Conserved Transcriptional Regulatory Programs Underlying Rice and Barley Germination

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Abstract
Germination is a biological process important to plant development and agricultural production. Barley and rice diverged 50 million years ago, but share a similar germination process. To gain insight into the conservation of their underlying gene regulatory programs, we compared transcriptomes of barley and rice at start, middle and end points of germination, and revealed that germination regulated barley and rice genes (BRs) diverged significantly in expression patterns and/or protein sequences. However, BRs with higher protein sequence similarity tended to have more conserved expression patterns. We identified and characterized 316 sets of conserved barley and rice genes (cBRs) with high similarity in both protein sequences and expression patterns, and provided a comprehensive depiction of the transcriptional regulatory program conserved in barley and rice germination at gene, pathway and systems levels. The cBRs encoded proteins involved in a variety of biological pathways and had a wide range of expression patterns. The cBRs encoding key regulatory components in signaling pathways often had diverse expression patterns. Early germination up-regulation of cell wall metabolic pathway and peroxidases, and late germination up-regulation of chromatin structure and remodeling pathways were conserved in both barley and rice. Protein sequence and expression pattern of a gene change quickly if it is not subjected to a functional constraint. Preserving germination-regulated expression patterns and protein sequences of those cBRs for 50 million years strongly suggests that the cBRs are functionally significant and equivalent in germination, and contribute to the ancient characteristics of germination preserved in barley and rice. The functional significance and equivalence of the cBR genes predicted here can serve as a foundation to further characterize their biological functions and facilitate bridging rice and barley germination research with greater confidence.

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Introduction
Seed germination is a biological process important to plant development, plant evolution and agricultural production. Strictly defined, germination begins with the uptake of water by dry quiescent seeds and ends with visible emergence of an embryo tissue from its surrounding tissues [1]. Seed germination is accompanied by many distinct metabolic, cellular and physiological changes. For example, upon imbibition, the dry quiescent seeds take up water and rapidly resume many fundamental metabolic activities such as respiration, RNA metabolism, and protein synthesis using surviving structures and components in the desiccated cells. These concerted biological activities transform a desiccated embryo with almost undetectable metabolism into one with vigorous metabolism culminating in growth [2,3].

Transcriptional regulatory program underlying seed germination and its associated biological pathways were investigated in divergent plant species [4,5,6,7,8,9,10,11]. Extremely complex transcriptional regulatory programs are activated over the course of seed germination. In barley germination and seedling growth, 50% of examined genes are expressed in dry and germinating seeds at a detectable level. Twenty-five percent of those examined genes are differentially regulated over the course of seed germination and seedling growth. Based on global and dynamic expression changes of the germination-regulated genes, the transcriptional regulatory program underlying barley seed germination is divided into early and late phases. Each phase is accompanied by differential expression of a distinct set of genes and biological pathways. For example, the early phase of seed germination is accompanied by transcriptional up-regulation of cell wall synthesis and regulatory components including transcription factors, signaling proteins, and post-translational modification proteins. During the late germination phase, histone families and many metabolic pathways are up-regulated. Stress related pathways and seed storage protein genes are down-regulated through the entire course of germination. Comparing transcriptomes of barley and Arabidopsis showed that high accumulation of many seed stored transcripts in Arabidopsis and barley dry seeds have been preserved for 200 million years of monocot-dicot divergence [9,11].

Barley and rice have been divergent for 50 million years, but share a great similarity in seed germination and seedling growth [3,12]. For example, both rice and barley are endospermic and...
starch cereal species, and have a highly conserved seed storage mobilization pathway. Both rice and barley produce hydrolytic enzymes in aleurome tissues during seed germination and seedling growth, and translocate the hydrolytic enzymes to starch endosperm for mobilizing seed storage reserves. Seed germination and its associated production of hydrolytic enzymes are induced by gibberellic acid through a highly conserved transduction pathway [10,13,14,15]. To gain an insight into transcriptional regulatory programs underlying the conserved characteristics of barley and rice germination, we determined transcriptomes of rice grains at start-, mid- and end-germination points, and developed a bioinformatic and evolutionary approach to compare them with our previously determined transcriptome of barley at the equivalent germination stages [9]. Genome-wide sequence comparison identified germination regulated rice and barley gene pairs with a strong sequence similarity. While a small percentage of these pairs showed similar expression patterns over the course of seed germination, a majority had divergent expression pattern. The analysis also identified a collection of germination regulated barley-rice gene sets. The rice and barley genes in each set shared strong similarities in protein sequences and expression patterns. Gene expression patterns and protein sequences changes quickly if there are no functional constraints [16,17,18,19,20,21,22]. Seed germination is accomplished through concerted activities of many gene products, which are mainly defined by their protein sequences and accumulation patterns. The preservation of germination-regulated expression patterns and protein sequences of the barley and rice genes in each set suggests that the barley and rice genes were functionally important and equivalent in germination, and likely contributed to the molecular and cellular processes conserved in barley and rice germination.

Results

Transcriptomes of Barley and Rice at Three Distinct and Equivalent Developmental Stages of Germination

An objective of this study was to compare transcriptomes of rice and barley over the course of germination and to identify germination regulated barley and rice genes with conserved protein sequences and expression patterns. Since expression of germination related genes are often differentially regulated with respect to specific developmental stages over the course of seed germination [6,9], it is critical to compare their transcript accumulation levels at distinct and equivalent physiological stages. Our previous studies showed that transcriptional regulatory program underlying seed germination is divided into early and late germination phases that are separated by the mid-time point of germination [9]. Transcriptomes of barley at start- (dry), middle- (9 hr) and end-points of germination (18 hr) were previously determined and used for the comparison [9]. It took 42 hours for radicles to emerge from rice grains at the germination condition identical to barley germination. To compare transcriptomes of germinating rice and barley grains at their equivalent stages of barley germination, we examined transcriptomes of rice at 0 (dry), 21 and 42 hours of germination as start-, middle- and end-stages of germination. Three independent biological replications were conducted for each stage in rice and barley transcriptome assays.

Both barley and rice transcriptome data used in this study were produced using the Affymetrix GeneChip technologies (GeneChip Barley Genome Array and GeneChip Rice Genome Array), and were analyzed using identical statistical approaches and parameters to reduce variation from different transcriptome assay platforms and statistical analysis. One-way ANOVA identified a total of 3599 barley and 18665 rice probe-sets that were differentially regulated between any two examined stages of germination with a false discovery rate less than 5%. Considering the potential that non-specific hybridization between paralogous genes could cause an inaccurate assignment of signal intensity to gene family members, the probe-sets flagged by Affymetrix as potentially cross-hybridizing probes were removed from further analysis. A total of 2537 barley and 13013 rice probe sets were identified as germination regulated genes, and were used for further comparative analysis. A much higher number of germination regulated probe-sets were identified in rice than in barley. It was partially caused by the fact that the GeneChip Rice Genome Array has two times as many probe-sets as the GeneChip Barley Genome Array. In addition, probe-sets on barley array were designed using EST sequences while the ones on the rice array were designed using genes predicted from genome sequence, which are likely to lead to a lower percentage of germination regulated genes on the barley array than on the rice array.

Conservation and Divergence of Transcriptional Regulatory Programs Underlying Barley and Rice Germination

A total of 1507 pairs of barley and rice genes (BRs) with protein sequence similarity at an e-value less than ~50 were identified among the germination regulated barley and rice genes. The BRs contained 805 barley and 1054 rice genes (Table 1). Pearson correlation coefficients (PCC) between log2 signal intensities of each paired barley and rice genes at start-, mid- and end-stages of germination were calculated to determine the similarity of their expression patterns. Sixty percent of the BRs had a PCC value higher than 0.5, indicating that the barley and rice genes in each of the BRs had a good similarity in their transcript accumulation patterns (Figure 1, Table 2). However, forty percent of the BRs had PCC value lower than 0.5, indicating that a significant percentage of BRs had low similarity or no similarity in their expression patterns. Thus, the BRs with high protein sequence similarity preferentially preserved their expression patterns after rice and barley diverged from their most recent ancestor.
A collection of randomly paired barley/rice genes were generated from the germination regulated barley and rice genes. The randomly paired BRs had a relatively symmetrical distribution of PCC value with a slightly higher percentage at a range of PCC value from 0.8 to 1.0 than that from −0.8 to −1.0. Interestingly, twenty-seven percent of the randomly paired BRs had a PCC value greater than 0.8 (Figure 1).

Percentage of BRs with similar expression patterns (PCC value from 0.5 to 1.0) positively correlated with their protein sequence similarities in the e value range of −5 to −100 (Table 2). However, there was little difference in distribution of PCC values between BRs with e value ranging from −50 to −100 and BRs with e value less than −100. Chi-square analysis was performed to compare distributions of PCC values between randomly paired BRs and BRs with a given range of e value. There was a significant difference in distribution of PCC values between BRs with e value from −50 to −100 and randomly paired BRs at P < 0.01 (Table 2). However, there was no significant difference in distribution of PCC values between BR genes with e value from −20 to −50 and random paired BRs at P value of 0.1. Thus, the BRs at e-values less than −50 were used for identification of BRs that had conserved expression patterns.

Barley and Rice Genes with Conserved Protein Sequences and Germination Regulated Expression Patterns (cBRs)

A total of 483 BRs with a PCC value higher than 0.9 were identified among the 1507 germination regulated BR genes. Those BRs accounted for 32% of the germination regulated BRs. The 483 BRs were comprised of 368 distinct barley genes and 388 distinct rice genes. Those genes represented a small percentage of the 2537 barley and 13813 rice germination regulated genes. Thus, majority of the germination-regulated genes had diverged beyond our thresholds in protein sequences, gene expression patterns or in both. The 483 BRs were further merged into 262 single-gene cBRs containing only one gene from each species and 60 multi-gene cBRs (Table 1 and Table 3). Barley and rice genes in each of those BRs were differentially regulated during seed germination, and shared strong similarity in both protein sequences and transcriptional expression patterns. We referred to the BRs as conserved BRs (cBRs). Each multi-gene cBR had at least three genes with one-to-many, many-to-one and many-to-many barley and rice gene relationship. Any pair of “orthologous” or paralogous genes in each multi-gene cBR had sequence similarity with an e-value less than −50 and expression pattern similarity with a PCC value higher than 0.9. The largest multi-gene cBR (cBR_M2) encoded a U-box domain containing RING protein family and had a total of 20 rice and barley genes (Table 3). However, the numbers of rice and barley genes in each cBRs were not always equally distributed. For example, the cBR_M2 was composed of 17 barley genes and 3 rice RING protein genes.

Diverse Gene Expression Patterns Were Preserved in Barley and Rice Germination

There are eight possible expression patterns based on up or down-regulations of a gene in early and late germination phases. All of the possible expression patterns were observed for the cBRs, and were preserved in both rice and barley since their divergence (Table 3 and 4). Table 4 summarized the cBRs in the eight expression patterns. A total of 71 cBRs showed up-regulated expression patterns in both early and late germination phases, and made up the largest group of cBRs (Group 1). Many cBRs in the Group 1 encoded the proteins related to cell wall metabolism, cell organization, chromatin structure, protein degradation, and signaling G-proteins.

Table 1. Summary of Germination Regulated BRs and cBRs.

| Species   | No. of BRs with an e-value less than −50 | No. of Distinct Genes |
|-----------|----------------------------------------|-----------------------|
| Barley    | 1507                                    | 805                   |
| Rice      |                                        | 1054                  |

| Species   | No. of BRs with PCC >0.9 and e-value ≤ −50 | No. of Distinct Genes |
|-----------|-------------------------------------------|-----------------------|
| Barley    | 483                                       | 368                   |
| Rice      |                                          | 388                   |

| Species   | No. of BRs in Single-gene cBRs | No. of BRs in multi-gene cBRs | Single-gene/Distinct |
|-----------|--------------------------------|-------------------------------|----------------------|
| Barley    | 288                            | 80                            | 78.26%               |
| Rice      | 358                            | 30                            | 92.27%               |

Table 2. Relationship Between Protein Sequence Similarity and Expression Similarity of Barley and Rice Genes.

| Sequence Similarity/PCC value | [−1,−0.5) | [−0.5,0) | [0,0.5) | [0.5,1) | p value |
|-------------------------------|-----------|----------|---------|---------|---------|
| BRs with e-value ≤ −100       | 18%       | 8%       | 14%     | 60%     | < 0.01  |
| BRs with e-value from −50 to −100 | 16%       | 9%       | 16%     | 59%     | < 0.01  |
| BRs with e-value from −20 to −50 | 23%       | 12%      | 12%     | 54%     | < 0.1   |
| BRs with e-value from −5 to −20 | 36%       | 15%      | 12%     | 37%     | < 1     |
| random                        | 38%       | 13%      | 12%     | 37%     |         |

Table 2. Relationship Between Protein Sequence Similarity and Expression Similarity of Barley and Rice Genes.
Table 3. The cBRs and Their Expression Patterns and Functions.

| cBR ID  | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | Early Phase | Late Phase | MapMan Functional Groups                      | Gene Annotation                                      |
|---------|---------------------|-------------------|-------------------|---------------------|------------|-----------|---------------------------------------------|--------------------------------------------------|
| cBR_1   | 1 1 2 4             | 1.2               | 8.1               | RNAtRNAregulation of transcription,Silencing Group | 4          | 1.2       | anti-silencing protein 1,                   |                                                  |
| cBR_2   | 1 1 2 6             | −1.5              | 3.0               | not assigned.       | 3.0        | 0         | expressed protein                           |                                                  |
| cBR_3   | 1 1 2 1             | 1.7               | 24.5              | DNAsynthesis/replication protein A 70 kDa DNA-binding subunit, | 23         | 0         | CCR4-NOT transcription complex subunit 7,    |                                                  |
| cBR_4   | 1 1 2 3             | 2.8               | −2.2              | RNAprocessing       | 1.1        | 12.4      | protein kinase,                              |                                                  |
| cBR_5   | 1 1 2 4             | 1.1               | 12.4              | not assigned.       | 12.4       | 0         | NO_MATCH                                    |                                                  |
| cBR_6   | 1 1 2 4             | 1.1               | 6.6               | not assigned.       | 6.6        | 0         | NO_MATCH                                    |                                                  |
| cBR_7   | 1 1 2 3             | 3.9               | −2.0              | protein posttranslational modification kinase receptor like cytoplasmic kinase VII | 3.0        | 0         | protein kinase,                              |                                                  |
| cBR_8   | 1 1 2 5             | −1.3              | −1.7              | misc.acid and other phosphatases | 1.1        | 1.7       | hydrolase/polymerase                           |                                                  |
| cBR_9   | 1 1 2 2             | 9.4               | −1.1              | protein degradation | 9.4        | 0         | subtilisin-like protease precursor,          |                                                  |
| cBR_10  | 1 1 2 8             | −2.0              | −4.1              | TCA/organic transformation | 1.1        | 0         | pyruvate dehydrogenase E1 component alpha subunit |                                                  |
| cBR_11  | 1 1 2 3             | 7.4               | −1.4              | misc.UDP glucosyl and glucoronyl transferases | 7.4        | 0         | transferase, transferring glycolyl groups,   |                                                  |
| cBR_12  | 1 1 2 2             | 1.7               | 3.0               | amino acid metabolism | 2.9        | 0         | threonine synthase, chloroplast precursor,   |                                                  |
| cBR_13  | 1 1 2 1             | 1.7               | 1.9               | protein degradation ubiquitin ubiquitin | 1.7        | 1.9       | polyubiquitin 2,                             |                                                  |
| cBR_14  | 1 1 2 8             | −3.1              | −6.4              | development late embryogenesis | 3.1        | 0         | late embryogenesis abundant protein D-34,    |                                                  |
| cBR_15  | 1 1 2 5             | 1.2               | −3.2              | development unspecified | 1.2        | 0         | expressed protein                           |                                                  |
| cBR_16  | 1 1 2 5             | 1.0               | −2.4              | not assigned.       | 1.0        | 0         | NO_MATCH                                    |                                                  |
| cBR_17  | 1 1 2 3             | 2.1               | −2.4              | signalling calcium   | 2.1        | 0         | granulatin,                                 |                                                  |
| cBR_18  | 1 1 2 8             | −1.5              | −2.6              | not assigned.       | −1.5       | 0         | early fruit mRNA,                            |                                                  |
| cBR_19  | 1 1 2 3             | 5.3               | −2.2              | stress abiotic cold | 5.3        | 0         | SRC2,                                       |                                                  |
| cBR_20  | 1 1 2 1             | 1.5               | 2.9               | not assigned.       | 2.9        | 0         | NO_MATCH                                    |                                                  |
| cBR_21  | 1 1 2 8             | −2.6              | −1.6              | protein degradation autophagy | 2.6        | 0         | autophagy-related protein 8 precursor,       |                                                  |
| cBR_22  | 1 1 2 1             | 2.2               | 1.7               | not assigned.       | 2.2        | 0         | nucleolar protein, Nop52 containing protein, |                                                  |
| cBR_23  | 1 1 2 4             | 1.2               | 0.8               | misc. peroxidases   | 1.2        | 0         | peroxidase 1 precursor,                      |                                                  |
| cBR_24  | 1 1 2 8             | −2.1              | −1.8              | not assigned.       | −2.1       | 0         | monoglyceride lipase,                        |                                                  |
| cBR_25  | 1 1 2 8             | −1.5              | −2.1              | stress biotic       | −1.5       | 0         | lectin precursor,                            |                                                  |
| cBR_26  | 1 1 2 8             | −2.2              | −1.4              | not assigned.       | −2.2       | 0         | expressed protein                           |                                                  |
| cBR_27  | 1 1 2 4             | 1.0               | 2.0               | protein synthesis   | 1.0        | 2.0       | 60S ribosomal protein L13a-2,                 |                                                  |
| cBR_28  | 1 1 2 7             | −2.6              | −1.1              | not assigned.       | −2.6       | 0         | NO_MATCH                                    |                                                  |
| cBR_29  | 1 1 2 8             | −1.9              | −2.4              | not assigned.       | −1.9       | 0         | lipid binding protein,                       |                                                  |
| cBR ID | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | Early Phase | Late Phase | MapMan Functional Groups | Gene Annotation |
|--------|---------------------|-------------------|-------------------|---------------------|-------------|-----------|--------------------------|----------------|
| cBR_30 | 1                   | 1                 | 2                 | 1                   | 5.0         | 3.1       | RNA regulation of transcription, unclassified | aspartic proteinase nepenthesin-1 precursor, |
| cBR_31 | 1                   | 1                 | 2                 | 8                   | −1.6        | −4.3      | stress, abiotaic cheat | heat shock protein 82, |
| cBR_32 | 1                   | 1                 | 2                 | 3                   | 16.6        | −5.1      | signalling, in sugar and nutrient physiology | phi-1-like phosphate-induced protein, |
| cBR_33 | 1                   | 1                 | 2                 | 1                   | 1.5         | 1.4       | protein targeting, chloroplast | signal peptidase I-1, |
| cBR_34 | 1                   | 1                 | 2                 | 6                   | −2.1        | 7.4       | miscellaneous short chain dehydrogenase/reductase (SDR) | estradiol 17-beta-dehydrogenase B, |
| cBR_35 | 1                   | 1                 | 2                 | 5                   | −1.2        | −2.1      | not assigned, no ontology | STAM-binding protein, |
| cBR_36 | 1                   | 1                 | 2                 | 7                   | −2.2        | −1.1      | not assigned, unknown | expressed protein |
| cBR_37 | 1                   | 1                 | 2                 | 3                   | 3.1         | −2.9      | not assigned, no ontology | abhydrolase domain-containing protein 5, |
| cBR_38 | 1                   | 1                 | 2                 | 8                   | −1.8        | −5.5      | stress, abiotaic cheat | heat shock 70 kDa protein 1, |
| cBR_39 | 1                   | 1                 | 2                 | 4                   | 1.1         | 1.6       | cell organisation | myosin le, |
| cBR_40 | 1                   | 1                 | 2                 | 1                   | 5.7         | 19        | secondary metabolism, flavonoids, flavonols | flavonol synthase/flavanone 3-hydroxylase, |
| cBR_41 | 1                   | 1                 | 2                 | 4                   | 1.3         | 5.6       | lipid metabolism, FA synthesis and FA elongation, long chain fatty acid CoA ligase | acyl-CoA synthetase, |
| cBR_42 | 1                   | 1                 | 2                 | 5                   | −1.2        | −2.6      | protein degradation, ubiquitin E3, HECT | thyroid receptor-interacting protein 12, |
| cBR_43 | 1                   | 1                 | 2                 | 8                   | −1.4        | −2.3      | development, storage proteins | protein COQ10 A, mitochondrial precursor, |
| cBR_44 | 1                   | 1                 | 2                 | 2                   | 13.3        | −1.1      | nucleotide metabolism, synthesis, purine, amidophosphoribosyltransferase | amidophosphoribosyltransferase, chloroplast precursor, |
| cBR_45 | 1                   | 1                 | 2                 | 4                   | −1.1        | 7.2       | transport, p- and v-ATPases, H+-transporting two-sector ATPase | vacuolar ATP synthase subunit E, |
| cBR_46 | 1                   | 1                 | 2                 | 8                   | −2.1        | −3.8      | protein degradation, AAA type | ATP binding protein, |
| cBR_47 | 1                   | 1                 | 2                 | 8                   | −1.7        | −1.5      | protein degradation, ubiquitin E3, RING | RING zinc finger protein, |
| cBR_48 | 1                   | 1                 | 2                 | 8                   | −2.4        | −2.6      | not assigned, unknown | expressed protein |
| cBR_49 | 1                   | 1                 | 2                 | 3                   | 2.5         | −2.3      | not assigned, unknown | NO_MATCH |
| cBR_50 | 1                   | 1                 | 2                 | 8                   | −2.5        | −1.7      | RNA regulation of transcription, HDA | histone deacetylase 11, |
| cBR_51 | 1                   | 1                 | 2                 | 1                   | 2.0         | 2.3       | stress, abiotaic cheat, salt | ankyrin protein kinase-like, |
| cBR_52 | 1                   | 1                 | 2                 | 8                   | −1.5        | −1.5      | not assigned, no ontology | retrotransposon protein, putative, Ty3-gypsy subclass |
| cBR_53 | 1                   | 1                 | 2                 | 1                   | 2.8         | 2.1       | cell organisation | actin-1, |
| cBR_54 | 1                   | 1                 | 2                 | 4                   | −1.0        | 1.9       | not assigned, unknown | expressed protein |
| cBR_55 | 1                   | 1                 | 2                 | 3                   | 2.9         | −2.7      | protein degradation, ubiquitin E3, SCF, FBOX | adagio protein 1, |
| cBR_56 | 1                   | 1                 | 2                 | 4                   | 1.3         | 8.3       | hormone metabolism, jasmonate synthesis, degradation, 12-Oxo-PDA reductase | 12-oxophytodienoate reductase 2, |
| cBR_57 | 1                   | 1                 | 2                 | 3                   | 1.8         | −2.4      | miscellaneous glutathione S transferases | glutathione S-transferase GSTU6, |
### Table 3. 

| cBR ID | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | MapMan Functional Groups | Gene Annotation |
|--------|---------------------|-------------------|-------------------|---------------------|-------------------------|-----------------|
| cBR_58 | 1                   | 1                 | 2                 | 4                   | 1.0 1.9                 | protein translation modification.kinase receptor like cytoplasmic kinase VII |
| cBR_59 | 1                   | 1                 | 2                 | 1                   | 3.6 1.4                 | signalling G-proteins |
| cBR_60 | 1                   | 1                 | 2                 | 4                   | -1.0 9.7                | not assigned.no ontology |
| cBR_61 | 1                   | 1                 | 2                 | 8                   | -2.0 -2.0               | not assigned.unknown |
| cBR_62 | 1                   | 1                 | 2                 | 1 1                 | 1.4 2.5                | not assigned.unknown |
| cBR_63 | 1                   | 1                 | 2                 | 8                   | -2.2 -2.1               | not assigned.no ontology |
| cBR_64 | 1                   | 1                 | 2                 | 4                   | -1.1 2.4               | not assigned.no ontology armadillo/beta-catenin repeat family protein |
| cBR_65 | 1                   | 1                 | 2                 | 1                   | 1.7 3.4                | misc.nitrilases, nitrile lyases, berberine bridge enzymes, reticuline oxidases, troponine reductases |
| cBR_66 | 1                   | 1                 | 2                 | 6                   | -1.9 1.4               | not assigned.no ontology pentatricopeptide (PPR) repeat-containing protein |
| cBR_67 | 1                   | 1                 | 2                 | 4                   | -1.3 3.6               | development storage proteins |
| cBR_68 | 1                   | 1                 | 2                 | 6.7                 | 12                     | misc.peroxidases |
| cBR_69 | 1                   | 1                 | 2                 | 2                   | 7.3 -1.3               | misc.peroxidases |
| cBR_70 | 1                   | 1                 | 2                 | 7                   | -2.5 -1.1              | not assigned.no ontology |
| cBR_71 | 1                   | 1                 | 2                 | 8                   | -1.4 -1.4              | signalling 14-3-3 proteins |
| cBR_72 | 1                   | 1                 | 2                 | 1                   | 7.7 14                 | misc.UDP glucosyl and glucoronyl transferases |
| cBR_73 | 1                   | 1                 | 2                 | 4                   | -1.0 7.2               | RNA regulation of transcription. DNA methyltransferases |
| cBR_74 | 1                   | 1                 | 2                 | 5                   | -1.2 -1.5              | signalling G-proteins |
| cBR_75 | 1                   | 1                 | 2                 | 5                   | -1.1 -1.9              | stress abiotic cheat |
| cBR_76 | 1                   | 1                 | 2                 | 7                   | -1.6 1.0               | not assigned.unknown |
| cBR_77 | 1                   | 1                 | 2                 | 3                   | 2.6 -1.6               | signalling calcium |
| cBR_78 | 1                   | 1                 | 2                 | 7                   | -2.4 -1.4              | not assigned.unknown |
| cBR_79 | 1                   | 1                 | 2                 | 3                   | 2.3 -2.3               | signalling G-proteins |
| cBR_80 | 1                   | 1                 | 2                 | 3                   | 2.3 1.3                | protein translation modification |
| cBR_81 | 1                   | 1                 | 2                 | 4                   | -1.2 3.0               | protein synthesis misc ribosomal protein |
| cBR_82 | 1                   | 1                 | 2                 | 8                   | -2.0 -3.8              | protein degradation cysteine protease |
| cBR_83 | 1                   | 1                 | 2                 | 8                   | -1.8 -1.6              | not assigned.unknown |
| cBR_84 | 1                   | 1                 | 2                 | 1                   | 2.4 4.8                | not assigned.unknown |
| cBR_85 | 1                   | 1                 | 2                 | 8                   | -1.7 -3.8              | misc cytochrome P450 |

Conservation of Germination
| cBR ID | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | MapMan Functional Groups | Gene Annotation |
|--------|---------------------|------------------|-------------------|---------------------|------------------------|----------------|
| cBR_86 | 1                   | 1                | 2                 | 1.4 1.7             | not assigned.unknown   | expressed protein |
| cBR_87 | 1                   | 1                | 2                 | 5.1 −1.2           | lipid metabolism, FA synthesis and FA elongation, beta ketoacyl CoA synthase | 3-ketoacyl-CoA synthase |
| cBR_88 | 1                   | 1                | 2                 | 12.1 −2.7          | development unspecified | tRNA 2-phosphotransferase |
| cBR_89 | 1                   | 1                | 2                 | 1.1 1.5            | misc. acid and other phosphatases | lipid phosphate phosphatase 3, chloroplast precursor |
| cBR_90 | 1                   | 1                | 2                 | 5.1 1.2            | protein synthesis, misc ribosomal protein | 60S acidic ribosomal protein P2A |
| cBR_91 | 1                   | 1                | 2                 | 1.1 1.5            | cell wall, cell wall proteins, AGPs | fasciclin-like arabinogalactan protein 10 precursor |
| cBR_92 | 1                   | 1                | 2                 | 1.3 2.1            | development unspecified | RNA 2-phosphotransferase |
| cBR_93 | 1                   | 1                | 2                 | 0.1 1.8            | protein posttranslational modification | calcium-dependent protein kinase |
| cBR_94 | 1                   | 1                | 2                 | 1.1 1.3            | misc. peroxidases | peroxidase 17 precursor |
| cBR_95 | 1                   | 1                | 2                 | 1.1 1.3            | not assigned.unknown | NO_MATCH |
| cBR_96 | 1                   | 1                | 2                 | 5.1 2.7            | protein posttranslational modification | EDR1 |
| cBR_97 | 1                   | 1                | 2                 | 1.1 1.8            | not assigned.unknown | mitochondrial prohibitin complex protein 2 |
| cBR_98 | 1                   | 1                | 2                 | 1.1 1.8            | not assigned.unknown | expressed protein |
| cBR_99 | 1                   | 1                | 2                 | 1.1 1.8            | cell organisation | ATP2-A13 |
| cBR_100| 1                   | 1                | 2                 | 1.1 1.8            | transport, peptides and oligopeptides | peptide transporter PTR2 |
| cBR_101| 1                   | 1                | 2                 | 1.1 1.8            | PS light reaction, ATP synthase | ATP synthase beta chain, mitochondrial precursor |
| cBR_102| 1                   | 1                | 2                 | 1.1 1.8            | not assigned.unknown | WD-repeat protein pop3 |
| cBR_103| 1                   | 1                | 2                 | 1.1 1.8            | not assigned.unknown | steroid nuclear receptor, ligand-binding |
| cBR_104| 1                   | 1                | 2                 | 1.1 1.8            | lipid metabolism, glycerol metabolism, Glycerol-3-phosphate dehydrogenase (NAD+) | glyceral-3-phosphate dehydrogenase |
| cBR_105| 1                   | 1                | 2                 | 1.1 1.8            | not assigned.unknown | protein YIF1A |
| cBR_106| 1                   | 1                | 2                 | 1.1 1.8            | not assigned.unknown | acid phosphatase/vanadium-dependent haloperoxidase related |
| cBR_107| 1                   | 1                | 2                 | 1.1 1.8            | protein degradation, ubiquitin, E3, SCF, FBOX | F-box/LRR-repeat MAX2 |
| cBR_108| 1                   | 1                | 2                 | 1.1 1.8            | nucleotide metabolism, phosphor transfer and pyrophosphatase, misc | ectonucleotide pyrophosphatase/phosphodiesterase 1 |
| cBR_109| 1                   | 1                | 2                 | 1.1 1.8            | transport, metal | metal tolerance protein C3 |
| cBR_110| 1                   | 1                | 2                 | 1.1 1.8            | protein degradation, ubiquitin, E3, SCF, FBOX | protein C3 |
| cBR_111| 1                   | 1                | 2                 | 1.1 1.8            | protein synthesis, misc ribosomal protein | 60S ribosomal protein L44 |
| cBR_112| 1                   | 1                | 2                 | 1.1 1.8            | protein degradation, ubiquitin, E3, SCF, FBOX | F-box domain containing protein, expressed |
| cBR_113| 1                   | 1                | 2                 | 1.1 1.8            | not assigned.unknown | EMB1374 |
| cBR_114| 1                   | 1                | 2                 | 1.1 1.8            | N-metabolism, nitrate metabolism, NR | cytochrome b5 |
| cBR ID | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | MapMan Functional Groups | Gene Annotation |
|--------|-------------------|------------------|-------------------|--------------------|------------------------|-----------------|
| cBR_115 | 1                 | 1                | 2                 | 6                 | −1.9                  | 2.0             | not assigned.unknown.expressed protein |
| cBR_116 | 1                 | 1                | 2                 | 4                 | 1.1                   | 2.2             | amino acid metabolism.synthesis.aromatic aa.tryptophan.tryptophan synthase |
| cBR_117 | 1                 | 1                | 2                 | 1                 | 3.3                   | 9.1             | misc.gluco-, galacto- and mannosidases |
| cBR_118 | 1                 | 1                | 4                 | −1.1              | 3.4                   | 1.2             | protein synthesis.misc ribosomal protein |
| cBR_119 | 1                 | 1                | 4                 | 1.0               | 6.6                   | secondary metabolism.isoprenoids.mevalonate pathway.HMG-CoA synthase |
| cBR_120 | 1                 | 1                | 8                 | −1.8              | −5.5                  | Biodegradation of Xenobiotics.lactoylglutathione lyase |
| cBR_121 | 1                 | 1                | 2                 | 2.0               | 2.0                   | Misc. short chain dehydrogenase/reductase (SDR) |
| cBR_122 | 1                 | 1                | 2                 | 4                 | 1.0                   | 9.1             | not assigned.no ontology.expressed protein |
| cBR_123 | 1                 | 1                | 2                 | 8                 | −1.8                  | −7.0            | Biodegradation of Xenobiotics.lactoylglutathione lyase |
| cBR_124 | 1                 | 1                | 2                 | 2                 | −2.0                  | −1.4            | cell cycle.peptidyl.prolyl isomerase,peptidyl-prolyl isomerase, |
| cBR_125 | 1                 | 1                | 2                 | 5                 | −1.3                  | −1.6            | not assigned.unknown.NO MATCH |
| cBR_126 | 1                 | 1                | 2                 | 3                 | 2.6                   | 2.5             | minor CHO metabolism.trehalose.TPP |
| cBR_127 | 1                 | 1                | 2                 | 5                 | 1.0                   | −2.4            | metal handling,selenium-binding protein, |
| cBR_128 | 1                 | 1                | 2                 | 1                 | 1.8                   | 2.2             | protein degradation.ubiquitin.proteasom |
| cBR_129 | 1                 | 1                | 2                 | 1                 | 3.25                  | 3.5             | cell wall modification.beta-expansin 1a precursor, |
| cBR_130 | 1                 | 1                | 2                 | 6                 | −1.4                  | 41.0            | stress abiots.unspecified.oxidate oxidase 2 precursor, |
| cBR_131 | 1                 | 1                | 2                 | 1                 | 4.3                   | 3.2             | not assigned.unknown.expressed protein |
| cBR_132 | 1                 | 1                | 2                 | 3                 | 2.1                   | −2.0            | misc.cytochrome P450 |
| cBR_133 | 1                 | 1                | 2                 | 6                 | −1.9                  | 1.5             | not assigned.unknown.NO MATCH |
| cBR_134 | 1                 | 1                | 2                 | 1                 | 1.4                   | 7.2             | C1-metabolism |
| cBR_135 | 1                 | 1                | 2                 | 1                 | 1.9                   | 3.7             | not assigned.no ontology.seed maturation protein, |
| cBR_136 | 1                 | 1                | 2                 | 5                 | −1.2                  | −2.1            | stress abiots.cold.Usp family protein, |
| cBR_137 | 1                 | 1                | 2                 | 8                 | −1.4                  | −3.4            | development.unspecified.caleosin 2, |
| cBR_138 | 1                 | 1                | 2                 | 4                 | 1.1                   | 2.2             | protein degradation.ubiquitin.proteasom |
| cBR_139 | 1                 | 1                | 2                 | 1                 | 1.4                   | 2.1             | not assigned.no ontology.translocon-associated protein beta containing protein, |
| cBR_140 | 1                 | 1                | 2                 | 5                 | −1.2                  | −2.2            | not assigned.unknown.holoacarboxylase synthetase, |
| cBR_141 | 1                 | 1                | 2                 | 2                 | 2.1                   | 1.3             | signalling.G-proteins |
| cBR_142 | 1                 | 1                | 2                 | 6                 | −1.7                  | 4.0             | not assigned.no ontology.wound/stress protein, |
| cBR ID  | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | MapMan Functional Groups | Gene Annotation |
|---------|---------------------|-------------------|-------------------|---------------------|------------------------|-----------------|
| cBR_143 | 1                   | 1                 | 2                 | 8                   | −1.5                   | not assigned,no ontology | nifU-like N-terminal domain containing protein, mitochondrial precursor, |
| cBR_144 | 1                   | 1                 | 2                 | 8                   | −2.2                   | minor CHO metabolism,others | aldose reductase, |
| cBR_145 | 1                   | 1                 | 2                 | 4                   | 1.1                    | major CHO metabolism,degradation,starch, starch cleavage | alpha-amylase precursor, |
| cBR_146 | 1                   | 1                 | 2                 | 1                   | 1.5                    | secondary metabolism,phenylpropanoids,lignin biosynthesis,COAOMT | caffeoyl-CoA O-methyltransferase 1, |
| cBR_147 | 1                   | 1                 | 2                 | 1                   | 2.6                    | cell organisation | tubulin alpha-1 chain, |
| cBR_148 | 1                   | 1                 | 2                 | 3                   | 4.1                    | glycolysis,pyrophosphate-fructose-6-P phosphotransferase | pyrophosphate-fructose 6-phosphate 1-phosphotransferase alpha subunit, |
| cBR_149 | 1                   | 1                 | 2                 | 2                   | 2.8                    | transport metabolite transporters at the envelope membrane | plasticid phosphate translocator-like protein1, |
| cBR_150 | 1                   | 1                 | 2                 | 8                   | −1.5                   | RNA,regulation of transcription,unclassified | DNL zinc finger family protein, expressed |
| cBR_151 | 1                   | 1                 | 2                 | 1                   | 2.8                    | misc,misc2 | glycosyltransferase 48 kDa subunit precursor, |
| cBR_152 | 1                   | 1                 | 2                 | 4                   | −1.1                   | misc,acid and other phosphatases | tartrate-resistant acid phosphatase type 5 precursor, |
| cBR_153 | 1                   | 1                 | 2                 | 4                   | 1.2                    | protein,aa activation,bifunctional aminoacyl-tRNA synthetase | bifunctional aminoacyl-tRNA synthetase, |
| cBR_154 | 1                   | 1                 | 2                 | 4                   | −1.0                   | protein,posttranslational modification | serine/threonine protein phosphatase 2A |
| cBR_155 | 1                   | 1                 | 2                 | 5                   | −1.3                   | not assigned,no ontology | BCL-2 binding anthanogene-1, |
| cBR_156 | 1                   | 1                 | 2                 | 1                   | 1.8                    | TCA/org. transformation,TCA, pyruvate DH,E1 | pyruvate dehydrogenase E1 component alpha subunit, |
| cBR_157 | 1                   | 1                 | 2                 | 8                   | −1.8                   | not assigned,no ontology | hypersensitive-induced response protein, |
| cBR_158 | 1                   | 1                 | 2                 | 4                   | 1.1                    | cell wall,precursor synthesis,phosphomannomutase | phosphomannomutase, |
| cBR_159 | 1                   | 1                 | 2                 | 4                   | −1.2                   | signalling,G-proteins | ras-related protein Rab11A, |
| cBR_160 | 1                   | 1                 | 2                 | 1                   | 1.6                    | signalling,G-proteins | ras-related protein ARA-3, |
| cBR_161 | 1                   | 1                 | 2                 | 1                   | 4.9                    | cell wall,degradation,cellulases and beta-1,4-glucanases | periplasmic beta-glucosidase precursor, |
| cBR_162 | 1                   | 1                 | 2                 | 4                   | −1.0                   | not assigned,unknown | NO_MATCH |
| cBR_163 | 1                   | 1                 | 2                 | 1                   | 2.3                    | metal handling,binding, chelation and storage | nicotianamine synthase 3, |
| cBR_164 | 1                   | 1                 | 2                 | 1                   | 1.8                    | DNA,synthesis,chromatin structure | DNA replication licensing factor mcm4, |
| cBR_165 | 1                   | 1                 | 2                 | 4                   | 1.1                    | lipid metabolism,exotics (steroids, squalene etc) | minor allergen Alt a 7, |
| cBR_166 | 1                   | 1                 | 2                 | 7                   | −1.6                   | not assigned,no ontology | INS1 C-terminus family protein, expressed |
| cBR_167 | 1                   | 1                 | 2                 | 1                   | 1.9                    | protein,degradation,cysteine protease | vgnain precursor, |
| cBR_168 | 1                   | 1                 | 2                 | 4                   | 1.2                    | secondary metabolism,phenylpropanoids,lignin biosynthesis,COAOMT | quercetin 3-O-methyltransferase 1, |
| cBR_169 | 1                   | 1                 | 2                 | 1                   | 2.9                    | development,unspecified | pollen-specific protein SF3, |
| cBR_170 | 1                   | 1                 | 2                 | 1                   | 2.7                    | not assigned,unknown | expressed protein |
| cBR_171 | 1                   | 1                 | 2                 | 8                   | −1.8                   | Biodegradation of Xenobiotics,jactoyleglutathione lyase | glyoxalase family protein superfamily, |
| cBR ID   | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | MapMan Functional Groups                                                                 | Gene Annotation                                                                 |
|----------|---------------------|-------------------|--------------------|---------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| cBR_172  | 1                   | 1                 | 2                  | 2                   | Early Phase: 4.2, Late Phase: 1.3; MapMan Functional Groups: lipid metabolism, FA synthesis and FA elongation, pyruvate DH | poyruvate dehydrogenase E1 component subunit beta, epoxide hydrolase 2, |
| cBR_173  | 1                   | 1                 | 2                  | 5                   | Early Phase: −1.1, Late Phase: −1.8; MapMan Functional Groups: misc. | miscellaneous, epoxide hydrolase 2, |
| cBR_174  | 1                   | 1                 | 2                  | 6                   | Early Phase: −1.6, Late Phase: 2.8; MapMan Functional Groups: amino acid metabolism, synthesis, branched chain group, leucine specific, 3-isopropylmalate dehydrogenase | 3-isopropylmalate dehydrogenase 2, chloroplast precursor, |
| cBR_175  | 1                   | 1                 | 2                  | 3                   | Early Phase: 7.8, Late Phase: −1.7; MapMan Functional Groups: protein, posttranslational modification, kinase, receptor-like, cytoplasmatic kinase VII | protein kinase, |
| cBR_176  | 1                   | 1                 | 2                  | 3                   | Early Phase: 1.7, Late Phase: −3.0; MapMan Functional Groups: secondary metabolism, isoprenoids, tocopherol biosynthesis, hydroxyphenylpyruvate dioxygenase | 4-hydroxyphenylpyruvate dioxygenase |
| cBR_177  | 1                   | 1                 | 2                  | 6                   | Early Phase: −1.5, Late Phase: 1.7; MapMan Functional Groups: not assigned, unknown | expressed protein, |
| cBR_178  | 1                   | 1                 | 2                  | 8                   | Early Phase: −2.1, Late Phase: −2.4; MapMan Functional Groups: transport, NDP-sugars at the ER | solute carrier family 35 member B3, |
| cBR_179  | 1                   | 1                 | 2                  | 6                   | Early Phase: −1.4, Late Phase: 1.8; MapMan Functional Groups: not assigned, unknown | NO_MATCH, |
| cBR_180  | 1                   | 1                 | 2                  | 8                   | Early Phase: −2.6, Late Phase: −1.9; MapMan Functional Groups: signalling, calcium | calmodulin-related protein 2, touch-induced, |
| cBR_181  | 1                   | 1                 | 2                  | 2                   | Early Phase: 2.0, Late Phase: 2.5; MapMan Functional Groups: not assigned, no ontology, C2 domain-containing protein | elicitor-responsive protein 3, |
| cBR_182  | 1                   | 1                 | 2                  | 4                   | Early Phase: −1.1, Late Phase: 5.2; MapMan Functional Groups: lipid metabolism, glycerol metabolism, glycerol kinase | glycerol kinase, |
| cBR_183  | 1                   | 1                 | 2                  | 1                   | Early Phase: 2.8, Late Phase: 13.3; MapMan Functional Groups: protein degradation, serine protease | serine carboxypeptidase 3 precursor, |
| cBR_184  | 1                   | 1                 | 2                  | 4                   | Early Phase: −1.2, Late Phase: 2.6; MapMan Functional Groups: not assigned, unknown | expressed protein, |
| cBR_185  | 1                   | 1                 | 2                  | 3                   | Early Phase: 1.7, Late Phase: −5.9; MapMan Functional Groups: not assigned, no ontology | WD-repeat protein-like, |
| cBR_186  | 1                   | 1                 | 2                  | 8                   | Early Phase: −3.1, Late Phase: −2.0; MapMan Functional Groups: not assigned, no ontology, C2 domain-containing protein | calcium lipid binding protein-like, |
| cBR_187  | 1                   | 1                 | 2                  | 3                   | Early Phase: 6.7, Late Phase: −3.8; MapMan Functional Groups: protein degradation, AAA type | cell Division Protein AAA ATPase family, |
| cBR_188  | 1                   | 1                 | 2                  | 3                   | Early Phase: 17.3, Late Phase: −11.1; MapMan Functional Groups: not assigned, unknown | nematode resistance protein, |
| cBR_189  | 1                   | 1                 | 2                  | 2                   | Early Phase: −1.3, Late Phase: −2.0; MapMan Functional Groups: not assigned, unknown | seed maturation protein PM23, |
| cBR_190  | 1                   | 1                 | 2                  | 4                   | Early Phase: 1.2, Late Phase: 4.3; MapMan Functional Groups: transporter, sugars | major myo-inositol transporter iota, |
| cBR_191  | 1                   | 1                 | 2                  | 5                   | Early Phase: −1.0, Late Phase: −2.8; MapMan Functional Groups: RNA regulation of transcription, AP2/EREBP, APETAL2/Ethylene-responsive element binding protein family | AP2/EREBP, APETAL2/Ethylene-responsive element binding protein family, |
| cBR_192  | 1                   | 1                 | 2                  | 4                   | Early Phase: 1.0, Late Phase: 5.0; MapMan Functional Groups: amino acid metabolism, synthesis, serine-glycine-cysteine group, serine-phosphoserine phosphatase | phosphoserine phosphatase, chloroplast precursor, |
| cBR_193  | 1                   | 1                 | 2                  | 2                   | Early Phase: 3.1, Late Phase: 13.3; MapMan Functional Groups: signalling, calcium | calmodulin, |
| cBR_194  | 1                   | 1                 | 2                  | 1                   | Early Phase: 1.4, Late Phase: 6.6; MapMan Functional Groups: N-metabolism, N-degradation, glutamate dehydrogenase | glutamate dehydrogenase, |
| cBR_195  | 1                   | 1                 | 2                  | 1                   | Early Phase: 2.9, Late Phase: 4.3; MapMan Functional Groups: not assigned, unknown | GPI-anchored protein At5g19240 precursor, |
| cBR_196  | 1                   | 1                 | 2                  | 6                   | Early Phase: −1.4, Late Phase: 2.3; MapMan Functional Groups: not assigned, unknown | brain protein 44-like protein, |
| cBR_197  | 1                   | 1                 | 2                  | 4                   | Early Phase: 1.0, Late Phase: 3.9; MapMan Functional Groups: not assigned, unknown | expressed protein, |
| cBR_198  | 1                   | 1                 | 2                  | 5                   | Early Phase: −1.1, Late Phase: −1.9; MapMan Functional Groups: cell vesicle transport | syntaptin 23, |
| cBR ID  | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | Gene Annotation |
|---------|---------------------|-------------------|-------------------|---------------------|----------------|
| cBR_199 | 1                   | 1                 | 2                 | 1                   | 6.4 8.3 cell wall degradation, mannan-xylose-arabinofucose, beta-D-xylosidase, NO MATCH |
| cBR_200 | 1                   | 1                 | 2                 | 1                   | 1.6 1.8 not assigned, unknown |
| cBR_201 | 1                   | 1                 | 2                 | 1                   | 8.6 3.8 Not assigned, mannose-6-phosphate isomerase |
| cBR_202 | 1                   | 2                 | 5                 | 1                   | 1.3 1.3 Proline synthesis, ribosomal protein |
| cBR_203 | 1                   | 1                 | 2                 | 1                   | 1.2 2.9 cell wall modification, protein posttranslational modification |
| cBR_204 | 1                   | 2                 | 1                 | 1                   | 1.6 2.4 cell wall modification, cell wall modification |
| cBR_205 | 1                   | 1                 | 2                 | 1                   | 6.4 2.4 Not assigned, unknown |
| cBR_206 | 1                   | 1                 | 2                 | 1                   | 6.4 2.4 Not assigned, unknown |
| cBR_207 | 1                   | 1                 | 2                 | 1                   | 6.4 2.4 Not assigned, unknown |
| cBR_208 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_209 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_210 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_211 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_212 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_213 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_214 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_215 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_216 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_217 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_218 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_219 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_220 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_221 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_222 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_223 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_224 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_225 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_226 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_227 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_228 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_229 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_230 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_231 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_232 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_233 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_234 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_235 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_236 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_237 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR_238 | 1                   | 1                 | 2                 | 1                   | 1.6 2.4 Not assigned, unknown |
| cBR ID | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | MapMan Functional Groups | Gene Annotation |
|--------|---------------------|-------------------|-------------------|---------------------|-------------------------|-----------------|
| cBR_229 | 1 | 1 | 2 | 4 | −1.2 | 4.1 | not assigned, unknown | NO_MATCH |
| cBR_230 | 1 | 1 | 2 | 8 | −1.4 | −2.0 | amino acid metabolism, synthesis, serine-glycine-cysteine group, cysteine SAT | serine acetyltransferase 2, |
| cBR_231 | 1 | 1 | 2 | 8 | −1.6 | −1.4 | not assigned, no ontology | hydrolase, NUDIX family protein, expressed |
| cBR_232 | 1 | 1 | 2 | 5 | −1.0 | −1.8 | not assigned, unknown | fos intronic gene CG761S-PA, |
| cBR_233 | 1 | 1 | 2 | 8 | −1.4 | −2.1 | cell cycle | cyclin-T1, |
| cBR_234 | 1 | 1 | 2 | 1 | 1.5 | 3.6 | N-metabolism, ammonia metabolism, unspecified | haloacid dehalogenase-like hydrolase domain-containing protein 1A, |
| cBR_235 | 1 | 1 | 2 | 8 | −1.7 | −2.8 | not assigned, unknown | NO_MATCH |
| cBR_236 | 1 | 1 | 2 | 6 | −1.4 | 2.7 | nucleotide metabolism, synthesis, purine, GMP synthetase | GMP synthase, |
| cBR_237 | 1 | 1 | 2 | 4 | 1.3 | 3.0 | not assigned, unknown | NO_MATCH |
| cBR_238 | 1 | 1 | 2 | 2 | 2.6 | −1.0 | protein posttranslational modification | CDNP-E like kinetochore protein, |
| cBR_239 | 1 | 1 | 2 | 2 | −1.3 | 3.7 | protein targeting, mitochondria | mitochondrial import inner membrane translocase subunit tim22, |
| cBR_240 | 1 | 1 | 2 | 1 | 1.5 | 5.3 | signalling, G-proteins | ADP-ribosylation factor-like protein 88, |
| cBR_241 | 1 | 1 | 2 | 1 | 3.4 | 2.1 | signalling, calcium | calcium-transporting ATPase 4, plasma membrane-type, |
| cBR_242 | 1 | 1 | 2 | 2 | 20.3 | 1.2 | N-metabolism, nitrate metabolism, NR | desaturase/cytochrome b5 protein, |
| cBR_243 | 1 | 1 | 2 | 3 | 3.2 | −2.1 | not assigned, unknown | expressed protein |
| cBR_244 | 1 | 1 | 2 | 5 | −1.3 | −1.9 | misc. glutathione S transferases | glutathione S-transferase GSTU6, |
| cBR_245 | 1 | 1 | 2 | 8 | −2.0 | −1.7 | protein degradation, ubiquitin E3 SCF FBOX | F-box domain containing protein, expressed |
| cBR_246 | 1 | 1 | 2 | 2 | −2.4 | −1.8 | signalling, G-proteins | ras-related protein Rab-18, |
| cBR_247 | 1 | 1 | 2 | 3 | 4.6 | −2.4 | cell wall pectin esterases, PME | pectinesterase-1 precursor, |
| cBR_248 | 1 | 1 | 2 | 7 | −4.5 | −1.0 | transport, metal | zinc transporter 10 precursor |
| cBR_249 | 1 | 1 | 2 | 8 | −2.1 | −2.7 | not assigned, unknown | expressed protein |
| cBR_250 | 1 | 1 | 2 | 1 | 2.8 | 1.5 | protein synthesis, ribosomal protein, prokaryotic | succinate dehydrogenase iron-sulfur protein, mitochondrial precursor, |
| cBR_251 | 1 | 1 | 2 | 4 | 1.1 | 4.7 | DNA synthesis, chromatin structure, histone | histone H2A, |
| cBR_252 | 1 | 1 | 2 | 4 | −1.0 | 6.1 | lipid metabolism, steroids, squalene etc | flavonol 4-sulfotransferase, |
| cBR_253 | 1 | 1 | 2 | 7 | −1.9 | −1.3 | protein degradation, serine protease | serine carboxypeptidase 1 precursor, |
| cBR_254 | 1 | 1 | 2 | 8 | −1.5 | −2.4 | cell vesicle transport | syntaxin 132, |
| cBR_255 | 1 | 1 | 2 | 1 | 3.1 | 8.0 | misc. oxidases - copper, flavone etc | L-ascorbate oxidase homolog precursor, |
| cBR_256 | 1 | 1 | 2 | 1 | 2.3 | 4.7 | transport, unspecified anions | UDP-glucose 6-dehydrogenase, |
| cBR ID   | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | Gene Annotation                                                                 |
|----------|---------------------|-------------------|--------------------|---------------------|--------------------------------------------------------------------------------|
| cBR_257  | 1                   | 1                 | 2                  | 1                   | transport.mp sequentially regulated protein, ATP-dependent isomerase, PPA-family protein, expressed at the mitochondrial membrane |
| cBR_258  | 1                   | 1                 | 2                  | 8                   | not assigned no ontology, ADP-ribose synthesis, ubiquitin-activating enzyme 1, L-AIAK |
| cBR_259  | 1                   | 1                 | 2                  | 5                   | not assigned no ontology, cell wall secretion synthesis, cell wall secretion synthesis, ribosomal protein |
| cBR_260  | 1                   | 1                 | 2                  | 8                   | not assigned no ontology, cell wall secretion synthesis, cell wall secretion synthesis, ribosomal protein |
| cBR_261  | 1                   | 1                 | 2                  | 6                   | not assigned no ontology, cell wall secretion synthesis, cell wall secretion synthesis, ribosomal protein |
| cBR_262  | 1                   | 1                 | 2                  | 8                   | not assigned no ontology, cell wall secretion synthesis, cell wall secretion synthesis, ribosomal protein |
| cBR_M1   | 1                   | 2                 | 3                  | 1                   | cell wall secretion synthesis, prokaryotic ribosomal protein, expressed |
| cBR_M2   | 17                  | 3                 | 20                 | 4                   | protein degradation, ubiquitin E3 RING U-box domain containing protein, expressed |
| cBR_M3   | 1                   | 2                 | 3                  | 6                   | protein degradation, ribosomal protein |
| cBR_M4   | 2                   | 1                 | 3                  | 4                   | GDSL-motif lipase esterase precursor |
| cBR_M5   | 1                   | 2                 | 3                  | 2                   | PS-light reaction, other electron carrier (ox/red) ferredoxin-1, chloroplast precursor |
| cBR_M6   | 1                   | 2                 | 3                  | 8                   | stress, biotic, tobamovirus multiplication 3 |
| cBR_M7   | 1                   | 2                 | 3                  | 3                   | protein degradation, AAA type ATPase 2 |
| cBR_M8   | 2                   | 1                 | 3                  | 5                   | cell organization myosin XI |
| cBR_M9   | 1                   | 2                 | 3                  | 4                   | signalling, G-proteins ras-related protein ARA-3 |
| cBR_M10  | 1                   | 3                 | 4                  | 1                   | cell organization tubulin beta-5 chain |
| cBR_M11  | 2                   | 2                 | 4                  | 4                   | not assigned unknown |
| cBR_M12  | 3                   | 2                 | 5                  | 8                   | ABA-induced plasma membrane protein PM 19 |
| cBR_M13  | 1                   | 2                 | 3                  | 4                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M14  | 1                   | 2                 | 3                  | 8                   | minor CHO metabolism, mannose synthase, galactinol synthase positive |
| cBR_M15  | 1                   | 6                 | 7                  | 1                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M16  | 3                   | 4                 | 8                  | 1                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M17  | 3                   | 5                 | 8                  | 1                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M18  | 3                   | 4                 | 7                  | 1                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M19  | 3                   | 4                 | 7                  | 1                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M20  | 3                   | 4                 | 7                  | 1                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M21  | 3                   | 4                 | 7                  | 1                   | signalling, receptor kinase, receptor-like protein kinase, galactinol synthase positive |
| cBR_M22  | 8                   | 2                 | 10                 | 6                   | DNA synthesis, chromatin structure histone H4 |
| cBR_M23  | 8                   | 2                 | 10                 | 6                   | DNA synthesis, chromatin structure histone H4 |
| cBR_M24  | 2                   | 1                 | 3                  | 4                   | DNA synthesis, chromatin structure histone H4 |
| cBR_M25  | 3                   | 6                 | 9                  | 1                   | DNA synthesis, chromatin structure histone H4 |
| cBR_M26  | 3                   | 6                 | 9                  | 1                   | DNA synthesis, chromatin structure histone H4 |
| cBR ID | No. of barley genes | No. of rice genes | No. of total genes | Expression Patterns | MapMan Functional Groups | Gene Annotation |
|--------|---------------------|-------------------|-------------------|---------------------|-------------------------|-----------------|
| cBR_M27 | 1 2 3 5             |                   |                   | −1.3                | −3.4                    | not assigned.no ontology |
| cBR_M28 | 1 2 3 5             |                   |                   | −1.1                | −2.3                    | protein degradation.ubiquitin.E3.RING |
| cBR_M29 | 1 2 3 6             |                   |                   | −2.0                | 1.4                     | not assigned.no ontology |
| cBR_M30 | 2 3 5 8             |                   |                   | −1.7                | −5.0                    | misc.short chain dehydrogenase/reductase (SDR) |
| cBR_M31 | 2 1 3 1             |                   |                   | 1.4                 | 2.1                     | stress.abiotic |
| cBR_M32 | 1 2 3 8             |                   |                   | −2.8                | −4.6                    | not assigned.unknown |
| cBR_M33 | 1 5 6 3             |                   |                   | 1.6                 | −1.9                    | protein posttranslational modification.kinase.receptor like cytoplasmatic kinase VII |
| cBR_M34 | 1 2 3 8             |                   |                   | 2.5                 | 2.2                     | transporter.membrane system unknown |
| cBR_M35 | 1 3 4 1             |                   |                   | 2.6                 | 1.7                     | signalling.14-3-3 proteins |
| cBR_M36 | 1 2 3 8             |                   |                   | 1.5                 | 53.3                    | DNA.synthesis/chromatin structure.histone |
| cBR_M37 | 1 2 3 1             |                   |                   | 1.5                 | 3.9                     | cell wall.modification |
| cBR_M38 | 1 3 4 4             |                   |                   | 1.3                 | 3.3                     | signalling.G-proteins |
| cBR_M39 | 1 2 3 2             |                   |                   | 1.5                 | 2.9                     | protein degradation.ubiquitin.E2 |
| cBR_M40 | 2 1 3 3             |                   |                   | 0.0                 | −5.2                    | cell wall.modification |
| cBR_M41 | 1 2 3 8             |                   |                   | 2.5                 | 1.7                     | cell wall.cellulose synthesis |
| cBR_M42 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | ubiquitin-conjugating enzyme E2 |
| cBR_M43 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.modification |
| cBR_M44 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M45 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M46 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M47 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M48 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M49 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M50 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M51 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M52 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M53 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M54 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M55 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M56 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M57 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
| cBR_M58 | 1 2 3 8             |                   |                   | 1.0                 | 1.2                     | cell wall.cellulose synthesis |
Interestingly, Group 3 had 28 cBRs that were transiently up-regulated in the early germination phase. Expression levels of most cBRs in Group 3 at the end of germination were down-regulated to levels at the dry seed stage. Preserving transient up-regulation in early germination followed by down-regulation in late germination in both barley and rice indicated that those genes likely participated in biological processes specific to early germination. Many cBRs in Group 3 encoded proteins involved in cell wall modification, protein degradation, protein modification, and signaling transduction. Cell wall modification is required to weaken cell walls during early germination to permit radicle protrusion and to provide access to stored metabolites in the endosperm [23]. Also in Group 3 were proteins such as F-box proteins, receptor-like kinases, G-proteins and calcium-dependent protein kinases, which play important roles in a variety of signaling transduction pathways. Those signaling components likely played roles in transducing a variety of signals in the early germination phase to initiate the biological pathways required in seed germination. Sixty-two cBRs in Group 8 were down-regulated in both early and late stages. They encoded proteins with a wide range of biological functions. Those cBRs highly accumulated in dry mature grains and their accumulation gradually decreased over the course of seed germination. This raises the possibility that these cBRs encoded proteins involved in seed development and maturation. The highly accumulated transcripts were degraded over the course of seed germination.

The cBRs Encoded Proteins in Diverse Biological Pathways

The genes represented on the rice and barley GeneChips are classified into 35 functional groups based on their functions in metabolic pathways, signaling pathways and gene families in MapMan and PageMan [24,25]. The cBRs encoded proteins in most of the functional groups (Figure 2 and Table 3). For examples, 13 cBRs encoded proteins in cell wall metabolic pathways while 22 cBRs were functionally related to signaling pathways. Eighty-nine cBRs encoded proteins that are not classified into any of the functional groups. cBRs in the same functional group often had diverse expression patterns. For example, cBRs in stress-related pathways had both up-regulated and down-regulated expression patterns in early phase of germination. Conversely, cBRs in several functional groups had similar expression patterns. For example, all three cBRs in the biodegradation of xenobiotics pathway were down-regulated in both early and late phases of germination while all eight cBRs

Table 4. Summary of cBR Expression Patterns.

| Group | early phase | late phase | No. of cBRs |
|-------|-------------|------------|-------------|
| 1     | Up          | Up         | 71          |
| 2     | Up          | No         | 18          |
| 3     | Up          | Down       | 28          |
| 4     | No          | Up         | 69          |
| 5     | No          | Down       | 36          |
| 6     | Down        | Up         | 17          |
| 7     | Down        | No         | 13          |
| 8     | Down        | Down       | 62          |

Note: The cut-off value for the Up, Down and No change of cBR expression in early and late germination phase is 1.4-fold change.

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except cBR_M23 in DNA related pathways were up-regulated in both early and late phase of germination (Figure 2 and Table 3). Interestingly, a large number of transcription factor genes are differentially regulated over the course of barley germination [9]. However, a limited number of cBRs encoded transcription factors. Only a PHD finger protein (cBR_207) and an AP2/EREBP protein (cBR_191) were down-regulated during seed germination (Table 3). Therefore, germination regulated transcription factor genes evolved quickly in either their protein sequences or/and their expression patterns.

### Biological Pathways Regulated by Conserved Transcriptional Regulatory Programs

Representation analysis of cBRs in each functional group showed that the cBRs in a number of biological pathways were preferentially regulated in conserved expression programs (Figure 3A). Early germination up-regulated cBRs were over-represented in cell wall metabolic pathways and peroxidase gene family (Figure 3A, 3B and 3C). A total of 13 cBRs such as arabinogalactan protein (AGP), cellulose synthase, beta-glucanase, beta-D-xilosidase, expansins and xyloglucan endotransglucosylase were identified in the cell wall metabolic pathway. All of the 13 cBRs were up-regulated during early germination, except that cBR_228 encoding beta-D-xylosidase was slightly down-regulated (Figure 3B). In addition, five cBRs encoded peroxidases; and four of them were up-regulated in the early germination phase (Figure 3C). Most of the peroxidase genes were also preferentially up-regulated in the late germination phase. It was reported previously that peroxidase activity increases significantly in the micropylar end of germinating tomato seeds [26]. The conserved up-regulation of peroxidase genes in barley and rice provides additional evidence supporting the functional importance of peroxidase in seed germination.

The cBRs encoding chromatin remodeling and structural proteins were preferentially up-regulated during the late germination phase. There were 8 cBRs in chromatin structure

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**Table 3. List of cBRs assigned to each functional group**

| BINCODE | Biological Pathways                           | No. of cBRs |
|---------|----------------------------------------------|-------------|
| 1       | PS                                           | 2           |
| 2       | major CHO metabolism                         | 2           |
| 3       | minor CHO metabolism                         | 3           |
| 4       | glycolysis                                   | 1           |
| 5       | fermentation                                 | 0           |
| 6       | gluconeogenesis/ glyoxylate cycle            | 0           |
| 7       | OPP                                          | 0           |
| 8       | TCA / org                                    | 1           |
| 9       | mitochondrial electron transport / ATP synthesis | 0           |
| 10      | cell wall                                    | 13          |
| 11      | lipid metabolism                             | 10          |
| 12      | N-metabolism                                 | 4           |
| 13      | amino acid metabolism                        | 5           |
| 14      | S-assimilation                               | 0           |
| 15      | metal handling                               | 0           |
| 16      | secondary metabolism                         | 0           |
| 17      | hormone metabolism                           | 1           |
| 18      | Co-factor and vitamin metabolism             | 1           |
| 19      | tetrapyrrole synthesis                       | 0           |
| 20      | stress                                       | 12          |
| 21      | redox                                        | 0           |
| 22      | polyamine metabolism                         | 0           |
| 23      | nucleotide metabolism                        | 0           |
| 24      | Biodegradation of Xenobiotics                | 0           |
| 25      | C1-metabolism                                | 0           |
| 26      | misc                                         | 40          |
| 27      | RNA                                          | 13          |
| 28      | DNA                                          | 8           |
| 29      | protein                                      | 51          |
| 30      | signalling                                   | 22          |
| 31      | cell                                         | 13          |
| 32      | microRNA/Natural Antisense                   | 0           |
| 33      | development                                  | 0           |
| 34      | transport                                    | 11          |
| 35      | not assigned                                 | 89          |

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**Figure 2. Distribution of cBRs and Their Expression Patterns in Biological Pathways.** All cBRs were assigned to 35 functional categories defined by MapMan tools. The log2 of average fold changes from dry seed over the course of germination for each cBR were graphed next to its functional categories. The number of cBRs assigned to each functional group was listed in the table.

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All of them were dramatically up-regulated during the late germination phase by more than 4.7 fold with an average of 30 fold. However, expression levels of those cBRs had no or little change during the early germination phase (Figure 3D). Thus, the specific and strong up-regulation of chromatin-related genes in the late germination phase was conserved in rice and barley. Five of the eight cBRs encoded histone proteins. For example, the cBR_M23 was composed of 8 barley and 2 rice histone H4 genes. Two of the eight cBRs encoding replication licensing factor MCM proteins were specifically up-regulated in late germination phase. MCM encodes a conserved minichromosome maintenance protein and plays an essential function as a helicase in DNA replication elongation in eukaryotes. MCM proteins also participate in other chromosome processes including transcription, chromatin remodeling, and genome stability [27].

Biological Pathways and Gene Families Containing cRBs with Diverse Expression Patterns

Interestingly, the cBRs in a number of signaling pathways and gene families had diverse expression patterns. The cBRs encoding 14-3-3 proteins, G-proteins, receptor kinases, calmodulin and calcium-dependent protein kinase in signaling pathways were identified. The expression patterns of those cBRs were highly diverse (Table 3 and Figure 4A). A total of 12 cBRs encoded G-proteins, but their expression patterns were highly diverse over the course of germination. For example, the cBR_M17 was up-regulated by 13-fold in the early germination phase. In contrast, another ras-related G protein cBR (cBR_246) was down-regulated by 2.4 fold in the early germination phase. Two cBRs (cBR-M37 and cBR_71) encoded 14-3-3 proteins. The cBR_71 was down-regulated while cBR-M37 was up-regulated over the course of seed germination. Fourteen cBRs encoded proteins in ubiquitin/26S proteasome-mediated protein degradation pathways, which often play important roles in a variety of signaling transduction pathways (Figure 4B). Most of the cBRs encoded E2 and E3 regulatory proteins such as E2, HECT, RING and F-BOX proteins, and had diverse expression patterns. For example, four cBRs encoding F-box proteins were differentially regulated by seed germination, and showed diverse expression patterns.

Both alpha- and beta-amylases are key enzymes required in seed storage starch mobilization during seed germination and seedling growth [1,23]. Interestingly, the cBRs encoding alpha- and beta-amylases had opposite transcriptional patterns. The alpha-amylase cBR was up-regulated in late germination stages while the beta-amylase cBR was down-regulated in late germination (Figure 4C). In addition, two cBRs encoding cysteine proteases and two cBRs encoding serine proteases were identified. Both cysteine and serine proteases were suggested to play a role in protein mobilization during seed germination and shown diverse expression patterns.

Discussions

Barley and rice diverged from their common ancestor 50 million years ago [12]. However, they share a great similarity
morphologically and physiologically in germination and seedling growth. In this study, we measured the transciptomes of germinating rice grains at dry, mid- and end points of seed germinations, which should represent the most distinct stages of the dynamic transcriptional changes over seed germination process. Having determined transcriptomes of rice at the three equivalent stages [9], we designed a systems and evolutionary strategy to compare the dynamic transcriptomic changes over the course of seed germination to gain an insight into divergence and conservation of gene regulatory programs underlying rice and barley germination.

One-Way ANOVA analysis of the transcriptomes revealed that 2537 barley and 13813 rice genes were differentially regulated over the course of seed germination. Comparing their encoding protein sequences and expression patterns identified 322 sets of conserved barley and rice genes (cBRs) sharing strong similarity in both protein sequences and gene expression patterns. The collection of cBRs contained 368 barley genes and 388 rice genes. Thus, only a very small percentage of the germination-regulated genes preserved their protein sequences and gene expression patterns; and a significant divergence occurred in transcriptional regulatory programs underlying rice and barley germination since the barley-rice divergence. As expected, protein sequence similarity of germination regulated barley and rice genes positively correlated to the similarity of their expression patterns, suggesting co-evolution of protein functions and gene expression patterns.

Biological functions of genes are mainly determined by their protein sequences and their expression patterns. Both protein sequences and expression patterns change quickly if the genes have no functional significance [17,29,30,31]. Therefore, we hypothesized that the germination regulated expression patterns and protein sequences of the barley and rice genes in each cBR have been preserved for 50 million years after the split of rice and barley from their common ancestor because the genes are functionally important to seed germination, and should contribute to the characteristics shared by rice and barley germination. Additionally, 60 of the 322 cBRs were multi-gene cBRs. Each multi-gene cBR contained at least one pair of paralogs. Duplicated paralogous genes are subjected to little functional constraints, and offer a great opportunity for their sub-functionalization or neo-functionalization through divergence of their protein sequence and/or expression patterns [17,19,20,21,32]. Preserving germination regulated expression patterns and protein sequences of those paralogous genes in the multi-gene cBRs suggests that they may be subjected to negative selection, and provides additional evidence supporting their functional significance in seed germination.

We identified a number of biological pathways enriched with cBRs of similar expression patterns, suggesting that their underlying transcriptional regulatory programs are highly conserved in rice and barley. Preserving coordinate regulation of their gene expression patterns across rice and barley in each of those pathways provided further evolutionary evidence for functional significance of those biological pathways in seed germination. As suggested, most of those biological pathways have been previously proposed to functionally important in seed germination based on a variety of evidences. For example, a total of 13 cBRs were identified in cell wall metabolic pathway; and 12 of the 13 cBRs were up-regulated during early germination. Cell wall metabolism plays an important role in germination for most angiosperm seeds. It is required for two important germination biological processes [33,34]: radicle elongation growth and endosperm weakening. It was previously reported that endosperm weakening is accompanied with the induction of cell wall remodeling enzymes in several species. They include endo-beta mannannase, beta-1,3-glucanases, expansins, xyloglucan endotransglycosylase, pectin methylesterase, polygalacturonase and arabinogalactan protein [34]. We identified cBR encoding each of these proteins. Three cBRs encoding expansins were up-regulated during early germination. Expansins are involved in modifying the cell wall matrix during plant growth and development, and have been demonstrated to have cell wall extension activity in vitro and in vivo [35]. It was proposed that expansins is involved in the expansion of cucumber hypocotyls [36]. During germination of tomato seeds, a specific alpha-expansin transcript accumulates in the endosperm cap, presumably in association with the weakening of cell walls that facilitates emergence of the radicle [37]. The functional significance of expansins in germination might be an importance force to preserve the early germination up-regulated expression patterns and protein sequences of the cBRs. Cell wall precursor synthesis, cellulose synthesis and cell cell modification genes are up-regulated during the early germination phase in barley [9]. A number of cell wall degradation related genes are preferentially expressed in after-ripening barley coleorhiza, and are likely to associate with breaking seed dormancy [7]. Preserving early germination up-regulation of those cell wall metabolic enzyme genes in barley and rice also provided further evidence supporting the hypothesis that the early germination process turns on the transcriptional regulatory programs underlying cell wall metabolism to weaken coleorhiza and facilitate root emergence.

The cBRs encoding chromatin remodeling and structural proteins were preferentially up-regulated during the late germination phase. There were 8 cBRs in chromatin structure pathways. All of them were dramatically up-regulated during the late germination phase by more than 4.7 fold with an average of 30 fold. Histone modification and chromatin remodeling play important roles in reprogramming transcriptional programs. Chromatin-based regulation of seed dormancy and germination was also reported [38,39,40]. Mutation of histone monoubiquitination genes in Arabidopsis reduces ubiquitinated forms of histone H2B and alters expression levels for several dormancy-related genes [39]. A transient histone deacetylation event occurs during seed germination one day after imbibition, and is likely to serve as a key developmental signal that affects the repression of a number of histone deacetylase regulated genes [40]. Preserving preferential up-regulated expression of cBRs in late germination phase suggests an important role for histone modification and chromatin remodeling in germination, which likely supports radicle elongation and quick seedling growth in late and post-germination phase.

Interestingly, a number of biological pathways and gene families contained cBRs with diverse expression patterns. The cBRs encoding proteins in signaling pathways such as G-proteins and kinases often had diverse germination regulated expression patterns. G-proteins are involved in seed germination [41]. Diverse expression patterns of those G-protein cBRs suggested...
that those G-protein cBRs may participate in diverse signaling pathways in seed germination process. Thus, those cBRs had distinct biological functions in the most recent ancestor of barley and rice, and their protein sequences and germination regulated expression patterns have been preserved after their split from the ancestor. In addition, two distinct regulatory programs controlling alpha- and beta-amylation production were conserved in barley and rice. Starch, a major storage reserve in rice and barley grains, is mobilized during seed germination to support seedling growth. Alpha- and beta-amyloses are key enzymes required in starch mobilization [1,23]. The alpha-amylose cBR was up-regulated in late germination stages while the beta-amylose cBR was down-regulated in late germination (Figure 3D). Alpha-amylose genes are up-regulated in cereal grain germination and seedling growth. They are also induced by GA in barley aleurone tissues [10,15,23,42,43]. Preserving up-regulation of alpha-amylose genes was consistent with its biological functions in starch degradation during seed germination and seedling growth [44]. In contrast, previous biochemical studies showed that beta-amylose is synthesized and stored exclusively in the starchy endosperm after seed maturation rather than in the aleurone after the initiation of germination [45,46]. Accumulation level of beta-amylose transcript does not respond to GA treatment in barley aleurone [10]. Thus, the alpha- and beta-amylose cBRs had two opposite expression patterns that had been preserved during barley and rice seed germination for 50 million years of barely-rice divergence. Two cBRs encoding protease also showed opposite expression patterns during seed germination. The functional and evolutionary significance in preserving the two opposite transcriptional regulatory programs for these functionally related genes remains to be explored.

We also hypothesized in the study that the barley and rice genes in each cBR have equivalent or similar biological functions because of their strong similarity in protein sequences and expression patterns. Rice serves as a model plant for monocot plant research, and has rich research resources such as a large collection of genetic mutants and substantial genomic information. barley germination has been extensively studied biochemically and physiologically. Identification of the functionally equivalent rice and barley genes should greatly facilitate integration of research resource and knowledge from rice and barley research. In addition, gene expression changes in response to a biological process are used to successfully predict functional involvement of a gene in the biological process. However, it is often limited to a single species. It is difficult or even impossible to distinguish coincidentally regulated genes from those that are physiologically important. We hypothesized that the evolutionary conservation in the expression patterns of the inter-species and intra-species homologous genes could be used to predict their biological functions with a higher confidence [47,48]. Overall, the evolutionary and systems strategies described in the manuscript have a broad application in predicting genes functionally important and equivalent in a biological process and translate the research and knowledge across plant species with a great confidence.

Materials and Methods

Plant Growth and Harvest

Oryza sativa L. ssp. japonica (cv. Nipponbare) seeds were used in the experiment. Plump and healthy seeds were imbibed in water for three hours and then germinated on water-saturated germination pack in the dark at 30°C. Twenty seeds were planted in each 15 cm diameter Petri dish and spaced evenly to reduce the variation. The seeds at each representative time point of 0 h (dry grains), 21 h and 42 h were harvested. Three replications were conducted for each time point. Each replication represented an independent germination experiment at identical growth condition. The seeds for each replication were pooled together and immediately frozen in liquid nitrogen, and then stored at -80 degree for RNA extraction.

RNA Purification

Plant tissue (2 g) was ground using a mortar and pestle in liquid nitrogen followed by adding 10 mL extract buffer (4% p- aminosalicylic disodium, 1% 1, 5-naphthalenedisulfonic acid) and 10 ml phenol. The mixture was inverted several times, and then 10 ml chloroform was added; and the solution was homogenized for 45 seconds using a Polytron. After centrifuging, the aqueous phase was transferred into a new tube. Calculin white (60 ul of 10% solution) was added, mixed thoroughly and centrifuged for another 15 min at 4°C, 12,000 rpm. RNA in the supernatant was precipitated using 1/10 volume of 3 M NaOAc, and 2 volume of 100% ethanol. After centrifuging, the pellet was dissolved in 8 ml water. 5 ml of 8 M LiCl was added and the solution incubated on ice overnight. The resulting RNA pellet, isolated after centrifugation, was dissolved in water. RNA quality and quantity was determined using a Nano-Drop AN1000 (NanoDrop, Wilmington, DE) and Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA).

Microarray Assay and Data Analyses

Preparation of cDNA and biotin-labeled cRNA were performed and analyzed as recommended by Affymetrix (Santa Clara, CA). According to the manufacturer’s protocol, 10 ug of total RNA was used in a reverse transcription reaction to generate first-strand cDNA using SuperScript II (Invitrogen, Clarssbad, CA). After second-stranded synthesis, double-strand cDNA was used for an in vitro transcription reaction to generate biotinylated cRNA. 10 ug of fragmented cRNA for each sample was used in the hybridization. Staining and scanning steps were performed according to the manufacturer’s recommended protocols (Affymetrix, Inc., Santa Clara, CA).

The GeneChip probe-level data were background-corrected, normalized and summarized based on GC-Robust Multi-Array Analysis (RMA) approach [49]. In this approach, quantile normalization was used to remove the variation introduced during sample preparation, manufacturing of the arrays, and the processing of arrays, so that GeneChips from different time points and replicates are comparable, and expression level value for each gene was derived from probe pairs based on a log scale linear additive model [50].

Then pre-normalized data were analyzed with Genespring 7.2 software (Silicon Genetics, Redwood City, CA). Within each array, a further “per gene normalize the median” (with cutoff 0.01) was applied. The most unreliable data with absent call across 9 chips based on analyzed result using Microarray Suite 5.0 (Affymetrix, Santa Clara, CA) were filtered out. Statistical analyses were performed using a one-way ANOVA provided in GeneSpring 7.2 software (With Parametric Test, Variances Assumed Equal Option; Benjamini and Hochberg multiple testing correction. FDR set at 0.05) to identify genes that were differentially expressed among samples at any two time points during seed germination.

Considering that the potential non-specific hybridization between homologous genes could lead to cause an inaccurate correlation of their expression profiles, we excluded probes flagged by Affymetrix as potentially cross-hybridizing. The flagged probe sets included the ones with _x_at, which designates probe sets
where it was not possible to select either a unique probe set or a probe set with identical probes among multiple transcripts, _s_at_, which designates probe sets with common probes among multiple transcripts from different genes and _l_at_, _g_at_, _f_at_, _r_at_.

Identification of Barley-Rice (BR) Genes

The exemplar sequences of all probe-sets on Barley Genome GeneChip and Rice genome GeneChip were downloaded for the GeneChips used (http://www.affymetrix.com/products/arrays). An all-against-all reciprocal tBLASTX search was used to identify BRs with a given sequence homology. Pearson correlation coefficients (PCCs) of log2 expression values were calculated between homologs in R. Barley and rice genes with significantly changed expression level during seed germination were permuted to produce 100,000 random pairs to determine the distribution of PCCs for the randomized population. Chi-square analysis was used for comparison of observed values between barley and rice genes in each BR and PCC values from randomized pairs. Chi-square analysis was used for comparison of expression values between observed and random pairs. The microarray data used in the studies were deposited in NCBI Gene Expression Omnibus database (GSE 23395).

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Disclaimer Note

Names are necessary to report factually on available data; however, the US Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. USDA is an equal opportunity provider and employer.

Author Contributions

Conceived and designed the experiments: YQCA LL. Performed the experiments: YQCA LL. Analyzed the data: YQCA LL ST. Wrote the paper: YQCA LL ST SK ZL.

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