Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2023.618.627

Research Article
Identification of Candidate SNPs and Genes Controlling Chalkiness in the Medium-Grain Rice (*Oryza sativa* L.)

[1]Bui Phuoc Tam, [2]Pham Thi Be Tu and [3]Nguyen Thi Pha

[1]Can Tho University, Ninh Kieu, Can Tho, Vietnam
[2]College of Agriculture, Can Tho University, Ninh Kieu, Can Tho, Vietnam
[3]Institute of Food and Biotechnology, Can Tho University, Ninh Kieu, Can Tho, Vietnam

Abstract

Background and Objective: Grain chalkiness is an important indicator of rice appearance quality. It is a multi-gene complex trait and understanding its genetic bases in medium rice provides the basis for improving grain quality. This study was conducted to identify new SNPs and genes controlling chalkiness in medium-grain rice. **Materials and Methods:** In this study, a rice diversity panel of 114 genotypes was applied to evaluate the percentage of chalkiness (PC) and identify its genetic characteristics. Genome-Wide Association Study (GWAS) and other bioinformatic tools (IRRI SNP-Seek, RiceFREND and ePlant databases) were utilized to identify significant SNPs and candidate genes related to the trait of grain chalkiness. **Results:** A total of 17 SNPs were identified on ten chromosomes and these SNPs explained 0.94-60.38% of the variations in the chalkiness among medium-grain rice varieties. Of these, three of them were updated in the IRRI SNP-seek database. Two predicted genes, *LOC_Os02g12580* on chromosome 2 and *LOC_Os11g07840* on chromosome 11, were associated with chalkiness in medium-grain rice varieties. **Conclusion:** The candidate genes play crucial roles during starch synthesis in the endosperm and are important in developing rice varieties of high-grain quality.

Key words: Chalkiness, Genome-Wide Association Study (GWAS), medium-grain rice, Quantitative Trait Loci (QTL), Single Nucleotide Polymorphism (SNP)

Citation: Tam, B.P., P.T.B. Tu and N.T. Pha, 2023. Identification of candidate SNPs and genes controlling chalkiness in the medium-grain rice (*Oryza sativa* L.). Asian J. Plant Sci., 22: 618-627.

Corresponding Author: Pham Thi Be Tu, College of Agriculture, Can Tho University, Ninh Kieu, Can Tho, Vietnam

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chalkiness is one of the most important indices of rice grain quality. It is an unfavorable trait since it affects both grain appearance and grain quality including physical and chemical characteristics, milled quality and cooking quality[1]. Chalkiness is the term used to describe the opaque portion of the rice endosperm brought on by the free starch granules, including white core, white belly and white back[1,2]. In addition, it is a complex trait controlled by multiple genes and affected by environmental factors. These genes are controlled by the endosperm and maternal cytoplasm[3-5]. Many genes and QTLs related to rice grain chalkiness were confirmed in previous studies, including *BEIIb*[6], *Chalk5*[7], *qPGWC-6*[8], *qPGWC-7*[9], *qPGWC-8*[2] and *qACE-9*[10]. Despite the fact that a greater number of QTLs for chalkiness have been reported across all 12 chromosomes, only a few of the QTLs have been fine-mapped or cloned to date[11].

For physical appearance, medium-grain rice falls between long-grain and short-grain rice. According to IRRI[12], medium-grain rice is from 5.51-6.60 mm in length and from 2.1-3.0 in the length-width ratio. In addition, some studies have suggested that the trait of grain chalkiness appeared much more in short-grain (*Japanica*) than long-grain (*Indica*)[3]. This implied that grain chalkiness in rice may be related to grain size.

Genome-Wide Association Study (GWAS) mapping makes it possible to look at a very large number of accessions at the same time for genetic differences that cause complex traits with many different causes. One of the best things about the GWAS design for rice is that most rice varieties are homozygous. This makes it possible to use a "genotype or sequence once and phenotype many times" strategy, which means that once the lines have been characterized genomically, the genetic data can be used many times in different phenotypes and environments[13]. In rice, GWAS is gaining widespread use and were reported by researchers[13-18].

The objectives of this study were: (1) To detect Single Nucleotide Polymorphisms (SNPs) in the whole of rice chromosomes related to chalkiness in the medium-grain rice using GWAS and (2) To identify the significant candidate genes for chalkiness in the medium-grain rice.

MATERIALS AND METHODS

The experiments were conducted at the experimental station of Can Tho University, Can Tho City, Vietnam from January to December, 2021.

Plant materials: A total of 114 medium-grain varieties of the rice diversity panel (NSF-TV Set) were provided by the Genetic Resources Center, International Rice Research Institute (IRRI).

Analysis of the percentage of chalkiness: The percentage of chalkiness (PC) is visually assessed based on the standards evaluation system for rice (SES) of IRRI. The PC in rice is classified into four scales: Scale 0 (non-chalkiness), scale 1 (chalkiness area less than 10%), scale 5 (chalkiness area from 11-20%), scale 9 (chalkiness area more than 20%)[19]. For each seed sample, 100 g of rice grains were milled and classified for each grain of rice. The percentage of chalkiness was determined by the formula:

$$\text{PC (\%)} = \frac{\text{Weight of chalkiness grains in scale 9}}{\text{Weight of milled rice}} \times 100$$

Investigation of Single Nucleotide Polymorphisms (SNPs) by GWAS: The 44,100 SNP genotyping data[13] were used for the association of SNP variants with the phenotypes. Genome-Wide Association (GWAS) by GAPIT was done using R-studio 3.2.4 software. The GAPIT tool optimized the Enriched Compressed Mixed Linear Model Approach (eCMLM). The significance threshold was set to $p<0.0001$ to combine association results. Information on SNPs was collected using the Rice SNP-Seek database[20]. The QTL mapping of related SNPs was made using the Ritchie Lab Visualization database[21]. The steps of GWAS analysis based on the GAPIT tool are depicted in Fig. 1.

Selecting the candidate genes for chalkiness in the medium-grain rice: Candidate genes were selected and manipulated following Fig. 2. The list of SNP-related candidate genes selected from GWAS results was collected using the Rice SNP-Seek database[20]. The Rice Genome Annotation Project database[22] allowed us to find information and function of candidate genes. To determine the co-expression of candidate genes, the network analysis of related genes was performed using the RiceFREND database[23]. Candidate genes concerned with the growth and development of the specific site in the plant (shoot, root, leaf, flower and seed) were defined using the ePlant database[24]. In this study, the selected candidate genes related to grain chalkiness and focused on the growth and development of seed.

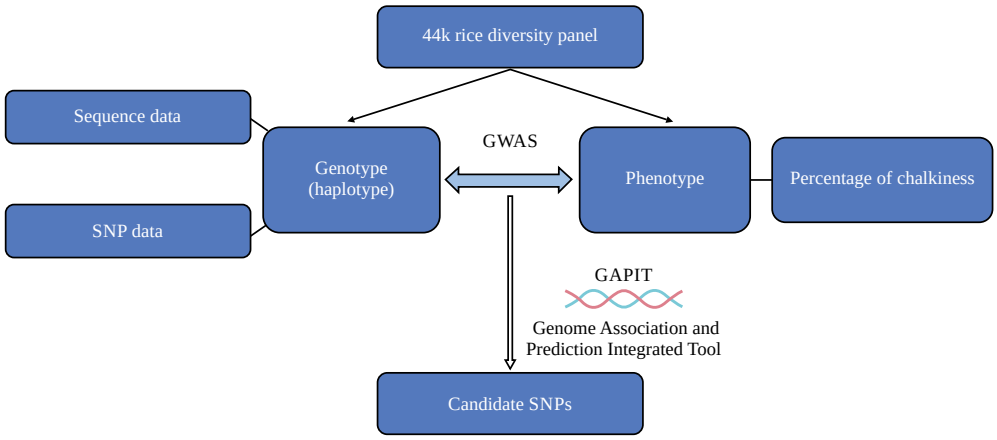


Fig. 1: Investigation of Single Nucleotide Polymorphism (SNP) for rice genotypes with different percentage of chalkiness on chromosomes of rice

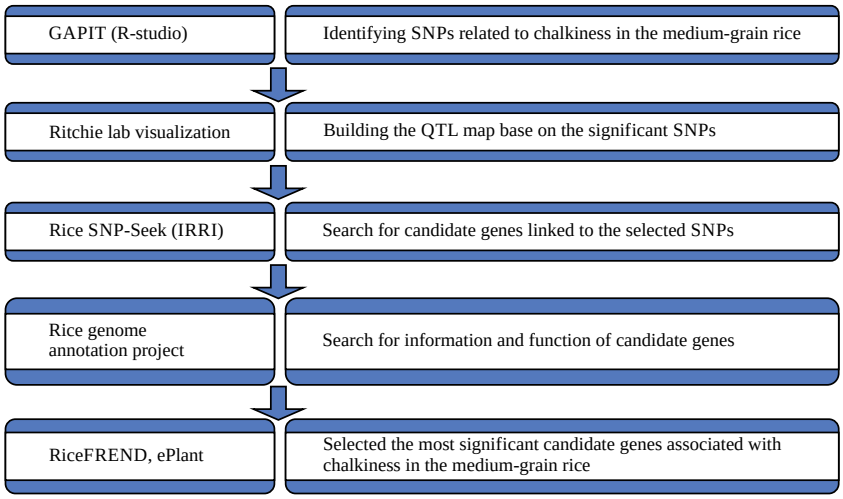


Fig. 2: Identification and selection of the significant SNPs and candidate related to chalkiness in the medium-grain rice

RESULTS AND DISCUSSION

Percentage of chalkiness in the medium-grain rice: The percentage of chalkiness (PC) was very different between different types of medium-grain rice. Most rice varieties were non-chalky (PC = 0%), however, some of the other rice varieties had a high rate of chalkiness, up to 86.7%. The average percentage of chalkiness in rice varieties was 15.23%. In this study, there were 33 rice varieties with non-chalkiness (scale 0), accounting for a fairly high rate of 28.9% of the total number of varieties. Continuously, rice chalkiness with low (scale 1), average (scale 5) and high (scale 9) percentages accounted for 35.1, 14.0 and 22.0%, respectively (Fig. 3). Thus, through the assessment of chalkiness in the medium-grain rice set, the varieties with non-chalkiness or a low percentage of chalkiness (which made up the high rate of 64%) were a potential basis for the breeding of new rice varieties with less chalkiness.

Identification of Single Nucleotide Polymorphisms (SNPs) by GWAS: Rice (*Oryza sativa*) is a good candidate system for GWAS because it can pollinate itself and has a lot of different genes[13,25]. In GWAS studies, the high resolution of SNPs covered the entire genome. The confidence interval of QTLs was only about 50-100 kb, depending on the genomic analysis method, instead of several megabases as in the previous method of mapping QTL. This made it simpler to localize and identify potential target genes[15]. Based on the phenotype of chalkiness and the 44,100 SNP database of Zhao *et al.*[13], GAPIT analysis was performed and GWAS results were shown in Fig. 4a-c. At log10 (p-value) 3, 17 significant LD-associated SNP positions were identified at $p \leq 0.001$ and allele

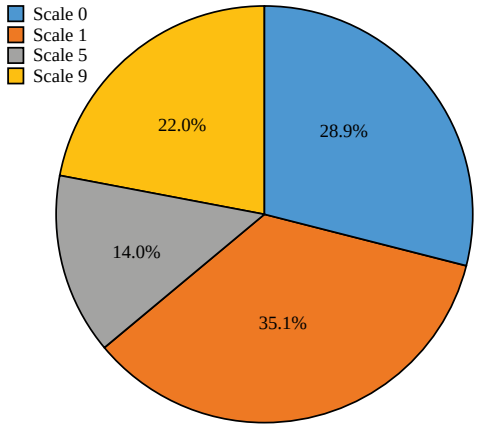


Fig. 3: Groups of classified chalkiness in the medium-grain rice set

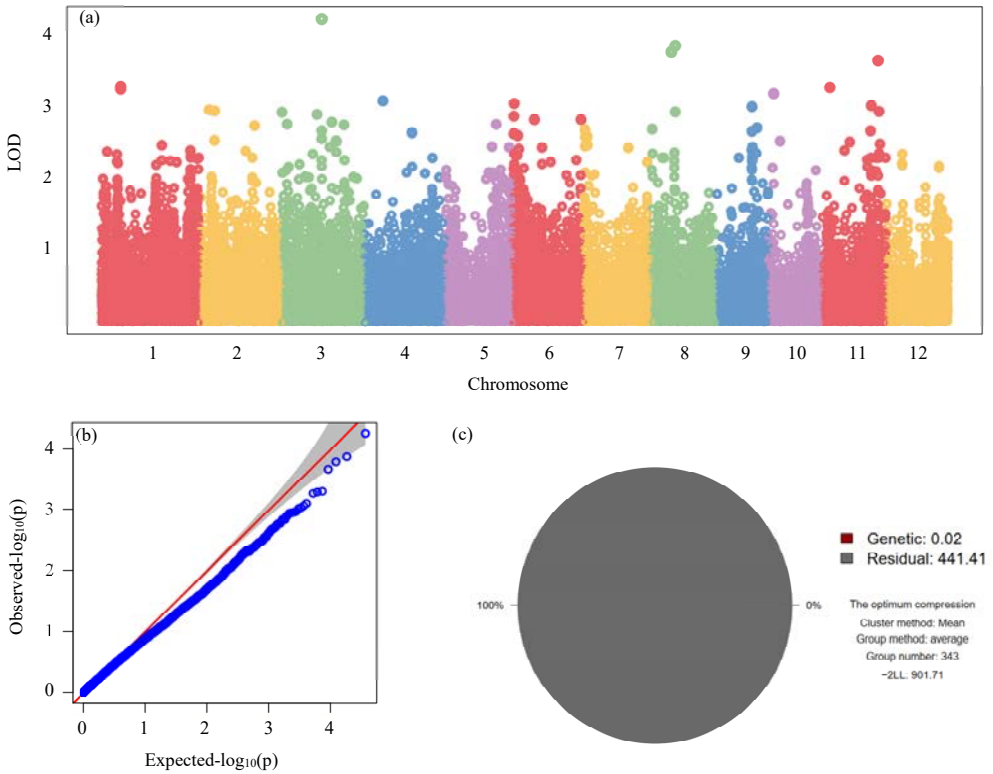


Fig. 4(a-c): Results of Single Nucleotide Polymorphism (SNP) based on Genome Wide Association Studies (GWAS) for the trait of percentage of chalkiness in the medium-grain rice, (a) Manhattan plot of eCMLM, (b) Quantile-quantile plot and (c) Optimum compression data of the eCMLM model for SES scores

Negative $\log_{10}$-transformed p-values from a genome wide scan are plotted against the position on each of the 12 chromosomes, Red horizontal dashed line indicates the genome wide set threshold while blue box indicates the significant SNP peaks

frequencies ranging from 0.94-60.38% (Table 1). The SNPs in this LD association were significantly responsible for the difference in the percentage of chalkiness among the medium-grain rice varieties. Among the 17 SNPs identified to be associated with rice grain chalkiness, three SNPs, id 2003372, id 9004794 and id 11001576, located on chromosomes 2, 9 and 11, respectively, had their genetic information updated in the rice SNP-Seek database. On chromosome 2, SNP id 2003372 has an A with a G substitution at position 6570949 bp and the frequency of the substitute

Table 1: Significant SNPs related to the percentage of grain chalkiness located on different chromosomes in the medium-grain rice

SNP id	Chr.	Position (bp)	p-value	LOD	Original allele	Substitute allele	Substitute allele frequency (%)	MAF	R^2
id3008630	3	17672562	5.36×10^{-5}	4.3	A	G	55.66	0.433	19.9
id8003399	8	10596010	1.28×10^{-4}	3.9	G	A	60.38	0.381	18.1
id8002781	8	8875284	1.57×10^{-4}	3.8	C	T	1.89	0.024	17.7
id11010079	11	25145185	2.08×10^{-4}	3.7	A	G	8.49	0.105	17.1
id1006455	1	8628373	4.84×10^{-4}	3.3	G	A	23.58	0.243	15.3
id11001576	11	3993497	4.98×10^{-4}	3.3	G*	A*	4.38	0.038	15.3
ud1000416	1	8633965	5.22×10^{-4}	3.3	C	T	24.53	0.252	15.2
id10000708	10	2476548	6.08×10^{-4}	3.2	T	G	26.42	0.400	14.9
id4002939	4	8118966	7.68×10^{-4}	3.1	G	A	4.72	0.048	14.4
id6000530	6	796654	8.38×10^{-4}	3.1	T	A	5.66	0.057	14.2
dd11001101	11	22175646	9.00×10^{-4}	3.0	T	C	0.94	0.019	14.1
id9004794	9	15882677	9.29×10^{-4}	3.0	G*	A*	58.79	0.324	14.0
id2002230	2	4143928	10.35×10^{-4}	3.0	G	A	0.94	0.010	13.8
id2003372	2	6570949	10.68×10^{-4}	3.0	A*	G*	24.00	0.338	13.7
id11010263	11	25578200	10.88×10^{-4}	3.0	C	T	4.72	0.052	13.7
id8003390	8	10591929	11.04×10^{-4}	3.0	G	T	59.43	0.386	13.7
id3000014	3	202169	11.14×10^{-4}	3.0	G	T	4.72	0.090	13.6

Chr.: Chromosome, SNP: Single nucleotide polymorphism, id: Identification, p-value: Calculated probability-value, LOD: Logarithm of the odds ratio, MAF: Minor allele frequency, *Substitute allele information was updated on the Rice SNP-Seek database (IRRI) and R^2: Ratio of expression of each QTL to the total expression of the corresponding characteristic

Table 2: List of candidate genes corresponding to selected SNPs

SNP	Candidate gene	Chr.	Position-start	Position-end	Function
id2003372	*LOC_Os02g12560*	2	6566133	6566588	Expressed protein
	LOC_Os02g12570	2	6568146	6572535	Pre-mRNA cleavage complex II protein Clp1, putative, expressed
	LOC_Os02g12580	2	6572759	6577608	OsPP2Ac-3-Phosphatase 2A isoform 3 belonging to family 1, expressed
id9004794	*LOC_Os09g26280*	9	15880529	15881668	Transposon protein, putative, CACTA, En/Spm sub-class, expressed
	LOC_Os09g26290	9	15888897	15890074	Amino acid transporter family protein, putative, expressed
id11001576	*LOC_Os11g07810*	11	3987849	3988694	Retrotransposon protein, putative, unclassified
	LOC_Os11g07820	11	3989476	3989871	Expressed protein
	LOC_Os11g07830	11	3994604	3995784	Dirigent, putative, expressed
	LOC_Os11g07840	11	3996633	3999897	Expressed protein

Chr: Chromosome

allele was 24.00%. This SNP explained 13.7% of the variations in the chalkiness of medium-grain rice. Similarly, SNP id9004794 at position 15882677 bp on chromosome 9 had a substitution of the G allele by the A allele with a very high frequency of 58.79% and explained 14.0% of the phenotypic variations. Lastly, SNP id 11001576 at position 3993497 bp on chromosome 11 had the substitution of the G allele by the A allele with a frequency of 4.38% and explained 15.3% of the variations in chalkiness in medium-grain rice. The QTL SNP associated with grain chalkiness on chromosome 2 has been documented by some researchers[26-28]. On chromosome 9, many studies also recorded significant SNPs related to chalkiness, e.g., Gao *et al.*[10], Bian *et al.*[26] and Wan *et al.*[29]. Yang *et al.*[11] and Qiu *et al.*[30] discovered significant SNPs on chromosome 11 for the QTLs associated with the trait of chalkiness. Thus, three significant SNPs related to chalkiness in medium-grain rice, ids 11001576, 9004794 and 2003372, were selected to identify the corresponding candidate genes in further studies. The QTL maps (Fig. 5) were made based on the SNPs related to chalkiness that were chosen from the GWAS analysis. This map showed the QTLs associated with chalkiness located on chromosomes 1, 2, 3, 4, 6, 8, 9, 10 and 11. The wide distribution of these QTLs on multiple chromosomes in rice could be explained by the fact that rice grain chalkiness is controlled by multiple genes and these polygenes have a reciprocal influence[31].

Selection of the candidate genes for chalkiness in the medium-grain rice: Using GWAS, 17 candidate SNPs associated with rice grain chalkiness explained 13.6-19.9% of the phenotypic variance, with an average of 15.2%. On this basis, it was confirmed that the previously stated SNPs could be linked to the target genes that regulate the chalkiness percentage of medium-grain rice. However, based on the Rice SNP-Seek database (IRRI), only three SNPs (id 2003372, id 9004794 and id 11001576) with information on allele substitution (or SNP variation) were updated, therefore, these SNPs were selected to be further exploited to screen for corresponding candidate genes. The candidate genes are identified corresponding to the above SNPs and the information about the candidate genes was shown in Table 2. For the candidate genes related to grain chalkiness, SNP id 2003372 identified three genes (*LOC_Os02g12560*, *LOC_Os02g12570* and *LOC_Os02g12580*) on chromosome 2.

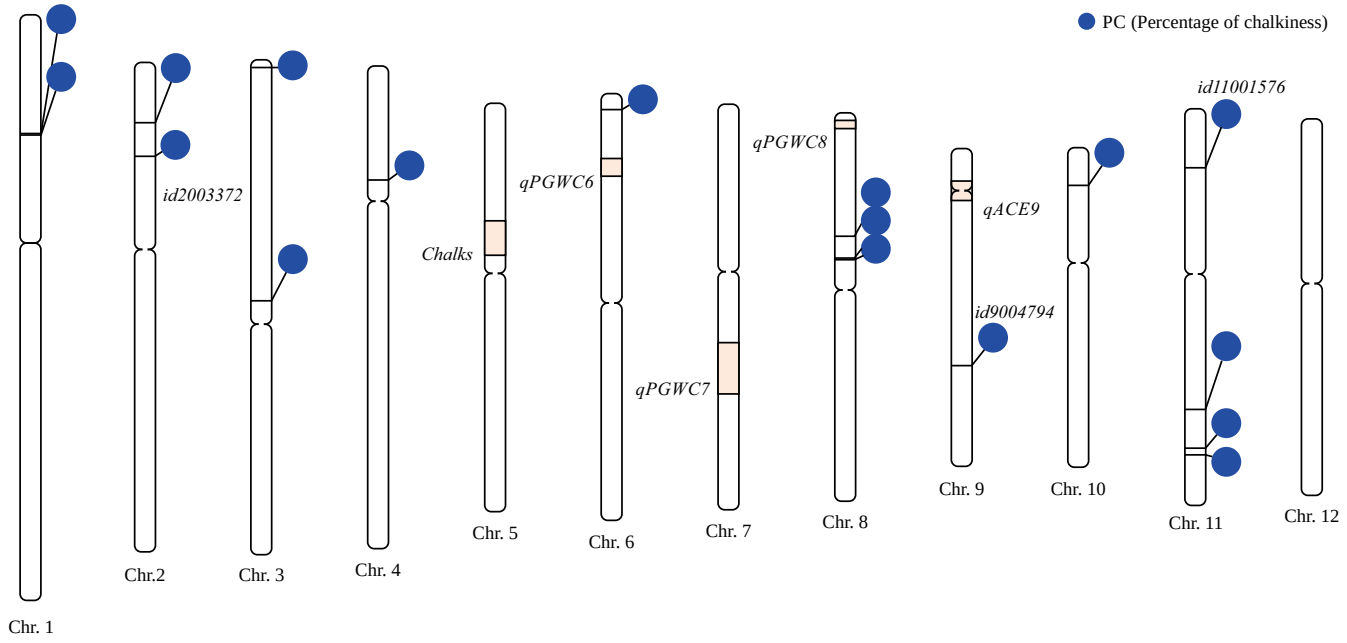


Fig. 5: QTL map of candidate SNPs for chalkiness in the medium-grain rice on all twelve chromosomes detected based on GWAS
chr.: Chromosome, PC: Percentage of chalkiness, pink areas were the gene regions associated with the trait of chalkiness suggested in previous studies, horizontal lines represented the SNP positions on the respective chromosome and colored round nodules represent specific SNPs

Table 3: Information of candidate genes updated by RiceFREND and ePlant databases

	From RiceFREND database			
Candidate gene name	Hierarchy	Mutual rank	Node	From ePlant database
LOC_Os02g12560	-	-	-	-
LOC_Os02g12570	3	7	92	Relate to inflorescence (P2), SAM
LOC_Os02g12580	3	7	47	Whole parts of plant
LOC_Os09g26280	-	-	-	-
LOC_Os09g26290	-	-	-	-
LOC_Os11g07810	-	-	-	-
LOC_Os11g07820	-	-	-	-
LOC_Os11g07830	3	10	96	Relate to root
LOC_Os11g07840	5	7	64	Relate to seed S3, S4 and S5

SAM: Sterile alpha motif domain

The SNP id9004794 identified two genes (*LOC_Os09g26280* and *LOC_Os09g26290*) on chromosome 9 and SNP id11001576 identified four genes (*LOC_Os11g07810*, *LOC_Os11g07820*, *LOC_Os11g07830* and *LOC_Os11g07840*) on chromosome 11. The RiceFREND and ePlant databases were used to assess the significance and ability of these candidate genes in relation to the target trait.

The Rice Functionally Related Gene Expression Network Database (RiceFREND) is a database that may be used to find information about gene coexpression in a variety of gene expression profiles[32]. This will enable the identification of gene modules with comparable expression profiles and serve as a basis for more precise gene function prediction. Meanwhile, the ePlant database helps to discover the large datasets from the model plant *Arabidopsis thaliana* for any given gene, gene product and the linkage between them[33]. In this study, the ePlant database was used to examine candidate genes related to the growth and development of rice plant parts. Therefore, genes involved in rice seed formation are desired.

The information about nine candidate genes in this study was shown in Table 3. Among the candidate genes, four genes with connections in the gene network with known functions on the RiceFREND database were recorded. These genes were involved in the growth and development of flowers, seeds and roots. In the study, the percentage of chalkiness was the main subject that was strongly related to rice grain formation, especially the grain maturity stages.

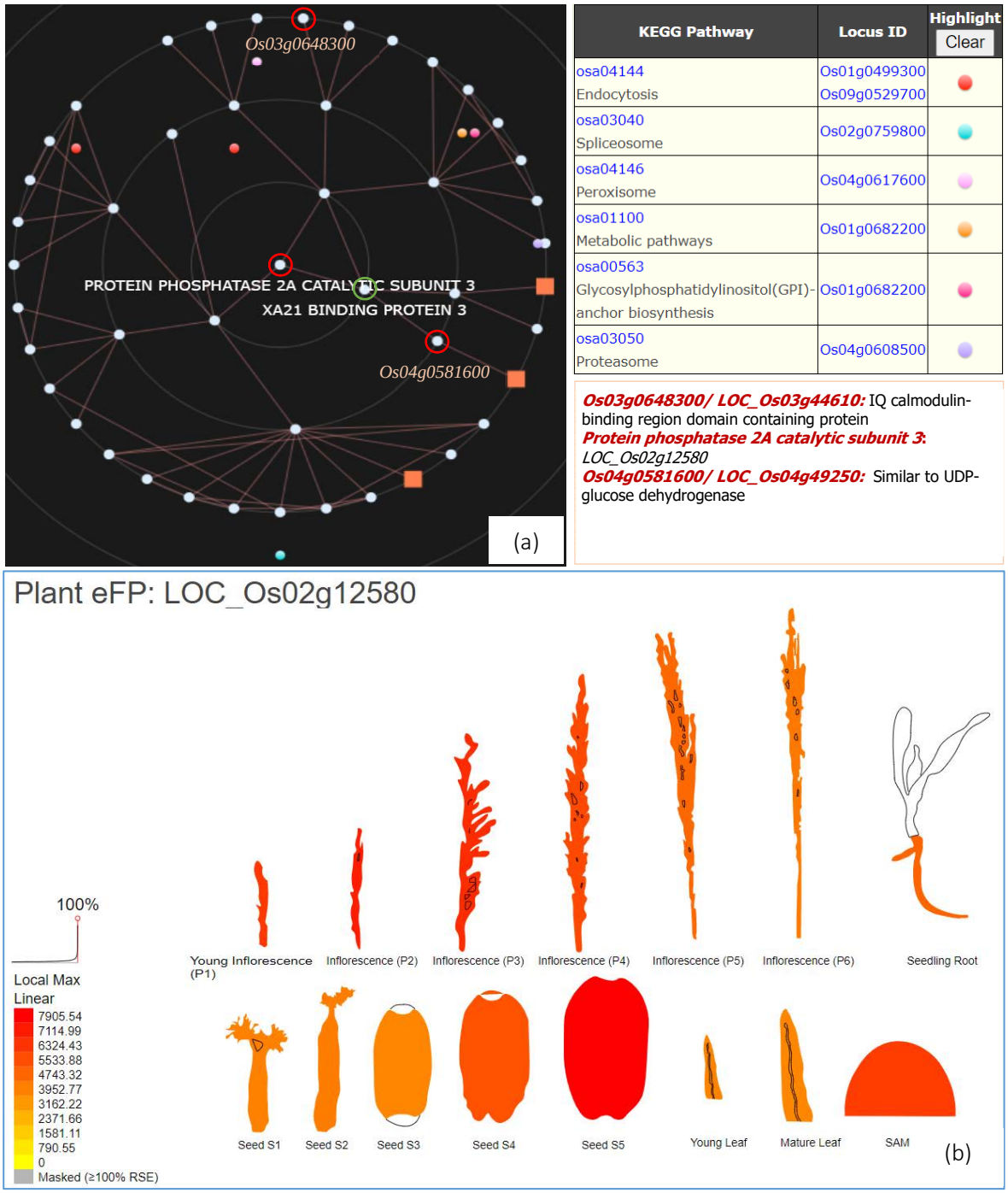
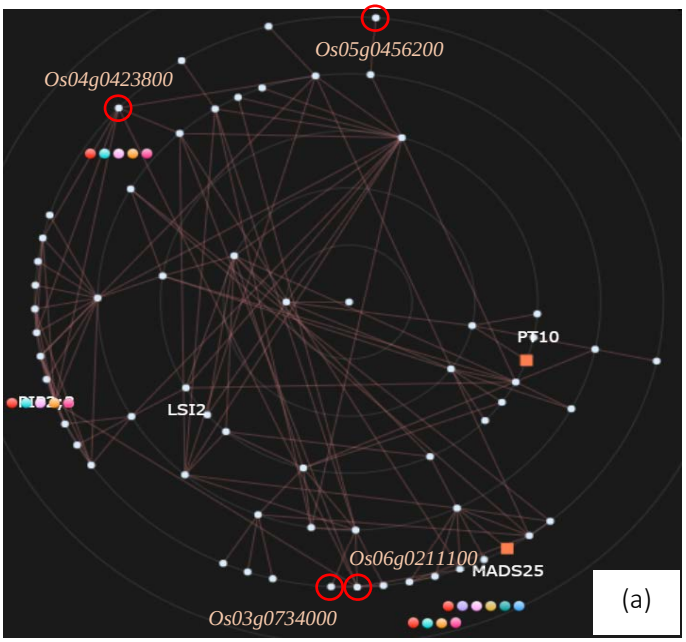


Fig. 6(a-b): Information of candidate gene *LOC_Os02g12580* on (a) RiceFREND and (b) ePlant database

Through the ePlant database, two genes, *LOC_Os02g12580* and *LOC_Os11g07840*, were identified as the two candidate genes that regulated the trait of chalkiness rate related to rice grain maturation.

The amount of chalkiness in medium-grain rice varieties was linked to the gene *LOC_Os02g12580* or *Os02g0217600*, which is on chromosome 2. The *LOC_Os02g12580* has been identified as the *PP2A-3* (*Protein Phosphatase 2A Catalytic Subunit-3*) gene. The *PP2A* gene is known to be a family of genes that carry many functions in plants, such as signal regulation (receptors and organelles), metabolic pathways and gene expression in biotic stress and plant immunity[34]. The protein phosphatase product is also thought to have a potential role in starch metabolism[35,36].



KEGG Pathway	Locus ID	Highlight Clear
osa01100 Metabolic pathways	Os01g0263000 Os04g0423800 Os06g0104900 Os07g0638400	
osa00680 Methane metabolism	Os01g0263000 Os04g0423800 Os07g0638400	
osa01110 Biosynthesis of secondary metabolites	Os01g0263000 Os04g0423800 Os06g0104900	
osa00360 Phenylalanine metabolism	Os01g0263000 Os04g0423800 Os07g0638400	
osa00940 Phenylpropanoid biosynthesis	Os01g0263000 Os04g0423800 Os07g0638400	
osa00010 Glycolysis / Gluconeogenesis	Os06g0104900	
osa00640 Propanoate metabolism	Os06g0104900	
osa00270 Cysteine and methionine metabolism	Os06g0104900	
osa00620 Pyruvate metabolism	Os06g0104900	

Os03g0734000/ LOC_Os03g52360: Pentatricopeptide repeat containing protein
Os04g0423800/ LOC_Os04g34630: Peroxidase (EC 1.11.1.7)
Os05g0456200: Similar to Aldose reductase
Os06g0211100/ LOC_Os06g10870: Similar to Glycine-rich cell wall structural protein 1 precursor.

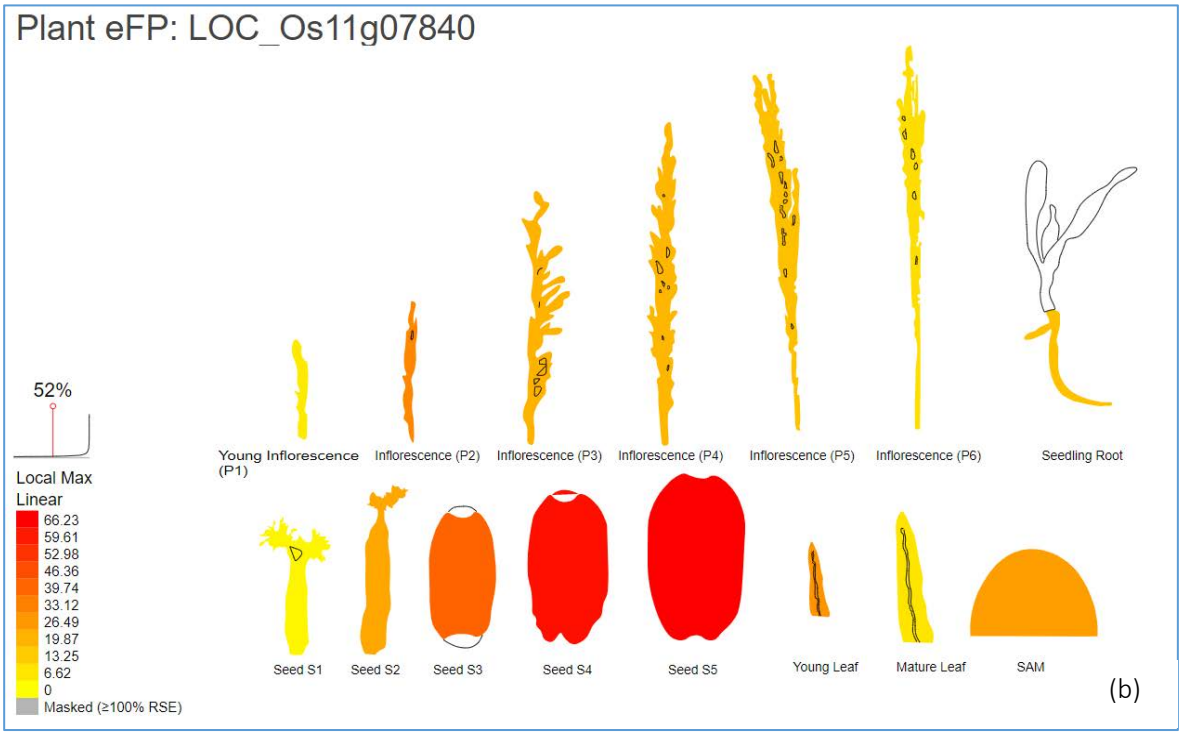


Fig. 7(a-b): Information of candidate gene *LOC_Os11g07840* on (a) RiceFREND and (b) ePlant database

At the same time, the *LOC_Os02g12580* gene was involved in t he KEGG pathway with functions like intracellular (endocytosis, osa04144), spliceosome (osa03400), peroxisome (osa04146), metabolic pathway (osa01100), GPI-anchor biosynthesis (osa00563) and proteasome (osa03500). Regarding the chalkiness trait, on the gene network,

LOC_Os02g12580 is co-expressed with the gene *LOC_Os03g44610*(or *Os03g0648300*, the protein binding site for IQ calmodulin) and *LOC_Os04g49250* (or *Os04g0581600*, similar to UDP-glucose dehydrogenase). These genes are thought to be associated with the formation of chalkiness in rice grains[37] (Fig. 6a). In addition, on the ePlant database, the *LOC_Os02g12580* gene was related to seed maturation (at stages S4, 5) and the gene expression level could reach 100% (Fig. 6b).

Similarly, the gene *LOC_Os11g07840* or *Os11g0180100* located on chromosome 11 is related to the percentage of chalkiness in medium-grain rice. On the gene network, this gene was connected to four other genes and was also involved in the KEGG pathway with nine different functions (Fig. 7a). Regarding grain chalkiness, the gene *LOC_Os11g07840* collaborates with the genes *LOC_Os03g52360* (or Os03g0734000, a pentatricopeptide repeat-containing protein), *LOC_Os04g34630* (or *Os04g0423800*, peroxidase), *Os05g0456200* (similar to aldose reductase) and *LOC_Os06g10870* (or *Os06g0211100*, similar to glycine-rich cell wall structural protein 1 precursor). These four genes were suggested to be related to the formation of white bellies in rice grains[37]. In addition, on the ePlant database, the *LOC_Os11g07840* gene was involved in seed maturation (at stages S4 and 5) and the gene expression level could reach up to 52% (Fig. 7b).

Through the RiceFREND and ePlant databases, it was found that two genes (*LOC_Os02g12580* and *LOC_Os11g07840*) related to how chalky rice grains are could be expressed in medium-grain rice varieties. These genes were selected to design binding molecular markers for further experiments.

Molecular markers play an important role in rice breeding by marker-assisted selection. Identification of new SNPs and genes helps to design new markers more specifically. Further research is needed to comprehend the haplotype of chalkiness and develop new specific markers for breeding medium-grain rice varieties using molecular markers.

CONCLUSION

There was an enormous variation in the percentage of chalkiness among the medium-grain rice varieties. Two putative genes, *LOC_Os02g12580* and *LOC_Os11g07840* on chromosomes 2 and 11, respectively, were associated with the percentage of chalkiness. Thus, these findings are really important in studying the genetic characteristics of chalkiness in medium-grain rice.

SIGNIFICANCE STATEMENT

The market value are affected by the grain chalkiness in rice. In this study, we first determined new SNPs and genes related to chalkiness in medium-grain rice. By genome-wide association study, 17 significant SNPs were identified with allele frequencies from 0.94-60.38%. Of which, three significant SNPs with id(s) 2003372, 9004794 and 11001576, located on chromosomes 2, 9 and 11, respectively, were selected with explaining 13.7-15.3% of the variations in chalkiness phenotype. Through the RiceFREND and ePlant databases, two candidate genes namely *LOC_Os02g12580* and *LOC_Os11g07840* linked to the SNP id(s) 2003372 and 11001576 were selected. In addition, these genes control seed formation and maturity. These two candidate genes play vital roles in controlling the chalkiness in medium-grain rice.

REFERENCES

1. Peng, B., L. Wang, C. Fan, G. Jiang, L. Luo, Y. Li and Y. He, 2014. Comparative mapping of chalkiness components in rice using five populations across two environments. BMC Genet., Vol. 15. 10.1186/1471-2156-15-49
2. Guo, T., X. Liu, X. Wan, J. Weng and S. Liu *et al*., 2011. Identification of a stable quantitative trait locus for percentage grains with white chalkiness in rice (*Oryza sativa*). J. Integr. Plant Biol., 53: 598-607.
3. Zhu, A., Y. Zhang, Z. Zhang, B. Wang and P. Xue *et al*., 2018. Genetic dissection of *qPCG1* for a quantitative trait locus for percentage of chalky grain in rice (*Oryza sativa* L.). Front. Plant Sci., Vol. 9. 10.3389/fpls.2018.01173.
4. Tanaka, H., E. Takasu, T. Aigaki, K. Kato, S. Hayashi and A. Nose, 2004. Formin3 is required for assembly of the F-actin structure that mediates tracheal fusion in *Drosophila*. Dev. Biol., 274: 413-425.
5. Zhou, S.R., L.L. Yin and H.W. Xue, 2013. Functional genomics based understanding of rice endosperm development. Curr. Opin. Plant Biol., 16: 236-246.
6. Nevame, A.Y.M., R.M. Emon, M.A. Malek, M.M. Hasan and Md. A. Alam *et al*., 2018. Relationship between high temperature and formation of chalkiness and their effects on quality of rice. BioMed. Res. Int., Vol. 2018 10.1155/2018/1653721.
7. Li, Y., C. Fan, Y. Xing, P. Yun and L. Luo *et al*., 2014. *Chalk5* encodes a vacuolar H^+-translocating pyrophosphatase influencing grain chalkiness in rice. Nat. Genet., 46: 398-404.
8. Li, Z.F., M.J. Wang, F.J. Xia and Q.H. Zhai, 2003. Mapping quantitative trait loci underlying appearance quality of rice grains (*Oryza sativa* L.). Yi Chuan Xue Bao., 30: 251-259.
9. Zhou, L., L. Chen, L. Jiang, W. Zhang and L. Liu *et al*., 2009. Fine mapping of the grain chalkiness QTL *qPGWC-7* in rice (*Oryza sativa* L.). Theor. Appl. Genet., 118: 581-590.

10. Gao, Y., C. Liu, Y. Li, A. Zhang and G. Dong *et al*., 2016. QTL analysis for chalkiness of rice and fine mapping of a candidate gene for *qACE9*. Rice, Vol. 9. 10.1186/s12284-016-0114-5.
11. Yang, W., J. Liang, Q. Hao, X. Luan and Q. Tan *et al*., 2021. Fine mapping of two grain chalkiness QTLs sensitive to high temperature in rice. Rice, Vol. 14. 10.1186/s12284-021-00476-x.
12. IRRI., 2014. Standard Evaluation System for Rice. 5th Edn., International Rice Research Institute (IRRI), Los Banos, Philippines, ISBN-13: 9789712203046, Pages: 57.
13. Zhao, K., C.W. Tung, G.C. Eizenga, M.H. Wright and M.L. Ali *et al*., 2011. Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. Nat. Commun., Vol. 2. 10.1038/ncomms1467.
14. Courtois, B., A. Audebert, A. Dardou, S. Roques and T. Ghneim-Herrera *et al*., 2013. Genome-wide association mapping of root traits in a japonica rice panel. PloS ONE, Vol. 8. 10.1371/journal.pone.0078037.
15. Huang, X., X. Wei, T. Sang, Q. Zhao and Q. Feng *et al*., 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. Nat. Genet., 42: 961-967.
16. Kumar, V., A. Singh, S.V.A. Mithra, S.L. Krishnamurthy and S.K. Parida *et al*., 2015. Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). DNA Res., 22: 133-145.
17. Ueda, Y., F. Frimpong, Y. Qi, E. Matthus and L. Wu *et al*., 2015. Genetic dissection of ozone tolerance in rice (*Oryza sativa* L.) by a genome-wide association study. J. Exp. Bot., 66: 293-306.
18. Vanniarajan, C., K.K. Vinod and A. Pereira, 2012. Molecular evaluation of genetic diversity and association studies in rice (*Oryza sativa* L.). J. Genet., 91: 9-19.
19. IRRI, 1996. Standard Evaluation System for Rice. 4th Edn., International Rice Research Institute, Manila, Philippines, Pages: 52.
20. Mansueto, L., R.R. Fuentes, F.N. Borja, J. Detras and J.M. Abriol-Santos *et al*., 2017. Rice SNP-seek database update: New SNPs, indels, and queries. Nucleic Acids Res., 45: D1075-D1081.
21. Wolfe, D., S. Dudek, M.D. Ritchie and S.A. Pendergrass, 2013. Visualizing genomic information across chromosomes with phenogram. BioData Mining, Vol. 6. 10.1186/1756-0381-6-18.
22. Kawahara, Y., M. de la Bastide, J.P. Hamilton, H. Kanamori and W.R. McCombie *et al*., 2013. Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. Rice, Vol. 6. 10.1186/1939-8433-6-4.
23. Sato, Y., N. Namiki, H. Takehisa, K. Kamatsuki and H. Minami *et al*., 2013. RiceFREND: A platform for retrieving coexpressed gene networks in rice. Nucleic Acids Res., 41: D1214-D1221.
24. Waese, J., J. Fan, A. Pasha, H. Yu and G. Fucile *et al*., 2017. ePlant: Visualizing and exploring multiple levels of data for hypothesis generation in plant biology. Plant Cell, 29: 1806-1821.
25. Flint-Garcia, S.A., J.M. Thornsberry and E.S. Buckler, 2003. Structure of linkage disequilibrium in plants. Ann. Rev. Plant Biol., 54: 357-374.
26. Bian, J., C. Li, H. He, H. Shi and S. Yan, 2014. Identification and analysis of QTLs for grain quality traits in rice using an introgression lines population. Euphytica, 195: 83-93.
27. Yuan, P.R., H.J. Kim, Q.H. Chen, H.G. Ju, S.D. Ji and S.N. Ahn, 2010. Mapping QTLs for grain quality using an introgression line population from a cross between *Oryza sativa* and *O. rufipogon*. J. Crop Sci. Biotechnol., 13: 205-212.
28. Zheng, L., W. Zhang, S. Liu, L. Chen and X. Liu *et al*., 2012. Genetic relationship between grain chalkiness, protein content, and paste viscosity properties in a backcross inbred population of rice. J. Cereal Sci., 56: 153-160.
29. Wan, X.Y., J.M. Wan, J.F. Weng, L. Jiang, J.C. Bi, C.M. Wang and H.Q. Zhai, 2005. Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. Theor. Appl. Genet., 110: 1334-1346.
30. Qiu, X., Y. Pang, Z. Yuan, D. Xing and J. Xu *et al*., 2015. Genome-wide association study of grain appearance and milling quality in a worldwide collection of *Indica* rice germplasm. PLoS ONE, Vol. 10. 10.1371/journal.pone.0145577.
31. Sun, W., Q. Zhou, Y. Yao, X. Qiu and K. Xie and S. Yu, 2015. Identification of genomic regions and the *Isoamylase* gene for reduced grain chalkiness in rice. PLoS ONE, Vol. 10. 10.1371/journal.pone.0122013.
32. Smita, S., A. Katiyar, V. Chinnusamy, D.M. Pandey and K.C. Bansal, 2015. Transcriptional regulatory network analysis of MYB transcription factor family genes in rice. Front. Plant Sci., Vol. 6. 10.3389/fpls.2015.01157.
33. Fucile, G., D. di Biase, H. Nahal, G. La and S. Khodabandeh *et al*., 2011. ePlant and the 3D data display initiative: Integrative systems biology on the World Wide Web. PLoS ONE, Vol. 6. 10.1371/journal.pone.0015237.
34. Durian, G., M. Rahikainen, S. Alegre, M. Brosché and S. Kangasjärvi, 2016. Protein phosphatase 2A in the regulatory network underlying biotic stress resistance in plants. Front. Plant Sci., Vol. 7. 10.3389/fpls.2016.00812.
35. Sokolov, L.N., J.R. Dominguez-Solis, A.L. Allary, B.B. Buchanan and S. Luan, 2006. A redox-regulated chloroplast protein phosphatase binds to starch diurnally and functions in its accumulation. Proc. Natl. Acad. Sci. U.S.A., 103: 9732-9737.
36. White-Gloria, C., J.J. Johnson, K. Marritt, A. Kataya, A. Vahab and G.B. Moorhead, 2018. Protein kinases and phosphatases of the plastid and their potential role in starch metabolism. Front. Plant Sci., Vol. 9. 10.3389/fpls.2018.01032.
37. Lin, Z., X. Zhang, X. Yang, G. Li and S. Tang *et al*., 2014. Proteomic analysis of proteins related to rice grain chalkiness using iTRAQ and a novel comparison system based on a notched-belly mutant with white-belly. BMC Plant Biol., Vol. 14. 10.1186/1471-2229-14-163.