Plant Mol Biol (2007) 63:337–349
DOI 10.1007/s11103-006-9092-0

Distinct reorganization of the genome transcription associates with organogenesis of somatic embryo, shoots, and roots in rice

Ning Su · Kun He · Yuling Jiao · Chen Chen · Junli Zhou · Lei Li · Sulan Bai · Xueyong Li · Xing Wang Deng

Received: 9 June 2006 / Accepted: 10 September 2006 / Published online: 28 October 2006

Abstract Most plant cells retain the capacity to differentiate into all the other cell and organ types that constitute a plant. However, genome-wide transcriptional activities underlying the process of cell differentiation are poorly understood, especially in monocot plants. Here we used a rice (*Oryza sativa*) cell culture system to generate somatic embryos, which were further induced into shoots and roots. The global transcriptional reorganization during the development of somatic embryos, shoots, and roots from cultured cells was studied using a rice whole genome microarray and verified by RNA blotting analysis of representative genes. Overall, only 1–3% of expressed genes were differentially regulated during each organogenesis process at the examined time point. Also metabolic pathways were minimally regulated. Thus the genes that dictating organ formation should be relatively small in number. Comparison of these three transcriptomes revealed little overlap during these three organogenesis processes. These results indicate that each organogenesis involves specific reorganization of genome expression.

Keywords Rice · Oligo chip · Development · Organogenesis · Transcriptome

Ning Su, Kun He, and Yuling Jiao have contributed equally to this work

Electronic supplementary material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s11103-006-9092-0 and is accessible for authorized users.

N. Su · K. He · S. Bai · X. W. Deng
Peking-Yale Joint Center of Plant Molecular Genetics and Agrobiotechnology, College of Life Sciences, Peking University, Beijing 100871, China

N. Su · K. He · Y. Jiao · J. Zhou · L. Li · S. Bai · X. Li · X. W. Deng (✉)
Department of Molecular, Cellular, and Development Biology, Yale University, Osborn Memorial Laboratories, Room 352A, 165 Prospect Street, P.O. Box 208104, New Haven, CT 06520-8104, USA
e-mail: xingwang.deng@yale.edu

C. Chen
Beijing Institute of Genomics of Chinese Academy of Sciences, Beijing Genomics Institute, Beijing 101300, China

C. Chen
James D. Watson Institute of Genome Sciences, Zhejiang University, Hangzhou 310008, China

Introduction

One of the fundamental aspects in developmental biology is organogenesis, i.e., how cells organize to form new organs. All plant cells have the remarkable capacity to differentiate into any cell or organ types that constitute the adult plant. During post-embryonic development, plants maintain two self-maintaining stem cell systems, i.e., the shoot apical meristem (SAM) and the root apical meristem (RAM) that were formed during embryogenesis. The two meristems are able to perpetuate existing organs and initiate new organ primordia such as lateral roots, leaves, and flowers that form the plant body plan during post-embryonic development (Steeves and Sussex 1989).

The organogenesis is a complex morphogenetic phenomenon in which multiple extrinsic and intrinsic

factors play important roles. Plant hormones are known to be among the critical factors in the developmental programs. The classic plant tissue culture studies showed that the developmental fate of the regenerating tissues, i.e., whether the undifferentiated tissue would form shoots, roots or remain as undifferentiated calli, could be governed by the balance of auxin and cytokinin in the tissue culture medium (Skoog and Miller 1957). The relationship of auxin and cytokinin has been widely utilized for vegetative propagation and regeneration procedures (Christianson and Warnick 1983; Mok and Martin 1994).

Recently the molecular basis of the organogenesis has begun to be examined. A set of genes involved in the cytokinin signal transduction pathway was identified in *Arabidopsis* (Haberer and Kieber 2002). The cytokinin signaling pathway has been shown to influence the shoot formation (Hwang and Sheen 2001). *cytokinin-insensitive* (CKI1) protein can serve as a potential regulator of shoot regeneration, and overexpression of the gene induces cytokinin-independent growth in *Arabidopsis* culture (Kakimoto 1996). Recently, a novel two-component phosphorelay signaling system has been demonstrated to be responsible for cytokinin signaling. The components of this signaling pathway including: cytokinin receptor histidine kinases (Inoue et al. 2001, Ueguchi et al. 2001a, b), Arabidopsis response regulator (ARRs, Sakai et al. 2001; Hwang et al. 2002), Arabidopsis histindine phosphotransfer proteins (AHPs) that responsible for transmiting the signal from the receptor to ARRs (Suzuki et al. 2000; Hwang and Sheen 2001).

In shoot meristem development, a number of regulators have been identified that include maize KNOTTED1 (Vollbrecht et al. 1991) and its homolog Arabidopsis SHOOT MERISTEMLESS (STM) (Long et al. 1996), WUSCHEL (*WUS*, Laux et al. 1996; Mayer et al. 1998) and three CLAVATA proteins (Leyser and Furner 1992; Clark et al. 1993, 1995; Kayes and Clark 1998; Fletcher et al. 1999). It has been shown that cytokinin can stimulate the expression of *STM* (Rupp et al. 1999; Teo et al. 2001). The mutants with defects in *STM*, *WUS* or a double mutant of *CUP-SHAPED COTYLEDON 1 and 2* (*CUC1* and *CUC2*) can block the formation of SAM (Aida et al. 1997; Takada et al. 2001). A microarray based genome profiling analysis in Arabidopsis demonstrated that the morphological changes during shoot formation when root explants preincubated on an auxin-rich callus induction medium (CIM) transferred to a cytokinin-rich shoot induction medium (SIM) are associated with extensive gene expression changes (Che et al. 2002).

It is well accepted that auxin plays an important role in root formation (Laskowski et al. 1995). The observation that *aberrant lateral root formation1* (*alf1*), *superroot1* (*sur1*), *rooty* (*rty*) mutants exhibited elevated levels of endogenous auxin and produced extra number of lateral roots strongly support that notion (Boerjan et al. 1995; Celenza et al. 1995; King et al. 1995). Mutations in a large number of genes that affecting auxin response also affects root development. Those include AUX1 (Bennett et al. 1996), *auxin resistant 4* (AXR4, Hobbie and Estelle 1995), *transport inhibitor response1* (*TIR1*) (Ruegger et al. 1998; Gray et al. 1999), the *Aux/IAA* gene family (Leyser et al. 1996; Tian and Reed 1999; Rogg et al. 2001; Fukaki et al. 2002), the auxin efflux transporter family PIN proteins (Gälweiler et al. 1998; Müller et al. 1998; Geldner et al. 2001; Friml et al. 2002a, b). Microarray profiling of *Arabidopsis* seed germination on different media suggested that lateral root formation is associated with expression changes in large number of genes (Himanen et al. 2004).

The plant tissue culture system has been extensively used in studying organogenesis in vitro at morphological, physiological, cellular, and biochemical levels (Goldberg et al. 1994; Laskowski et al. 1995; Malamy and Benfey 1997; Rinne and Schoot 1998; Gisel et al. 1999; Schoot and Rinne 1999; Casimiro et al. 2001). However, a systematic analysis of genome expression was not reported in a model organism with full genome sequence available. Revealing those underlying genome expression changes associated with organogenesis will be very useful in understanding regulatory mechanisms involved. An in vitro cell culture system is a good model for studying the molecular events in embryogenesis and lateral organogenesis since it is easy to harvest a large amount of synchronized culture samples of giving organogenesis state. Here we report a characterization of the global transcriptional events during somatic embryogenesis and the shoot and root organogenesis from somatic embryo in rice (*Oryza sativa*). The associated genome expression changes during those organogenesis processes and its implications have been a focus of this study.

Materials and methods

Plant material

The dehusked seeds of *O. sativa* L. subsp *indica* cv 93-11 were surface sterilized and cultured in petri dishes containing MS medium (Murashige and Skoog

1962) with 3% maltose and 2 mg/l 2,4-D, solidified with 0.8% (w/v) agar. After 4–5 weeks in the dark at 26–28 °C, small, loosely attached globular calli were carefully selected and placed into a 50 ml Erlenmeyer flask containing 50 ml liquid SZ medium (Zhang 1995) supplemented with 3% maltose and 2 mg/l 2,4-D and 0.2 mg/l kinetin, 300 mg/l casein enzymatic hydrolysate (N-Z-Amine A), 500 mg/l each of proline and glutamine. The medium was completely replaced at intervals for 1–5 days for 2–3 weeks. Liquid cultures were maintained in 125-ml Erlenmeyer flasks containing 50 ml SZ medium at intervals of 7 days subculture on a gyratory shaker at 100–120 rpm. Seven months cell cultural suspension was passed through a 1,000 μm nylon mesh (spectrumlabs) to separate out small cell clumps. The cell clumps greater than 1,000 μm were used as somatic embryo induction on liquid SZ medium [3% maltose, 5 mg/l ABA, 0.5 mg/l NAA 2 mg/l Kinetin, 300 mg/l casein enzymatic hydrolysate (N-Z-Amine A), 500 mg/l each of proline and glutamine] in dark. After subculturing in petri dishes containing NB medium [3% maltose, 5 mg/l ABA, 0.5 mg/l NAA, 2 mg/l Kinetin, 300 mg/l casein enzymatic hydrolysate (N-Z-Amine A), 500 mg/l each of proline and glutamine, 3 g/l Phytelgel] for 2 days, the somatic embryos were used to induce shoots on NB medium [3% maltose, 2.5 mg/l Kinetin, 0.05 mg/l NAA, 300 mg/l casein enzymatic hydrolysate (N-Z-Amine A), 500 mg/l each of proline and glutamine] at 28 °C under the continuous light and roots on NB medium [3% maltose, 0.5 mg/l NAA, 300 mg/l casein enzymatic hydrolysate (N-Z-Amine A), 500 mg/l each of proline and glutamine] at 28 °C in dark.

Histology

For histological studies, the tissues were fixed in Formalin/glacial acetic acid/ethanol (FAA) at a ratio of 5:5:90 (v/v/v) for 48 h, dehydrated through a grated ethanol-xylene series and embedded in paraffin wax. The tissue were sectioned at 10 μm thickness and stained with 1% safranin O. The section were observed and photographed with a Zeiss Axiophot microscope.

Microarray experiments

A rice 70-mer genome-wide oligo microarray (Ma et al. 2005) was used throughout this work. Microarray probe labeling, hybridization, washing, and scanning were carried out as described previously (Jiao et al. 2005; Ma et al. 2005). Hybridized microarray slides were scanned with a GenePix 4000B scanner (Axon, Whipple road, CA, USA), and independent TIFF images for both Cy3 and Cy5 channels were used for subsequent analysis.

Microarray data processing

To identify and remove systematic sources of variation, spot intensities from the GenePix Pro output files of all repeats of a given sample pair were normalized using the limma package (Smyth 2004) in *R*. The print-tip lowess normalization method was used. A common used strategy (Rinn et al. 2003) was adopted to define whether a gene is expressed or not with minor adjustment. First, in each microarray replicate, the oligos with a normalized intensity higher than 90% of the intensity values of 156 negative controls on each slide were selected as expressed genes (oligos). Second, only the genes with the majority (two of two, or at least two of three) of corresponding spots from multiple replicates showed detectable expression defined in the first criterion were kept as expressed genes. To identify differentially expressed genes, a linear model and empirical Bayes method were adopted to assess discrepancies in expression levels (Smyth 2004). Criteria for differentially expressed genes included having a *P*-value of less than 0.05 and at least a twofold change in expression.

Hierarchical clustering analysis was applied to the differentially expressed genes that at least presented in one of the three different experiments (Supplemental Table 1). The hierarchical clusters were done with the cluster 3.0 (http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/software.htm#ctv) on a Redhat Linux server and visualized with the Java Treeview program (http://genetics.stanford.edu/~alok/TreeView/).

RT-PCR cloning and mRNA blot analysis

A random group of selected genes based on microarray data were used for mRNA blot analysis (Supplemental Table 2). Probes used in RNA gel blot hybridization were cloned by RT-PCR. Reverse transcription was performed using Oligo (dT) 19-mer, Reverse Transcriptase (Promega, Madison, WI, USA), and 50 ng mRNA, generating cDNA for PCR. The information of primers is in the supplemental data (Supplemental Table 2). The PCR products were visualized on 1.0% agarose gels. The cloned PCR product was used as probes after sequencing.

RNA gel blot hybridization

RNA (10 μg of total RNA per sample) was separated by electrophoresis on 1.2% agarose gels

containing 6% formaldehyde and blotted onto nylon membranes (Hybond-N; Amersham Pharmacia Biotech, Piscataway, NJ, USA). Duplicate RNA gels were stained with ethidium bromide to verify RNA quality and to ensure equal loading. DNA probes were labeled with ^{32}P-labeled DNA probes were prepared using a Rediprime II Random Prime Labeling System (RPN 1633, Amersham Biosciences) according to the manufacturer's instructions. Hybridization signals were visualized by autoradiography with Kodak XAR film.

Results

Optimization of rice organ induction conditions

We selected *indica* rice cultivar 93-11 for callus tissue preparation and organogenesis analysis because of the microarray design (Ma et al. 2005). Since *indica* rice varieties may differ from other varieties in their response to in vitro culture conditions, we tested different culture conditions to set up a stable and synchronized culture system for this cultivar. Four mediums, R2 medium (Ohira et al. 1973), AA medium (Müller and Grafe 1978), N6 basal salts supplemented with Gamborg's B5 vitamins (Chu et al. 1975), and SZ medium (Zhang 1995), are commonly used for rice tissue culture. We found that while the SZ medium was suitable for the cell suspension culture and somatic embryo induction, N6 basal salts supplemented with Gamborg's B5 medium had the best results in shoot and root induction.

In this study, an established suspension culture cell line of ~7 months old was used. Somatic embryos were consistently induced in high efficiency after hormone treatment (see Method section), which increases the embryogenic ability and provides a signal for synchronization. Series of combination of hormone concentrations under the light or in darkness were tested for the induction of somatic embryos, shoots, and roots. The optimal conditions were determined for somatic embryo (SZ medium with 5 mg/l ABA, 0.5 mg/l NAA, and 2 mg/l Kinetin, 300 mg/l casein enzymatic hydrolysate, 500 mg/l each of proline and glutamine, 3% maltose, at 28°C in darkness), somatic embryo to shoot (NB medium with 2.5 mg/l Kinetin, 0.05 mg/l NAA, 300 mg/l casein enzymatic hydrolysate, 500 mg/l each of proline and glutamine, 3% maltose, at 28°C under continuous light), and somatic embryo to root (NB medium with 0.5 mg/l NAA, 300 mg/l casein enzymatic hydrolysate, 500 mg/l each of proline and glutamine, 3% maltose, at 28°C in darkness) induction.

In our analysis, we found that the 7-day-old somatic embryos (from the onset of hormone induction) were the in best stage for shoots or roots induction with the highest induction rates (≥98%). Using these somatic embryos, the mean number of shoot plantlets can be formed per embryo was 6.5 ± 2.0 and the mean number for root plantlets was 15.0 ± 3.0. Microscopic examination revealed that the surface of somatic embryos was smooth and had nodular appearance, which are distinct from the 7-month suspension cell clusters or calli (Fig. 1a, b).

An anatomic analysis of rice *in vitro* organogenesis

As an initial step to study the in vitro organogenesis processes in rice, morphological and histological changes during somatic embryo, shoot, and root formation were examined. The microscopic observation at different time points during shoot induction showed that there was no observable changes occurred during the first 3 days from the onset of shoot induction from somatic embryos. After 3 days and most evident from 6 days onward, numerous green patches were observed on the surface of somatic embryos. A histological examination indicated that 3–6 days after shoot induction, coleoptile-like structures appeared (Fig. 1c, d). The green shoot buds formation was evident after 9-day induction and histological examination revealed the presence of obvious SAM and leave primordia (Fig. 1e). After 28 more days, multiple shoots can be observed protruding out of each individual somatic embryo (Fig. 1i). As transcription reprogramming likely happens before morphological changes, we selected somatic embryos subjected to 6-day shoot induction for microarray analysis with somatic embryos without induction as the control.

Morphological analysis of root induction indicated that after 3 days of root induction, spindly root-like cells became apparent in those somatic embryos (Fig. 1f). The root hair like surface structures appeared after 6-day root induction of somatic embryos, with waving surface appearance (Fig. 1g). Evident root tips formed 9 days after root induction (Fig. 1h). Usually each somatic embryo could form several roots as showed in Fig. 1j. Again, the samples subjected to 6-day root induction were chosen for gene expression analysis.

Identification of genes differentially expressed during formation of somatic embryos, shoots, and roots

A 70-mer oligo microarray described previously (Ma et al. 2005), which represents 36,926 unique

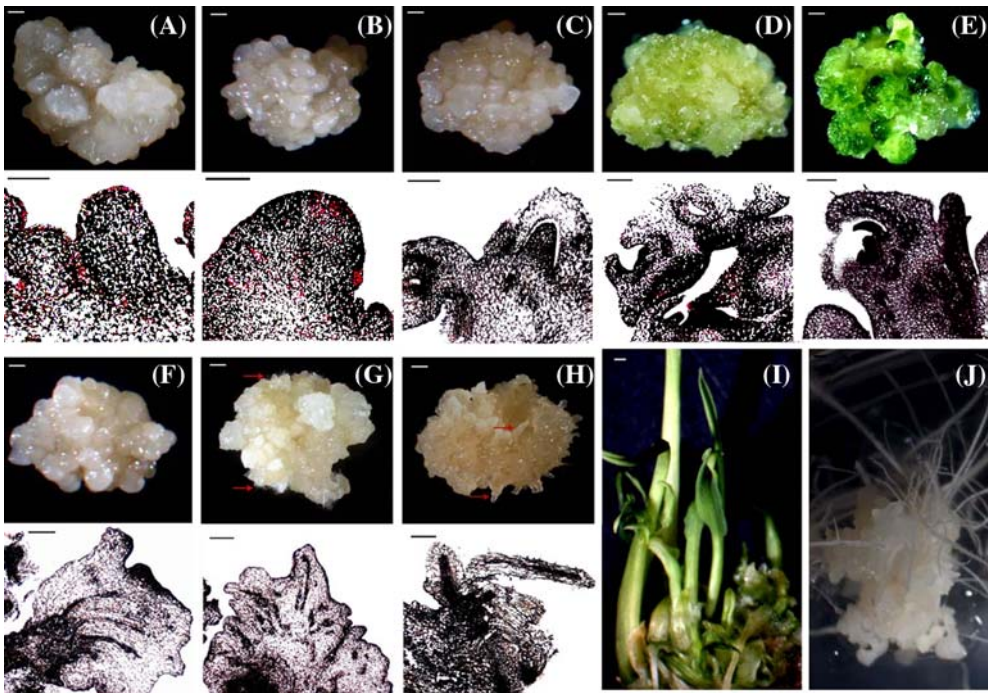


Fig. 1 Morphological changes during three organogenesis processes: from callus to embryo colonies and the further induction into shoots and roots in *Oryza sativa* L. subsp. *indica* (var 9311). (**a**) Callus (7 months culture). (**b**) Embryo colonies (induction for 7 days). (**c**–**e**) The induction of shoot from embryo colonies, from left to right as 3, 6, and 9-day induction. (**f**–**h**) The induction of root from embryo colonies, from left to right as 3, 6, and 9 days induction. (**i**) Shoot after 28-day induction. (**j**) Root after 15-day induction. The upper half of each panel is from microscopy analysis, while the lower half of each panel is from histological analysis for panel (a)–(h). *Bar* in each panel is 1 mm for microscopy analysis and 0.1 mm for histology analysis

known and predicted *indica* rice genes, was used for the analysis of genome expression during organogenesis. Three pair-wise comparisons were made that involve 7-day induced somatic embryos compared to suspension cells without induction, 6-day induced shoots or roots compared to the somatic embryos. Total RNA from three independent biological samples was fluorescence-labeled and hybridized to the microarray and dye-swap was performed in each of the three comparisons.

Examination of the expression ratios of genes indicated that a relatively small portion of the rice genome was differentially expressed in each organogenesis process. We found that 383 genes were induced and 607 genes were repressed by at least twofold, with a *P*-value below 0.05 when suspension cells were induced into somatic embryos (Fig. 2). During shoot induction, 433 genes were up-regulated and 397 genes were down-regulated. Whereas 157 genes were induced and 203 genes were repressed while somatic embryos were induced into roots. Taken together, transcript levels of only 1–3% of the rice genes represented on the microarray were significantly altered in each of these in vitro organogenesis processes at the examined time point. Detailed lists of the differentially expressed genes are available in Supplemental Table 1 and a summary is presented in Fig. 3.

Clear specificities for each organogenesis process at transcriptional level

A surprising finding was that a rather limited overlap was found among these three organogenesis processes, as shown in a Venn diagram (Fig. 4). There were only nine genes commonly reduced among totally 1,103 down-regulated genes during organogenesis of somatic embryos, shoots or roots. Similarly, among 917 up-regulated genes, only three genes were commonly involved. Slightly bigger overlaps, 59 repressed and 32 induced genes, were found between shoots induction and roots induction from somatic embryos. The results suggest that distinct genome reprogramming is specifically involved in each organogenesis process.

To further compare the transcriptome changes during three organogenesis processes, we analyzed detailed expression profiles using a Hierarchical clustering algorithm (Fig. 5). The cluster results revealed that the embryogenesis was more distinct

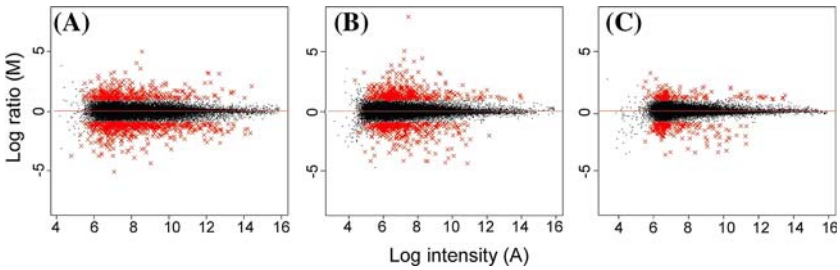


Fig. 2 The overall assessment of differential gene expression during the three organogenesis processes using the genome-wide oligonucleotide microarray. Expression profiles of somatic embryos were compared with callus (**a**), induced shoots were compared with somatic embryos (**b**), and induced roots were compared with somatic embryos (**c**). Plots were generated from experimental data of three replicates after normalization and replicate integration analysis (see Methods for details). log(-ratio) = $\log_2 (I_1/I_2)$; log(intensity) = 0.5 $\log_2 (I_1 \cdot I_2)$, where I_1 and I_2 are median signal intensities for a given element in the first or the second sample of the sample pair, respectively. The differential expressed genes were defined as the log (2) ratio greater than 1 and highlighted in *red color*

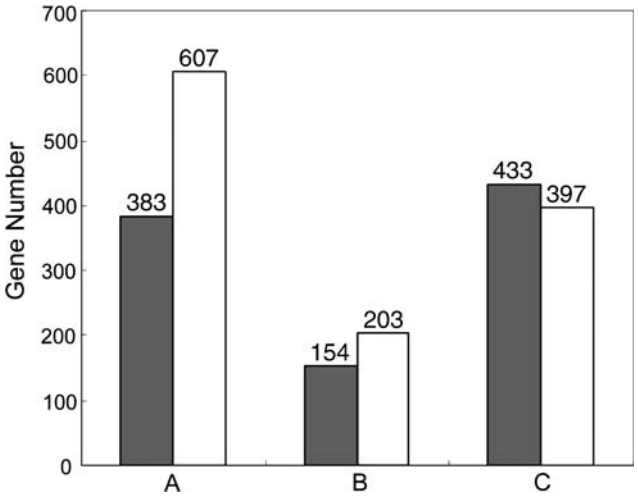


Fig. 3 The number of differential expressed genes in the three organogenesis processes. (**a**) Differentially expressed genes from 7-day post-induction somatic embryos versus callus. (**b**) Differentially expressed genes from 6-day induced roots versus somatic embryos. (**c**) Differentially expressed genes from 7-day induced shoots versus somatic embryos. The *shaded bars* represents up-regulated genes while the *open bars* represent down-regulated genes. The differential expressed genes were defined as the log (2) ratio greater than 1 and $P- < 0.05$

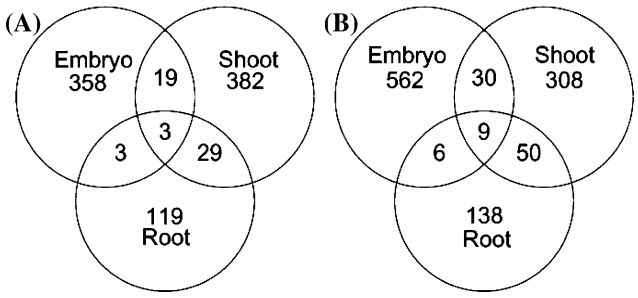
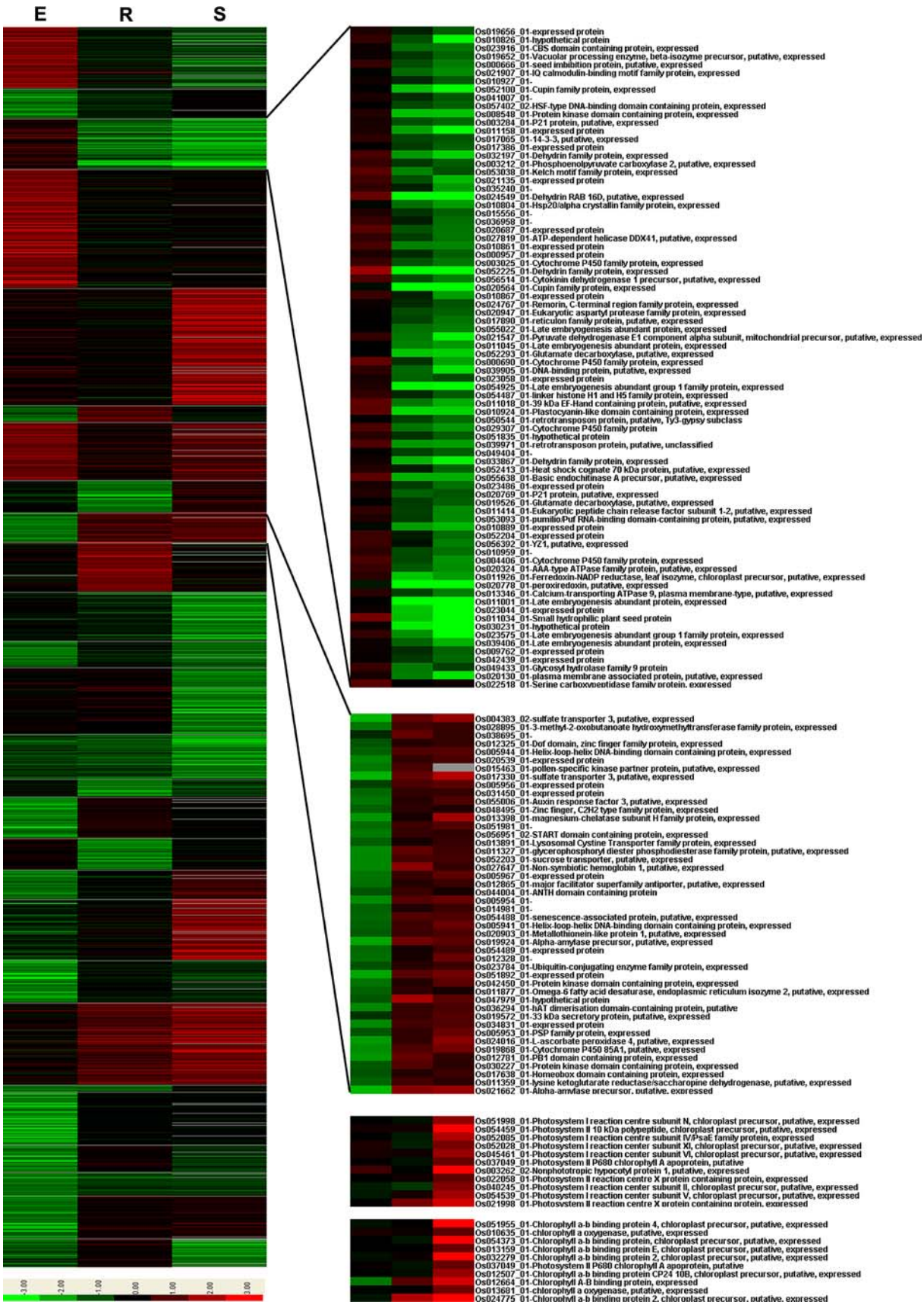


Fig. 4 Venn diagrams to show the number of differentially expressed genes and the shared genes. (**a**) Up-regulated genes; and (**b**) Down-regulated genes

from root or shoot induction. Such a cluster analysis also allowed us to reveal those genes weakly regulated. We found only 6% of the differentially regulated genes during root induction had similar regulation but with a weaker magnitude (with a 1.5-fold change and *P*-value of 0.05 cut-off) in the other two organogenesis processes. While only 3% of the differentially regulated genes during shoot induction have similar regulation at a low magnitude in the other two organogenesis processes. There are slightly more similarity between shoot and root induction, 12% of the differentially regulated genes during root induction shared with those differentially expressed genes during shoot induction, including a group of late embrogenesis genes (dehydrin family and late embrogenesis abundant family) that were down-regulated in both root and shoot induction (Fig. 5). It is interesting to note that 11 photosystem reaction related genes and ten ChlorophyII related genes are found specifically up-regulated during the shoot induction, but not or only weakly regulated in other two organogenesis processes. Fifty-one and twenty genes exhibited organ-specific and opposite regulation between embryogenesis and roots or shoots induction, respectively. While only three genes were oppositely regulated during root and shoot induction.

Fig. 5 Hierarchical clustering display of differentially expressed genes in the three organogenesis processes. The 7-day post-induction somatic embryos (*E*) versus callus, the 6-day post-induction root (*R*) versus somatic embryos, and 6-day –post-induction shoot (*S*) versus somatic embryos were displayed together. Only those genes that exhibited twofold or greater differential expression in at least one sample pair among the three tested were included for comparison. A total of 1,963 genes were included in the cluster analysis ▶


E
R
S
Os019656_01-expressed protein
Os010826_01-hypothetical protein
Os023916_01-CBS domain containing protein, expressed
Os019652_01-Vacuolar processing enzyme, beta-isozyme precursor, putative, expressed
Os000666_01-seed inhibition protein, putative, expressed
Os021907_01-IQ calmodulin-binding motif family protein, expressed
Os010927_01-
Os052100_01-Cupin family protein, expressed
Os041007_01-
Os057402_02-HSF-type DNA-binding domain containing protein, expressed
Os008548_01-Protein kinase domain containing protein, expressed
Os003284_01-P21 protein, putative, expressed
Os011158_01-expressed protein
Os017065_01-14-3-3, putative, expressed
Os017386_01-expressed protein
Os032197_01-Dehydrin family protein, expressed
Os003212_01-Phosphoenolpyruvate carboxylase 2, putative, expressed
Os053038_01-Kelch motif family protein, expressed
Os021135_01-expressed protein
Os035240_01-
Os024549_01-Dehydrin RAB 16D, putative, expressed
Os010804_01-Hsp20/alpha crystallin family protein, expressed
Os015556_01-
Os036958_01-
Os020687_01-expressed protein
Os027819_01-ATP-dependent helicase DDX41, putative, expressed
Os010861_01-expressed protein
Os000957_01-expressed protein
Os003025_01-Cytochrome P450 family protein, expressed
Os052225_01-Dehydrin family protein, expressed
Os056514_01-Cytokinin dehydrogenase 1 precursor, putative, expressed
Os020564_01-Cupin family protein, expressed
Os010867_01-expressed protein
Os024767_01-Remorin, C-terminal region family protein, expressed
Os020947_01-Eukaryotic aspartyl protease family protein, expressed
Os017890_01-reticulon family protein, putative, expressed
Os055022_01-Late embryogenesis abundant protein, expressed
Os021547_01-Pyruvate dehydrogenase E1 component alpha subunit, mitochondrial precursor, putative, expressed
Os011045_01-Late embryogenesis abundant protein, expressed
Os052293_01-Glutamate decarboxylase, putative, expressed
Os000690_01-Cytochrome P450 family protein, expressed
Os039905_01-DNA-binding protein, putative, expressed
Os023058_01-expressed protein
Os054925_01-Late embryogenesis abundant group 1 family protein, expressed
Os054487_01-linker histone H1 and H5 family protein, expressed
Os011018_01-39 kDa EF-Hand containing protein, putative, expressed
Os010924_01-Plastocyanin-like domain containing protein, expressed
Os050544_01-retrotransposon protein, putative, Ty3-gypsy subclass
Os029307_01-Cytochrome P450 family protein
Os051835_01-hypothetical protein
Os039971_01-retrotransposon protein, putative, unclassified
Os049404_01-
Os033867_01-Dehydrin family protein, expressed
Os052413_01-Heat shock cognate 70 kDa protein, putative, expressed
Os055638_01-Basic endochitinase A precursor, putative, expressed
Os023486_01-expressed protein
Os020769_01-P21 protein, putative, expressed
Os019526_01-Glutamate decarboxylase, putative, expressed
Os011414_01-Eukaryotic peptide chain release factor subunit 1-2, putative, expressed
Os053093_01-pumilio/Puf RNA-binding domain-containing protein, putative, expressed
Os010889_01-expressed protein
Os052204_01-expressed protein
Os056392_01-YZ1, putative, expressed
Os010959_01-
Os004406_01-Cytochrome P450 family protein, expressed
Os020324_01-AAA-type ATPase family protein, putative, expressed
Os011926_01-Ferredoxin-NADP reductase, leaf isozyme, chloroplast precursor, putative, expressed
Os020778_01-peroxiredoxin, putative, expressed
Os013346_01-Calcium-transporting ATPase 9, plasma membrane-type, putative, expressed
Os011001_01-Late embryogenesis abundant protein, expressed
Os023044_01-expressed protein
Os011034_01-Small hydrophilic plant seed protein
Os030231_01-hypothetical protein
Os023575_01-Late embryogenesis abundant group 1 family protein, expressed
Os039406_01-Late embryogenesis abundant protein, expressed
Os009762_01-expressed protein
Os042439_01-expressed protein
Os049433_01-Glycosyl hydrolase family 9 protein
Os020130_01-plasma membrane associated protein, putative, expressed
Os022518_01-Serine carboxypeptidase family protein, expressed
Os004383_02-sulfate transporter 3, putative, expressed
Os028895_01-3-methyl-2-oxobutanoate hydroxymethyltransferase family protein, expressed
Os038695_01-
Os012325_01-Dof domain, zinc finger family protein, expressed
Os005944_01-Helix-loop-helix DNA-binding domain containing protein, expressed
Os020539_01-expressed protein
Os015463_01-pollen-specific kinase partner protein, putative, expressed
Os017330_01-sulfate transporter 3, putative, expressed
Os005956_01-expressed protein
Os031450_01-expressed protein
Os055006_01-Auxin response factor 3, putative, expressed
Os048495_01-Zinc finger, C2H2 type family protein, expressed
Os013398_01-magnesium-chelatase subunit H family protein, expressed
Os051981_01-
Os056951_02-START domain containing protein, expressed
Os013891_01-Lysosomal Cystine Transporter family protein, expressed
Os011327_01-glycerophosphoryl diester phosphodiesterase family protein, putative, expressed
Os052203_01-sucrose transporter, putative, expressed
Os027647_01-Non-symbiotic hemoglobin 1, putative, expressed
Os005967_01-expressed protein
Os012865_01-major facilitator superfamily antiporter, putative, expressed
Os044004_01-ANTH domain containing protein
Os005954_01-
Os014981_01-
Os054488_01-senescence-associated protein, putative, expressed
Os005941_01-Helix-loop-helix DNA-binding domain containing protein, expressed
Os020903_01-Metallothionein-like protein 1, putative, expressed
Os019924_01-Alpha-amylase precursor, putative, expressed
Os054489_01-expressed protein
Os012328_01-
Os023784_01-Ubiquitin-conjugating enzyme family protein, expressed
Os051892_01-expressed protein
Os042450_01-Protein kinase domain containing protein, expressed
Os011877_01-Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2, putative, expressed
Os047979_01-hypothetical protein
Os036294_01-hAT dimerisation domain-containing protein, putative
Os019572_01-33 kDa secretory protein, putative, expressed
Os034831_01-expressed protein
Os005953_01-PSP family protein, expressed
Os024016_01-L-ascorbate peroxidase 4, putative, expressed
Os019868_01-Cytochrome P450 85A1, putative, expressed
Os012701_01-PB1 domain containing protein, expressed
Os030227_01-Protein kinase domain containing protein, expressed
Os017638_01-Homeobox domain containing protein, expressed
Os011359_01-lysine ketoglutarate reductase/saccharopine dehydrogenase, putative, expressed
Os021662_01-Alpha-amylase precursor, putative, expressed
Os051998_01-Photosystem I reaction centre subunit N, chloroplast precursor, putative, expressed
Os054459_01-Photosystem II 10 kDa polypeptide, chloroplast precursor, putative, expressed
Os052085_01-Photosystem I reaction centre subunit IV/PsaE family protein, expressed
Os052028_01-Photosystem I reaction center subunit XI, chloroplast precursor, putative, expressed
Os045461_01-Photosystem I reaction center subunit VI, chloroplast precursor, putative, expressed
Os037049_01-Photosystem II P680 chlorophyll A apoprotein, putative
Os003262_02-Nonphototropic hypocotyl protein 1, putative, expressed
Os022058_01-Photosystem II reaction centre X protein containing protein, expressed
Os040245_01-Photosystem I reaction center subunit II, chloroplast precursor, putative, expressed
Os054539_01-Photosystem I reaction center subunit V, chloroplast precursor, putative, expressed
Os021998_01-Photosystem II reaction centre X protein containing protein, expressed
Os051955_01-Chlorophyll a-b binding protein 4, chloroplast precursor, putative, expressed
Os010635_01-chlorophyll a oxygenase, putative, expressed
Os054373_01-Chlorophyll a-b binding protein, chloroplast precursor, putative, expressed
Os013159_01-Chlorophyll a-b binding protein E, chloroplast precursor, putative, expressed
Os032279_01-Chlorophyll a-b binding protein 2, chloroplast precursor, putative, expressed
Os037049_01-Photosystem II P680 chlorophyll A apoprotein, putative
Os012507_01-Chlorophyll a-b binding protein CP24 10B, chloroplast precursor, putative, expressed
Os012664_01-Chlorophyll A-B binding protein, expressed
Os013681_01-chlorophyll a oxygenase, putative, expressed
Os024775_01-Chlorophyll a-b binding protein 2, chloroplast precursor, putative, expressed
-3.00
-2.00
-1.00
0.00
1.00
2.00
3.00

Organogenesis involves genes with diverse functions

Among those specifically regulated genes, we identified clusters of photosystem enzyme genes induced during shoot genesis. Other gene functional groups, however, did not show a clear enrichment either in commonly regulated genes by two processes or in those specific genes for only one organogenesis process. In fact each of these groups included genes with various functions in regulatory roles, such as transcription and signal transduction (including kinases), and in specific biochemical processes. There also lacked an enrichment of those regulatory genes. For example, we identified 116 transcription factors regulated in at least one organogenesis process. These transcription factor genes correspond to about 5% of all transcription factors predicted in rice, which is a similar portion to the overall count (Supplemental Table 1). Moreover, these regulated transcription factors distribute similar to other genes in distinct regulated gene groups.

Still we were able to identify genes with different regulation patterns. Examples of previously studied genes are shown in Fig. 6. We found a general consistency between our microarray data and isolated reports. For example, *Em* is a gene reported to abundantly expressed during embryogenesis and is enhanced by exogenous abscisic acid (ABA) (William and Tsang 1991; Hattori et al. 1995). Our microarray expression data showed a consistent induced expression during embryo induction. The *ZmOCL* homologous gene in rice is down regulated, consistent with reported expression in a distinct region of the embryonic protoderm during the early development (Ingram et al. 2000). Rice *ZWILLE* gene was down-regulated during shoots induction, consistent with its function in maintaining stem cells in the developing shoot meristem during the transition from embryonic development to repetitive postembryonic organ formation (Moussain et al. 1998).

A special attention was given to cytokinin and auxin signaling pathway genes, since these two phytohormones play crucial roles during in vitro organogenesis. We found three rice homologs for *Arabidopsis* cytokinin signaling genes were significantly regulated. All of them showed a clear induction during embryogenesis, whereas the regulation during roots or shoots induction was limited (Fig. 6). Another three *Arabidopsis* auxin signaling gene homologous were found significantly or barely regulated (Fig. 6). These expression profiles were distinct from each other and were involved in not only embryogenesis but also roots and shoots induction. Regulations were some time in the opposite direction in different organogenesis processes. For example, *ARF3* homolog was repressed in expression during embryogenesis but was induced during other two organogenesis processes.

Mutagenesis studies identified many genes essential for *Arabidopsis* embryogenesis (Tzafrir et al. 2004). We found 242 of them have significant rice homologous covered by this microarray. Among genes expressed in somatic embryo, we found the expression in 211 (87%) of them, suggesting that not only embryogenesis is an evolutionarily conserved process, but also a high similarity between normal embryogenesis and the in vitro embryogenesis process.

Kinetics of transcription regulation during organogenesis

To gain insights into the kinetics of organogenesis and also to further verify differentially expressed genes identified by microarray analysis, a total of 28 selected genes were cloned, sequenced, and used as probes for RNA blot analysis (Supplemental Table 2). Total RNA samples were prepared from multiple time points during each of the three organogenesis processes, while roots and shoots RNA samples from the 10-day and 60-day rice plants were used as positive controls. In all those 28 genes, the RNA gel blot analysis confirmed trends of microarray data (Fig. 7).

Limited regulation of metabolic pathways during organogenesis

To examine the involvement of different functional groups of genes during organogenesis, we explored the regulation of various gene functional categories. By functional assignment using Gene Ontology (GO) terms (Gene Ontology Consortium 2000), we found that several major gene functional categories were regulated (see Supplemental Figure 1). For the majority of these categories, induced and repressed gene members were similarly distributed.

The annotation of metabolic pathway genes is more definitive, in general, than other gene groups. Therefore, we initially focused on metabolic pathways to understand the effect of organogenesis on genome expression. Moreover, metabolic pathway genes are often the targets of signaling cascades. We followed the standardized AraCyc-defined metabolic pathways (Mueller et al. 2003), which currently include 1,759 Arabidopsis enzyme genes, to identify genes in each pathway. In this analysis, rice genes were grouped into

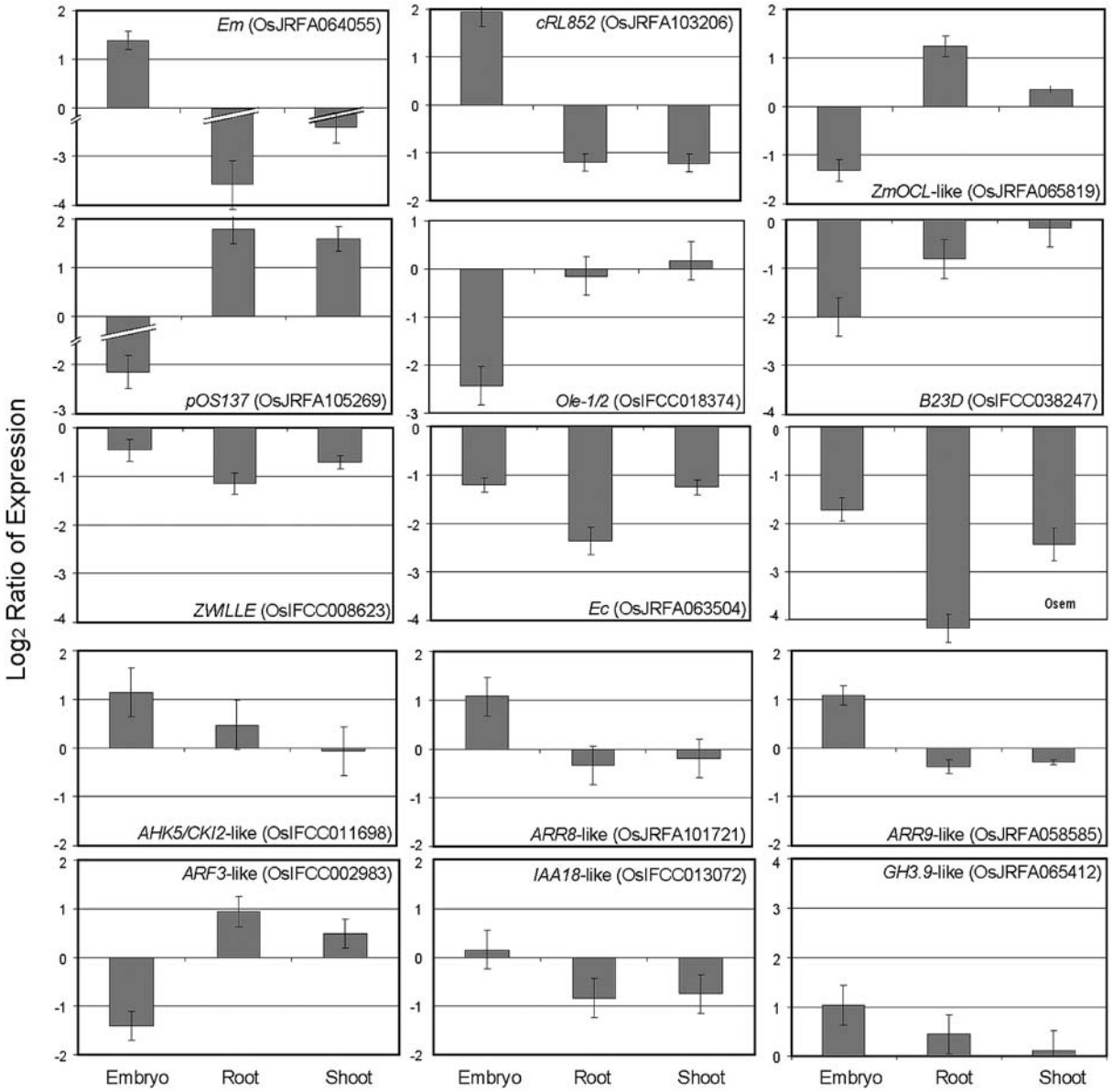


Fig. 6 Expression changes of representative genes known to be differentially regulated during specific organogenesis. Nine previously reported genes involved in organogenesis (*Em*, *cRL852*, *ZmOCL*-like, *pOS137*, *Ole-1/2*, *B23D*, *ZWILLE*, *Ec, and Osem) and six regulated cytokinin and auxin pathway genes are shown (see text for more detail)*

pathways based on their best homologous in the Arabidopsis genome as described previously (Jiao et al. 2005).

To examine similarities and differences among these three organogenesis processes, entire biosynthetic pathways were analyzed (Fig. 8). By comparing regulation of several biosynthetic/assimilation pathways, degradation pathway, and energy pathway, we found these pathways were largely unaffected in all three processes. However, a few pathway steps are induced or repressed in almost each pathway. Despite the general similarity, the highly regulated steps in the pathway may be different in each organogenesis process. For example, in the gluconeogenesis pathway, the genes encoding enzyme catalyzing the 1, 3-diphosphateglycerate to D-glyceraldehyde-3-phosphate step were only weakly regulated during embryogenesis but repressed during roots induction and induced during shoots induction.

To compare comprehensively the transcriptional regulation of major metabolic pathways among three distinct organogenesis processes, we aligned ratios of expression of all enzyme genes during three processes in related pathways (Supplemental Table 3). Pathways for

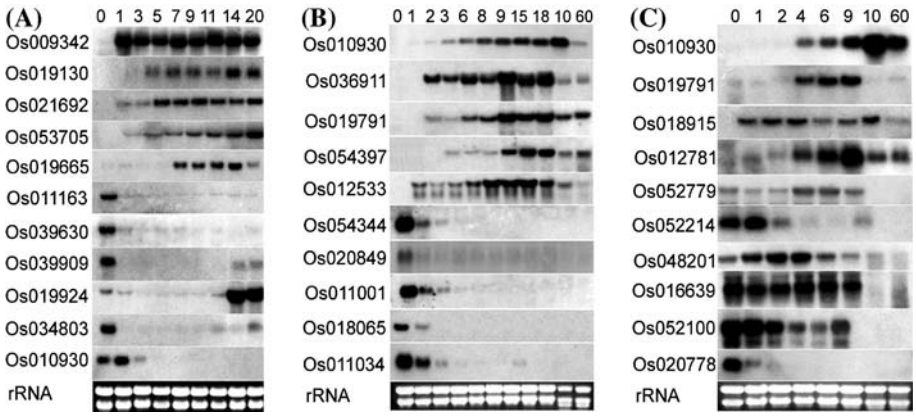


Fig. 7 RNA gel blotting analysis of representative genes shown to be differentially regulated during organogenesis by our microarray analysis. (**a**) The transcript levels during somatic embryo induction. (**b**) The transcript levels during shoot induction. (**c**) The transcript levels during root induction. During the shoot and root induction, the RNA samples of the time course marked 10 and 60 on the top of each panel are from the 10-day-old shoots and 60-day-old roots of rice plants. The transcripts in different induced time (day) on the top of each panel were detected with the probes selected according to the microarray data on the left panel

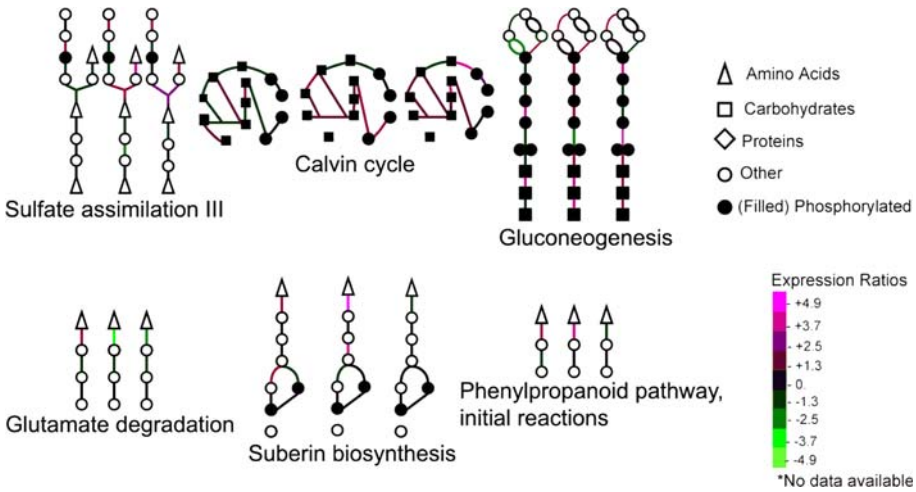


Fig. 8 Diagram of representative biosynthesis pathways affected during organogenesis. Each pathway is shown as glyphs consisting of nodes, which represent the metabolites, and lines, which represent the reactions. Expression-level change of each reaction is shown in a *color* relative to the expression level, as indiacted in *color scale bar* in bottom right. Missing gene expression data, which may come from lack of annotated enzyme, lack of microarray probe, or lack of expression, are represented by *gray lines*. From left to right: embryogenesis, root induction, and shoot induction

the biosynthesis of most primary and secondary metabolites, utilization pathways, and energy pathways (Fig. 9) show variable degrees of similarity in light-regulated expression during these three processes. Again, only a limited number of genes were regulated in all processes. Interestingly, sugars and polysaccharides biosynthesis pathways had more genes induced among all three processes than other pathways. Cell structure pathways had relatively larger portion repressed in all processes. In addition, cases exist where regulation of genes occurs in one process but not or in different direction in others. Interestingly, hormone biosynthesis genes were barely regulated in either process.

Discussion

Organogenesis is a complex developmental process and it is still insufficiently understood. In parallel with in planta studies, cell culture system has been demonstrated as an efficient system to understand organogenesis (Howell et al. 2003). This is especially true for embryogenesis since it is difficult to harvest enough normal embryo samples for embryogenesis. In contrast, cell culture system provides an ideal alternative as it can supply large amounts of homogeneous and reproducible sample. In this study, we used in vitro cultured cell system to understand the transcriptome

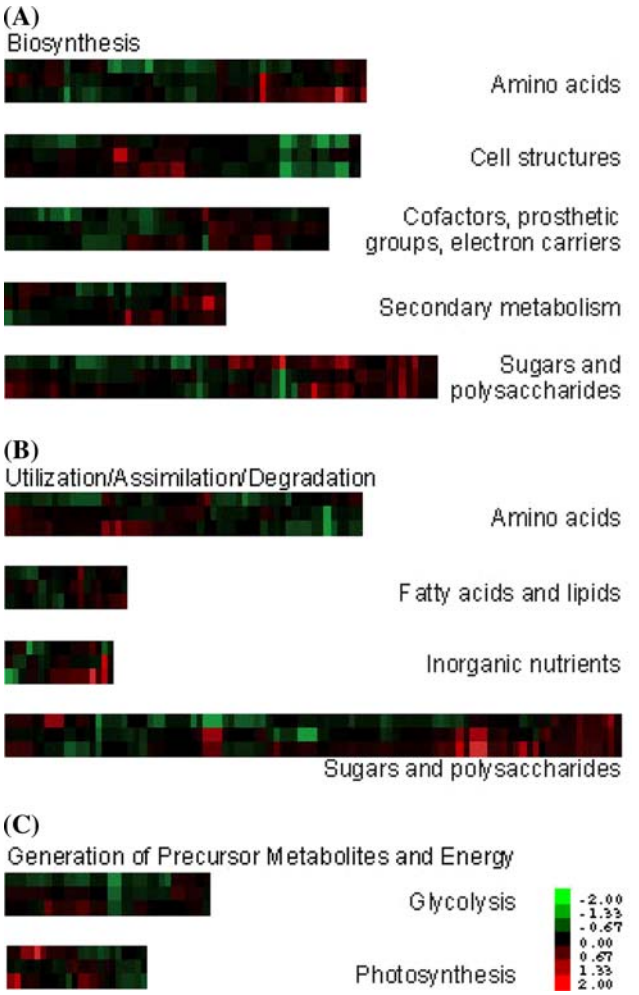


Fig. 9 Transcriptional signature of expression in major metabolic pathways. (**a**) Five representative biosynthesis pathways. (**b**) Four representative utilization/assimilation/degradation pathways. (**c**) Two representative precursor metabolites and energy pathways. Patterns in the gene expression data sets were identified from homologs to *Arabidopsis*. For each pathway, *rectangular blocks* represented the measured changes in expression of each gene. All genes in each pathway are shown. In each pathway block, data from embryogenesis are in the top row, followed by roots induction data and shoots induction data. Expression-level change of each reaction is shown in a *color* relative to the expression level, as indiacted in *color scale bar* in bottom right

reprogramming during rice embryogenesis and shoot and root organogenesis using a whole-genome oligomer microarray covering 36,962 rice genes.

In our in vitro embryogenesis and subsequent organogensis experiments, we found the transcription of a large portion (60–70%) of the genome in all explored conditions. Specifically, 29,409 genes in induced embryos, 23,946 genes in induced roots, and 26,984 genes in induced shoots were detected out of 36,962 genes we monitored. However, only a small portion (1–3%) of these expressed genes was differentially regulated after the in vitro treatments for organ specification (Fig. 2). Moreover, few biochemical pathways exhibit significant differential transcriptional regulation during either process, although we observed expected photosynthetic genes highly expressed in induced shoots. Thus it is tempting to speculate that organogenesis may be regulated by the coordination of a small set of key genes, which lead to the dramatic morphological changes during these organogenesis processes.

A comparison of differentially regulated genes during these three processes revealed that the overlap is very limited, especially between embryogenesis and two other organogenesis processes (Fig. 4). We therefore, conclude that each of these processes is highly diverged from each other. Examination of the roles of key plant hormones illustrates the point. For instance, we found several cytokinin signaling pathway genes were similarly induced during embryogenesis but not during roots or shoots formation. On the other hand, several auxin pathway genes were differentially regulated albeit in distinct manners among the three organogenesis processes, suggesting that auxin may play multiple roles during organogenesis (Fig. 6).

To illustrate similarity and difference between somatic and normal zygotic embryogenesis, we compared the transcriptomes of the somatic embryos and zygotic embryo. We compared all genes in somatic embryogenesis defined in this study with all the public available information about embryo expressed-related genes from *Arabidopsis*. This comparison indicates that 87% (211 of 242) known embryogenesis related genes in *Arabidopsis* have homologous rice genes expressed in somatic embryos. This clearly indicates that a high similarity between the somatic embryos and zygotic embryos. The comparison between rice and *Arabidopsis* also imply that the embryogenesis-related genes are highly conserved between the monocots and dicots.

Overall, the observed gene expression patterns here are in good agreement with published data. It provides a general description of the transcriptional events underlying the morphological changes during those in vitro organogenesis processes. While the reiterative formation of new organs is a very complex process, the genes differentially expressed in the process as defined in this study can provide a starting point to examining roles of new genes involved in the processes.

Acknowledgments We thank Valerie J. Karplus for reading and commenting on this manuscript, and the Yale DNA microarray laboratory of the Keck Biological Resource Center for the printing of the rice 70-mer oligo microarray slides used in this study (http://info.med.yale.edu/wmkeck/dna_arrays.htm). This research was supported by grants from National Institutes of

Health (GM-47850) and National Science Foundation Plant Genome Program (DBI-0325821) to XWD, and by the Ministry of Science and Technology of China special rice functional genomics program. Kun He is supported by Monsanto Fellowship of PYC center, the National Key Basic Research Program of China (2003CB715900), and the National Natural Science Foundation of China (30170232).

References

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997) Genes involved in organ separation in *Arabidopsis*: an analysis of the *cup-shaped cotyledon* mutant. Plant Cell 9:841–857

Bennett MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA (1996) *Arabidopsis AUX1* gene: a permiase-like regulator of root gravitropism. Science 273:948–950

Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Onckelen HV, Montagu MV, Inze D (1995) *Superroot*, a recessive mutation in *Arabidopsis*, confers auxin overproduction. Plant Cell 7:1405–1419

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inzé N, Sandberg G, Casero PJ, Bennett M (2001) Auxin transport promote *Arabidopsis* lateral root initiation. Plant cell 13:843–852

Celenza JL, Grisafi PL, Fink GR (1995) A pathway for lateral root formation in *Arabidopsis thaliana*. Genes Dev 9:2131–2142

Che P, Gingerich DJ, Lall S, Howell SH (2002) Global and hormone-induced gene expression changes during shoot development in *Arabidopsis*. Plant cell 14:2771–2785

Christianson ML, Warnick DA (1983) Competence and determination in the process of in vitro shoot organogenesis. Dev Biol 95:288–289

Chu CC, Wang CC, Sun CS, Hus C, Yin KC, Chu CY (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci Sin 18:659–668

Clark SE, Running MP, Meyerowitz EM (1993) CLAVATA1, a regulator of meristem and flower development in *Arabidopsis*. Development 119:397–418

Clark SE, Running MP, Meyerowitz EM (1995) CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. Development 121:2057–2067

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Communication of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. Science 283:1911–1914

Friml J, Benkova E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K (2002a) AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell 108:661–673

Friml J, Wisniewska J, Benkova E, Mendgen K, Palme K (2002b) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature 415:806–809

Fukaki H, Tameda S, Masuda H, Tasaka M (2002) Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsis*. Plant J 29:153–168

Gälweiler L, Guan C, Muller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. Science 282:2226–2230

Geldner N, Friml J, Stierhof YD, Jurgens G, Palme K (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413:425–428

Gisel A, Barella S, Hempel FD, Zambryski PC (1999) Temporal and spatial regulation of symplastic trafficking during development in *Arabidopsis thaliana* apices. Development 126:1879–1889

Goldberg RB, De Paiva G, Yadegari R (1994) Plant embryogenesis: zygote to seed. Science 266:605–614

Gray WM, del Pozo JC, Walker L, Hobbie L, Risseeuw E, Banks T, Crosby WL, Yang M, Ma H, Estelle M (1999) Identification of an SCF ubiquitin–ligase complex required for auxin response in *Arabidopsis thaliana*. Genes Dev 13:1678–1691

Haberer G, Kieber JJ (2002) Cytokinins. New insights into a classic phytohormone. Plant Physiol 128:354–362

Hattori T, Terada T, Hamasuna S (1995) Regulation of the osem gene by abscisic and the transcriptional activator VP1: analysis of cis-acting promoter elements required for regulation by abscisic acid and VP1. Plant J 7:913–925

Himanen K, Vuylsteke M, Vanneste S, Vercruysse S, Boucheron E, Alard P, Chriqui D, Van Montagu M, Inze D, Beechman T (2004) Transcript profiling of early lateral root initiation. PNAS 101(14):5146–5151

Hobbie L, Estelle M (1995) The *axr4* auxin-resistant mutants of *Arabidopsis thaliana* define a gene important for root gravitropism and lateral root initiation. Plant J 7:211–220

Howell SH, Lall S, Che P (2003) Cytokinins and shoot development. Trends Plant Sci 8:453–459

Hwang I, Sheen J (2001) Two-component circuitry in *Arabidopsis* cytokinin signal transduction. Nature 413:383–289

Hwang I, Chen HC, Sheen J (2002) Two-component signal transduction pathways in *Arabidopsis*. Plant Physiol 129:500–515

Ingram GC, Boisnard-Lorig, Dumas C, Rogowsky PM (2000) Expression patterns of genes encoding HD-ZipIV homeo domain proteins define specific domains in maize embryos and meristems. Plant J 22:401–414

Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K, Kakimoto T (2001) Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. Nature 409:1060–1063

Jiao Y, Jia P, Wang X, Su N, Yu S, Zhang D, Ma L, Feng Q, Jin Z, Li L, Xue Y, Cheng Z, Zhao H, Han B, Deng XW (2005) A tiling microarray expression analysis of rice chromosome 4 suggests a chromosome-level regulation of transcription. Plant Cell 17(6):1641–1657

Kakimoto T (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. Science 274:982–985

Kayes JM, Clark SE (1998) *CLAVATA2*, a regulator of meristem and organ development in *Arabidopsis*. Development 125:3843–3851

King JJ, Stimart DP, Fisher RH, Bleecker AB (1995) A mutation altering auxin homeostasis and plant morphology in *Arabidopsis*. Plant Cell 7:2023–2037

Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM (1995) Formation of lateral root meristems is a two-stage process. Development 121:3303–3310

Laux T, Mayer KFX, Berger J, Jürgens G (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development 122:87–96

Leyser HMO, Furner IJ (1992) Characterization of three shoot apical meristem mutants of *Arabidopsis thaliana*. Development 116:397–403

Leyser HMO, Pickett FB, Dharmasiri S, Estelle M (1996) Mutations in the *AXR3* gene of *Arabidopsis* result in altered

auxin response including ectopic expression from the *SAUR-AC1* promoter. Plant J 10:403–413
Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis*. Nature 379:66–69
Ma L, Chen C, Liu X, Jiao Y, Su N, Li L, Wang X, Cao M, Sun N, Zhang X, Bao J, Li J, Pedersen S, Bolund L, Zhao H, Yuan L, Wong GKS, Wang J, Deng XW, Wang J (2005) A microarray analysis of the transcriptome and its comparison to *Arabidopsis*. Genome Res 15(9):1274–1283
Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development 124:33–44
Mayer KF, Schoof H, Haecker A, Lenhard M, Jürgens G, Laux T (1998) Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell 95:805–815
Mok DWS, Martin RC (1994) Cytokinin metabolic enzymes. In: Mok DWS, Mok MC (eds) Cytokinins: Chemistry, activity and function, chap. 24, CRC Press, pp 129–138
Moussian B, Schoof H, Haecker A, Jurgens G, Laux T (1998) Role of the ZWILLE gene in the regulation of the central shoot meristem cell fate during *Arabidopsis* embryogenesis. Plant J 17:1799–1809
Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K (1998) AtPIN2 defines a locus of *Arabidopsis* for root gravitropism control. EMBO J 17:6903–6911
Müller AJ, Grafe R (1978) Isolation and characterization of cell lines of *Nicotiana tabacum* lacking nitrate reductase. Mol Gen Genet 161:67–76
Mueller LA, Zhang P, Rhee SY (2003) AraCyc: a biochemical pathway database for *Arabidopsis*. Plant Physiol 132:453–460
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497
Ohira K, Ojima K, Fijiwara A (1973) Studies on the nutrition of rice cell culture. Plant Cell Physiol 14:1113–1121
Rinn JL, Euskirchen G, Bertone P, Martone R, Luscombe NM, Hartman S, Harrison PM, Nelson FK, Miller P, Gerstein M (2003) The transcriptional activity of human chromosome 22. Genes Dev 17:529–540
Rinne PLH, Schoot C (1998) Symplastic fields in the tunica of the shoot apical meristem coordinate morphogenetic events. Development 125:1477–1485
Rogg LE, Lasswell J, Bartel B (2001) A gain-of-function mutation in *IAA28* suppresses lateral root development. Plant Cell 13:465–480
Ruegger M, Dewey E, Gray WM, Hobbie L, Turner J, Estelle M (1998) The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast Grr1p. Genes Dev 12:198–207
Rupp HM, Frank M, Werner T, Strnad M, Schmuelling T (1999) Increased steady state mRNA levels of the *STM* and *KNAT1* homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. Plant J 18:557–563
Sakai H, Honma T, Aoyama T, Sato S, Kato T, Tabata S, Oka A (2001) ARR1, a transcription factor for genes immediately responsive to cytokinins. Science 294:1519–1521
Schoot C, Rinne P (1999) Networks for shoot design. Trends Plant Sci 4:31–37
Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 3(1): [Article 3]
Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp Soc Exp Biol 11:118–131
Steeves T, Sussex I (1989) Patterns in plant development, 2nd edn. Cambridge University Press, Cambridge, NY
Suzuki T, Sakurai K, Imamura A, Nakamura A, Ueguchi C, Mizuno T (2000) Compilation and characterization of histindine-containing phosphotransmitters implicated in His-to Asp phosphorelay in plants: AHP signal transducers of *Arabidopsis thaliana*. Biosci Biotechnol Biochem 64:2486–2489
Takada S, Hibara K, Ishida T, Tasaka M (2001) The *CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. Development 128:1127–1135
Teo W, Kumar PP, Goh CJ, Swarup S (2001) The expression of *Brostm*, a *KNOTTED1*-like gene, marks the cell type and timing of in vitro shoot induction in *Brassica oleracea*. Plant Mol Biol 46:567–580
Tian Q, Reed JW (1999) Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. Development 126:711–721
The Gene Ontology Consortium (2000) Gene Ontology: tool for the unification of biology. Nat Genet 25:25–29
Tzafrir I, Pena-Muralla R, Dickerman A, Berg M, Rogers R, Hutchens S, Sweeney TC, McElver J, Aux G, Patton D, Meinke D (2004) Identification of genes required for embryo development in *Arabidopsis*. Plant Physiol 135:1206–1220
Ueguchi C, Koizumi H, Suzuki T, Mizuno T (2001a) Novel family of sensor histidine kinase genes in *Arabidopsis thaliana*. Plant Cell Physiol 42:231–235
Ueguchi C, Sato S, Kato T, Tabata T (2001b) The *AHK4* gene involved in the cytokinin-signaling pathway as a direct receptor molecule in *Arabidopsis thaliana*. Plant Cell Physiol 42:751–755
Vollbrecht E, Veit B, Sinha N, Hake S (1991) The developmental gene *Knotted-1* is a member of a maize homeobox gene family. Nature 350:241–243
William B, Tsang A (1991) A maize gene expressed during embryogenesis is abscisic acid- inducible and highly conserved. Plant Mol Biol 16:919–923
Zhang SP (1995) Efficient plant regeneration from *Indica* (group1) rice protoplasts of one advanced breeding line and three varieties. Plant Cell Rep 15:68–71