Uncovering Genomic Regions Regulating the Superoxide Dismutase, Flavonoids, Anthocyanins, Carotenoids, γ -Oryzanol and Antioxidant Activity in Rice Through Association Mapping

E Pandit

ICAR-National Rice Research Institute

P Sanghamitra

ICAR-National Rice Research Institute

SR Barik

ICAR-National Rice Research Institute

R Bastia

ICAR-National Rice Research Institute

SP Mohanty

ICAR-National Rice Research Institute

A Behera

ICAR-National Rice Research Institute

A Moharana

ICAR-National Rice Research Institute

N Basak

ICAR-National Rice Research Institute

SK Pradhan (✉ pradhancrri@gmail.com)

ICAR-National Rice Research Institute

Research Article

Keywords: ABTS, antioxidant capacity, anthocyanins, association mapping, carotenoids, flavonoids, γ -oryzanol, superoxide dismutase

Posted Date: June 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-558142/v1>

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Abstract

Consumption of antioxidants rich rice has impressive health benefits. Five antioxidant traits viz., superoxide dismutase, flavonoids, anthocyanins, γ -oryzanol and ABTS were mapped in a panel population using 136 SSR markers through association mapping. A panel population containing 120 germplasm lines by including genotypes from all the phenotypic groups of the six antioxidant traits from the original shortlisted 270 rice genotypes. Donor lines rich in multiple antioxidant compounds were identified from the population. The population was classified into 4 genetic groups and showed fair degree of correspondence with the antioxidants content. A total of 14 significant marker-trait associations for antioxidants were detected of which 3 QTLs namely *qANC3*, *qPAC12-2* for anthocyanin content and *qAC12* for ABTS activity were validated in the population. Eleven putative QTLs such as *qTAC1.1* and *qTAC5.1* for anthocyanin content; *qSOD1.1*, *qSOD5.1* and *qSOD10.1* for SOD; *qTFC6.1*, *qTFC11.1* and *qTFC12.1* for TFC; *qOZ8.1* and *qOZ11.1* for γ -oryzanol and *qAC11.1* for ABTS were detected to be novel loci. Co-localization of the QTLs detected for *OZ11.1*, *TFC11.1* and *AC11.1* regulating γ -oryzanol, flavonoid and anthocyanin content, respectively while *PAC12.2* for anthocyanin content remained closer to *TFC12.1* for flavonoid content. These QTLs will be useful in the antioxidant improvement programs in rice.

Background

Nutritional health of a country has significant effect on economic performance of that country¹ (Alsan et al., 2006). About half of the global population is dependent on rice for their primary diet, but majority of them are affected by malnutrition, Fe and Zn deficiency, and oxidative stress-related health problems such as stroke, psoriasis, type II diabetes, heart diseases, obesity, cancers, dermatitis, and rheumatoid arthritis^{2,3}. Thus, enrichment of staple foods with Fe, Zn, and antioxidant compounds have been prioritized through biofortification^{4,5,6,7,8}. Since rice is the primary source of nutrition to meet daily calorie and protein intake of more than two billion people around the world⁹ improving nutraceutical value of rice grain enriching with antioxidant compounds could be the best option to onslaught the stress related disorders and to utilize rice as health promoting food to improve human health. Also in recent years, consumption of whole grain pigmented rice enriched with antioxidant compounds is gaining popularity in developed and developing countries as it is advised to improve the health by reducing the risk of such chronic diseases^{10,11}. Thus, rice breeding programs need to focus on the development of nutrient dense rice for which improvement of the antioxidant traits is a necessity to meet the nutritional quality standards.

Antioxidants are present in plants both in enzymatic and non-enzymatic forms: enzymatic antioxidants are catalases, peroxidases, superoxide dismutases, glutathione and other proteins and non-enzymatic antioxidant includes phenolic defense compounds (vitamin E, flavonoids, phenolic acids and other phenols); nitrogen compounds (alkaloids, amino acids and amines), carotenoids and chlorophyll derivatives^{12,13}. The enzymatic antioxidants protect the plant cell from damage caused by reactive oxygen species and acts as defense system for maintaining the structural and functional integrity of a cell by inhibiting the oxidative deterioration to macromolecules like lipid, protein and nucleic acid^{13,14,15}. Hence, improvement of these traits will lead to development of superior quality seed. Non-enzymatic antioxidants such as phenolic acids, flavonoids, anthocyanins and proanthocyanidins, tocopherols and tocotrienols (vitamin E) and γ -oryzanol reported to be higher in pigmented rice (rice with red, black and purple pericarp). The antioxidants show impressive health benefits like reducing oxidative stress and cholesterol levels in human body, lowering the chances of type-2 diabetes, obesity, cancer etc.^{16,17,18}. These antioxidant traits are complex traits, polygenic in nature and quantitatively inherited¹⁹. Understanding the genetic bases of these complex antioxidant traits and identification of major QTLs are essential for marker-assisted breeding for improvement of these phytochemicals to ameliorate the increasing nutrition problems of the rice-eating population and seed quality as well.

Increasing carotenoid content in rice grains remained an important breeding objective over the past decades due to its antioxidative, anti-inflammatory properties and most importantly as dietary source of provitamin A²⁰. Identification of QTLs/gene for higher carotenoid content and development of functional markers is slow in rice as carotenoids are not detected in rice endosperm²⁰. However, there exist a huge potential in the exploitation of genetic variability of the carotenoid content in red and black rice that accumulate high carotenoid content, while white rice accumulates very little^{21,22}. Color of the pigmented rice has several health promoting effect due to two primary pigments, anthocyanidin and proanthocyanidin that are present in the pericarp and aleurone layer of rice grain. The proanthocyanidin content in rice which impart red colour to rice pericarp was considered to be controlled by interaction of *Rc* and *Rd* genes^{23,24,25}. Whereas anthocyanidins that impart purple-black pericarp to rice grain is controlled by two loci, *Pb* and *Pp*²⁶. The *Pb* gene responsible for purple colour has been fine mapped and it was suggested that the *Ra* gene present in the 25 kb of delimited region of the *Pb* locus may be the *Pb* gene, and the purple pericarp characteristic of rice is caused by a two bases deletion (GT) within exon 7 of the *Ra*^{27,28,29}. Two genes, dihydro flavonol reductase (*DFR*), and anthocyanin synthase (*ANS*) located on chromosome 1 controlled the anthocyanin content³⁰. However, a recent study reported that A1 (*Kala1/Rd/OsDFR*), C1 (*OsC1*) determine purple colour of the grain and pattern of anthocyanin pigmentation is determined by the allelic status of A1, C1, and S1 (*OsANST*)³¹. *Kala 4/OsB2/Pb* gene was mainly responsible for black pigmentation in rice pericarp^{32,33,34,35,36}. Although several genes responsible for pericarp pigmentation have already been identified but possibility of additional genes and variants associated with these traits exist and need to be discovered.

The genetic analyses for identification and fine mapping of genes and QTLs for pericarp pigmentation in rice have been published by using various mapping populations^{10,25,35,37,39,40}. However, few reports on QTL mapping are available on gamma-oryzanol, total phenolics content (TPC), total flavonoids content (TFC), ABTS (Azinobis 3-ethyl benzothiazoline-6-sulfonic acid) and SOD (Super oxide dismutase) activity in rice. Flavonoids are the major class of phenolic compounds responsible for color in rice. Rice bran contains seven flavonoids of which Tricin is the key compound. Antioxidants protect the cells against free radicals, which may cause diseases in human. The QTLs, *qPH-12*, *qFL-2-1* and *qAC-1* The genetic control of phenolic content, flavonoid content, and antioxidant capacity in rice have reported by various workers^{10,37,38,40,41}. Mapping report of γ -oryzanol content in rice identified eight QTLs from a single publication⁴². A recent report indicated the possibility of marker-trait association for phenolics, carotenoids, anthocyanin, γ -oryzanol and other antioxidant contents in rice⁴³.

Enhancement of anthocyanin content has been attempted through genetic engineering of anthocyanin biosynthesis transgenes that has generated 'Purple Endosperm Rice'⁴⁴. Attempts have been made to develop few pigmented rice varieties like 'Rubi' and 'Onix' of Brazil⁴⁵ and 'Rice berry' of Thailand⁴⁶. Also, some advanced breeding lines are available^{33,36,47,48,49}. Though several studies reported about genes for these antioxidant traits of which some genes have been fine mapped in certain cases (*Rc*, *Rd*, *Pb*) and each adopted a different gene coding system which is still confusing for which more firm genes/loci should be identified to explain these complex traits¹⁹. There by development of trait-specific markers will accelerate the efforts to breed high yielding pigmented rice varieties.

The genetic diversity and structure of the population will be helpful for detecting marker-trait association which could be useful for trait enhancement in molecular breeding programs. In order to avoid spurious association of marker-phenotype in a population, population structure (Q) with relative kinship (K) analyses is essential to check the correctness of the panel population composition for linkage disequilibrium (LD) mapping analyses^{50,51,52}. Thus, association estimate based on both the models of Generalized linear model (GLM) and Mixed linear model (MLM) is considered appropriate for mapping complex traits that have shown to perform better than other model analyses.

Association mapping based on linkage disequilibrium has emerged as a powerful alternative strategy for identifying genes or quantitative trait loci (QTL) for various complex traits in plants in a natural variable population by examining the marker–trait associations. In the present study, we have identified the marker loci/QTLs underlying the naturally occurring variations of antioxidant traits and antioxidant activity by association mapping. We have used a panel population of 120 genotypes (67 white and 53 red grain) shortlisted from 270 diverse genotypes (121 white and 149 red grain) of India through phenotyping of six physio-biochemical traits (carotenoids, SOD, total anthocyanins, γ -oryzanol, TFC, and ABTS). Rice microsatellite markers were used to study the population genetic structure, diversity and association of molecular markers with these phytochemical traits to improve nutritional quality of rice.

Materials And Methods

Seed material

The study material includes 270 genotypes (landraces and cultivars) of 121 white and 149 colored rice grains. The initial population was shortlisted on the basis of maturity duration (upto 135 days) and kernel color (red, black, purple and white) from about 1000 germplasm lines. Seeds of these germplasm were collected from Gene bank, ICAR-NRRI, Cuttack and were grown in the experimental plot of the Institute during wet season, 2018. The genotypes were grown in randomized complete block design in three rows each following spacing of 20 x 15 cm in two replications following recommended package and practices. Each replication is divided into 5 blocks accommodating 54 germplasm lines in each block. Panicles of middle row plants of each replication were harvested, sun dried for 4-5 days to reduce the moisture content to 11-12 %, stored for three months to remove dormancy and then used for estimation of superoxide dismutase, flavonoids, anthocyanins, carotenoids, γ -oryzanol and antioxidant activity. A representative panel population containing 120 germplasm lines was prepared from the original 270 germplasm lines (120 genotypes consisting of 67 white and 53 red grain rice). The panel population was raised during wet seasons, 2019 and 2020 and the antioxidants were estimated. The panel population (120) is used for genotyping for association mapping of antioxidant traits (Table 1). The plant experiments were performed in accordance with relevant **guidelines and regulations**.

Phenotyping for antioxidant traits

The seed samples were dehulled by the Satake rice huller, Japan and were ground into flour by a grinding machine (Glenmini grinder) and sieved through 100 mesh size and then stored at 4^o C for the experiment. Analysis of all the traits were based on dry matter basis except for carotenoid content which were estimated based on fresh weight basis. Leaf samples from 10 days old seedling grown on petriplate at 30^o C were used for estimation of carotenoids (mg g⁻¹) by following the protocol of Davis⁶⁸. Seed enzymatic antioxidant, super oxide dismutase (SOD: unit g⁻¹) was estimated as per the procedure of Madamanchi et al.⁶⁹. Non-enzymatic antioxidant, total anthocyanin content (TAC: mg 100 g⁻¹) was estimated by the procedure of Fuleki and Francis⁷⁰. Estimation of γ -Oryzanol (GO:mg 100 g⁻¹) was done according to Bucci et al.⁷¹ with minor modifications. Total flavonoids content (TFC) was estimated as per the procedure of Eberhardt et al.⁷² and expressed as catechine equivalent (mg CEt 100 g⁻¹). Antioxidant activity, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging was assayed by modified protocol of Serpen et al.⁷³ and expressed as % inhibition.

Statistical analysis

Cropstat software 7.0. was used for analysis of variance (ANOVA) for each trait including the estimation of mean, range, and coefficient of variation (CV %). Pearson's correlation coefficients were analyzed to find out the relationship among the various antioxidant traits, based on the mean values of the 120 genotypes and presented in correlation matrix heatmap by using PAST software. The germplasm lines were classified into six groups as very high, high, medium, low and very low category based on mean values antioxidant traits.

Genomic DNA isolation, PCR analysis and selection of SSR markers

The genomic DNA was isolated from 15 days-old seedlings of the germplasm lines by adopting CTAB method⁷⁴ (Murray and Thompson 1980). The 136 SSR (simple sequence repeat) markers were taken from the database (<http://gramene.org/>) available in the public domain (Supplementary Table 5). The DNA fragments were resolved in gel electrophoresis for quantification of isolated DNA. PCR analysis was done using the markers selected based on positions covering all the chromosomes to illustrate the diversity and to identify the polymorphic loci among the 120 rice germplasm lines (Table 1). Conditions of reaction was set to initial denaturation step (2 min, 95^o C), followed by 35 cycles of denaturation (30 s, 95^o C) and annealing/extension (30 min, 55^o C), extension (2 min, 72^o C), final extension (5 min, 72^o C) and store at 4^o C (infinity). The PCR products were electrophoresed using 3 % agarose gel containing

0.80 g ml⁻¹ ethidium bromide and. 50 bp DNA ladder was used to determine the size of amplicons. The gel was run for 4 h at 2.5 V cm⁻¹ and photographed using a Gel Documentation System (SynGene). Earlier publications of ^{75,76,77} were followed for DNA isolation, electrophoresis and imaging techniques.

Molecular data analysis

Presence or absence of amplified products obtained on the basis genotype-primer combination was used to score the data. A binary data matrix was used as discrete variables for the entry of our result data. The parameters namely polymorphic information content (PIC), observed heterozygosity (H), number of alleles (N), major allele frequency (A) and gene diversity (GD) for each SSR locus⁷⁸ was analysed by using, 'Power Marker Ver3.25' software. A Bayesian model based clustering approach STRUCTURE 2.3.6 software was used to analysis genetic data and obtain population structure⁷⁹. STRUCTURE software was run with K varying from 1 to 10, with 10 iterations for each K value to derive the ideal number of groups. A high throughput parameter set of burn-in period of the 150,000 followed by 150,000 Markov Chain Monte Carlo (MCMC) replications was adapted during the running period. Highest value of ΔK was pick up from Evanno table used to detect the subpopulation groups from the panel of populations in the next step. The maximal value of L (K) was identified using the exact number of sub-populations. The model choice criterion to detect the most probable value of K was ΔK , an ad-hoc quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by STRUCTURE⁸⁰. For estimation of the ΔK -value as function of K showing a clear peak as the optimal K-value Structure Harvester was used. The principal coordinate analysis of all the genotypes and unweighted neighbor joining unrooted tree for Nei coefficient dissimilarity index⁸² with bootstrap value of 1,000 were obtained by using DARwin5 software⁸³. Analysis of molecular variance (AMOVA) using GenAlEx 6.5 software was used to estimate the presence of molecular variance across the whole population, within a population and between the sub-population structures (FIT, FIS, FST) calculated by the deviation from Hardy-Weinberg expectation. The procedures followed in earlier publications were adopted for molecular data analysis^{51,67,84}.

To analyze the marker-trait association for mapping study of the seed antioxidant traits in rice, the software "TASSEL 5.0" was used. General linear model and Mixed linear model in TASSEL 5.0 were used to perform the genetic association between the phenotypic traits and molecular markers⁸⁵. By considering the significant p-value and r^2 value convincing associated markers were identified. The associations of markers were further confirmed by the Q-Q plot generated by the software. Linkage disequilibrium plot was obtained using LD measured r^2 , between pair of markers is plotted against the distance between the pair. Also, the accuracy of the marker-trait association by estimating the FDR adjusted p-values (q-values) using R software as described in the earlier publications^{6,61,67}.

Results

Phenotyping of the population for antioxidant content in rice

A total of six antioxidant compounds including one antioxidant enzyme namely superoxide dismutase, flavonoids, anthocyanins, carotenoids, γ -oryzanol and ABTS were estimated from 270 genotypes during wet seasons of 2019 (Supplementary Table 1). Wide variation exists among the germplasm lines for 6 antioxidant compounds. The germplasm lines were classified into 5 groups based on the phenotyping results of each compounds (Fig. 1). The frequency distribution of germplasm lines showed various groups or populations for each compound and enzyme (Fig. 1). A panel population was prepared by selecting 120 genotypes representing each group and trait from the original population of 270 germplasm lines (Table 1; Fig. 2). The mean estimates of 6 antioxidants obtained from the representative panel population showed wide variation among the genotypes for each trait (Table 1). Very high values of carotenoid content was found in grmplasm lines Ac. 44598, Ac. 44597, and Ac. 9005. Very high TAC content was estimated from Ac. 43670, Ac. 43660 and Ac. 43675. Germplasm lines namely Ac. 9063, Ac. 20371 and Ac-20627 showed very high level of γ -oryzanol in the seed. Good donor lines having very high TFC content, namely Ac. 43670, Ac. 43660, Ac. 44646, Ac. 44592, Ac. 44595, Ac. 43737, Ac. 43738 and Ac. 43676 were identified. The SOD level was very high in the seeds of genotypes such as Ac. 20317, Palinadhan-1, Ac.20362, Ac. 20328, Gochi, Chatuimuchi, Ac. 20770, Ac. 20920, Ac. 20907, Magra and Chinamal. The potential donors identified from the population for showing very high level of ABTS were Ac. 44592, Ac. 43670, Ac.4460, Ac. 44595, Ac. 44588, Ac. 43660, Ac. 43738 and Ac. 43732. However, a higher level of antioxidants such as more than 3 compounds in Ac. 44592, Ac. 44646, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac.43669 were indentified from the germplasm lines.

Relatedness among germplasm lines for antioxidant compounds through Genotype-by-trait biplot analysis

The scatter diagram was plotted taking the first two principal components to generate genotype-by-trait biplot graph for the 6 antioxidants estimated from the 120 genotypes present in the panel (Fig. 3). The first and second principal components showed 68.3 and 19.8 % of the total variability with eigen values of 8064 and 2342, respectively (Supplementary Fig. 1). γ -oryzanol contributed maximum diversity among the 6 antioxidant compounds in the genotypes present in the panel followed by TFC and ABTS (Fig. 3). The scattering pattern of genotypes in the 4 quadrants indicated that genotypes containing high estimates of antioxidants are placed in the quadrant I (top right) and II (bottom right). Higher estimates of antioxidants with multiple compounds containing genotypes have been encircled in the figure (Fig. 3). The top right (1st quadrant) and bottom right (2nd quadrant) accommodated majority of the genotypes containing high estimates of antioxidant compounds. The 3rd (bottom left) quadrant kept most of the germplasm lines with moderate in antioxidant compounds while the 4th quadrant (top left) accommodated majority of poor in antioxidant compounds containing germplasm lines (Fig. 3).

Nature of association among antioxidant traits

The association among 6 antioxidant traits revealed a strong positive correlation ($r \geq 0.7$) of TAC with TFC and TFC with ABTS. Moderate positive correlation (r : 0.5-0.7) of TAC with ABTS and weak positive correlation ($r < 0.5$) was observed for carotenoid with GO (Fig. 4). These antioxidant traits positively or negatively correlated may be controlled by the closely linked genes or because they might be structurally related. Therefore, a variety that accumulates high concentrations of one antioxidant may contain higher quantity of other correlated antioxidants.

Genetic diversity parameters analysis

The panel population containing 120 germplasm lines which exhibited wide variation for the antioxidants was genotyped using 136 SSR markers. The genetic diversity parameters estimated from the panel population are presented in the Table 2. Genotyping results showed a total of 508 markers alleles from the population exhibiting mean alleles of 3.74 per locus. The number of alleles per locus ranged from 2 to 7 per marker. The highest numbers of alleles were produced by the marker RM493 in the studied panel for antioxidants content in rice. The measure for the variation by a marker in the population was analysed by the of availability major allele frequency parameter. The average major allele frequency linked to the polymorphic markers was computed to be 0.561 which showed a range of 0.279 (RM8044) and 0.925 (RM6054) (Table 2). The informativeness of a genetic marker is estimated by the PIC value. It ranged from 0.137 (RM6045 and 6054) to 0.787 (RM493) with average value of 0.496. The observed mean heterozygosity (H_o) in the population was 0.116 which varied from 0.00 to 0.958 (RM3735). Twenty marker loci showed 0.00 H_o value in the panel population. The gene diversity (H_e) which gives a measure of genetic diversity in the panel population ranged from 0.142 (RM6054) to 0.813 (RM493) with a mean value of 0.555.

Population Genetic Structure Analysis

The diverse population for antioxidants was genotyped for genetic structure and analyzed by adopting probable sub-populations (K) and selecting higher delta K-value by applying the STRUCTURE 2.3.6 software. The rate of change in the log probability of data between successive K values is the delta K value used in the analysis. The panel population was categorized into two sub-populations with a high ΔK peak value of 264.2 at K = 2 among the assumed K (Supplementary Table 2; Supplementary Fig. 2). The two subpopulations were in the proportion of 0.277 and 0.723 for population 1 and population 2, respectively. But, the subpopulations showed poor correspondence with antioxidants containing genotypes present in the studied population. Therefore, next ΔK peak at K=3 was compared in which the population was classified into 3 subpopulations. The 3 subpopulations showed genotypes in the proportion of 0.208, 0.689 and 0.103 in the inferred clusters for the sub-population 1, 2, and 3, respectively. The F_{st1} , F_{st2} and F_{st3} values were 0.3392, 0.1664 and 0.3701 for the sub-population 1, 2, and 3, respectively (Supplementary Table 2; Supplementary Fig. 3). The ancestry value of $\geq 80\%$ obtained in a genotype grouped the genotype into the particular subpopulation.

The assumed subpopulations at K=3 differentiated the germplasm lines for antioxidant compounds but did not clearly separate for the SP2 and SP3 subpopulations. Hence, next ΔK peak at K=4 was considered for the subpopulations in which the population was classified into 4 genetic groups. The antioxidant compounds present in the studied population showed a fair correspondence at K=4 with inferred structure values in the subpopulations. Majority of the germplasm lines with high to very high antioxidants were present in the subpopulations 4. The moderate value antioxidant containing germplasm lines are present in the subpopulation 2. Poor and moderate antioxidants containing germplasm lines were in subpopulation 1 while very poor to poor types are in the subpopulation 3 (Table 3; Fig. 5). The alpha value of the panel showed a low value ($\alpha=0.0578$) estimated by the structure analysis at K=4. Positively skewed leptokurtic distributions were observed for the mean alpha-value while normally skewed leptokurtic distributions detected for all the 4 F_{st} values for the panel population showing a distinct variation in the distribution among the F_{st} values (Supplementary Fig. 4).

Molecular variance (AMOVA) and LD decay plot analysis

The closely related plants among themselves in a population are grouped into different subpopulations. The genetic variations obtained within and between the subpopulations at K=4 were estimated by the analysis of molecular variance (AMOVA) (Table 4). The genetic variations estimated at K=4 was computed to be 6 % among the populations, nil among individuals and 94 % variation within individuals of the panel population. Wright's F statistics was used to know the deviation from Hardy-Weinberg's prediction. The parameter F_{IS} was used to know the uniformity of individuals within the subpopulation and F_{IT} for individual within the total population for differentiation of the population. The F_{IT} and F_{IS} of the total population and within population estimated on the basis of 136 marker loci showed -0.148 and -0.235, whereas the total population had F_{ST} value of 0.071 between the 4 subpopulations. F_{st} is used to know the subpopulations or population differentiation within the total population. A clear differentiation among the 4 subpopulations was observed from each other for their distribution pattern was based on the F_{st} values (Supplemental Fig. 4).

The association of alleles by different loci in a nonrandom manner is utilized in the marker-trait association analysis. Existence of marker-trait association is dependent on the LD decay rate in a population over a time period. The LD decay rate will indicate the possibility of new genes or allelic variants controlling the antioxidant compounds associated with molecular markers for these traits. Syntenic r^2 value was used to plot the linkage disequilibrium decay of the population versus the physical distance in million base pair (Fig. 6A). Tightly linked markers had higher r^2 value and the average r^2 values rapidly decreases for increase in linkage distance. In the LD plot it is observed that the LD decay in the beginning was delayed in the studied panel populations. However, a decline of LD decay was noticed in the curve for the associated markers at about 1-2 mega base pair and thereafter a gradual and very slow decay was noticed from the graph. The graph clearly indicated the continuance of linkage disequilibrium decay in the population for the studied antioxidant compounds in the rice population. The limitation for LD decay depends on non-random mating, mutation, selection, migration or admixture, and genetic drift will influence the estimates of LD. This LD decay plot also provided clue for creation of genetic admixture groups for various antioxidants compounds in the normal population. A similar trend was also noticed in the marker 'P' versus marker 'F' and marker R^2 (Fig. 6B) curve. The detected markers from this study indicated the strength of the markers for the studied antioxidant compounds.

Principal coordinates and cluster analyses for genetic relatedness among the germplasm lines

The two dimensional plot for principal coordinate analysis (PCoA) was constructed based on the genotyping results of 136 SSR markers which classified the 120 germplasm lines as per the genetic relatedness among the lines (Fig. 7). The inertia showed by component 1 was 11.73 % while 7.49 % exhibited by the component 2. The germplasm lines were allotted different spots in the four quadrants forming 3 major groups (Fig. 7). The biggest group accommodated all the germplasm lines of the subpopulation 2 and 3 together and clustered in the 2nd (bottom right) quadrant. The genotypes in the 1st quadrant are divided into

2 groups of which one group on the top of the 1st quadrant forms the sp3 subpopulation with low to very low in antioxidant content in the seeds. The other group near to the axis1 is all admix type of germplasm lines. Few germplasm lines of quadrant 2 and closer to the axis 1 are also admix genotypes. The admix genotypes present on both sides of axis 1 are depicted in black colour (Fig. 7).

The germplasm lines containing high to very high mean values of antioxidants are grouped together forming the subpopulation 4. This subpopulation is present on the quadrant 3 (top left) and 4 (bottom left) and encircled in red color. The germplasm lines rich in antioxidants are placed both side of the axis 1 on the quadrant 3 and 4 (Fig. 7). The PCoA distributed all the germplasm lines into the four quadrants classifying into 4 clusters and a separate admixture group. The subpopulations clustered by PCoA showed correspondence with the population structure (Fig. 7). Germplasm lines namely Ac. 44594, Ac. 43669, Ac. 44597, Ac. 44588, Ac. 43737, Ac. 44595, Ac. 43676, Ac. 44597, Ac. 44592, Ac. 43738, and Ac. 44646 are placed together in one structure group present in the quadrant III & IV and are rich in antioxidants. The PCoA placed germplasm lines in the Quadrant II which were mostly average in antioxidant content. This quadrant formed the group by placing all the germplasm lines of subpopulation 1 and 2.

The Wards's clustering broadly grouped all the genotypes into two major groups. The largest cluster, cluster 1 accommodated 111 germplasm lines in which all poor to average containing antioxidants were present. The cluster II had 9 germplasm lines only. The dendrogram placed all the germplasm lines in this cluster II which were rich in antioxidant content. This cluster again subdivided into 2 sub groups which further divided into 6 sub-sub clusters. The cluster I was divided into two main sub clusters which finally divided into 32 small groups. All the clusters and small groups accommodated in the Ward's clustering approach were based on the antioxidant compounds content in the germplasm lines (Fig. 8A).

The cluster analysis discriminated the germplasm lines on the basis of genotyping of 136 SSR markers and placed the genotypes into different clusters which corresponded with the studied antioxidant traits. The unweighted-neighbour joining tree differentiated the genotypes into 4 different clusters (Fig. 8B). The Cluster for Subpopulation 4 was differentiated from SP₂ by the presence of germplasm lines containing high antioxidants in it while moderate to high containing genotypes were in the subpopulation 2. The green coloured portion of the tree is designated as SP4 while blue for SP2. The very poor antioxidants containing germplasm lines were in the subpopulation 3 depicted in red colour in the tree. Majority of the germplasm present in the subpopulation 1 were poor to medium in antioxidant content and shown in pink colour. The germplasm lines with admix type of population are depicted in black color in the neighbour joining tree neighbour joining tree neighbour joining tree (Fig. 8B).

Marker-trait association for antioxidant compounds in rice

Marker-trait associations was computed for 6 antioxidant compounds by using Generalized Linear Model (GLM) and Mixed Linear Model (MLM/ K+Q model)) in the TASSEL 5 software. The marker-trait association values were compared at less than 1% error *i.e.* 99% confidence ($p < 0.01$). A total of 57 and 23 significant marker-trait associations were detected for 5 antioxidant compounds by GLM and MLM, respectively at $p < 0.01$. The range for marker R^2 values was from 0.0477 to 0.159 by GLM while 0.0607 to 0.1169 detected by Mixed Linear Model (Supplementary Table 3; Supplementary Table 4). A total of 15 significant marker-trait associations were detected by both the models for 5 antioxidant compounds present in the seed at $p < 0.01$ (Fig. 9A). Significant association of 5 SSR markers with TAC; 3 with SOD, TFC; 2 with GO, and ABTS were detected. Five antioxidant compounds present in the studied germplasm lines showed higher marker R^2 (> 0.1) with low p (< 0.01) values in the associations study includes SOD with RM405 and GO with RM3701 (Table 5; Fig. 9A). The Q-Q plot also confirmed the association of these markers with the associated antioxidant traits in rice (Fig. 9B).

Four markers namely RM440, RM5638, RM253 and RM5626 showed significant associations with compound, TAC detected by GLM and MLM models at $p < 0.01$ showing > 0.05 marker R^2 value. The QTLs controlling anthocyanin content in these genotypes are detected to be located markers RM440, RM5638, RM253 and RM5626 at 92.7, 86, 37 and 99 cM on the chromosome 5, 1, 6 and 3, respectively. Three markers namely RM582, RM467 and RM405 located at 66.4, 46.8 and 28.6 cM positions on chromosome 1, 10 and 5, respectively were associated with the compound, SOD. TFC content was detected to be associated with markers RM 3701, RM235 and RM494 present at 45.3, 101.8 and 124.4 cM on chromosome 1, 11 and 12, respectively. The QTLs for ABTS showed significant associations with RM3701 and RM235 on chromosome 1 and 11, respectively. The marker RM216 showed association with SOD at very low p -value and high marker R^2 value of > 0.10618 analyzed by the GLM only. The QTLs for antioxidant compound, OZ showed significant associations with RM3701 and RM502 on chromosome 1 and 8, respectively (Table 5; Fig. 9A). The Q-Q plot also confirmed the associations of these markers with the estimated antioxidant compounds in rice (Fig. 9).

Association mapping study for antioxidant compounds in rice seeds identified co localization of QTLs controlling antioxidant compounds in rice. It is observed that same marker showed significant associations with different antioxidant compounds in rice by both the models (Table...). Significant associations of marker RM3701 with antioxidant compounds GO, TFC, and ABTS present in the germplasm lines were detected. In addition, it was also detected association of RM235 with compounds TAC, TFC, and ABTS by both the models at $< 1\%$ error and $p < 0.01$ (Table 5). While considering marker association analyzing by GLM, the marker RM494 showed association with both carotenoids and TFC. In addition, RM494 was associated with both SOD and TFC content analyzed by MLM.

Discussion

The genotypes shortlisted for antioxidants mapping showed variation from each other with respect to 6 antioxidant compounds (Supplementary Table 1; Table 1). Also, few compounds showed higher correlation values among themselves. This provides enough clues for improvement of the antioxidant compounds through the correlated compounds based on the coefficients of the correlations and the availability of higher genetic variations for the antioxidants in the population (Table 1; Table 4). Earlier reports of high variations for antioxidants were also published by few researchers^{17,43,53,54,55}. The

available diversity in the population based on 136 markers data and 6 antioxidants showed clear cut groups in the studied population (Table 2). A moderate to high PIC value coupled with better informative markers in the studied population will be useful for improvement of antioxidants breeding program. The Jayapur tract of Odisha, known for secondary centre of origin of rice, germplasms from this tract has also been included in this study. Also, the shortlisted germplasm lines used as materials in this study were collected from the states known for their rich rice genetic diversity⁴³.

The genotypes rich in multiple antioxidant compounds were identified in Ac. 44646, Ac. 44594 and Ac. 44595; 7 in Ac. 43660, and Ac. 20282 and 6 in Ac. 43738, Ac. 43660, and Ac. 44592. These germplasm lines will be good source materials in the antioxidant improvement programs (Table 1; Supplementary Table 2). Therefore, it is expected that breeding program with inclusion of parental lines from this population will be effective for antioxidants improvement. Structure analysis categorized the population into 4 subpopulations showing different *F_{st}* values supported the availability of linkage disequilibrium groups in the population. Detection of a low alpha value and existence of many genetic admix types germplasm lines in the population indicated that the antioxidants content evolved from a single source initially during evolution of the trait. Different antioxidant compounds were subsequently formed different admix genotypes with different ancestry value during evolutionary process. A good correspondence of genetic structure and different traits was previously published by many researchers^{55,56,43}. Additionally, publications on phenotype of various traits and structure correlation have been published by many workers^{51,52,57,58,59,60,61}.

Five antioxidant compounds were found to be associated with 12 SSR markers analyzed by both GLM and MLM approaches (Table 5). The markers association detected by both the models at *p*<0.01 and low 'p' value are considered to be very robust and useful markers for improvement program. The strongly associated SSR markers namely RM440, RM235, RM5638, RM253 and RM5626 for TAC; RM582 and RM467 for SOD; RM 3701, RM235 and RM494 for TFC; RM3701 and RM235 for ABTS; RM3701 and RM502 for GO will be useful markers for selection of antioxidant compounds (Table 5). The Q-Q plot also confirmed the associations of these markers with the antioxidant compounds in rice (Fig. 9B).

The QTLs for anthocyanin and proanthocyanin content in rice was reported by earlier researchers^{23,35,39,62}. In the present investigation, the QTLs for total anthocyanin content were detected on chromosome 1, 3, 5 and 12. The QTLs on chromosome 1 and 3 were at position 86 cM and 99 cM, respectively. The genes, *qANC3* and *qPAC12-2* reported by Xu et al.³⁹ were at the same position as in the present investigation. Therefore, these two QTLs were validated in our study. However, another two QTLs located on chromosome 1 and 5 detected in this investigation are not reported by earlier researchers. These two QTLs may be new loci which affect TAC in rice and designated as *qTAC1.1* and *qTAC5.1*. Three markers namely RM582, RM405 and RM467 showed association with SOD and located on chromosome 1, 5 and 10 at 66.4, 28.6 and 46.8 cM, respectively. The QTLs for SOD located in these locations were not reported in earlier mapping studies. These QTLs designated as *qSOD1.1*, *qSOD5.1* and *qSOD10.1* may be new loci controlling the SOD activities in rice seeds. Total flavonoids content (TFC) is detected to be associated with 3 regions on the chromosome 6, 11 and 12. Earlier publication showed the presence of QTLs on chromosome 4, 7, 8, 9 and 10¹⁰. The main flavonoids structural genes located on chromosome 11 for CHS⁶²; on chromosome 3 for CHI⁶³; on chromosome 4 for F3H⁶⁴; on Chromosome 1 for DFR⁶⁵ and ANS⁶⁶. The gene CHS on chromosome 11 was at 3.3 cM. We detected at 45.3 cM. Therefore, all these 3 detected QTLs which affect total flavonoids are new loci and designated as *qTFC 6.1*, *qTFC11.1* and *qTFC12.1*.

Food containing γ-oryzanol (OZ) is well recognized for health benefit. This is a mixture of several compounds present in the rice bran layer. γ-oryzanol content in this study showed significant association with markers on the chromosomes 8 and 11. However, QTLs previously reported by earlier workers reported on the chromosome 1, 5 and 9 in Asominori/IR24 RILs⁴². But they detected another 5 QTLs for OZ in the backcross lines of Sasanishiki/Habataki//Sasanishiki. These two new loci detected in this investigation are new loci controlling γ-oryzanol and designated as *qOZ8.1* and *qOZ11.1*. The QTLs for ABTS showed significant associations with RM3701 and RM235 on chromosome 11 and 12, respectively. The candidate gene controlling ABTS and present on the chromosome 11 is not reported by earlier researchers. Hence, the detected QTL for ABTS on chromosome 11 at 45.3 cM position is a new locus controlling the trait and designated as *qAC11.1*. However, the other detected association for the trait on chromosome 12 is located at 26.1 Mb position. In earlier mapping publication reported the gene on the chromosome 12 at 25.2 Mp position³⁸. As our detected QTL position for ABTS is close to the reported QTL *qAC12*, this QTL is validated in our mapping population and can be useful in the marker-assisted breeding for ABTS improvement.

Two markers were observed to be associated with more than one antioxidant traits analyzed by both the models at <1% error and *p*<0.01. Marker RM3701 showed associations with GO, TFC, and ABTS present in the germplasm lines. Also, RM235 was associated with traits, TAC, TFC, and ABTS by both the models (Table 5). These observations indicated the close location of the candidate genes and simultaneous inheritance of these QTLs. Hence, simultaneous improvement of both these antioxidant traits will be effective. In recent publications also suggested easy improvement of the co-localized genes controlling various traits in rice^{6,51,61,67}. Results of the present investigation showed that association mapping is an effective method to find more potential loci for antioxidant compounds and antioxidant capacity in rice. The detected loci will further be fine mapped for application in marker-assisted breeding for improvement of antioxidants in rice.

Conclusion

Consumption rice containing higher content of antioxidants has many health benefits. Donor lines rich in antioxidants compounds with > 3 antioxidant compounds were identified in higher level of antioxidants. The germplasm lines Ac. 44592, Ac. 44646, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac. 43669 were identified for rich germplasm lines carrying more than 3 antioxidants. Five antioxidant traits viz., superoxide dismutase, flavonoids, anthocyanins, γ-oryzanol and ABTS were mapped in the panel population using 136 SSR markers through association mapping. Wide variations were observed for the six antioxidants content in the population. The population was classified into 4 genetic structure groups by the structure analysis. The existence of linkage disequilibrium for the antioxidant traits was established based on the population's fixation indices. The population was classified into four subpopulations which showed fair degree of correspondence with the antioxidants content. A total of 14 significant marker-trait associations for antioxidants were detected of which 3 QTLs namely *qANC3*, *qPAC12-2* for anthocyanin content and *qAC12* for ABTS activity were validated in the population.

These 3 QTLs are useful in the marker-assisted breeding programs. Eleven putative QTLs such as *qTAC1.1* and *qTAC5.1* for anthocyanin content; *qSOD1.1*, *qSOD5.1* and *qSOD10.1* for SOD; *qTFC6.1*, *qTFC11.1* and *qTFC12.1* for TFC; *qOZ8.1* and *qOZ11.1* for γ -oryzanol and *qAC11.1* for ABTS were detected to be novel loci. Co-localization of the QTLs detected for *OZ11.1*, *TFC11.1* and *AC11.1* regulating γ -oryzanol, flavonoid and anthocyanin content, respectively while *PAC12.2* for anthocyanin content remained closer to *TFC12.1* for flavonoid content. These strongly associated QTLs will be useful in the antioxidants improvement programs in rice.

Declarations

Ethics approval and consent to participate: The authors declare that this study complies with the current laws of the country in which the experiments were performed.

Consent for publication: Not applicable

Availability of data and material: The data generated or analyzed in this study are included in this article.

Competing interest: The authors declare no conflicts of interest

Funding: No externally aided funding was utilized for this research work

Authors contribution: SK Pradhan and E Pandit conceived the study. SR Barik, R Bastia, A Behera, A Moharana, and E Pandit conducted the genotyping work. P Sanghamitra, R Bastia, SP Mohanty, N Basak and SK Pradhan performed the phenotyping work. SR Barik analyzed the data. SK Pradhan and P Sanghamitr wrote the manuscript. All the authors read and approved the final version.

Acknowledgement

The authors acknowledge the support of Director, ICAR-National Rice Research Institute, Cuttack for providing all the necessary facilities including the funding for conducting the experiment.

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Tables

Table 1. Mean values of carotenoids, SOD, TAC, GO, TFC and ABTS antioxidants estimated from 120 genotypes present in the panel population

Sl.No.	Kernel colour	Genotype/Vernacular name/Accession no.	Carotenoids	SOD	TAC	GO	TFC	ABTS
1	White	AC5993	0.115	0.239	0.209	43.750	12.333	8.853
2	White	AC6221	0.423	0.101	0.159	47.375	13.333	8.952
3	White	AC6183	0.182	0.015	0.102	47.125	13.889	14.119
4	White	AC6170	1.165	0.176	0.090	52.250	13.333	11.063
5	White	AC6023	0.112	0.280	0.143	33.313	13.000	10.522
6	White	AC6172	0.297	0.181	0.225	34.125	13.444	7.569
7	White	AC6027	0.133	0.175	0.141	38.188	12.333	7.983
8	White	AC6007	0.287	0.192	0.027	32.125	13.111	7.983
9	White	AC9006	1.014	0.284	0.064	70.438	17.889	11.412
10	White	AC9021	0.444	0.199	0.083	76.313	22.000	11.769
11	Red	AC9028	0.776	0.216	0.250	87.500	45.556	36.976
12	White	AC9030	0.686	0.150	0.123	39.563	18.667	11.555
13	White	AC9035	0.262	0.196	0.117	49.500	17.667	11.698
14	Red	AC9038	0.371	0.241	0.459	28.438	47.000	41.341
15	White	AC9043	0.308	0.175	0.061	39.688	18.444	8.131
16	White	AC9044A	0.713	0.221	0.048	49.938	17.556	15.906
17	Red	AC20920	1.264	0.312	0.325	54.125	43.889	26.061
18	Red	AC20907	0.919	0.308	0.551	64.750	52.444	26.501
19	White	AC20845	1.257	0.265	0.102	61.250	18.889	6.442
20	Red	AC20770	1.379	0.313	0.568	62.688	62.333	35.959
21	Red	AC20627	1.164	0.245	0.451	93.375	45.333	22.694
22	White	AC20686	0.968	0.290	0.073	43.188	21.778	4.539
23	White	AC20664	0.828	0.256	0.070	51.938	19.778	7.028
24	Red	AC20614	0.727	0.273	0.609	85.250	62.841	38.448
25	White	Jhagrikartik	0.080	0.209	0.167	39.688	15.222	10.623
26	White	Dadghani	0.411	0.206	0.130	51.875	16.000	12.606
27	White	Shayam	0.455	0.196	0.170	58.313	19.333	12.677
28	White	Basumati-B	0.091	0.177	0.124	55.750	20.667	15.935
29	Red	Bharati	0.108	0.235	0.442	41.250	35.667	33.669
30	White	Joha	0.094	0.248	0.155	41.688	17.000	11.402
31	Red	Adira-1	0.350	0.137	0.943	46.750	114.222	39.115
32	Red	Adira-2	0.511	0.094	0.901	54.313	80.111	38.316
33	Red	Adira-3	0.472	0.039	2.996	48.750	79.667	38.099
34	Red	PK6	0.217	0.112	1.168	46.125	62.222	33.091
35	Red	Vachaw	0.388	0.078	1.568	47.563	54.111	39.317
36	Red	Kozhivalan	0.476	0.007	0.684	51.500	67.667	27.279
37	Red	Marathondi	0.479	0.059	0.501	45.188	45.556	35.626
38	Red	Ezhoml-2	0.234	0.035	0.801	46.688	85.667	33.512
39	Red	Jyothi	0.437	0.062	0.901	56.750	58.889	31.916
40	Red	Kantakapura	0.947	0.068	0.417	39.000	62.333	36.994
41	Red	Kantakaamala	1.202	0.116	0.451	34.875	60.111	31.503
42	Red	Kapanthi	0.989	0.177	0.451	10.813	41.444	41.757
43	White	Karpurkanti	1.052	0.155	0.079	44.625	18.333	12.645

44	Red	Kathidhan	0.087	0.143	0.601	25.750	35.222	28.107
45	Red	Kundadhan	0.489	0.008	0.876	30.063	56.556	39.595
46	Red	Champaeisiali	0.360	0.222	0.534	20.688	31.444	30.275
47	White	Latamahu	0.493	0.189	0.141	23.375	21.444	13.584
48	Red	Latachaunri	0.507	0.211	1.018	19.875	50.444	30.925
49	White	AC10608	0.427	0.087	0.108	43.125	12.333	10.414
50	White	AC10187	0.395	0.159	0.085	30.063	37.111	12.981
51	Red	AC10162	0.259	0.192	0.526	45.000	81.333	32.397
52	White	AC7282	0.136	0.047	0.084	37.063	20.667	10.335
53	White	AC7269	0.119	0.005	0.204	43.938	14.111	10.189
54	White	AC7134	0.418	0.144	0.118	46.438	17.333	6.841
55	White	AC7008	0.913	0.011	0.078	42.438	22.444	9.534
56	White	AC9093	0.357	0.215	0.061	45.750	17.000	11.270
57	White	AC9090	0.255	0.221	0.079	48.438	16.667	10.556
58	White	AC9076A	0.899	0.159	0.048	43.688	22.889	12.126
59	Red	AC9065	0.353	0.176	0.359	44.875	61.778	30.485
60	Red	AC9063	0.860	0.235	0.375	110.563	52.222	23.538
61	White	AC9058	0.573	0.126	0.055	5.313	23.222	11.698
62	White	AC9053A	0.154	0.159	0.053	42.000	17.333	9.415
63	Red	AC9050	0.395	0.191	0.388	28.313	54.889	32.411
64	White	AC9005	1.612	0.268	0.126	47.375	24.333	14.622
65	White	AC20389	1.247	0.279	0.035	66.250	19.333	10.102
66	White	AC20371	0.839	0.284	0.083	110.000	32.000	6.149
67	Red	AC20423	0.713	0.182	0.434	46.625	53.000	33.031
68	White	AC20362	0.811	0.312	0.077	68.750	19.222	10.688
69	White	AC20328	1.331	0.312	0.078	67.500	22.000	6.076
70	White	AC20317	0.870	0.332	0.102	79.063	23.444	10.542
71	Red	AC20282	1.118	0.201	1.043	84.500	76.889	42.167
72	Red	AC20246	1.083	0.279	2.846	67.875	69.333	41.947
73	Red	AC20347	1.188	0.292	0.272	57.313	23.778	27.906
74	White	Palinadhan-1	0.094	0.342	0.150	38.313	21.000	14.589
75	White	Chatuimuchi	0.525	0.322	0.120	49.875	18.778	16.714
76	White	Uttarbangalocal-9	0.098	0.296	0.060	51.500	18.333	15.439
77	White	Gochi	0.098	0.323	0.118	42.000	22.000	14.731
78	White	Sugandha-2	0.273	0.278	0.127	57.125	19.444	11.615
79	White	Jhingesal	0.423	0.209	0.163	39.750	19.000	13.456
80	Red	Cheruvirippu	0.315	0.114	0.676	37.313	89.667	37.205
81	Red	Mahamaga	0.343	0.187	0.584	38.000	40.778	33.861
82	White	Jaya	0.091	0.079	0.093	43.688	14.778	16.255
83	Red	D1	0.164	0.153	0.451	81.938	73.111	37.997
84	Red	Pk-21	0.269	0.169	0.568	40.000	44.222	32.964
85	White	Gandhakasala	0.066	0.250	0.129	66.750	17.000	13.353
86	Red	Sreyas	0.217	0.148	0.618	57.375	119.889	31.495
87	Red	Gondiachampeisiali	0.762	0.213	0.626	24.750	54.556	24.855

88	White	Chinamal	0.748	0.300	0.111	18.313	22.222	9.104
89	White	Magra	0.146	0.311	0.119	19.875	17.111	9.971
90	Red	Landi	1.380	0.142	0.918	28.000	63.111	29.480
91	White	Lalgundi	0.353	0.289	0.124	10.563	22.222	11.272
92	White	Balisaralaktimachi	0.234	0.253	0.116	18.750	39.111	11.922
93	White	Laxmibilash	0.289	0.191	0.211	40.813	18.667	12.139
94	Red	Kaniar	1.027	0.214	0.651	39.000	16.778	21.532
95	White	Kanakchampa	0.129	0.272	0.159	39.313	16.444	15.795
96	White	Magura-s	0.210	0.295	0.134	43.063	16.000	13.512
97	White	AC44603	1.098	0.227	0.110	60.875	43.889	13.088
98	Red	AC44585	0.693	0.188	0.918	61.000	80.111	38.705
99	White	AC44598	1.938	0.124	0.224	59.313	28.889	11.618
100	Red	AC44592	1.032	0.118	2.320	64.938	242.000	50.515
101	Red	AC44646	1.025	0.251	10.407	63.938	316.889	58.750
102	White	AC44604	1.259	0.203	0.149	60.313	28.889	13.015
103	White	AC44597	1.735	0.075	0.116	54.875	40.654	13.015
104	White	AC44638	0.801	0.161	0.104	77.250	55.667	9.559
105	Red	AC44595	1.014	0.145	6.618	66.500	334.111	69.412
106	Red	AC44588	0.910	0.223	1.302	59.750	227.778	50.368
107	Red	AC44591	1.158	0.206	0.818	47.188	124.111	35.147
108	Red	AC44594	0.986	0.191	3.388	60.563	183.222	35.735
109	Red	AC43737	0.136	0.295	11.934	37.375	230.222	48.544
110	White	AC43660	1.197	0.292	0.220	41.250	26.778	12.955
111	White	AC43732	0.665	0.257	0.079	31.063	33.778	35.239
112	White	AC43661	0.164	0.281	0.107	43.000	50.778	24.600
113	Red	AC43738	0.164	0.274	11.274	47.500	246.000	53.566
114	White	AC43669	1.028	0.243	0.115	55.063	31.505	40.175
115	White	AC43663	0.154	0.269	0.217	40.625	62.667	15.429
116	Red	AC43658	0.325	0.269	19.796	38.688	79.778	52.475
117	White	AC43662	0.112	0.258	0.079	36.375	66.222	13.028
118	Red	AC43670	0.115	0.282	28.375	56.813	358.444	81.441
119	White	AC43675	0.168	0.238	0.115	40.875	24.444	32.678
120	Red	AC43676	0.161	0.186	10.280	34.188	226.333	46.288
Mean			0.586	0.200	1.924	48.209	61.059	20.678
CV			12.25	3.100	12.800	1.810	6.700	6.200
LSD5%			0.174	0.0582	0.389	3.523	7.833	2.421

Carotenoids (mg g⁻¹); SOD: super oxide dismutase (unit g⁻¹); TAC: total anthocyanin content (mg 100g⁻¹);

GO: ga γ-oryzanol (mg 100 g⁻¹); TFC: Total flavonoids content (mg catechine or CEt 100 g⁻¹) and

ABTS : 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (% inhibition).

Table 2. Estimation of genetic diversity parameters based on 136 SSR marker loci in a panel containing 120 rice germplasm lines

Sl. No	Marker	No. of Alleles	Range of amplicon (bp)	Major allele frequency	Gene diversity	Heterozygosity	PIC	Inbreeding coefficient (f)
1	RM5310	4	140-190	0.783	0.367	0.033	0.343	0.910
2	RM582	4	210-245	0.708	0.466	0.033	0.433	0.929
3	RM13335	4	160-180	0.563	0.532	0.008	0.435	0.984
4	RM6275	4	140-160	0.721	0.447	0.058	0.411	0.870
5	RM50	4	190-205	0.400	0.689	0.025	0.630	0.964
6	RM85	4	80-110	0.413	0.675	0.125	0.615	0.816
7	RM222	4	210-250	0.629	0.557	0.025	0.519	0.956
8	RM247	5	140-200	0.500	0.597	0.067	0.519	0.889
9	RM328	3	185-200	0.567	0.580	0.000	0.513	1.000
10	RM337	6	155-400	0.446	0.668	0.117	0.612	0.827
11	RM340	5	100-220	0.713	0.454	0.100	0.415	0.781
12	RM470	5	60-140	0.463	0.690	0.833	0.644	-0.203
13	RM472	3	290-410	0.513	0.508	0.092	0.387	0.821
14	RM506	3	120-130	0.683	0.459	0.133	0.390	0.712
15	RM1812	3	130-140	0.442	0.607	0.000	0.523	1.000
16	RM3701	4	160-260	0.675	0.484	0.492	0.428	-0.012
17	RM6947	3	150-160	0.883	0.212	0.000	0.199	1.000
18	RM14978	3	240-250	0.417	0.639	0.000	0.563	1.000
19	RM18776	3	175-200	0.846	0.267	0.025	0.242	0.907
20	RM22034	3	75-85	0.917	0.155	0.000	0.147	1.000
21	RM24161	4	270-290	0.542	0.612	0.117	0.552	0.811
22	RM223	5	110-170	0.654	0.536	0.058	0.504	0.892
23	RM440	5	160-210	0.408	0.689	0.258	0.634	0.628
24	RM201	4	150-160	0.467	0.645	0.217	0.581	0.666
25	RM216	4	145-160	0.513	0.639	0.125	0.583	0.806
26	RM258	3	140-150	0.383	0.652	0.000	0.576	1.000
27	RM286	4	100-130	0.471	0.632	0.100	0.562	0.843
28	RM3735	4	135-500	0.333	0.725	0.958	0.674	-0.318
29	RM1347	3	100-110	0.517	0.566	0.000	0.475	1.000
30	RM7571	3	130-140	0.713	0.433	0.008	0.373	0.981
31	RM14723	4	220-250	0.492	0.643	0.200	0.581	0.691
32	RM103	3	255-330	0.492	0.559	0.767	0.461	-0.369
33	RM315	3	135-140	0.867	0.235	0.000	0.214	1.000
34	RM225	3	135-150	0.525	0.547	0.183	0.449	0.667
35	RM486	3	130-140	0.654	0.469	0.108	0.380	0.770
36	RM256	3	110-150	0.721	0.411	0.058	0.339	0.859
37	RM1113	3	150-180	0.671	0.457	0.058	0.373	0.873
38	RM3423	3	125-140	0.500	0.575	0.000	0.484	1.000
39	RM6100	3	170-180	0.442	0.643	0.033	0.569	0.949
40	RM590	3	140-150	0.725	0.431	0.067	0.384	0.846
41	RM5793	3	115-130	0.633	0.525	0.017	0.464	0.969

42	RM405	3	100-110	0.675	0.491	0.000	0.441	1.000
43	RM547	5	190-300	0.471	0.573	0.167	0.481	0.711
44	RM7364	5	180-250	0.621	0.573	0.167	0.541	0.711
45	RM205	3	130-180	0.621	0.532	0.025	0.467	0.953
46	RM167	4	130-180	0.704	0.463	0.100	0.421	0.786
47	RM229	5	120-140	0.358	0.710	0.133	0.657	0.814
48	RM20A	3	230-240	0.625	0.533	0.017	0.472	0.969
49	RM235	5	100-145	0.396	0.719	0.175	0.671	0.758
50	RM7003	4	100-110	0.667	0.502	0.083	0.453	0.835
51	RM5436	4	155-190	0.442	0.621	0.058	0.545	0.907
52	RM25181	5	130-160	0.379	0.710	0.167	0.660	0.767
53	RM469	3	100-110	0.621	0.524	0.042	0.452	0.921
54	RM6547	3	155-165	0.867	0.240	0.017	0.226	0.931
55	RM152	4	145-155	0.508	0.628	0.017	0.565	0.974
56	RM148	2	140-150	0.675	0.439	0.083	0.342	0.812
57	RM421	3	250-260	0.458	0.631	0.000	0.555	1.000
58	RM2634	3	100-120	0.379	0.658	0.025	0.584	0.962
59	RM248	4	75-115	0.346	0.732	0.117	0.684	0.842
60	RM7179	5	50-250	0.325	0.765	0.358	0.727	0.535
61	RM215	3	155-165	0.617	0.491	0.017	0.392	0.966
62	RM324	4	220-260	0.542	0.635	0.158	0.590	0.753
63	RM317	3	150-160	0.725	0.403	0.000	0.328	1.000
64	RM174	3	230-270	0.508	0.621	0.067	0.551	0.893
65	RM556	3	190-210	0.842	0.279	0.033	0.260	0.881
66	RM257	4	130-155	0.408	0.663	0.233	0.595	0.651
67	RM502	3	260-265	0.808	0.318	0.000	0.281	1.000
68	RM331	4	95-115	0.483	0.664	0.058	0.611	0.913
69	RM403	4	110-130	0.596	0.570	0.083	0.515	0.855
70	RM309	3	180-190	0.696	0.460	0.025	0.405	0.946
71	RM6641	3	140-145	0.567	0.583	0.000	0.517	1.000
72	RM3	3	110-120	0.383	0.663	0.033	0.589	0.950
73	RM594	3	300-320	0.588	0.558	0.008	0.488	0.985
74	RM3392	4	160-180	0.504	0.615	0.108	0.545	0.825
75	RM1278	3	135-150	0.783	0.361	0.067	0.329	0.817
76	RM168	3	95-125	0.625	0.510	0.150	0.431	0.708
77	RM3375	3	190-200	0.567	0.576	0.033	0.506	0.943
78	RM282	3	140-150	0.725	0.436	0.000	0.395	1.000
79	RM26632	4	450-550	0.363	0.701	0.158	0.644	0.776
80	RM1341	3	170-190	0.613	0.529	0.025	0.455	0.953
81	RM4112	3	160-170	0.488	0.623	0.158	0.549	0.748
82	RM20377	4	300-380	0.771	0.369	0.067	0.326	0.821
83	RM210	5	130-180	0.363	0.734	0.700	0.687	0.051
84	RM218	4	130-160	0.583	0.585	0.033	0.531	0.943
85	RM494	5	130-180	0.383	0.717	0.025	0.670	0.965

86	RM336	5	105-160	0.383	0.711	0.092	0.661	0.872
87	RM3475	4	135-160	0.450	0.656	0.042	0.591	0.937
88	RM480	4	190-210	0.538	0.618	0.025	0.561	0.960
89	RM566	4	150-200	0.433	0.656	0.017	0.591	0.975
90	RM11701	3	210-230	0.642	0.471	0.000	0.375	1.000
91	RM220	6	85-130	0.358	0.745	0.183	0.703	0.756
92	RM488	6	155-200	0.321	0.750	0.192	0.708	0.746
93	RM6374	6	130-160	0.338	0.771	0.075	0.737	0.904
94	RM233	5	130-160	0.350	0.727	0.233	0.680	0.681
95	RM112	3	130-135	0.875	0.222	0.000	0.204	1.000
96	RM13600	4	105-130	0.479	0.662	0.100	0.607	0.850
97	RM495	3	145-165	0.600	0.560	0.033	0.499	0.941
98	RM493	7	180-250	0.283	0.813	0.558	0.787	0.317
99	RM444	5	180-240	0.321	0.773	0.158	0.737	0.797
100	RM468	3	210-220	0.771	0.379	0.025	0.346	0.935
101	RM6054	3	120-130	0.925	0.142	0.017	0.137	0.883
102	RM509	3	165-170	0.758	0.395	0.000	0.360	1.000
103	RM5638	6	190-240	0.613	0.587	0.133	0.558	0.775
104	RM8044	6	240-300	0.279	0.761	0.233	0.721	0.695
105	RM8271	5	180-250	0.404	0.723	0.133	0.679	0.817
106	RM171	4	380-420	0.517	0.633	0.058	0.575	0.909
107	RM16686	3	90-100	0.417	0.655	0.000	0.581	1.000
108	RM434	4	250-280	0.567	0.595	0.025	0.537	0.958
109	RM6091	4	70-80	0.817	0.318	0.000	0.299	1.000
110	RM209	4	145-175	0.542	0.612	0.000	0.552	1.000
111	RM245	4	145-155	0.583	0.577	0.000	0.518	1.000
112	RM1089	4	210-260	0.417	0.637	0.067	0.565	0.896
113	RM228	4	110-170	0.625	0.544	0.192	0.491	0.650
114	RM401	3	250-300	0.754	0.398	0.058	0.360	0.855
115	RM11	3	140-160	0.463	0.590	0.008	0.502	0.986
116	RM3351	3	170-190	0.583	0.517	0.000	0.420	1.000
117	RM5749	3	130-160	0.588	0.504	0.025	0.400	0.951
118	RM335	2	100-110	0.721	0.402	0.075	0.321	0.815
119	RM144	3	200-210	0.588	0.516	0.158	0.419	0.695
120	RM300	3	125-145	0.867	0.238	0.017	0.221	0.930
121	RM1132	4	90-125	0.358	0.724	0.033	0.674	0.954
122	RM400	4	210-260	0.367	0.717	0.467	0.665	0.353
123	RM471	3	100-120	0.800	0.338	0.000	0.309	1.000
124	RM243	3	120-140	0.575	0.554	0.017	0.475	0.970
125	RM467	3	200-210	0.558	0.575	0.000	0.502	1.000
126	RM564	4	250-300	0.450	0.599	0.100	0.515	0.834
127	RM8007	3	130-150	0.767	0.385	0.000	0.352	1.000
128	RM441	4	160-200	0.475	0.627	0.567	0.557	0.100
129	RM518	3	150-170	0.542	0.537	0.000	0.437	1.000

130	RM253	4	130-170	0.554	0.594	0.083	0.530	0.861
131	RM274	3	75-80	0.667	0.477	0.000	0.406	1.000
132	RM242	4	200-240	0.575	0.591	0.017	0.536	0.972
133	RM3231	4	170-550	0.346	0.703	0.650	0.645	0.080
134	RM5687	4	160-500	0.417	0.687	0.650	0.630	0.059
135	RM5626	3	165-180	0.583	0.512	0.733	0.411	-0.430
136	RM452	3	240-250	0.475	0.618	0.000	0.541	1.000
	Mean	3.74	–	0.561	0.555	0.116	0.496	0.793

Table 3. The inferred ancestry value and population structure of individual member in the panel population with their antioxidants classification

Sl. No.	Accession No./ Vernacular name of germplasm line	Inferred ancestry value at K=4				Group	Antioxidants content in each germplasm line
		Q1	Q2	Q3	Q4		
1	AC. 5993	0.986	0.009	0.003	0.003	SP1	high SOD
2	AC. 6221	0.984	0.006	0.003	0.007	SP1	Low
3	AC. 6183	0.945	0.003	0.003	0.049	SP1	Low
4	AC. 6170	0.994	0.002	0.002	0.002	SP1	high Carotenoid
5	AC. 6023	0.978	0.009	0.002	0.012	SP1	high SOD
6	AC. 6172	0.963	0.005	0.002	0.03	SP1	Low
7	AC. 6027	0.012	0.002	0.983	0.002	SP3	Low
8	AC. 6007	0.994	0.002	0.002	0.003	SP1	Low
9	AC. 9006	0.973	0.006	0.009	0.012	SP1	high
10	AC. 9021	0.927	0.053	0.005	0.015	SP1	Low
11	AC. 9028	0.924	0.006	0.003	0.066	SP1	high GO& SOD
12	AC. 9030	0.989	0.005	0.001	0.005	SP1	Low
13	AC. 9035	0.959	0.021	0.017	0.003	SP1	Low
14	AC. 9038	0.982	0.015	0.001	0.002	SP1	high SOD
15	AC. 9043	0.95	0.046	0.002	0.002	SP1	Low
16	AC. 9044	0.987	0.006	0.004	0.003	SP1	high SOD
17	AC. 20920	0.51	0.48	0.007	0.004	Admix	high SOD & Carotenoid
18	AC. 20907	0.866	0.131	0.001	0.002	SP1	high SOD
19	AC. 20845	0.087	0.907	0.001	0.005	SP2	high Carotenoid
20	AC. 20770	0.966	0.025	0.008	0.002	SP1	high SOD & Carotenoid
21	AC. 20627	0.378	0.619	0.001	0.002	Admix	high Carotenoid & SOD
22	AC. 20686	0.432	0.564	0.002	0.002	Admix	high SOD
23	AC. 20664	0.006	0.99	0.001	0.003	SP2	Medium
24	AC. 20614	0.109	0.887	0.003	0.001	SP2	high SOD
25	Jhagrikarti	0.97	0.02	0.002	0.008	SP1	high GO
26	Dadghani	0.963	0.03	0.003	0.004	SP1	high SOD
27	Shayam	0.004	0.002	0.993	0.002	SP3	Very low
28	Basumati	0.128	0.005	0.862	0.005	SP3	Very low
29	Bharati	0.551	0.444	0.004	0.001	Admix	high SOD
30	Joha	0.973	0.023	0.002	0.002	SP1	high SOD
31	Adira-1	0.586	0.02	0.364	0.03	Admix	Medium
32	Adira-2	0.992	0.004	0.002	0.002	SP1	Medium
33	Adira-3	0.256	0.327	0.413	0.004	Admix	Medium
34	PK6	0.985	0.002	0.01	0.003	SP1	Low
35	Vachaw	0.803	0.154	0.041	0.002	SP1	Medium
36	Kozhivalan	0.988	0.008	0.001	0.002	SP1	Low
37	Marathondi	0.017	0.486	0.464	0.033	Admix	Medium
38	Ezhoml-2	0.862	0.135	0.002	0.001	SP1	Medium
39	Jyothi	0.973	0.025	0.001	0.001	SP1	Medium

40	Kantakopura	0.521	0.476	0.002	0.001	Admix	Medium
41	Kantakaamal	0.055	0.585	0.207	0.153	Admix	Medium
42	Kapanthi	0.032	0.296	0.333	0.339	Admix	Low
43	Karpurkanti	0.001	0.042	0.956	0.001	SP3	Very low
44	Kathidhan	0.426	0.475	0.005	0.094	Admix	Medium
45	Kundadhan	0.005	0.992	0.001	0.002	SP2	Low
46	Champaeisia	0.005	0.991	0.002	0.002	SP2	high SOD
47	Latamahu	0.016	0.977	0.002	0.005	SP2	Medium
48	Latachaunri	0.028	0.966	0.002	0.005	SP2	high SOD
49	AC. 10608	0.981	0.013	0.001	0.005	SP1	Low
50	AC. 10187	0.944	0.005	0.002	0.049	SP1	Low
51	AC. 10162	0.941	0.012	0.021	0.026	SP1	Low
52	AC. 7282	0.003	0.002	0.995	0.001	SP3	Very low
53	AC. 7269	0.994	0.003	0.001	0.002	SP1	Very low
54	AC. 7134	0.749	0.032	0.21	0.009	Admix	Low
55	AC. 7008	0.94	0.057	0.001	0.002	SP1	Low
56	AC. 9093	0.99	0.005	0.004	0.001	SP1	high SOD
57	AC. 9090	0.958	0.022	0.016	0.004	SP1	high SOD
58	AC. 9076A	0.844	0.148	0.001	0.007	SP1	Low
59	AC. 9065	0.923	0.012	0.061	0.004	SP1	Low
60	AC. 9063	0.667	0.324	0.001	0.008	Admix	GO & SOD
61	AC. 9058	0.992	0.005	0.001	0.001	SP1	Low
62	AC. 9053A	0.852	0.007	0.014	0.127	SP1	Low
63	AC. 9050	0.894	0.097	0.007	0.002	SP1	Low
64	AC. 9005	0.985	0.009	0.003	0.004	SP1	high SOD
65	AC. 20389	0.963	0.004	0.008	0.026	SP1	high Carotenoid &SOD
66	AC. 20371	0.976	0.019	0.001	0.004	SP1	high GO&SOD
67	AC. 20423	0.975	0.019	0.001	0.005	SP1	Medium
68	AC. 20362	0.968	0.013	0.006	0.013	SP1	high SOD,
69	AC. 20328	0.804	0.172	0.014	0.009	SP1	high SOD
70	AC. 20317	0.882	0.089	0.027	0.003	SP1	high SOD
71	AC. 20282	0.536	0.339	0.009	0.116	Admix	high GO &SOD,
72	AC. 20246	0.639	0.262	0.069	0.03	Admix	high SOD & Carotenoid
73	AC. 20347	0.927	0.029	0.002	0.042	SP1	high SOD& Carotenoid
74	Palinadhan-	0.321	0.038	0.381	0.26	Admix	high SOD
75	Chatuimuchi	0.001	0.001	0.996	0.001	SP3	high SOD
76	Uttarbangal	0.743	0.155	0.002	0.101	Admix	high SOD
77	Gochi	0.943	0.007	0.007	0.043	SP1	high SOD
78	Sugandha-2	0.003	0.002	0.995	0.001	SP3	high SOD
79	Jhingesal	0.365	0.631	0.001	0.002	Admix	high SOD
80	Cheruviripp	0.852	0.142	0.002	0.004	SP1	Low
81	Mahamaga	0.548	0.399	0.002	0.051	Admix	Very low
82	Jaya	0.928	0.064	0.001	0.007	SP1	Low
83	D1	0.89	0.042	0.019	0.049	SP1	Low

84	PK21	0.705	0.27	0.002	0.023	Admix	Low
85	Gandhakasal	0.002	0.086	0.908	0.004	SP3	high SOD,
86	Sreyas	0.909	0.085	0.003	0.002	SP1	Medium
87	GondiAc.hamp	0.011	0.986	0.002	0.002	SP2	high SOD
88	Chinamal	0.229	0.761	0.008	0.002	Admix	high SOD
89	Magra	0.267	0.726	0.005	0.003	Admix	high SOD
90	Landi	0.011	0.986	0.002	0.002	SP2	Low
91	Lalgundi	0.005	0.988	0.004	0.003	SP2	high SOD
92	Balisaralak	0.004	0.99	0.002	0.003	SP2	VL,L,SOD
93	Laxmibilash	0.005	0.465	0.527	0.003	Admix	Very low
94	Kaniar	0.03	0.958	0.006	0.007	SP2	high Carotenoid &SOD
95	Kanakchampa	0.037	0.95	0.009	0.004	SP2	high SOD
96	Magura-S	0.003	0.984	0.012	0.001	SP2	high SOD
97	AC. 44603	0.014	0.017	0.001	0.967	SP4	high Carotenoid &SOD
98	AC. 44585	0.005	0.003	0.012	0.981	SP4	Low
99	AC. 44598	0.02	0.003	0.01	0.968	SP4	high Carotenoid
100	AC. 44592	0.001	0.001	0.014	0.984	SP4	high Carotenoid ,TFC,ABTS
101	AC. 44646	0.002	0.001	0.001	0.996	SP4	high Carotenoid ,TAC,TFC,SOD, ABTS
102	AC. 44604	0.028	0.004	0.012	0.956	SP4	high Carotenoid &SOD
103	AC. 44597	0.002	0.003	0.001	0.994	SP4	high TFC &Carotenoid
104	AC. 44638	0.001	0.001	0.701	0.297	Admix	Low
105	AC. 44595	0.007	0.003	0.011	0.978	SP4	high SOD, Carotenoid,ABTS,
106	AC. 44588	0.002	0.001	0.001	0.995	SP4	High ABTS
107	AC. 44591	0.002	0.002	0.001	0.995	SP4	high Carotenoid & SOD
C08	AC. 44594	0.011	0.006	0.002	0.981	SP4	high SOD
109	AC. 43737	0.003	0.002	0.002	0.993	SP4	high TAC &SOD
110	AC. 43660	0.003	0.003	0.001	0.993	SP4	high Caro,TAC,TFC,SOD, ABTS
111	AC. 43732	0.002	0.001	0.001	0.995	SP4	high SOD &ABTS
112	AC. 43661	0.006	0.004	0.001	0.989	SP4	high SOD,
113	AC. 43738	0.002	0.004	0.002	0.992	SP4	high SOD, ABTS,TAC,
114	AC. 43669	0.006	0.004	0.003	0.987	SP4	high Caro,TAC,TFC,SOD,
115	AC. 43663	0.002	0.002	0.002	0.994	SP4	high SOD
116	AC. 43658	0.001	0.001	0.001	0.997	SP4	High TAC &SOD
117	AC. 43662	0.004	0.002	0.027	0.967	SP4	High SOD
118	AC. 43670	0.003	0.003	0.18	0.815	SP4	High SOD, ABTS, TAC,
119	AC. 43675	0.003	0.002	0.014	0.98	SP4	High TAC,SOD
120	AC. 43676	0.007	0.015	0.043	0.935	SP4	High SOD

Table 4. Analysis of molecular variance (AMOVA) of the sub-populations of the panel population for antioxidant content 120 rice genotypes

Source of variation	AMOVA for the four subpopulations at K = 4			
	df.	Mean sum of squares	Variance components	Percentage variation
Among populations	4	551.634	2.575	6%
Among individuals (accessions) within population	115	2983.721	0.000	0%
Within individuals (accessions)	120	5027.000	41.892	94%
Total	239	8562.354	44.467	100%
F-Statistics	Value	P-value		
F _{ST}	0.071	0.001		
F _{IS}	-0.235	1.000		
F _{IT}	-0.148	1.000		
F _{ST} max.	0.501			
F' _{ST}	0.141			

Table 5. Marker-trait associations with antioxidant content in the panel population detected by both the models of GLM and MLM at p<0.01.

SL.No	Antioxidant compounds	Marker	Position (cM)	GLM				MLM				
				Marker_F	Marker_p	Marker_R ²	q-value	Marker	Marker_F	Marker_p	Marker_R ²	q-value
1	SOD	RM582	66.4-66.4cM	7.51326	0.00713	0.0617	0.0617	RM582	10.35724	0.00169	0.09191	0.005
2	SOD	RM405	28.6-28.6cM	8.28345	0.00479	0.06759	0.06759	RM405	12.0128	7.52E-04	0.10661	0.005
3	SOD	RM467	46.8-46.8cM	9.70831	0.00233	0.07829	0.07829	RM467	9.70377	0.00234	0.08612	0.005
5	TAC	RM440	92.7-92.7cM	10.07764	0.00194	0.06646	0.06646	RM440	9.06064	0.00323	0.08013	0.005
6	TAC	RM5638	86-86cM	12.02036	7.47E-04	0.07803	0.07803	RM5638	11.04573	0.0012	0.09768	0.005
7	TAC	RM253	37-37cM	11.30677	0.00106	0.07443	0.07443	RM253	10.51261	0.00157	0.09297	0.005
8	TAC	RM5626	99-99cM	9.36875	0.00276	0.06215	0.06215	RM5626	9.35822	0.00278	0.08276	0.005
9	GO	RM3701	45.3-45.3cM	14.94433	1.87E-04	0.11729	0.11729	RM3701	9.33336	0.00282	0.08155	0.005
10	GO	RM502	121.8-121.8cM	21.52493	9.54E-06	0.15935	0.15935	RM502	8.35407	0.00463	0.073	0.006
11	TFC	RM3701	45.3-45.3cM	11.62841	9.06E-04	0.06613	0.06613	RM3701	8.95629	0.00341	0.07279	0.005
12	TFC	RM235	101.8-103.8cM	16.06018	1.11E-04	0.08746	0.08746	RM235	9.20885	0.003	0.07484	0.005
13	TFC	RM494	124.4-124.4cM	9.85164	0.00217	0.05638	0.05638	RM494	9.64481	0.00241	0.07839	0.005
14	ABTS	RM3701	45.3-45.3cM	12.55463	5.79E-04	0.08346	0.08346	RM3701	10.97479	0.00125	0.09699	0.005
15	ABTS	RM235	101.8-103.8cM	8.08868	0.0053	0.05533	0.05533	RM235	7.06457	0.00902	0.06243	0.005

Figures

Figure 1

Frequency distribution of germplasm lines for each of the studied antioxidant traits namely carotenoids, superoxide dismutase, anthocyanins, γ -oryzanol flavonoids, and ABTS present in the shortlisted 270 germplasm lines.

Figure 2

Antioxidant content of 120 genotypes and their frequency distribution in the panel population. A. Spider graph showing the TFC content & ABTS activity. B. the TAC & γ -oryzanol content, C. Carotenoid and SOD content D. Frequency distribution of germplasm lines for carotenoids, superoxide dismutase, anthocyanins, γ -oryzanol, flavonoids, and ABTS in the panel population

Figure 3

Genotype-by-trait bi-plot diagram showing 120 germplasm lines in two PCs for 6 antioxidant traits.

Figure 4

Heat map showing Pearson's correlation coefficients for physiological traits. Significant correlations are colored either in red (negative) or blue (positive). Shades of blue indicate increasing positive correlation coefficient; shades of red indicate increasing negative correlation coefficient. CART: Carotenoids (mg g⁻¹); SOD: super oxide dismutase (unit g⁻¹); TAC: total anthocyanin content (mg 100g⁻¹); GO: γ-oryzanol (mg 100 g⁻¹); TFC: Total flavonoids content (mg catechine or CEt 100 g⁻¹); ABTS: 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (% inhibition).

Figure 5

A) Graph of ΔK value, to the rate of change in the log probability of data between successive K values; B) Population structure of the panel population based on membership probability fractions of individual genotypes at K = 4. The genotypes with the probability of $\geq 80\%$ membership proportions were assigned as subgroups while others grouped as admixture group. The numbers in the diagram depict the serial number of the germplasm lines listed in the Table 1.

Figure 6

A. The physical distance (base pairs, bp) between pairs of loci on chromosomes against linkage disequilibrium (LD) decay (r^2) curve plotted in rice. The decay started in million bp estimated by taking 95th percentile of the distribution of r^2 for all unlinked loci. B. the marker 'P' versus marker 'F' and marker R2

Factorial analysis: (Axes 1 / 2)

Figure 9

A. Positions of the QTLs on the chromosomes for antioxidant content detected by association mapping in rice. B. Distribution of marker-trait association and quantile-quantile (Q-Q) plot generated from Generalized Linear Model analysis for six antioxidant traits at (A) $p < 0.05$ and (B) at $p < 0.01$.

Supplementary Files

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