Sequence variation and expression analysis of seed dormancy- and germination-associated ABA- and GA-related genes in rice cultivars

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INTRODUCTION

Seed dormancy has been defined as a temporary failure of seed germination under conditions that favor germination (Bewley, 1997). The initiation of dormancy can either occur in the dry state (after-ripening) or be triggered by imbibition under defined conditions in mature seeds (Gubler et al., 2005). Pre-harvest sprouting of cereal grain leads to reduced grain yield and poor quality products, resulting in economic losses of cultivars. Strong dormancy also prevents grain such as barley from being re-planted immediately after harvest, causing delayed or non-uniform germination. Both situations would result in poor crop establishment and grain performance.

Seed dormancy and germination are regulated by nearly all plant hormones. Several studies have shown that ethylene, auxin, and brassinosteroids promote the germination of dormant seeds, but it is now generally accepted that ABA and GA are the leading regulators (Koornneef et al., 2002; Gubler et al., 2005). ABA deficiency during seed development is associated with the absence of primary dormancy in mature seeds, whereas over-expression of ABA biosynthesis genes can increase seed ABA content and enhance seed dormancy or delay germination. ABA plays important roles in many cellular processes including seed development, dormancy, germination, vegetative growth, and environmental stress responses. ABA level can increase significantly during seed maturation and in response to environmental stresses. In addition to hormone content, the transition from the dormant to the non-dormant state of many seeds is characterized by a decrease in ABA sensitivity and an increase in GA sensitivity (Chiwocha et al., 2005).

Recently, the major enzymes involved in ABA and GA metabolism pathways have been identified. ABA metabolic genes have been identified such as ABA biosynthetic gene NCED that encodes a 9-cis-epoxycarotenoid dioxygenase (NCED) and ABA catabolic gene CYP707A that encodes a ABA 8′-hydroxylase (CYP707A; Seo and Koshiba, 2002; Lefebvre et al., 2006; Okamoto et al., 2006; Yang and Guo, 2007). Genes associated with GA metabolism have also been identified such as GA biosynthetic gene GA20ox that encodes a GA 20-oxidase (GA20ox), GA3ox that encodes a GA 3-hydroxylase (GA3ox), and GA catabolic gene GA2ox that encodes a GA 2-oxidase (GA2ox; Yamauchi et al., 2004; Finch-Savage et al., 2007). However, no potential receptors that could perceive this increase in abscisic acid had been identified until recent reports of four abscisic acid binding proteins: the chloroplast protein magnesium protoporphyrin-IX chelatase H subunit (Shen et al., 2006), the membrane-associated protein G protein coupled receptor 2 (Liu et al., 2007; Verslues and Zhu, 2007), two GPCR-type G proteins (GTG1 and GTG2) (Ma et al., 2009; Pandey et al., 2009), and the PYR/PYL/RCAR family proteins (Ma et al., 2009; Park et al., 2009). Since there have been controversies over structural and functional classification of ABA receptors, we carried out analyses on the recently available recent receptor candidates in this study.
The identification of GA receptors has also improved our understanding of GA function in relation to germination (Nakajima et al., 2006).

Rice is one of the most important cereal crops, providing food for billions of people. In addition, it is also a model plant with various cultivars and mutants for the study of heterosis and domestic process. 9311 is a cultivar of Oryza sativa L. ssp. indica – the major rice subspecies grown in Asia-Pacific regions. Pei-Ai 64s (PA64s) has a major background of indica and a minor background of japonica and javanica, and the first two are two other commonly cultivated subspecies in China. 9311 and PA64s as parental lines show significant difference in their phenotype of seed dormancy. Their F1 offspring, an elite super hybrid rice LYP9, also shows significant heterosis including weak dormancy like its paternal line 9311. Compared to its wild ancestor O. rufipogon, cultivated rice typically exhibits reduced dormancy (Veasey et al., 2004; Sweeney and Mccouch, 2007). Therefore, to elucidate the molecular mechanisms regulating rice seed dormancy and germination is of importance for both plant biology and crop development. Toward this end, we identified genes encoding ABA and GA metabolic enzymes and found that there were many single nucleotide polymorphisms (SNPs) and Indels in both protein-coding and regulatory regions between different rice cultivars. We also found differentially expressed transcription units in the seed of our rice model 9311 and PA64s. We demonstrate that seed dormancy state in rice may be influenced by both gene sequence variation as well as expression patterns of ABA and GA metabolic genes.

MATERIALS AND METHODS

PLANT MATERIALS

Mature seeds from fresh harvest of 9311, PA64s, IR24, SHOEMED, AZUCENA, DOURADO AGULHA, MAKALIOKA34, and IR36 were harvested at the maturing stage then dried and stored at room temperature. The seeds of 9311 and PA64s were provided by the Chinese National R&D Center on Hybrid Rice, Changsha, China. The seeds of IR24, SHOEMED, AZUCENA, DOURADO AGULHA, MAKALIOKA34, and IR36 were provided by the International Rice Research Institute (IRRI). Six months after harvesting the seeds were used for the experiments. Mature rice embryos were separated manually from their seeds using scalpels, and then stored in –80°C immediately before RNA extraction.

GERMINATION EXPERIMENTS

For the germination experiment, we started with 50 seeds in a Petri dish with multiple layers of wet filter papers (sterile double-distilled water) at 37°C. The germination rate was calculated daily based on radical emergence. Experiments were performed in triplicate for each cultivar examined.

DETERMINATION OF ABA AND GA LEVELS

Mature rice embryos were homogenized in 80% (v/v) acetone. After adding internal standards 13C-abscisic acid (ABA) and 3H-labeled GA3, the homogenate was shaken for 10 h on ice in darkness and then centrifuged at 2,000g for 30 min. The precipitate was then extracted, and the combined supernatant was evaporated to remove residual acetone. After a series of organic extractions, the extracts were purified through C18 column. ABA was methylated with diazomethane, whereas GA3 were trimethylsilylated with BSTFA at 100°C for 60 min. Gas chromatography–electron impact ionization mass spectrometry was carried out to determine ABA and GA concentrations. The following mass-to-charge ratio peaks were used for quantification: for ABA, 192 (labeled) and 190 (endogenous); and for GA3, 506 (labeled) and 504 (endogenous). ABA and GA concentrations of 9311 and PA64s from each individual assay of the triplicate are provided in Data Sheet S1 in Supplementary Material.

SEQUENCE SIMILARITY ANALYSIS AND ALIGNMENT OF AMINO ACID SEQUENCES

We acquired sequences of seed dormancy- and germination-associated genes from Arabidopsis and rice databases (Goff et al., 2002; Yu et al., 2002). We analyzed ABA metabolism-related genes from rice Nipponbare including OsNCED1 (AY388987), OsNCED2 (AY388988), OsNCED4 (AY388989), OsNCED3 (AY388990), OsNCED5 (AY388901), OsCYP707A5 (AB277270), OsCYP707A6 (NM_001068556), OsCYP707A7 (NM_001069901), and OsGPCR (CM000147), and homologs from Arabidopsis including AtNCED1 (AT3G63520), AtNCED2 (AT4G18350), AtNCED3 (AT3G14440), AtNCED4 (AT4G19170), AtNCED5 (AT1G30100), AtNCED6 (AT3G24420), AtNCED9 (AT1G78390), AtCYP707A1 (AT4G19230), AtCYP707A2 (AT2G29090), AtCYP707A3 (AT5G43540), AtCYP707A4 (AT3G19270), and AtGCR2 (AT1G52920) (Note: not all NCED enzymes are involved in ABA biosynthesis in Arabidopsis). GA metabolism-related genes from rice are OsGA20ox1 (AC096690), OsGA20ox2 (NM_001051549), OsGA20ox3 (AP005840), OsGA3ox1 (NM_001048899), OsGA3ox2 (AC119288), OsGA2ox (AC119288), OsGA2ox1 (NC_001048899), OsGA2ox3 (NM_001050827), OsGA2ox4 (AC132485), OsGA2ox5 (NM_001062199), OsGA3ox1 (NM_001047821), OsGA3ox2 (AC144738), OsGA2ox1 (AC119288), OsGA2ox2 (NM_001048899), OsGA2ox3 (NM_001050827), OsGA2ox4 (AC132485), OsGA2ox5 (NM_001062846), and OsGID1 (AB211399), and homologs from Arabidopsis are AtGA2ox1 (At1g25420), AtGA2ox2 (At5g51810), AtGA2ox3 (At5g07200), AtGA3ox1 (At1g15550), AtGA3ox2 (At1g80340), AtGA3ox3 (At4g21690), AtGA2ox1 (AT1G78440), AtGA2ox2 (AT1G30040), AtGA2ox3 (AT2G34555), and AtGID1 (AT3G05120). Alignment of sequences and phylogenetic analysis were carried out by using ClustalW with default parameters and MEGA4.1 with neighboring joining method (Tamura et al., 2007). All sequences of 9311, PA64s, and Nipponbare used in this study are provided in Data Sheet S2 in Supplementary Material. For each gene pair, we calculated the number of non-synonymous substitutions per non-synonymous site (Ka) and the number of synonymous substitutions per synonymous site (Ks) using the maximum-likelihood method (Goldman and Yang, 1994). Ka/Ks < 1 indicates neutral evolution, where the number of non-synonymous changes at each possible non-synonymous site is the same as the number of synonymous changes per synonymous site. Ka/Ks > 1 suggests purifying selection, where selection generally eliminates deleterious mutations; Ka/Ks > 1 indicates positive selection, where selection bring more amino acid changes.

TEMPLATE PREPARATION AND REAL-TIME PCR VALIDATION

Total RNA was purified from each sample using Trizol (Invitrogen) according to the manufacturer's instructions. Eight microgram total RNA of each sample was used for first-strand cDNA synthesis in 25 μl reaction containing 5 μl 5 × RT buffer, 2.5 μl 10 mM dNTP, 50 ng random primer, 50 ng oligoDT(15), 2.5 μl RNase inhibitor
(20 U/μl), 4 μl reverse transcriptase (50 U/μl; Invitrogen), 2.5 μl DTT. Reverse transcription was performed at 42°C for 60 min with a final denaturation at 70°C for 15 min.

Specific primers for Real-time PCR in the experiments are listed as follows:

- OsNED1: (upper: 5′-TGGAGCAGGAGGCTACTG-3′, lower: 5′-GCCAGTGACGTCATCTT-3′; OsNED2: (upper: 5′-GCTTGACTTTGCTGTCT-3′, lower: 5′-CGGTTGGATGGAATGTT-3′; OsNED3: (upper: 5′-GCCATTCCTTTGCTTGA-3′, lower: 5′-AGAGTTGAGCAGAAGC-3′; OsNED4: (upper: 5′-GCTTGACTTTGCTGTCT-3′, lower: 5′-AGAGTTGAGCAGAAGC-3′; OsNED5: (upper: 5′-GAGTGGTACGGAAGGAG-3′, lower: 5′-GCCATTCCTTTGCTTGA-3′).

Reverse transcription was performed at 42°C (20 U/μl), 4 μl reverse transcriptase (50 U/μl; Invitrogen), 2.5 μl Ks ratios. Statistical significance \( p \) was determined based on the \( t \)-test in GraphPad Prism 5. Wilcoxon rank sum test was performed to compare SNPs and Indels between coding regions and regulatory regions, also between paralogs and orthologs.

**RESULTS**

**GERMINATION TEST AND ABA/GA CONCENTRATION MEASUREMENT OF RICE SEEDS FROM VARIOUS CULTIVARS**

We initiated our study by carrying out a germination experiment using seeds from various cultivars (9311, PA64s, IR24, SHOEMED, AZUCENA, DOURADO AGULHA, MAKALIOKA34, and IR36) with diverse genetic backgrounds (Figure 1). Although all seeds started to germinate after 1 day rehydration, we observed significant differences among the cultivars. For instance, IR24 showed the highest germination rate (82%) and about half of the seeds from 9311, SHOEMED, IR36, and AZUCENA germinated within 36-h imbibition. In contrast, only 29% of MAKALIOKA34, 14% of DOURADO, and less than 10% of PA64s seeds germinated at this time period.

Since rice germination rate is highly associated with the ABA/GA ratio (Nonogaki et al., 2010), we quantified ABA and GA in 9311 and PA64s dry seeds (see Materials and Methods). The endogenous ABA level of 9311 was obviously lower than those of PA64s and the GA/ABA ratio was much higher in 9311 than in PA64s, which is consistent with our experimental results that PA64s exhibited stronger dormancy than 9311 (Figure 2).

**IDENTIFICATION OF RICE ABA AND GA METABOLIC GENES**

Because ABA and GA metabolic genes and their receptors are not well-annotated in the rice genome assemblies, we extracted well-annotated *Arabidopsis* genes from the public databases and searched for orthologs and paralogs from the rice databases. To confirm the predicted protein.

**STATISTICAL ANALYSIS**

We used the unpaired Student’s \( t \)-test to analyze expression levels of ABA and GA, transcripts of ABA- and GA- related genes in dry seeds when comparing the two groups of 9311 and PA64s, and Ka/Ks ratios. Statistical significance \( p < 0.01 \) was determined based on the \( t \)-test in GraphPad Prism 5. Wilcoxon rank sum test was performed to compare SNPs and Indels between coding regions and regulatory regions, also between paralogs and orthologs.

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1www.ncbi.nlm.nih.gov/blast/
sequences, we used PFAM database\(^2\) to identify conserved domains and found that rice has five NCED, three CYP707A, four GA20ox, two GA3ox, and five GA2ox gene family members. They are distributed on chromosome 1 (OsGA20ox2, OsGA3ox2, OsGA2ox2, and OsGA2ox3), chromosome 2 (OsNCED1 and OsCYP707A5), chromosome 3 (OsNCED3 and OsGA20ox1), chromosome 5 (OsGA20ox4, OsGA3ox1, OsGA2ox1, OsGA2ox4, and OsGA2ox5), chromosome 7 (OsNCED4), chromosome 8 (OsCYP707A6), chromosome 9 (OsCYP707A7), and chromosome 12 (OsNCED2, OsNCED5, and OsGA2ox3). Our annotations were further confirmed in GBROWSE\(^3\) (Table 1).

**PHYLOGENIC ANALYSIS OF ABA AND GA METABOLIC GENES**

We identified five NCED genes in rice and nine in *Arabidopsis* (Schwartz et al., 2003; Tan et al., 2003; Kushiro et al., 2004). OsNCED2 and OsNCED5 are both located on chromosome 12 and OsNCED1, OsNCED3, and OsNCED4 are located on chromosomes 2, 3, and 7, respectively. The amino acid sequence of *Arabidopsis* AtNCED4 showed higher similarities with OsNCED1 and OsNCED2 (51–57% identities) but relatively lower identities with OsNCED3 to OsNCED5 (ranging between 33 and 36%). Phylogenetic analysis (Figure 3A) revealed that OsNCED3 and OsNCED5, which were more similar to each other (80% identity) than any other reported NCED proteins from *Arabidopsis*, were also closely related to AtNCED2, AtNCED3, AtNCED5, AtNCED6, and AtNCED9 (59–63% identities).

Since *Arabidopsis* CYP707A has four paralogs (AtCYP707A1, AtCYP707A2, AtCYP707A3, and AtCYP707A4), we tried really hard to find each of their orthologs in rice. However, we only identified three rice CYP707A genes; one of them is identical to what was reported previously (Saika et al., 2007). OsCYP707A5, OsCYP707A6, and OsCYP707A7 reside on different chromosomes: 2, 8, and 9, respectively. We observed independent gene duplication events in both rice and *Arabidopsis* (Figure 3A).

As to GA3ox in rice, we identified only two previously reported genes (Itoh et al., 2001), matching to four GA3ox orthologs in *Arabidopsis* (Hedden et al., 2001). OsGA3ox1 and OsGA3ox2 are located on chromosomes 5 and 1, respectively. The amino acid sequence of OsGA3ox1 and OsGA3ox2 displayed about 37% similarities to *Arabidopsis* GA3ox proteins. Phylogenetic analysis (Figure 3B) revealed that GA3ox genes duplicated separately after the monocot and dicot divide.

We identified four OsGA20ox genes; two were previously reported in rice (Sasaki et al., 2002) and five were reported in *Arabidopsis* (Hedden et al., 2001). In rice, OsGA20ox1, OsGA20ox2, OsGA20ox3, and OsGA20ox4 are located on chromosomes 3, 1, 12, and 5, respectively. The amino acid sequence of OsGA20ox1 has the highest homology with *Arabidopsis* GA20ox proteins (52–57% identities). In comparison, OsGA20ox2 and OsGA20ox3 have 46–51% identities, respectively. Phylogenetic analysis (Figure 3B) revealed that GA20ox proteins were subdivided into two groups. OsGA20ox2 and OsGA20ox1 are more closely related (64% identity) than the other OsGA20ox proteins, and they formed one separate branch, but then OsGA20ox1 and OsGA20ox3 are grouped with AtGA20ox1, AtGA20ox2, and AtGA20ox3 to form another.

We identified five GA20ox genes in rice as compared to seven previously reported *Arabidopsis* counterparts (Schoenburg et al., 2003); four (OsGA2ox1, OsGA2ox2, OsGA2ox3, and OsGA2ox4) of them were reported previously (Sakamoto et al., 2001; Sakai et al., 2003). OsGA2ox1, OsGA2ox4, and OsGA2ox5 are located on chromosome 5, and OsGA2ox2 and OsGA2ox3 are on chromosome 1. Although some OsGA2ox genes are localized on the same chromosomes, they are well separated with large phylogenetic distance that suggests early duplication events. OsGA2ox3 showed highest similarity to *Arabidopsis* GA2ox (49–51% identities), whereas the other OsGA2ox showed identities between 35 and 48% with the *Arabidopsis* counterparts. Phylogenetic analysis (Figure 3B) revealed that OsGA2ox1 has lower identities with those of *Arabidopsis* counterparts, constituting a separate clade.

**COMPARATIVE ANALYSIS OF ABA AND GA METABOLIC GENES AMONG RICE CULTIVARS**

Using a threshold of nucleotide identity over 95% and coverage over 80%, we defined orthologous gene pairs in 9311, PA64s, and *Nipponbare* (also including a 3-kb upstream sequence containing promoters and UTRs) for comparative analysis (Table 2). Although 9311, PA64s, and *Nipponbare* share a common ancestor of *O. sativa*, there are many sequence variations between orthologs that may be a result of artificial selection. In general, there are significantly more SNPs and Indels in coding regions between paralogs than between orthologs (Wilcoxon rank sum test: p < 0.01). Compared to coding regions, regulatory regions have significantly higher numbers of

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\(^1\)http://www.sanger.ac.uk/resources/databases/pfam.html

\(^2\)http://gbrowse.ncpgr.cn/cgi-bin/gbrowse/japonica/

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**FIGURE 2 | Quantification of endogenous ABA and GA levels in dry seeds of 9311 and PA64s.** Measurements are averaged over three replicates with error bars representing SE. The GA/ABA ratio is significantly different in 9311 and PA64s (t-test: **p < 0.01).**
substitutions and Indels (Wilcoxon rank sum test: \( p < 0.01 \)), which may also collectively contribute to the differential expression of these genes.

Based on the degree of sequence variations, we can readily identify the gene family members and their relationship with regard to obvious inheritances from their ancestor subspecies, indica or japonica. For instance, in NCED family, three genes (OsNCED1, OsNCED3, and OsNCED5) of PA64s and 9311 were inherited from the same indica ancestor as shown in Figure 4, which is consistent with lower variation between 9311 and PA64s (Table 2). In another gene family, OsCYP707A6 and OsCYP707A7 of PA64s were from indica (Figure 4B). In GA20ox family, PA64s inherited OsGA20ox2 from indica, whereas other genes were not obviously separated into indica or japonica (Figure 4D). In GA3ox family, both OsGA3ox1 and OsGA3ox2 were from indica background, and in GA2ox family, PA64s inherited OsGA2ox4 from its indica background (Figures 4E and 4F). ABA receptor (OsGPCR) of PA64s was most likely from japonica or javanica rather than from indica background. Both ABA and GA receptors were found with large amount of variations between 9311 and PA64s due to frequent hybridization with complex genetic background during rice domestication and breeding. We concluded that the dormancy trait of PA64s was mainly inherited from indica, though it also maintained a partial background of japonica and javanica. The mixed background may contribute to its significant difference in germination when compared with 9311.

**Table 1 | ABA and GA metabolism-related genes in Nipponbare.**

| Number | Gene name | Entry name | DNA sequence length (bp) | Annotation | Chromosome |
|--------|-----------|------------|-------------------------|------------|------------|
| **ABA BIOSYNTHESIS** | | | | | |
| 1 | OsNCED1 | AY838897 | 1917 | 9-cis-epoxy carotenoid dioxygenase 1 | II |
| 2 | OsNCED2 | AY838898 | 1731 | 9-cis-epoxy carotenoid dioxygenase 2 | XII |
| 3 | OsNCED3 | AY838899 | 1827 | 9-cis-epoxy carotenoid dioxygenase 3 | III |
| 4 | OsNCED4 | AY838900 | 1749 | 9-cis-epoxy carotenoid dioxygenase 4 | VII |
| 5 | OsNCED5 | AY838901 | 1842 | 9-cis-epoxy carotenoid dioxygenase 5 | XII |
| **ABA CATABOLISM** | | | | | |
| 6 | OsCYP707A6 | AB277270 | 1416 | ABA 8′-hydroxylase 1 | II |
| 7 | OsCYP707A6 | NM_001068556 | 1521 | Cytochrome P450 family protein | VIII |
| 8 | OsCYP707A7 | NM_001069901 | 1503 | Cytochrome P450 family protein | IX |
| **GA BIOSYNTHESIS** | | | | | |
| 9 | OsGA3ox1 | AC096690 | 1113 | Putative gibberellic 20-oxidase | III |
| 10 | OsGA20ox2 | NM_001051549 | 1170 | Gibberellin 20-oxidase | I |
| 11 | OsGA20ox3 | AP005840 | 1104 | Putative gibberellin 20-oxidase | XII |
| 12 | OsGA20ox4 | NM_001062119 | 1332 | Gibberellin 20-oxidase | V |
| 13 | OsGA3ox1 | NM_001048721 | 1113 | GA 3 beta-hydroxylase | V |
| 14 | OsGA3ox2 | AC144738 | 1155 | Putative gibberellic 3 beta-hydroxylase | I |
| **GA CATABOLISM** | | | | | |
| 15 | OsGA2ox1 | AC119288 | 1149 | Gibberellin 2-oxidase | V |
| 16 | OsGA2ox2 | NM_001048899.1 | 1008 | Gibberellin 2-oxidase | I |
| 17 | OsGA2ox3 | NM_001050827 | 984 | Gibberellin 2-oxidase | I |
| 18 | OsGA2ox4 | AC132485 | 729 | Putative gibberellin 2-oxidase | V |
| 19 | OsGA2ox5 | NM_001062846 | 1062 | Gibberellin 2-oxidase | V |
| **ABA AND GA PERCEPTION** | | | | | |
| 20 | OsGPCR | CM000147 | 1386 | Lanthionine synthetase C-like | X |
| 21 | OsGID1 | AB211399 | 1065 | Gibberellin insensitive dwarf 1 | V |

**SEQUENCE AND FUNCTIONAL ANALYSIS OF ABA AND GA METABOLIC GENES OF 9311, PA64s, AND NIPPONBARE**

We essentially identified two classes of differences among the three cultivars: SNPs and Indels (Table 2). Some genes are obviously more conserved (by and large due to selection) than others, such as OsGA3ox2 and OsGA20ox3, who have no Indel or SNP discovered in either 9311 or PA64s (Table 2). One possible reason is that these genes play major functional roles and therefore are strongly selected. Another explanation is that these genes were recently duplicated and therefore less mutation was accumulated. Other genes in 9311, PA64s, and Nipponbare appear high variable, such as OsGA20ox4 (41 SNPs and 2 Indels with a total length of 540 bp) and OsGA2ox5 (65 SNPs and 9 Indels with a total length of 154 bp) (Table 2). One possible reason for the variability is that they are relative older or less conserved due to weaker selection.

We have further scrutinized some SNPs and Indels of protein domains of the related genes to look for potential changes in protein structure and function. Ninety-seven candidate SNPs and 618 candidate Indels involving amino acid changes were found to lie in domains of RPE membrane protein, cytochrome P450, 2OG-Fe(II) oxygenase superfamily, lanthionine synthetase C-like protein, and alpha/beta hydrolase fold, as well as other residues associated with specific functions predicted by pfam (Table 3). We found that 2OG-Fe(II) oxygenase of OsGA3ox family is highly conserved but alpha/beta hydrolase fold of OsGID1 has 24 SNPs and 172 amino acid Indels (Figure 5). Compared to the sequences of PA64s and...
are required to confirm their precise roles. causes for functional variation, although further functional tests that differ between the three subspecies may be one of the direct sensitivity to GA. Sequence polymorphisms in the functional domains fold (Ollis et al., 1992), and this insertion may lead to enhanced sen-

Nipponbare, 9311 has a large insertion in the alpha/beta hydrolase family members also confirmed the complexity of the regulatory network, also footnoted by higher variations in regulatory regions. Indeed, a correlation between the expression level of GA metabo-

DISCUSSION
Studying the plasticity of dormancy and germination is of necessity in plant biology and crop breeding (Roberts, 1961). We engaged our study on seed germination and discovered that dormancy states are different among rice cultivars, consistent with previous publications (Seshu and Dadlani, 1991; Gu et al., 2004, 2006; Vea
eys et al., 2004). Aligning sequences in coding and regulatory regions, we observed large amount of Indels and SNPs in genes that are related to regulate the ABA and GA metabolic pathways. For instance, two Indels of 306 bp in the coding sequence and two Indels of 260 bp in the upstream regulatory region of OsCYP707A5 between 9311 and rice. We calculated non-synonymous and synonymous substitution rate (Ka and Ks) for each pair (Figure 6). As indicated by Ka/Ks < 1, all of pairwise comparisons appear to undergo purifying selection. Interestingly, the Ka/Ks ratio of OsGA2ox2 is significantly different from other members of OsGA2ox family (t-test: p < 0.01), indicating that it may evolve faster than others. This difference may be either a result of positive selection or relaxed purifying selection. Our results suggest that genes with multiple copies tend to evolve in different patterns, consistent with the view that one of the duplicates may undergo purifying selection after gene duplication while the other may enjoy more relaxed selective pressure. Furthermore, we also found that some of these genes (such as OsNCED1 and OsGA3ox2) had different Ka/Ks values between different rice cultivars.

**EXPRESSION ANALYSIS OF NCED, CYP707A, GA20ox, GA3ox, AND GA2ox GENES IN 9311 AND PA64s**
In imbibing seeds, ABA and GA contents are regulated by ABA biosynthetic enzyme NCED, ABA catabolic enzyme CYP707A, GA biosynthetic enzymes GA20ox and GA3ox, and GA catabolic enzyme GA2ox. We detected expression levels of these genes which are important for the regulation of ABA and GA contents (Xiong and Zhu, 2003; Welsch et al., 2008). We also performed real-time PCR to confirm the expression levels of 19 genes in 9311 and PA64s, including 6 genes (OsNCED3, OsCYP707A5, OsGA2ox2, OsGA3ox2, OsGA2ox, and OsGA2ox5) high-abundance and 13 (OsNCED1, OsNCED2, OsNCED4, OsNCED5, OsCYP707A6, OsCYP707A7, OsGA2ox1, OsGA2ox4, OsGA2ox3, OsGA3ox1, OsGA2ox2, OsGA2ox3, OsGA2ox4) low-abundance transcripts. Our results showed that the expression of ABA and GA metabolic genes was relative low and that there were significant differences between 9311 and PA64s (Figure 7). For instance, the expression of ABA synthetic gene OsNCED3 was 3.9-folds higher (t-test: p < 0.01) and catabolic gene OsCYP707A5 was 3.5-folds lower (t-test: p < 0.01) in PA64s than in 9311; this result implied a lower ABA level in 9311 (Barrero et al., 2006). The GA synthetic gene OsGA3ox2 was expressed at a much higher level (2.8-folds) in 9311 than in PA64s. In contrast, both of GA catabolic genes OsGA2ox1 and OsGA2ox5 were expressed at a much higher level in PA64s than in 9311; this result suggested higher GA level or less dormant seeds in 9311. Indeed, a correlation between the expression level of GA metabolism genes and the content of active GA has been reported (Busov et al., 2003; Oh et al., 2006). The differential expression among gene family members also confirmed the complexity of the regulatory network, also footnoted by higher variations in regulatory regions.

**DIFFERENT FUNCTIONAL CONSTRAINTS ON ABA AND GA METABOLIC GENES BETWEEN 9311, PA64s, AND NIPONBARE**
Following a gene duplication event, the two duplicates may be subjected to different selective constraints and even new functions. To investigate whether or not different rice cultivars have undergone different types of artificial selection, we identified reciprocal best matches – orthologs – among ABA and GA metabolic genes from

**FIGURE 3** | Neighbor-joining trees for ABA (A) and GA (B) metabolic genes were constructed by using MEGA4.1 and generated based on full-length amino acid sequences from rice and Arabidopsis.
| Gene name   | Genes on chromosome | SNP no. | SNP rate | Indel no. | Indel length |
|-------------|---------------------|---------|----------|-----------|--------------|
| OsNCED1     | Chr02_3173          | 5|60 | 0.026|0.0026|141|41|48|239|189|
| OsNCED2     | Chr12_1047          | 10|90 | 0.057|0.0057|0|0|0|0|0|0|
| OsNCED3     | Chr03_3073          | 8|168 | 0.004|0.0088|0.004|0|2|2|129|129|
| OsNCED4     | Chr07_0366          | 19|8 | 0.007|0.0022 |0.007|0|0|0|0|0|0|
| OsNCED5     | Chr12_2021          | 1|41 | 0.007|0.007 |0.007|0|2|2|306|306|
| OsCYP707A5  | Chr02_3170          | 1|20 | 0.001|0.0001 |0.001|0|0|0|0|0|0|
| OsCYP707A6  | Chr08_2087          | 1|20 | 0.001|0.001 |0.001|0|0|0|0|0|0|
| OsCYP707A7  | Chr09_1329          | 1|20 | 0.001|0.001 |0.001|0|0|0|0|0|0|
| OsGA20ox1   | Chr01_0837          | 19|147 | 0.001|0.0129 |0.001|7|7|7|42|42|
| OsGA20ox2   | Chr03_3066          | 36|93 | 0.0117|0.0059 |0.0117|6|9|6|9|9|9|
| OsGA20ox3   | Chr06_2528          | 36|93 | 0.012|0.0011 |0.012|6|6|6|10|10|
| OsGA20ox5   | Chr06_2587          | 36|93 | 0.05375|0.0079 |0.05375|80|11|8|184|340|228|
| OsGPCR      | Chr10_1648          | 10|41 | 0.0111|0.0057 |0.0111|7|7|7|42|42|
| OsGID1      | Chr05_1857          | 10|41 | 0.0111|0.0057 |0.0111|7|7|7|42|42|

*Matched genes of 9311 and PA64s with chromosome and gene ID information.
*Number of SNPs between the rice cultivars | Number of SNPs between PA64s and Nipponbare | Number of SNPs between 9311 and PA64s. NA strands for poor similarity.
*Rate of SNPs between 9311 and Nipponbare | Rate of SNPs between PA64s and Nipponbare | Rate of SNPs between 9311 and PA64s.
*Number of Indels between 9311 and Nipponbare | Number of Indels between PA64s and Nipponbare | Number of Indels between 9311 and PA64s.
*Total length (bp) of Indels between 9311 and Nipponbare | Total length (bp) of Indels between PA64s and Nipponbare | Total length (bp) of Indels between 9311 and PA64s.
and PA64s may affect the function of OsCYP707A5 and the binding of its regulatory transcriptional factors. In addition, we compared genes differentially expressed in dry seed between 9311 and PA64s, and hypothesized that different cellular concentrations of ABA and GA may reflect the genetic variations among these genes, especially those related to the ABA and GA pathways (Xiong and Zhu, 2003; Welsch et al., 2008).

As rice cultivars have been under strong artificial selection during domestication and the loss of seed dormancy is one of the most important traits in the domestication syndrome, dormancy- and germination-associated genes are absolutely the targets of selection. Thus, we further calculated \( Ka/Ks \) values for the ABA and GA metabolism-related genes and found that all were lower than 0.5; the result suggests that they are most likely undergoing purifying selection despite the fact that most of them belong to multi-gene families. Compared to other OsNCED family members, OsNCED3, due to their high expression levels, may play a major role in controlling dormancy and germination (Lefebvre et al., 2006; Hwang et al., 2010), which is consistent with that the ectopic expression of OsNCED3 in Arabidopsis leads to a delay in seed germination (Hwang et al., 2010). Expression analysis of CYP707A genes suggests that OsCYP707A5 may play a key role in seed dormancy and germination (Kushiro et al., 2004; Millar et al., 2006; Okamoto et al., 2006). One SNP and several Indels with a total length of 59 amino acids were identified in functional regions of OsCYP707A5, indicating its potential involvement in regulating ABA concentration.
| Gene        | Change | Position | Indel length (aa) | Domain/family                               | Domain/family position |
|-------------|---------|----------|-------------------|---------------------------------------------|------------------------|
| OsNCED1     | E/K     | 448      | 63                | RPE (retinal pigment epithelial) membrane protein | 131–630                |
|             | G/R     | 465      |                   |                                             |                        |
| OsNCED2     | I/L     | 188      | 0                 | RPE membrane protein                         | 68–569                 |
|             | V/A     | 199      |                   |                                             |                        |
|             | R/Q     | 359      |                   |                                             |                        |
| OsNCED3     | R/A     | 238      | 43                | RPE membrane protein                         | 108–601                |
|             | A/G     | 240      |                   |                                             |                        |
|             | C/W     | 241      |                   |                                             |                        |
|             | G/R     | 242      |                   |                                             |                        |
|             | D/E     | 245      |                   |                                             |                        |
|             | N/Y     | 254      |                   |                                             |                        |
|             | L/F     | 257      |                   |                                             |                        |
|             | V/I     | 258      |                   |                                             |                        |
|             | D/G     | 282      |                   |                                             |                        |
|             | C/R     | 492      |                   |                                             |                        |
| OsNCED4     | P/S     | 87       | 59                | RPE membrane protein                         | 76–575                 |
|             | V/L     | 109      |                   |                                             |                        |
|             | G/A     | 116      |                   |                                             |                        |
|             | V/I     | 125      |                   |                                             |                        |
|             | A/T     | 131      |                   |                                             |                        |
|             | G/S     | 549      |                   |                                             |                        |
| OsNCED5     | A/V     | 260      | 0                 | RPE membrane protein                         | 110–606                |
| OsCYP707A5  | A/T     | 237      | 59                | Cytochrome P450                               | 40–432                 |
| OsCYP707A6  | NA      | NA       | 4                 | Cytochrome P450                               | 52–489                 |
| OsCYP707A7  | N/H     | 69       | 0                 | Cytochrome P450                               | 45–474                 |
| OsGA2ox1    | NA      | NA       | 0                 | ZOG-Fe(II) oxygenase superfamily             | 208–307                |
| OsGA2ox2    | NA      | NA       | 0                 | ZOG-Fe(II) oxygenase superfamily             | 225–324                |
| OsGA2ox3    | NA      | NA       | 9                 | ZOG-Fe(II) oxygenase superfamily             | 199–304                |
|             | S/N     | 304      |                   |                                             |                        |
|             | C/S     | 312      |                   |                                             |                        |
|             | S/A     | 313      |                   |                                             |                        |
|             | I/T     | 315      |                   |                                             |                        |
| OsGA2ox4    | R/L     | 316      | 49                | ZOG-Fe(II) oxygenase superfamily             | 234–369                |
|             | L/V     | 318      |                   |                                             |                        |
|             | L/F     | 320      |                   |                                             |                        |
|             | T/R     | 321      |                   |                                             |                        |
|             | T/Q     | 331      |                   |                                             |                        |
|             | Q/S     | 347      |                   |                                             |                        |
| OsGA3ox1    | NA      | NA       | 0                 | ZOG-Fe(II) oxygenase superfamily             | 226–327                |
| OsGA3ox2    | NA      | NA       | 0                 | ZOG-Fe(II) oxygenase superfamily             | 212–304                |
| OsGA2ox1    | NA      | NA       | 0                 | ZOG-Fe(II) oxygenase superfamily             | 190–321                |
| OsGA2ox2    | NA      | NA       | 0                 | ZOG-Fe(II) oxygenase superfamily             | 179–287                |
| OsGA2ox3    | NA      | NA       | 0                 | ZOG-Fe(II) oxygenase superfamily             | 174–278                |
| OsGA2ox4    | 25      | /        | 113               | ZOG-Fe(II) oxygenase superfamily             | 113–177                |
| OsGA2ox5    | F/P     | 206      | 0                 | ZOG-Fe(II) oxygenase superfamily             | 179–292                |
|             | G/R     | 207      |                   |                                             |                        |
| OsGPCR      | 18      | /        | 47                | Lanthionine synthetase C-like protein         | 120–461                |
| OsGID1      | 24      | /        | 172               | alpha/beta hydrolase fold                    | 116–330                |

*Amino acid changes in functional regions are indicated as well as Indels numbers.

The position of amino acid changes in functional regions. NA stands for no change. Positions of three proteins, OsGA2ox4, OsGPCR, and OsGID1 were not listed due to a high number of changes.

/ represents that there are too many SNPs to list in the Table.
rice cultivars exhibit different dormancy states due to differential expression of ABA and GA metabolic genes. Environmental conditions, such as high temperature and light (Toh et al., 2008), can enhance expression of specific ABA biosynthesis genes (OsNCED family) and GA catabolic genes (OsGA2ox family), resulting in seed dormancy through induction of ABA dominance. Low temperature (Finch-Savage et al., 2007) can also enhance expression of specific ABA catabolic genes (OsCYP707A family) and GA biosynthesis genes (OsGA20ox and OsGA3ox families). In order to further illustrate molecular mechanisms of embryonic dormancy in rice, additional studies should be carried out, focusing on comparative sequence analysis and expression profiling of dormancy-associated genes among large samples of rice cultivars to construct gene regulation networks covering all the stages of seed dormancy and germination.

In OsGA20ox and OsGA3ox families, OsGA20ox2 and OsGA3ox2 may play major roles in the regulation of GA synthesis (Calvo et al., 2004); both are highly conserved as no SNP/Indel was found in their functional regions. Therefore, their differential expression may control the cellular concentration of GA (Mitchum et al., 2006; Rieu et al., 2008). By the same token, OsGA2ox1 and OsGA2ox5 of OsGA2ox family may play major roles in regulating GA catabolism (Mitchum et al., 2006) so do OsGPCR and OsGID1 (Aleman et al., 2008; Hirano et al., 2008; Murase et al., 2008).

A balanced ABA/GA ratio governed by dynamics of hormone synthesis and catabolism regulates dormancy and germination through changing seed sensitivity to the external environment (Cadman et al., 2006; Seo et al., 2006). We propose a model extending the ABA/GA balance theory to address changes in gene function and expression (Figure 8). According to this model, different rice cultivars exhibit different dormancy states due to differential expression of ABA and GA metabolic genes. Environmental conditions, such as high temperature and light (Toh et al., 2008), can enhance expression of specific ABA biosynthesis genes (OsNCED family) and GA catabolic genes (OsGA2ox family), resulting in seed dormancy through induction of ABA dominance. Low temperature (Finch-Savage et al., 2007) can also enhance expression of specific ABA catabolic genes (OsCYP707A family) and GA biosynthesis genes (OsGA20ox and OsGA3ox families). In order to further illustrate molecular mechanisms of embryonic dormancy in rice, additional studies should be carried out, focusing on comparative sequence analysis and expression profiling of dormancy-associated genes among large samples of rice cultivars to construct gene regulation networks covering all the stages of seed dormancy and germination.
CONCLUSION
In this study, we identified rice ABA and GA metabolism-related genes based on comparative and phylogenetic analyses among rice cultivars. We found that there are many SNPs and Indels in the coding and regulatory sequences of these genes due to their redundancy and diverse genetic background. We further traced the variations on the genome sequences of 9311 and PA64s and showed that sequence variations may lead to functional variations and variable seed dormancy states regulated by ABA and GA. In addition, we also surveyed and compared differentially expressed genes between 9311 and PA64s and demonstrated that differential expression of related genes may also play roles in the variable dormancy and germination states in rice. Although precise correlation between sequence variations and dormancy states need further experimental verification, sequence and expression analyses of ABA and GA metabolism-related genes among rice cultivars pave a way to study molecular mechanism of seed dormancy and germination.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at http://www.frontiersin.org/Plant_Genetics_and_Genomics/10.3389/fpls.2011.00017/abstract

Data Sheet S1 | ABA and GA concentrations of 9311 and PA64s from each individual assay of the triplicate.

Data Sheet S2 | DNA sequences of 9311, PA64s, and Nipponbare used in this study.
REFERENCES

Alemán, L., Kitamura, J., Abdel-Mageed, H., Lee, J., Sun, Y., Nakajima, M., Ueguchi-Tanaka, M., Matsuoka, S., and Allen, R. D. (2008). Functional analysis of cotton orthologs of GA signal transduction factors GID1 and SLR1. Plant Mol. Biol. 68, 1–16.

Barrero, J. M., Rodríguez, P. L., Quesada, V., Piqueras, P., Ponce, M. R., and Micó, J. L. (2006). Both abscisic acid (ABA)-dependent and ABA-independent pathways govern the induction of NCED3, AAO3 and ABA1 in response to salt stress. Plant Cell Environ. 29, 2000–2008.

Bewley, J. D. (1997). Seed germination and dormancy. Plant Cell 9, 1055–1066.

Busov, V. B., Meilan, R., Pearce, D. W., Ma, C., Rood, S., and Strauss, S. H. (2003). Activation tagging of a dominant gibberellin catalysis (GA 2-oxidase) from poplar that regulates tree stature. Plant Physiol. 132, 1209–1219.

Cadman, C. S., Toorop, P. E., Hilhorst, H. W., and Finch-Savage, W. E. (2006). Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J. 46, 805–822.

Calvo, A. P., Nicolas, C., Nicolas, G., and Rodriguez, D. (2004). Evidence of a cross-talk regulation of a GA 20-oxidase (FsGA20ox1) by gibberellins and ethylene during the breaking of dormancy in Fagus sylvatica seeds. Physiol. Plant. 120, 623–630.

Chiwocha, S. D., Cutler, A. J., Abrams, S. R., Ambrose, S. J., Yang, J., Ross, A. R., and Kermode, A. R. (2005). The etr1-2 mutation in Arabidopsis thaliana affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy via moist-ripening and germination. Plant J. 42, 35–48.

Finch-Savage, W. E., Cadman, C. S., Toorop, P. E., Lynn, J. R., and Hilhorst, H. W. (2007). Seed dormancy release in Arabidopsis Cvi by dry after-ripening: low temperature, nitrate and light environment specifically influence the endosperm is synthesized in the endosperm is involved in the induction of seed dormancy. Plant J. 45, 309–319.

Liu, X., Yue, Y., Li, B., Nie, Y., Li, W., Wu, W. H., and Li, M. (2007). A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. Science 315, 1712–1716.

Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A., and Grill, E. (2009). Regulators of PP2C phosphate activity function as abscisic acid sensors. Science 324, 1064–1068.

Milla, A. A., Jacobsen, J. V., Ross, J. J., Hilliwell, C. A., Poole, A. T., Scofield, G., Reid, J. B., and Gubler, F. (2006). Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8-hydroxylase. Plant J. 45, 942–954.

Mitchum, M. G., Yamaguchi, S., Hanada, A., Kuwahara, A., Yoshioke, Y., Kato, T., Tabata, S., Kiami, Y., and Sun, T. P. (2006). Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. Plant J. 45, 804–818.

Murase, K., Hirano, Y., Sun, T. P., and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456, 459–463.

Nakajima, M., Shimada, A., Takashi, Y., Kim, Y. C., Park, S. H., Ueguchi-Tanaka, M., Suzuki, H., Kato, E., Iuchi, S., Kobayashi, M., Maeda, T., Matsuoka, M., and Yamaguchi, I. (2006). Identification and characterization of Arabidopsis gibberellin receptors. Plant J. 46, 880–889.

Nonogaki, H., Bassel, G. W., and Bewley, J. D. (2010). Germination – still a mystery. Plant Sci. 179, 374–581.

Oh, E., Yamaguchi, S., Kiami, Y., Bae, G. (2006). Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. Plant J. 47, 124–139.

Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., Kiami, Y., Koshiba, T., and Nambara, E. (2006). CYP707A1 and CYP707A2, which encode abscisic acid 8-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. Plant Physiol. 141, 97–107.

Ollis, D. L., Cheah, E., Cygler, M., Dijkstra, T., Koornneef, M., Bentsink, L., and Hilhorst, H., Lee, J., Sun, Y., Nakajima, M., Shimada, A., Takashi, Y., and Matsuoka, M. (2007). Ethylene promotes submergence-induced expression of OsABA6ox1, a gene that encodes ABA 8-hydroxylase in rice. Plant Cell Physiol. 48, 287–298.

Saikai, M., Sakamoto, T., Saito, T., Matsuoka, M., Tanaka, H., and Kobayashi, M. (2003). Expression of novel rice gibberellin 2-oxidase gene is under homeostatic regulation by biologically active gibberellins. J. Plant Res. 116, 161–166.

Salomon, T., Kobayashi, M., Itoh, T., Tagiri, A., Kayano, T., Saito, H., Iwahori, S., and Matsuosa, M. (2001). Expression of a gibberellin 2-oxidase gene around the shoot apex is related to phase transition in rice. Plant Physiol. 125, 1508–1516.

Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, N., Ishimura, A., Swapan, D., Ishiyama, K., Saito, T., Kobayashi, M., Khush, G. S., Kitano, H., and Matsuosa, M. (2002). Green revolution: a mutant gibberellin-synthesis gene in rice. Nature 416, 701–702.

Schnoburg, F. M., Bizell, C. M., Lee, D. J., Zeevaart, J. A., and Asamino, R. M. (2003). Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. Plant Cell 15, 151–163.

Schroeder, J. I., and Zeevaart, J. A. (2003). Elucidation of the indirect pathway of abscisic acid biosynthesis.
by mutants, genes, and enzymes. Plant Physiol. 131, 1591–1601.

Seo, M., Hanada, A., Kuwahara, A., Endo, A., Okamoto, M., Yamauchi, Y., North, H., Marion-Poll, A., Sun, T.P., Koshiba, T., Kamiya, Y., Yamaguchi, S., and Nambara, E. (2006). Regulation of hormone metabolism in Arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. Plant J. 48, 354–366.

Seo, M., and Koshiba, T. (2002). Complex regulation of ABA biosynthesis in plants. Trends Plant Sci. 7, 41–48.

Seshu, D. V., and Dadlani, M. (1991). Mechanism of seed dormancy in rice. Seed Sci. Res. 1, 187–194.

Shen, Y. Y., Wang, X. F., Wu, F. Q., Du, S. Y., Cao, Z., Shang, Y., Wang, X. L., Peng, C. C., Yu, X. C., Zhu, S. Y., Fan, R. C., Xu, Y. H., and Zhang, D. P. (2006). The Mg-chelatase H subunit is an abscisic acid receptor. Nature 443, 823–826.

Sweeney, M., and McCouch, S. (2007). The complex history of the domestication of rice. Seed Sci. Res. 17, 951–957.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tan, B. C., Joseph, L. M., Deng, W. T., Liu, L., Li, Q. B., Cline, K., and McCarty, D. R. (2003). Molecular characterization of the Arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. Plant J. 35, 44–56.

Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Jikumaru, Y., Hanada, A., Aso, Y., Ishiyama, K., Tamura, N., Iuchi, S., Kobayashi, M., Yamaguchi, S., Kamiya, Y., Nambara, E., and Kawakami, N. (2008). High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. Plant Physiol. 146, 1368–1385.

Veasey, E. A., Karasawa, M. G., Santos, P. P., Rosa, M. S., Mamani, E., and Oliveira, G. C. (2004). Variation in the loss of seed dormancy during after-ripening of wild and cultivated rice species. Ann. Bot. 94, 875–882.

Verslues, P. E., and Zhu, J. K. (2007). New developments in abscisic acid perception and metabolism. Curr. Opin. Plant Biol. 10, 447–452.

Welsch, R., Wust, F., Bar, C., Al-Babili, S., and Beyer, P. (2008). A third phytoene synthase gene (SgNCED1) from Stylosanthes guianensis and its expression in response to abiotic stresses. Plant Cell Rep. 26, 1383–1390.

Yu, J., Hu, S., Wang, J., Wong, G. K., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen, Y., Huang, X., Lin, W., Ye, C., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Huang, X., Li, W., Li, J., Liu, Z., Li, L., Liu, J., Qi, Q., Liu, J., Li, L., Li, T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X., Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He, S., Zhang, J., Xu, J., Zhang, K., Zheng, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Ren, X., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Xia, H., Bao, Q., Li, G., Gao, H., Cao, T., Wang, J., Zhao, W., Li, P., Chen, W., Wang, X., Zhang, Y., Hu, J., Wang, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, C., Zhu, Z., Chen, R., Hao, J., Zheng, W., Chen, S., Guo, W., Li, G., Liu, S., Tao, M., Wang, J., Zhu, L., Yuan, L., and Yang, H. (2002). A draft sequence of the rice genome (Oryza sativa L. ssp. indica). Science 296, 79–92.

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