Transcript profiling of crown rootless1 mutant stem base reveals new elements associated with crown root development in rice

Yoan Coudert1, Martine Bès2, Thi Van Anh Le3,4, Martial Prê3, Emmanuel Guiderdoni2 and Pascal Gantet1,3,4,5*

Abstract

Background: In rice, the major part of the post-embryonic root system is made of stem-derived roots named crown roots (CR). Among the few characterized rice mutants affected in root development, crown rootless1 mutant is unable to initiate crown root primordia. CROWN ROOTLESS1 (CRL1) is induced by auxin and encodes an AS2/LOB-domain transcription factor that acts upstream of the gene regulatory network controlling CR development.

Results: To identify genes involved in CR development, we compared global gene expression profile in stem bases of crl1 mutant and wild-type (WT) plants. Our analysis revealed that 250 and 236 genes are down- and up-regulated respectively in the crl1 mutant. Auxin induces CRL1 expression and consequently it is expected that auxin also alters the expression of genes that are early regulated by CRL1. To identify genes under the early control of CRL1, we monitored the expression kinetics of a selected subset of genes, mainly chosen among those exhibiting differential expression, in crl1 and WT following exogenous auxin treatment. This analysis revealed that most of these genes, mainly related to hormone, water and nutrient, development and homeostasis, were likely not regulated directly by CRL1. We hypothesized that the differential expression for these genes observed in the crl1 mutant is likely a consequence of the absence of CR formation. Otherwise, three CRL1-dependent auxin-responsive genes: FSM (FLATTENED SHOOT MERISTEM)/FAS1 (FASCIATA1), GTE4 (GENERAL TRANSCRIPTION FACTOR GROUP E4) and MAP (MICROTUBULE-ASSOCIATED PROTEIN) were identified. FSM/FAS1 and GTE4 are known in rice and Arabidopsis to be involved in the maintenance of root meristem through chromatin remodelling and cell cycle regulation respectively.

Conclusion: Our data showed that the differential regulation of most genes in crl1 versus WT may be an indirect consequence of CRL1 inactivation resulting from the absence of CR in the crl1 mutant. Nevertheless some genes, FAS1/FSM, GTE4 and MAP, require CRL1 to be induced by auxin suggesting that they are likely directly regulated by CRL1. These genes have a function related to polarized cell growth, cell cycle regulation or chromatin remodelling. This suggests that these genes are controlled by CRL1 and involved in CR initiation in rice.

Background

Molecular mechanisms underlying initiation of new roots have been extensively studied in the plant model Arabidopsis thaliana (Arabidopsis) (for a recent review see [1]). In the embryonic primary root of Arabidopsis, new root meristems derive from pericycle founder cells. These meristems give rise to post-embryonic roots named lateral roots (LR). Several genes related to auxin, involved in the mitotic competence of pericycle cells, in LR primordia initiation, patterning and LR emergence have been identified [2-7]. In cereals, the major part of the root system is made of post-embryonic stem-derived roots named crown roots (CR). Rice (Oryza sativa L.) is a relevant model to study the genetic control of CR development [8]. To date only a few rice mutants with less or no crown root have been identified. One of those is altered in CRL4 (CROWN ROOTLESS4)/OsGNOM1 that encodes an ARF-GEF (ADP-RIBOSYLATION FACTOR-GUANIDINE EXCHANGE FACTOR) [9,10] and is homologous to Arabidopsis AtGNOM1 [5].
AtGNOM1 regulates the intracellular traffic of PIN1 (PINFORMED1) auxin efflux carrier proteins [11], and by consequence modulates polar auxin transport involved in cell division events leading to the differentiation of LR meristem [1]. The study of the crl4/Osgnom1 mutant in rice suggested that OsPIN may regulate polarised auxin transport that controls the first divisions of the ground meristem, the tissue giving birth to CR [9,10]. Two other mutants named adventitious rootless1 (arl1) and crown rootless1 (crl1) are devoid of crown root [12,13]. They are both affected in the ARL1/CRL1 gene (referred as CRL1 hereafter) that encodes an AS2/LOB (ASYMMETRIC LEAVES2/LATERAL ORGAN BOUNDARIES)-domain transcription factor [14]. CRL1 is expressed in parenchyma cells adjacent to the peripheral vascular cylinder of the stem that is the area of CR initiation [12]. CRL1 expression is induced by auxin likely via direct binding of an ARF (AUXIN RESPONSE FACTOR) transcription factor (TF) to its promoter [12]. CRL1 is homologous to Arabidopsis LBD16 (LATERAL ORGAN BOUNDARIES-DOMAIN PROTEIN 16) and LBD29 that are directly induced by auxin via ARF7 and ARF19 and necessary for lateral root initiation [15].

The absence of CR primordia in crl1 mutant suggests that the CRL1 transcription factor acts upstream of the gene regulatory network that control the early steps of CR primordia differentiation. In order to identify genes involved in this process, we compared global gene expression profiles in stem bases of crl1 and wild-type (WT) plants, using rice expression arrays (Affymetrix). We identified 486 genes differentially expressed in the crl1 mutant. To arrange molecular events downstream of auxin and CRL1, we analysed expression kinetics of a selected subset of 47 genes in response to auxin treatment in crl1 and WT. This allowed to identify 3 CRL1-dependent auxin responsive genes. Two of them or their orthologues in A. thaliana, FSM (FLATENNED SHOOT MERISTEM)/FAS1 (FASCIATA1) and GTE4 (GENERAL TRANSCRIPTION FACTOR GROUP E4) were already reported to be involved in chromatin remodelling and to affect shoot and root development, meristem differentiation and functioning. The third one encodes a MAP (MICROTUBULE-ASSOCIATED PROTEIN) that may be involved in the control of cell division. Our results support the conclusion that these genes, and the related biological processes, are likely involved in crown root differentiation and are under the control of CRL1.

Methods
Plant material and growth conditions
Hulled seeds of wild-type and crl1 mutant rice (Oryza sativa cultivar Taichung 65) were disinfected and inoculated under sterile conditions in square Petri dishes (Corning, NY, USA) containing half strength Murashige and Skoog (MS) medium (Duchefa Biochemie B.V., The Netherlands) and 0.8% agar type II (Sigma, MO, USA) and were incubated in a vertical position at 26°C and under a 12 h light/12 h dark photoperiod. Stem bases used for transcriptome analysis were collected from 7 day-old plantlets. For auxin treatment, seven day-old axenic seedlings were transferred in flasks containing half strength MS liquid medium, the culture medium was supplemented with 10^{-3} M indole acetic acid (IAA) (Sigma) one day after transfer. The crl1 mutant is a kind gift of Pr. Makoto Matsuoka (Nagoya University, Japan) [12].

RNA extraction and preparation
For each biological replicate, twenty stem bases (Figure 1) were collected and immediately frozen in liquid nitrogen. RNA were extracted with Plant RNeasy (Qiagen, The Nederlands) and treated on column with Dnase (Qiagen). They were quantified with a NanoQuant at 260 nm wavelength and analysed for quality on a BioAnalyzer 2100 (Agilent, CA, USA). 200 ng of total RNA were used for a single amplification. Antisense RNA strand were then biotynilated according the Affymetrix (Affymetrix, CA, USA) IVT Express protocol.

Array hybridisation and analysis
All steps were performed according manufacturer’s instructions (Affymetrix, CA, USA) unless otherwise stated. Equipments were provided by Affymetrix company.

![wild type](image_url) ![crown rootless1](image_url)

**Figure 1** Portion of stem base collected for transcriptome analysis. Stem bases of wild type (WT) and crown rootless1 (crl1) mutant. White arrows show post-embryonic crown roots. Blue arrow shows emergence point of embryonic seminal root. Red dashed lines delimit sampled zone where CR initiate in WT. Scale bar, 4 mm.
Twelve μg of biotin-labelled antisens RNAs were fragmented and hybridized to GeneChip Rice Genome Arrays (Affymetrix) for 16 h at 45°C and 60 rpm in a Hybridisation Oven 645. Arrays were washed, labelled with phycoerythrin on the Fluidic Station 450 and read with the Scanner 3000 7G. Data acquisition was done with the GeneChip Command Console. Array pictures were analysed with MAS5 algorithm of the Expression Console software (Affymetrix). Default parameters were applied, global scaling method was used to normalise data (TGT value set at 100). A comparative analysis was carried out for each biological replicate with MAS5 algorithm in the GeneChip Operating software (Affymetrix). Probes with a “Present” Detection Call were kept for subsequent analysis, “Absent” or “Marginal” were rejected. The three biological replicates were compared. Expression of a gene was considered as differentially increased or decreased when its signal ratio was consistently superior to 2 or lower than 0.5 respectively with a p-value P ≤ 0.01 in the three replicates. Orygenes Database http://orygenesdb.cirad.fr/ [16] was used to retrieve gene annotation in rice and in Arabidopsis corresponding to selected Affymetrix probes. Microarray data obtained in this study are available in the gene expression omnibus (GEO) database under the reference GSE30818.

RT-qPCR (Reverse Transcription-quantitative Polymerase Chain Reaction)
cDNA synthesis was performed using RQ DNase I-treated total RNA preparations (see above) and SuperScript III reverse transcriptase kit (Invitrogen). Relative transcript abundance of selected genes (See Additional file 1, Table S1) was determined using the Roche LightCycler 480 system and the LC480 SYBR Green I Master kit (Roche Applied Sciences). The range of primer efficiencies observed for the couples of primers used was comprised between 1.77 and 2. Measurements were taken for three or four biologically independent sets of samples. A technical replicate was performed for each replicate. Expression level of EXP (Os06g11070) reference gene was used to normalize gene expression level between the different timepoints [17]. We verified that both in crl1 mutant and WT, the Ct value of the EXP gene remained stable at different times after auxin treatment and was comprised for different independent biological and technical repetition of the experiment between 27.5 and 29. In addition, LightCycler melting curves were obtained for the reactions, revealing single peak melting curves for all amplification products. The amplification data were analysed using the second derivative maximum method, and resulting Cp values were converted into relative expression values using the comparative Ct method [18]. Mean values of expression levels obtained from different biological repetitions were statistically compared using a Student’s t-test.

Results and discussion

Transcript profiling of crownrootless1 stem base

Total RNAs were extracted from WT and crl1 stem bases of 7 day-old seedlings. The sampled zone was located just above the zone of emergence of CR in the WT, which correspond to the zone where CR primordia differentiate (Figure 1). RNAs were amplified, labelled and hybridised on GeneChip Rice Genome Arrays (Affymetrix). Transcript profiling of three independent biological replicates obtained from WT and crl1 were compared. Only genes exhibiting a significant induction or repression in the three replicates were selected for further analysis. We identified 250 and 236 genes down- and up-regulated respectively at least twofold in the crl1 mutant (p-value, P ≤ 0.01). In both groups, about 200 genes were differentially regulated less than 5-fold on average. About 10% of the genes were differentially regulated more than 5-fold in crownrootless1 relative to wild-type (Figure 2A). Genes were annotated automatically with function prediction in rice and Arabidopsis and categorised manually according to their putative molecular function (Figure 2B). About one third of differentially regulated genes had no predicted function and about one quarter was involved in metabolism. Most of the up-regulated genes in crl1 (UPIC) fell into transduction, transcription factor and cell cycle/DNA categories whereas down-regulated genes in crl1 (DOIC) were rather distributed in transduction, post-translation/ proteolysis and transport categories (Figure 2B).

For all genes, and the most similar genes in Arabidopsis, we searched for published data relative to their characterised functions. The rice genome sequence has been available since 2005 [19]. Despite an important effort of the scientific community to assign to each gene a function, very few genes have been functionally characterised yet. Precise information was found for only 32 of the 486 genes identified, mostly in reference to a known function of the Arabidopsis ortholog (Figure 3). Interestingly, most of these genes had an assigned function related to signal transduction in particular auxin or to gene expression regulation (transcription factor, chromatin remodelling factors) associated with root development or meristem differentiation and functioning.

Auxin-induced CRL1 expression was used to determine CRL1-dependent auxin-response genes

In order to determine how the genes identified by transcript profiling operate in the gene regulatory network downstream of CRL1, we took advantage of the known transcriptional regulation of CRL1 by auxin. Auxin (Indole Acetic Acid, IAA) activates CRL1 expression
within one hour following exogenous treatment (Figure 4). As CRL1 is a transcriptional activator (our unpublished results), we anticipated that expression of CRL1 target genes would increase between 2 and 6 hours following auxin treatment in WT plants, but not in crl1 plants. We selected a subset of 47 genes, including most of the 32 genes previously mentioned. These genes were chosen according to a differential regulation in crl1 mutant relative to wild-type, their molecular function or their putative role in a developmental process based on available literature data. Expression kinetics of the selected genes in response to IAA treatment (0, 1, 3 or 6 hours) in WT and crl1 stem base was measured by RT-qPCR.

As expected, CRL1 expression was highly induced 1 hour following auxin treatment and was consistently observed among all the biological replicates with a magnitude ranging between 7 and 12-fold. CRL1 expression level decreased following one hour, as a probable result of a negative feedback loop. Expression of OsIAA4 (INDOLE ACETIC ACID4), an early auxin-responsive gene, was induced in the same time frame than CRL1 thereby confirming the efficiency of the auxin treatment [12,20].

**Auxin homeostasis may be altered in crl1 stem base**

AUX/IAA genes encode regulatory proteins involved in auxin signal transduction that interact with ARF TF and repress their activity in absence of auxin [21]. TIR1-dependent auxin perception leads to the degradation of AUX/IAA via the ubiquitin pathway and to the regulation of expression of auxin-responsive genes by ARF. This mechanism participates in the regulation of LR differentiation in Arabidopsis involving IAA14-mediated ARF7 and ARF19 repression [3,15,22]. In rice, OsARF16 is able to bind in vitro an auxin response element in the CRL1 promoter. Moreover the expression in rice of a mutated form of OsIAA31 altered in its ubiquitination site causes the inhibition of the induction of CRL1 expression by auxin [12] which results in a reduced number of CR [23]. Here we found that OsIAA14 was DOIC and that OsIAA11 and OsIAA24 were UPIC.
OsIAA11 belongs to the same expression cluster than OsIAA31 and is specifically expressed in differentiated roots and stems [20]. OsIAA14 is mostly expressed in plumule and floral organs whereas OsIAA24 is expressed in various organs including radicle or root [20]. Further RT-qPCR analysis showed that OsIAA14 and OsIAA24 were early auxin responsive genes both in WT and crl1 stem bases (Figure 5). This indicates that their regulation by auxin does not require CRL1. It is likely that the absence of root meristem in the stem base of crl1 mutant modifies the auxin balance and results in differential regulation of auxin regulated genes independently of CRL1. This also holds true for the DOIC Os09g09370 gene which encodes OsBTBN18 (Bric-a-Brac, Tramtrack, Broad Complex BTB domain with non-phototropic hypocotyl 3 NPH3 domain) a protein presenting homology with NPH3 family proteins. In Arabidopsis the NPY1 (NAKED PINS IN YUC MUTANTS1) gene belongs to the NPH3 gene family and regulates auxin-mediated plant development [24]. NPY genes are highly expressed in root tips and contribute to root gravitropism response [25]. Further expression analysis showed that Os09g09370 is an early auxin responsive gene but this response to auxin did not require CRL1 (Figure 5). In this study we also characterized two late auxin-responsive genes regulated independently of CRL1: OsMIP1 (MADS-BOX INTERACTING PROTEIN1) and OsPRR95 (PSEUDO RESPONSE REGULATOR95). The DOIC OsMIP1 (Os03g55890) gene is very similar to Antirrhinum ternary complex factor MIP1 gene. MIP1 interacts with MADS-box TF involved in meristem determination during floral transition [26]. The DOIC OsPRR95 (Os09g36220) gene is orthologous to the Arabidopsis APRR5 gene belonging to the APRR1/TOC1 (TIMING OF CAB EXPRESSION 1) quintet that participates in the circadian regulation of numerous genes notably involved in flowering time and light response [27,28]. The latter results suggest that auxin-regulated genes involved in the control of meristem functioning are differentially regulated in the crl1 mutant, but are not under the direct control of CRL1.

Expression of nutrient and water status related genes varies in crl1 stem base

The absence of CR in the crl1 mutant may also modify the nutrient and water status of the plant. We further analysed the differential expression pattern of genes related to these statuses. Genes involved in mineral homeostasis and in the response to water deficit indeed appear to be differentially regulated in crl1 (Figure 3 and Table 1). The DOIC Os09g03860 gene encodes a protein orthologous to AtSPX2 (SPX: SYG1/Pho81/XPR1: SYG1, suppressor of yeast gpal; Pho81, CDK inhibitor in yeast PHO pathway; XPR1, xenotropic and polytropic retrovirus receptor). In Arabidopsis, AtSPX2 with AtSPX1, AtSPX3 and AtSPX4 are involved in phosphate (P) homeostasis [29]. In rice OsSPX1 is a negative regulator of P uptake but the function of the other ones is still unknown [30]. Other genes putatively involved in ion transport such as Os02g57240 that encodes the KOB1 (K⁺ CHANNEL BETSUB UNIT1) have been found to be differentially regulated between WT and crl1 [31].

Several UPIC genes with a function related to osmotic stress or abscissic acid were identified. Os07g05570 encodes the OsERD4 (EARLY RESPONSIVE DEHYDRATION4) protein orthologous to ZmERD4 that enhances drought and salt tolerance when constitutively expressed in Arabidopsis [32]. Os05g41070 encodes a bZIP transcription factor homologous to AtAREB3 (ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3)
that is involved in abscisic acid signal transduction [33]. The DOIC Os05g14550 gene encodes a protein orthologous to the conserved eukaryotic TOR (TARGET OF RAPAMYCIN) kinase that promote cell growth in response to favourable conditions. In Arabidopsis AtTOR is involved in the inhibition of root growth in response to osmotic stress and excess of nitrate [34]. The absence of CR of the crl1 mutant may influence the nutrient and water status of the plant, and consequently modulate the expression of nutrient and stress related genes.

Genes related to meristem differentiation are deregulated in crl1 and some of them are CRL1-dependent auxin responsive genes

Several genes already identified to regulate processes involved in cell or meristem differentiation are differentially regulated in crl1. The DOIC Os08g40620 gene encodes a MAP and is a CRL1-dependent auxin responsive gene (Figure 6). This suggests that it can be a component of the gene regulatory network directly controlled by CRL1. Its function has not been determined yet either in rice or in Arabidopsis. Nevertheless the function of other MAP has been characterized in Arabidopsis. For example, AtMAP70-5 is involved in the maintenance of cellular polarity and ensures regular extension of plant organs [35]. The MAP Spiral2 mutant is defective in directional cell elongation and exhibits right-handed helical growth in longitudinally expanding organs such as the roots [36]. This suggests that the MAP encoded by Os08g40620 may be involved in polarized cellular growth during early steps of CR meristem organization regulated by CRL1.

Different genes have a function related with root hair or root meristem differentiation. The UPIC Os03g42750 gene encodes an Exocyst Complex Component SEC3 family protein orthologous to maize ROOTHAIRLESS1 protein [37,38]. Several UPIC genes encode WD domain G-beta repeat domain containing proteins that can interact with TF or chromatin associated proteins to regulate gene expression and cell or meristem differentiation processes. One of them, OsTTG1 (TRANSPARENT TESTA GLABRA1) (Os02g45810), is orthologous to AtTTG1 that is involved
Table 1 Selected genes for expression analysis by RT-qPCR in response to auxin

| TIGR Id | FC | Annotation | BBMH Arabidopsis | Annotation | References |
|---------|----|-----------|------------------|------------|-----------|
| **UPIC (up-regulated in crf1 relative to WT)** | | | | | |
| Os05g43820 | 11,11 | ras-related protein | At4g28950 | ROP9 (RHO-RELATED PROTEIN FROM PLANTS 9) | [54] |
| Os08g02490 | 8,64 | AT hook motif domain containing protein | At4g12080 | DNA-binding family protein | |
| O Os02g24740 | 8,14 | OsSAUR9 - Auxin-responsive SAUR gene family member | At4g34760 | auxin-responsive family protein | [55,56] |
| O Os03g51580 | 6,89 | helix-loop-helix DNA-binding domain containing protein | At2g22750 | basic helix-loop-helix (bHLH) family protein | |
| O Os07g22534 | 5,78 | WD domain G-beta repeat domain containing protein | At3g49180 | RID3 (ROOT INITIATION DEFECTIVE 3); nucleotide binding | [40,41] |
| O Os02g02600 | 5,06 | serine/threonine-protein kinase Cx32chloroplast precursor | At2g17220 | protein kinase | |
| O Os12g41900 | 4,94 | SET domain containing protein | At5g42400 | SDG25 (SET DOMAIN PROTEIN 25)/ATXR7/MDH9 | [46] |
| O Os02g45810 | 4,72 | WD domain G-beta repeat domain containing protein | At5g24520 | TTT1 (TRANSPARENT TESTA GLABRA 1) | [57] |
| O Os03g20720 | 3,85 | GTPase-activating protein | At1g08680 | ZIGA4 (ARF GAP-like zinc-finger-containing protein ZIGA4) | [58] |
| O Os08g05660 | 3,32 | ZOS8-11 - C2H2 zinc finger protein | At2g27100 | SERRATE (SE) | [44] |
| O Os06g05530 | 2,96 | whirly transcription factor domain containing protein | At2g02740 | WHY3 (WHIRLY 3); DNA binding; PTAC11 | [59] |
| O Os01g62760 | 2,88 | protein phosphatase 2C | At5g59220 | protein phosphatase 2CA | [73,74] |
| O Os05g14550 | 2,86 | Phosphatidylinositol kinase and FAT containing domain protein | At1g50030 | TOR (TARGET OF RAPAMYCIN) | [60] |
| O Os12g06610 | 2,54 | nucleolar complex protein 2 | At3g55510 | RBL (REBELOTE) | [45] |
| O Os12g01140 | 2,39 | ACG kinases include homologs to PKAPK and PKC | At3g45780 | PHOT1 (PHOTOTROPIN 1); protein serine/threonine kinase | [72] |
| O Os01g49160 | 2,33 | MBF8 family transcription factor | | | |
| O Os05g41070 | 2,31 | bZIP transcription factor | At3g56850 | AREB3 (ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3) | [33] |
| O Os07g08460 | 2,30 | OsIAA24 - Auxin-responsive Aux/IAA gene family member | | | [20,61] |
| O Os03g43890 | 2,24 | WD domain G-beta repeat domain containing protein | At5g38230 | MSII (MULTICOPY SUPPRESSOR OF IRA1) | [62] |
| O Os01g69850 | 2,13 | OsMADS65 - MADS-box family gene with MIKC type-box | | | |
| O Os06g49510 | 2,06 | zinc knuckle family protein | At4g19190 | zinc knuckle (CCHC-type) family protein | [63] |
| O Os07g74820 | 2,05 | acyl-CoA dehydrogenase family member 10 | At3g06810 | IBR3 (IBA-RESPONSE 3) | [64] |
| O Os03g43400 | 2,05 | OsIAA11 - Auxin-responsive Aux/IAA gene family member | | | [20,61] |
| O Os03g42750 | 2,02 | roothairless 1 | At1g47550 | AtSec3a (Exocyst complex) | |
| **DOIC (down-regulated in crf1 relative to WT)** | | | | | |
| O Os03g24930 | 25,48 | tyrosine protein kinase domain containing protein | At1g61590 | protein kinase | [65] |
| O Os03g18810 | 15,00 | II-Diaminopimelate Aminotransferase | At4g33680 | AGD2 (ABERRANT GROWTH AND DEATH 2); aminotransferase | [66] |
| O Os11g11790 | 8,16 | NBS-LRR type disease resistance protein | | | |
| A O Os03g55890 | 8,14 | ternary complex factor MIP1 | At5g66600 | unknown protein | [26] |
| O Os03g07450 | 4,24 | HOX21 homeobox associated leucine zipper | At1g69780 | ATHB13 | [67] |
| A O Os09g36220 | 3,60 | OsPRR95 - response regulator receiver domain containing protein | At5g24470 | APRR5 (ARABIDOPSIS PSEUDO-RESPONSE REGULATOR 5) | [68] |
Table 1 Selected genes for expression analysis by RT-qPCR in response to auxin (Continued)

| Accession   | Fold change | Description                      | Accession   | Fold change | Description                      |
|-------------|-------------|----------------------------------|-------------|-------------|----------------------------------|
| Os08g41340  | 3,10        | ras-related protein              | At2g31680   | 8,21        | AtRABA5d (Arabidopsis Rab GTPase homolog Asd) |
| Os09g38890  | 2,81        | T-complex protein                | At5g18820   | 6,50        | EMB3007 (embryo defective 3007)   |
| Os09g09370  | 2,64        | BTB18 - BTB domain with non-phototropic hypocotyl 3 domain | At5g47800   | 3,10        | BTB - NPH3 domain                |
| Os09g55560  | 2,62        | AP2 domain containing protein    | At4g36920   | 7,61        | PLETHORA-like transcription factor |
| Os01g67100  | 2,61        | OsFSM, expressed protein         | At1g65470   | 4,50        | FAS1 (FASCIATA 1)                |
| Os08g40620  | 2,59        | rabGAP/TBC domain-containing protein | At1g29950   | 3,64        | microtubule-associated protein   |
| Os03g58350  | 2,40        | OsAA14 - Auxin-responsive Aux/IAA gene family member | At1g36070   | 3,64        | ATLIP (TUBBY LIKE PROTEIN 8)     |
| Os02g08310  | 2,23        | Tubby-like protein 4             | At1g16070   | 4,62        | SPX (SYG1/Pho81/XPR1) domain-containing protein |
| Os06g03860  | 2,28        | uncharacterized membrane protein | At4g22990   | 5,71        | GTE4 (GLOBAL TRANSCRIPTION FACTOR GROUP E 4) |
| Os01g07630  | 2,18        | BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase | At1g60800   | 5,61        | NIK3 (NSP-INTERACTING KINASE 3) |
| Os07g04700  | 2,16        | MYB family transcription factor  | At3g18100   | 5,62        | MYB4R1 (myb domain protein 4R1)  |
| Os12g37780  | 2,13        | tetrapartiteptide repeat domain containing protein | At2g45300   | 5,71        | NPG1 (no pollen germination 1); calmodulin binding |
| Os02g15220  | 2,13        | bromodomain containing protein, expressed | At1g06230   | 5,71        | GTE4 (GLOBAL TRANSCRIPTION FACTOR GROUP E 4) |
| Os04g26850  | 2,10        | SAD2                             | At5g47800   | 6,50        | EMB3007 (embryo defective 3007)   |
| Os10g37640  | 2,04        | HIT zinc finger domain containing protein | At1g60800   | 5,71        | NIK3 (NSP-INTERACTING KINASE 3) |
| Os02g25990  | 2,04        | OsSAUR12 - Auxin-responsive SAUR gene family member | At5g63830   | 6,50        | unknown protein                  |

*O* a gene mentioned in Figure 3. *A* indicates a CRL1-independent auxin responsive gene. *CA* indicates a CRL1-dependent auxin responsive gene. ID; identifier. BBMH; Best Blast Mutual Hit. TIGR; Rice Genome Annotation Resource [52-74].
altered development including a reduced number of CR, a reduced seminal root growth and strong defects in root meristem structure. OsFSM is also expressed in root tips [50]. The other DOIC and CRL1-dependent auxin responsive gene (Os02g15220) encodes a bromodomain-containing protein homologous to the Arabidopsis GTE4. GTE4 is involved in the maintenance of the mitotic cell cycle and may be related to chromatin remodelling. In gte4 mutants, root development is delayed and the number of lateral root is reduced. In addition, in this mutant, a partial loss of identity is observed for quiescent centre cells and the division pattern of initial cells is disrupted [51]. FSM and GTE4 play important roles in the control of root meristem differentiation and maintenance. The differential expression of FSM and OsGTE4 in response to auxin in crl1 mutant versus WT suggests that these genes are early CRL1 target genes and stress that chromatin structure modulation and cell cycle regulation may be essential parameters in early steps of CR meristem differentiation in rice.

Conclusions

First hypotheses toward the arrangement of the crown root differentiation gene regulatory network controlled by CRL1

The differential transcriptome analysis of crl1 versus WT allowed the identification of a set of genes differentially regulated in crl1 stem base. Further RT-qPCR analysis of the response to auxin of a subset of genes in WT and crl1 was used to determine their dependence to CRL1. Some genes such as OsIAA and NPH3-like, which are involved in auxin response and auxin-mediated plant development are CRL1-independent auxin responsive genes. It is likely that the differential regulation of these genes in crl1 versus WT results from the absence of CR meristems that may modify the auxin balance in the crl1 mutant stem base tissues. The absence of CR in crl1 may also modify the nutrient and water status of the plant and result in the differential expression of genes involved in nutrient uptake or water stress response. Similarly the absence of CR differentiation in crl1 may deregulate the expression of genes involved in the control of meristem differentiation and cell division.

Three genes were found to be CRL1-dependent auxin responsive genes. They are likely directly regulated by CRL1. This concern Os08g40620 which encodes a microtubule-associated protein (MAP) that could be involved in asymmetric control of cell division during early steps of CR meristem differentiation, and the two genes FSM and GTE4 that encode chromatin
remodelling and cell cycle regulation factors known to be involved in rice or Arabidopsis on the proper cell division patterning and maintenance of the root meristem.

Figure 7 summarizes these hypotheses. Among all the genes that we have identified to be misregulated in the crl1 mutant, other CRL1-dependent auxin responsive genes remain to be identified. Their involvement in CR initiation and development will be further investigated with corresponding insertion mutants.

Additional material

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Author details
1. Université Montpellier 2, UMR DAP, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France. 2CIRAD, UMR DAP, Avenue Agropolis TA A-96/03, 34398 Montpellier Cedex 5, France. 3Université Montpellier 2, UMR DIADE, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France. 4University of Sciences and Technology of Hanoi, Department of Biotechnology, Ha Noi, Viet Nam. 5IRD, UMR DIADE, Avenue Agropolis, 34398 Montpellier Cedex 5, France.

Authors’ contributions
YC designed the research, performed the experiments and drafted the manuscript. MB, TYVAL, MP participated in the RT-qPCR analysis. EG helped in drafting the manuscript. PG designed and coordinated the research and drafted the manuscript. All authors read and approved the final manuscript.

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