Screening out the phytochemicals and colour values in white and black rice lines and its interrelationship

Abstract:

In the study, twenty three breeding rice lines from the cross between black kavuni and Co 50 were investigated for colour values and phytochemical *viz.*, total phenols content (TPC), total flavonoids content (TFC) and total anthocyanin content (TAC). Twenty five rice samples were grouped as black and white rice based on colour of aleuronic layer by visual inspection. The black phenotype rice had low value in *L** (lightness), *b*(*yellowness), *C* (chroma), *H*° (hue) and high values in *a** (redness) than the white color rice. In the black rice group, 145-6 breeding line had darker black aleuronic layer than other black rice. The TPC, TFC and TAC ranged between 0.74 -3.24 mg Gallic acid equivalent/gram of rice flour (mg GAE/g), 12.79 – 82.58 mg Catechin equivalent/100 gram of rice flour (mg CATE/100g) and 0.4-223.76 mg Cyanidin 3-glucoside equivalent/100 gram of rice flour (c3-glucoside/100g), respectively. High level of TPC, TFC and TAC were recorded in black phenotypic rice, where as lower levels in white rice. The maximum amount of TPC and TAC were observed in 145-6 rice line, which on par with parent black kavuni rice. The maximum level TFC was observed in 144-2 rice line than the parent rice kavuni. The TPC, TFC and TAC were negatively correlated with *L**, *b**, *C* and *H*° values and positively correlated with *a** values at 0.01 probability level. Among the 23 rice lines, based on TPC, TAC and TFC the superior breeding rice lines were 145-6 and 144-2 respectively. The results indicate that it is possible to develop advanced breeding lines for improvement of quality of black rice in terms of therapeutic properties.

Key words: Black rice, white rice, phytochemical and breeding lines

Introduction

Rice (Oryza sativa L.) is a seed of *Poaccae* grass family [1] and most economical staple food crops, that helps in poverty alleviation in the developing countries [2]. White rice is a widely consumed in the form of milled and parboiled [3]. It does not contain β- carotene, lutein and zeaxanthin. There are many special cultivars of rice that contain different color pigments, such as red rice and black rice [4]. Pigmented rice has phytochemical compounds higher than the white rice [5].

Recent years pigmented rice varieties had received increased attention among the consumers, due to the presence of health-promoting compounds and therapeutic properties such as anti-oxidative, anti-inflammatory and anti-carcinogenic effect [6]. Historically many indigenous pigmented rice varieties were used as improves vocal clarity, eyesight, fertility and mitigating rashes, etc., [7]. In addition to this traditional rice varieties represents as a gene pool for valuable traits and also considered a warehouse of novel genes for controlling stress tolerance, valuable nutritive and therapeutic traits [8].

Kavuni is a blackish purple pigmented and traditional rice genotype of Tamil Nadu. Match up to other rice, it has mild earthy flavor and slight bitter taste cause [9]. It is rich in iron, zinc, calcium, copper, sodium, potassium, magnesium [10]. Also found to possess several functional advantages like low total soluble sugars and fat with high level of dietary fiber, β-carotene, lutein, polyphenols, high anti-oxidant activity and inhibitory activity against enzymes *viz.*α-amylase and α-glucosidase [11].

These phytochemical and micronutrient components played a major role for reducing the incidence of non-communicable diseases *viz.,* cardiovascular diseases, diabetes, cancer and stroke [12]. In-spite of the undesirable agronomical features on photosensitivity, long duration, poor tillering and low yield, it was not cultivated widely. The farmer's need of high yield variety lead to disappearance of valuable traditional rice varieties. So there is an urgent need to conserve these varieties by a way of bio-fortification to the traditional varieties with highest yield varieties gene.

Therefore, the high yield new rice variety with good nutritional quality are expected to be breed through the hybrid between the traditional pigmented rice variety (kavuni) and high yield rice variety (CO 50). The Co 50 was chosen as a female because of high yield, tolerant to BLB (bacterial leaf blight) and photo insensitive characters. Recently, 23 breeding lines have been developed through hybrid technique between the kavuni and C0-50. The objective of this study was to investigate and compare the total phenols, total flavonoids and total anthocyanin and color values of 23 samples and their correlation with colour of aleuronic layer of rice.

Materials and methods

2.1. Samples

The 23 white and black phenotype breeding rice lines derived from the cross between male traditional rice (kavuni) and female white rice (Co 50) were used form the Crop Breeding and Molecular Biology, Tamil Nadu Agricultural University, Coimbatore. The dehulled and unpolished grains of 23 breeding lines and the one pair of parent samples could be divided into two classes according to their colour shades by visual:

White phenotype traits rice (Co50, 131-4, 31-3, 13-3, 13-5 and 13-2) and

Black phenotype traits rice (Kavuni, 31-3, 31-6, 32-2, 35-2,35-3, 39, 40, 144,144-1,144-2, 144-3,144-5,145-2, 145-3, 145-6, 148-2, 163-5 and 271-2).

Colour determination

The color of rice grain sample was measured by using Lovibond Tintometer and it's performed in triplicate value. The colour was expressed as tristimulus parameters *L**, *a**, *b**. *L** indicates lightness (100=white and 0= black), *a** indicates redness- greenness (positive =red) and *b** indicates yellowness-blueness (positive=yellow). In addition, the chroma *(C)* value indicates color intensity or saturation, calculated as $C=(a^{*2}+b^{*2})^{1/2}$, and hue angle was calculated as $H^{\circ}\tan^{-1}(b^{*}/a^{*})$ [13].

Extraction of rice samples

Rice flour (0.5g) was mixed with 50ml acidified methanol (95% methanol: 1M HCL as 85:15 (v/v). The solutions were centrifuged at 2500g for 10min and supernatants were collected and filtered it. The filtered supernatants were concentrated by evaporation at 45°C [14]. Then the concentrated filtrate diluted with 10 ml of acidified methanol and stored for further analysis.

Determination of total phenols content (TPC)

The total phenols content of rice lines was quantified using the Folin-Ciocalteu method [15].The total phenols content was expressed as milligrams of gallic acid equivalent per gram of rice (mg of GA/g) . The amount of total phenolic compounds in the extracts was determined by extrapolating the absorbance of the sample extract on the calibration curve (y = 0.0025x - 0.0044, R^2 = 0.9968) obtained with gallic acid as standard.

Determination of total flavonoids content (TFC)

Total flavonoids content assayed was using a UV spectrophotometer [16]. The results were expressed as milligrams of catechin equivalent per 100 gram of dry rice flour (mg CAE/100g). The amount of total flavonoids compounds in the extracts was determined by extrapolating the absorbance of the sample extract on the calibration cure ($y = 0.0024x - 0.0013$, $R^2 = 0.9967$) obtained with catechin as standard.

Determination of total anthocyanin content (TAC)

Total anthocyanin content was estimated by using the pH differential assay of [17]. Absorbance was measured in a spectrophotometer at 520 and 700 nm in buffers at pH 1.0 and 4.5 using absorbance (A) = $(A_{520}-A_{700})_{\text{pH } 1.0}-(A_{520}-A_{700})_{\text{pH } 4.5}$, with a molar extinction coefficient of cyaniding-3-glucoside. Results were expressed as mg Cyanidin 3-glucoside equivalent per 100 gram of dry weight (c3g equivalent/100g).

2.4. Statistical analysis

Data were reported as mean ± standard deviation (SD) for triplicate determination of each sample. Analysis of variance (ANOVA), correlation analysis was performed in SPSS (Software Version 11.1). Statistical significance is defined to be at level of $p<0.05$, 0.01.

3. Results

3.1. Colour of rice lines

Table 1. Colour values (*L, *a**, *b**, *H*° and *C*) of rice samples**

	Name	Colour parameters				
		L*	***a****	***b****	***C***	***H°***
	White rice					
1.	**CO 50 (Parent)**	**187.53 ± 1.27^{a}**	**0.46±0.0^{t}**	**47.92±0.61^{a}**	**49.52± 0.60^{a}**	**170.08±2.43^{a}**
2.	**13-2**	**167.56 ± 2.73^{b}**	**0.49±0.00 ^{t}**	**40.96 ± 1.03^{b}**	**41.1 ± 0.25^{b}**	**93.63± 1.11^{b}**
3.	**13-3**	**134.02± 4.28^{d}**	**0.95±0.01^{s}**	**30.18±0.32^{c}**	**22.2±0.49^{d}**	**85.14 ±2.02^{c}**
4.	**13-5**	**140.95±4.28 ^{c}**	**0.63±0.00^{t}**	**40.96±1.11^{b}**	**32.66±0.06^{c}**	**86.86±1.53^{c}**
5.	**31-3**	**121.18±2.38^{e}**	**1.38±0.01^{r}**	**21.52±0.49^{d}**	**21.59± 0.10 ^{e}**	**84.99±0.11^{c}**
6.	**131-4**	**104.02±1.41^{f}**	**1.88±0.01^{q}**	**20.06±0.34^{e}**	**19.6±0.62^{f}**	**80.83±0.12^{d}**
	Black rice					
4.	**Kavuni (Parent)**	**24.50± 0.71^{r}**	**12.49±0.18^{a}**	**1±0.03^{r}**	**3.59±0.10^{t}**	**45.94±1.37^{o}**
5.	**31-6**	**37.61±0.97^{jk}**	**2.58±0.07^{n}**	**14.89 ±0.17^{j}**	**13.37±0.04^{k}**	**75.48±1.43^{gh}**
6.	**32-2**	**29.77± 0.26^{pq}**	**8.9±0.28^{f}**	**8.3±0.06^{o}**	**8.57±0.04^{q}**	**61.06±1.28^{m}**
7.	**35-2**	**37.25±0.50^{jkl}**	**2.81± 0.01^{m}**	**13±0.42^{k}**	**13.37±0.14^{k}**	**75.42±0.97^{gh}**
8.	**35-3**	**28.7±0.27^{pq}**	**9.51 ±0.19^{d}**	**7± 0.06^{p}**	**5.82± 0.01^{s}**	**60.79±1.61^{m}**

9.	**39**	**34.87±1.11^{lmn}**	**6.46±0.18^{j}**	**11.54±0.05^{l}**	**11.99 ± 0.11^{m}**	**70.89±0.67^{j}**
10.	**40**	**29.53±0.56^{pq}**	**9.12±0.22^{e}**	**8.14±0.00^{o}**	**8.05±0.04^{r}**	**60.82±1.69^{m}**
12.	**143-1**	**33.76 ±0.16 ^{mn}**	**7.22 ±0.15^{i}**	**10.50± 0.07^{m}**	**11.55 ±0.10^{n}**	**68.32±1.16 ^{k}**
13.	**144**	**35.66±1.09^{klm}**	**3.96±0.06^{k}**	**2.48±0.29^{k}**	**12.69±0.35^{l}**	**71.81±0.63^{ij}**
14.	**144-1**	**38.99± 0.18^{j}**	**2.46±0.07^{no}**	**15.5±0.22^{i}**	**14.92±0.12^{j}**	**77.02±0.73^{fg}**
15.	**144-2**	**27.66±0.41^{q}**	**11.05±0.23^{c}**	**5.45±0.10^{q}**	**5.73±0.08^{s}**	**60.48±1.56^{m}**
16.	**144-3**	**37.21±1.11^{jkl}**	**3.05±0.02^{l}**	**12.75±0.19^{k}**	**13.73±0.44^{k}**	**73.58±1.00^{hi}**
17.	**144-5**	**54.88±0.55^{h}**	**2.23±0.03^{p}**	**17.65±0.02^{g}**	**18.70±0.33^{h}**	**77.88±2.06^{ef}**
18.	**145-2**	**74.15±2.37^{g}**	**2.15±0.05^{p}**	**18.87±0.36^{f}**	**19.12±0.53^{g}**	**79.5±0.10^{de}**
19.	**145-3**	**31.12±0.78^{op}**	**8.67±0.15^{gh}**	**9.68±0.01^{n}**	**9.89±0.10^{o}**	**64.25±0.78^{l}**
20.	**145-6**	**25.27±0.51^{r}**	**12.15±0.02^{b}**	**5.36±0.06^{q}**	**0.18±5.59^{s}**	**53.03±1.15^{n}**
21.	**163-5**	**44.17±0.57^{i}**	**2.3±0.05^{op}**	**16.36±0.24^{h}**	**0.21±17.76^{i}**	**77.88±2.43^{ef}**
22.	**148-2**	**29.82±0.14^{pq}**	**8.72±0.28^{fg}**	**9.58±0.12^{n}**	**9.32±0.27^{p}**	**64.05±1.00^{l}**
23.	**271-2**	**32.84±0.93^{no}**	**8.51±0.15^{h}**	**9.89±0.30^{n}**	**9.93±0.27^{o}**	**67.51±1.65^{k}**

Values are means ± standard deviation with triplicates. Data followed by the different letter in the same column are significantly different ($p < 0.05$) by Duncan's test.

The lightness (*L**), redness (*a**), yellowness (*b**), chroma (*C*) and hue (*H°*) values of 25 black and white rice are shown in table 1. The results indicated that the *L** value of white and black phenotype rice ranged from 104.02 to 187.53 and from 24.53 to 74.15, respectively. The *b** values ranged from 20.06 to 47.92 and from 1 to 18.87, respectively for white and black phenotype rice. The whiteness (*L**) and yellowness (*b**) was more dominant in the CO 50 white color parent rice followed by 13-2 >13-5 >13-3 >31-3 >131-4.

While the black rice group had a lowest *L** (145-2 >144-5>163-5>144-1>31-6) and *b** (145-2 >144-5>163-5>144-1>31-6) values at $p < 0.05$, this indicating that white phenotype breeding rice lines had higher lightness and yellowness who analyzed than black phenotype breeding rice lines. The findings were similar to the results obtained [18] that whiteness (L*) was more dominant in the white rice group followed by the red rice group, while the black rice group gave the lowest of L* values in "chemometric classification of pigmented rice varieties" and [19] also opined that the white rice had higher lightness and yellowness than light-purple and black rice.

The redness (a^*) values of twenty five white and black phenotype rice ranged from 0.46 to 1.88 and from 2.23 to 12.49 respectively. The positive a^* values was highest in kavuni and 145-6>144-2>35-3>40>32-2>145-3 lines belongs to the group of black color phenotype breeding. Nevertheless, the high levels of white phenotype traits resulted in lower redness values. The observation was comparable with the results found by [20] who reported that red rice have the highest a* values; it may be due to more reddish color of the external layers than the white rice varieties.

The *C* (Chroma) and *H* (Hue) values of twenty five rice samples ranged from 3.59 to 49.52 and 45.94 to 170.08, respectively. The hue angle was higher for the group of white phenotype rice than black rice group, which indicated that the black rice varieties were more in red colour than that of the white rice varieties and the findings were agreement with who found that [21] the pigmented rice gave lowest hue angle it indicating that pigmented rice was more redness than non-pigmented rice. Furthermore, the chroma was high in the white colour phenotype rice group, while the black color phenotype rice group presented low value of chroma, due to white and yellow color that appeared on white color phenotype grains as different from the other of black color phenotype grain.

3.2. Total phenols, total anthocyanin and total flavonoids content of rice samples

Table 2. Total phenols, total anthocyanin and total flavonoids content of rice samples

S. No	Name	TPC (mg of GA/g)	TFC (mg CAE/100g)	TAC(mg c3g/100g)
	White rice			
1.	CO 50 (Parent)	0.4±0.01^{j}	0.74 ±0.01^{t}	12.79±0.40^{r}
2.	13-2	0.53±0.01^{j}	0.87 ±0.00^{s}	15.43±0.43^{q}
3.	13-3	0.56±0.00^{j}	1.05 ±0.00^{r}	15.58±0.13^{q}
4.	13-5	0.55±0.00^{j}	1.08 ±0.00^{r}	15.31±0.34^{q}
5.	31-3	0.73±0.01^{j}	1.05 ±0.01^{r}	17.26±0.22^{p}
6.	131-4	0.73 ±0.01^{j}	1.15 ±0.00^{q}	16.82±0.35^{po}
	Black rice			
1.	Kavuni (Parent)	3.24 ±0.05^{a}	68.58±2.13^{b}	223.76±3.34^{a}
2.	31-6	1.54 ±0.03^{m}	43.71±1.35^{f}	129±4.12^{g}

3.	**32-2**	$\mathbf{2.67 \pm 0.08^{e}}$	$\mathbf{35.54 \pm 1.39^{ij}}$	$\mathbf{190 \pm 0.12^{e}}$
4.	**35-2**	$\mathbf{1.61 \pm 0.02^{l}}$	$\mathbf{33.47 \pm 0.53^{l}}$	$\mathbf{130 \pm 2.21^{g}}$
5.	**35-3**	$\mathbf{2.78 \pm 0.06^{d}}$	$\mathbf{65.14 \pm 0.00^{c}}$	$\mathbf{201 \pm 6.15^{c}}$
6.	**39**	**2.01 ±0.00i**	$\mathbf{43.61 \pm 0.80^{f}}$	$\mathbf{132 \pm 2.60^{g}}$
7.	**40**	$\mathbf{2.77 \pm 0.04^{d}}$	$\mathbf{35.43 \pm 0.40^{d}}$	$\mathbf{196.99 \pm 5.62^{d}}$
8.	**143-1**	$\mathbf{2.08 \pm 0.04^{h}}$	$\mathbf{39.86 \pm 1.21^{g}}$	$\mathbf{132 \pm 3.50^{g}}$
9.	**144**	$\mathbf{1.75 \pm 0.01^{j}}$	$\mathbf{24.21 \pm 0.76^{n}}$	$\mathbf{134 \pm 1.52^{g}}$
10.	**144-1**	$\mathbf{1.43 \pm 0.02^{n}}$	$\mathbf{48.55 \pm 0.72^{g}}$	$\mathbf{107 \pm 2.40^{h}}$
11.	**144-2**	$\mathbf{2.88 \pm 0.01^{c}}$	$\mathbf{80.85 \pm 1.98^{a}}$	$\mathbf{204 \pm 1.25^{c}}$
12.	**144-3**	$\mathbf{1.69 \pm 0.02^{k}}$	$\mathbf{35.12 \pm 0.43^{ij}}$	$\mathbf{131 \pm 0.08^{g}}$
13.	**144-5**	$\mathbf{1.23 \pm 0.01^{p}}$	$\mathbf{33.37 \pm 0.54^{kl}}$	$\mathbf{105 \pm 0.64^{hi}}$
14.	**145-2**	$\mathbf{1.63 \pm 0.03^{k}}$	$\mathbf{37.51 \pm 0.93^{h}}$	$\mathbf{102 \pm 2.0^{1i}}$
15.	**145-3**	$\mathbf{2.58 \pm 0.00^{f}}$	$\mathbf{65.21 \pm 1.33^{c}}$	$\mathbf{164 \pm 3.01^{f}}$
16.	**145-6**	$\mathbf{2.94 \pm 0.02^{b}}$	$\mathbf{49.27 \pm 1.77^{e}}$	$\mathbf{209.73 \pm 5.85^{b}}$
17.	**163-5**	$\mathbf{1.33 \pm 0.042^{o}}$	$\mathbf{36.78 \pm 1.11^{h}}$	$\mathbf{106 \pm 0.36\ ^{hi}}$
18.	**148-2**	$\mathbf{2.64 \pm 0.01^{e}}$	$\mathbf{59.13 \pm 0.37^{d}}$	$\mathbf{165 \pm 0.44^{f}}$
19.	**271-2**	$\mathbf{2.52 \pm 0.04^{g}}$	$\mathbf{32.52 \pm 0.05^{m}}$	$\mathbf{133 \pm 3.43^{g}}$

Values are means ± standard deviation with triplicates. Data followed by the different letter in the same column are significantly different ($p < 0.05$) by Duncan's test.

The TPC, TFC and TAC of six white rice and nineteen black rice samples were analyzed and presented in Table 2. Among the all rice accessions, TAC ranged from 0.4 to 223.76 mg c3g equivalent/100g**,** while the lower values presented in the white rice and higher values were found in black rice. This values was comparable to that found by [22] who reported TAC in black kavuni rice (244.45 mg c3g equivalent/100g) .Variations found within the white rice samples and black rice samples, ranging from 0.4 to 0.73 and from 102 to 223.76 mg c3g equivalent/100g, respectively. Among twenty five rice samples, the black phenotype rice contained higher levels of TAC in the order of kavuni (223.76 mg c3g equivalent/100g) followed by 145-6 (209.73 mg c3g equivalent/100g) and 144-2 (204 mg c3g equivalent/100g), where lower in white rice samples.

Among the rice samples, black rice had the highest TPC from 1.23 to 3.24 mg of GAE/g. The kavuni (3.24 ± 0.05 mg GAE/g) parent contained the highest TPC, followed by the breeding rice lines 145-6 (2.94 ±0.02 mg GAE/g), 144-2 (2.88 ± 0.01 mg GAE/g), 40 (2.77 ± 0.04 mg GAE/g), 32-2 (2.67 ± 0.08 mg GAE/g), 148-2 (2.64 ±0.01) and 145-3 (2.58 ± 0.00 mg GAE/g). The above findings were in harmony with information gathered by [23] declared the TPC of kavuni rice (3.33 ± 0.02 mg GAE/g). Compare to black rice samples white rice samples got the lowest TPC.

Results showed that the significant difference (P<0.05) were observed in the selected samples. The TFC was highest in kavuni (62.58 mg CAE/g) and lowest in CO 50 (12.79 mg CAE/g). The white rice and black rice lines containing the TFC ranged from 12.79 to 42.58 mg CAE/g. The black rice possessed the highest TFC in the rice samples 144-2>kavuni>145-3>35-3>148-2>145-6, whereas lower levels were found in white rice sample (31-3>131-4>13-3>13-2>13-5>CO 50). The obtained results were in harmony with the findings of [24] who observed the TFC level was observed in black kavuni (34.50 ± 1.24 mg QE/g).

3.3. Relationship between TPC, TFC, TAC and colour values

Table 3. Correlation among colour, TPC, TFC and TAC

	TPC	TFC	TAC
*L**	**-0.76****	**-0.79****	**-0.89****
*a**	**0.77****	**0.77****	**0.87****
*b**	**-0.80****	**-0.76****	**-0.87****
H°	**-0.75****	**-0.92****	**-0.81****
C	**-0.72****	**-0.66****	**-0.71****

,**,were significant at 0.05, 0.01 and 0.001 probability level respectively.**

The TPC, TFC and TAC were detectable in all the breeding rice grains, among which were the black phenotype rice lines having most abundant form. Apparently, the black rice had *L**, *b**,*C* and *H°* values of colour parameters which was less than the white rice.

Among total rice accessions the four colour parameters such as *L**, *b**, *C* and *H°* values were significantly negatively correlated with TPC, TFC and TAC. The negative correlation found in between lightness (*L*)* value with TAC (p= -0.89**) as highest, and the chroma (*C*) with TAC (p= -0.71) as lowest and the findings were agreement with who found that [25] " that soluble-conjugated phenolics, TFC, and anthocyanins were negatively correlated with L*, b*, C and H° values.

Among the rice samples, the black rice had redness (*a**) values of color parameters higher than the white rice. The color parameter *a** value highly positively significantly correlated with TPC, TFC and TAC. The highest positive correlation was found in the TAC with *a** (redness) color value (p=0.87**). Among the rice accessions, the relation of TFC and TFC were poorly positive correlated with *a** (redness) color value with (p=0.77).

Conclusion

Bio-fortification is one of the strategies to improve the nutritional quality of the rice grains. It is noticeable that TPC, TFC and TAC were differing from the black and white rice. Therefore breeding efforts for better improved 23 breeding line samples were used to investigated the colour values (*L**,*a**,*b**,*C* and *H*°), TPC, TFC and TAC content. Among the breeding rice samples the 145-6 line having highest TPC and TAC and the 144-2 line had highest TFC. In conclusion this study shows the quality information to the rice breeders and eventually commercial producers with new opportunities to promote the production of pigmented rice to enhanced levels of the phytochemical. Rice genotypes rich in phytochemical may be incorporated into development of product and functional foods. In future research to find out the scope of i*n-vivo* capability the best breeding line.

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