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Full Length Article

Analysis of Anthocyanins and Proanthocyanidins Synthesis Related Genes by Genome Re-sequencing of Radiation-induced Mutation in Rice

Yining Huang[1,2], Lihua Chen[3], Zuxin Cheng[1], Xinying Huang[1], Ming Xu[1] and Jingui Zheng[1]*
[1]*College of Crop Science, Fujian Agriculture and Forestry University, Fuzhou Fujian, China*
[2]*Zhangzhou Institute of Technology, Zhangzhou Fujian, China*
[3]*Jinshan College of Fujian Agriculture and Forestry University, Fuzhou Fujian, China*
*For correspondence: zzuliuqi@hotmail.com

Abstract

Anthocyanins and proanthocyanidins, as important functional substances in black rice and red rice, respectively, with special health care effects. In order to gain a deeper understanding of the genes involved in the synthesis of anthocyanins and proanthocyanidins in rice, this study was carried out for genome re-sequencing of three strains of mutant black rice and four strains of mutant red rice derived from mutation of ^{60}Co-γ ray irradiation, wild type 9311. Results showed that there were 9345 large effect variation sites in seven rice mutants, involving 3533 genes. The biosynthesis pathway for 42 mutant genes to participate in beneficial substances or pigment accumulation was predicted by mutant gene analysis. These mutations have potential research value in providing new information for rice grain pigmentation research. © 2020 Friends Science Publishers

Keywords: Radiation-induced mutation; Re-sequencing; Red rice; Black rice; Anthocyanins

Introduction

Rice, as one of the important food crops (Khush, 2001), is also a model plant for functional gene research. Red rice and black rice have been extensively studied as important cultivars in rice. Due to deposition of extensive flavonoids in grain peel, black rice and red rice grains demonstrate different color from ordinary rice. The flavonoids in black rice peel are mainly anthocyanins, and the grains are black or purple (Yamuangmorn *et al*., 2019). The flavonoids in red rice peel are mainly proanthocyanidins and the grain is red. Both anthocyanins and proanthocyanidins are secondary metabolites in higher plants, and have physiological functions such as anti-oxidation, hypolipidemic, anti-inflammatory effect and inhibition of cancer cells (Mattei *et al*., 2015; Zhu, 2018).

In plants both anthocyanins and proanthocyanidins are produced by flavonoid synthesis pathway (Shih *et al*., 2008). Studies on flavonoid synthesis have been carried out in many plants such as *Arabidopsis thaliana*, *Zea mays*, and *Oryza sativa*, and its synthetic pathways are well known (Forkmann, 2010). Some genes related to the synthesis of anthocyanins and proanthocyanidins have been found in rice (Ithal and Reddy, 2004; Shih *et al*., 2008; Gross *et al*., 2010). Studies have found that structural genes and transcription factors affect the synthesis of black rice anthocyanins and red rice proanthocyanidins (Ithal and Reddy, 2004). It is shown that *OsC1-MYB*, *OsS1-bHLH* and *OsDFR* in rice determine the color of black rice grains together. The transcription factors *OsC1-MYB* and *OsS1-bHLH* are the key genes deciding color formation in black rice seeds (Sun *et al*., 2018). Red rice grain color is controlled by two complementary genes, *Rc* and *Rd* (Zhu *et al*., 2019). Numerous studies have revealed that transcription factors bHLH and MYB are indispensable in the formation of anthocyanins and proanthocyanidins (Oikawa *et al*., 2015; Q *et al*., 2017; Sun *et al*., 2018). MYB family of transcription factors plays a central role in the coordinated activation of anthocyanins and proanthocyanidin synthesis pathway-specific genes, regulating these complex pathways (Dixon *et al*., 2013). So far, the research on the synthesis of anthocyanins and proanthocyanidins in rice mainly focuses on known gene verification or application, and undiscovered genes with the same function are less studied.

Radiation-induced mutation technology has been successfully applied to germplasm innovation in plants such as rice and maize (Shu *et al*., 2012), and more than 1,000 major crop vaieties have been developed so far (http://mvd.iaea.org). As the mutation sites of radiation-induced mutation are random (Li *et al*., 2016), it is possible to have a more comprehensive understanding of genes associated with rice pigmentation by re-sequencing black rice and red rice resulting from radiation-induced mutation and finding unreported genes that may cause phenotypic changes.

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In this study, main rice cultivar Yangdao 6 (9311) was irradiated with ^{60}Co, and seven red rice and black rice of different strains with consistent genetic backgrounds and stable traits were obtained by self-crossing. The progeny of these strains was subjected to genome re-sequencing to find related genes that may lead to peel color variation, thus supplementing the current genes related to the synthesis of anthocyanins and proanthocyanidins in rice, which is of great significance for the research and breeding of rice functional genomics.

Materials and Methods

Plant Material

The seeds of Yangdao 6 (9311) irradiated with ^{60}Co (300 Gy, 50 min) were planted in rice fields. The seeds of M1 generation were collected, and the seeds with black or red mutant peel were retained and selfed for six generations until stable variation. Finally, four mutant red rice strains of 9311-4C478, 9311-4C487, 9311-4C481, 9311-4C483, and three mutant black rice strains of 9311-4C498, 9311-4C503 and 9311-4C513 were obtained. The content of flavonoids and color value of dried red and black rice grains were higher than that of wild type 9311 (Table 1). In July 2013, seven colored rice and one wild type 9311 obtained from mutation were planted in Zhao'an, Fujian Province. Eight rice lines were planted separately and managed in convention. One leaf of each line was collected on the 7th day of the grouting period for DNA extraction.

Rice Genome Re-sequencing

Healthy and intact leaves were collected and cleaned, and DNA was extracted using CTAB method (Zhang *et al*., 2012). After the quality assessment, the extracted genomic DNA was randomly broken by ultrasound (range controlled between 150–800 bp), and DNA fragment of the required length was recovered by electrophoresis to construct a Pair-end library. Finally, the se-quencing was performed on the Hiseq 2500, and the data quality was detected.

The Nipponbare (Assembly version IRGSP-1.0, Gene Set Annotated Version MSU 7) was used as a reference genome, and the reads were aligned to the reference genome by Bwamem (version 0.7.15). The Samtools Stats Program (version 1.6) was used to analyze and calculate information like PCR repetition ratio, mapped rate and single positioning ratio.

Identification of DNA Polymorphisms (SNP and Indel)

Whole genome re-sequencing data were combined for samples 9311-4C477, 9311-4C478, 9311-4C487, 9311-4C481, 9311-4C483, 9311-4C498, 9311-4C503, 9311-4C513, 9311_BGI-1, 9311_BGI-2 (where 9311_BGI-1, 9311_BGI-2 were derived from the NCBISRA public database). Ten sets of genomic data were subject to pairwise alignment through Bwamem (version 0.7.15) software to obtain DNA polymorphisms (SNP and InDel) and informative marker set of samples. The distribution of SNP and InDel on the whole genome chromosome was visualized to plot variation density profile.

Table 1: Contents Color value of anthocyanins and total flavonoids

Sample name	Anthocyanins color value (μ/g)	Total flavonoids (g/100 g)	Peel color
9311-4C477	9.24±0.26	3.16±0.06	White
9311-4C478	32.54±0.12	4.26±0.09	Red
9311-4C481	37.78±0.34	5.12±0.16	Red
9311-4C483	38.11±0.58	4.14±0.08	Red
9311-4C487	42.51±0.82	4.56±0.11	Red
9311-4C498	46.55±0.72	5.68±0.09	Black
9311-4C503	52.56±1.21	6.12±0.17	Black
9311-4C513	43.15±0.78	5.24±0.20	Black

Mutation SNP and InDel Identification

Each mutant was compared with 9311 wild-type material (4C477, BGI-1, BGI-2) and other mutants (as cross-reference) using CausalFinder, and each mutant-specific homozygous genotype variation site was screened by CausalFinder (including SNP and InDel). The mutation sites with large variation effect in the Non-TE related gene were further filtered, specifically by removing mutation of intron, mutation of the exon UTR (untranslated region) and small effect mutation like synonymous mutations from the results. The genes that may affect the grain color were screened by analysis.

Results

Mapping of Reads to the Nipponbare Genome

Whole genome re-sequencing of eight rice strains resulted in 522,020,732 sequences (Total reads), clean data of 65.25G in total, and more than 5G clean data was obtained from each sample. With Q20>93.5%, Q30>87.5% in all samples, sequencing quality was high. The coverage rate of sequencing sample was 95.17–97.01% for genome of Nipponbare, the ratio of duplicated reads was 1.57–18.37%, mapped rate was 92.02–97.01%, and unique mapped rate was 76–81.04%. The average coverage depth of each sample at the genome-wide level ranged from 7.78 to 30.02.

Detection of DNA Polymorphisms

Polymorphisms (including SNPs and InDels) of the 9311 wild-type and mutant strains were screened using Bwamem (version 0.7.15) software. A total of 949,354 polymorphs were identified from genomic data of 10 samples (including the NCBISRA public database 9311_BGI-1, 9311_BGI-2). There were 828,182 SNPs, accounting for 87.2%, transition/transversion (Ts/Tv) of SNP is 2.46; there are

95,828 InDel accounting for 10.1% and 50,979 1bp InDels, with the specific distribution (Fig. 1).

Distribution of SNP and InDel Variations Across Rice Genome

Polymorphisms (including SNPs and InDels) show uneven distribution on each chromosome, and the most SNPs (96,367) and the most InDel (11,477) were distributed on chromosome 4, while the fewest SNPs and InDel were distributed on chromosome 9. SNP and InDel display similar distribution patterns (Fig. 2). The average distance between adjacent SNP variant sites was 451 bp. That is, at the genome-wide level, there is averagely one mutation in interval of 451 bp, and each kb has 2.217 mutations on average. The average distance of adjacent variation sites of InDel was 3884 bp, which means there were an average of 0.257 mutations per kb.

Mutation Site Analysis of Each Mutant

Each mutant was compared to 9311 wild-type material (9311-4C477, 9311_BGI-1, 9311_BGI-2) and each mutant-specific homozygous genotype variant site (including SNP and InDel) was screened using CausalFinder (version1.1, coding by Xiamen Jointgene Biotechnology Co.,Ltd.). Further, each mutant was used as a sample for comparison with 9311 wild type material (9311-4C477, 9311_BGI-1, 9311_BGI-2) and other mutants, and each mutant-specific homozygous genotype variation site (including SNP and InDel) was screened by CausalFinder (Table 2). In this round of screening, the resulting mutation was unique to this sample which did not exist in other samples and belonged to homozygous genotype in this sample.

Mutation SNP and Mutation InDel Analysis

For the last round of sample-specific mutation sites, we removed mutations in introns, mutations in exon UTR (untranslated regions) and small effect variation such as synonymous mutations to screen variation sites with large variation effects. The results indicated that there were 9345 large effect variation sites in the 7 rice mutants, involving 3533 genes (Table 3). By analyzing the mutant gene, the anthocyanin synthesis-related structural genes and transcription factors were screened according to the annotation of rice MSU, and a total of 42 genes possibly related to anthocyanin synthesis were obtained (Table 4).

Discussion

The proanthocyanidins synthesis of red rice and the anthocyanins synthesis of black rice share the upstream of the flavonoid synthesis pathway, which are separated on the branch with anthocyanins/leucocyanidins as the reaction substrate. So there exists a certain relationship between

Table 2: Statistics of mutant-specific homozygous genotype variation site

Sample	Variation site	Intronic	Exon
9311-4C478	10839	3532	1824
9311-4C481	9954	2948	1329
9311-4C483	9933	3017	1697
9311-4C487	36798	11415	4994
9311-4C498	25399	8800	4525
9311-4C503	53699	19030	10083
9311-4C513	28973	9027	4434

Table 3: Large effect variation sites in mutant

Sample	Retained variation sites after screening	Involved genes
9311-4C478	544	224
9311-4C481	381	169
9311-4C483	371	170
9311-4C487	1505	535
9311-4C498	1811	587
9311-4C503	3310	1338
9311-4C513	1423	510

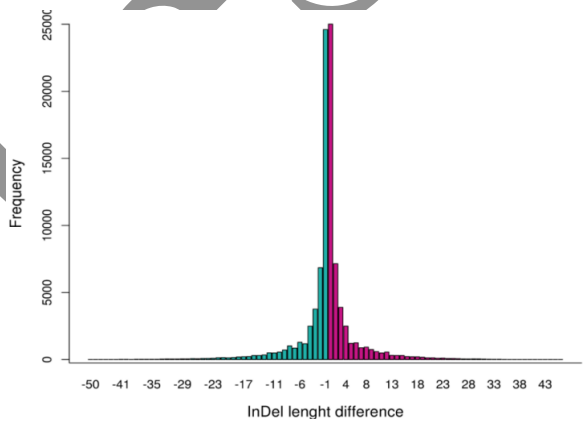

Fig. 1: InDel length distribution

proanthocyanidins synthesis and anthocyanin synthesis (Q *et al*., 2017). For instance, DFR (dihydroflavonol-4-reductase) mutation-resulting black or red rice grains turn brown (Furukawa *et al*., 2007; Sun *et al*., 2018). Seven mutants in this study were obtained with the mutation of 9311, including three black rice and four red rice genotypes. This represented the first time of conjoint analysis on genome of red rice and black rice with the same genetic background. Mutations related to grain color were easier to find in the case of consistent genetic background.

Studies have shown that radiation-induced mutations are random (Li *et al*., 2016), and consistent post-radiation mutations belong to small probability events. Screened radiation-induced mutations (including SNPs and InDels) are unique to each mutant. Based on this premise, we designed a cross-checking method to remove the same polymorphisms of the mutant and find the mutation unique to each mutant. Such checking method with more similar genetic background material can more easily eliminate interference, bringing more accurate results (Alexander, 2011).

Table 4: Genes involved in mutation of pigmentation

8	Gene-ID	Annotation	Variation site position	Reference Gene Sequence	Mutation Sequence
9311-4C478	LOC_Os05g42040	UDP-glucosyl transferase domain containing protein	24605726	C	T
	LOC_Os10g39140	flavonol synthase/flavanone 3-hydroxylase	20890899	C	A
	LOC_Os08g43680	glutathione S-transferase	27611160	G	A
	LOC_Os04g53800	leucoanthocyanidin reductase	32059606	TCGGCGGTTGAGAGGAAGAC	TC
9311-4C481	LOC_Os08g05510	MYB family transcription factor	2939114	G	A
	LOC_Os08g05520	myb-like DNA-binding domain containing protein	2949436	T	C
	LOC_Os08g06100	O-methyltransferase	3340639	C	T
	LOC_Os08g06110	MYB family transcription factor	3366191	GCATTTTTTTTT	GT
	LOC_Os08g06240	MYB family transcription factor	3438728	G	T
	LOC_Os08g33800	MYB family transcription factor	21155705	G	T
9311-4C483	LOC_Os08g15020	MYB family transcription factor	9069182	TCTGAGAGAGACTGAGAGAGAT	TCTGAGAGAGAT
	LOC_Os08g15330	anthocyanidin 3-O-glucosyltransferase	9333502	G	A
	LOC_Os08g17520	flavonol sulfotransferase-like	10726485	G	T
9311-4C487	LOC_Os03g49524	anthocyanidin 3-O-glucosyltransferaseexpressed	28196184	T	C
	LOC_Os03g49550	glucosyltransferase	28219507	C	T
	LOC_Os03g50130	microsomal glutathione S-transferase 3	28594810	C	G
	LOC_Os06g10860	glucosyltransferase	5664560	GC	GCTTCGGCC
	LOC_Os06g35140	MYB family transcription factor	20454850	G	A
	LOC_Os06g37410	bHLH family transcription factor	22120060	C	A
9311-4C498	LOC_Os01g09280	myb-related transcription activator	4718392	CCCTCCTCCTCCTC	CCCTCCTCCTCCTCCTC
	LOC_Os01g09640	Myb transcription factor	4972013	A	G
	LOC_Os11g32580	chalcone synthase	19227924	C	T
	LOC_Os11g32610	chalcone and stilbene synthases	19237316	C	G
	LOC_Os11g32620	chalcone synthase	19244037	C	T
	LOC_Os11g45740	MYB family transcription factor	27671072	C	G
9311-4C503	LOC_Os01g72610	glycosyltransferase	42129894	T	G
	LOC_Os02g36770	galactosyltransferase family protein	22177270	C	G
	LOC_Os02g42280	UDP-glucoronosyl/UDP-glucosyl transferase family protein	25424394	G	C
	LOC_Os04g09654	O-methyltransferase	5178930	TGG	TG
	LOC_Os04g09670	O-methyltransferase	5190042	T	C
	LOC_Os05g02530	glutathione S-transferase	877499	C	T
	LOC_Os11g15210	bHLH family transcription factor (Lc protein)	8584822	C	A
	LOC_Os11g19140	methyltransferase domain containing protein	15497917	G	T
	LOC_Os11g26950	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein	15497917	G	T
	LOC_Os11g33300	O-methyltransferase	19694988	A	G
9311-4C513	LOC_Os01g08160	MYB family transcription factor	3953442	AGGCGGCGGCGGCG	AGGCGGCGGCGGCGGCG
	LOC_Os02g11110	flavonol-3-O-glycoside-7-O-glucosyltransferase 1	5956395	T	C
	LOC_Os02g11670	glucosyltransferase	6003144	T	A
	LOC_Os02g15760	helix-loop-helix DNA-binding domain containing protein	8881237	G	A
	LOC_Os02g17190	MYB family transcription factor	9848295	A	T
	LOC_Os03g25550	myb-like DNA-binding domain containing protein	14598656	GCGCCGCCGCCGCCGCCGCCGCA	GCGCCGCCGCCGCA
	LOC_Os10g22310	glutathione S-transferase GST 26	11538575	A	C

Previous studies have shown that the accumulation of pigment in red rice or black rice is affected by transcription factor and structural gene, in which transcription factors play a key role. In particular, MYB family transcription factors play a core role in the synergistic activation of anthocyanins and proanthocyanidin pathway-specific genes (Dixon *et al*., 2013). The transcription factors regulating black rice anthocyanins are mainly MYB and bHLH (Q *et al*., 2017), while the transcriptional regulators of red rice proanthocyanidins are mainly bHLH (Furukawa *et al*., 2007). Analysis of the mutant genes screened from three black rice genotypes revealed no mutation in the three genes *C1*, *S1* and *A1*. Sun *et al.* (2018) reported to cause blackening of the grain color. Since MYB transcription factors and bHLH transcription factors play an indispensible role in the synthesis of anthocyanins (Petroni and Tonelli, 2011), it is speculated that other gene mutations with similar functions to C1, S1 and A1 lead to grain color phenotype changes. We found mutation of six MYB family genes and two bHLH family genes in the three black rice mutants.

Fig. 2: SNP and InDel mutation density distribution
Note: a is SNP density distribution map and b is InDel density distribution map. The y-axis represents density, which is the number of variances per 1 kb (the number of intra-window variances divided by the window size), and the x-axis is the physical location on each chromosome (in unit Mb)

Lc gene belonging to bHLH family found in 9311-4C503 has been demonstrated as relevant with anthocyanin accumulation (Li *et al*., 2013), while 6 MYB family genes and 1 bHLH family were found in 9311-4C498, 9311-4C513. These seven genes are not reported earlier and can be used as targets for further validation.

Analysis of four red rice mutant genes revealed 19 genes possibly associated with grain surface color, including 7 MYB family genes and 2 bHLH family genes. According to the report, the phenotype of red rice is caused by mutation of Rc gene (bHLH gene family) (Furukawa *et al*., 2007), but mutation of the gene was not found in red rice in this study. So the reason for grain color to turn red is possibly mutations in other bHLH family genes.

In addition, mutations in related structural genes were also found in black and red rice mutants. Such structural genes have an effect on the accumulation of anthocyanins and proanthocyanidins. For example, there is correlation between presence of glycosyltransferase and anthocyanin content. For higher glycosyltransferase activity, anthocyanin content is higher and grain color is darker (Yamazaki *et al*., 2002). These genes may affect the accumulation of anthocyanins or proanthocyanidins, which may be used as objects for further study on quantitative traits of proanthocyanidins or anthocyanins.

Conclusion

It is revealed that 42 genes that may be involved in the synthesis of anthocyanins and proanthocyanidins, including 25 structural genes and 17 transcription factors (13 MYB and 4 bHLH family genes). For 17 transcription factors found here, except that LOC_Os11g15210 was demonstrated as relevant with the synthesis of anthocyanins, the rest were reported to be involved in the synthesis of anthocyanins or proanthocyanidins. These genes provide important basis for further study on the synthesis of anthocyanins and proanthocyanidins. The current theory on rice pigmentation synthesis mechanism can be enriched by further validation of these genes.

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References

Alexander I., 2011. *Introduction to Mathematical Methods in Bioinformatics*. Science Press, Beijing, China

Dixon, R.A., C. Liu and J.H. Jun, 2013. Metabolic engineering of anthocyanins and condensed tannins in plants. *Curr. Opin. Biotechnol.*, 24: 329–335

Forkmann, G., 2010. Flavonoids as flower pigments: the formation of the natural spectrum and its extension by genetic engineering. *Plant Breed.*, 106: 1–26

Furukawa, T., M. Maekawa, T. Oki, I. Suda, S. Iida, H. Shimada, I. Takamure and K. Kadowaki, 2007. The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant J*., 49: 91–102

Gross, B.L., F.T. Steffen and K.M. Olsen, 2010. The molecular basis of white pericarps in African domesticated rice: Novel mutations at the Rc gene. *J. Evol. Biol.*, 23: 2747–2753

Ithal, N. and A.R. Reddy, 2004. Rice flavonoid pathway genes, OsDfr and OsAns, are induced by dehydration, high salt and ABA, and contain stress responsive promoter elements that interact with the transcription activator, OsC1-MYB. *Plant Sci*., 166: 1505–1513

Khush, G.S., 2001. Green revolution: the way forward. *Nat. Rev. Genet.*, 2: 815–822

Li, S., Y. Zheng, H. Cui, H. Fu, Q. Shu and J. Huang, 2016. Frequency and type of inheritable mutations induced by γ rays in rice as revealed by whole genome sequencing. *J. Zhejiang Univ. Ser. B. Biomed. Biotechnol.*, 17: 905

Li, Y., T. Zhang, Z.W. Shen, Y. Xu and J.Y. Li, 2013. Overexpression of maize anthocyanin regulatory gene Lc affects rice fertility. *Biotechnol. Lett.*, 35: 115–119

Mattei, J., V. Malik, N.M. Wedick, F.B. Hu, D. Spiegelman, W.C. Willett, H. Campos and I. Global, Nutrition Epidemiologic Transition. 2015. Reducing the global burden of type 2 diabetes by improving the quality of staple foods: The Global Nutrition and Epidemiologic Transition Initiative. *Global Heal.*, 11: 23

Oikawa, T., H. Maeda, T. Oguchi, T. Yamaguchi, N. Tanabe, K. Ebana, M. Yano, T. Ebitani and T. Izawa, 2015. The Birth of a Black Rice Gene and Its Local Spread by Introgression. *Plant Cell*, 27: 2401–2414

Petroni, K. and C. Tonelli, 2011. Recent advances on the regulation of anthocyanin synthesis in reproductive organs. *Plant Sci*., 181: 219–229

Q, Z., Y. S, Z. D, L. H, W. H, Y. Z, X. X, S. R, T. J and L. H, 2017. Development of "Purple Endosperm Rice" by Engineering Anthocyanin Biosynthesis in the Endosperm with a High-Efficiency Transgene Stacking System. *Mol. Plant*, 10: 918–929 (Please add complete author's names)

Shih, C.H., H. Chu, L.K. Tang, W. Sakamoto, M. Maekawa, I. K. Chu, M. Wang and C. Lo, 2008. Functional characterization of key structural genes in rice flavonoid biosynthesis. *Planta*, 228: 1043–1054

Shu, Q.Y., B.P. Forster and H. Nakagawa, 2012. *Plant Mutation Breeding and Biotechnology*. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. International Atomic Energy Agency, Vienna, Austria

Sun, X., Z. Zhang, C. Chen, W. Wu, N. Ren, C. Jiang, J. Yu, Y. Zhao, X. Zheng, Q. Yang, H. Zhang, J. Li and Z. Li, 2018. The C-S-A gene system regulates hull pigmentation and reveals evolution of anthocyanin biosynthesis pathway in rice. *J. Exp. Bot*., 69: 1485–1498

Yamazaki, M., E. Yamagishi, Z. Gong, M. Fukuchi-Mizutani, Y. Fukui, Y. Tanaka, T. Kusumi, M. Yamaguchi and K. Saito, 2002. Two flavonoid glucosyltransferases from Petunia hybrida: molecular cloning, biochemical properties and developmentally regulated expression. *Plant Mol. Biol.*, 48: 401–411

Yamuangmorn, S., B. Dell, X. Du, Y. Ren and C. Prom-u-thai, 2019. Simultaneous quantification of anthocyanins and phenolic acids in pigmented rice (*Oryza sativa*) using UPLC-PDA/ESI-Q-TOF. *Intl. J. Agric. Biol*., 21: 590–596

Zhang, L., Z. Cheng, R. Qin, Y. Qiu, J.L. Wang, X. Cui, L. Gu, X. Zhang, X. Guo, D. Wang, L. Jiang, C.Y. Wu, H. Wang, X. Cao and J. Wan, 2012. Identification and characterization of an epi-allele of FIE1 reveals a regulatory linkage between two epigenetic marks in rice. *Plant Cell*, 24: 4407–4421

Zhu, F., 2018. Anthocyanins in cereals: Composition and health effects. *Food Res. Intl*., 109: 232–249

Zhu, Y., Y. Lin, S. Chen, H. Liu, Z. Chen, M. Fan, T. Hu, F. Mei, J. Chen, L. Chen and F. Wang, 2019. CRISPR/Cas9-mediated functional recovery of the recessive *rc* allele to develop red rice. *Plant Biotechnol. J*., in press

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