation (Singh et al. 2014), and the genotype × environment interaction (GE) (Singh et al. 2003). But, a little evaluation of pigmented rice (*O. sativa* L.) germplasm for genetic variation and GE interaction for grain quality and yield traits has been reported. Besides, the quality traits of 11 important pigmented rice lines of north eastern states of India used in present research work is not documented systematically elsewhere. Therefore, the aim of this study was (1) to characterise important pigmented rice lines with respect to variation in major grain quality, yield traits and to get information on the environmental impact on these traits, (2) to calculate broad sense heritability (BSH) and the expected genetic gain (GA) in order to get information on achievable improvement of respective traits by breeding, (3) correlation between grain quality and yield traits to know the association type and magnitude among the traits (4) to identify promising genotypes.

Materials and methods

Plant material

Eleven diverse pigmented rice (*Oryza sativa* L.) genotypes available at ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack were tested. These 11 lines are rice landraces collected from North-Eastern states of India. The grain colours vary from slightly reddish to blackish in colour. These genetic materials were grown in the wet season, 2014 and dry season, 2014–2015 at the experimental field, ICAR-NRRI (20.45°N, 85.93°E), Cuttack, Odisha, India. The experiments were laid out in a randomised complete block design with three-replications. All required agronomic practices and plant protection measures against pests and diseases to raise a successful crop were followed. Seeds were harvested at maturity and sub samples (100 g) of dry rice seeds (10–12% moisture) were collected from each of the three replicated field plots of each genotype and thoroughly homogenised to obtain one composite sample for each genotype in each season.

Observations

Observations of quality and yield traits were recorded in both the seasons. The quality traits of pigmented rice we have considered the antioxidant capacity as ascorbic acid equivalent per gram (AAE g^{-1}), anthocyanin content (mg 100 g^{-1}), flavonoids content as catechine equivalent per 100 g (mg CEt 100 g^{-1}), oryzanol (mg 100 g^{-1}), phenolics as catechol equivalent per 100 g (mg CE 100 g^{-1}), phytic acid (%), amylose content (%), head rice recovery (%) and gel consistency (mm). The agronomic or yield traits comprised of grain fertility (%), grain yield $plant^{-1}$, plant height (cm), test weight (g) and number of tillers. Weather variables for the experimental period was also recorded and presented in Table 1.

Methods for estimation of Grain quality

For the analysis of grain quality traits, grains were subsampled (100 g) from dry rice grains at standard moisture content (10–12% moisture) from each genotype and thoroughly homogenized to obtain one composite sample. Two samples from each replicate were averaged for quality trait analysis and data were calculated on a dry matter basis.

ABTS radical scavenging assay

ABTS radical assay has been widely used to evaluate antioxidant activities of different food components (Sengupta et al. 2015). For chemical estimation, grain samples were milled to flour. Total antioxidant capacity of ABTS + radical scavenging was estimated by standard method (Serpen et al. 2008) with some modifications. The ABTS + solution was prepared with ABTS (7 mM) and potassium persulfate (2.45 mM) in distilled water and was kept for constant agitation for 16h in dark at normal room temperature. This reaction mixture was further dissolved in the mixture of ethanol: water (50:50, v/v) to adjust the absorbance to 0.700 ± 0.020 at 734 nm The ABTS + reagent (6 ml) was added to 10 mg of rice flour and was

Table 1 Monthly weather variables of WS-2014 and DS-2014-15 at experimental station. *Source*: Meteorological Station of ICAR-NRRI, Cuttack, Odisha, India

Season	Months	Temperature		Rainfall (mm)	Relative humidity (%)		Wind speed (km^{-1} h)	Evaporation (mm)	Sun shine (h)
		Max. (°C)	Min. (°C)		7 a.m.	2 p.m.			
WS	July	30.07	23.94	469.7	93.61	78.86	5.39	3.3	1.93
	August	31.41	24.2	356.1	93.87	75.42	5.65	3.47	4
	September	30.56	24.16	349.3	95.27	78.87	4.04	3.68	4.13
	October	30.7	23.39	144.4	94.81	69.84	3.34	3.46	6.18
	November	29.44	18.3	0	93.23	55.33	1.8	2.47	7.55
	Average	30.436	22.798	263.9	94.158	71.664	4.044	3.276	4.758
DS	December	26.71	13.67	0	91.58	44.19	2.29	2.13	5.82
	January	26.73	14.91	13.5	95.35	44.48	3.08	1.98	7.21
	February	30.69	18.64	0	95.63	41.54	3.34	3.54	7.47
	March	35.42	21.3	0	94.58	42.23	4.21	6.32	7.55
	April	39.65	24.62	0	93.21	40.23	3.25	5.38	7.42
	Average	31.84	18.628	2.7	94.07	42.534	3.234	3.87	7.094

Max. Maximum, *Min*. minimum, *WS* wet season, *DS* dry season

vortexed for 1.5 min to perform the surface reaction and centrifuged at 9200*g* for 2 min. The absorbance was measured after 30 min at 734 nm and the antioxidant capacity was expressed as micromole of ascorbic acid equivalent (AAE) g^{-1} of rice flour.

Anthocyanin content

Total anthocyanin content was determined as per standard method (Fuleki and Francis 1968) with slight modifications. 1 g of brown rice flour was homogenised with 5 ml acidified organic solvent (95% methanol: 1.5 N HCL (85:15, v:v) and was centrifuged at 4 °C at 15,000*g* for 15 min. The residue was re-extracted twice with the acidified organic solvent to ensure the complete extraction of the total anthocyanins. All the supernatants were pooled to adjust the volume up to 10 ml with the solvent and absorbance was measured at 535 nm. The result was calculated as mg total anthocyanin 100 g^{-1} of sample using a multiplication factor of 16.73.

Flavonoid content

Total flavonoid content was assayed by colorimetric method (Eberhardt et al. 2000). The absorbance was measured at 510 nm using catechine as a standard and the result was expressed as mg CEt (catechine equivalent) 100 g^{-1} of rice flour.

γ-oryzanol content

γ-oryzanol extraction was performed as per standard procedure (Chen et al. 2005) with some simplification. 0.5 g of samples (brown rice flour) were mixed with 5 ml of HPLC-grade isopropanol, vortexed for 2 min at 25 °C, centrifuged at 4500*g* for 10 min and the supernatant was collected. After 2–3 times repetition, supernatant fractions were evaporated under hot water bath and then extracts were dissolved in 5 ml of HPLC-grade isopropanol. It was followed by filtration through a 0.45 µm membrane. 20 µL aliquots were injected into the column (C18-Phenomenex Column) and was separated by an analytical Shimadzu High Performance Liquid Chromatography (RP-HPLC) system equipped with an LC-20AT pump and PDA detector (Shimadzu, Kyoto, Japan). The composition of the mobile phase was 35% acetonitrile, 55% methanol and 10% isopropanol and operated in low pressure gradient mode.

Phenolic content

Folin-Cio-calteau a standard procedure for total phenolic estimation was used (Zilic et al. 2011) with slight modifications. The brown rice flour sample (0.3 g) was homogenized in 70% acetone at room temperature to ensure maximum recovery of all the phenolic compounds followed by centrifugation at 4 °C at 10,000*g* for 20 min. The extract (200 µL) was diluted with 0.5 ml with distilled water and 0.25 ml of Folin-Ciocalteu reagent. To the above reaction mixture, 1.25 ml of 20% sodium carbonate was added and mixed thoroughly. The absorbance was measured after 40 min at 725 nm using catechol (CE) as a standard and the result was expressed as mg catechol 100 g^{-1}.

Phytic acid content

Phytic acid content was estimated using standard procedure (Gao et al. 2007) with little modification. Brown rice flour sample (1 g) was extracted in 10 ml of 2.4% HCL, the solution allowed to shaking at 220 rpm for 16 h in an incubator shaker at 50 °C. Then centrifuged at 10,062*g* in a table-top centrifuge (Remi, India) at 25 °C for 20 min. To the supernatant NaCl (1 g) was added and allowed to shake constantly for 20 min at 350 rpm followed by storage at − 20 °C for 20 min. Then, it was again centrifuged at 3000*g* for 20 min. Finally, the supernatant was collected and was diluted (25 times). 3 ml diluted sample was added with 1 ml Wade reagent (0.03% $FeCl_3 \cdot 6H_20$ + 0.3% Sulpho-salicylic acid) and mixed thoroughly by vortexing for 30 min. The absorbance of supernatant was measured at 500 nm using sodium phytate as standard so that absorbance of the blank should be 0.453 ± 0.002. The phytic acid (%) was calculated as: PA% = {(0.453 − Abs.) × 25 V}/(22.05 × M). Where Abs. is absorbance; V = final volume (ml); M = weight of sample (g).

Physico-chemical and cooking properties of rice

Head rice yield

Milling of rice is usually measured quantitatively by head rice yield (Saleh and Meullenet 2015). 100 g of rice seeds were de-hulled and milled using a standard de-husker and miller, and the head rice recovery (HRR in %) was calculated as percentage of milled rice.

Gel consistency

Gel consistency (GC) was estimated following universal procedure (Cagampang et al. 1973) and was measured as length of the gel (mm) spreading of the tubes, laid horizontally on the ml graph for 1 h.

Amylose content

Amylose content (AC) was determined using Iodine Colorimetric Method (Juliano 1985) by measuring the absorbance at 620 nm using potato amylose as standard.

Statistical analysis

Analysis of variance for individual character (ANOVA) including the estimation of mean, range, and coefficient of variation (CV%) was estimated using a statistical R software (Version 3.4.0) package. The test of significance was performed using Fisher's (*F*) test. The average mean of the genotype (G) was considered as fixed effect whereas, seasons (S), and the interaction of GS considered to be random. To find out the relationship among the various grain quality and yield traits, Pearson's correlation coefficients was analysed based on the mean values of the 11 genotypes. Genetic parameters were also estimated to understand genetic variations among the test genotypes and to determine genetic and environmental effects on different characters. These parameters include the genotypic and phenotypic variance, environmental variance and their coefficient, broad sense heritability (BSH) and genetic advance (GA) which were calculated by already published procedure (Singh and Chaudhary 1977; Allard 1960). The formulas used for the phenotypic (PCV), genotypic (GCV), and environmental (ECV) coefficient of variation are $PCV = \frac{\sqrt{\sigma^2 p}}{\mu} \times 100$, $GCV = \frac{\sqrt{\sigma^2 g}}{\mu} \times 100$, $ECV = \frac{\sqrt{\sigma^2 e}}{\mu} \times 100$ where, $\sigma^2 p$ = phenotypic variance, $\sigma^2 g$ = genotypic variance, $\sigma^2 e$ = environmental variance, μ = population mean of the trait. Thereafter, the common formula for estimating BSH (Johnson et al. 1955) was used $h^2 = \sigma^2 g/\sigma^2 p \times 100$. Where, $\sigma^2 g$ is the genotypic variance (variance component for genotype), and $\sigma^2 p$ is the phenotypic variance.

Genetic advance (GA) generally refers to the possible improvement in the genotypic value of selected individuals over the parental population. It was influenced by genetic variability, heritability and selection intensity. It was calculated standard procedure (Lush 1949). GA = $k\sigma p h^2$, where, h^2 = heritability in broad sense, σp = phenotypic standard deviation, k is a constant called selection differential. For the purpose of the present study, 'k' has the value, 2.06 which is the expectation in case of 5% selection in a normally distributed population. DMRT test was done to differentiate the mean performance between the two seasons for all the traits.

Results

Genotypic mean and variation

The average mean, range and coefficients of variation (CV) of grain quality and agronomic traits of pigmented rice lines tested are shown in Table 2. The average grain yield $plant^{-1}$

Table 2 Estimation Genetic variables for grain quality and grain yield traits of 11 pigmented rice genotypes

Parameters	Antioxidant capacity	Anthocyanin	Flavonoids	Oryzanol	Phenolics	Phytic acid	Amylose content
Range	1191.12–3210.98	0.72–94.23	73.88–307.96	34.09–83.94	230.85–661.19	0.18–0.29	9.98–25.00
Pooled mean	2955.54	10.44	169.9	42.89	382.5	0.28	19.63
CV% (at 5%)	9.01	39.45	3.32	1.10	2.36	0.97	3.71
ECV	23.06	285.86	47.53	19.66	27.44	19.98	19.37
GCV	24.28	239.14	41.66	31.12	30.11	4.56	24.22
PCV	26.05	266.1	45.96	32.14	32.12	9.34	25.05
h^2	0.87	0.81	0.82	0.94	0.88	0.24	0.93
GA as % of (1%)	59.77	567.39	99.7	79.56	74.5	5.87	55.23

Parameters	Head rice recovery	Gel consistency	Grain fertility	Grain yield plant^{-1}	Plant height	Test weight	Number of tillers
Range	17.50–60.50	32–70	61.00–87.46	5.46–25.45	94.48–178.86	20.05–30.36	6–22.66
Pooled mean	44.80	50.77	75.46	16.14	107.63	25.16	15.53
CV% (at 5%)	5.28	9.71	9.37	15.36	14.31	6.93	11.19
ECV	25.52	16.98	12.16	37.83	12.12	12.33	26.52
GCV	34.75	21.99	10.43	33.12	21.58	16.53	36.49
PCV	38.58	23.87	11.55	36.55	22.14	17.28	38.06
h^2	0.62	0.79	0.82	0.82	0.95	0.92	0.92
GA as % of (1%)	54.38	71.47	24.86	79.25	55.52	41.74	92.35

CV coefficient of variation; *ECV* environmental coefficient of variation; *GCV* genotypic coefficient of variation; *PCV* phenotype coefficient of variation, *h2* heritability; *GA* genetic advance

of 11 genotypes was 16.13 g. This yield was contributed by yield attributing traits for which various agronomic traits such as average grain fertility (75.46%), average plant height (107.63 cm), average test weight (26.16 g) and average number of tillers (15.53) were measured. Further, quality traits of 11 genotypes were also measured viz; average antioxidant capacity was 2955.54 AAEg^{-1} which was very high, anthocyanin content was 10.44 mg 100 g^{-1}, flavonoids was 169.90 mg CEt 100 g^{-1}, oryzanol was l42.89 mg 100 g^{-1}, phenolic compound was 382.50 mg CE 100 g^{-1}, phytic acid was 0.28%, amylose was 19.63%, head rice recovery was 44.80%, and gel consistency was 50.77 mm. The mean performance over seasons ranged from 1191.12 to 3210.98 AAEg^{-1} for antioxidant, 0.72 to 94.24 mg 100 g^{-1} for anthocyanin, 73.88 to 307.96 mg CEt 100 g^{-1} for flavonoids, 34.09 to 83.94 mg 100 g^{-1} for oryzanol, 230.85 to 661.19 mg CE 100 g^{-1} for phenolics, 0.18–0.29% for phytic acid, 9.98 to 25.00% for amylose, 17.50–60.50% for head rice recovery, 32 to 70 mm for gel consistency, 62.47 to 83.87% for grain fertility, 5.46 to 25.45 g for grain yield plant^{-1}, 84.55 to 163.50 cm for plant height, 20.05 to 30.36 g for test weight and 6 to 22.66 for number of tiller (Table 2). This showed that genotypes were diverse for yield and quality traits. The F-test of ANOVA for genotypes of individual season revealed significant differences between genotypes for all agronomic and quality traits (Table of individual season not presented). Variability in grain yield plant^{-1} was high ranging from 5.46 to 25.45 g plant^{-1} and anthocyanin content ranged between 0.72 and 94.23 mg 100 g^{-1}. This becomes obvious in a high CV (%) for the grain yield plant^{-1} (15.36%), anthocyanin content (39.45%), which was the highest for all examined traits.

Relative comparison of mean of genotypes

The quality traits of pigmented rice are presented in Tables 3, 4. The antioxidant capacity and flavonoids were the highest for Jool (3158.93 AAE g^{-1}) and Mamihunger (3152.29 AAE g^{-1}). Similarly, Mamihunger possessed the highest anthocyanin content (93.67 mg 100 g^{-1}), oryzanol (73.47 mg 100 g^{-1}), phenolics compound (704.63 mg 100 g^{-1}), and the lowest phytic acid (0.18%). Hence, the genotype Mamihunger was good in above quality traits.

Generally, amylose content decides the stickiness and softness of cooked rice and was taken as important traits for eating and cooking quality. Highly significant difference for amylose content for both the seasons especially for Nalbora genotype was observed. Accordingly, three categories were recognised, genotypes Assambiroin, Balam, Mamihunger, Nalbora had low amylose content (< 12–20%), Annapurna, Jool, Lalbora, Mornodoiga, Sathi and Setka-36 had intermediate amylose content (20–25%), and, PB-140 had high amylose content (> 25%). However, amylose content alone did not explain all of the variations

Table 3 Genotypic means and interaction effect of seed quality traits, based on 11 genotypes of rice

Variety	Antioxidant capacity (AAE g^{-1})			Anthocyanin (mg 100 g^{-1})			Flavonoids (mg CEt 100 g^{-1})			Oryzanol (mg 100 g^{-1})			Phenolics (mg CE 100 g^{-1})		
	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled
Annapurna	1278.22a	3179.18a	2228.70a	0.66a	5.09b	2.88b	105.67ab	184.20a	144.94	25.13c	36.88b	31.01b	438.64ab	477.61ab	458.13ab
Assambiroin	3029.64a	3105.90a	3067.77a	0.79a	3.34b	2.07b	125.44ab	226.00a	175.72	43.19abc	53.25ab	48.22ab	348.58ab	191.25b	269.92b
Balam	2765.15a	3110.20a	2937.68a	1.01a	2.99b	2.00b	92.33ab	284.80a	188.57	40.75bc	80.38ab	60.57ab	324.72ab	270.46b	297.59b
Jool	3106.12a	3211.73a	3158.93a	1.96a	2.46b	2.21b	55.43b	190.40a	122.92	72.31a	42.31ab	57.31b	414.21ab	774.21ab	594.21ab
Lalbora	1192.28a	3210.25a	2201.27a	0.85a	2.07b	1.46b	95.89ab	153.20a	124.55	47.56abc	26.50b	37.03b	440.06ab	548.18ab	494.12ab
Mamihunger	3121.35a	3183.23a	3152.29a	3.62a	183.71a	93.67a	169.33a	348.13a	258.73	53.50abc	93.44a	73.47a	453.98ab	955.28a	704.63a
Mornodoiga	1149.57a	3042.89ab	2096.23a	0.97a	4.76b	2.87b	97.11ab	267.80a	182.46	35.25bc	74.44ab	54.85ab	386.93ab	355.46b	371.20ab
Nalbora	1232.69a	3208.77a	2220.73a	1.31a	1.29b	1.30b	102.44ab	186.40a	144.42	47.19abc	32.63b	39.91b	364.49ab	611.59ab	488.04ab
PB140	2698.94a	3210.25a	2954.60a	1.06a	2.12b	1.59b	77.46b	179.80a	128.63	62.06ab	35.56b	48.81ab	284.38b	533.86ab	409.12ab
Sathi	1264.34a	3104.47a	2184.41a	3.01a	2.69b	2.85b	113.33ab	176.20a	144.77	61.94ab	32.75b	47.35ab	266.76b	320.68b	293.72b
Setka36	1110.24a	2818.07a	1964.16a	1.10a	2.70b	1.90b	46.67b	167.80a	107.24	46.81abc	53.50ab	50.16ab	478.69a	432.73ab	455.71ab
Mean	1995.32	3125.9	2560.61	1.49	19.38	10.43	98.28	214.98	156.63	48.7	51.06	49.88	381.95	497.39	439.67
S	20,981,288***			5302.88***			223,890.9			91.71***			231,709***		
G	1,394,930***			4572.28***			11,162.40***			829.04***			105,462.90***		
GS	1,167,426***			4342.06***			3340.68			1205.04***			59,203.66***		

a, b, c represent for means comparison. Means represented by two or more letters in common indicate that the difference is not significant or weakly significant

*** represent significance at $p \leq 0.001$

AAE g^{-1} ascorbic acid equivalent per gram; *mg 100* g^{-1} milligram per 100 g; *mgCEt100* g^{-1} catechine equivalent per 100 g; *mg CE 100* g^{-1} catechol equivalent per 100 g; *WS* wet season, *DS* dry season

Table 4 Genotypic means and interaction effect of grain quality traits, based on 11 genotypes of rice

Variety	Phytic acid (%)			Amylose content (%)			Head rice recovery (%)			Gel consistency (mm)		
	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled
Annapurna	0.22abc	0.21ab	0.22ab	21.22ab	19.72b	20.47bc	14.00e	21.00e	17.50e	31.50d	47.50b	39.50d
Assambiroin	0.19bc	0.21ab	0.20ab	11.47c	13.42d	12.45cd	55.00ab	58.00b	56.50ab	47.00c	71.00a	59.00bc
Balam	0.23abc	0.23ab	0.23ab	16.57b	18.72bc	17.65	57.00a	63.00a	60.00a	71.00ab	45.00b	58.00bc
Jool	0.26ab	0.28a	0.27ab	22.01a	25.64a	23.83a	61.00a	60.00a	60.50a	65.00ab	32.00c	48.50cd
Lalbora	0.26ab	0.25ab	0.26ab	22.46a	22.57a	22.52b	48.00ab	52.00bc	50.00bc	49.50c	34.00c	41.75cd
Mamihunger	0.23abc	0.13b	0.18b	16.76b	15.60c	16.18c	43.50bc	48.50c	46.00bc	69.50ab	44.00b	56.75bc
Mornodoiga	0.24abc	0.33a	0.29a	22.65a	23.21a	22.93ab	24.80	35.50d	30.15d	75.00a	65.00a	70.00a
Nalbora	0.18c	0.27a	0.23ab	7.24d	12.71d	9.98d	47.00ab	51.00bc	49.00bc	62.00ab	44.50b	53.25bc
PB140	0.25abc	0.19ab	0.22ab	24.80a	25.20a	25.00a	30.00d	45.00cd	37.50cd	30.00d	34.00c	32.00d
Sathi	0.25abc	0.26ab	0.26ab	23.17a	22.16ab	22.67ab	36.00bcd	52.00bc	44.00bc	62.00b	28.50c	45.25cd
Setka36	0.28a	0.30a	0.29a	21.11ab	23.55ab	22.33ab	33.40cd	50.00bc	41.70cd	63.50ab	45.50b	54.50bc
Mean	0.23	0.24	0.24	19.04	20.23	19.63	40.88c	48.73bc	44.80c	56.91	44.64b	50.77c
S	0			17.54**			16.74***			31.40***		
G	0.008***			49.71***			18.84***			94.19***		
GS	0.005***			3.97**			2.09***			6.28***		

a, b, c, d represent for means comparison. Means represented by two or more letters in common indicate that the difference is not significant or weakly significant

*** represent significance at $p \leq 0.001$

WS wet season, *DS* dry season

for eating and cooking quality, as genotypes with similar amylose content possessed different eating and cooking quality. The other quality traits like head rice recovery was observed to be higher for Jool (60.50%), Balam (60.00%) and Assambiroin (56.50%). Similarly, the highest gel consistency was gained by Assambiroin (59.00 mm) and Mornodoiga (70.00 mm).

The mean of agronomic traits of 11 pigmented rice genotypes of two season's evaluation is presented in Table 5. Maximum and significant grain fertility percentage was recorded for genotype Annapurna (83.87%) but, the magnitude was higher for PB140 (83.86%) and Assambiroin (80.04%). This difference in grain fertility was large in both the season except for Lalbora where, the magnitude difference between both the seasons was very less, which showed stable performance of this trait over the season. High and significant grain yield plant^{-1} was obtained in observed Annapurna (28.99 g) and Nalbora (23.84 g) but, test weight difference among them (Annapurna, 20.01 g and Nalbora, 25.59 g) was significantly. Similarly, minimum grain yield plant^{-1} was obtained for Sathi (4.25 g) and Setka36 (8.54 g). Taller plants in wet season than in dry season due to presence of optimum uniform temperature (around 30 °C) from July to October where as in the dry season gradual increased in temperature from December to March reduced the growth and stem elongation of the plant (Table 1). The tallest plant over the season was observed for Assambiroin (163.50 cm) and Sathi (84.55 cm) was the shortest. Significant higher tillers was developed by Setka36 (20.23) and Sathi (17.77) whereas, low tiller in Mamihunger (4.10). It was also seen that tiller number were high in dry than wet season. Thus, Annapurna considered to be good for agronomic traits, since it possessed high grain fertility, moderate tillering, and highest grain yield plant^{-1}.

Comparison of genetic component of variance

Comparison of variance components of the E, G, and GS for each trait shows their contribution to the total variance. Variance components for S were the largest for plant height, test weight, antioxidant, anthocyanin, flavonoids and phenolics. Which indicate influenced of season on grain quality and yield. For grain fertility, grain yield plant^{-1}, plant height, test weight, antioxidant capacity, anthocyanin, oryzanol, phenolic and phytic acid, GS was significant. Suggesting the contribution of GS variance on phenotypic expression for these traits. Zero variance components for phytic acid suggested that under the experimental conditions, season made little influence on this traits. Further, for grain yield plant^{-1}, plant height, test weight, number of tillers, antioxidant, anthocyanin, flavonoids, phenolics and phytic acid content, the variance component of the G was higher in comparison to G × S, suggesting a comparable high genetic variation for these traits.

Table 5 Genotypic means and interaction effect of agronomy traits, based on 11 genotypes of rice

Variety	Grain fertility (%)			Grain yield per plant (g)			Plant height (cm)			Test weight (g)			Number of tillers		
	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled	DS	WS	Pooled
Annapurna	97.27ab	70.47ab	83.87a	29.10a	28.87a	28.99a	83.67b	88.93b	86.30b	19.79b	20.22a	20.01b	15.27bc	16.40a	15.84ab
Assambiroin	77.67a	82.40a	80.04ab	17.43ab	22.97ab	20.20abc	140.80a	186.20a	163.50a	20.31b	30.56a	25.44ab	12.60bc	8.73ab	10.67ab
Balam	51.53a	77.58a	64.56ab	18.30a	27.93a	23.12abc	119.07ab	171.53ab	145.30ab	21.12b	21.11a	21.12b	10.47bc	6.47ab	8.47ab
Jool	83.30a	72.97a	78.14ab	12.30abc	18.83abc	15.57abc	122.87ab	159.47ab	141.17ab	20.29b	30.41a	25.35ab	11.20bc	10.53ab	10.87ab
Lalbora	70.17ab	70.47ab	70.32ab	14.40bc	8.57bc	11.49abc	115.13ab	180.67a	147.90ab	29.64a	30.19a	29.92a	12.60bc	11.27ab	11.94ab
Mamihunger	80.49a	74.51a	77.50ab	11.87abc	15.04abc	13.46abc	120.07ab	101.67ab	110.87ab	19.74b	29.91a	24.83ab	5.20c	3.00b	4.10b
Mornodoiga	77.43b	47.51b	62.47b	16.33abc	12.13abc	14.23abc	117.67ab	126.60ab	122.14ab	21.07b	30.11a	25.59ab	10.47bc	9.00ab	9.74ab
Nalbora	68.80a	87.10a	77.95ab	19.87a	27.80a	23.84ab	128.87a	161.47ab	145.17ab	20.96b	30.21a	25.59ab	11.73bc	15.93a	13.83ab
PB140	91.62a	76.09a	83.86a	15.40abc	12.40abc	13.90abc	108.93ab	138.27ab	123.60ab	20.74b	30.53a	25.64ab	15.93bc	9.60ab	12.77ab
Sathi	75.32a	82.25a	78.79ab	3.93c	4.57c	4.25c	80.03b	89.07b	84.55b	20.77b	19.95a	20.36b	19.33ab	16.20a	17.77a
Setka36	58.84a	86.43a	72.64ab	10.37bc	6.70bc	8.54bc	108.27ab	123.20ab	115.74ab	20.81b	30.32a	25.57ab	28.93a	11.53ab	20.23a
Mean	75.67	75.25	75.46	15.39	16.89	16.14	113.22	138.82	126.02	21.38	27.59	24.49	13.98	10.79	12.38
S	2.43			34.90			11,001.13***			645.40***			137.31		
G	307.96***			316.62***			3882.24***			50.54***			137.92		
GS	579.54***			44.14***			879.457***			36.79***			32.30		

a, b, c represent for means comparison. Means represented by two or more letters in common indicate that the difference is not significant or weakly significant

*** represent significance at $p \leq 0.001$

WS wet season, *DS* dry season

Genetic variables assessment

Genotypic and phenotypic coefficient of variation (GCV and PCV), BSH and GA were estimated and are given in Table 2. The high PCV was observed for anthocyanin content (266.10%), grain yield plant^{-1} (36.55%), no. of tillers (38.06%), flavonoids (45.96%), oryzanol (32.14%) and phenolics (32.12%). Relatively moderate PCV were recorded for grain fertility (11.55%), plant height (22.14%), test weight (17.28%) and antioxidant capacity (26.05%). Low PCV was observed for phytic acid (9.34%). GCV was near to PCV for plant height, number of tillers, test weight and oryzanol indicating a higher contribution of genetic constitution of plants to phenotypic expression and very little effect of GS interaction for these traits. This is further evidenced by the high values of BSH for these characters, which was higher than $h^2 = 90\%$ (0.90). Lowest values of BSH were recorded for phytic acid ($h^2 = 24\%$). Thus, efficiency of selection can be improved by judging the variability of the trait and high heritability. Both parameters were combined in the term of GA. GA was relatively high for flavonoids (99.7%) and number of tillers (92.35%) indicating a good chance for genetic improvement by breeding. For anthocyanin content it was exceptionally very high (567.39%), due to the combination of high heritability (81%) with a very large PCV (266.1%).

Association analysis

Pearson's correlation coefficients between agronomic and grain quality were calculated are presented in Table 6. Only 14 of the 120 correlation coefficients values were significantly different from zero. Among grain quality parameters, antioxidant was significantly and negatively correlated with phytic acid content at $p \leq 0.05$. Similarly, anthocyanin content was positive associated with antioxidant (higher magnitude $r = 0.40$), flavonoids, oryzanol and phenolics content. Phytic acid content was significant positive associated with amylose content. Yield was not significantly associated with any parameters chosen for this experiment, but, had higher degree positive association with plant height and test weight. The significant associated traits between the agronomic and quality traits were negative association of grain fertility and head rice recovery, positive association of plant height with head rice recovery; negative association of number of tillers with antioxidant, anthocyanin, flavonoids and oryzanol content.

Identification of promising genotypes

Superior lines of pigmented rice for each traits have been identified. In the present study we have considered the good pigmented rice for quality traits should possesses high level of antioxidant, anthocyanin, flavonoids, oryzanol, phenolics, head rice recovery, gel consistency, medium level of amylose, and low level of phytic acid. Jool and Mamihunger possessed high level antioxidant and phenolics. Mamihunger was selected as donor for quality traits since it showed significant performance for maximum number quality traits (highest anthocyanin, oryzanol, phenolics, and lowest phytic acid). On the basis of yield parameters Annapurna was selected as good donor for yield improvement in pigmented rice since it possess high grain fertility, grain yield and average tiller numbers with considerable test weight.

Discussion

The variation in pigmented rice with respect to range, mean for quality and yield traits was higher in different genotypes. This variation was an ideal to initiate the breeding programme. Such variation in pigmented rice were also observed by other researcher (Sanghamitra et al. 2017). Similarly, variation in amylose content was reported (Singh et al. 2003; Bao et al. 2004). In general, antioxidant capacity, anthocyanin content, flavonoids and head rice recovery were high in wet than dry season. Whereas, oryzanol, phenolics, phytic acid, amylose content, and gel consistency were varied with genotype to genotypes between seasons due to difference in genotypic buffering capacity and seasonal changes. Variation in relation to season, environmental parameters like temperature and genotypes for quality traits was also observed earlier (Singh et al. 2003, 2014; Kaur et al. 2016a, Pal et al. 2016.

Genotypic performance with S can be predicted using linear relationship for traits with significant G × S such as, grain fertility, grain yield plant^{-1}, plant height, test weight, antioxidant capacity, anthocyanin, oryzanol, phenolic and phytic acid. But, this perdition will be more strengthened if genetic contribution will be more for expression of traits. This contribution of genetic and non-genetic factors was calculated by the ratio of the genotypic variance component to the sum of the G and variance components of GS. We found genetic contribution was high for all traits except grain fertility and oryzanol content. Which suggested that these traits were under relatively strong genetic control and that the ranking of genotypes across environments was relatively constant for these traits. These findings closely correspond to previous evaluation results about G × S interaction of *O. sativa* grain quality traits (Fasahat et al. 2014) and yield traits (Vanisri et al. 2016). Similarly the grain fertility and oryzanol content had non-genetic control or highly influenced by environment in most of the genotypes. It was also reported that higher temperatures during

Table 6 Pearson's correlation coefficients among seed quality and agronomic traits

Parameters	GF	GYP	PH	TW	NT	ANT	ANH	FL	OR	PHE	PHY	AC	HRR	GC
GF	1.00													
GYP	0.14	1.00												
PH	− 0.28	0.24	1.00											
TW	− 0.19	0.28	0.61*	1.00										
NT	0.26	0.22	− 0.39	− 0.19	1.00									
ANT	0.26	0.19	0.39	0.00	− 0.69*	1.00								
ANH	0.09	− 0.12	− 0.21	0.03	− 0.61*	0.40	1.00							
FL	− 0.19	0.15	− 0.02	− 0.18	− 0.80**	0.45	0.80**	1.00						
OR	− 0.30	− 0.28	0.09	− 0.01	− 0.65*	0.60	0.67*	0.68	1.00					
PHE	0.19	− 0.03	− 0.09	0.34	− 0.33	0.21	0.66*	0.20	0.30	1.00				
PHY	− 0.51	− 0.47	− 0.06	0.19	0.50	− 0.58*	− 0.55	− 0.61	− 0.14	− 0.16	1.00			
AC	− 0.10	− 0.53	− 0.42	0.03	0.27	− 0.17	− 0.23	− 0.43	− 0.02	0.02	0.59*	1.00		
HRR	− 0.19	− 0.13	0.72*	0.28	− 0.34	0.54	0.02	0.07	0.41	− 0.01	− 0.04	− 0.31	1.00	
GC	− 0.67*	0.04	0.29	0.05	− 0.37	− 0.02	0.19	0.52	0.50	− 0.12	0.17	− 0.39	0.21	1.00

GF grain fertility; *GYP* grain yield par plant; *PH* plant height; *TW* test weight; *NT* number of tillers; *ANT* antioxidant capacity; *ANH* anthocyanin content; *FL* flavonoid content; *OR* Oryzanol; *PHE* phenol content; *PHY* phytic acid; *AC* amylose content; *HRR* head rice recovery; *GC* gel consistency

* significance at $p \leq 0.05$ level

grain-filling stage of plant development resulted in chalky kernels (Kaur et al. 2016a, b).

The genotypes expressing larger proportion of genetic variability (genotypic variance) for particular character or group of characters may be more amenable to selection. But, presence of genetic variability does not imply which traits to be selected. So heritability was estimated to separate the proportion of heritable variation from total phenotypic variation which is transmissible to progeny. Heritability (h^2) was higher for all traits (except phytic acid) may be due to additive nature of genes, easily transmissible to progeny and pre-requisite for breeders. Thus, improvement in grain quality and agronomic traits in pigmented rice absolutely possible. Similar results were reported by Rafii et al. (2014). Low h^2 traits like phytic acid, were not easily inherited character in the pigmented rice.

In the present study, high genetic gain values for most of the traits indicated that improvement could be made in the aforesaid characters. The high GA for traits was because of extreme variation in the material investigated, and smaller values for GA (grain fertility and phytic acid) was expected in further selection cycles in a more improved material. Selection on the basis of phenotypic performance of highly heritable traits or low heritable traits or traits with high genetic advance not promise to improve the trait performance after simple selection. Hence, heritability together with genetic advance was used to predict the probable response after selection and to quantify genetic gain possible after selection for the traits. The highest values for both variables (h^2 and GA) were obtained for antioxidant capacity, anthocyanin content, flavonoids, oryzanol, phytic acid, test weight, grain yield plant^{-1}, no. of tillers, which can facilitate the improvement of traits to many folds. Verma et al. (2014) also observed the high genetic gain for quality traits and grain yield like volume expansion ratio and gel consistency.

In order to change the pattern of grain quality composition and to improve grain yield, knowledge of correlations among the characters was useful. In this study, test weight and plant height were positively correlated (acceptable magnitude of ‘r’) with grain yield plant^{-1} and head rice recovery. Tiller number was also an important parameter for increasing the grain yield but, higher tillers were negatively correlated with quality traits like antioxidant, anthocyanin, flavonoids and oryzanol. Further, lower tillering habit genotypes may not preferential for higher grain yield. However, overall quality traits had significant positive correlation between each other like, anthocyanin content with flavonoids, oryzanol and phenol. Similarly, amylose content was correlated with phytic acid. The positive correlations of anthocyanin with antioxidant was also reported by Sanghamitra et al. (2017). The genotype Mamihunger possessed high-quality grain and suitable donor for transfer of most of the quality traits. But, grain yield per plant and tiller numbers of Mamihunger comparatively lower than others. Similarly, for higher grain yield attributing traits Annapurna is the better. Therefore, yield improvement can be made by hybridization among them and selection for high antioxidant capacity, anthocyanin content, high grain fertility, test weight of grain and the moderate number of tiller in plants as a useful trait for selection in young generations of pigmented rice.

Conclusion

This study has shown that potential of improving pigmented rice for quality enhancement in rice grain for the food industry, and the above-selected gene pool fulfil the donor of the high-quality rice grain (Mamihunger) and yield traits (Annapurna) for the breeding programme. However, seasonal variation was seen in quality traits so that genetic base should be broadened by utilizing these lines for breeding progress in the future. Genetic gain in the genotypes now possible and enhance by selection and hybridization of identified traits and genotypes in this research i.e. high antioxidant, anthocyanin, test weight and moderate tillering for cultivar development. Selection for anthocyanin content helps in indirect selection for antioxidant, flavonoids, oryzanol and phenolics.

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