Association Mapping for Quantitative Trait Loci Controlling Superoxide Dismutase, Flavonoids, Anthocyanins, Carotenoids, \( \gamma \)-Oryzanol and Antioxidant Activity in Rice

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Abstract: Antioxidant-rich rice is a cheaper way to solve stress-related disorders and other health benefits for the global rice-eating population. Five antioxidant traits, namely, superoxide dismutase, flavonoids, anthocyanins, \( \gamma \)-oryzanol and 2,2‘-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) activity were mapped using a representative panel population through association mapping. Potential landraces carrying multiple antioxidant compounds were identified from the population. The population represented four genetic groups and correspondence for presence of antioxidants traits in each group was noticed. The population showed linkage disequilibrium for the studied traits based on the Fst values. A total of 14 significant marker–trait associations were detected for these antioxidant traits. The study validated the QTLs, \( qANC3 \) and \( qPAC12-2 \) for anthocyanin content and \( qAC12 \) for ABTS activity will be useful in marker-assisted breeding. Eleven QTLs such as \( qTAC1.1 \) and \( qTAC5.1 \) controlling anthocyanin content, \( qSOD1.1 \), \( qSOD5.1 \) and \( qSOD10.1 \) for superoxide dismutase (SOD), \( qTFC6.1 \), \( qTFC11.1 \) and \( qTFC12.1 \) for total flavonoids content (TFC), \( qOZ8.1 \) and \( qOZ11.1 \) for \( \gamma \)-oryzanol (OZ) and \( qAC11.1 \) for ABTS activity were detected as novel loci. Chromosomal locations on 11 at 45.3 cM regulating GO, TFC and TAC, and on the chromosome 12 at 101.8 cM controlling TAC and ABTS activity, respectively, were detected as antioxidant hotspots.

Keywords: ABTS; antioxidant activity; anthocyanins; association mapping; flavonoids; \( \gamma \)-oryzanol; superoxide dismutase

1. Introduction

Rice is a principal food for more than half of the global population. The crop is mostly produced and consumed in Asiatic countries. However, the majority of rice-consuming people are observed to suffer problems such as 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 [1,2]. Antioxidants protect cells against free radicals, which may cause diseases in human. As the majority of the global population is dependent on rice, enriching the grains with Fe, Zn, and antioxidant compounds are the priority areas of rice research [3–7]. Consumption of rice rich in antioxidants is a better and cheaper option for combatting the stress-related disorders and gaining other health benefits [7]. Enhancing the nutraceutical value of the antioxidant compounds in rice though biofortification is the best and cheapest way of achieving health...
benefits for the people in a country [8]. In recent years, consumption of wholegrain pigmented rice enriched with antioxidant compounds has been gaining popularity in developed and developing countries due to its health benefits of reducing the risk of many chronic diseases [9–11]. 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. Therefore, locating the genes and QTLs regulating these antioxidants in rice grain is very important research to conduct before starting an improvement program for these traits.

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 antioxidants include 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 act as a defense system for maintaining the structural and functional integrity of a cell by inhibiting the oxidative deterioration to macromolecules such as lipid, protein, and nucleic acid [13–15]. Hence, improvement of these traits in rice will lead to the development of better-quality rice. Non-enzymatic antioxidants such as phenolic acids, flavonoids, anthocyanins and proanthocyanidins, tocopherols and tocotrienols (vitamin E), and γ-oryzanol have been reported to be higher in pigmented rice (rice with red, black, and purple pericarp). The antioxidants show impressive health benefits such as reducing oxidative stress and cholesterol levels in human body, lowering the chances of type II diabetes, obesity, cancer, etc. [16–18]. These antioxidant traits are complex traits, polygenic in nature and quantitatively inherited [19]. Understanding the genetic bases of these complex antioxidant traits and identification of major QTLs are essential for the improvement of these phytochemicals through molecular breeding to ameliorate the increasing nutrition problems of the rice-eating population and seed quality, as well.

Identification of QTLs genes for higher carotenoid content and development of functional markers is slow in rice as reports of carotenoids are not available in rice [20]. Wide genetic variation for carotenoid content exists in rice. White rice accumulates a very small quantity of carotenoid [21,22]. Color-providing pigments, anthocyanidin and proanthocyanidin, are present in the pericarp and aleurone layer of rice grain. The proanthocyanidin content in rice imparts red color to rice pericarp is controlled by the interaction of Rc and Rd genes [23–25]. Whereas, anthocyanidins impart purple-black pericarp to rice grain which is controlled by two loci, Pb and P [26]. Two genes, dihydroflavonolreductase (DFR) and anthocyanin synthase (ANS), present on chromosome 1 regulate the anthocyanin content in rice seeds [27]. However, a recent study reported that A1 (Kal1/Rd/OsDFR) and C1alleles (OsC1) determine the purple color of grain, and the pattern of anthocyanin pigmentation in grain is determined by the allelic status of A1, C1, and S1 (OsANS1) [28]. Kala 4/ OsB2/Pb gene was mainly responsible for black pigmentation of rice pericarp [29,30].

The genetic analyses for identification and fine mapping of genes and QTLs for pericarp pigmentation in rice have been published by many workers using various mapping populations [10,19,25,30,31]. However, few reports on QTL mapping are available for γ-oryzanol, total phenolics content (TFC), total flavonoids content (TFC), ABTS (Aznobis 3-ethyl benzothiazoline-6-sulfonic acid), and SOD (Super oxide dismutase) traits 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. The QTLs, qPH-12, qFL-2-1 and qAC-1, control the phenolic content, flavonoid content, and antioxidant capacity, respectively, in rice [10,31–33]. A mapping study on γ-oryzanol content in rice was reported by Kato et al. [34]. In addition, recent reports indicated the possibility of marker–trait association for phenolics, carotenoids, anthocyanin, γ-oryzanol, and other antioxidant contents in rice [9,35]. In addition, the antioxidant traits are reported to be regulated by different pathways, viz. Phenylpropanoid biosyn-
thesis pathway for flavonoids [36,37]; Methylerithritol 4-phosphate (MEP) pathway for carotenoids [38,39]; Mevalonate (MVA) pathway [40]; chromogen activator and tissue-specific regulator (CAP) regulatory pathway [41]; Phenyl propanoid metabolic pathway [42], or Phenyl alanine pathway [43] for anthocyanins; Esterification of hydroxyl sterols for Gamma-oryzanols [42] and Mitogen activated protein (MAP) kinase pathway for superoxide dismutase [44], from which insights into molecular mechanisms of the traits are possible.

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 by analyzing natural variable population. 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 are essential to check the adequacy of the panel population composition for linkage disequilibrium (LD) mapping analyses [45,46]. Thus, association estimates based on both the models of Generalized linear model and Mixed linear model are considered appropriate for mapping complex traits that have been shown to perform better than other model analyses. Although several genes for these antioxidant traits have been reported, more genes/loci are still to be identified to explain the complex regulation of carotenoids, SOD, total anthocyanins, \(\gamma\)-oryzanols, TFC, and ABTS in rice grains. In the present study, we have mapped these six antioxidant traits through association mapping in a highly variable representative set of 120 rice population representing the landraces and cultivars (67 white and 53 red grain) from an original population of 270 germplasm lines using 136 rice microsatellite markers.

2. Materials and Methods

2.1. Seed Materials

The study material comprised of 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-National Rice Research Institute, Cuttack and were grown in the experimental plot of the Institute during wet season, 2019. The genotypes were grown in a randomized complete block design in three rows, each with a spacing of 20 × 15 cm, in two replications, by following recommended package and practices. Each replication is divided into 5 blocks by accommodating 54 germplasm lines in each block. Panicles from 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, \(\gamma\)-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 season, 2019 and 2020. The harvested seeds from both years were used for the estimation of antioxidant traits. The panel population (120) was used in the genotyping for association mapping of antioxidant traits (Table 1).

2.2. Phenotyping for the 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 a 100-mesh-size sieve, and then stored at 4 °C. Analyses of all the traits were based on dry matter basis, except for carotenoid content, which was estimated on a fresh-weight basis. Leaf samples from 10 days old seedling grown on aPetri dish at 30 °C were used for estimation of carotenoids (mg g\(^{-1}\)) by following the protocol of Davis [47]. Seed enzymatic antioxidant, super oxide dismutase (SOD: unit g\(^{-1}\)), was estimated as per the proce-
dure of Madamanchi et al. [48]. Non-enzymatic antioxidant, total anthocyanin content (TAC: \( \text{mg} \ 100 \ \text{g}^{-1} \)) was estimated by the procedure of Fuleki and Francis, [49]. Estimation of \( \gamma \)-Oryzanol (GO: \( \text{mg} \ 100 \ \text{g}^{-1} \)) was performed according to Bucci et al. [50] with minor modifications. Total flavonoids content (TFC) was estimated as per the procedure of Eberhardt et al. [51] and expressed as catechin equivalent (\( \text{mg} \ \text{CEt} \ 100 \ \text{g}^{-1} \)). Antioxidant activity, 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging was assayed by the modified protocol of Serpen et al. [52] and expressed as % inhibition.

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 Color | Genotype/ Vernacular Name/Accession No. | Carotenoids | SOD | TAC | GO | TFC | ABTS |
|---------|--------------|----------------------------------------|-------------|-----|-----|----|-----|------|
| 1       | White        | Ac. 5993                               | 0.115       | 0.239 | 0.209 | 43.750 | 12.333 | 8.853 |
| 2       | White        | Ac. 6221                               | 0.423       | 0.101 | 0.159 | 47.375 | 13.333 | 8.952 |
| 3       | White        | Ac. 6183                               | 0.182       | 0.015 | 0.102 | 47.125 | 13.889 | 14.119 |
| 4       | White        | Ac. 6170                               | 1.165       | 0.176 | 0.090 | 52.250 | 13.333 | 11.063 |
| 5       | White        | Ac. 6023                               | 0.112       | 0.280 | 0.143 | 33.313 | 13.000 | 10.522 |
| 6       | White        | Ac. 6172                               | 0.297       | 0.181 | 0.225 | 34.125 | 13.444 | 7.569 |
| 7       | White        | Ac. 6027                               | 0.133       | 0.175 | 0.141 | 38.188 | 12.333 | 7.983 |
| 8       | White        | Ac. 6007                               | 0.287       | 0.192 | 0.027 | 32.125 | 13.111 | 7.983 |
| 9       | White        | Ac. 9006                               | 1.014       | 0.284 | 0.064 | 70.438 | 17.889 | 11.412 |
| 10      | White        | Ac. 9021                               | 0.444       | 0.199 | 0.083 | 76.313 | 22.000 | 11.769 |
| 11      | Red          | Ac. 9028                               | 0.776       | 0.216 | 0.250 | 87.500 | 45.556 | 36.976 |
| 12      | White        | Ac. 9030                               | 0.686       | 0.150 | 0.123 | 39.563 | 18.667 | 11.555 |
| 13      | White        | Ac. 9035                               | 0.262       | 0.196 | 0.117 | 49.500 | 17.667 | 11.698 |
| 14      | Red          | Ac. 9038                               | 0.371       | 0.241 | 0.459 | 28.438 | 47.000 | 41.341 |
| 15      | White        | Ac. 9043                               | 0.308       | 0.175 | 0.061 | 39.688 | 18.444 | 8.131 |
| 16      | White        | Ac. 9044A                              | 0.713       | 0.221 | 0.048 | 49.938 | 17.556 | 15.906 |
| 17      | Red          | Ac. 20920                              | 1.264       | 0.312 | 0.325 | 54.125 | 43.889 | 26.061 |
| 18      | Red          | Ac. 20907                              | 0.919       | 0.308 | 0.551 | 64.750 | 52.444 | 26.501 |
| 19      | White        | Ac. 20845                              | 1.257       | 0.265 | 0.102 | 61.250 | 18.889 | 6.442 |
| 20      | Red          | Ac. 20770                              | 1.379       | 0.313 | 0.568 | 62.688 | 62.333 | 35.959 |
| 21      | Red          | Ac. 20627                              | 1.164       | 0.245 | 0.451 | 93.375 | 45.333 | 22.694 |
| 22      | White        | Ac. 20686                              | 0.968       | 0.290 | 0.073 | 43.188 | 21.778 | 4.539 |
| 23      | White        | Ac. 20664                              | 0.828       | 0.256 | 0.070 | 51.938 | 19.778 | 7.028 |
| 24      | Red          | Ac. 20614                              | 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 |
Table 1. Cont.

| Sl. No. | Kernel Color | Genotype/ Vernacular Name/Accession No. | Carotenoids | SOD | TAC | GO | TFC | ABTS |
|---------|--------------|----------------------------------------|-------------|-----|-----|----|-----|------|
| 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          | Ezholi-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          | Champaaisiali                            | 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          | Latchaunri                              | 0.507       | 0.211 | 1.018 | 19.875 | 50.444 | 30.925 |
| 49      | White        | Ac. 10608                               | 0.427       | 0.087 | 0.108 | 43.125 | 12.333 | 10.414 |
| 50      | White        | Ac. 10187                               | 0.395       | 0.159 | 0.085 | 30.063 | 37.111 | 12.981 |
| 51      | Red          | Ac. 10162                               | 0.259       | 0.192 | 0.526 | 45.000 | 81.333 | 32.397 |
| 52      | White        | Ac. 7282                                | 0.136       | 0.047 | 0.084 | 37.063 | 20.667 | 10.335 |
| 53      | White        | Ac. 7269                                | 0.119       | 0.005 | 0.204 | 43.938 | 14.111 | 10.189 |
| 54      | White        | Ac. 7134                                | 0.418       | 0.144 | 0.118 | 46.438 | 17.333 | 6.841  |
| 55      | White        | Ac. 7008                                | 0.913       | 0.011 | 0.078 | 42.438 | 22.444 | 9.534  |
| 56      | White        | Ac. 9093                                | 0.357       | 0.215 | 0.061 | 45.750 | 17.000 | 11.270 |
| 57      | White        | Ac. 9090                                | 0.255       | 0.221 | 0.079 | 48.438 | 16.667 | 10.556 |
| 58      | White        | Ac. 9076A                               | 0.899       | 0.159 | 0.048 | 43.688 | 22.889 | 12.126 |
| 59      | Red          | Ac. 9065                                | 0.353       | 0.176 | 0.359 | 44.875 | 61.778 | 30.485 |
| 60      | Red          | Ac. 9063                                | 0.860       | 0.235 | 0.375 | 110.563 | 52.222 | 23.538 |
| 61      | White        | Ac. 9058                                | 0.573       | 0.126 | 0.055 | 5.313  | 23.222 | 11.698 |
| 62      | White        | Ac. 9053A                               | 0.154       | 0.159 | 0.053 | 42.000 | 17.333 | 9.415  |
| 63      | Red          | Ac. 9050                                | 0.395       | 0.191 | 0.388 | 28.313 | 54.889 | 32.411 |
| 64      | White        | Ac. 9005                                | 1.612       | 0.268 | 0.126 | 47.375 | 24.333 | 14.622 |
| 65      | White        | Ac. 20389                               | 1.247       | 0.279 | 0.035 | 66.250 | 19.333 | 10.102 |
| 66      | White        | Ac. 20371                               | 0.839       | 0.284 | 0.083 | 110.000 | 32.000 | 6.149  |
| 67      | Red          | Ac. 20423                               | 0.713       | 0.182 | 0.434 | 46.625 | 53.000 | 33.031 |
| 68      | White        | Ac. 20362                               | 0.811       | 0.312 | 0.078 | 67.500 | 22.000 | 6.076  |
| 69      | White        | Ac. 20328                               | 1.331       | 0.312 | 0.102 | 79.063 | 23.444 | 10.542 |
| 70      | White        | Ac. 20317                               | 0.870       | 0.332 | 0.102 | 79.063 | 23.444 | 10.542 |
| 71      | Red          | Ac. 20282                               | 1.118       | 0.201 | 1.043 | 84.500 | 76.889 | 42.167 |
| 72      | Red          | Ac. 20246                               | 1.083       | 0.279 | 2.846 | 67.875 | 69.333 | 41.947 |
| 73      | Red          | Ac. 20347                               | 1.188       | 0.292 | 0.272 | 57.313 | 23.778 | 27.906 |
Table 1. Cont.

| Sl. No. | Kernel Color | Genotype/ Vernacular Name/Accession No. | Carotenoids | SOD | TAC | GO | TFC | ABTS |
|---------|--------------|----------------------------------------|-------------|-----|-----|----|-----|------|
| 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        | Balisalaralaktimachi                   | 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        | Ac. 44603                              | 1.098       | 0.227 | 0.110 | 60.875 | 43.889 | 13.088 |
| 98      | Red          | Ac. 44585                              | 0.693       | 0.188 | 0.918 | 61.000 | 80.111 | 38.705 |
| 99      | White        | Ac. 44598                              | 1.938       | 0.124 | 0.224 | 59.313 | 28.889 | 11.618 |
| 100     | Red          | Ac. 44592                              | 1.032       | 0.118 | 2.320 | 64.938 | 242.000 | 50.515 |
| 101     | Red          | Ac. 44646                              | 1.025       | 0.251 | 10.407 | 63.938 | 316.889 | 58.750 |
| 102     | White        | Ac. 44604                              | 1.259       | 0.203 | 0.149 | 60.313 | 28.889 | 13.015 |
| 103     | White        | Ac. 44597                              | 1.735       | 0.075 | 0.116 | 54.875 | 40.654 | 13.015 |
| 104     | White        | Ac. 44638                              | 0.801       | 0.161 | 0.104 | 77.250 | 55.667 | 9.559  |
| 105     | Red          | Ac. 44595                              | 1.014       | 0.145 | 6.618 | 66.500 | 334.111 | 69.412 |
| 106     | Red          | Ac. 44588                              | 0.910       | 0.223 | 1.302 | 59.750 | 227.778 | 50.368 |
| 107     | Red          | Ac. 44591                              | 1.158       | 0.206 | 0.818 | 47.188 | 124.111 | 35.147 |
| 108     | Red          | Ac. 44594                              | 0.986       | 0.191 | 3.388 | 60.563 | 183.222 | 35.735 |
| 109     | Red          | Ac. 43737                              | 0.136       | 0.295 | 11.934 | 37.375 | 230.222 | 48.544 |
| 110     | White        | Ac. 43660                              | 1.197       | 0.292 | 0.220 | 41.250 | 26.778 | 12.955 |
| 111     | White        | Ac. 43732                              | 0.665       | 0.257 | 0.079 | 31.063 | 33.778 | 35.239 |
| 112     | White        | Ac. 43661                              | 0.164       | 0.281 | 0.107 | 43.000 | 50.778 | 24.600 |
| 113     | Red          | Ac. 43738                              | 0.164       | 0.274 | 11.274 | 47.500 | 246.000 | 53.566 |
Table 1. Cont.

| Sl. No. | Kernel Color | Genotype/ Vernacular Name/Accession No. | Carotenoids | SOD | TAC | GO | TFC | ABTS |
|---------|--------------|----------------------------------------|-------------|-----|-----|----|-----|------|
| 114     | White        | Ac. 43669                              | 1.028       | 0.243 | 0.115 | 55.063 | 31.505 | 40.175 |
| 115     | White        | Ac. 43663                              | 0.154       | 0.269 | 0.217 | 40.625 | 62.667 | 15.429 |
| 116     | Red          | Ac. 43658                              | 0.325       | 0.269 | 19.796 | 38.688 | 79.778 | 52.475 |
| 117     | White        | Ac. 43662                              | 0.112       | 0.258 | 0.079 | 36.375 | 66.222 | 13.028 |
| 118     | Red          | Ac. 43670                              | 0.115       | 0.282 | 28.375 | 56.813 | 358.444 | 81.441 |
| 119     | White        | Ac. 43675                              | 0.168       | 0.238 | 0.115 | 40.875 | 24.444 | 32.678 |
| 120     | Red          | Ac. 43676                              | 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 100 g\(^{-1}\)); GO: ga \(\gamma\)-oryzanol (mg 100 g\(^{-1}\)); TFC: Total flavonoids content (mg catechin or CEt 100 g\(^{-1}\)) and ABTS: 2,2\(^{'}\)-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (% inhibition).

2.3. Statistical Analysis

Cropstat software7.0 developed by IRRI was used for analysis of variance (ANOVA) for each trait and for the estimation of mean, range, and coefficient of variation (CV\%). Pearson’s correlation coefficients were analyzed to identify the relationship among the various antioxidant traits, based on the mean values of the 120 genotypes and presented in a correlation matrix heatmap by using PAST 4.03 software (Oyvind Hammer). The germplasm lines were classified into five groups as very high, high, medium, low, and very low categories based on the mean values of the antioxidant traits.

2.4. Genomic DNA Isolation, PCR Analysis, and Selection of SSR Markers

The genomic DNA was isolated from 15-day-old seedlings of the germplasm lines by adopting CTAB method [53]. A total of 136 SSR (simple sequence repeat) markers were selected from the database (http://gramene.org/, accessed on 24 August 2022) available in the public domain and used for genotyping of the panel population (Supplementary Table S5). The DNA fragments were resolved in gel electrophoresis for quantification of the isolated DNA. PCR analysis was performed 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). The conditions of reaction were set to initial denaturation step (2 min, 95 °C), followed by 35 cycles of denaturation (30 s, 95 °C) and annealing/extension (30 min, 55 °C), extension (2 min, 72 °C), final extension (5 min, 72 °C) and store at 4 °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 molecular analysis were followed for DNA isolation, electrophoresis, and imaging techniques [54–57].

2.5. 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 number of alleles (N), major allele frequency (A), polymorphic information content (PIC), observed heterozygosity (H), and gene diversity (GD) for each SSR locus were analyzed by using ‘Power Marker Version3.25’ software [57]. A Bayesian model-based clustering approach STRUCTURE 2.3.6 software.
was used to analyze genetic data and obtain population structure [58]. STRUCTURE software was run with K-values 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. The highest value of ΔK was obtained from the 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 STRUCTURE [59]. For estimation of the ΔK-value as a function of K showing a clear peak, the optimal K-value Structure Harvester was used [60].

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 calculate the genetic association between the phenotypic traits, and molecular markers were adopted as per Bradbury et al. [65]. By considering the significant p-value and r² 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 r², between pairs of markers plotted against the distance between the pair. Additionally, the accuracy of the marker–trait association was established by estimating the FDR adjusted p-values (q-values) using R software as described in the earlier publications [9,35,46].

3. Results

3.1. Phenotyping of the Population for the Six Antioxidant Traits

A total of five antioxidant compounds and one antioxidant enzyme, viz., superoxide dismutase, flavonoids, anthocyanins, carotenoids, γ-oryzanol, and ABTS, were estimated from the 270 germplasm lines during wet season, 2019 (Supplementary Table S1). Wide genetic variation was observed for the six antioxidant traits in the germplasm lines. The genotypes were classified into five groups based on the phenotyping results of each compounds (Figure 1). The frequency distribution of germplasm lines showed various groups or populations for each compound and enzyme (Figure 1). A panel population was prepared by selecting 120 genotypes which represented each group and trait from the original population of 270 germplasm lines (Table 1; Figure 2). The mean estimates of six antioxidant traits obtained from the representative panel population showed wide variation among the genotypes for each trait (Table 1). Very high values of carotenoid content were found in germplasm lines Ac. 44598, Ac. 44597, and Ac. 9005. Additionally, very high TAC content was estimated from the lines Ac. 43670, Ac. 43660, and Ac. 43675. Germplasm lines namely Ac. 9063, Ac. 20371, and Ac. 20627 showed very high level of γ-oryzanol content in the seeds. Good donor lines were identified carrying very high TFC content, viz., Ac. 43670, Ac. 43660, Ac. 44646, Ac. 44592, Ac. 44595, Ac. 43737, Ac. 43738, and Ac. 43676. The SOD level was found very high in the seeds of germplasm lines 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 for exhibiting very high level of ABTS were Ac. 44592, Ac. 43670, Ac. 4460, Ac. 44595, Ac. 44588, Ac. 43660, Ac. 43738, and Ac. 43732. However, germplasm lines (Ac. 44592, Ac. 44646, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac. 43669) were identified for possessing a higher level of more than three antioxidant traits.
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. Estimate of 6 antioxidant traits in the 120 genotypes and their frequency distribution in the panel population. (A) Spider graph showing TFC content and ABTS activity. (B) TAC and 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.
3.2. Genotype-by-Trait Biplot Analysis for the Six Antioxidant Traits in the Germplasm Lines

The scatter diagram was plotted using the first two principal components to generate genotype-by-trait biplot graph for the six antioxidant traits in the 120 germplasm lines present in the panel (Figure 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 Figure S1). The compound, γ-oryzanol content contributed maximum diversity, followed by TFC and ABTS, among the six antioxidant traits estimated from the genotypes present in the panel (Figure 3). The scattering pattern of the germplasm lines in the four quadrants indicated that genotypes containing high estimates of the studied antioxidants are placed in quadrants I (top right) and II (bottom right). Higher estimates of the antioxidant traits with multiple compounds containing genotypes have been encircled in the figure (Figure 3). The top right (quadrant I) and bottom right (quadrant II) accommodated the majority of the genotypes containing high estimates of the antioxidant traits. The quadrant III (bottom left) kept most of the germplasm lines as moderate in the studied antioxidant traits, while the 4th quadrant (top left) accommodated the majority of poor germplasm lines for the antioxidants (Figure 3).

![Figure 3](image-url)

**Figure 3.** Genotype-by-trait biplot diagram showing 120 germplasm lines in two PCs for 6 antioxidant traits.

3.3. Nature of Association among the Antioxidant Traits

The association study provides information for correlation among the traits in which the correlated complex traits are useful in improvement programs. The association among the six 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 a weak positive correlation ($r < 0.5$) were observed for carotenoid with γ-oryzanol content (Figure 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 also contain a larger quantity of other correlated antioxidants.
The studied panel population exhibiting wide genetic variation in 120 germplasm lines for the six antioxidant traits was genotyped using 136 SSR markers. The genetic diversity parameters estimated from the panel population are depicted in 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 largest numbers of alleles were produced by the marker RM493 in the studied panel population. The measure for the variation by a marker in the population was analyzed by the availability of 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 an average value of 0.496. In case of low predicted heterozygosity of alleles in a population, the population may be shifting towards inbreeding for that trait. If it is higher than the predicted heterozygosity, that may be the effect of mixing of two genetic populations. Here, the observed mean heterozygosity (Ho) in the population was 0.116 which varied from 0.00 to 0.958 (RM3735). Twenty marker loci showed 0.00 Ho value in the panel population. The gene diversity (He), 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.
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                  | 0.599          | 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                     |
| Sl. No | Marker   | No. of Alleles | Range of Amplicon (bp) | Major Allele Frequency | Gene Diversity | Heterozygosity | PIC | Inbreeding Coefficient (f) |
|-------|----------|----------------|------------------------|------------------------|---------------|---------------|-----|---------------------------|
| 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    | RM418    | 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    | RM392    | 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                     |
| Sl. No | Marker | No. of Alleles | Range of Amplicon (bp) | Major Allele Frequency | Gene Diversity | Heterozygosity | PIC | Inbreeding Coefficient (f) |
|--------|--------|---------------|-----------------------|------------------------|---------------|---------------|-----|--------------------------|
| 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                  | 0.822         | 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     | RM4444 | 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    | RM4343 | 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                    |
### Table 2. Cont.

| Sl. No | Marker | No. of Alleles | Range of Amplicon (bp) | Major Allele Frequency | Gene Diversity | Heterozygosity | PIC | Inbreeding Coefficient (f) |
|--------|--------|----------------|------------------------|------------------------|----------------|---------------|-----|--------------------------|
| 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                    |

#### 3.5. Population Genetic Structure Analysis

The diverse population for the studied antioxidant traits was genotyped for genetic structure and analyzed by adopting probable sub-populations (K) and selecting higher ΔK-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 by considering a high ΔK peak value of 362.4 at K = 2 among the assumed K (Supplementary Table S2; Supplementary Figure S2). The two subpopulations were in the proportion of 0.277 and 0.723 for population 1 and population 2, respectively. However, the subpopulations showed poor correspondence with the six antioxidant traits in the germplasm present in the studied population. Therefore, the next ΔK peak at K = 3 was compared in which the population was classified into three subpopulations. The three subpopulations showed genotypes in the proportion of 0.277 and 0.723 for population 1 and population 2, respectively. However, the subpopulations showed poor correspondence with the six antioxidant traits in the germplasm present in the studied population. Therefore, the next ΔK peak at K = 3 was compared in which the population was classified into three subpopulations. The three 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 Fst1, Fst2, and Fst3 values were 0.3392, 0.1664, and 0.3701 for the sub-population 1, 2, and 3, respectively (Supplementary Table S2; Supplementary Figure S3). The ancestry value of ≥80% obtained in a genotype grouped the genotype into the particular subpopulation.

The assumed subpopulations at K = 3 differentiated the germplasm lines based on the six antioxidant traits, but did not clearly separate the SP2 and SP3 subpopulations. Hence, the next ΔK peak at K = 4 was considered for the subpopulations in which the population was classified into four genetic groups. The six antioxidant traits in the studied population showed a fair degree of correspondence at K = 4 with inferred structure values in the
subpopulations. The majority of the germplasm lines with high to very high antioxidant-carrying germplasms were present in subpopulation 4. The germplasm lines showing moderate value of the antioxidant estimates are present in subpopulation 2. Germplasm lines with poor and moderate levels of antioxidant estimates were in subpopulation 1, while very poor to poor types are in subpopulation 3 (Table 3; Figure 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 Fst values for the panel population showing a distinct variation in the distribution among the Fst values (Supplementary Figure S4).

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

| Sl. No. | Accession No./ Vernacular Name of Germplasm Line | Inferred Ancestry Value at K = 4 | Antioxidants Content in Each Germplasm Line |
|---------|---------------------------------------------------|----------------------------------|---------------------------------------------|
| 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 0.003          | 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                                           | 0.866 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                                |
Table 3. Cont.

| Sl. No. | Accession No./ Vernacular Name of Germplasm Line | Inferred Ancestry Value at K = 4 | Q1 | Q2 | Q3 | Q4 | Group |
|---------|------------------------------------------------|---------------------------------|-----|-----|-----|-----|-------|
| 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      | Champaesia                                      | 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 |
| Sl. No. | Accession No./Vernacular Name of Germplasm Line | Inferred Ancestry Value at K = 4 | Antioxidants Content in Each Germplasm Line |
|---------|------------------------------------------------|---------------------------------|-------------------------------------------|
|         |                                                 | Q1  | Q2  | Q3  | Q4  | Group         |                           |
| 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      | Gondiachampeisiali                              | 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     |
| 108     | 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 | 0.272 | 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                  |
3.6. Molecular Variance (AMOVA) and LD Decay Plot Analysis

The closely related plants among themselves in a population are grouped into isolated 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 there was 94% variation within individuals of the panel population. Wright’s F statistics was used to obtain the deviation from the Hardy–Weinberg prediction. The parameter $F_{IS}$ was used to analyze the uniformity of individuals within the subpopulation and $F_{IT}$ for individuals 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 a $F_{ST}$ value of 0.071 between the four subpopulations. $F_{ST}$ is used to identify the subpopulations or population differentiation within the total population. A clear differentiation among the four subpopulations was observed for the $F_{ST}$ values from each other in their distribution pattern (Supplementary Figure S4).

![Graph of $\Delta K$ value](image1)

![Population structure of the panel population based on membership probability fractions of individual genotypes at K = 4.](image2)

**Figure 5.** (A) Graph of $\Delta K$ 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 were grouped as admixture group. The numbers in the diagram depict the serial number of the germplasm lines listed in the Table 1.
Table 4. Analysis of molecular variance (AMOVA) of the sub-populations of the panel population for the antioxidant traits in the 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 |
|--------------|-------|---------|
| Fst          | 0.071 | 0.001   |
| F<sub>IS</sub> | −0.235 | 1.000 |
| F<sub>IT</sub> | −0.148 | 1.000 |
| F<sub>ST</sub> max. | 0.501 |
| F<sub>ST</sub>' | 0.141 |

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. A syntenic r<sup>2</sup> value was used to plot the linkage disequilibrium decay of the population versus the physical distance in million base pair (Figure 6A). Tightly linked markers had higher r<sup>2</sup> values and the average r<sup>2</sup> 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 there, after a gradual and very slow decay, this can be noticed in the graph. The graph clearly indicates the continuance of linkage disequilibrium decay in the population for the studied antioxidant traits in the population. The limitation for LD decay depends on non-random mating, mutation, selection, migration or admixture, and genetic drift, which will influence the estimates of LD. This LD decay plot also provides a clue for the creation of genetic admixture groups for various antioxidants traits in the population. A similar trend was also noticed in the marker ‘P’ versus the marker ‘F’ and marker R<sup>2</sup> (Figure 6B) curve. The detected markers from this study indicated the strength of the markers for the studied antioxidant compounds.
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 the 95th percentile of the distribution of $r^2$ for all unlinked loci. (B) The marker ‘P’ versus marker ‘F’ and marker $R^2$.

3.7. Principal Coordinates and Cluster Analyses for Genetic Relatedness among the Germplasm Lines

The two-dimensional plot for the principal coordinate analysis (PCoA) was constructed based on the genotyping data of 136 SSR markers which classified the 120 germplasm lines as per the genetic relatedness among the lines (Figure 7). The inertia showed by component 1 was 11.73%, while 7.49% exhibited by component 2. The germplasm lines were allotted different spots in the four quadrants forming 3 major groups (Figure 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 which showed mostly low to very low estimates for the antioxidant traits in the seeds. The other group near to the axis1 is for all the admix types of the germplasm lines. Few germplasm lines of quadrant II and closer to the axis 1 are also admix genotypes. Then, admix genotypes present on both sides of axis 1 are depicted in black color (Figure 7).
The germplasm lines containing high to very high estimates of antioxidant traits are grouped together, forming subpopulation 4. This subpopulation is present on quadrant III (top left) and IV (bottom left) and encircled in red color. The germplasm lines rich in antioxidants are placed on both sides of the axis 1 on the quadrant III and IV (Figure 7). The PCoA distributed all the germplasm lines into the four quadrants classifying them into 4 clusters and a separate admixture group. The subpopulations clustered by PCoA showed correspondence with the population structure (Figure 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 quadrants III and IV and are rich in antioxidants. The PCoA placed germplasm lines in quadrant II which were mostly average in the antioxidant traits. This quadrant formed the group by placing all the germplasm lines of subpopulations 1 and 2.

As per the Ward clustering, all the germplasm lines were broadly grouped into two major groups. The largest cluster, cluster 1, accommodated 111 germplasm lines in which most of the lines showed poor to average for the antioxidant estimates. The cluster II had nine germplasm lines only. The dendrogram placed all the germplasm lines in this cluster II which were rich for the antioxidant traits. This cluster again subdivided into
2 subgroups, which were further divided into six sub-subclusters. 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 clustering approach were based on the antioxidant traits estimates in the germplasm lines (Figure 8A). The cluster analysis discriminated the germplasm lines on the basis of markers data of 136 SSR markers and placed the genotypes into different clusters which corresponded with the studied antioxidant level in the germplasm. The unweighted-neighbor joining tree differentiated the genotypes into four different clusters (Figure 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 subpopulation 2. The green-colored portion of the tree is designated as SP4 while blue for SP2. The very poor in antioxidant traits in the germplasm lines were in the subpopulation 3 those depicted in red color in the tree. The majority of the germplasm lines present in subpopulation 1 were poor to medium in antioxidant value and are shown in pink color. The germplasm lines with admix type of population are depicted in black color in the neighbor joining tree (Figure 8B).

Figure 8. (A) Grouping of the panel germplasm lines. A. Ward’s clustering based on the antioxidant content (B) Unrooted tree using unweighted-neighbor joining method depicting clustering pattern of 120 germplasm lines with respect to 136 molecular markers colored on the basis of subpopulations obtained from structure analysis (SP1: Pink; SP2: Blue; SP3: Red; SP4: Green, and Admix: Black).

3.8. Marker–Trait Association for Antioxidant Traits in the Rice Panel Population

Marker–trait associations were computed for the six antioxidant traits 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 five antioxidant traits 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 S3; Supplementary Table S4). A total of 14 significant marker–trait associations were detected by both the models for five antioxidant traits present in the seed at \( p < 0.01 \) (Figure 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 presented a higher marker \( R^2 \) (>0.1) with low \( p \)-values (<0.01) in the associations study includes SOD with RM405 and GO with
RM3701 (Table 5; Figure 9A). The Q-Q plot also confirmed the association of these markers with the associated antioxidant traits in rice (Figure 9B).

![Diagram of QTLs on chromosomes for antioxidant content detected by association mapping in rice.](image)

**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 $p < 0.01$. 
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_\(F\) Marker_\(p\) Marker_\(R^2\) q-Value |
| 1      | SOD                    | RM582  | 66.4–66.4 cM  | 7.51326 0.00713 0.0617 0.0617 RM582 | 10.35724 0.00169 0.09191 0.005571 |
| 2      | SOD                    | RM405  | 28.6–28.6 cM  | 8.28345 0.00479 0.06759 0.06759 RM405 | 12.0128 7.52 \times 10^{-4} 0.10661 0.005571 |
| 3      | SOD                    | RM467  | 46.8–46.8 cM  | 9.70831 0.00233 0.07829 0.07829 RM467 | 9.70377 0.00234 0.08612 0.005571 |
| 4      | TAC                    | RM440  | 92.7–92.7 cM  | 10.07764 0.00194 0.06646 0.06646 RM440 | 9.06064 0.00323 0.08013 0.005726 |
| 5      | TAC                    | RM5638 | 86–86 cM      | 12.02036 7.47 \times 10^{-4} 0.07803 0.07803 RM5638 | 11.04573 0.0012 0.09768 0.005571 |
| 6      | TAC                    | RM253  | 37–37 cM      | 11.3067 0.00106 0.07443 0.07443 RM253 | 10.51261 0.00157 0.09297 0.005571 |
| 7      | TAC                    | RM5626 | 99–99 cM      | 9.36875 0.00276 0.06215 0.06215 RM5626 | 9.35822 0.00278 0.08276 0.005571 |
| 8      | GO                     | RM3701 | 45.3–45.3 cM  | 14.94433 1.87 \times 10^{-4} 0.11729 0.11729 RM3701 | 9.33336 0.00282 0.08155 0.005571 |
| 9      | GO                     | RM502  | 121.8–121.8 cM| 21.52493 9.54 \times 10^{-6} 0.15935 0.15935 RM502 | 8.35407 0.00463 0.073 0.006936 |
| 10     | TFC                    | RM3701 | 45.3–45.3 cM  | 11.62841 9.06 \times 10^{-4} 0.06613 0.06613 RM3701 | 8.95629 0.00341 0.07279 0.005782 |
| 11     | TFC                    | RM235  | 101.8–103.8 cM| 16.06018 1.11 \times 10^{-4} 0.08746 0.08746 RM235 | 9.20885 0.003 0.07484 0.005571 |
| 12     | TFC                    | RM494  | 124.4–124.4 cM| 9.85164 0.00217 0.05638 0.05638 RM494 | 9.64481 0.00241 0.07839 0.005571 |
| 13     | ABTS                   | RM3701 | 45.3–45.3 cM  | 12.55463 5.79 \times 10^{-4} 0.08346 0.08346 RM3701 | 10.97479 0.00125 0.09699 0.005571 |
| 14     | ABTS                   | RM235  | 101.8–103.8 cM| 8.08868 0.0053 0.05533 0.05533 RM235 | 7.06457 0.00902 0.06243 0.009257 |
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 near the markers present at 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 activity showed significant associations with RM3701 and RM235 on chromosomes 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 chromosomes 1 and 8, respectively (Table 5; Figure 9A). The Q-Q plot also confirmed the associations of these markers with the estimated antioxidant compounds in rice (Figure 9).

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

4. Discussion

The genotypes shortlisted for the six antioxidant traits mapping exhibited wide genetic variation among themselves (Supplementary Table S1; Table 1). In addition, significant correlation was observed between few antioxidant traits viz., TAC with TFC, TFC with ABTS, and TAC with ABTS. Existence of genetic variation and correlation for these traits provide enough insight about the possibility for improvement of the antioxidant traits in rice (Tables 1 and 4). Earlier reports of high variations for antioxidant traits were also published by few researchers [17,35,45,66–68]. The available diversity in the population based on 136 markers data for the six antioxidant traits represented 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 the antioxidant breeding program. The Jeypore tract of Odisha is known for being a secondary center of origin of rice, and germplasms from this tract were also included in this study. Additionally, the shortlisted germplasm lines used as materials in this study were collected from states known for their rich rice genetic diversity [35,45,69]. The genotypes rich in multiple antioxidant traits were estimated from the germplasm lines Ac. 44592, Ac. 44464, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac. 43669. These germplasm lines will be good source materials in the antioxidant improvement programs (Table 1; Supplementary Table S2). Therefore, it is expected that the breeding program with inclusion of parental lines from this population will be effective in terms of antioxidants’ improvement in rice. The assumed subpopulations at $K = 3$ differentiated the members different subpopulations for the 6 antioxidant traits but did not clearly separate the SP2 and SP3 subpopulations. Therefore, the next $\Delta K$ peak at $K = 4$ was considered for the subpopulations in which the population was classified into four genetic groups. The six antioxidant traits in the studied population showed a fair degree of correspondence at $K = 4$ with inferred structure values in the subpopulations. Structure analysis categorized the population into four subpopulations ($K = 4$), showing different $Fst$ values, supporting the availability of the linkage disequilibrium groups in the population. The detection of a low alpha value and the existence of many genetic admix-type germplasm lines in the population indicated that the antioxidant traits evolved from a
single source initially during evolution of the trait. Different antioxidant compounds were subsequently formed by admix genotypes with different ancestry value during evolutionary process. A similar view of the evolution of complex traits was reported by earlier publications based on the admix genotypes [5,8,9,70]. Population genetic structure group and its correspondence with the traits in each group are important for obtaining a marker–trait association. A good correspondence of genetic structure and different traits was previously published by many researchers [36,61,71]. Additionally, publications on the phenotype of various traits and structure correlation have been published by many workers [45,46,66,67,72].

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 carrying plants (Table 5). The Q-Q plot also confirmed the associations of these markers with the antioxidant compounds in rice (Figure 9B).

The QTLs for anthocyanin and proanthocyanin content in rice were reported by earlier researchers [19,23,30]. In the present investigation, the QTLs for total anthocyanin content were detected on chromosomes 1, 3, 5, and 12. The QTLs on chromosomes 1 and 3 were at position of 86 cM and 99 cM, respectively. The genes \( qANC3 \) and \( qPAC12-2 \) reported by Xu et al. [19] were at the same position as in the present investigation. Therefore, these two QTLs were validated in our study using the present mapping population. However, another two QTLs located on chromosome 1 and 5 detected in this investigation were not reported by earlier researchers. These two QTLs may be new loci which affect TAC in rice and are designated as \( qTAC1.1 \) and \( qTAC5.1 \). Three markers, namely, RM582, RM405, and RM467, showed an association with SOD and were located on chromosomes 1, 5 and 10 at 66.4, 28.6, and 46.8 cM, respectively. The QTLs reported by [23,27] for anthocyanin content in rice were at different position than the locations detected by us on chromosomes 1, 3, and 6. Saini et al. [73] reported 23 QTLs located on chromosome 3,5,6,7, and 9. We detected QTLs for the trait on chromosome 1, 5, and 10. The detected QTLs by Saini et al. [73] on chromosome 5 were quite away from the QTLs detected by us. In addition, no report of QTL from the earlier studies on chromosome 1 and 10 which were detected by us at 15.34 Mb and 13.48 Mb positions, respectively. Therefore, all the 3 QTLs were not reported in earlier studies. The QTLs designated as \( qSOD1.1 \), \( qSOD5.1 \), and \( qSOD10.1 \) may be new loci controlling the SOD activities in rice seeds. The total flavonoids content (TFC) is detected to be associated with three regions on chromosomes 6, 11, and 12. The earlier publication of Shao et al. [10] showed the presence of QTLs on chromosome 4, 7, 8, 9, and 10 [10]. The main flavonoids structural genes located on chromosome 11 for CHS [74]; on chromosome 3 for CHI [36]; on chromosome 4 for F3H [75]; on chromosome 1 for DFR [75] and ANS [72]. The gene CHS on chromosome 11 was at 3.3 cM. We detected it at 45.3 cM. Therefore, all these three detected QTLs which affect total flavonoids are new loci and are designated as \( qTFC6.1 \), \( qTFC11.1 \), and \( qTFC12.1 \). Zhang [76] reported four QTLs controlling flavonoid content in rice grain located on chromosome 4. However, we detected three QTLs on chromosomes 6, 11, and 12. Therefore, the detected QTLs by us regulating flavonoid content in rice were not reported in earlier studies.

Food containing \( \gamma \)-oryzanol (OZ) is well recognized for its health benefits. This is a mixture of several compounds present in the rice bran layer. The \( \gamma \)-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 RILs [34]. However, 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 \( \gamma \)-oryzanol, and are designated as \( qOZ8.1 \) and \( qOZ11.1 \).
QTLs for ABTS activities showed significant associations with RM3701 and RM235 on chromosomes 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 it is designated as qAC11.1. However, the other detected association for the trait on chromosome 12 is located in the 26.1 Mb position. An earlier mapping publication reported the gene on the chromosome 12 at 25.2 Mb position [33]. As our detected QTL position for ABTS activity 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 trait analyzed by both the models at <1% error and \( p < 0.01 \). Marker RM3701 showed associations with antioxidant traits, GO, TFC, and ABTS present in the germplasm lines. Additionally, RM235 was associated with traits, TAC, TFC, and ABTS by both models (Table 5). These observations indicated the close location of the candidate genes and simultaneous inheritance of these QTLs are expected in the progenies. Hence, simultaneous improvement of both these antioxidant traits will be effective. These genomic locations are considered as chromosome hot spots and are very useful in improvement programs. Recent publications have also suggested easy improvement of the co-localized genes controlling various traits in rice [6,45,76]. Results of the present investigation showed that association mapping is an effective method to detect potential loci for antioxidant traits in rice. The detected loci will further be fine-mapped for application in maker-assisted breeding for improvement of antioxidant traits in rice.

5. Conclusions

Consumption of rice containing a higher content of antioxidants has many health benefits. Donor lines rich in more than three antioxidant traits were identified from the population. The germplasm lines, namely, Ac. 44592, Ac. 44646, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac. 43669, presented high results for three antioxidant traits. Antioxidant traits such as superoxide dismutase, flavonoids, anthocyanins, \( \gamma \)-oryzanol, and ABTS were mapped in a representative panel population using 136 SSR markers through association mapping. Wide genetic variations were observed for the studied six antioxidant traits in the population. The population was classified into four 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 a fair degree of correspondence with the antioxidant traits present in each subpopulation. A total of 14 significant marker–trait associations for the antioxidant traits were detected of which 3 QTLs namely qANC3, qPAC12-2 for anthocyanin content and qAC12 for ABTS activity were validated in the population. These three 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 \( \gamma \)-oryzanol, and qAC11.1 for ABTS, were detected as novel loci. Co-localization of the QTLs detected for OZ11.1, TFC11.1, and AC11.1 regulating \( \gamma \)-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 antioxidant improvement programs in rice.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123036/s1, Figure S1: Scree plot and loadings generated by the six antioxidant traits and eigen values % in the 120 rice germplasm lines. Figure S2: (A) Graph of \( \Delta K \) value, to the rate of change in the log probability structure of the 120 germplasm lines of the panel population based on membership probability fractions of individual genotypes at K of data between successive K values; (B) Population = 2. Figure S3: (A) Graph of \( \Delta K \) value, to the rate of change structure of the 120 germplasm lines of the panel population based on membership probability fractions ge in the log probabil-
ity of data between successive K values; of individual genotypes at K (B) Population = 3. Figure S4: The distribution pattern of alpha value and Fst values in the 4 subpopulations at K = 4. Table S1: Mean vaules of carotenoids, SOD, TAC, GO, TFC and AABTS antioxidants in 270 rice germplasm line. Table S2: The inferred ancestry value and population structure of individual member with their antioxidants classification in the panel population at K = 2 & K = 3. Table S3: Marker-trait associations with antioxidant content in the panel population detected by the model GLM at p < 0.01. Table S4: Marker-trait associations with antioxidant content in the panel population detected by the model MLM at p < 0.01. Table S5: Markers information of the selected 136 SSR markers used for antioxidant content in indica rice.

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