Genetic Variation And Association Mapping of Phenolic, Flavonoid Content And Antioxidant Capacity In USDA Rice Mini-Core Collection

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Abstract

Genotypic diversity of total phenolics, flavonoid content and antioxidant capacity in the USDA rice mini-core collection was analyzed. Wide genotypic variation was found in total phenolics, flavonoid content and ABTS antioxidant capacity. Genome-wide association mapping between the three antioxidant traits and 155 SSR markers was conducted using Q + K model which takes both population structure (Q) and relative kinship (K) into consideration. A total of 23 marker trait association were identified with markers from *Rc* gene showed the strongest association with the three antioxidant traits. Rid12, RM484, RM162, RM5371 were commonly detected for phenolic content, flavonoids content and antioxidant capacity.

Introduction

For better eating quality, rice seeds are usually consumed as white rice which was obtained by removing the bran layer in polishing brown rice (whole rice grain). However, protein, fiber, oil, minerals, and phytochemicals which mainly concentrate in the bran layer were largely lost in this process (Yokoyama et al. 2004). There are plenty of studies showing that consumption of brown rice is associated with reduced risk of developing chronic disease, such like cardiovascular disease, type II diabetes, obesity and cancer (Anderson et al. 2003; Hudson et al. 2000; Seal 2006; Okater & Liu, 2010; Venn and Mann, 2004). The brown rice has been attracting increased research interest because of its better nutrition quality and health benefits.

The phenolic compounds include phenolic acids, flavonoids, condensed tannins and many other (Dykes and Rooney, 2007) chemicals containing a benzene ring with one or more hydroxyl groups (– OH). It is a group of phytochemicals that have antioxidant properties and can promote health by protecting cells against oxidative damage. The red rice is pigmented by condensed tannins, also called proanthocyanidin (Oki et al. 2002), which consists of polymerized flavanol units and demonstrated higher antioxidant activity in vitro compared to monomeric phenolic compounds (Hagerman et al., 1998). The purple and black rice are pigmented by anthocyanins which is a type of water-soluble flavonoids (Reddy et al. 1995). Generally, the pigmented rice (i.e. red, purple and black rice) has higher total phenolic, flavonoids content and antioxidant capacity than non-pigmented rice, which was revealed by several studies (Goffman and Bergman 2004; Shen et al. 2009). Therefore, The color parameters of rice grain are positively correlated with the total phenolic, flavonoid content and antioxidant capacity (Shen et al., 2009).

A good understanding of the genetic basis is a prerequisite for improvement of antioxidant property in rice. Classical genetic analysis suggested that two genes needed to produce red rice pericarp: *Rd* (red pericarp and seed coat) and *Rc* (brown pericarp and seed coat). The red pericarp was only produced when the two genes present together, otherwise, brown pericarp was produced when *Rc* is present with an absence of *Rd*, and non-pigmented pericarp was produced when *Rd* present alone (Kato and Ishikawa, 1921). In a later study, Sweeney et al. (2006) revealed how the sequence mutation of *Rc* gene affected the pigmentation in rice pericarp. For purple and black rice pericarp, classical genetic analysis suggested that two loci, *Pb* (*Prp-b*) and *Pp* (*Prp-a*) which were located on chromosome 4 and 1 respectively, responsible

for anthocyanins deposition in rice pericarp (Yoshimura et al., 1997). A later study mapped the *Pb* gene on chromosome 4 and suggested the *Pb* gene may be the *Ra* gene (Wang and Shu et al. 2007). In a QTL mapping study using a recombinant inbred line population, Tan et al. (2001) reported several QTLs for the color parameters of lightness (L^*), redness (a^*) and yellowness (b^*). Jin et al. (2009) used a doubled haploid population to map genes for rice grain color, total phenolics and flavonoid contents and antioxidant capacity, and found each trait was controlled by several QTLs with minor effects. On the other hand, association analysis between molecular marker and traits in antioxidant property was limited. There is one association mapping study used 416 rice germplasm accessions and 100 SSR markers which confirmed that *Ra* and *Rc* genes were main-effect loci for rice grain color and antioxidant properties and identified several high trait-marker associations.

Reports on genotypic diversity of antioxidants in large rice population was limited. Shen et al. (2009) reported the phenotypic variations in total phenolic, flavonoid content and antioxidant capacity in 481 rice genotypes and the high correlation of these three parameters with color parameters. Goffman and Bergman (2004) reported the phenolic content of 133 colored rice cultivars. The USDA rice mini-core collection containing 217 accessions is a good representative of the whole germplasm resource in the USA gene bank (Agrama et al. 2010). Our previous studies revealed its high genotypic diversity in starch physicochemical properties, but its diversity in antioxidant properties in mini-core is still unknown. In the present study, we aimed to reveal the genotypic diversity in antioxidant properties (i.e. total phenolic, flavonoid content and antioxidant capacity) contained in USDA rice mini-core collection, and to conduct a genome wide association analysis based on 155 SSR markers in order to find makers for antioxidant properties. The result should be helpful for parental material selection in mini-core for improvement of antioxidant properties and should contribute to a better understanding of genetic basis of antioxidant properties in rice.

Materials And Methods

Materials

The seeds of USDA rice mini-core collection were provided by USDA-ARS and all were grown in Hainan island (18°N), China from December 2013 to April 2014. After harvest, rice seeds were de-hulled and ground to whole grain flour. A total of 164 rice accessions including 132 white rice, 31 red rice and 1 black rice were used for analysis.

Extraction

0.5 g rice flour was extracted with 10 ml of 80% methanol twice. Each time, the mixture was shaken on a shaker for 30 minutes at room temperature. The mixture was then centrifuged at 4000g for 15 min and the supernatants were collected.

Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu colorimetric method with minor modifications (Cai et al. 2004). Generally, 200 μ L of standard solution or appropriately diluted extracts were mixed with 1.5 ml 10-fold diluted Folin-Ciocalteu reagent, and then the mixture was neutralized with 1.5 ml saturated sodium carbonate (75 g/L). After 2h of reaction at room temperature in the dark, the absorbance was measured at 725 nm. Gallic acid solution with concentrations ranging from 25 to 150 mg/L was used to produce a calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalent (mg GAE) per 100g of dry rice flour.

Determination of total flavonoid content

Total flavonoid content was determined by colorimetric method with slight modifications (Bao et al. 2005). 500 μ L of appropriately diluted extracts or standard solutions was mixed with 150 μ L of 5% NaNO_2 for 5 min, then 150 μ L of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added and the mixture was blend for 5 min before 1 mL of 1 M NaOH and 3 mL H_2O were added. The finally mixture was shaken thoroughly and 10 min reaction was allowed before the absorbance was measured at 510 nm. The catechin solution with concentrations ranging from 50 to 250 mg/L was used to make a standard curve and the total flavonoid content was expressed as milligrams of catechin equivalent (mg CAE) per 100 g of dry rice flour.

Determination of ABTS radical scavenging activity

Total antioxidant capacity of $\text{ABTS}^{\cdot+}$ radical scavenging activity was measured according to the procedure described before (Shen et al. 2009). 100 μ L of appropriately diluted extracts or standard solution was mixed thoroughly with 3.9 mL $\text{ABTS}^{\cdot+}$ solution. After 6 mins' reaction, the absorbance was measured at 734 nm. Trolox standard solution was prepared and assayed following the same procedure to get a standard curve, the total antioxidant capacity was expressed as μ mol of Trolox equivalent antioxidant capacity (μ mol TEAC) per 100 g of dry rice flour.

Association mapping

Population structure (Q) and kinship (K) of 164 rice accessions were calculated based on 155 SSR markers reported by Li et al. (2010). Association analysis between 155 SSR markers and total phenolic, flavonoid content and antioxidant capacity was conducted using TASSEL (Version 2.1) software, taking the gross level population structure and kinship into account. The association between a marker and a trait was determined by the $q\text{FDR}$ value (marker) ($q\text{FDR} < 0.01$).

Statistical analysis

All the measurements were performed in duplicate. Data analyses were performed with the SAS program version 8 (SAS Institute Inc., Cary, NC, USA). The Student-Newman-Keuls test was performed for comparison of means at $P < 0.05$.

Results

Total phenolic, flavonoid contents and antioxidant capacity

Wide range of variations was observed in total phenolic, flavonoid contents and antioxidant capacity in mini core (Table 1). Among 164 rice accessions, TPC ranged from 91.8 (Plant Identification: SORNAVARI; Ancestry: AUS) to 1329.8 (Karayal, AUS) mg GAE/100g. The mean value was 272.4 mg GAE/100g with a CV of 97.0%. TPC ranged from 130.2 (Tauli, AUS) to 1329.8 (Karayal, AUS) mg GAE/100g in red rice with a mean value of 755.2 mg GAE/100g and 34.1% for CV. In white rice, the range was from 91.8 (SORNAVARI, AUS) to 250.8 (LUSITANO, TEJ) mg GAE/100g with mean value of 155.5 mg GAE/100g and CV of 20.6%. The mean value in red rice was significantly higher than that of white rice (755.2 versus 155.5), however, some red rice still showed lower total phenolic content than white rice.

Table 1 Means and ranges of antioxidant traits in 164 sampled accessions from the mini-core collection¹

Groups	TPC				TFC				ABTS			
	Mean $\pm$ SD	Min	Max	CV	Mean $\pm$ SD	Min	Max	CV	Mean $\pm$ SD	Min	Max	CV
White rice (n = 132)	155.5 ^b $\pm$ 32.0	91.8	250.8	20.6%	67.6 ^b $\pm$ 18.9	43.4	153.3	28.0%	415.9 ^b $\pm$ 77.2	187	621.2	18.6%
Red rice (n = 31)	755.2 ^a $\pm$ 257.7	130.2	1329.8	34.1%	254.1 ^a $\pm$ 77.5	54	411.9	30.5%	4038.2 ^a $\pm$ 1344.7	1707	8286.8	33.3%
Black rice (n = 1)	731.5				144.3				679.3			
Total (n = 164)	272.38 $\pm$ 264.09	91.81	1329.8	97.0%	103.3 $\pm$ 82.25	43.41	411.89	79.6%	1118.8 $\pm$ 1546.1	187	8286.8	138.2%

¹Different letters in the same column indicate significant difference at $P < 0.05$ level.

TFC in 164 accessions ranged from 43.4 (SORNAVARI, AUS) to 411.9 (Romeno, TEJ) mg CAE/100g with mean value of 103.3 mg CAE/100g and CV of 79.6%. The range in white rice was from 43.4 (SORNAVARI, AUS) to 153.3 (4595, IND) mg CAE/100g with an average of 67.6 mg CAE/100g and CV of 28.0%; and from 54.0 (Dhan, IND) to 411.9 (Romeno, TEJ) mg CAE/100g with a mean value of 254.1 mg CAE/100g and CV of 30.5% in red rice. Not all red rice had higher TFC although the mean value in red rice was significantly higher than that of white rice (254.1 versus 67.6).

The total antioxidant capacity varied greatly in 164 rice accessions. The range was from 187 (BHIM DHAN, Admix) to 8286.8 (Nang Bang Bentre, AUS) μ M TEAC with an averaged at 1118.8 μ M TEAC and CV of 138.2%. Among the white rice, the ABTS⁺ ranged from 187 (BHIM DHAN, Admix) to 621.2 μ M (GPNO 1106, TRJ) TEAC and averaged at 415.9 μ M TEAC with CV of 18.6%, the red rice had a significantly higher mean value at 4038.2 μ M TEAC with a range from 1707.0 (Tauli, AUS) to 8286.8 μ M (Nang Bang Bentre, AUS) TEAC and CV of 33.3%.

Antioxidant properties in different ancestry groups

The 164 accessions were divided into six ancestry groups in present study according to Li et al. (2010) and the total phenolic, flavonoid and ABTS⁺ antioxidant capacity were compared among groups (Table 2). The AUS group had the highest mean TPC, TFC and ABTS⁺ antioxidant capacity, followed by TEJ group. It showed be pointed out that the AUS and TEJ rice had the highest and the second highest percentage of pigmented rice respectively (20 out of 35 accessions in AUS group and 5 out of 22

accessions in TEJ group are red rice, respectively), indicating that the significant higher values in parameters observed in AUS and TEJ rice may be attributed to its relatively higher percentage of red rice.

Table 2 Means and ranges of antioxidant traits in rice accessions of different ancestry groups^{1, 2}

Ancestry group	No. of accessions	TPC				TFC				ABTS			
		Mean $\pm$ SD	Min	Max	CV (%)	Mean $\pm$ SD	Min	Max	CV (%)	Mean $\pm$ SD	Min	Max	CV (%)
AUS	35	455.27 ^a $\pm$ 319.32	91.8	1329.8	70.1	159.46 ^a $\pm$ 96.63	43.4	377.4	60.6	2331.1 ^a $\pm$ 2023.9	212.1	8286.8	86.8
TEJ	22	365.21 ^{ab} $\pm$ 342.62	128.0	1124.6	93.8	130.71 ^{ab} $\pm$ 120.23	48.8	411.9	92	1469.3 ^{ab} $\pm$ 1906.5	373.1	5673.4	129.8
Admix	12	227.60 ^b $\pm$ 254.36	108.1	1031.5	111.8	90.51 ^b $\pm$ 67.28	54.3	300.1	74.3	807.6 ^b $\pm$ 1451.7	187.0	5409.4	179.8
TRJ	31	210.73 ^b $\pm$ 198.32	114.9	1110.6	94.1	78.78 ^b $\pm$ 54.02	46.0	345.8	68.6	690.6 ^b $\pm$ 980.4	254.3	5112.4	142
IND	59	180.71 ^b $\pm$ 159.51	102.6	888.8	88.30%	78.73 ^b $\pm$ 51.23	46.6	301.4	65.1	614.7 ^b $\pm$ 868.3	284.6	4822	141.3
ARO	5	155.26 ^b $\pm$ 24.91	118.8	180.4	16.00%	62.00 ^b $\pm$ 10.52	51.4	74.7	17	439.9 ^b $\pm$ 46.5	382.3	489.2	105.7

¹Different letters in the same column indicate significant difference at $P < 0.05$ level.

²IND *indica*, AUS *aus*, TEJ *temperate japonica*, TRJ *tropical japonica*, ARO *aromatic*, Admix *accessions with mixed ancestry*.

Compare to other groups, the ARO group showed lower mean values in antioxidant traits and smaller extent of variation (CV) which was probably because of its small number of accessions. However, the Admix group still showed wide variations in TPC and ABTS although its number of accessions was not high neither (n=12).

When red rice accessions were excluded, there was no significant difference in TFC among ancestry groups; TEJ rice showed significant high value in TPC compared to all the other groups and the highest antioxidant capacity among groups.

Correlations among total phenolic, flavonoid content, antioxidant capacity and apparent amylose content

Pairwise correlation analysis showed that the three parameters of antioxidant property were positively correlated with each other ($r = 0.923 - 0.953$, $P < 0.0001$) in 164 rice accessions, which was also observed in 31 red rice (Table 3). However, the correlation between TPC and ABTS, TFC and ABTS were higher in all accessions ($r = 0.944, 0.923$ respectively) than that of red rice ($r = 0.684, 0.656$ respectively). Also, the correlation between parameters in white rice was pretty weak. This difference may be due to the smaller variation in values of parameters in red rice and white rice, compared to that in the total rice accessions.

Table 3 Correlation analysis of antioxidant traits and amylose content

Corr	164 accessions			Red rice		White rice	
	TFC	ABTS	AAC	TFC	ABTS	TFC	ABTS
TPC	0.953 ^{****}	0.944 ^{****}	0.155	0.936 ^{****}	0.684 ^{****}	-0.169	0.487 ^{****}
TFC		0.923 ^{****}	0.161 [*]		0.656 ^{****}		0.094
ABTS			0.183 [*]				

**** indicate significance at $P < 0.0001$ level.

The relationship between red pericarp and AAC, AAC and antioxidant traits

25 out of 31 red rice had AAC > 23%, In 164 rice accessions, apparent amylose content positively correlated with TPC, TFC ($P < 0.05$) and antioxidant ($P < 0.05$) (Table 3).

Association mapping in 164 rice accessions

Association mapping between three antioxidant traits and 155 SSR markers (Li et al., 2010) in 164 rice accessions was performed based on Q + K model. The results were shown in Table 4 and Figure 1. Overall, a total of 12 different markers from 7 chromosomes were detected in 23 significant marker-trait associations. Four markers, Rid12, RM484, RM162, RM5371 were commonly detected for phenolic content, flavonoids content and antioxidant capacity. The Rid12 marker which targeted an InDel at the *Rc* locus showed the strongest association with all three traits, this confirmed the *Rc* gene acting as one main loci responsible for antioxidant traits in rice grain.

Table 4 Marker-trait associations detected in MLM analysis (Q + K model)

Trait	Locus	Chro. No.	Position	<i>P</i>	<i>qFDR</i>	<i>R</i>²
TPC	Rid12	7	6.1	1.40 × 10 ⁻³⁰	2.14 × 10 ⁻²⁸	0.4759
	RM484	10	20.8	2.21 × 10 ⁻⁶	1.69 × 10 ⁻⁴	0.2023
	RM162	6	24	2.38 × 10 ⁻⁶	1.21 × 10 ⁻⁴	0.2827
	RM5371	6	25.83	4.65 × 10 ⁻⁶	1.78 × 10 ⁻⁴	0.1729
	RM125	7	5.4	1.24 × 10 ⁻⁴	3.79 × 10 ⁻³	0.1843
	RM404	8	15.3	3.03 × 10 ⁻⁴	7.73 × 10 ⁻³	0.3122
	RM208	2	35.1	3.80 × 10 ⁻⁴	8.31 × 10 ⁻³	0.2087
	RM490	1	6.7	5.16 × 10 ⁻⁴	9.87 × 10 ⁻³	0.1482
	RM510	6	2.8	7.07 × 10 ⁻⁴	1.20 × 10 ⁻²	0.1473
	RM510	6	2.8	7.07 × 10 ⁻⁴	1.20 × 10 ⁻²	0.1473
ABTS	Rid12	7	6.1	1.70 × 10 ⁻²⁶	2.60 × 10 ⁻²⁴	0.4077
	RM484	10	20.8	2.26 × 10 ⁻⁷	1.73 × 10 ⁻⁵	0.2137
	RM507	6	0.1	4.76 × 10 ⁻⁵	2.43 × 10 ⁻³	0.0973
	RM162	6	24	7.56 × 10 ⁻⁵	2.89 × 10 ⁻³	0.2332
	LJSSR1	9	21.5	1.51 × 10 ⁻⁴	4.62 × 10 ⁻³	0.1105
	RM490	1	6.7	2.41 × 10 ⁻⁴	6.15 × 10 ⁻³	0.1541
	RM5371	6	25.83	2.92 × 10 ⁻⁴	6.38 × 10 ⁻³	0.1279
	RM404	8	15.3	7.21 × 10 ⁻⁴	1.38 × 10 ⁻²	0.2567
TFC	Rid12	7	6.1	2.89 × 10 ⁻³¹	4.42 × 10 ⁻²⁹	0.484
	RM484	10	20.8	1.61 × 10 ⁻⁷	1.23 × 10 ⁻⁵	0.2326
	RM162	6	24	7.40 × 10 ⁻⁶	3.77 × 10 ⁻⁴	0.2816
	RM404	8	15.3	2.05 × 10 ⁻⁵	7.84 × 10 ⁻⁴	0.3357
	RM125	7	5.4	2.94 × 10 ⁻⁵	9.00 × 10 ⁻⁴	0.2081
	RM5371	6	25.83	1.16 × 10 ⁻⁴	2.96 × 10 ⁻³	0.1453
	RM44	8	11.8	2.09 × 10 ⁻⁴	4.57 × 10 ⁻³	0.2182
	RM25766	10	20.2	2.54 × 10 ⁻⁴	4.86 × 10 ⁻³	0.1666
	RM208	2	35.1	6.06 × 10 ⁻⁴	1.03 × 10 ⁻²	0.2136

Most markers detected in association with antioxidant traits were on chromosome 6, including two of the four common markers detected for TPC, TFC and ABTS. Furthermore, three markers on chromosome 6 were identified for ABTS antioxidant capacity.

Discussion

Consumption of whole grain cereals containing the antioxidants is associated with the reduced risk of developing cardiovascular disease and certain cancers (Liu 2004, 2007; Dykes and Rooney 2007). To investigate the genotypic diversity of antioxidant traits in rice is helpful to identify rice cultivars with good antioxidant properties and to select suitable parental material for cultivar improvement. In the present study, the genotypic diversity of antioxidant traits (total phenolic content, total flavonoid content and antioxidant capacity) in 164 accessions of USDA rice mini-core collection was investigated. The total phenolic content ranged from 91.8 to 1329.8 mg GAE/100g (Table 1) while Goffman and Bergman (2004) reported a much lower range of phenolic content from 25 to 535 mg GAE/100g in 133 rice including brown, purple and red rice. The narrower range was probably due to the experimental design factors as pointed out by Shen et al. (2009) who reported wide range from 108.1 to 1244.9 mg GAE/100 g in 481 rice genotypes ranged which is similar to the present report. Goufo et al. (2013) also reported a similar range from 100.1 to 1640.6 mg GAE/100 g for phenolic content in 106 genotypes. The total flavonoid content ranged from 43.4 to 411.9 mg CE/100 g in the present study, since Shen et al. (2009) used rutin equivalent rather than catechin equivalent to express flavonoid content, it is difficult to compare the results between the previous study and our present study. Nevertheless, Goufo et al. (2013) reported TPC ranged from 30 to 293 mg CE/100 g in non-pigmented rice and from 108.7 to 473 mg CE/100 g in red rice, which was similar to the present report. Apparently, the measured phenolic content and flavonoid content in the present study is reasonable and a wide range of antioxidant traits contained in mini-core rice was revealed, which is favorable for rice breeders to select suitable materials that could be used to improve antioxidant properties in rice. Furthermore, the wide genotypic diversity of antioxidant traits is also suitable for a genome wide association mapping in order to find molecular markers to be used in marker assisted selection.

The pigmented rice (red and black) had higher mean values in total phenolic, flavonoid content and antioxidant capacity than non-pigmented rice (Table 1), this is expected since coloration in rice is due to accumulation of condensed tannins in red rice and anthocyanins in purple and black rice (Oki et al., 2002; Sweeney et al., 2006; Reddy et al., 1995). Condensed tannins is formed by polymerized flavanol units and showed higher antioxidant activity than monomeric phenolic compounds in vitro (Hagerman et al. 1998); anthocyanins is a type of water-soluble flavonoids. However, not all red rice was higher in antioxidant traits than all white rice, as revealed by (Shen et al. 2009) previously. In the present study, the red rice “Tauli” and “Dhan” showed lower TPC and TFC than many non-pigmented rice accessions, this indicated that other phenolic and flavonoid compositions may be higher in white rice. Nevertheless, grain color could be used as an efficient indirect index in selection for high antioxidants and antioxidant capacity. In the present study, when rice accessions were grouped into ancestry groups, the *Aus* rice which contains the highest percentage of pigmented rice exhibited the highest mean phenolic, flavonoid content and antioxidant capacity.

The genetic basis for the phenolic content, flavonoid content and antioxidant capacity in rice grain was previously studied by linkage mapping using doubled haploid population by Jin et al., 2009, and by association mapping using 416 rice germplasm and 100 SSR markers (Shao et al., 2011). However, these two available reports are very different in results with only few possible common loci on chromosome 6

identified for color parameters L^* and b^* . Compare the findings of the present study to that of Jin et al. (2009), a similar locus on chromosome 2 for phenolic content and flavonoid content was found in both studies. That is the marker RM208 positioned at 35.1 cM on chromosome 2 in the present study and the interval region between two markers CT87 and G1234, positioned at 21.4 cM and 31.8 cM on chromosome 2 respectively in the previous study. This genomic region was not reported as putative QTL locus for color parameters in any of previous studies, suggesting its association with phenolic content and flavonoid content may be not a link effect exerted by grain color. Compare the present study to Shao et al. (2011), both studies reported several markers associated with phenolic content, flavonoid content and ABTS.⁺ antioxidant capacity. First of all, both studies agreed markers from *Rc* gene have the strongest association with three antioxidant traits; Besides, both studies found several markers on chromosome 7, 8 and 10 associated with the antioxidant traits. However, it should be pointed out, in the previous study, these markers were also reported associated with color parameters of rice grain (Shao et al., 2011), suggesting their association with the three antioxidant traits may be because of link effect with grain color. Apart from markers from chromosome 7, 8 and 10, two markers were consistently detected for all three traits in the present study, RM162 and RM5371, positioned at 24 cM and 25.83 cM on chromosome 6 respectively. However, Jin et al. (2009) mapped a similar region as a putative QTL locus for L^* of color parameters. Compare to these two previous studies, new genomic regions were found for antioxidant traits in the present study, for example, RM490 on chromosome 1 for TPC and ABTS, LJSSR1 from chromosome 9 for antioxidant capacity.

Interestingly, Shao et al (2011) identified *Wx* gene for the color parameters lightness (L^*), redness (a^*) and hue angle (H^p) in association analysis. However, this association would could be because of linkage effect of red rice AAC. In 31 red rice accessions used in the present study, 25 of 31 of them had AAC > 23% and our previous study revealed these red rice all had GAC or GAT *Wx* haplotype. Furthermore, the red rice usually have higher values in antioxidant traits. In this case, the association between *Wx* gene antioxidant traits was predictable. and Although color parameters were not analyzed by the present study, antioxidant traits were studied. The correlation analysis showed the TPC, TFC and antioxidant capacity all positively correlated with AAC.

Conclusion

The present study revealed wide genotypic diversity in total phenolic, flavonoid content and antioxidant capacity contained in the USDA mini-core collection. These data should be helpful for rice breeders to select suitable parental material from mini-core to be used in rice breeding practice aiming to enhance antioxidant properties. Genome wide analysis confirmed *Rc* gene is the major gene responsible for antioxidant traits, and other SSR markers having high association were also identified, these markers would be useful in marker assisted breeding. For further study, quantification and genome wide mapping of individual compositions of phenolic acids and flavonoids in mini-core would be meaningful.

Declarations

Conflicts of Interest: The authors declare no conflict of interest.

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Figures

Figure 1

Locations of markers associated with antioxidant traits. Distances are in Kosambi centiMorgans (cM) on the left of each chromosome. Blue, red and green arrows pointing to markers associated with total phenolic content, ABTS.+ antioxidant capacity and total flavonoid content respectively.