Analysis and comparison of the γ -oryzanol content based on phylogenetic groups in Korean landraces of rice (*Oryza sativa* L.)

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ABSTRACT Brown rice contains beneficial phytonutrients such as antioxidants, anthocyanins and oryzanol, and oryzanol is one of the major components. However, the information of oryzanol contents and genetic data are limited in Korean rice landraces to use the genetic resources. This study was conducted to investigate varietal differences of the oryzanol content and analyze the genetic diversity using SSR marker about 196 Korean rice landraces. Among tested germplasm, the total γ -oryzanol values showed the high variation ranged from 9.8 to 55.9 mg 100g⁻¹ and an average content value was 27.2 mg 100g⁻¹. Particularly, IT007903, IT007714, IT006622 and IT006125 accessions were showed γ -oryzanol contents higher than 50mg 100g⁻¹. The 24-methylene cycloartenyl ferulate was the most prevalent with an average value of 29.9% among the total γ -oryzanol components, and followed by cycloartenyl ferulate (26.7%). Genetic diversity among 196 landrace accessions was evaluated based on 46 SSR markers carrying total 396 alleles. The mean values of observed (H_o) and expected heterozygosities (H_e) were 0.009 and 0.497, respectively, indicating a considerable amount of polymorphism within this collection. A genetic distance-based phylogeny grouped into seven clusters with genetic distance (GD) value was 0.6. According to the phylogenetic analysis, roughly 7 clusters were divergent, and the γ -oryzanol content values showed statistical differences by the four groups ($P < 0.001$). These traits of the selected accession would be helped broadening for parent materials selection to improve the γ -oryzanol content through the rice breeding.

Keywords γ -oryzanol, Genetic diversity, Genetic conservation, Microsatellite, Rice landrace

INTRODUCTION

Rice as harvested from the field is called paddy that has several layers. Milling is processed and rice grains are transformed into a form that suitable for human consumption. Only the outermost layer, the hull, is removed at first commercial milling process and we call brown rice. Brown rice further was milled to produce white rice. Rice is mostly consumed in polished and whole milled form with little or no bran remaining on the endosperm. However, it is known that valuable nutrients such as dietary fiber, ferullic acid, isovitexin, phytic acid, γ -oryzanol, tocopherol, γ -amino butyric acid are concentrated in the germ and outer layer of the starchy endosperm and these compounds are well-known

for functions as antioxidant activity (Chae 2002; Oh and Oh 2004; Fardet et al. 2008). It is reason that brown rice is widely valued as a healthy food, and for good reason.

γ -oryzanol is one of the major valuable nutrients in rice bran as an antioxidant compound (Kim et al. 1995; Lee et al. 2011). γ -oryzanol is a mixture of phytosteryl ferulates (Scavariello and Arellano 1998) and it may help lower elevated LDL (low density lipoprotein)-cholesterol levels and inhibition of LDL-cholesterol synthesis (Nakayama et al. 1987; Scavariello and Arellano 1998) and increase HDL cholesterol levels (Cicero and Gaddi 2001). Xu and Godber (1999) found that 24-methylene cycloartanyl ferulate, cycloartanyl ferulate, campesteryl ferulate, β -sitosteryl ferulate and campestanol ferulate which have been identified

Received Mar 14, 2013; Revised Mar 23, 2013; Accepted Mar 25, 2013; Published March 31, 2013

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as the major components and were found to have antioxidant activity 10 times greater than the major tocopherol and tocotrienol components of vitamin E.

Some reports have been published about γ -oryzanol contents in rice bran (Lee et al. 2011; Boonsit et al. 2010), but little is known regarding of its levels in Korean rice landraces. Landraces have been continuously maintained by farmers within different agricultural areas as well as their local environments. Landraces have distinct identity and lack formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems (Villa et al. 2006). The landraces of crop has been valued as a source of traits that can be used in breeding programs and to improve the productivity and the quality of new crop varieties. Therefore, it is very important to acquire knowledge of the genetic diversity and relationships among landraces germplasm. Studies of genetic diversity using molecular markers are necessary to understand the genetic relationships and structures of populations and to orientate effective strategies of landraces germplasm conservation (Ganapathy et al. 2011). With many other crop species, SSR markers are being widely used in rice studies. SSR markers are highly efficient molecular tools for plant variety characterization or diversity assessment of rice landraces germplasm because of their high levels of polymorphism and high reproducibility (Park et al. 2008; Verma et al. 2010).

The germplasm of crop has a great diversity for morphological traits like grain size, leaf shape and height. This diversity offers opportunities to develop unique cultivars for agronomic applications. An assessment of relationship between oryzanol contents and genetic diversity among landraces germplasm will be useful to screen the germplasm. The information of high oryzanol content about genetic resources will be a valuable data to the people who substantially need it and in the breeding program. The objectives of this study were to investigate the γ -oryzanol contents in rice landraces collected from Korea and to evaluate genetic diversity and relationships between landrace accessions with genetic variation.

MATERIALS AND METHODS

Plant materials and γ -Oryzanol contents analysis

The collection contained a total of 196 landrace accessions of Korea (*Oryza sativa* L.) were acquired from Agrobiodiversity Center (NAAS, RDA, Korea). A list of germplasm accessions used in this study is given in Table 1. Seed samples were cultivated in Suwon city, Korea, in 2009 and 2010 according to standard rice cultivation Manual of RDA and harvested seeds of two years were separately analyzed.

Gamma-oryzanol analysis was performed by Xu and Godber. The γ -oryzanol was extracted from dehulled rice with 50 ml of dichloromethane-methanol (2:1, v/v) and total γ -oryzanol analysis was performed using an Agilent HPLC series 1200 (Agilent, Waldbronn, Germany). Agilent HPLC series 1200 (Agilent, Waldbronn, Germany) were used to determine the concentration of γ -oryzanol using Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm i.d. and 5- μ m particle size) (Agilent, Waldbronn, Germany) at 40 °C according to Lee et al. (2011). The mobile phase consisted of methanol, acetonitrile, dichloromethane and acetic acid (50:44:3:3, v/v). The separation was carried out with a Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm i.d. and 5- μ m particle size) (Agilent, Waldbronn, Germany) operated at 40 °C. UV detection wavelength was set at 315 nm. Quantitation was based on the linear calibration curve of the sum of the area of all fractions versus molarities of gamma-oryzanol dilutions at 315 nm wavelength of UV detector. Standard chemicals were obtained from Sigma-Aldrich (St. Louis, Mo, USA).

DNA extraction and assess of microsatellite markers

Genomic DNA was extracted from the young leaves of samples using Plant DNAzol reagent (Invitrogen). The DNA concentration was determined using a NanoDrop (ND-1000; NanoDrop, Wilmington, DE, USA). The final concentration of each DNA sample was adjusted to 20 ng/ μ l in TE buffer before conducting PCR. The M13-tail at the 5'-end region PCR method was used to measure the sizes of the amplified products (Schuelke 2000). For genotyping analysis, primers were chosen from the Gramene database (<http://www.gramene.org/markers/microsat/ssr.html>) and

Table 1. List and total γ -oryzanol contents of 196 rice landrace accessions.

Stock No. of genebank	Accession name	γ -oryzanol (mg 100g ⁻¹)	Stock No. of genebank	Accession name	γ -oryzanol (mg 100g ⁻¹)
IT004688	Ggaebyeo	22.55	IT005743	Daegoldo-2	36.88
IT004692	Gasanbyeo	24.45	IT005754	Daegwando	26.65
IT004694	Gaksijeomjo	22.51	IT005756	Daeguna	18.5
IT004753	Gangdodo	26.74	IT005762	Daegudo	35.31
IT004760	Gangreungdo	38.73	IT005835	Daejodo	41.19
IT004768	Gangsan byeo	17.3	IT005882	Danduna	22.69
IT004769	Gangbaedo	27.1	IT005893	Dangdo	21.79
IT004770	Gangwondo	39.27	IT005908	Dorae	31.05
IT004771	Gangwonna	37.14	IT005915	Doaji	25.24
IT004775	Gangcheongdo	20.86	IT005946	Dongsanjo-1	18.12
IT004811	Ge	14.63	IT005948	Dongsanjo-2	31.94
IT004839	Gyeongjobaekjo	20.77	IT005970	Dongobyeo	21.96
IT004899	Gwaksanjo	21.62	IT005980	Dudo	39.16
IT004914	Gwansansaek	21.42	IT005987	Duchungjong-1	31.91
IT005044	Guwangdo	29.52	IT005989	Duchungjong-2	33.33
IT005046	Guwoldo	14.88	IT005994	Deokjeokjodo	35.77
IT005051	Gujungdo-1	22.56	IT006000	Deulleongdeulchigi	19.05
IT005052	Gujungdo-2	34.31	IT006010	Ddangjo	23.43
IT005057	Gucheondo	11.92	IT006066	Maekjo	29.33
IT005068	Guhwangdo-1	21.19	IT006078	Monajo	23.8
IT005070	Guhwangdo-2	13.91	IT006084	Modo-1	28.93
IT005076	Gunjo	31.71	IT006087	Modo-2	25.78
IT005095	Gwido	22.87	IT006089	Mojo	25.28
IT005126	Geumdo	36.03	IT006100	Monggeunchanarak	22.82
IT005133	Geumjeomdo	27.04	IT006103	Mudaraegi	31.15
IT005142	Geumchangdo	34.62	IT006112	Musando	47.34
IT005205	Na-1	27.17	IT006114	Musaek Jojeokjo	25.71
IT005206	Na-2	26.53	IT006116	Muando	41.51
IT005216	Naengdo	20.54	IT006119	Muyeopseoldo	27.2
IT005223	Namgangbaekjo	29.53	IT006125	Mujudo	50.04
IT005500	Noindari	46.09	IT006129	Migwang	30.74
IT005504	Noinjo-1	35.22	IT006138	Mido	30.37
IT005505	Noinjo-2	20.29	IT006151	Mijo	35.5
IT005506	Noindo-1	34.05	IT006242	Mitdarae	31.59
IT005508	Noindo-2	25.62	IT006243	Badol byeo	29.77
IT005509	Noindo-3	27.97	IT006247	Baramdungguri	32.08
IT005657	Nokdudo-1	11.47	IT006258	Bandalbyeo-1	32.07
IT005660	Nokdudo-2	20.77	IT006260	Bandalbyeo-2	43.56
IT005677	Neuseubyeo	18.59	IT006266	Banchonjo	41.91
IT005678	Neutdakbyeo	13.19	IT006298	Baekkiongzo	23.18
IT005679	Dadajo-1	27.22	IT006302	Baekgogna	34.52
IT005681	Dadajo-2	35.46	IT006310	Baekgwangok	20.57
IT005682	Dadajo-3	26.37	IT006328	Baekmangjo	30.57
IT005683	Dadajo-4	27.79	IT006354	Baekseok	19.67
IT005689	Dadeogbereum	35.16	IT006366	Baekjanggun	18.9
IT005691	Dadoaek	38.78	IT006372	Baekjo	26.22
IT005693	Dadujo	20.68	IT006376	Baekjicheongbyeo	14.33
IT005694	Damagung	24.16	IT006380	Baekchalbyeo	24.3
IT005716	Dabaekjo	28.59	IT006385	Baecheon-1	31.61
IT005718	Daigolbyeo	28.46	IT006386	Baecheon-2	25.3
IT005736	Daigolna	38.75	IT006396	Baekhaedal	19.35
IT005742	Daegoldo-1	22.56	IT006397	Baekhyangjo	26.11

Table 1. Continued.

Stock No. of genebank	Accession name	γ -oryzanol (mg 100g ⁻¹)	Stock No. of genebank	Accession name	γ -oryzanol (mg 100g ⁻¹)
IT006400	Beodeulbyeo	18.45	IT007532	Aedal	29.34
IT006404	Beonjo	15.48	IT007559	Aengmi	21.46
IT006410	Beobpanhwa	25.43	IT007570	Yangdo	32.42
IT006424	Boribyeo	11.32	IT007578	Eoreumbyeo	15.33
IT006483	Bujari	22.63	IT007585	Yeobyeeo	30.13
IT006520	Buldo	30.03	IT007592	Yeosubyeo	36.25
IT006522	Buljo	26.87	IT007596	Yeoussalbyeo	19.32
IT006538	Saducho	25.1	IT007598	Yeonanjo	17.08
IT006551	Sandadagido	22.74	IT007604	Yeolna	24.13
IT006554	Sando-1	22.2	IT007605	Yeolsulbyeo	20.66
IT006556	Sando-2	37.91	IT007622	Yejo	25.98
IT006559	Sandudo-1	27.19	IT007629	Orido-1	31.97
IT006560	Sandudo-2	34.37	IT007630	Orido-2	24.66
IT006577	Ssanmadeuragi	19.21	IT007631	Orido-3	17.13
IT006578	Ssalbyeo	13.09	IT007633	Obaekjo-1	17.7
IT006596	Samgyeongjo	33.09	IT007634	Obaekjo-2	31.39
IT006620	Sangdo-1	30.73	IT007684	Olmuge	39.49
IT006622	Sangdo-2	50.05	IT007688	Olbyeoo	23.04
IT006657	Seogandodo	29.2	IT007693	Olwaedu	16.29
IT006663	Seorianjeunbaengi	24.23	IT007714	Waengchal byeoo	54.72
IT006684	Seoksanna	30.11	IT007717	Oegukbyeoo	29.37
IT006687	Seoksanjo	23.34	IT007721	Waejo	25.82
IT006699	Seondal	22.47	IT007740	Yonamjo	18.77
IT006735	Sodujo	21.54	IT007742	Yongmyeonheuk	25.35
IT006768	Soemeoribyeoo	14.62	IT007746	Yongcheon-1	25.69
IT006772	Soemeorijijang	31.71	IT007747	Yongcheon-2	29.38
IT006776	Soebenchigi	24.38	IT007792	Wonjabyeoo	28.43
IT006818	Susangjo	42.87	IT007801	Woljo	30.26
IT007245	Suwonjo	19.55	IT007807	Yu	34.74
IT007254	Sujungjo	22.49	IT007900	Yukwoljo	37.21
IT007268	Ssubyeoo	30.24	IT007903	Eumeuchal	55.85
IT007270	Sukna-1	22.04	IT007975	Eunjo	20.12
IT007274	Sukna-2	20.83	IT007981	Eumjo	21.97
IT007278	Sulsuldo	31.81	IT007999	Irakdo	38.85
IT007282	Sutdarkbyeoo	31.45	IT008189	Icheonchunggubyeoo	22.22
IT007286	Seungsiljo	13.85	IT008196	Inbujido	32.97
IT007290	Sseundegi	22.16	IT008199	Inbujinado	29.44
IT007389	Sinbaekseok	27.9	IT008255	Jandadagi	31.56
IT007436	Agabyeoo	23.21	IT008267	Jangsamdo	30.35
IT007442	Agudo	26.07	IT008268	Jangsamdo	22.39
IT007446	Agukdo	26.9	IT008277	Jangjo-1	43.77
IT007458	Arongbyeoo	23.22	IT008278	Jangjo-2	45.48
IT007460	Anna	45.2	IT008286	Jaeraesuyeom	16.54
IT007464	Annamjo	30.02	IT008289	Jaeraedo	19.34
IT007486	Anjeunbaengi-1	19.35	IT008293	Jaeraejodo-1	17.24
IT007487	Anjeunbaengi-2	9.78	IT008295	Jaeraejodo-2	19.18

the genome-wide SSR markers used in this study are listed in Table 3. Amplified fluorescent-labeled PCR products were analyzed on an ABI-Prism 3130x1 Genetic Analyzer (Applied Biosystems). Fragments were sized and scored into alleles using GeneMapper v4.0 (Applied Biosystems).

Diversity statistics

The total number of alleles (N_A), expected (H_E) and observed heterozygosities (H_O) and polymorphic information content (PIC) for each SSR locus were calculated with PowerMarker version 3.25 (Liu and Muse 2005). An unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering were performed based on the matrix of genetic similarity estimates according to the procedures of the PowerMarker software. The tree to visualize the phylogenetic distribution of accessions was constructed using the software MEGA (Tamura et al. 2007). Analysis of variance (ANOVA) was performed to statistical analysis for the difference of total γ -oryzanol contents among the groups classified by phylogenetic tree using SAS (version 9.2) software.

RESULTS

Analysis of γ -oryzanol contents

The physicochemical trait about γ -oryzanol contents was analyzed using HPLC and the γ -oryzanol contents of 196 rice landrace accessions are presented in Table 1. In this study, 10 kinds of γ -oryzanols were detected and the compositions in the γ -oryzanol were as follows: Δ 7-Stigmastenyl (0.3 to 3.7%), Stigmastenyl (0.5 to 2.1%), Cycloartenyl (12.4 to 38.4%), 24-Methylene Cycloartenyl (20.3 to 45.8%), Δ 7-Campestenyl ferulate (0 to 11.7%), Campesteryl (8.3 to 36.4%), Δ 7-Sitostenyl (0 to 0.9%), Sitosteryl (5.3 to 16.4%), Campestanyl (0 to 14.6%) and Sitostanyl (1.3 to 13.1%) (Fig. 2). These results indicated that the averages of individual components of γ -oryzanol among the total γ -oryzanol were: 24-methylenecycloartanyl ferulate (29.9%), and followed by cycloartenyl ferulate (26.7%) and campesteryl ferulate (20.3%). The minor component group, sitosteryl ferulate, showed high proportions of 11.4%. The main components are 24-methylenecycloartanyl, cycloartenyl, and campesteryl with about 80% of the γ -oryzanol (Fig. 2). There is a similarity in the γ -oryzanol

Table 2. List of 10 accessions containing highest total γ -oryzanol ($\text{mg } 100\text{g}^{-1}$) and the contents (%) of ten compositions in the γ -oryzanol among 196 rice landrace accessions.

Stock No. of genebank	1	2	3	4	5	6	7	8	9	10	γ -oryzanol ($\text{mg } 100\text{g}^{-1}$)	Clusters
IT007903	2.0%	1.5%	23.9%	31.8%	2.1%	18.1%	0.7%	11.9%	5.2%	2.8%	55.85	Cluster 2
IT007714	2.3%	1.6%	23.2%	31.1%	2.0%	18.7%	0.7%	12.0%	5.3%	3.0%	54.72	Cluster 2
IT006622	0.9%	1.1%	27.7%	27.1%	4.8%	8.6%	0.8%	6.6%	12.0%	10.3%	50.05	Cluster 1
IT006125	1.2%	1.3%	28.1%	31.4%	0.8%	20.6%	0.5%	12.4%	2.3%	1.5%	50.04	Cluster 3
IT006112	0.3%	1.2%	21.7%	31.5%	1.9%	8.5%	0.7%	10.3%	10.7%	13.1%	47.34	Cluster 6
IT005500	0.7%	1.1%	29.9%	34.3%	1.4%	17.4%	0.5%	9.8%	3.3%	1.6%	46.09	Cluster 2
IT008278	1.9%	1.2%	23.0%	31.8%	2.0%	22.5%	0.0%	11.3%	4.3%	2.0%	45.48	Cluster 3
IT007460	1.0%	1.1%	36.2%	27.1%	0.7%	18.2%	0.4%	11.6%	2.2%	1.5%	45.2	Cluster 4
IT008277	1.9%	1.1%	23.2%	34.3%	2.0%	21.3%	0.0%	11.2%	3.4%	1.7%	43.77	Cluster 3
IT006260	0.9%	1.1%	26.8%	32.2%	1.0%	19.7%	0.8%	12.7%	2.8%	2.0%	43.56	Cluster 3

1, Δ 7-Stigmastenyl; 2, Stigmastenyl; 3, Cycloartenyl; 4, 24-Methylene Cycloartenyl; 5, Δ 7-Campestenyl ferulate; 6, Campesteryl; 7, Δ 7-Sitostenyl; 8, Sitosteryl; 9, Campestanyl; 10, Sitostanyl.

content in the previous study (Miller et al. 2006).

The Distribution of rice landrace accessions based on the γ -oryzanol content showed in the Fig. 1. Among tested germplasm, the total average γ -oryzanol values were observed considerable variations, which ranged from 9.8 to 55.9 mg 100g⁻¹ with a mean value of 27.2 mg 100g⁻¹ in 196 rice landraces. The total γ -oryzanol content of this study was more or less than in other studies (Boonsit et al. 2010, Lee et al. 2011, Yoshie et al. 2009). Most of cultivars were in the range of 20-40 mg 100g⁻¹ (Lee et al. 2011; Yoshie et al. 2009) in common rice grains and 40-73 mg 100g⁻¹ (Boonsit et al. 2010) in purple rice. A hundred forty-six (74.4%) of all accessions revealed the γ -oryzanol content to range from 20 to 40 mg 100 g⁻¹ in this study.

The four rice landraces in this study that yielded the highest total γ -oryzanol content were IT007903 (55.85 mg 100 g⁻¹), IT007714 (54.72 mg 100 g⁻¹), IT006622 (50.05 mg 100 g⁻¹) and IT006125 (50.04 mg 100 g⁻¹) (Table 2), while thirty-six accessions (18.4%) showed the γ -oryzanol contents less than 20 mg 100 g⁻¹ and more than 40 mg 100 g⁻¹ in fourteen accessions (7.1%) (Fig. 1). There was the

interaction among the compositions of γ -oryzanol. It can be seen that the content of campesterol increased, whereas the sitosterol was decreased. Although our extracts in landraces were averagely lower than those of colored rice (Boonsit et al. 2010), the total average γ -oryzanol values were observed high variations, and the used germplasm in this study contained a higher amount of γ -oryzanol than in common rice.

Genetic Diversity Statistics

The average number of alleles, the frequency of the major allele and gene diversity are given in Table 3. All the 46 genome-wide SSR primers were used for genetic diversity analysis and detected 396 alleles among 196 rice landrace accessions. The average alleles per primer pair was 8.6, ranging from a minimum of 2 alleles for RM6165 and RM12676 on chromosome 2 to a maximum of 33 for RM206 on chromosome 11. In general, higher values both of H_o and H_e revealed a higher genetic variability among the germplasm accessions. The values of H_o and H_e ranged from 0.000 to 0.046 (mean 0.009) and from 0.021 to 0.928

Fig. 1. Distribution of rice landrace accessions according to γ -oryzanol content.

Fig. 2. Comparison of the means of proportions expressed as the percentage of total γ -oryzanol in individual components isolated from grain of the rice germplasms.

(mean 0.497), respectively and the highest H_0 and H_E were revealed by RM3766 (0.046) on chromosome 3 and RM206 (0.928) on chromosome 11, respectively. The PIC values, a reflection of allele diversity and frequency among the used germplasm, also varied from one locus to another. The PIC values ranged from 0.021 to 0.924, with an average of 0.467. Markers with PIC values of more than 0.5 are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (DeWoody et al. 1995). Twenty-six of 46 genome-wide SSR markers observed in this study were higher than the PIC value of 0.5 and the genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. The genetic variation among the accessions revealed by SSRs reflected a high level of polymorphism at the DNA level. The results suggest that these SSR markers of rice landraces would be a valuable marker resource for the genetic diversity analysis of rice germplasm.

Phylogenetic relationship

We divided into seven clusters in the landraces germplasm to assess the genetic relationship among the rice landraces

and to evaluate the genetic differentiation among the clusters by UPGMA cluster analysis of the similarity matrix. However, the landraces originally from various regions of Korea did not form distinct clusters by SSR markers. These were interspersed with each another in the classified clusters, which confirmed no association between the landraces patterns by SSR and their γ -oryzanol content. The landraces with high variation of the contents were mixed and distributed throughout the seven clusters.

The UPGMA dendrogram has classified seven clusters (Cluster 1, Cluster 2, Cluster 3, Cluster 4, Cluster 5, Cluster 6 and Cluster 7) in the 196 accessions at 0.49 similarity coefficient (Fig. 3). To determine the variation of γ -oryzanol content among the phylogenetic clusters in rice landraces, we performed ANOVA analysis using SAS program. According to the phylogenetic analysis, roughly 7 clusters were divergent, and the γ -oryzanol content values showed statistical differences by the four groups ($P < 0.001$) (Table 4). The first group included 24 landraces, which are Cluster 1 with average γ -oryzanol contents ($33.2 \text{ mg } 100\text{g}^{-1}$) and Cluster 2 with $32.6 \text{ mg } 100\text{g}^{-1}$. The second group contained 21 accessions, which is Cluster 3 with $34.5 \text{ mg } 100\text{g}^{-1}$ and the third group contained 40 accessions, which is Cluster 4

Table 3. Genetic parameters obtained from the 46 SSR markers that were used to evaluate the 196 rice landrace accessions.

SSR marker	Chromosome	M _{AF}	N _A	H _E	H _O	PIC
RM1	1	0.546	9	0.636	0.005	0.594
RM5	1	0.451	5	0.682	0.036	0.628
RM580	1	0.230	11	0.843	0.031	0.824
RM246	1	0.341	7	0.729	0.000	0.680
RM174	2	0.972	3	0.055	0.005	0.055
RM048	2	0.350	25	0.836	0.000	0.825
RM3857	2	0.301	13	0.816	0.025	0.793
RM6165	2	0.990	2	0.021	0.010	0.021
RM12676	2	0.563	2	0.492	0.000	0.371
RM135	3	0.979	4	0.041	0.010	0.041
RM3766	3	0.309	13	0.775	0.046	0.743
RM231	3	0.833	6	0.295	0.000	0.281
RM232	3	0.244	15	0.855	0.000	0.841
RM252	4	0.833	4	0.282	0.015	0.249
RM349	4	0.644	6	0.516	0.005	0.457
RM241	4	0.464	12	0.714	0.000	0.680
RM6629	4	0.968	3	0.062	0.000	0.061
RM16427	4	0.932	3	0.127	0.000	0.120
RM13	5	0.829	4	0.289	0.010	0.257
RM249	5	0.211	17	0.892	0.000	0.883
RM3322	5	0.797	5	0.335	0.000	0.297
RM19159	5	0.655	11	0.524	0.040	0.483
RM103	6	0.964	4	0.071	0.010	0.070
RM253	6	0.500	7	0.667	0.031	0.622
RM253	6	0.541	6	0.642	0.000	0.602
OSR21	6	0.709	5	0.432	0.000	0.367
RM197	6	0.990	3	0.021	0.000	0.021
WxOligo	6	0.549	7	0.546	0.000	0.455
RM418	7	0.439	11	0.753	0.026	0.729
RM1306	7	0.337	21	0.851	0.036	0.842
RM214	7	0.259	21	0.879	0.000	0.870
RM3718	7	0.516	5	0.524	0.000	0.412
RM149	8	0.567	7	0.599	0.015	0.545
RM044	8	0.495	12	0.708	0.000	0.683
RM310	8	0.316	12	0.820	0.045	0.801
RM23455	8	0.834	3	0.290	0.000	0.270
RM444	9	0.922	6	0.148	0.000	0.145
RM257	9	0.544	8	0.595	0.000	0.530
RM171	10	0.961	3	0.075	0.005	0.074
RM228	10	0.526	8	0.650	0.000	0.608
RM6144	10	0.927	2	0.135	0.000	0.126
RM021	11	0.317	11	0.764	0.000	0.727
RM206	11	0.143	33	0.928	0.000	0.924
RM519	12	0.982	4	0.035	0.005	0.035
RM235	12	0.922	5	0.148	0.000	0.144
RM247	12	0.356	12	0.739	0.000	0.701
Mean		0.610	8.6	0.497	0.009	0.467
Min		0.143	2	0.021	0.000	0.021
Max		0.990	33	0.928	0.046	0.924

Fig. 3. An UPGMA tree showing the genetic relationships among the 196 Korean landrace accessions. Triangle; accessions with high oryzanol content, Quadrangle; accessions with low oryzanol content.

with $28.3 \text{ mg } 100\text{g}^{-1}$. The fourth group contained 111 accessions, which are Cluster 5 with $24.2 \text{ mg } 100\text{g}^{-1}$, Cluster 6 with $26.1 \text{ mg } 100\text{g}^{-1}$ and Cluster 7 with $23.6 \text{ mg } 100\text{g}^{-1}$ (Table 4). Clear relationship between Korean landraces contained variations of the total average γ -oryzanol contents and the clusters classified by cluster analysis was not found in this study. Only the clusters were revealed the total γ -oryzanol contents difference. As in the cluster analysis, Korean landraces showed genetical variations, although the accessions used in this study were not classified obviously with oryzanol contents. However, the four

accessions, IT007903, IT007714, IT006622 and IT005500, containing the highest the total γ -oryzanol contents belonged to Cluster 1 and Cluster 2 (Table 2 and Fig. 3.). It inferred that Korean landraces have diverse genetic bases and can be utilized in future breeding.

DISCUSSION

Brown rice is widely known as a staple and valuable food in many diets around the world because it contains

Table 4. ANOVA test of γ -oryzanol content based on the pylogenetic clusters.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	2926.4	487.7	8.34	<.0001
Cluster	N	Total (γ -oryzanol)		Duncan grouping ²⁾	
		Mean	Std Dev		
Cluster 1	6	33.2	10.3	AB	
Cluster 2	18	32.6	11.6	AB	
Cluster 3	21	34.5	8.2	A	
Cluster 4	40	28.3	6.0	BC	
Cluster 5	29	24.2	7.1	C	
Cluster 6	24	26.1	7.5	C	
Cluster 7	58	23.6	6.9	C	

²⁾Means with the same letters are not significantly different at $p < 0.001$ as determined by Duncan's multiple test.

higher levels of gamma-tocotrienol, ferullic acid, dietary fiber and gamma-oryzanol. One of these compounds, γ -oryzanol, functions as lipid-soluble antioxidants that can reduce blood levels of LDL-cholesterol (Nakayama et al. 1987) and due to its antioxidant effects, it has been shown to be beneficial in patients with menopausal symptoms (Ishihara et al. 1982) and controlling blood pressure (Chae 2002). Therefore, a rice genotype producing high levels of γ -oryzanol would be commercially valuable (Boonsit et al. 2010)

The long tradition of rice cultivation in Korea had allowed the evolution of many landraces adapted to restricted areas. Nowadays, in response to market demands, landraces have been gradually replaced by improved cultivars, because it can contain some valuable alleles not common in modern germplasm. However, landraces still one of the important genetic resources for breeding area, because they contain huge genetic variability. Variation in landraces can be used to complement and is helpful for broadening the crop gene pool (Kobayashi et al. 2006), but little is known about its levels in Korean rice landraces. The objective of in this paper was to investigate the content of γ -oryzanol in rice landrace genotypes domesticated in South Korea. Compared to previous studies reported that the oryzanol contents with range from 16-20 mg 100g⁻¹ (Lee et al. 2011), our results could indicate the considerable variations in γ -oryzanol content among the rice genotypes cultivated from Korea. We obtained that the total γ -oryzanol content of landraces showed high variation ranged from 9.8 to 55.9 mg 100 g⁻¹ and a mean was 27.2 mg 100 g⁻¹ in this study. Furthermore, some landrace accessions, IT007903 (55.85 mg 100g⁻¹), IT007714 (54.72 mg 100g⁻¹), IT006622 (50.05 mg 100g⁻¹) and IT006125 (50.04 mg 100g⁻¹), have higher contents of total γ -oryzanol, it will be good the genetic resources to rice breeding to improve the γ -oryzanol and quality.

An UPGMA dendrogram showed that the genotypes that are derivatives of genetically similar types clustered together, and landraces in the same subgroup mostly shared a high proportion of ancestry and agronomic features such as plant height, maturity, seed length, etc (Pervaiz et al. 2010; Odile et al. 2011). However, it is difficult to distinguish relationship of γ -oryzanol contents according

to physiological, ecological classifications and agronomic characters of rice. Therefore, in molecular aspects, we performed to the genetic differentiation and patterns of phylogenetic relationship among a diverse set of rice landrace accessions collected from South Korea using the UPGMA cluster analysis based on genome-wide SSR markers, and then we checked the association between classified clusters and γ -oryzanol contents of individual landrace accession. The results indicated that the phylogenetic clustering showed difference of genetic variability among all 196 rice landrace accessions and the landrace accessions revealed the presence of 7 possible clusters. However, the UPGMA dendrogram did not observe clear grouping of the accessions according to high or low γ -oryzanol contents. Only it was revealed the difference of average γ -oryzanol contents among the clusters.

We analyzed the γ -oryzanol content in rice landraces, and compared the content values by the phylogenetic clusters of Korean landraces using ANOVA test. Based on the phylogenetic analysis, roughly 7 clusters were divergent, and the γ -oryzanol content values showed statistical differences by the 4 groups ($P < 0.001$). The results revealed that the dendrogram showed the complex distribution pattern among 196 landraces. However, 14 landrace accessions with high γ -oryzanol content were closely located within three clusters, Cluster 1, Cluster 2 and Cluster 3. Previous studies have been reported that rice bran consists of the outer layers (pericarp, seed coat, and aleurone) and the embryo or germ (Rohrer et al. 2004), and environment as well as genetics affects the contents and composition of γ -oryzanols in rice seed (Bergman et al. 2003; Miller et al. 2006). Therefore, it will be further study that the content of γ -oryzanol was affected according to the embryo size and pericarp thickness of the landraces seed in this study.

The assessment of genetic variability among genotypes is useful for the conservation of genetic resources and for cultivar protection (Yuzbaşıoglu et al. 2006). The information obtained here would be useful to evaluate genetic resources of rice accessions and for the utilization of these plants for the rice breeding of parent materials selection. This study might be the basis for association analysis of γ -oryzanol in diverse rice landraces.

ACKNOWLEDGEMENTS

This study was supported by a grant (Code no. PJ0083-682013) from the National Academy of Agricultural Science, RDA, Republic of Korea.

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