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ORIGINAL RESEARCH

Transcriptomics Analysis Identified Candidate Genes Colocalized with Seed Dormancy QTLs in Rice (*Oryza sativa* L.)

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Abstract Rice seed dormancy is an important trait related to the preharvest sprouting resistance of rice and is controlled by a polygene network. To identify the genes involved in this process, transcriptome analysis was applied to strong seed dormancy indica cultivar N22 and its weak dormancy mutant Q4646. The results showed that 280 genes were significantly upregulated and 244 genes significantly downregulated in the seed of Q4646 as compared to N22 during 25 to 28 days after heading. These genes were mainly involved in stress response, C-compound metabolism, plant development, DNA processing, and lipid metabolism. Some of these genes were colocalized with several reported dormancy QTLs, suggesting that they are possibly candidate genes underlying rice seed dormancy. Our work provides important clues for future effort to clone seed dormant genes in rice.

Keywords Rice · Seed dormancy · Mutant · Microarray · Quantitative trait loci (QTL)

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Introduction

Seed dormancy is a state in which seeds do not germinate even under favorable environmental conditions. Seed dormancy is especially important for cereal crops because it is associated with preharvest sprouting (PHS) which frequently happens in China and Southeast Asia and causes great loss of yield and reduction of grain quality (Ringlund 1993). Heavy PHS may occur in more than 5% of the rice in the field and in some year may be up to 20–30% for hybrid rice (Jiang et al. 2005). Seed dormancy is a complex trait controlled by polygenes and modified by the genetic background and environmental factors during the late stage of ripening (Ikehashi 1972; Koornneef et al. 2002; Gu et al. 2004). There are many reports on the genetics of rice seed dormancy. Several isozyme loci, such as *pgi1* (chromosome 3), *Amp3* and *C* (chromosome 6), *Est9* (chromosome 7), and *Acp2* (chromosome 12), have been found to be linked with dormancy genes (Wan et al. 1997). More than five regions were mapped on the RFLP map of chromosomes 3, 5, 7, and 8, respectively, which were supposed to harbor seed dormancy genes (Lin et al. 1998). Gu et al. (2004) identified six quantitative trait loci (QTLs), controlling seed dormancy from the weedy rice strain SS18-2. Wan et al. (2006) found four dormancy QTLs (*qSdn-1*, *qSdn-5*, *qSdn-7*, *qSdn-11*) from traditional indica cultivar N22. The QTL *qSdn-1* was simultaneously detected in almost the same region on chromosome 1 with relatively high LOD values of 4.59, 4.05, and 3.53, and percentages of the variance explained by the QTLs (PVE) of 8.6%, 18.7%, and 12.0% in genetic populations: Nanjing35/N22//Nanjing35, USSR5/N22//USSR5, and USSR5/N22, respectively. To our knowledge, although over 50 QTLs for seed dormancy were mapped to rice chromosomes, only *qSD12* was able to

be narrowed down to a genomic region of smaller than 75 kb (Gu et al. 2009), in which two genes, *PIL5* and a *bHLH* gene, are predicted to be likely the candidate genes.

Despite of this, the molecular basis of seed dormancy was poorly understood. We obtained a weak dormancy mutant line Q4646 that was derived by gamma ray irradiation from the strong seed dormancy indica cultivar N22. We then applied Affymetrix GeneChip® Rice Genome Arrays analysis on these two lines using RNAs from spikes of late stage of seed maturation and studied their global gene expression pattern differences. Several differentially expressed genes can be mapped to previously reported QTL loci, suggesting that they may be good candidates for seed dormancy genes. Our results provide useful clues for future rice seed dormancy gene identification.

Materials and Methods

Plant Materials Growth Conditions

N22 seeds were treated with 400-Gy ^{60}Co gamma radiations. We screened the offspring of mutant populations from M1 to M5 generation and got a seed-dormancy-reduced mutant Q4646. All plants were grown in the greenhouse at Nanjing Agricultural University (Weigang, Nanjing), and heading date of each plant was marked by emergence of the first panicle from the leaf sheath. The seeds at 25–28 days after heading (DAH) were harvested for RNA extraction.

Seed Dormancy Test

The dormancy levels of N22 and Q4646 were assessed following the method by Wan et al. (1997, 2006). Filled grains were collected on 35 days after heading, 50 seeds from plants were placed on doubled sheets of filter papers and moistened with distilled water in a Petri dish of 6-cm diameter, maintained at 30°C and 100% relative humidity for 7 days. Germination tests had three replications. Germination rate was determined by the emergence of radicle or/and plumule, and the degree of seed dormancy was scored routinely as the mean percentage of germinated seeds.

RNA Preparation, Probe Labeling, and Microarray Hybridization

Total RNA was isolated with RNA Extraction Kit (Auto-Lab, China). The RNA was further purified according to an RNAeasy Mini kit (Qiagen, Germany). Total RNA was used to synthesize double-stranded cDNA and then produced biotin-tagged cRNA using MessageAmp™ II aRNA Amplification Kit. The resulting biotin-tagged cRNA were fragmented to strands of 35 to 200 bases in length according to Affymetrix's protocols. The fragmented cRNA was hybridized to Affymetrix rice Genome 230 2.0 Array (Capital BioCorp, China) containing over 51,279 transcripts representing two rice source of japonica cultivar and indica cultivar. Subsequently, hybridization was performed at 45° C with rotation for 16 h (Affymetrix GeneChip Hybridization Oven 640) to probe sets present on an Affymetrix rice genome array. The GeneChip arrays were washed and then stained (streptavidin–phycoerythrin) on an Affymetrix Fluidics Station 450 followed by scanning on GeneChip Scanner 3000. There were three independent biological replicates for N22 and Q4646, and six chips performed the analysis of six RNA samples altogether.

Data Acquisition and Analysis

The hybridization data were analyzed using GeneChip Operating software (GCOS 1.4). The scanned images were firstly assessed by visual inspection and then analyzed to generate raw data files saved as CEL files using the default setting of GCOS 1.4. A global scaling procedure was performed to normalize the different arrays using dChip software, which incorporates a statistical model for expression array data at the probe level (Yang et al. 2002). We applied two class unpaired method in the SAM (significant analysis of microarray) software to identify genes that are expressed differently in N22 and Q4646 (Tusher et al. 2001). Genes with intensity ratios (fold change; N22/Q4646) of $\geq$2.0 or $\leq$0.5 (q value$\leq$5%) were considered to be significantly differentially changed. Putative functions of the changed genes were obtained from the Institute for Genomic Research (http://tigrblast.tigr.org/euk-blast/) and NCBI (http://www.ncbi.nlm.nih.gov) databases. Functional categorization of significantly regulated genes was carried out manually based on the MIPS (Munich Information Center for Protein Sequences) functional catalog (FunCat).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Validation

In order to validate the results of the microarray experiment, we randomly selected genes to confirm their expression by RT-PCR. Nine selected genes were small heat shock protein (Os03g14180), 17.4 kDa class I heat shock protein (Os03g16040), glutamine synthetase (Os04g56400), Zn-finger (Os01g44250), UDP-glucosyl transferase family protein (Os07g30760), auxin response factor family (Os04g56850), plant thionin family protein (Os06g31960), alpha-expansin 1 precursor (Os05g39990), and glutelin type-B 4 precursor (Os02g14600). Total RNA

Table 1 Primers used for RT-PCR

Locus	Forward primer (5–3)	Reverse primer (5–3)
Os03g14180	TCTCGTCAGCCGTGTCTCGC	GATGACCTTGCGCTCCACCT
Os03g16040	CCCTTCTCCCTCGACCT	GGCTTCTTGGGCTCTTCCTT
Os04g56400	GGCAAGCTCCAGGAGAAGATAGT	AGTCATGGCGAAGTGATAGGTTTAG
Os01g44250	CGATAAAATTGCAGACAAA	GGGAGAAGACGGAGATG
Os07g30760	GCACGGGCGGTCATACTCAACACGG	CTCCATCGCCTCCCTCACCATCCTC
Os04g56850	TGCTGTCGCCACCAAAGGTAGAAT	CTTCGCACTTCGGTCAAGGCTGT
Os06g31960	ATGGAAGGAGTGAAGAGTTTGAT	TAGGAAACAACGGTGACAGTCTC
Os08g36910	TCAAGCACAGGTCCTCTTCCAGGGTT	GCCGTTGTAGCTCAGCGAGTTCCATA
Os05g39990	GTTCTACGGCGGCGGCGATGCTT	CTGCCAGTTCTGGTCCCAGTTCC
Os02g14600	CCCTAATCATGCTGATACTTACA	CCTCTTGAGTCTCACTTTCGTTT

was isolated from the same seed as used in the microarray. The total RNA was subjected to DNase I (TaKaRa, Japan) digestion to remove any contaminating genomic. First-strand cDNA was synthesized from 2 μg total RNA for each sample with AMV reverse transcriptase (TaKaRa, Japan) according to the supplier's manual. Rice actin gene was used as the inner control for RT-PCR analysis. All primers for the genes and the actin gene were designed by the Primer 3 program (http://redb.ncpgr.cn/modules/redbtools/primer3.php) and sequences are shown in Table 1. General PCR was conducted with the following program: an initial denaturation at 94°C for 4 min, followed by 25–30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 5 min and held at 4°C. RT-PCR products were detected by 1% agarose gel in 1.0% TAE buffer with ethidium bromide.

Mapping Differentially Expressed Genes with QTLs for Seed Dormancy

To integrate the microarray data in regions predicted to contain QTLs for seed dormancy cited from the literature, map locations of QTLs for seed dormancy were determined or verified by searching a public genome database (Gramene; http://www.gramene.org/). We identified those genes that had more than twofold change within the QTLs marker intervals or 100 kb neared to closed markers.

Results

Generation of the Mutant Line Q4646 with Reduced Seed Dormancy

The traditional indica cultivar N22 has very strong seed dormancy, in which intact seeds harvested at 35 days after heading display <2% germination at 7 days after imbibition (Gu et al. 2003). The Q4646 mutant was originally isolated from aγ -irradiation mutagenesis screen on the basis of its reduced seed dormancy phenotype. We analyzed the germination of Q4646 mutants in more detail and confirmed its reduced dormancy (Fig. 1). The wild-type N22 has a high level of seed dormancy, which has a germination rate of 1% in 7-day germination and 2.3% in 15-day germination. In contrast, Q4646 mutant has 41% germination rate in 7 days and 45% in 15 days, indicating that its dormancy was significantly reduced.

General Features of Differentially Expressed Genes

In total, 524 probes expression levels were changed more than twofold between Q4646 and N22. As shown in Fig. 2a, most of the gene expression levels were not

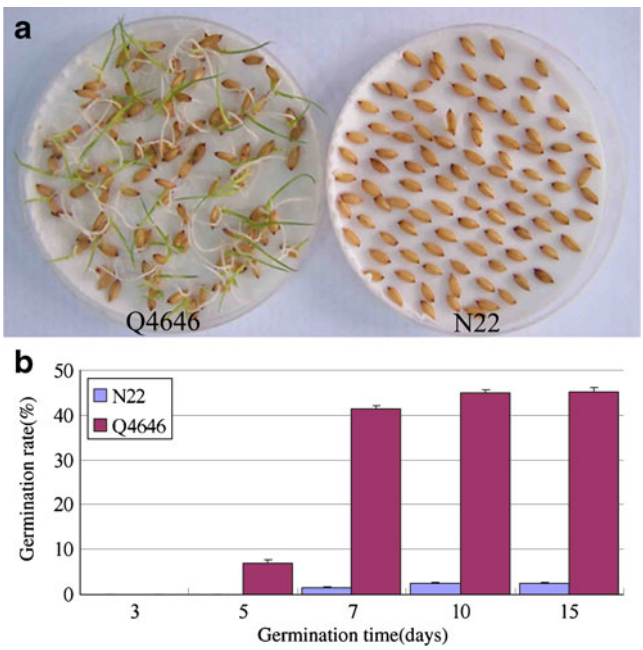

Fig. 1 Germination characteristics of indica cultivar N22 and Q4646 mutant seeds. **a** The Q4646 and N22 seeds after imbibition for 7 days. **b** Seed germination rate of the N22 and Q4646 mutant from 3 to 15 days after imbibition

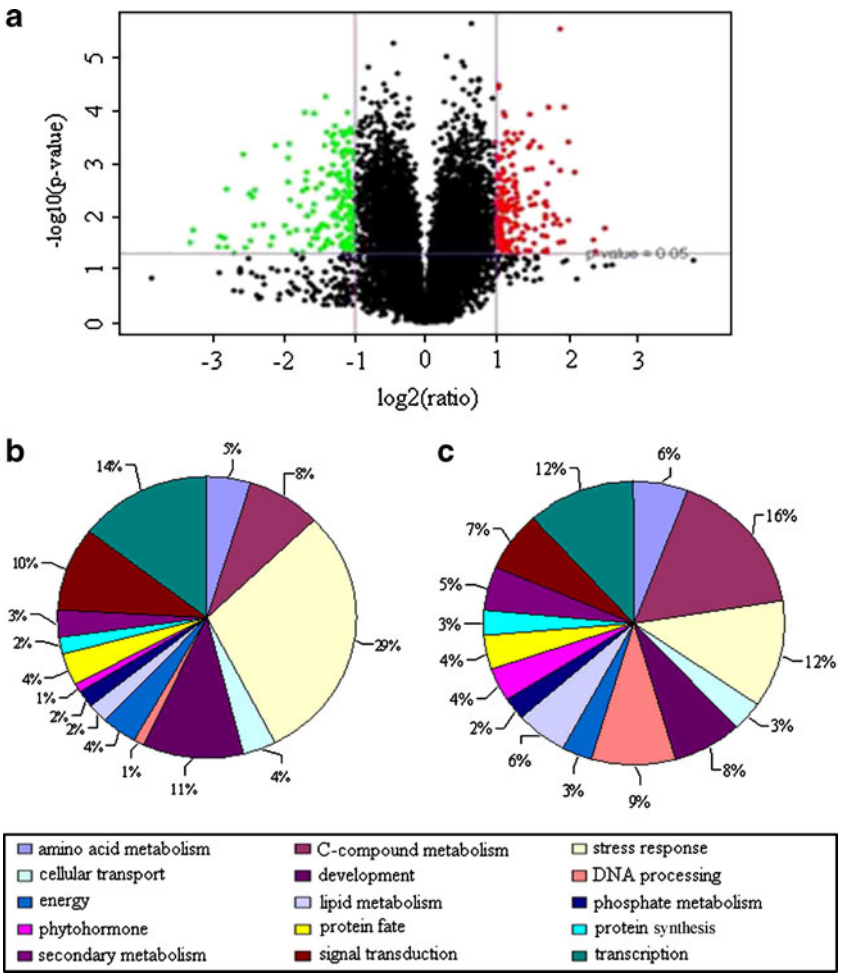

Fig. 2 Similarities and differences in gene expression between N22 and Q4646. **a** Volcano plot of log2-transformed expression ratios (N22/Q4646) plotted against the negative log10-transformed *p* value from a one-sample *t* test. Red and green highlight the genes showing a statistically significant difference in gene expression of at least 2.0-fold. **b** Functional category distribution of transcriptional changed genes in N22. **c** Functional category distribution of transcriptional changed genes in Q4646. The FunCat Scheme version 2.0 Web service at the Munich Information Center for Protein Sequences was used for the analysis

changed (black color); only a few gene expression levels were changed more than twofold (red and green color). Detailed analysis of the differentially expressed probes revealed that 244 upregulated probes were identified in N22 and 280 upregulated probes were identified in Q4646.

The classification of these differentially expressed genes into functional groups according to the Munich Information Center for Protein Sequences (MIPS) was investigated. All 524 genes were then classified according to their functions, as shown in Fig. 2b, c. Major upregulated or downregulated genes included those involved in amino acid metabolism, C-compound metabolism, stress response, cellular transport, development, DNA processing, energy, lipid metabolism, phosphate metabolism, phytohormone, protein fate, protein synthesis, secondary metabolism, signal transduction, and transcription. Stress-related genes were more numerous in the N22; metabolism and energy were relatively more numerous in the Q4646. Especially, 13 heat shock proteins (HSPs) were upregulated in N22. Gibberellin 20 oxidase (Os03g63970) and alpha-amylase isozymes (Os08g36910, Os02g52710, and Os09g28400) were upregulated in Q4646.

Validation of the Microarray Data

In order to confirm microarray results, we randomly selected nine genes and analyzed their expression by RT-PCR. Most results were consistent between RT-PCR and the microarray assay (Fig. 3). For example, in the micro-

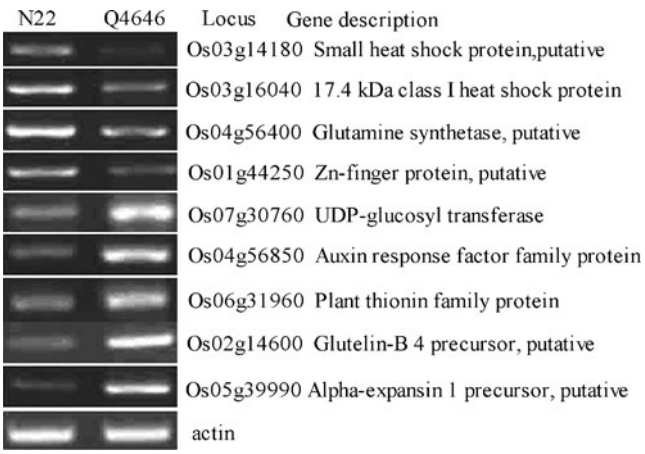

Fig. 3 Verification of microarray results by RT-PCR

array results, genes encoding for small heat shock protein (Os03g14180), 17.4 kDa class I heat shock protein (Os03g16040), glutamine synthetase (Os04g56400), and Zn-finger (Os01g44250) were upregulated in N22. They were also upregulated in N22 in the RT-PCR results. On the other hand, genes encoding for UDP-glucosyl transferase family protein (Os07g30760), auxin response factor family (Os04g56850), plant thionin family protein (Os06g31960), alpha-expansin 1 precursor (Os05g39990), and glutelin type-B 4 precursor (Os02g14600) were upregulated in Q4646 in the microarray results. Similar results were obtained from the RT-PCR. These data indicated that our microarray data were reliable.

Integrating QTLs and Transcriptomics Data to Identify Putative Seed Dormancy Genes

To map differentially expressed genes to seed dormancy QTL loci, we first collected the information of QTLs for seed dormancy cited from the literature (Table 2). Then, genes in these regions were compared with the list of genes that were differentially expressed between Q4646 and N22. Colocalized genes were listed in Table 3 and Fig. 4.

As shown in Fig. 4, 27 genes with more than twofold change were mapped to 14 regions with seed dormancy QTLs. In the *qSdn-1* region, the gene encoding for glucose-1-phosphate adenylyltransferase large subunit 1 (Os01g44220) was downregulated in N22 (0.46-fold), whereas GRAS family transcription factor containing protein gene (Os01g45860) was upregulated (2.11). The CPR5 protein gene (Os01g68970) at the region of *qSD-1* was also upregulated for a 2.46-fold change. In the region of *qDOR-2-1* and *qDOR-2-2*, genes encoding the unknown expressed protein (Os02g068300) and the nodulin MtN3 family protein (Os02g30910) had 2.65- and 0.25-fold of change, respectively. In the *qSD-3-1* region, the heat shock protein DnaJ gene (Os03g18870) was upregulated in N22, while the long cell-linked locus protein gene (Os03g19070) was downregulated when compared with its expression level in Q4646. In the region of *qDOR-3-3*, chaperonin-like RbcX (Os03g59320) and bZIP transcription factor family protein (Os03g59460) were upregulated in N22. In the region of *qDOR-6-2*, soluble starch synthase (Os06g12450) was upregulated in Q4646; Hsp20/alpha crystallin family protein (Os06g14240) was upregulated in N22. In the region of *qSD-7*, genes coding for F-box domain containing protein (Os07g37400), chlorophyll a–b binding protein of LHCII type III (Os07g37550), cotton fiber expressed protein (Os07g37620), and unknown expressed protein (Os07g39210) were upregulated in N22; LOB domain protein (Os07g40000) and transposon protein (Os07g40130) were downregulated. In the region of *qSD-9*, the C3HC4-type RING finger family protein gene (Os09g36500) was upregu-

Table 2 Reported QTLs of rice seed dormancy

Chr.	QTL	Closest marker or marker interval	Physical location (Mb)	LOD	Phenotypic variance explained (%)	Additive effect (%)	Cross	Allele increasing seed dormancy	Reference
1	qSdn-1	RM9-RM1297	23.65–28.94	4.61	9.1	25.33	Nanjing35/N22//Nanjing35	N22	Wan et al. 2006
1	qSD-1	RM104	40.49	2.1	4	12.3	IR50/Tachimimochi//Miyukimochi	IR50	Wan et al. 2005
2	qDOR-2-1	G365	3.47	2.49	9.82	7.091	Kinmaze/DV85	DV85	Tang et al. 2004
2	qDOR-2-2	XNpb132	18.49	2.01	8.37	6.25	Kinmaze/DV85	DV85	Tang et al. 2004
3	qSD-3	RM282	12.38	9.2	21	27.2	IR50/Tachimimochi//Miyukimochi	IR50	Wan et al. 2005
3	qDOR-3-3	R1927	33.78	NA	13.7	NA	Pei-kuh/W1944	W1944	Cai and Morishima 2000
3	qSD-3-1	XNpb62	10.66	2.75	12.3	10	Asominori/IR24	Asominori	Jiang et al. 2003
5	qSD-5	C597-C249	0.23–5.89	3.1	13.6	13.6	Nipponbare/Kasalath//Nipponbare	Kasalath	Miura et al. 2002
6	qDOR-6-2	R2171-RZ144	6.71–8.06	NA	12.6	NA	Pei-kuh/W1944	W1944	Cai and Morishima 2000
7	qSD-7	R1357-C1412	22.24–25.31	1.49	6.8	10.7	Nipponbare/Kasalath//Nipponbare	Kasalath	Miura et al. 2002
9	qSD-9	C1263	21.11	3.37	13	12	Asominori/IR24	Asominori	Jiang et al. 2003
11	qDOR-11-1	G24-RZ141	0.26–4.07	NA	8.1	NA	Pei-kuh/W1944	W1944	Cai and Morishima 2000
11	qDOR-11-6	RG1109-RZ536	23.11–28.37	NA	12.6	NA	Pei-kuh/W1944	W1944	Cai and Morishima 2000

NA not available

Table 3 Up/downregulated genes within regions of the rice genome predicted to contain quantitative trait loci (QTL) for seed dormancy

QTL	Accession no.	Location	Putative function	Fold change (N22/Q4646)
qSdn-1	Os01g44220	25681643	Glucose-1-phosphate adenylyltransferase large subunit 1	0.4647
	Os01g45860	26371079	GRAS family transcription factor containing protein	2.1099
qSD-1	Os01g68970	40395803	CPR5 protein	2.463
qDOR-2-1	Os02g06830	3441728	unknown expressed protein	2.6567
qDOR-2-2	Os02g30910	18448536	nodulin MtN3 family protein, putative	0.2538
qSD-3-1	Os03g18870	10550724	heat shock protein DnaJ, putative, expressed	2.1976
	Os03g19070	10656726	long cell-linked locus protein, putative	0.1753
qSD-3	Os03g21650	12338090	expressed protein	2.449
qDOR-3-3	Os03g59320	33710689	Chaperonin-like RbcX	3.1197
	Os03g59460	33783156	bZIP transcription factor family protein	2.4279
qSD-5	Os05g04170	1844907	AMP-binding enzyme family protein	0.4741
	Os05g04700	2197126	Uncharacterized protein family protein	2.1696
qDOR-6-2	Os06g12450	6747358	Soluble starch synthase 2–3	0.451
	Os06g14240	7939954	Hsp20/alpha crystallin family protein	3.7185
qSD-7	Os07g37400	22409529	F-box domain containing protein	5.0999
	Os07g37550	22485739	Chlorophyll a–b binding protein of LHCII type III	2.1621
	Os07g37620	22541752	Cotton fiber expressed protein	2.419
	Os07g39210	23478202	Expressed protein	2.0632
	Os07g40000	23994819	LOB domain protein 37, putative	0.494
	Os07g40130	24076493	Transposon protein, putative, CACTA, En/Spm subclass	0.4578
qSD-9	Os09g36500	21056063	Zinc finger (C3HC4-type RING finger) family protein	0.1558
qDOR-11-1	Os11g01790	421277	protein phosphatase 2c, putative	0.4721
	Os11g02010	536010	Transposon protein, putative, CACTA, En/Spm sub-class	0.4504
	Os11g02330	679036	Expressed protein	0.4095
	Os11g03110	1118746	GRAS family transcription factor containing protein	0.479
qSdn-11	Os11g32650	18776050	Chalcone synthase, putative, expressed	2.1308
qDOR-11-6	Os11g42510	25095104	Tyrosine aminotransferase, putative, expressed	0.4752

lated in Q4646. In the region of *qSdn-11*, the chalcone synthase gene (Os11g32650) was upregulated in N22. In the region of *qDOR-11-1*, genes for protein phosphatase 2C (Os11g01790), transposon protein (Os11g02010), expressed protein (Os11g02330), and GRAS family transcription factor (Os11g03110) were upregulated in Q4646. In the region of *qDOR-11-6*, the tyrosine aminotransferase (Os11g42510) gene was upregulated in Q4646. Therefore, these genes may be good candidates for seed dormancy and their functions should be investigated with further experimentation.

Discussion

Seed dormancy is an important physiological phenomenon and a key agronomic trait related to the quality of seed. Seed dormancy is controlled by intrinsic hormonal and metabolic pathways and influenced by external environmental factors (Finch-Savage and Leubner-Metzger 2006; Kucera et al. 2005; Holdsworth et al. 2008). However, the molecular mechanisms of rice seed dormancy are still poorly understood. In this study, we applied genome-scale gene expression analysis to strong seed dormancy N22 and its weak dormant mutant Q4646. Among the differentially expressed genes, there was a greater representation of the stress-related genes more highly expressed in N22 than in the Q4646. Meanwhile, genes related to metabolism and energy were more highly expressed in the mutant Q4646 compared with wild-type N22, such as starch metabolism and glucosyltransferase which may help reduce seed dormancy of N22. GA biosynthesis and catabolism are clearly linked to seed germination (Ogawa et al. 2003) and the key enzymes in GA biosynthesis have been well characterized (Olszewski et al. 2002). GA 20-oxidase catalyzes the penultimate steps in the production of bioactive GA and GA 3-b-dioxygenase catalyzes the production of bioactive GAs from these precursors. In our study, gibberellin 20 oxidase (Os03g63970) was upregulated in Q4646 (more than twofold change), indicating that this gene may be involved in promoting the seed dormancy release and germination in Q4646.

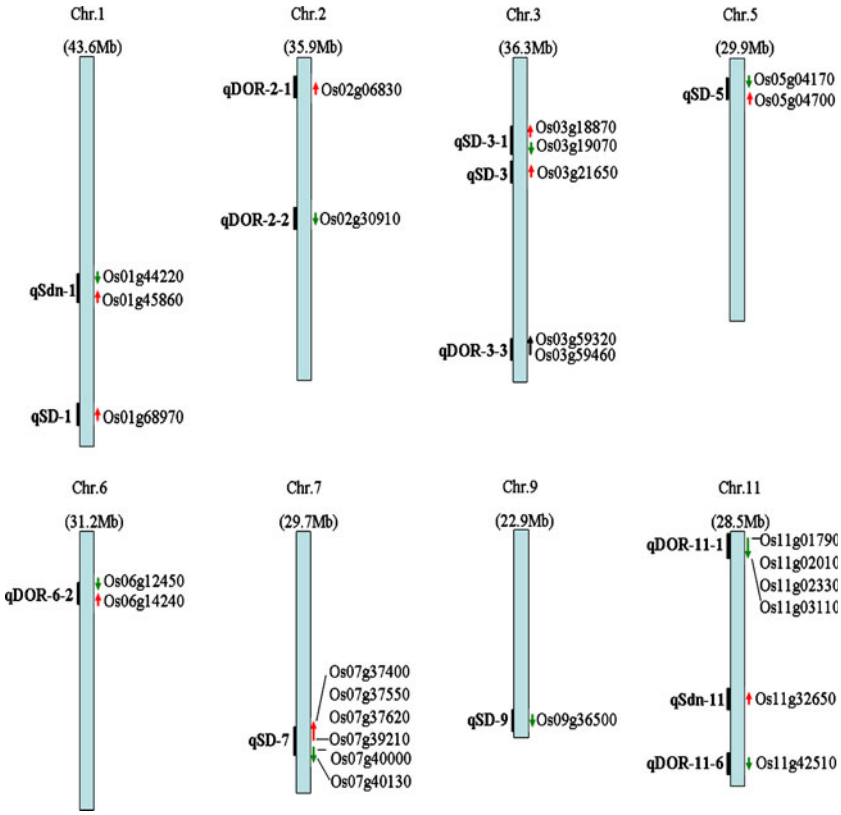

Fig. 4 Integration of the reported QTLs for rice seed dormancy with the genes significantly changed in the microarray data. Upregulated gene (*upward arrows*) downregulated gene (*downward arrows*)

To further investigate the possibility that some of the differentially expressed genes play an important role in seed dormancy, we compared the genomic locations of these genes with those of reported rice QTLs for seed dormancy. Many regions were found to be colocalized with genes of important functions. In the region of *Sdn-1*, for example, the GRAS family transcription factor containing protein gene (Os01g45860) was upregulated more than twofold in N22. GRAS family genes are known to be involved in signal transduction and meristem maintenance and development (Bolle 2004). *SLR1* gene, with sequence homology to members of the plant-specific *GRAS* gene family, for example, is a mediator of the GA signal transduction process and has enhanced capacity for abscisic acid level (Ikeda et al. 2002). GRAS family transcription factor (Os01g45860) may enhance the abscisic acid level in N22 and keep seed dormancy. In the region of *qSdn-11*, chalcone synthase (Os11g32650) was upregulated in N22. Flavonoids play important roles in plants including UV protection, defense against pathogens and pests, pollen fertility, signaling with microorganisms, auxin transport regulation, and pigmentation (Winkel-Shirley 2001). The chalcone synthase is a key enzyme that catalyzes the first dedicated reaction of the flavonoid pathway in higher plants. Accumulation of flavonoids could keep N22 in deep seed dormancy state.

In the region of *qDOR-6-2*, Hsp20/alpha crystallin family protein (Os06g14240) was upregulated in N22. HSPs play a central role not only in the protection against stress damage but also in the folding, intracellular distribution, and degradation of proteins. Small HSPs (sHSPs) with molecular size ranging from 15 to 30 kDa (Vierling 1991) represent the major family of HSPs induced by heat temperature in plants (Waters et al. 1996). *sHsp* genes were expressed constitutively in vegetative tissues and during panicle or seed development and may be involved in cellular functions under nonstress and stress conditions as well as during developmental processes (Sarkar et al. 2009). Especially, transcripts of OsHsp20 were accumulated differentially during vegetative and reproductive developmental stages (Ouyang et al. 2009). In our study, the Hsp20/alpha crystallin family protein gene (Os06g14240) was upregulated in N22. This means that these genes may play an important role in the protection against stress damage and heat temperature and in keeping N22 seed in deep dormancy.

In the region of *qSD-9*, zinc finger gene (Os09g36500) was significantly upregulated in Q4646. The zinc finger genes are involved in various signal transduction pathways and regulatory pathways, such as cold and salinity (Seong et al. 2007), plant growth (Zeba et al. 2009), and seed development (Xu and Li 2003). In Q4646, the zinc finger gene (Os09g36500) may play a role in enhancing seed germination.

In summary, a great number of genes have been identified through microarray analysis to be differentially expressed between N22 and Q4646. Colocalization and integration of these genes with QTLs indicate that these genes are good candidates for seed dormancy genes. Our work provided useful information that will aid in the cloning of important genes underlying the seed dormancy in the near future.

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