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.

Keywords  Rice · USDA mini-core · Antioxidant properties · GWAS

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 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 2003; Hudson et al. 2000; Seal 2006; Okater and Liu 2010; Venn and Mann 2004). The brown rice has been attracting increased research interest because of its better nutrition quality and health benefits (Saleh et al. 2019; Mir et al. 2020; Brotman et al. 2021).

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 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 that 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. 2009). 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, 110° E), 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

Rice flour (0.5 g) was extracted with 10 ml of 80% methanol twice. Each time, the mixture was shaken on a shaker for 30 min at room temperature. The mixture was then centrifuged at 4000 g for 15 min and the supernatants were collected (Shen et al. 2009).
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 µL of standard solution or appropriately diluted extracts were mixed with 1.5 ml tenfold diluted Folin-Ciocalteu reagent, and then the mixture was neutralized with 1.5 ml saturated sodium carbonate (75 g/L). After 2 h 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 100 g 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 µL of appropriately diluted extracts or standard solutions was mixed with 150 µL of 5% NaNO₂ for 5 min. Subsequently, 150 µL of AlCl₃·6H₂O was added and the mixture was blend for 5 min before 1 mL of 1 M NaOH and 3 mL H₂O 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 ABTS⁺ radical scavenging activity was measured according to the procedure described before (Shen et al. 2009). Appropriately diluted extracts or standard solution 100 µL was mixed thoroughly with 3.9 mL ABTS⁺ solution. After reaction for 6 min, the absorbance was measured at 724 nm. Trolox standard solution was prepared and assayed following the same procedure to get a standard curve, the total antioxidant capacity was expressed as µmol of Trolox equivalent antioxidant capacity (µmol 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 qFDR value (marker) (qFDR < 0.01).

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/100 g. The mean value was 272.4 mg GAE/100 g with a CV of 97.0%. TPC ranged from 130.2 (Tauli, AUS) to 1329.8 (Karayal, AUS) mg GAE/100 g in red rice with a mean value of 755.2 mg GAE/100 g and 34.1% for CV. In white rice, the range was from 91.8 (SORNAVARI, AUS) to 250.8 (LUSTANO, TEJ) mg GAE/100 g in red rice with a mean value of 755.2 mg GAE/100 g and 34.1% for CV. In white rice, the range was from 91.8 (SORNAVARI, AUS) to 250.8 (LUSTANO, TEJ) mg GAE/100 g with mean value of 155.5 mg GAE/100 g 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.

Total flavonoid content in 164 accessions ranged from 43.4 (SORNAVARI, AUS) to 411.9 (Romeno, TEJ) mg CAE/100 g with mean value of 103.3 mg CAE/100 g and CV of 79.6%. The range in white rice was from 43.4 (SORNAVARI, AUS) to 153.3 (4595,
Table 1  Means and ranges of antioxidant traits in 164 sampled accessions from the mini-core collection

| Groups          | TPC          | CV (%)         | TFC          | CV (%)         | ABTS         | CV (%)         |
|-----------------|--------------|----------------|--------------|----------------|--------------|----------------|
|                 | Mean ± SD    | Min | Max   | Mean ± SD    | Min | Max   | Mean ± SD    | Min | Max   | Mean ± SD    | Min | Max   |
| White rice      | 155.5 ± 32.0 | 91.8 | 250.8 | 20.6         | 67.6 ± 18.9  | 43.4 | 153.3 | 28.0         | 415.9 ± 77.2 | 187 | 621.2 | 18.6 |
| Red rice        | 755.2 ± 257.7| 130.2| 1329.8| 34.1         | 254.1 ± 77.5 | 54   | 411.9 | 30.5         | 4038.2 ± 1344.7| 1707 | 8286.8 | 33.3 |
| Black rice      | 731.5        | 144.3| 144.3| 144.3        |              |             |              |               |                |       |       |
| Total           | 272.3 ± 264.09| 91.8 | 1329.8| 97.0         | 103.3 ± 82.25| 43.4 | 411.89| 79.6         | 1118.8 ± 1546.1| 187 | 8286.8 | 138.2 |

Different letters in the same column indicate significant difference at \( P < 0.05 \)

*TPC* total phenolic content, *TFC* total flavonoid content, *ABTS* ABTS \(^+\) antioxidant capacity

Table 2  Means and ranges of antioxidant traits in rice accessions of different ancestry groups

| Ancestry group | No. of accessions | TPC          | CV (%)         | TFC          | CV (%)         | ABTS         | CV (%)         |
|----------------|-------------------|--------------|----------------|--------------|----------------|--------------|----------------|
|                |                   | Mean ± SD    | Min | Max   | Mean ± SD    | Min | Max   | Mean ± SD    | Min | Max   | Mean ± SD    | Min | Max   |
| AUS            | 35                | 455.27 ± 319.32| 91.8 | 1329.8 | 70.1         | 159.46 ± 96.63| 43.4 | 377.4 | 60.6         | 2331.1 ± 2023.9| 212.1 | 8286.8 | 86.8 |
| TEJ            | 22                | 365.21 ± 342.62| 128.0| 1124.6| 93.8         | 130.71 ± 120.23| 48.8 | 411.9 | 92           | 1469.3 ± 1906.5| 373 | 5673.4 | 129.8 |
| Admix          | 12                | 227.60 ± 254.36| 108.1| 1031.5| 111.8        | 90.51 ± 67.28 | 54.3 | 300.1 | 74.3         | 807.6 ± 1451.7| 187 | 5409.4 | 179.8 |
| TRJ            | 31                | 210.73 ± 198.32| 114.9| 1110.6| 94.1         | 78.78 ± 54.02 | 46.0 | 345.8 | 68.6         | 690.6 ± 980.4 | 254.3 | 5112.4 | 142 |
| IND            | 59                | 180.71 ± 159.51| 102.6| 888.8 | 88.30%       | 78.73 ± 51.23 | 46.6 | 301.4 | 65.1         | 614.7 ± 868.3 | 284.6 | 4822 | 141.3 |
| ARO            | 5                 | 155.26 ± 24.91 | 118.8| 180.4 | 16.00%       | 62.00² ± 10.52| 51.4 | 74.7  | 17           | 439.9 ± 46.5 | 382.3 | 489.2 | 105.7 |

Different letters in the same column indicate significant difference at \( P < 0.05 \)

*IND* indica, *AUS* aus, *TEJ* temperate japonica; *TRJ* tropical japonica, *ARO* aromatic, *Admix* accessions with mixed ancestry, *TPC* total phenolic content, *TFC* total flavonoid content, *ABTS* ABTS \(^+\) antioxidant capacity
IND) mg CAE/100 g with an average of 67.6 mg CAE/100 g and CV of 28.0%; and from 54.0 (Dhan, IND) to 411.9 (Romeno, TEJ) mg CAE/100 g with a mean value of 254.1 mg CAE/100 g 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) l M TEAC with an averaged at 1118.8 l M TEAC and CV of 138.2%. Among the white rice, the ABTS$^+$ ranged from 187 (BHIM DHAN, Admix) to 621.2 l M (GPNO 1106, TRJ) TEAC and averaged at 415.9 l M TEAC with CV of 18.6%, the red rice had a significantly higher mean value at 4038.2 l M TEAC with a range from 1707.0 (Tauli, AUS) to 8286.8 l 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 that 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.

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.

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

A total of 25 out of 31 red rice had AAC more than 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 Fig. 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.

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.
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/100 g (Table 1) while Goffman and Bergman (2004) reported a much lower range of phenolic content from 25 to 535 mg GAE/100 g 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 which is similar to the present report. Goufo and Trindade (2014) also reported a similar range from 100.1 to 1640.6 mg GAE/100 g for phenolic content in 106 genotypes ranging which is similar to the present report. The total flavonoid content ranged from 91.8 to 1329.8 mg GAE/100 g while Goffman and Bergman (2004) reported a much lower range of flavonoid content from 25 to 48 mg CE/100 g 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 which is similar to the present report. Goufo and Trindade (2014) also reported a similar range from 100.1 to 1640.6 mg GAE/100 g for flavonoid content in 106 genotypes ranging which is similar to the present report. The total antioxidant capacity ranged from 91.8 to 1329.8 mg GAE/100 g while Goffman and Bergman (2004) reported a much lower range of antioxidant capacity from 25 to 146 mg CE/100 g 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 which is similar to the present report. Goufo and Trindade (2014) also reported a similar range from 100.1 to 1640.6 mg GAE/100 g for antioxidant capacity in 106 genotypes ranging which is similar to the present report.

### Table 3: Correlation analysis of antioxidant traits and amylose content

| All 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     |
| TFC            | 0.923*  | 0.161*     | 0.183*    | 0.656*  | 0.183*     | 0.094   |
| ABTS           |         |            |          |         |            |         |

*Indicate significance at $P < 0.0001$

TPC: total phenolic content, TFC: total flavonoid content, ABTS: antioxidant capacity, AAC: apparent amylose content

### 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/100 g (Table 1) while Goffman and Bergman (2004) reported a much lower range of phenolic content from 25 to 535 mg GAE/100 g 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 which is similar to the present report. Goufo and Trindade (2014) also reported a similar range from 100.1 to 1640.6 mg GAE/100 g for phenolic content in 106 genotypes ranging which is similar to the present report. The total flavonoid content ranged from 91.8 to 1329.8 mg GAE/100 g while Goffman and Bergman (2004) reported a much lower range of flavonoid content from 25 to 48 mg CE/100 g 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 which is similar to the present report. Goufo and Trindade (2014) also reported a similar range from 100.1 to 1640.6 mg GAE/100 g for flavonoid content in 106 genotypes ranging which is similar to the present report. The total antioxidant capacity ranged from 91.8 to 1329.8 mg GAE/100 g while Goffman and Bergman (2004) reported a much lower range of antioxidant capacity from 25 to 146 mg CE/100 g 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 which is similar to the present report. Goufo and Trindade (2014) also reported a similar range from 100.1 to 1640.6 mg GAE/100 g for antioxidant capacity in 106 genotypes ranging which is similar to the present report.

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 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. In line with Shao et al. (2011), the present study 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 to be associated with color parameters of rice grain (Shao et al. 2011), suggesting their association

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

\( TPC \) total phenolic content, \( TFC \) total flavonoid content, \( ABTS \) \( ABTS^{+} \) antioxidant capacity
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 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. As compared to Shao Fig. 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
et al. (2011) and Jin et al. (2009), 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, such as lightness ($L^*$), redness ($a^*$) and hue angle ($H^*$) in association analysis. However, this association could be because of a linkage effect rather than a causal effect. In 31 red rice accessions used in the present study, 25 of 31 of them had AAC more than 23%, and our previous study had revealed these red rice all carrying GAC or GAT Wx haplotype (Li et al. 2017). The red rice usually have higher values in antioxidant traits. As suggested by the correlation analysis in the present study, the TPC, TFC and antioxidant capacity all positively correlated with AAC (Table 3). In this case, a positive association between Wx gene and color parameters was predictable.

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.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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