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Genome-Wide Analysis of a Radiation-Induced Lesion Mimic Mutant in Rice

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ABSTRACT. Lesion mimic mutations (LMMs) are very useful genetic tools in plants, however, it is difficult to uncover genome-wide mutations using traditional technology, the development of whole genome sequencing (WGS) provides a means by which to identify genome-wide LMMs. In this study, WGS was performed to reveal the molecular mechanisms behind LMMs in rice. An LMM JA001 was derived from indica rice IR64 by treating with 60Coγ-radiation. Solexa sequencing technology was applied to uncover the mutations. In the JA001 genome, 7.93% of the genome sequences were altered in the JA001 genome, among which were 351323 single nucleotide polymorphisms and 32,648 bp indel variations in 19,937 genes. Gene ontology clustering revealed that genes that are associated with binding, kinase activity, the oxoglutarate dehydrogenase complex, the beta-galactosidase complex, protein phosphorylation, and cell–pollen recognition had a high mutation rate. It was also predicted that five mutated genes were involved in the porphyrin and chlorophyll metabolism,

carotenoid biosynthesis, and plant–pathogen interaction pathways. Genome-wide analysis of mutations provides new insights into the molecular mechanisms of LMMs mutagenesis

Keywords: Genome-Wide; Radiation-Induced; Lesion Mimic Mutant; Rice; Molecular Mechanisms.

Abbreviations: LMMs: Lesion mimic mutations; WGS: Whole genome sequencing; HR: hypersensitive response; SNPs: single nucleotide polymorphisms; Indel: Insertion and deletion; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

INTRODUCTION

Plants have naturally developed complex signaling and defense mechanisms to protect themselves against invading pathogens. The hypersensitive response (HR) is one of the most common and effective defense responses to pathogen attack (Chen et al. 2013). HR is regarded as the most efficient defense mechanism because of its rapid physiological changes that arrest the multiplication or expansion of the pathogens in the host cell to prevent their further spread to adjacent cells (Sun et al. 2014). Research has found that HR-like symptoms can also be activated in the absence of a pathogen attack, resulting in lesion-like appearances (Huang et al. 2010). Plants capable of developing such spontaneous lesions are referred to as disease "lesion mimic mutants" or having lesion mimic mutations (LMMs), and have been identified in a variety of plants, such as Arabidopsis (Guo et al. 2013), barley (Persson et al. 2008), maize (Wang et al. 2013), and rice (Feng et al.2013); therefore, LMMs are very useful genetic tools by which to determine the molecular mechanisms of programmed cell death and defense responses in plants.

In rice, more than 50 LMMs have been genetically determined and 13 rice LMM genes (SL, SPL5, SPL7, SPL11, SPL18, SPL28, RLIN1, OsPti1, OsLSD1, TTM1, NPR1, acdr1, and edr1) have been isolated (Fujiwara et al. 2010; Chen et al. 2012; Yamanouchi et al 2002; Zeng et al. 2004; Mori et al. 2007; Qiao et al. 2010; Sun et al.2011; Takahashi et al. 2007; Wang et al. 2005; Takahashi et al. 2007; Chern et al., 2005; Kim et al. ,2009; Shen et al., 2011). These LMM genes encode different proteins with distinct functions. For example, SL encodes a cytochrome P450 monooxygenase (Fujiwara et al. 2010), SPL5 encodes a putative splicing factor 3b subunit 3 (SF3b3) (Chen et al. 2012), SPL7 is a heat-stress transcription factor protein (Yamanouchi et al. 2002), SPL11 encodes a U-box/armadillo repeated protein with the activity of E3 ubiquitin ligase (Zeng et al. 2004), SPL18 encodes an acyltransferase (Mori et al. 2007), SPL28 encodes a clathrin-associated adaptor protein complex 1, medium subunit μ1 (AP1M1) (Qiao et al. 2010), RLIN1 encodes a putative coproporphyrinogen III oxidase (Sun et al. 2011), OsPti1a encodes an active protein kinase (Takahashi et al. 2007), and OsLSD1 encodes a zinc-finger protein (Wang et al. 2005). Thus, those genes not only participate in multiple biochemical metabolic pathways, but also form HR-like mechanisms to protect plants from biotic and abiotic stresses.

Even given this knowledge, it is difficult to uncover genome-wide mutations using traditional technology. Previous studies on the molecular mechanisms of LMM mutagenesis focused only on small-scale variations in a mutated plant genome by anglicizing the known gene variations, using molecular markers or protein electrophoresis to scan mutative loci, or using mutated plants to locate target genes. Whole genome sequencing technology (WGS) allows for the detection of individual mutations and has been applied to rice (Huang et al. 2012), maize (Lai et al. 2010), and other plants (Krishnamurthy et al. 2015). The development of WGS provides a means by which to identify genome-wide LMMs.

JA001 is a variety of LMM derived from rice IR64 using 60COγ–radiation. The mutation begins to appear as yellow–brown lesion mimic spots on rice-plant leaves after the four-leaf stage. Although the genome-wide mutation in LMMs is not fully understood it is necessary for functional genomic research on rice. Here, we used Solexa sequencing technology to study the WG mutation of JA001, the results of which could be useful in further studies on the mechanisms involved in the hypersensitive response and resistant diseases in plants.

MATERIALS AND METHODS

Plant materials

The seeds of indica cultivar IR64 were irradiated with 60Coγ-radiated and sowed in the paddy field. The M1 seeds were bred and an interesting phenotype was inbred as follows: small yellow–brown lesion mimic spots appeared on the mutant leaves after the four-leaf stage (Figure 1); the target trait had been stably expressed for six generations in ji'an, Jiangxi, Sanya, and Hainan, China; the last generation was designated as JA001. The original control IR64 and mutant were cultivated under normal field management following essential agricultural practices at Jinggangshan University, Ji'an, China, in 2015.

(A) Seedling at the four-leaf stage; (B) Rice plants at the heading stage; (C) The leaf sheath of JA001; (D): The stem of JA001.

Figure 1. The LMM phenotype of the mutant at the seedling stage and the heading stage.

DNA isolation and Solexa sequencing

Young leaves without insect damage were harvested and washed for genomic DNA extraction using the CTAB/Sarkosyl procedure (Sun et al. 2011). Following quality assessment, the genomic DNA was randomly fragmented by sonication and size-fractionated by electrophoresis. DNA fragments of the desired lengths were gel purified and ligated to adapters for further Solexa sequencing.

Solexa sequencing of the sample was performed using the Hiseq 2500 (Illumina, San Diego, CA, USA) sequencing platform. According to the characteristics of the low mass fraction of sequencing, data were distributed at the end of sequence. Trim Galore! (Babraham Bioinformatics, Cambridge, UK) was used to analyze the sequence data from three to the end, and the quality control of the data was analyzed by FastQC (Babraham Bioinformatics, Cambridge, UK).

Analysis of genome-wide mutations

The genomic data containing 9311 reference sequences were downloaded from the Rice Information System. Short reads were aligned to reference sequences, and adapter sequences or low-quality reads were filtered using the Short Oligonucleotide Analysis Package (SOAP) aligner (http://soap.genomics.org.cn/soapsnp.html). After filtration, the clean data were assembled and estimated using a Bayesian model. Detection and annotation of single nucleotide polymorphisms (SNPs), Insertion and deletion (indels) were performed using SOAPsnp and SOAPindel found by Cheng (2014). The information on the genes with SNPs and indels was downloaded from the Ensembl Plants database (http://plants.ensembl.org/index.html).

Blast of mutative genes and ontology annotation

Gene ontology (GO) analysis was conducted on SNPs and indels in the genome for annotation and classification. Any gene having an SNP and indel was aligned to the Natural Center for Biotechnical Information (NCBI) database using Blast2go (BioBam, Valencia, Spain) JavaScript. GO numbers were downloaded from the Ensembl Plants database and imported to the Web Gene Ontology (WEGO) database (http://wego.genomics.org.cn/cgi-bin/wego/index.pl.) for clustering analysis.

Prediction of genes involved in lesion-related pathways

The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was conducted on SNPs and indels in the KEGG database pathway (http://www.genome.jp/keg) for gene functional annotation and classification. The KEGG pathway analysis method is similar to GO function-rich product analysis. Genes that were involved in the lesion-related pathway of porphyrin and chlorophyll metabolism, carotenoid biosynthesis, and plant–pathogen interaction pathways were selected and imported to KEGG for pathway mapping.

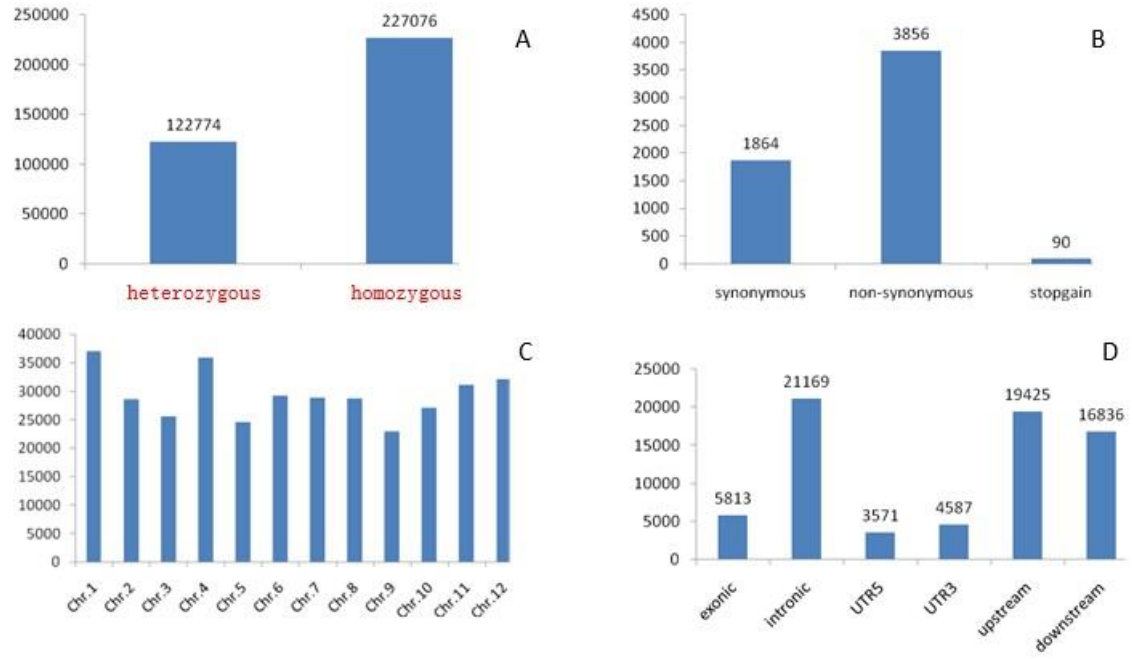
RESULTS

Genome-wide identification of genetic mutations in JA001

We sequenced the whole genome of the mutant and wild-type species with >24 GB of clean data. The average coverage rate >80% was more than tenfold; 75% coverage rate was more than twentyfold (Tab. Ⅰ). The results showed that the genomic guanine–cytosine content was 41.82%, and 43.26% of the JA001 genome sequences were identical to that of IR64.

SNPs and indels in the JA001genome

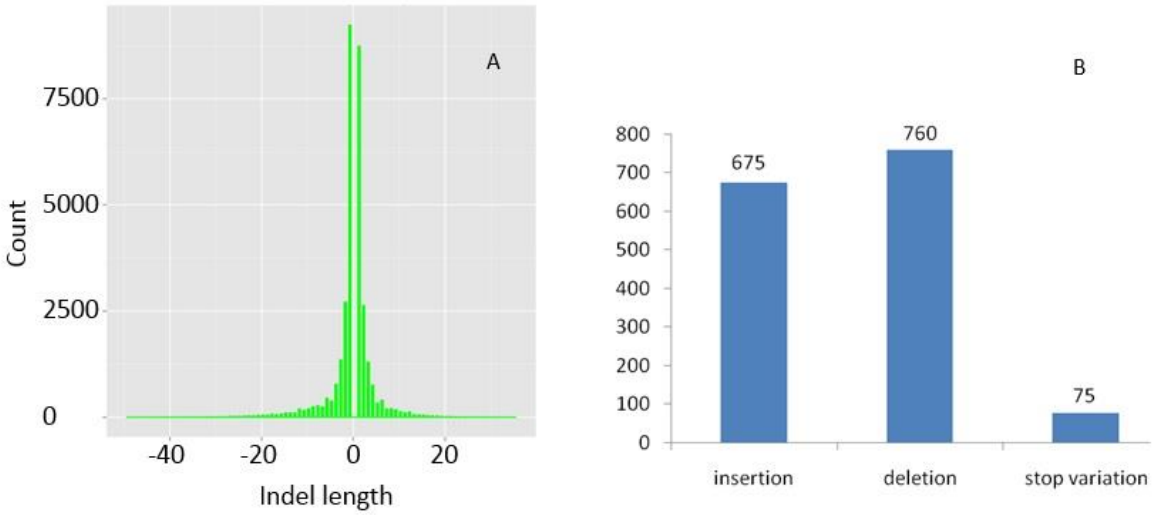
There were 351,323 SNPs (122,774 heterozygous and 227,076 homozygous) identified in the JA001 genome. There were 1,864 synonymous and 3,856 non-synonymous SNPs, and 90 SNPs that might lead to premature stops. The SNPs were distributed among 15,255 genes and enriched in chromosomes 1, 4, 11, and 12. Annotation analysis showed that 5,813 SNPs were located in exonic regions, 21,169 were located in intronic regions, 8,158 were located in untranslated regions, and 36,261 were located in stream regions (Figure 2).



(A) Numbers of homozygous and heterozygous SNPs; (B) numbers of synonymous, nonsynonymous, and stopgain SNPs; (C) distribution in the JA001 rice 12 chromosome; (D) distribution in exonic, intronic, untranslated (UTR), and stream regions. Indels in the JA001 genome.

Figure 2. Single nucleotide polymorphism (SNP) distribution in the JA001 rice genome.

We uncovered 32,648 indels, which were distributed among 4,682 genes. There were fewer indels than SNPs, and those that were1.0 bp long accounted for 55.23% of the whole genome. There are 368 large fragments insertion/deletion in exon (larger than 10bp) (Table 1). The number of indels also decreased sharply as indel length increased. Annotation analysis showed that 1,510 indels were located in the exonic regions, comprising 675 insertions, 760 deletions, and 75 stop variations (Figure 3).



(A) Numbers of indels at different indel lengths; (B) numbers of insertions, deletions, and stop variation SNPs.

Figure 3. Indel distribution in the JA001 rice genome.

Table 1. The data on large fragment insertion/deletion in exon

Sample	Average depth	Coverage (≥10)	Coverage (≥20)	GC-rate (%)	Production	
					Reads (M)	Bases (G)
IR64	58.06	85.49	80.42	43.26	169.60	24.47
JA001	55.91	84.98	77.51	41.82	166.61	24.01

Functional clustering of mutative genes

Functional clustering of mutative gene GO annotation was conducted and plotted using WEGO for gene function clustering (Figure 4).

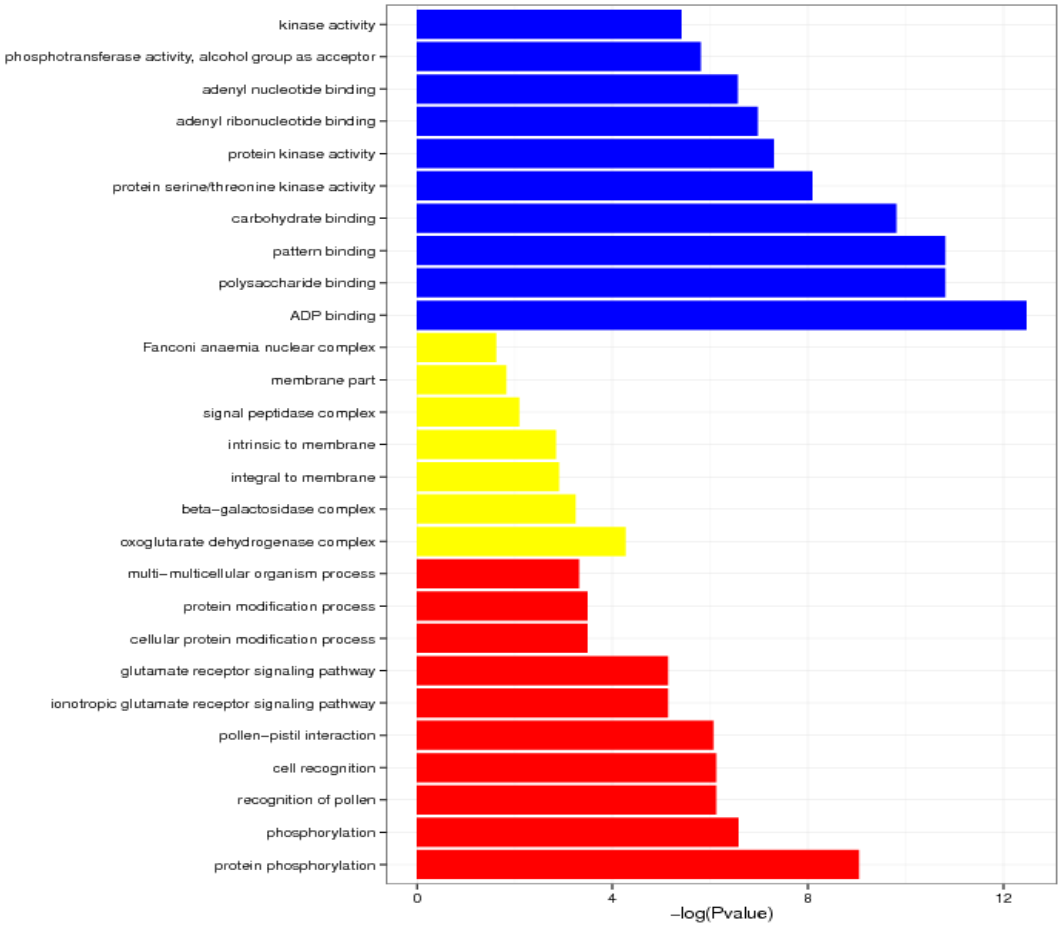


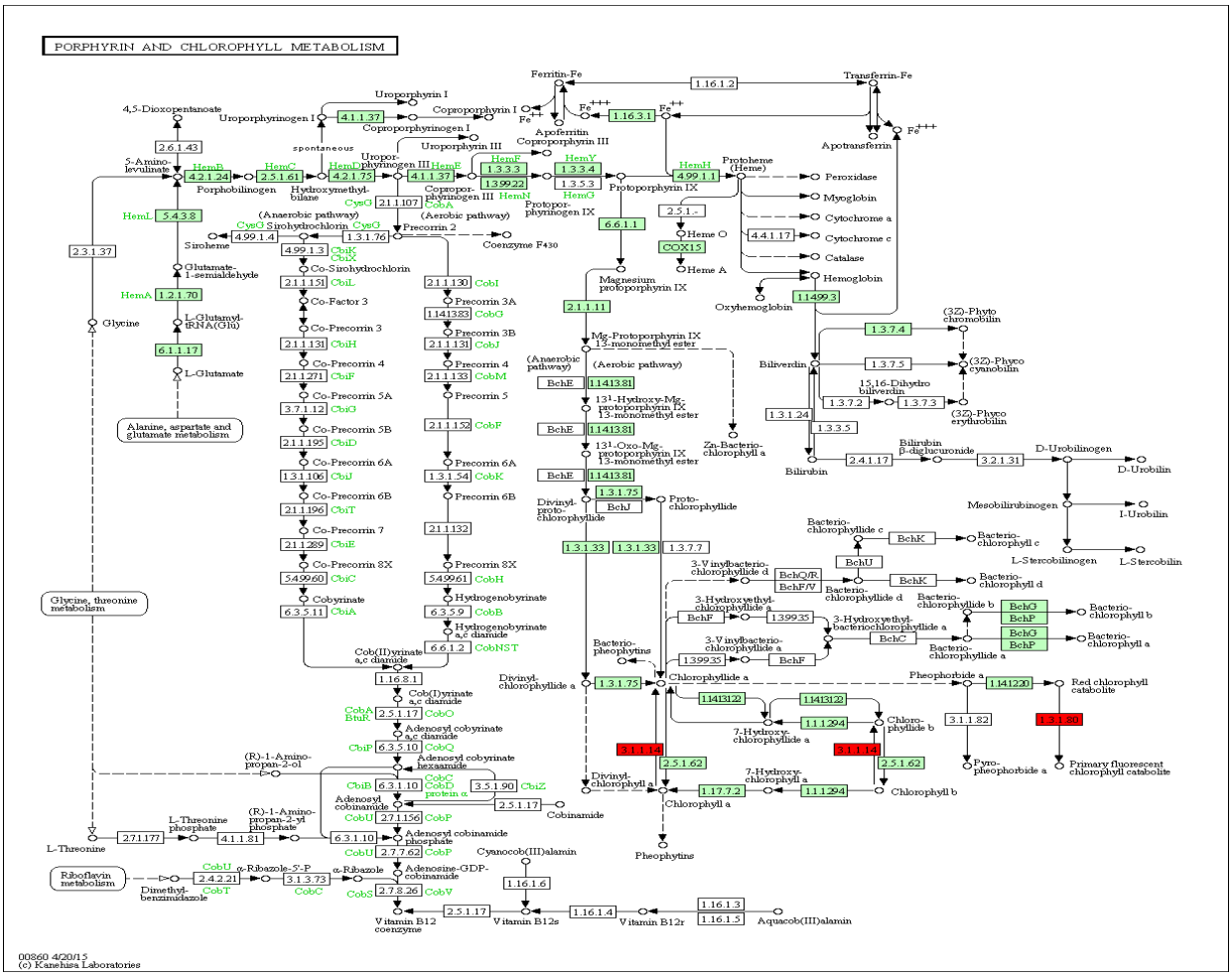
Figure 4. Web Gene Ontology database (WEGO) clustering of genes with three types of mutations. (Blue: Molecular function; Yellow: cellular component; Red: Biological process).

The results showed that SNPs and indels were distributed among different GOs. In molecular function ontology, binding and kinase activity has a higher mutation rate. In cellular component ontology, the GO term of the oxoglutarate dehydrogenase and beta-galactosidase complexes contains the majority of mutative genes. GO

terms associated with membrane structure contain membrane parts intrinsic and integral to the membrane. In biological process ontology, protein phosphorylation and cell–pollen recognition have a high mutation rate. The secondary pollen–pistil interaction, the ionotropic glutamate receptor signaling pathway, the cellular protein modification process, and the multi-multicellular organism process also contains some mutations, suggesting that some mutative genes might have an effect on the distinguishing traits between JA001 and IR64.

The pathways analysis of mutative genes

The mutated genes were blasted against the NCBI database using Blast2go (BioBam, Valencia, Spain). Because JA001 has the lesion mimic phenotype, we focused on those genes in the three KEGG pathways of porphyrin and chlorophyll metabolism, carotenoid biosynthesis, and plant–pathogen interaction. OS10g0419600 (EC: 3.1.1.14) and OS10g0389200 (EC: 1.3.1.80) were mapped to the porphyrin and chlorophyll metabolism pathways (Figure 5A), OS02g0817900 was mapped to the carotenoid biosynthesis pathway (Figure 5B), and Os01g0547000 and OS01g0505600 were mapped to plant–pathogen interaction pathways (Figure 5C). These genes variations might be the main reason for the mutant traits (Table 2).



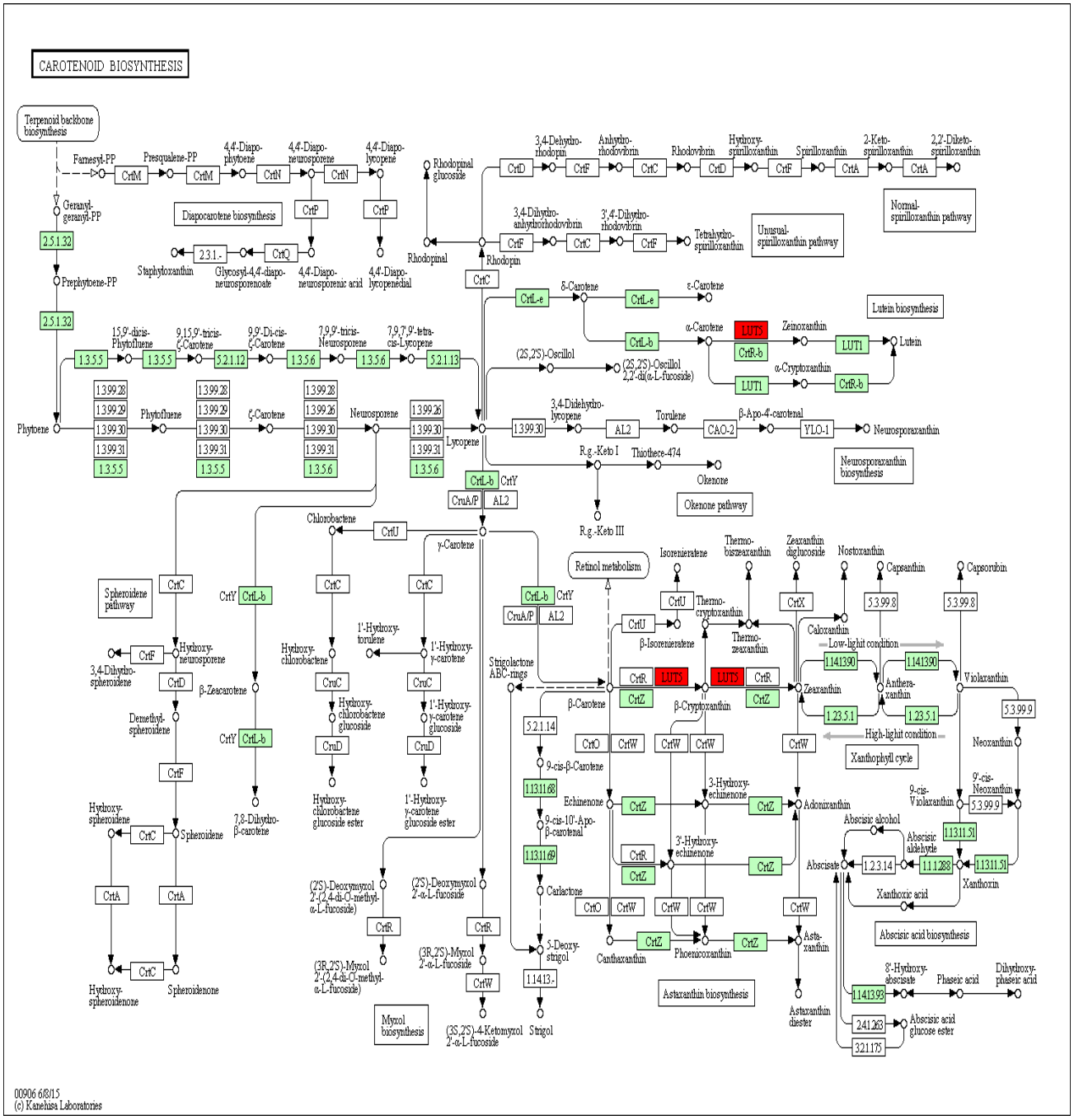


Figure 5. Mutative genes involved in the three Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. (A) Porphyrin and chlorophyll metabolism; (B) carotenoid biosynthesis; and (C) plant–pathogen interaction. Red boxes depict that mutated gene transcription of the corresponding difference was significant. Green boxes depict that mutated gene transcription of the corresponding difference was not significant. White squares depict that there was no difference in the mutant transcription of the corresponding gene

DISCUSSION

Classical genetics presumes that LMM mutants affect one or two major genes and keep other genes intact (Ahloowalia et al. 2001); however, we uncovered many mutation sites in the LMM mutant JA001. In our study, whole genome sequencing technology was used to analyze the genetic mutations in JA001, which was derived by exposing the IR64 rice to 60COγ–radiation. We found that 7.93% of the genome sequences were different between JA001 and the wild type. This is the first report using whole genome sequencing technology to study

LMMs, which provides new insights into the mechanisms by which genome-wide LMMs are identified. However, although there are a lot of mutation sites happened in the mutant, the fact might be only few involves in the LMM development.

Table 2. Selected gene mutations in JA001

Genes	Chromosomes	Variation type
OS10g0419600	10	3 non-synonymous SNPs
OS10g0389200	10	7 non-synonymous SNPs
OS02g0817900	2	3 non-synonymous SNPs
Os01g0547000	1	2 bp deletion
OS01g0505600	1	1 non-synonymous SNP

SNPs and indels are present in different individuals of the same plant and affect their gene expression and phenotypes in the genome (Huang et al. 2012). We identified 3,856 non-synonymous and 90 stopgain SNPs. These alterations could lead to the failure of functional gene expression, decreases in intron size, or shortened open reading frames. The proportion of 1.0-bp indels was observed to be the highest, suggesting that 60COγ-radiation might easily cause an insertion or deletion of a single base in the rice genome and result in a frameshift mutation. Our study also showed that there are 10 times more SNPs than indels, and that SNPs could account for most of the genetic mutations, which explains that point mutations are the main type of variation induced by radiation. A similar result was seen in a rice mutant induce by γ-radiation (Cheng et al. 2014).

WEGO clustering of mutative genes showed that the many mutations induce functional changes, which might cause physiological changes in JA001. In molecular function ontology, mutations occurred most frequently in the binding and kinase domains. Many of these domains are involved in regulating signal transduction in plants (Nguyen et al. 2012). In the cellular component, it appears that the genes that are associated with the oxoglutarate dehydrogenase complex, beta-galactosidase complex, and membrane structure preferentially change. Because LMM is a complex mutant, all genes appear to have an even chance of mutating; however, because the reason is not known, it is difficult to explain. We found that some DNA domains related to pollen (cell) recognition and pollen–pistil interaction have a high non-synonymous SNP ratio. In previous reports, the reason that the yield of the mutant was lower than that of the wild type was that HR caused cell death in part of the mutant leaves, which decreased photosynthate (Feng et al. 2013). We found that some generative-related genes were changed in the JA001 mutation, which is the main reason for the decrease in the LMM mutant. Further studies should be conducted to determine whether the HR response or generative-related genes vary.

CONCLUSION

LMM could affect the qualitative or quantitative traits through many pathways (Chen et al. 2013). Although there a lot of genes were mutant in JA001, the lesion mimic phenotype of JA001 may be due to one or two major genes. Based on the phenotype of the hypersensitive response, we focused on the three pathways of porphyrin and chlorophyll metabolism (OS10g0419600, OS10g0389200), carotenoid biosynthesis (OS02g0817900), and plant–pathogen interaction (Os01g0547000, OS01g0505600) for KEGG analysis, these genes variations might be the main reason for the mutant traits. RNA-Seq would be better to characterize the genes involved in the LMM development and investigate the mechanism of LMM, further research RNA-Seq will be used, which expected to pinpoint the causative genes for mutant JA001, as well as to our knowledge of gene functions and the mechanisms underlying phenotypic expression.

COMPETING INTERESTS

The authors have declared that they are no competing interests.

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