Acta Biochim Biophys Sin 2011, 43: 688–697 | © The Author 2011. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmr068.
Advance Access Publication 31 July 2011

Original Article

Structural characterization and expression pattern analysis of the rice *PLT* gene family

Peng Li and Hongwei Xue*

National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China
*Correspondence address. Tel: +86-21-54924059; Fax: +86-21-54924060; E-mail: hwxue@sibs.ac.cn

***Plethora* (*PLT*) genes encode transcription factors containing AP2-domain, and have been shown to maintain the activity of stem cells and regulate the root development of dicot *Arabidopsis thaliana*. Through genome-wide analysis, 10 *OsPLT* members were identified in monocot rice. Quantitative real-time polymerase chain reaction analysis of the expression patterns of these *OsPLT* genes showed that *OsPLTs1−5*, *OsPLTs7−9*, and *OsPLT10* were preferentially expressed in root, stem, or seed, respectively, and *OsPLT6* was preferentially expressed both in root and seed. Further analysis by *in situ* hybridization showed that six root-expressed *OsPLT* genes (1−6) were all expressed in the primodium of crown root, and most of them were expressed in the initial cells adjacent to quiescent center of primary, crown, and lateral roots. In addition, *OsPLT* genes were regulated by multiple hormones, suggesting that OsPLTs might play an important role in the regulation of hormone-mediated development of main, crown, and lateral roots of rice.**

Keywords *OsPLTs*; *Oryza sativa*; expression pattern; root development

Received: February 24, 2011 Accepted: May 3, 2011

Introduction

Root is a key determinant of nutrient and water uptake efficiency for yield increase of crops. Rice is the most commonly cultivated cereal species and the root system of rice is composed of primary roots (also known as main or radical root), crown roots (also known as adventitious root), and lateral roots (LR) that develop from the radicle, stem, and root. In contrast with *Arabidopsis*, in which adventitious roots are rarely formed, rice produces numerous crown roots, a kind of adventitious root that is dominant in the root system of cereals. Four distinct root types can be observed during the life cycle of rice [1]. Early during embryogenesis, root apical meristem (RAM) differentiates and generates the radicle root, followed by five embryonic crown roots emerging from the node of the coleoptile. Subsequently post-embryonic crown roots are formed from the nodes of the main stem and tillers. Both the main and crown roots can branch to generate lateral roots that are initiated by the local activation of pericycle cells from opposite protophloem poles in the differentiation zone [1–3]. Despite that four distinct root types origin from different tissues, RAM in the root tip plays a crucial role during root development.

Similar to *Arabidopsis*, rice RAM also consists of four histogenic initials: epidermis/lateral root cap initial, cortex and endodermis initial, vascular/pericycle initial, and columella initial [1,4]. Quiescent center (QC) is surrounded by these four types of initial cells and is a ubiquitous feature of all angiosperm RAMs, consisting of a population of slowly dividing cells [5]. QC functions as an organizing center to maintain the adjacent, rapidly dividing initial cells as stem cells. Depending on the species and the age of individual root, QC may vary in size from four cells in *Arabidopsis* to 800–1200 cells in maize [6]. Some molecular data suggest that rice QC is much smaller than that of maize [1].

PLT encodes AP2-domain-containing transcription factor, and is essential for stem cell specification and maintenance in the RAM [7,8]. In *Arabidopsis*, four members (*PLT1−3* and *AtBBM*) have been reported to be expressed in root. *PLT1* is mainly expressed in QC, surrounding stem, and columella cells, while *PLT2* has relatively weak signals in QC, surrounding stem cells [7]. *PLT3* accumulates strongly in the columella stem cell layer; whereas the transcript of *AtBBM* (*BABY BOOM*) is detected in both QC and columella stem cells [8]. The overlapping expression patterns imply the additive and dosage dependent activities of these *PLTs*. *plt1* or *plt2* single mutant displays increased numbers of columella tiers and cells, while in *plt1plt2* double mutants the root growth is extremely reduced with more cells and diatribes structure in columella [7]. The *plt1plt2plt3* triple mutant is rootless, and the

plt1plt2plt3bbm quadruple mutant is complete lack of root and hypocotyls [8].

Many genes have been demonstrated to be involved in controlling the expression regions of *PLTs* during root development in *Arabidopsis*. The joint action of five *PIN* genes restricts the *PLT* mRNA expression in the basal embryo region to initial root primordium formation [9]. Auxin binding protein 1 (ABP1) controls the developmental transition between the meristem and the elongation zones by modulating the expression area of *PLT* gradient [10]. In addition, WUSCHEL-related homeobox 5 (WOX5) is required for the activity of *PLT* in defining differentiation of the distal stem cells by acting upstream of PLTs [11]. Recent study has demonstrated that *PLT1* and *PLT2* genes are direct targets of the transcriptional co-repressor TOPLESS and PLT1/2 that are necessary for the homeotic conversion of shoots to roots in *tpl-1* mutants [12]. Root meristem growth factors (RGFs), a redundant family of sulfated peptides, have been shown to maintain the post-embryonic root stem cell niche by defining expression levels and patterns of *PLT* mainly at the post-transcriptional level and act independently of the auxin pathway [13].

In addition, multiple hormones including auxin [14,15], cytokinin [16,17], and ethylene [18,19] have been shown to participate in the regulation and maintenance of stem cell identities. For the lateral root, besides auxin, the key stimulatory hormone in lateral root formation [20,21], cytokinin [22–24] and ABA [25,26] negatively regulate LR formation, whereas ethylene has positive and negative roles during LR formation [27,28]. In addition, auxin and cytokinin have been reported to coordinately regulate crown root initiation and development [29,30].

Although studies have revealed the fundamental role of PLTs in RAM maintenance in *Arabidopsis*, the functions of the homologous genes in monocot rice remains less known. We here reported the identification and comprehensive analysis of rice *PLT* family, which could provide insights into the elucidation of their physiological functions.

Materials and Methods

Plant materials and hormone treatments

Rice plants (*Oryza sativa*, japonica cultivar *Zhonghua 11*) were germinated in sterilized water and then grown in a phytotron with a light/dark cycle of 12 h at 28°C/12 h at 24°C. Primary roots, crown roots, and stem-base were collected from 7-day-old seedlings germinated in water. Old crown roots, leaves, stems, and flowers were collected from 6-week-old plants. Seeds were collected 8 days after flowering.

For hormone treatments, seeds were germinated and grown for 7 days, then treated with IAA (50 μM), ABA (100 μM), Zt (10 μM), and ACC (100 μM) for 1, 3, 8, 12, and 24 h, respectively.

Phylogenetic and protein structure analysis

To identify the *PLT* homologs in rice, *PLT* sequences of *Arabidopsis* from The *Arabidopsis* Information Resource (TAIR; http://www.Arabidopsis.org) were used as query, and the TIGR (http://www.tigrblast.tigr.org/euk-blast), the Knowledge-based Oryza Molecular Biological Encyclopedia (KOME, http://www.cdna01.dna.affrc.go.jp/cDNA) [31], and the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/BLAST) databases were searched by BLASTN and TBLASTN. cDNAs with their corresponding genomic DNA sequences were searched in the GRAMENE Rice Protein Database (http://www.gramene.org/). Chromosomal location and segmental genome duplication events analysis were performed in the website (http://rice.plantbiology.msu.edu/segmental_dup/duplication_listing.html). Domain and motif searches were carried on with the protein sequences in SMART (http://smart.embl-heidelberg.de/) and Pfam (http://www.sanger.ac.uk/software/ pfam). To study the phylogenetic relationship among the *Arabidopsis* and rice PLTs proteins, multiple alignments were prepared using ClustalX2 2.0.12 program and neighbor-joining phylogenetic trees were generated using Mega 5.0 software.

Cis-element analysis of promoter regions of *OsPLT1−6*

Promoter regions of *OsPLT1−6* were analyzed for known consensus *cis*-regulatory elements using Signal Scan Search (http://www.dna.affrc.go.jp/htdocs/PLACE/). The 3-kb upstream sequences of ATG were extracted and analyzed except for *OsPLT2* of which the upstream sequence is only 2523 bp. In addition, manual searches were performed for the auxin response element (TGTCTC, GAGACA, CTCTGT) [32], ABA response element (MACGYGB), cytokinin response element (AGATT), and ethylene response element (GCC box) [33] by TOUCAN software (http://homes.esat.kuleuven.be/~saerts/software/toucan.php).

Quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from various tissues using TRizol reagent (Invitrogen, Carlsbad, USA) and reverse-transcripted according to the manufacturer's instructions (SuperScript pre-amplification system; Promega, Madison, USA). The primers used were as follows (positions after ATG): *OsPLT1* (in the linker region, 5'-TGACACGCC AGGAGTTTGTA-3', 662–681 bp; 5'-TGCCGAATGTT CCGAGGTAG-3', 807–826 bp); *OsPLT2* (in the 3' end, 5'-AGCTACTCCGGTAACAACAT-3', 1254–1273 bp; 5'-CTACTCCATCCCAAACAAAT-3', 1391–1410 bp); *OsPLT3* (in the 3' UTR, 5'-CCATGGGATGATCGATGAAG-3',

2093–2112 bp; 5'-CACCCAGTCAAGCAAAACCT-3', 2236–2255 bp); *OsPLT4* (in the linker region, 5'-AAGGCATAGATGGACAGGAAGAT-3', 852–874 bp; 5'-TTGTCATAACCGCCCAAATAAA-3', 938–959 bp); *OsPLT5* (in the linker region, 5'-CGCAAGGGTCGTCAA GTCTA-3', 934–953 bp; 5'-GATGCACCTCTGGAGAAA CC-3', 1132–1151 bp); *OsPLT6* (in the 3' end, 5'-GG CTACACCCACAACTTCTTC-3', 1327–1347 bp; 5'-CA CATTACGGCTGCCATACA-3', 1562–1581 bp); *OsPLT7* (in the 3' UTR, 5'-TAGGCGAGGTCCATAGCCACTT-3', 2106–2127 bp; 5'-GGTGATGAACGAGGCAAACG-3', 2201–2220 bp); *OsPLT8* (in the 3' UTR, 5'-CGCCT GGACCGACGCCTAAT-3', 927–946 bp; 5'-TCGCCGA GCAACCAAGAACC-3', 1059–1078 bp); *OsPLT9* (in the 3' UTR, 5'-GAGTGCCATTGCTCATCTCC-3', 1881–1900 bp; 5'-CACCAGCCTAGTGCTTACCA-3', 2064–2083 bp); *OsPLT10* (in the 5' end, 5'-TGGTTGGCTT GGCTTCTCCTTGTC-3', 15–38 bp; 5'-GCTTGGGGTC TTTGGCCTCTGC-3', 199–220 bp).

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a RotorGene 3000 system (Corbett Research Pty Ltd, Sydney, Australia) using a SYBR green detection protocol according to the manufacturer's instructions (SYBR Premix Ex Taq System; TOYOBO, Tokyo, Japan). A linear standard curve and threshold cycle number versus log (designated transcript level) were constructed using a series of dilutions of each PCR product; and the levels of the transcript in all unknown samples were determined according to the standard curve. The *ACTIN1* was used for normalizing cDNA concentration variations. Three independent biological replicates were performed.

In situ hybridization analysis

Wild-type primary roots (4 days), crown roots (7 days), and stem-base (7 days) were fixed in a formaldehyde solution (4%), dehydrated through an ethanol series, embedded in paraffin (Sigma-Aldrich, St Louis, USA), and sectioned at 8 μm using an Leica RM2126RT rotary microtome (Bannockburn, Germany). Gene-specific region of *OsPLTs* are amplified by PCR, including a 417-bp region of *OsPLT1* (primers 5'-CGGGATCCCTGCAGT CAGTAGCAACCTTCCAATCG-3' and 5'-CGGAATTC AAGCTTAGCTGTTGTTGGAGCTTTGTG-3'), a 358-bp region of *OsPLT2* cDNA (primers 5'-AGCTACTCCGG TAACAACA-3' and 5'-AATGAGCTTGAATTAGAAAC-3'), a 352-bp of *OsPLT3* cDNA (primers 5'-CCACCAA CCATGGAAACACC-3' and 5'-CACCCAGTCAAGCAA AACCT-3'), a 204-bp region of *OsPLT4* (primers 5'-AGGCAACCCACAGCATCACC-3' and 5'-AAACCCC TCATCGCCCAATC-3'), a 376-bp region of *OsPLT5* (primers 5'-CCACGAGCAGCAGCGACAT-3' and 5'-CT CCATTTCTACCCTTCTAT-3'), and a 255-bp region of *OsPLT6* (primers 5'-GGCTACACCCACAACTTCTTC-3' and 5'-CACATTACGGCTGCCATACA-3'). The amplified DNA fragments were subcloned into a pGEM-T easy vector (Promega) in two orientations; the sense and antisense probes were synthesized with T7 RNA polymerase and used to generate the RNA probe. *In situ* hybridization was performed as described previously [34].

Results

Identification of rice *PLT* genes

To identify *PLT* genes in rice, the TBLASTN search at the TIGR database, the KOME and the NCBI resources was performed using the amino acid sequences of *Arabidopsis PLT* genes as query. Ten homologs sharing similarity with that of *Arabidopsis* were identified in different chromosomes and were designated as *OsPLT1* to *OsPLT10*. The corresponding TIGR gene locus IDs (release 6.1) for all these 10 members are listed in **Table 1**. Among the 10 members, most of them have been annotated as AP2/EREBP transcription factor BABY BOOM, AP2-like ethylene-responsive transcription factor AINTEGUMENTA, and AP2-like ethylene-responsive transcription factor PLETHORA 2.

Structural organization, chromosomal distribution, and protein structure of *OsPLTs*

To clarify the gene structure of *OsPLTs*, the full-length cDNA sequences were compared with their corresponding genomic DNA sequences by Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/index.php) and searched in the GRAMENE Rice Protein Database. The results showed that similar to *Arabidopsis*, the coding sequences of *OsPLTs* consisted of nine exons [**Fig. 1(A)**]. The third to the eighth exon are of the same size in all of these 10 members, haboring 83, 9, 89, 74, 51, and 77 base pairs, respectively. The cDNA sizes of *OsPLTs* lie in the length between the first two exons and the last one (**Table 1**).

In general, Os*PLT* family was larger than that of *Arabidopsis* and these genes were distributed on 7 of the 12 chromosomes (**Table 1**). Genome duplication events are supposed to be occurred throughout the process of plant evolution [35,36] and large-scale duplication events have been recently uncovered in the rice genome with 10 duplicated blocks on 12 chromosomes containing 47% of the total predicted genes [37,38]. Indeed, chromosomal location and segmental genome duplication events analysis showed that *OsPLT3* and *OsPLT4*, *OsPLT7*, and *OsPLT8* were caused by chromosome duplications between chromosome 2 and 4, and chromosome 3 and 7, respectively, and they are located in the third and the sixth blocks, respectively.

Based on available databases, rice PLT peptide sizes range from 314 to 703 amino acids. Further analysis of

Table 1 *PLT* gene family of *Oryza sativa*

Gene	Accession no.	TIGR locus ID	Chromosome	No. of exon	Gene (bp)	ORF length (bp)	Protein (aa)	AP2 domain
OsPLT1	AK111891	LOC_Os04g55970	4	9	3887	1485	495	2
OsPLT2	AK105909	LOC_Os06g44750	6	9	3363	1407	469	2
OsPLT3	AK112070	LOC_Os02g40070	2	9	4145	2109	703	2
OsPLT4	AK287726	LOC_Os04g42570	4	9	3874	1974	658	2
OsPLT5	AK240892	LOC_Os01g67410	1	9	3676	2085	695	2
OsPLT6	AK287621	LOC_Os11g19060	11	9	4863	1677	559	2
OsPLT7	AK241712	LOC_Os03g56050	3	9	3394	1965	655	2
OsPLT8	AK109848	LOC_Os07g03250	7	7	4689	942	314	1
OsPLT9	AK106306	LOC_Os03g12950	3	9	3722	1926	643	2
OsPLT10	-	LOC_Os03g07940	3	9	4148	1647	549	2

TIGR locus numbers are listed. The protein lengths and protein sequences are obtained from the TIGR and GRAMENE databases.

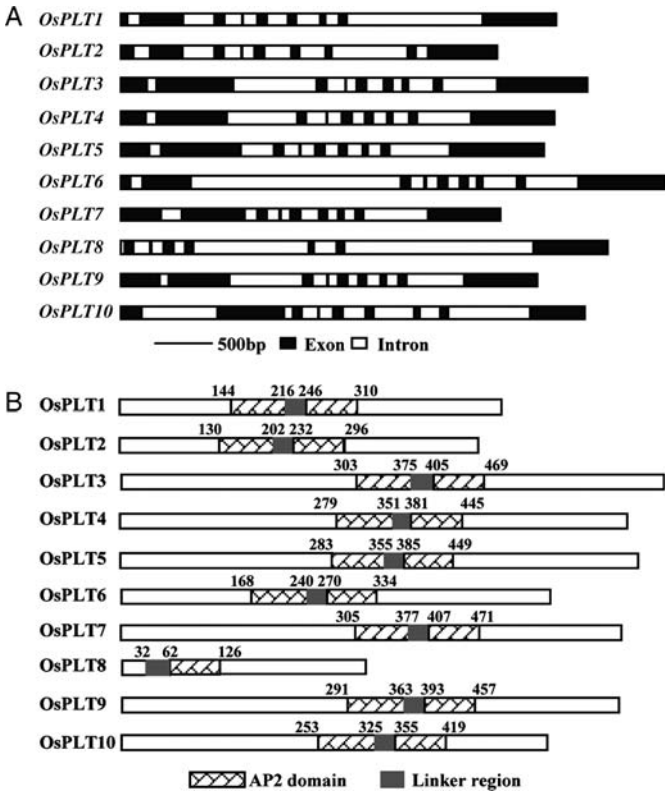

Figure 1 Gene structure and domain structure of *OsPLTs* (A) Exon–intron structures of Os*PLT* genes. White and black boxes indicated the intron or exon, respectively. (B) Schematic representations of the domain and motif structures of OsPLT proteins. Conserved domain and motifs were determined through analyzing OsPLT protein in PFAM and SMART website. Hatch box, AP2 domain; gray box, linker region; white box, other region without any domain.

domain and motif structure by SMART and Pfam website showed that consistent with *Arabidopsis*, all rice PLTs except OsPLT8 contain two repeats of the conserved AP2 DNA binding domain (one is 73 aa and the other 65 aa) and a 29 aa-linker region [**Fig. 1(B)**]. Analysis by KOME and TIGR databases showed that *OsPLT8* (945-bp CDS) lacks the first two exons, indicating that *OsPLT8* encoded a truncated protein lacking the N-terminal consisting of only the linker and the C-terminal AP2 domain. However, comparison analysis revealed the presence of a 984-bp cDNA coding sequence existing before ATG, which might encode the N-terminal AP2 domain.

Further, multiple sequence alignment of both the whole amino acid sequences and the conserved AP2 domain were performed by GENEDOC software and a pairwise analysis of the whole OsPLT protein sequences indicated that the overall identities range from 17 to 76% (**Supplementary Table S1**). The amino acid identity within the conserved AP2 domains is extremely high ranging from 91 to 100% similarity and 84 to 97% identity (**Supplementary Table S2**).

Phylogenetic analysis of OsPLTs

To examine the phylogenetic relationship among the *Arabidopsis* and rice PLT proteins, an unrooted tree was constructed from alignments of the whole protein sequences (**Fig. 2**). The sequences formed separate clusters and were grouped into two major groups (Groups A and B) with well-supported bootstrap values. Six *Arabidopsis* PLTs (PLT1−3, AtBBM, AIL5, and AIL7) and six OsPLTs (OsPLT1−6) proteins were included in Group A. Two *Arabidopsis* PLTs (AINTEGUMENTA and AIL1) and four OsPLTs (OsPLT7−10) were clustered in Group B. According to the results of phylogenetic tree and sequence identity and similarity analysis, OsPLT3 and OsPLT4 in Group A, OsPLT7 and OsPLT8 in Group B are well conserved, consisting with the analysis of chromosome duplication.

Expression pattern of *OsPLT*s

To study the tissue-specific expression pattern of *OsPLT* genes, qRT-PCR was performed to examine the expression of *OsPLTs* in primary root, crown root, old crown root, seeding, stem, stem-base, leaf, flower, and seed (**Fig. 3**). According to the analysis, expression pattern of *OsPLT* genes could be classified into three types: root-preferential

(*OsPLT1−5*), stem-preferential (*OsPLT7−9*), and seed-preferential (*OsPLT6* and *OsPLT10*). *OsPLT1−6* were mainly expressed in primary root, crown root, old crown root, stem-base, and seed. Among them, *OsPLT2−5* showed similar expression pattern with high transcription level in primary root, crown root, old crown root, and stem-base, and relative low level in the seed. *OsPLT1* exhibited high expression in primary root, crown root, old crown root, stem-base, and seed and the highest transcription level of *OsPLT6* was mainly observed in seed. Expressions of *OsPLT7−9* were mainly detected in seeding, stem, and stem-base, while *OsPLT10* was expressed preferentially in seed.

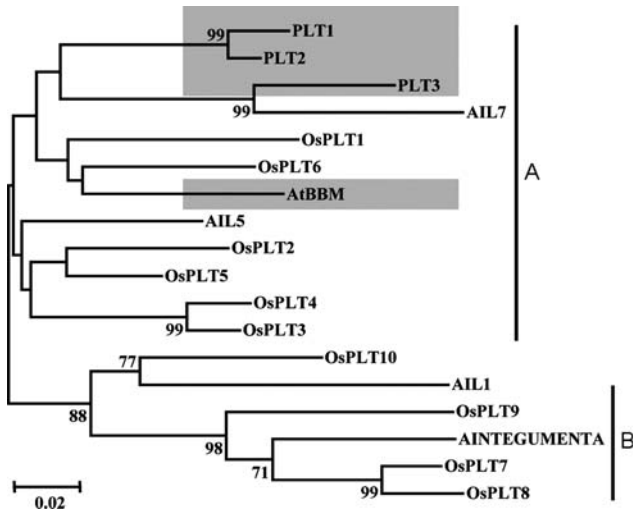

Figure 2 Phylogenetic relationship among the *Arabidopsis* and rice PLT proteins Bootstrap values (above 50%) from 1000 replicates were indicated at each node. PLT1−3 and AtBBM were highlighted in shade.

Expression of *OsPLTs* in root tissue

As PLTs have been shown to be crucial in root development of *Arabidopsis*, we further analyzed the exact expression of root-expressed *OsPLTs* (*1−6*) in root by *in situ* hybridization analysis (**Fig. 4**). Relatively strong signals were detected of *OsPLT1*, *OsPLT2*, *OsPLT3*, and *OsPLT5*, whereas low signals were detected of *OsPLT4* and *OsPLT6*. In root tip, all *OsPLT1−6* genes showed similar expression patterns both in primary root [**Fig. 4(A)**] and crown root [**Fig. 4(B)**]. *OsPLT1* was highly expressed in the stele initial cells of the meristemic zone. *OsPLT2* was moderately expressed in the stele initial cells of the

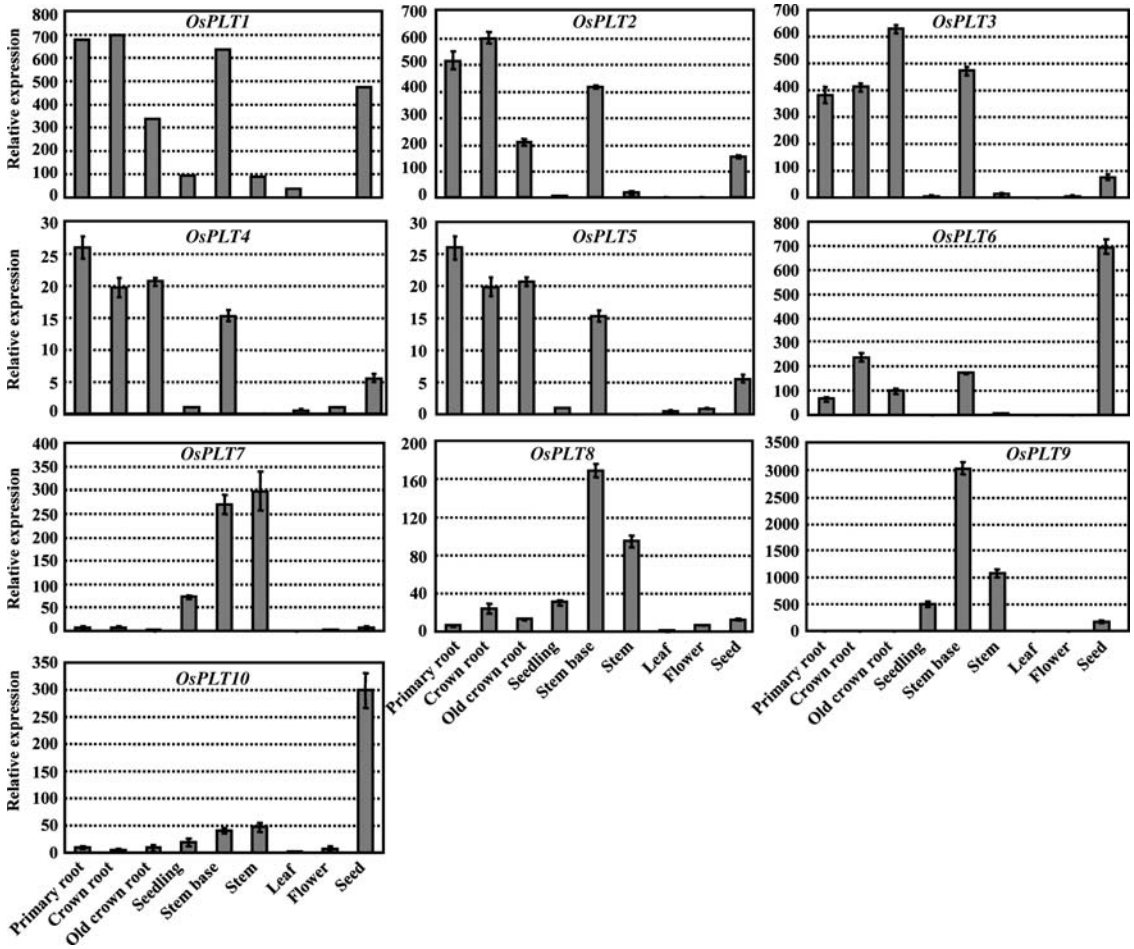

Figure 3 Expression of *OsPLTs* in various tissues qRT-PCR analysis revealed the high expression of *OsPLT1-5* in primary root, crown root, old crown root, stem-base, and seed, and high expression of *OsPLT7−9* in seedling, stem-base, and stem. *OsPLT6* and *OsPLT10* were predominately expression in seed. The experiments were repeated three times with biological replicates. Data were presented as means ± SD.

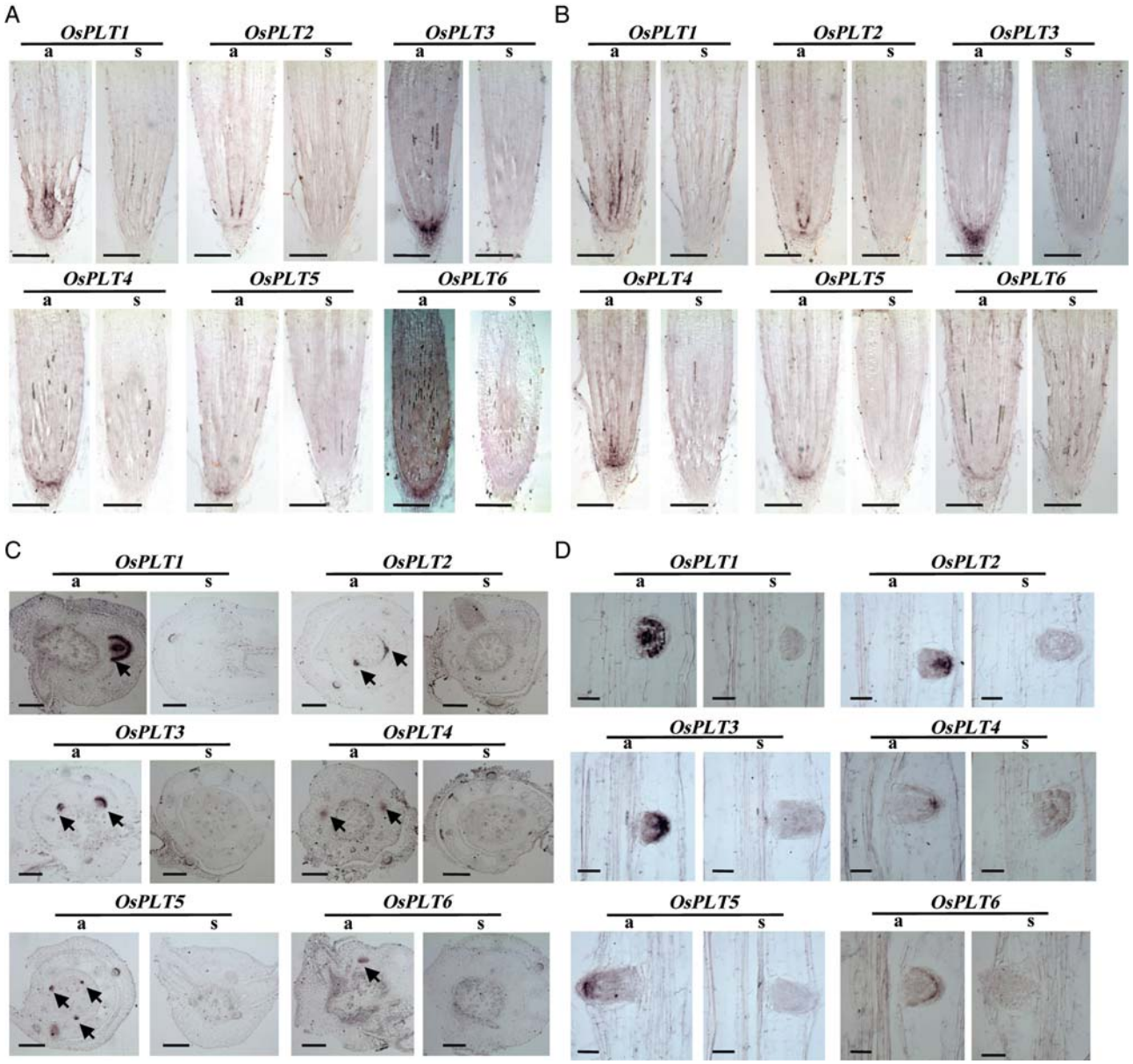

Figure 4 Detailed expression pattern of root-expressed *OsPLT*s (*OsPLT1−6*) *In situ* hybridization analysis of the expression patterns of root-expressed *OsPLT*s in root tip of main root (A), root tip of crown root (B), and cross-sections of stem-base (C) (primodium of crown root is indicated by black arrow), and lateral root (D). 'a' or 's' indicates the hybridization results by using antisense or sense probes. In D, bar = 50 μm.

meristemic zone and lowly expressed in the QC. *OsPLT3* and *OsPLT5* were highly expressed in QC and surrounding initial cells, whereas *OsPLT4* was relatively lowly expressed in QC. Being consistent with the low expression of *OsPLT6* in root by qRT-PCR, *OsPLT6* exhibited the least expression in the QC region of primary root.

Analyses of cross-sections at stem-base were performed to study the expression of *OsPLTs* during initiation of crown root. Consistent with high transcription in stem-base by qRT-PCR analysis, strong signals were detected in the stem-base at the initial cells of crown root for *OsPLT1−5* [**Fig. 4(C)**], while *OsPLT6* exhibited relatively low signal in the primodium of crown root. These results implied that OsPLT1-6 might play an important and redundant role in crown root initiation.

In lateral root, *OsPLT1*, *OsPLT2*, *OsPLT3*, and *OsPLT5* exhibited high expression in QC and initial cells adjacent to QC. *OsPLT4* and *OsPLT6* showed relatively low expression in QC region [**Fig. 4(D)**]. These results suggested that *OsPLT* genes might play distinct and overlapping roles in the differentiation of lateral roots.

Regulation of *OsPLTs* by hormones

Plant hormones including auxin, ABA, cytokinin, and ethylene have been shown to be involved in the regulation of RAM maintenance, initiation, and development of

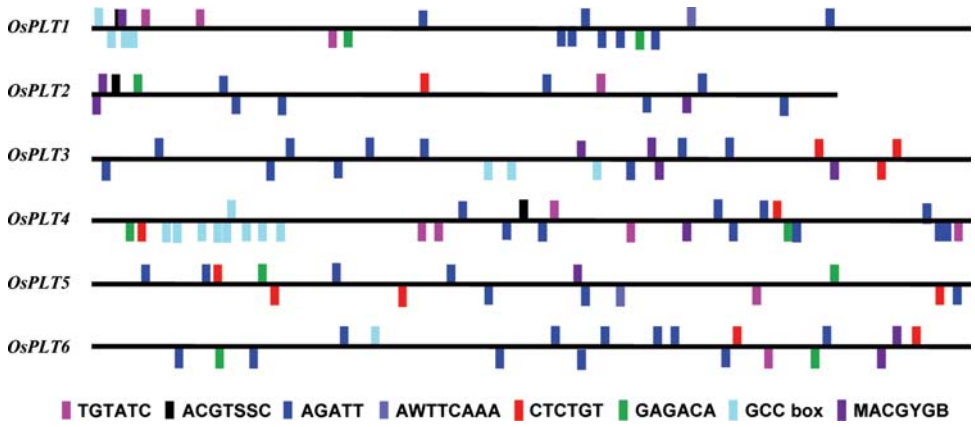

Figure 5 Analysis of hormone response elements in the promoter of *OsPLT1−6* *OsPLT1−6* possessed a number of *cis*-elements in their promoter regions. Computational analyses of promoter sequences were performed using analysis tools from the PLACE website, http://www.dna.affrc.go.jp/htdocs/PLACE/) and manual searches for *cis*-regulatory elements are performed by TOUCAN software. Elements of TGTATC, ACGTSSC, AGATT, AWTTCAAA, CTCTGG, GAGACA, GCC box, and MACGYGB are shown.

Table 2 Auxin-, ABA-, cytokinin-, and ethylene-regulated *cis*-elements in promoters of *OsPLT1-6*

Gene	TGTATC	CTCTGT	GAGACA	MACGYGB	AGATT	GCC BOX
OsPLT1	3	0	2	1	8	4
OsPLT2	1	1	1	3	7	0
OsPLT3	0	3	0	4	10	3
OsPLT4	5	2	2	1	10	9
OsPLT5	1	4	2	1	7	0
OsPLT6	1	2	2	2	11	1

Numbers of *cis*-elements are listed. Auxin-response element: TGTATC, CTCTGT, and GAGACA. ABA-response element: MACGYGB. Cytokinin-response element: AGATT. Ethylene-response element: GCC box.

lateral and crown roots. Analysis of the promoter regions of the root-expressed *OsPLTs* (*1−6*) by PLACE program or by TOUCAN software showed that some auxin response element (AuxRE) [37], ABA response elements (ABRE), ethylene response elements, and cytokinin response element [38] exist in promoter regions (**Fig. 5, Table 2**). These included two TGTCTC and two GAGACA in *OsPLT1*, two GACACA and one reverse sequences CTCTGT in *OsPLT2*, two CTCTGT in *OsPLT3*, one TGTCTC and one CTCTGT in *OsPLT4*, one CTCTGT in *OsPLT5*, one TGTCTC and two CTCTGT in *OsPLT6*, and some ABA-response elements in the promoter region of *OsPLTs*, suggesting that they might be regulated by auxin, ABA, cytokinin, and ethylene.

Then we examined the expression levels of *OsPLT* genes in 7-day-old roots (including primary root, crown root, and lateral root) under treatment with auxin, ABA, cytokinin, and ethylene by qRT-PCR. The results showed that all of these root-expressed *OsPLTs* were induced by auxin. *OsPLT1* responses rapidly and were induced at 1 h but suppressed after 8 h treatment. *OsPLT5* and *OsPLT6* were highly induced at 12 and 8 h, respectively. *OsPLT2−4* were relatively lowly induced [**Fig. 6(A)**].

All *OsPLT1−6* were up-regulated by ABA and *OsPLT1* was the most highly induced one [**Fig. 6(B)**]. The expression of *OsPLT1*, *OsPLT4*, *OsPLT5*, and *OsPLT6* were down-regulated by Zt, while *OsPLT2* and *OsPLT3* were induced at first and then repressed. After 24 h of treatment, the transcriptions of all *OsPLT1−6* genes decline to the lowest level [**Fig. 6(C)**]. Regarding regulation by ethylene, the responses to ACC treatment were different. The transcription level of *OsPLT1* was down-regulated, while *OsPLT2−4* and *OsPLT6* were up-regulated with the maximal induction 24 h after treatment. *OsPLT5* did not show any regulation by ACC [**Fig. 6(D)**]. These results revealed that root-expressed genes *OsPLT1−6* were possibly involved in the hormone-mediated root development.

Discussions

OsPLTs are highly conserved with that of *Arabidopsis* and show differential expression patterns in various tissues especially in roots

In this study, 10 rice *PLT* genes were identified and shared high sequence similarity with their orthologs in *Arabidopsis*, especial the conserved AP2 domain. Similar

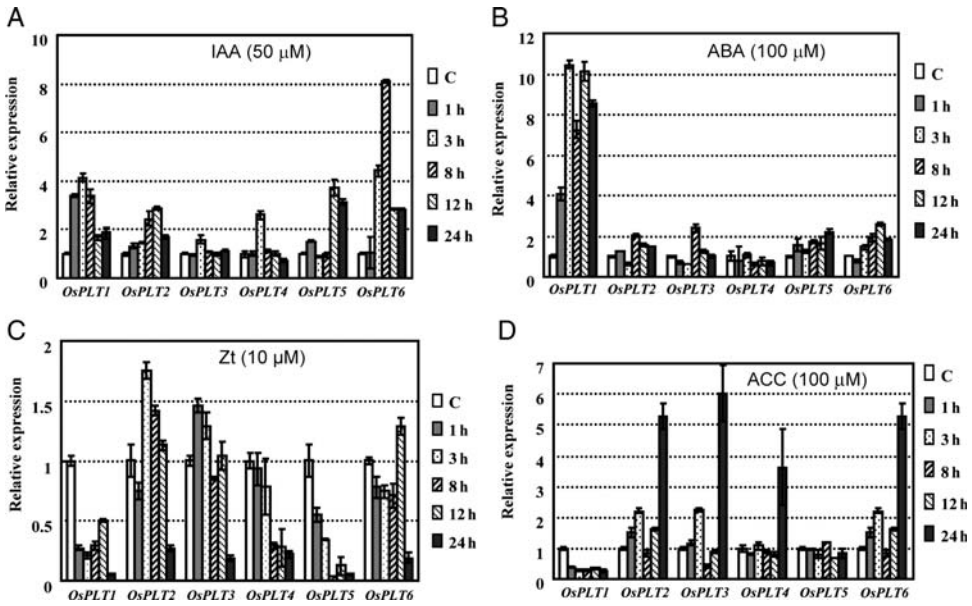

Figure 6 Expression of root-expressed *OsPLTs* (*OsPLT1−6*) under hormone treatments The transcription levels of *OsPLT1-6* under treatments of exogenous IAA (A), ABA (B), Zt (C), and ACC (D) were examined by qRT-PCR analysis. Seven-day-old seedlings were treated and whole roots (including primary root, crown root, and lateral root) were harvested for analysis. The relative transcription levels were shown by setting the expression of corresponding *OsPLT* without treatment as 1.0. The experiments were repeated three times with biological replicates, and the data were presented as means ± SD.

exon−intron organization, protein domain structure, and close phylogenetic relationship between *OsPLTs* and their *Arabidopsis* orthologs suggested that the evolution of PLTs in different species was well conserved, and supported the hypothesis that *PLT* genes in higher plants should be diverged from a single ancestral sequence.

OsPLTs showed complexity of specific and overlapping expression patterns in various tissues except leaf and flower (**Fig. 3**), indicating that they might perform distinct functions or act redundantly in development of various tissues. Being consistent with the phylogenetic analysis that *OsPLT1−6* and *OsPLT7−9* belonged to the distinct groups, the tissue expression pattern analysis showed the root- (*OsPLT1−6*) or stem-expressed (*OsPLT7−9*) patterns, indicating that the evolutionary relationship was to a certain extent related to variation of *OsPLTs* function.

In situ hybridization study further revealed the exact expression pattern of the root-expressed *OsPLTs*. Consistent with *PLTs* functions in *Arabidopsis*, *OsPLT1−5* were highly expressed in the initial cells adjacent to QC region, indicating the conserved functions of *PLTs* in maintaining activity of stem cell around QC both in rice and *Arabidopsis*. As *PLTs* in *Arabidopsis*, *OsPLT1−4* also exhibited strong signal in the QC region of lateral root. However, unlike the dicot plant *Arabidopsis* with tap root system, the root of monocot rice is characterized as a dense fibrous root system producing numerous crown roots from stem dominant in the root system of cereals. The observations that *OsPLTs* exhibited high expression in the initial cells of the crown root suggested their important roles in regulating the development of the crown root.

Functions of OsPLTs in mediating the hormone-regulated root development

Similar structure, expression, and close phylogenetic relationship suggested that PLT proteins classified in the same groups might have similar functions in events common to both monocot and dicot plants. *Arabidopsis PLT* genes are mainly expressed in QC and surrounding stem cells [7,8] and similar expression pattern were detected for *OsPLTs* in primary and lateral root. Functional studies showed that *Arabidopsis PLT* genes play a critical role in both primary and lateral root development [7,8] and are redundantly required for distal cell division patterns and stem cell maintenance in the root meristem [7], which suggests that OsPLTs may also play crucial roles in rice root development. In addition, the expression of *OsPLTs* in the crown root initial cells of stem base indicates their important and different roles from *Arabidopsis* in crown root development. It is proposed that OsPLTs on the one hand have the similar function as *Arabidopsis* in maintaining stem cell niche adjacent to QC in main and lateral roots, and on the other hand, they possess special function particular for monocot rice in crown root formation and development.

Plant hormones play a crucial role in regulating plant development and the plasticity shaping of the plant architecture. Many hormones including auxin, cytokinin, and ethylene have been shown to participate in the regulation

and maintenance of stem cell identities [39]. *Cis*-regulatory elements for auxin, ABA, cytokinin, and ethylene are present in the promoter region of *OsPLT1−6*, indicating these hormones may interact to act on the development of rice root. In *Arabidopsis*, *PLT* genes are not involved with auxin accumulation but rather act downstream of auxin. Accumulation of *PLT* transcripts to auxin occurs significantly later (between 5 and 24 h after IAA application) than that of primary auxin response genes and is dependent on Auxin response factor transcription factors [7]. In rice, the transcription levels of *OsPLT1−6* were up-regulated by auxin treatment, and also induced by ABA (mainly for *OsPLT1*). Similar to that *PLT1* and *PLT2* genes in *Arabidopsis* are down-regulated by cytokinin [16], *OsPLTs* are also repressed by cytokinin. Interestingly, most of the *OsPLTs* are induced by ethylene. These results suggest that OsPLTs probably participate in many processes including RAM differentiation, lateral and crown roots formation, and development via hormone-mediated pathway.

Supplementary Data

Supplementary data are available at *ABBS* online.

Funding

The work was supported by grants from the National Natural Science Foundation of China (30600048) and Chinese Academy of Sciences (KSCX2-YW-N-016).

References

1 Coudert Y, Périn C, Courtois B, Khong NG and Gantet P. Genetic control of root development in rice, the model cereal. Trends Plant Sci 2010, 15: 219–226.
2 Chen CW, Yang YW, Lur HS, Tsai YG and Chang MC. A novel function of abscisic acid in the regulation of rice (*Oryza sativa* L.) root growth and development. Plant Cell Physiol 2006, 47: 1–13.
3 Hochholdinger F and Zimmermann R. Conserved and diverse mechanisms in root development. Curr Opin Plant Biol 2008, 11: 70–74.
4 Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K and Scheres B. Cellular organisation of the *Arabidopsis thaliana* root. Development 1993, 119: 71–84.
5 Jiang K and Feldman LJ. Root meristem establishment and maintenance: the role of auxin. J Plant Growth Regul 2003, 21: 432–440.
6 Jiang K, Meng YL and Feldman LJ. Quiescent center formation in maize roots is associated with an auxin-regulated oxidizing environment. Development 2003, 130: 1429–1438.
7 Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C and Nussaume L, *et al*. The PLETHORA genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 2004, 129: 119–120.
8 Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R and Scheres B. PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. Nature 2007, 449: 1053–1057.
9 Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J and Heidstra R, *et al*. The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 2005, 433: 39–44.
10 Tromas A, Braun N, Muller P, Khodus T, Paponov IA, Palme K and Ljung K, *et al*. The AUXIN BINDING PROTEIN 1 is required for differential auxin responses mediating root growth. PLoS One 2009, 4: e6648.
11 Ding Z and Friml J. Auxin regulates distal stem cell differentiation in *Arabidopsis* roots. Proc Natl Acad Sci USA 2010, 107: 12046–12051.
12 Smith ZR and Long JA. Control of *Arabidopsis* apical-basal embryo polarity by antagonistic transcription factors. Nature 2010, 464: 423–426.
13 Matsuzaki Y, Ogawa-Ohnishi M, Mori A and Matsubayashi Y. Secreted peptide signals required for maintenance of root stem cell niche in *Arabidopsis*. Science 2010, 329: 1065–1067.
14 Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J and Benfey P, *et al*. An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 1999, 99: 463–472.
15 Friml J, Benková E, Blilou I, Wisniewska J, Hamann T, Ljung K and Wood YS, *et al*. AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell 2002, 108: 661–673.
16 Dello Ioio R, Nakamura K, Moubayidin L, Perilli S, Taniguchi M, Morita MT and Aoyama T, *et al*. A genetic framework for the control of cell division and differentiation in the root meristem. Science 2008, 322: 1380–1384.
17 Werner T, Motyka V, Laucou V, Smets R, Van Onckelen H and Schmülling T. Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. Plant cell 2003, 15: 2532–2550.
18 Ortega-Martínez O, Pernas M, Carol RJ and Dolan L. Ethylene modulates stem cell division in the *Arabidopsis thaliana* root. Science 2007, 317: 507–510.
19 Swarup R, Perry P, Hagenbeek D, Van Der Straeten D, Beemster GT, Sandberg G and Bhalerao R, *et al*. Ethylene upregulates auxin biosynthesis in *Arabidopsis* seedlings to enhance inhibition of root cell elongation. Plant cell 2007, 19: 2186–2196.
20 Fukaki H, Tameda S, Masuda H and Tasaka M. Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of *Arabidopsis*. Plant J 2002, 29: 153–168.
21 Hirota A, Kato T, Fukaki H, Aida M and Tasaka M. The auxin-regulated AP2/EREBP gene PUCHI is required for morphogenesis in the early lateral root primordium of *Arabidopsis*. Plant Cell 2007, 19: 2156–2168.
22 Li X, Mo X, Shou H and Wu P. Cytokinin-mediated cell cycling arrest of pericycle founder cells in lateral root initiation of *Arabidopsis*. Plant Cell Physiol 2006, 47: 1112–1113.
23 Laplaze L, Benkova E, Casimiro I, Maes L, Vanneste S, Swarup R and Weijers D, *et al*. Cytokinins act directly on lateral root founder cells to inhibit root initiation. Plant cell 2007, 19: 3889–3900.
24 Kuderová A, Urbánková I, Válková M, Malbeck J, Brzobohaty B, Némethová D and Hejátko J. Effects of conditional IPT-dependent cytokinin overproduction on root architecture of *Arabidopsis* seedlings. Plant Cell Physiol 2008, 49: 570–582.
25 Brady SM, Sarkar SF, Bonetta D and McCourt P. The ABSCISIC ACID INSENSITIVE 3 ABI3) gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in *Arabidopsis*. Plant J 2003, 34: 67–75.
26 De Smet I, Signora L, Beeckman T, Inzé D, Foyer CH and Zhang H. An abscisic acid-sensitive checkpoint in lateral root development of *Arabidopsis*. Plant J 2003, 33: 543–555.
27 Ivanchenko MG, Muday GK and Dubrovsky JG. Ethylene-auxin interactions regulate lateral root initiation and emergence in *Arabidopsis thaliana*. Plant J 2008, 55: 335–347.
28 Negi S, Ivanchenko MG and Muday GK. Ethylene regulates lateral root formation and auxin transport in *Arabidopsis thaliana*. Plant J 2008, 5: 175–187.

29 Hirose N, Makita N, Kojima M, Kamada-Nobusada T and Sakakibara H. Overexpression of a type-A response regulator alters rice morphology and cytokinin metabolism. Plant Cell Physiol 2007, 48: 523–539.
30 Zhao Y, Hu Y, Dai M, Huang L and Zhou DX. The WUSCHEL-related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. Plant Cell 2009, 21: 736–748.
31 Kikuchi S, Satoh K, Nagata T, Kawagashira N, Doi K, Kishimoto N and Yazaki J, *et al*. Collection, mapping, and annotation of over 28,000 cDNA clonesfrom japonica rice. Science 2003, 301: 376–379.
32 Hagen G and Guilfoyle T. Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 2002, 49: 373–385.
33 Ross EJ, Stone JM, Elowsky CG, Arredondo-Peter R, Klucas RV and Sarath G. Activation of the *Oryza sativa* non-symbiotic haemoglobin-2 promoter by the cytokinin-regulated transcription factor, ARR1. J Exp Bot 2004, 55: 1721–1731.
34 Luo D, Carpenter R, Vincent C, Copsey L and Coen E. Origin of floral asymmetry in Antirrhinum. Nature 1996, 383: 794–799.
35 Kent WJ, Baertsch R, Hinrichs A, Miller W and Haussler D. Evolution'scauldron: duplication, deletion, and rearrangement in the mouse and human genomes. Proc Natl Acad Sci USA 2003, 100: 11484–11489.
36 Cannon SB, Mitra A, Baumgarten A, Young ND and May G. The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. BMC Plant Biol 2004, 4: 10.
37 Wang X, Shi X, Hao B, Ge S and Luo J. Duplication and DNA segmental loss in the rice genome: implications for diploidization. New Phytol 2005, 165: 937–946.
38 Yu J, Wang J, Lin W, Li S, Li H, Zhou J and Ni P, *et al*. The Genomes of *Oryza sativa*: a history of duplications. PLoS Biol 2005, 3: e38.
39 Benková E and Hejátko J. Hormone interactions at the root apical meristem. Plant Mol Biol 2009, 69: 383–396.