Title: Genome-Wide High Resolution Expression Map and Functions of Key Cell Fate Determinants Reveal the Dynamics of Crown Root Development in Rice

Running head: Regulation of Rice de Novo Root Organogenesis

Author's Name:

Tushar Garg[1,#], Zeenu Singh[1,#], Anuj K. Dwivedi[2,¥], Vijina Varapparambathu[3,¥], Raj Suryan Singh[1], Manoj Yadav[1], Divya Chandran[4], Kalika Prasad[3], Mukesh Jain[2], and Shri Ram Yadav[1,*]

Author's Affiliations:

[1] Department of Biotechnology, Indian Institute of Technology, Roorkee, Uttarakhand, India

[2] School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi, India

[3] School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram, Kerala, India

[4] Laboratory of Plant-Microbe Interactions, Regional Center for Biotechnology, NCR Biotech Science Cluster, Faridabad, Haryana, India

[#] These authors contributed equally

[¥] These authors contributed equally

[*]**Corresponding Author:**

Dr. S. R. Yadav

Fax: +91-1332-286151

E-mail: shri.yadav@bt.iitr.ac.in

ABSTRACT

Root architecture is a major determinant of plant productivity. Adventitious/crown roots (CRs) developing from non-root tissues shape up the root architecture in rice. Mechanisms underlying initiation and subsequent outgrowth of CR primordia (CRP) remain under explored. Here, we provide genome-wide dynamics of gene expression patterns at distinct developmental stages of CRP formation. Our analyses reveal that early regulated transcription of potential epigenetic modifiers, key transcription factors and cell division regulators prime the initiation of CRP followed by progressive activation of auxin signaling modules ensure their outgrowth. In depth analysis of spatio-temporal expression patterns of key cell fate determinants and the functional analyses of rice *WUSCHEL RELATED HOMEOBOX10* (*OsWOX10*) and *PLETHORA* (*OsPLT*) genes reveal their unprecedented role in CRP development. Our study suggests that *OsWOX10* activates *OsERF3* and *OsCRL1* expression during CRP initiation and *OsPLTs* expression to accomplish their outgrowth. Interestingly, *OsPLT* genes, when expressed in the transcriptional domain of root-borne lateral root primordia of *Arabidopsis plt* mutant, rescued their outgrowth demonstrating the conserved role of *PLT* genes in root primordia outgrowth irrespective of their developmental origin. Together, these findings unveil the molecular framework of cellular reprogramming during trans-differentiation of shoot tissue to root leading to culmination of robust root architecture in monocot species which got evolutionary diverged from dicots.

INTRODUCTION

In rice (*Oryza sativa*), the mature root system is composed of a pole-borne primary/seminal root (PR/SR), shoot-borne adventitious/crown roots (ARs/CRs) and root-borne lateral roots (Itoh et al. 2005, Rebouillat et al. 2009). The origin of various post-embryonic roots is highly diversified in plant species. For example, *Arabidopsis* lateral roots (LRs) originate from the xylem pole pericycle cells of PR (Lavenus et al. 2013) whereas rice LRs originate from endodermal and pericycle cells located opposite to the protophloem (Rebouillat et al. 2009, Bellini et al. 2014). Similarly, ARs in *Arabidopsis* naturally originates from the pericycle cells at xylem pole of the hypocotyl whereas, in rice, ARs/CRs are developed from the innermost ground meristem cells peripheral to the vascular cylinder at the stem base (Itoh et al. 2005, Rebouillat et al. 2009, Bellini et al. 2014). Despite gross morphological similarities among various root types in cereals, both conserved and diverged root-type specific regulatory mechanisms exist. For example, an additive rootless phenotype is seen in rice *radicleless1* (*ral1*) and *crown rootless5* (*crl5*) double mutants (Kitomi et al. 2011a, Orman-Ligeza et al. 2013). Similarly, *OsCRL5*, *WUSCHEL-RELATED HOMEOBOX 11* (*OsWOX11*), *ADVENTITIOUS ROOTLESS 1* (*ARL1*)/*CROWN ROOTLESS 1* (*OsCRL1*), and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3* (*OsSPL3*) regulate CR development and do not significantly affect PR and LR development, whereas *OsLRT1, OsLRT2/OsCYP2, OsIAA11,* and *OsIAA13* are specific regulators of LR development (Meng et al., 2019).

The establishment of founder cells for rice adventitious/crown root primordia (ARP/CRP) requires an induction phase of cell cycle reactivation and cell division in a localized domain of the innermost ground tissues to produce initial cells for the CRP (Itoh et al., 2005; Guan et al., 2015). These initial cells originate from shoot tissues and trans-differentiate to produce root tissues. Different developmental events of *de novo* CR organogenesis have been divided into seven stages, starting from the initial cell establishment for CRP until their emergence (Itoh et al., 2005). Still, only a handful of CRP expressed genes are identified and global gene architecture during CRP development is not explored. Rice *QUIESCENT-CENTER-SPECIFIC HOMEOBOX* (*QHB*) and *SCARECROW* (*OsSCR*) have been identified as the earliest marker genes which are first expressed in the outer layer cells and later on *QHB* expression is restricted to the quiescent center (QC) cells, whereas *OsSCR* expression is limited to the endodermis layer (Kamiya et al. 2003a; 2003b). *OsARL1/OsCRL1* is amongst the early regulators of rice CRP development as in the *crl1* mutants

early cell division is suppressed (Inukai et al., 2001; 2005; Liu et al. 2005). Other TFs, such as *ETHYLENE-RESPONSIVE FACTOR3* (*OsERF3*), *OsTOC168/OsAP2/ERF-40*, *OsCRL5*, and *OsWOX11*, *CYTOKININ RESPONSE REGULATOR 2* (*OsRR2*) and *OsSPL3* play key roles at different stages of CR development in rice (Kitomi et al. 2011a; 2011b, Zhao et al. 2009, 2015, Neogy et. al., 2019, Shao et. al., 2019).

A cross-talk between transcription factors (TFs) and hormonal signaling pathways are instrumental for root development (Lavenus et al. 2013; Lakehal and Bellini, 2019, Meng et al., 2019). Different auxin modules regulate expression of many genes, including TFs during different stages of LR specification and emergence in *Arabidopsis* (De Rybel et al. 2010, Yadav et al. 2010, Lavenus et al. 2013). Mutation in rice genes regulating auxin distribution and signaling such as *CROWN-ROOTLESS4/OsGNOM1* (*OsCRL4/OsGNOM1*) and *PINFORMED1* (*OsPIN*) display defects in CR development (Kitomi et al. 2008, Liu et al. 2009, Li et al., 2019). Most of the TFs regulating CR developments function downstream of auxin signaling pathway. They, in turn, regulate and integrate the signaling pathway during CR organogenesis (Inukai et al. 2005, Liu et al. 2005, Kitomi et al. 2011b, Zhao et al. 2009, 2015, Coudert et al. 2015, Zhang et al., 2018, Lavarenne et al., 2019, Neogy et al., 2019; Mao et al., 2020). In this study, we have investigated the global gene networks operational during *de novo* CRP specification and differentiation in rice. Our genome-wide transcriptome analysis of the CRP collected at the stages of their initiation and outgrowth using laser capture microdissection (LCM) revealed a genetic blueprint for the localized developmental reprogramming during the trans-differentiation process and uncovered dynamic expression patterns of genetic regulators specifically regulated during CRP initiation and outgrowth. We also unfolded conserved functions of *OsWOX10* and *OsPLTs* for promoting post-embryonic root development in dicot and monocot plant species.

RESULTS

Laser Capture Microdissection and Global Gene Expression Profile During CRP Initiation and Outgrowth

To dissect out the molecular determinants and genetic networks during rice CRP development, we performed LCM coupled with RNA sequencing (LCM-seq) to profile transcripts from CRP during their initiation and outgrowth. During the initiation stage, CRP initials are established, which

eventually produce initials for epidermis-endodermis, root cap, and central stele (Figure 1A-1C). During CRP outgrowth, further tissue differentiation leads to the establishment of fundamental organization and growth of CRP (Figure 1A and 1D). Tissues from eleven initiation-stage (Figure 1E and 1E') and ten outgrowth-stage CRP were collected (Figure 1F and F'). The innermost ground tissues peripheral to the vascular cylinder have the competency to initiate a CR-specific developmental program when proper cues are perceived, therefore these tissues were collected as a control (Figure 1G and 1G'). Total RNAs extracted from the LCM collected cells were subjected to RNA sequencing. To uncover the common patterns of gene expression from the high throughput expression data, we performed fuzzy c-means clustering of the global data. The number of clusters was empirically determined as studied earlier (Harrop et al., 2016). Our analysis generated eight clear cluster cores with distinct expression pattern (Figure 1H).

To identify differential gene expression (DEG) patterns, $\log_2$ fold-change ≥ 1.0 ($q<0.05$) and ≤ -1.0 ($q<0.05$) was considered for up- and down-regulation, respectively (Supplemental Dataset 1). A total of 3744 (1619 induced and 2125 repressed) genes were commonly de-regulated during CRP initiation and outgrowth (Figure 1I; Supplemental Dataset 2). The expression of 2429 genes (1213 induced and 1216 repressed) was specifically de-regulated during CRP initiation as compared to control tissues (Figure 1I; Supplemental Dataset 2). Similarly, expression levels of 1294 genes (786 up-regulated and 508 down-regulated) were exclusively altered during CRP outgrowth (Figure 1I; Supplemental Dataset 3). The expression of 1035 genes was higher in the initiation-stage CRP as compared to outgrowing CRP whereas an opposite pattern was observed for 1333 genes (Supplemental Dataset 1). This reveals the dynamic expression patterns of the genes as CRP progress from its initiation to outgrowth. We next analyzed the expression pattern of DEGs across the different zones of growing CRs using an established framework for comparative genomics (Proost and Mutwil, 2018). The activated genes during CRP development showed a high level of expression in the actively dividing meristematic zone, and reduced expression in the differentiation zone (Figure 1J). On the other hand, the CRP repressed genes showed higher expression in the differentiation zone as compared to the meristematic zone (Figure 1K). Thus, our data show that a dynamic transcriptional reprogramming is instrumental for the cell fate change and indicate that the positive regulators of stem cell activities are activated, whereas differentiation promoting genes are repressed during CRP development.

Specific Induction of Epigenetic Modifiers, and Cell Cycle Genes During CRP Initiation

To gain deeper insights into the various biological processes associated during CRP development, gene ontology (GO) enrichment analysis was performed for the DEGs. The biological processes such as regulation of hormonal levels, transcription pre-initiation, RNA processing, cell cycle, cell division, and organ development were enriched with genes exclusively induced in the initiating CRP whereas GO terms related to metabolic processes are associated with the genes exclusively induced during CRP outgrowth (Figure 2A). However, the genes associated with GO terms related to regulation of gene expression and biological processes, hormonal metabolism and signaling, nucleic acid metabolism, cell cycle, and cell division, and developmental processes including post-embryonic root organogenesis have higher expression in the initiating CRP and their expression reduced in the outgrowing CRP (Figure 2B). Collectively, this suggests that primary biological and regulatory processes required for establishment and differentiation of CRP are associated with genes highly expressed during CRP initiation whereas metabolic processes were enriched in outgrowing CRP. Further, the geneset enrichment analysis (GSEA) of transcriptional regulators showed that several members of PHD, SWI/SNF, SET, GNAT, and Jumonji gene families, involved in epigenetic and chromatin-remodelling mediate pre-transcriptional gene regulation, are largely induced during CRP initiation (Figure 3A; Supplemental Table 1).

Transcriptional Regulators Specifically Expressed During CRP Initiation and Outgrowth

The expression of 368 TFs (112 induced and 256) was commonly regulated during CRP initiation and outgrowth (Figure 4B; Supplemental Dataset 3). However, the expression of 191 TFs (83 induced and 108 repressed) was specifically altered during CRP initiation whereas 96 TFs (41 up-regulated and 55 down-regulated) was exclusively de-regulated during CRP outgrowth (Figure 3B; Supplemental Dataset 4). Furthermore, the TFs activated during CRP development are preferentially expressed in the meristematic zone (Figure 3C), whereas the TFs repressed during CRP development are abundant in the differentiation zone (Figure 3D), suggesting activation of meristem-specific TFs and repression of differentiation associated TFs during CRP initiation and outgrowth.

TFs such as *OsCRL1* (LOB-domain), *OsCRL5* (AP2-domain), *OsERF3* (AP2-domain) and *OsRR2* (type-A response regulator) promote CRP initiation (Inukai et al., 2005; Kitomi et al., 2011a; Zhao et al., 2015). Together with *OsCRL1* and *OsERF3*, we identified 83 TFs specifically activated during CRP initiation, of which gene expression of 15 TFs, including *OsCRL1, OsERF3, OsHOX12, OsMFS1, OsHOX1, OsSta2* was induced by auxin in the rice base whereas the expression of remaining TFs including *FREEZY PANICLE* (*FZP*), CRL1-like *OsCRL1L2, OsLBD37/ASL39, QHB, ROC4, OsRR24* and *OsHB34* is not regulated by auxin signaling (Supplemental Figure 1A; Supplemental Dataset 5). Similarly, 6 of 41 TFs, specifically induced during CRP outgrowth, involving *OsAP2/EREBP127, OsNAC10, HSFB4A* and *OsRR5,* are auxin inducible (Supplemental Figure 1B; Supplemental Dataset 5). However, 16 of 112 TFs whose expression was activated during both stages, including *OsDH1, OsNAC039, OsERF61, OsbHLH1, bZIP78* and *OsNAC139* are targets of auxin signaling whereas remaining TFs like *OsPLTs, OsGATA10, 15, OsLBD1-8, OsGRFs* and *OsMADS17* are not regulated by auxin (Supplemental Figure 1C; Supplemental Dataset 5). Thus, our study has identified common and stage-specific, auxin-dependent and auxin-independent transcriptional regulators of CRP development.

Regulatory Modules Controlling Establishment and Positioning of Stem Cell Niches During CRP Initiation

Establishment, positioning and maintenance of SCN close the QC cells in the root meristem is essential for growth and are regulated by WOX5-CLAVATA3 (CLV3)-like, CLE40, CLAVATA1/ARABIDOPSIS CRINKLY4, ACR4 (WOX5-CLE1/ACR4-CLE40) signaling, the *SHORT ROOT-SCARECROW* (SHR-SCR) and Auxin-*PLETHORA* (PLT)-mediated pathways (Sabatini et al., 2003; Aida et al., 2004; Dinneny and Benfey, 2008; Stahl et al., 2009; 2013). The expression of *OsCR4* and *QHB*, putative rice homologs of *ACR4,* and *WOX5,* respectively, was induced in the developing CRP (Figure 3E). However, the expression of both paralogous *SCR* genes (*OsSCR1* and *OsSCR2*) and six *PLT* genes (*OsPLT1-6*) was strongly induced in the developing CRP (Figure 3F and 3G). Further, the expression levels of *OsSHR1, OsSHR2,* and *OsPLT7-OsPLT9,* were higher during CRP initiation and is reduced in the outgrowing CRP (Figure 3F and 3G; Supplemental Dataset 3), suggesting a mechanism with conserved regulators might be involved in establishing functional SCN in the developing CRP.

OsCRL1 is a master regulator of CRP initiation, therefore we analyzed expression pattern of genes activated by *OsCRL1* during CRP development (Coudert et al., 2011; 2015; Lavarenne et al., 2019). Consistent with the expression pattern of *OsCRL1*, the expression of 29 *OsCRL1*-activated TFs but none of the *OsCRL1*-repressed TFs were induced during CRP development (Supplemental Dataset 6). Of these, 11 TFs including *QHB*, *OsFZP*, *OsRR24*, *HOX12* and *OsLBD37* were specifically induced during CRP initiation, 5 TFs including *OsRSR1* and *OsARF8* were exclusively activated during CRP outgrowth whereas 13 TFs including *OsPLT3* and *OsNAC039* were induced both during CRP initiation and outgrowth (Supplemental Figure 2A-2C; Supplemental Dataset 6), revealing distinct *OsCRL1*-dependent regulatory modules during CRP initiation and outgrowth.

Temporal Regulation of Auxin Homeostasis and Transport During CRP Development

Generating local auxin maxima is an early event to activate auxin signaling in the localized domains to trigger post-embryonic root developmental programs (Benková et al., 2003; Dubrovsky et al., 2008; Yadav et al., 2011). The transcription of four putative auxin biosynthesis genes of the YUCCA gene family (*OsYUC1*, *4*, *7*, and *9*) was activated during CRP initiation (Figure 4A; Supplemental Dataset 7). The expression levels of *OsYUC4*, *7*, and *9* were progressively increased, but *OsYUC1* was reduced as CRP progress from initiation to outgrowth stage. In contrast, expression of five GH3 genes (*OsGH3.1*, *OsGH3.2*, *OsGH3.4*, *OsGH3.8*, and *OsGH3.13*) was activated during CRP initiation, but all except *OsGH3.2* were progressively reduced in outgrowing CRP (Figure 4A). The expression of *OsGH3.5* and *OsGH3.9* was exclusively induced during CRP outgrowth (Figure 4A). Besides local auxin biosynthesis and homeostasis genes, the expression of *OsPIN8* and *OsPIN10A*, regulators of polar auxin transport, was specifically induced during CRP initiation, whereas *OsPIN2* was activated and *OsPIN1D* was repressed only during CRP outgrowth (Figure 4A). These together suggest local and stage-specific YUC-GH3-PIN modules for auxin homeostasis and transport.

Dynamic Expression Pattern of Auxin Signaling During CRP Initiation and Outgrowth

The Aux/IAA proteins negatively regulate auxin signaling by post-translationally repressing auxin response factors (ARFs). The expression of 5 *OsIAA* genes was specifically induced and only *OsIAA13* is repressed during CRP initiation whereas 4 *OsIAA* genes were exclusively repressed and none was specifically induced in outgrowing CRP (Supplemental Dataset 7). Furthermore, the

expression levels of 11 *OsIAA* genes were repressed and 3 genes were induced when CRP progress from initiation to outgrowth stage (Supplemental Dataset 7). Further, we show transcriptional activation of *OsARF16* exclusively during CRP initiation, whereas *OsARF8* and *OsARF75* were exclusively induced in the outgrowing CRP (Figure 4A; Supplemental Dataset 7). However, *OsARF10* transcription is induced during both the stages, whereas transcription of *OsARF22* was progressively induced and the expression of a set of *OsARFs* including *OsARF19* was decreased in the developing CRP (Figure 4A; Supplemental Dataset 7).

Further, we studied the spatio-temporal auxin response during rice CRP development using DR5-erYFP reporter. Our RNA *in situ* hybridization and immunohistochemistry analysis revealed that auxin response is initiated in a localized domain at very early stage of CRP specification and no signal was detected in CRP of wild-type plant (Figure 4B and 4B1; Supplemental Figure 3A). During later stages of CRP outgrowth, auxin signaling is more at the tip of CRP (Figure 4B2-4D), which eventually gets restricted to QC, columella and initial cells of the emerged and growing root tip (Yang et al., 2017). Our qRT-PCR analysis validated auxin induction of few selected CRP expressed genes upon IAA treatment (Figure 4E; Supplemental Figure 3A-3D). All these observations together suggest that auxin signaling is activated progressively during induction and transition of CRP program from initiation to outgrowth stage.

Spatio-Temporal Expression Pattern of Transcription Factors in Developing CRP

Next, we studied the detailed temporal and spatial expression of a few selected genes exclusively activated during CRP initiation in LCM-seq data to uncover the onset and dynamic expression pattern during CRP development. We selected an AP2 domain-containing transcription factor, *OsERF3,* an auxin response factor, *OsARF16,* a cytokinin response regulator, *OsRR24,* and an auxin-responsive homeobox-containing transcription factor, *OsHOX1* for RNA *in situ* hybridization using DIG-UTP labeled anti-sense and sense RNA probes. We observed that all of these genes were specifically and strongly expressed in developing CRP, and other tissues did not show any expression above the background level (Figure 5A-5L). The onset of expression of *OsERF3, OsARF16,* and *OsHOX1* was in the localized domains of tissues peripheral to the vascular tissues during CRP establishment (Figure 5A, 5D and 5J) and they continue to express in the developing CRP (Figure 5B, 5E, 5K). The expression of *OsRR24* was detected at slightly later

stages when CRP is already established (Figure 5G). The expression of *OsERF3* and *OsRR24* is throughout the early CRP, but in the outgrowing CRP, the expression is reduced in the root cap tissues (Figure 5A and 5B; 5G and 5H). The expression of *OsARF16* was initiated at the early stage of CRP specification and continued to express in the differentiating CRP. The expression was more restricted towards the apical region of the CRP as compared to the base of the CRP (Figure 5D and 5E). In the outgrowing CRP, the expression of *OsRR24* was reduced in the QC and surrounding initials of the ground and vascular tissues, and their immediate daughter cells (Figure 5H). The *OsHOX1* has a very strong expression in the site of CRP specification and relatively uniform expression in the late CRP (Figure 5J and 5K). However, the cross-sections hybridized with sense probes did not see any signal above the background levels (Figure 5C, 5F, 5I, and 5L). This study confirms that the expression of these TFs is confined to developing CR primordia and suggests a strict necessity of their spatial regulation during CR development.

OsWOX10 **Function is Required to Promote Adventitious Root Formation**

Arabidopsis WOX11 and *WOX12*, members of WOX gene family, regulate first-step cell fate transition during root organogenesis (Liu et al. 2014; Hu and Xu, 2016). We studied function of a related WOX gene, *OsWOX10* whose expression is specifically activated during early stage of CRP initiation (Figure 6A; Lian et al., 2014). Our detailed temporal and spatial expression pattern analysis demonstrated that *OsWOX10* transcription is specifically activated in the founder cells of CRP, prior to their establishment (Figure 6B) and continue to express in the initiating and outgrowing CRP (Figure 6C and 6D).

To investigate function of *OsWOX10* during CR development, an inducible RNA-interference (RNAi) construct was used to down-regulate endogenous expression of *OsWOX10* in rice. Its partial knock-down resulted in a significant reduction of root regeneration in the plantlets when transferred to the rooting media (Figure 6E; Supplemental Figure 4A). In contrast, ectopic over-expression of *OsWOX10* in transgenic rice caused extensive root formation (Figure 6F-6I). Rice plantlets initiate robust rooting even in the shoot regeneration media (Figure 6G) and also develop significantly higher number of roots in the rooting media (Figure 6I). Further, qRT-PCR analysis confirmed partial down-regulation and over-expression of *OsWOX10* in these lines (Supplemental Figure 4B and 4C) and also demonstrate that *OsWOX10* induced the expression of two early

regulators of CRP initiation (*OsERF3* and *OsCRL1*) and *OsPLTs* (*OsPLT2*, *OsPLT3* and *OsPLT4*) but not related genes, *OsCRL1L2* and *OsMFS1* (Figure 6J; Supplemental Figure 4D). These observations together suggest that *OsWOX10* is necessary and sufficient for rice adventitious root development. Next, we studied if molecular function of *OsWOX10* is conserved across plant species. Ectopic over-expression of *OsWOX10* in wild-type *Arabidopsis* produced an extensive root system (Figure 6K). Importantly, *OsWOX10* over-expression in *wox11-2* and *wox12-1* single mutants, and *wox11-2 wox12-1* double mutant caused similar effects as in wild-type, suggesting that function *OsWOX10* in *Arabidopsis* is independent of *WOX11* and *WOX12.*

***PLETHORA* Genes Are Required for Adventitious Root Development in Rice**

The expression of few rice *PLETHORA* (*PLT*) genes were regulated by *OsWOX10,* hence we studied the function of *OsPLTs* during rice CR development. Rice genome encodes 10 PLT genes and *OsPLT1*-*OsPLT6* are expressed in the developing CRP (Li and Xue, 2011). *OsPLT8* (*OsCRL5*) function during CR initiation (Kitomi et al., 2011b). We analysed temporal and spatial expression pattern of *OsPLT7,* a close relative of *OsPLT8,* during CRP development. *OsPLT7* expression was activated very early at onset of CRP specification (Figure 7A), with continued expression throughout the CRP during their development (Figure 7B and 7C) and in the meristem of the emerged CRs (Figure 7D).

To uncover function of *OsPLTs* during rice CR development, an inducible double RNAi construct was generated to down-regulate *OsPLT1* and *OsPLT4.* In the transgenic lines, where either of the genes were partially down-regulated, the CR development was compromised (Figure 7E-7I), suggesting that *OsPLT1* and *OsPLT4* are required for establishment of proper root architecture in rice. In *Arabidopsis, PLTs* regulate lateral root outgrowth (Du and Scheres, 2017). We next studied if rice *PLTs* function is specific to root-type and plant species. To analyze whether the role of *PLT*s in lateral root primordia outgrowth from root tissue is conserved across species, we expressed the *OsPLT* genes under *Arabidopsis* regulatory element in *plt3;plt5-2;plt7* triple mutant. The *plt3;plt5-2;plt7* triple mutant is characterized by aberrant lateral root primordia which are defective in outgrowth (Figure 7J2). Strikingly, the lateral root outgrowth defect in the *plt3;plt5-2;plt7* triple mutant was rescued by *OsPLT1* when it expressed in *Arabidopsis PLT3* domain (Figure 7J3-7J4). Similarly, *OsPLT2* expression under Arabidopsis *PLT5* promoter (*plt 3;5;7; AtPLT5::OsPLT2-*

YFP) also rescues lateral root outgrowth in the triple mutant wherein upon reconstitution lateral root formation resembles the wildtype (Supplemental Figure 5; Radhakrishnan et al., 2020). The broader domain of *OsPLT2* expression near the emerging LRP when reconstituted in the *AtPLT5* domain can be attributed to the transcriptional activity of *AtPLT5* promoter (Supplemental Figure 5; Hofhuis et al.,2013). This suggests the role of *PLT* genes in lateral root outgrowth is conserved across species.

DISCUSSION

The process of CRP development begins with an induction phase where innermost ground tissues of rice stem base re-enter in the cell cycle in a localized domain to establish founder cells for CRP (Itoh et al., 2005; Guan et al., 2015). This would require a genetic reprogramming in the cells competent for CRP initiation in response to proper endogenous cues. Our study not only provides a global gene expression map of the CRP at the stage of their initiation and outgrowth but also reveals regulatory role of key cell fate determinants at different stages of CRP development. Importantly, our functional study reveal conserved role of rice WOX and PLT genes in controlling the root architecture across the plant species.

Auxin-Mediated Gene Regulatory Modules During CRP Development

Consistent with known role of auxin maxima and auxin signaling in *Arabidopsis* post-embryonic root development, we find localized auxin maxima, progressive surge of local auxin biosynthesis genes and converse pattern of negative regulators of auxin signaling during CRP development. Different regulatory modules involving components of the auxin signaling pathway and transcription factors are known to regulate post-embryonic root development in plants. The IAA28–ARF5, 6, 7, 8, 19-GATA23 module is important for the specification of the LR founder cells whereas IAA14-ARF7, 19-LBD16, 18, PUCHI module is required for regulating LR initiation in *Arabidopsis* (De Rybel et al., 2010, Lavenus et al., 2013, Kang et al., 2013). In growing roots, the IAA17/AXR3-ARF10/16 module functions in the root meristem to restrict expression of *WOX5* to QC and expression of *PLT1* (Ding and Friml, 2010). We observed dynamic expression pattern of related genes during rice CRP development with plausible regulatory divergence. For example, unlike in *Arabidopsis*, the expression of *PUCHI* homolog, *OsFZP* is not induced by auxin signaling. Instead, *OsFZP*-related *OsERF3* and *OsMFS1* are auxin inducible. Based on the

reported functions of the putative homologous genes in LR development, phylogenetic relationship and their co-expression pattern in our LCM-seq analysis, a possible regulatory module could be OsIAA13/30-OsARF16/19-OsWOX10, OsCRL1 during CRP initiation and OsIAA11/23/30-OsARF8/22-OsDH1 during CRP outgrowth (Jain et al., 2006; Wang et al., 2007; Jun et al., 2011, Kitomi et al., 2012). However, OsIAA30-OsARF10-OsCRL1, QHB might functions during meristem maintenance (Lavarenne et al., 2019). A detailed protein-protein interaction and combinatorial mutant analysis would be required to demonstrate species and root-type specificity in regulatory modules.

Epigenetic Regulation During CRP Development

The induction phase during CRP establishment would require the acquisition of pluripotency through genetic reprogramming in response to localized developmental cues. Stable but reversible modification of DNA and histone proteins along with chromatin remodeling factors provide epigenetic regulation of gene expression to control the crucial balance between stem cell self-renewal, cellular patterning, and tissue-specific differentiation during plant growth and development, and various stresses (Takatsuka and Umeda, 2015; Servet et al. 2010; Ojolo et al. 2018; Singh et al., 2020). *Arabidopsis* GCN5-related N-acetyltransferases family (GNAT), SWI2/SNF2 factors, and PHD domain-containing factors play a key role in positioning the SCN by epigenetically regulating expression domain of *PLTs* and *WOX5* (Kornet and Scheres, 2009; Servet et al., 2010; Sang et al., 2012; Napsucialy-Mendivil et al., 2014; Zhang et al., 2015). In rice, WOX11 recruits the GCN5-ADA2 complex to activate the expression of genes required for cell division in the CR meristem (Zhou et al., 2017). A global epigenetic control of auxin signaling is evident from differential H3K27me3 pattern associated with genes involved in auxin biosynthesis, distribution, and signaling between dividing and differentiated cells or during the acquisition of pluripotency (Lafos et al., 2011; He et al., 2012; Chen et al., 2016; Yamamuro et al., 2016; Mateo-Bonmatí et al., 2019). Members of CHD3 family, *PICKLE* (*PKL*) regulates LR development through the IAA14-ARF7/19 auxin signaling module (Fukaki et al., 2006) and *OsCRL6/OsCHR4* controls CR development by epigenetically regulating expression of YUCCA genes (Wang et al., 2016; Guo et al., 2019). Our genome-wide LCM-seq data suggests involvement of epigenetic regulation during cellular de-differentiation and re-differentiation required for genetic reprogramming during cell fate change during trans-differentiation.

OsWOX10 primes the initiation and *OsPLTs* promote outgrowth of CRP

Auxin-responsive *Arabidopsis WOX11* and *WOX12* redundantly regulate first-step cell fate transition from competent cell to root founder cells during *de novo* root organogenesis (Liu et al., 2014). Our functional studies along with auxin induced activation of *OsWOX10* in the CRP founder cells uncovers the non-redundant function of *OsWOX10* in priming developmental program for root organogenesis in the localized domain of competent tissues. Our studies place *OsWOX10* upstream of *OsPLT2, OsPLT3* and *OsPLT4. OsPLTs* regulate CRP formation and can rescue of LRP outgrowth defects in *Arabidopsis plt3plt5plt7* mutants (Du and Scheres, 2017) suggesting their function in promoting the outgrowth of root primordia during post-embryonic root development. The function of *OsWOX10* in priming CRP initiation is further supported by fact that it activates expression of *OsCRL1,* a regulator of first periclinal cell division during CRP establishment (Inukai et al., 2001; 2005; Liu et al. 2005) and *OsERF3,* a regulator of CRP initiation (Zhao et al., 2015), thus functions upstream of these early regulators. Eventually, the OsWOX10-OsPLTs in conjunction with SHR-SCR-QHB generates a robust regulatory module to maintain the SCN in the vicinity of the QC during CRP outgrowth. A genome-wide target identification of *OsWOX10* and *OsPLTs* would provide a comprehensive regulatory networks during CR organogenesis.

OsWOX10 and *OsPLTs* regulate root architecture across the plant species

Root architecture and origin of post-embryonic roots are diverged across the plant species. In *Arabidopsis,* both ARs and LRs originate from the xylem pole pericycle cells of hypocotyl and primary root, respectively whereas, in rice, ARs/CRs are developed from the innermost ground tissues of shoot (Itoh et al. 2005, Rebouillat et al. 2009, Bellini et al. 2014). Thus, the developmental context of LR in *Arabidopsis* and CR in rice is distinct. Interestingly, over expression of *OsWOX10* is sufficient to trigger the robust post-embryonic root development in Arabidopsis suggesting that root promoting function of OsWOX10 protein is conserved. Functional conservation of rice root fate determinant is further evident from rescue of root pericycle originated LRPs outgrowth in *Arabidopsis plt3,5,7* mutant by delivering OsPLT1 or OsPLT2 in LRP transcriptional domain. It is likely that conserved root promoting factors such as WOX and PLTs have acquired species specific function in two evolutionary diverged plants

species, rice and Arabidopsis, largely by modulating the cis-regulatory sequences but not the function of protein. Taken together, our studies provide CRP stage-specific temporal gene expression map, and discover OsWOX10-OsPLT regulatory module in controlling the root architecture in rice (Figure 7K; Supplemental Figure 6).

METHODS

Plant Material, Treatment and Laser Capture Microdissection

Oryza sativa var. IR-64 seed germination, growth, auxin treatment and sample collection was done as described by Neogy et al., (2019). For LCM, the stem base tissue from 6-day old rice seedling was harvested in Carnoy's fluid (ethanol: chloroform: acetic acid glacial; 6:3:1), infiltrated twice under mild vacuum and dehydrated through graded ethanol series followed by replacement with xylene. The tissue was embedded in Paraplast (Sigma-Aldrich) and 8-µm thin sections were cut using RM2125 microtome (Leica) and sections were taken on PEN membrane slides (Carl-Zeiss, Germany). The CRP were micro-dissected on PALM Microbeam (Carl-Zeiss). The *Arabidopsis* mutants *wox11-2* (SALK_004777), *wox12-1* (SALK_087882) and *wox11-2 wox12-1* seeds were kindly provided by Dr. Lin Xu used by Liu et al., (2014).

RNA Extraction, Library Preparation and RNA Sequencing

RNA was isolated from LCM collected CRP using ARCTURUS PicoPure RNA Isolation Kit (ThermoFisher, Waltham, MA, USA) according to manufacturer's protocol. The extracted RNA was assayed for RNA integrity on 2100 Bioanalyzer using RNA 6000 Pico Kit (Agilent Technologies, Santa Clara, CA, USA). RNA samples were depleted for ribosomal RNAs (rRNAs) using Ribo-Zero rRNA Removal Kit (Illumina, San Diego, CA, USA) and were used for cDNA synthesis and library preparation using SMARTer universal low input RNA kit (Clontech, Mountain View, CA). Library fragment size distribution was checked on the Agilent 2100 Tapestation System with the High Sensitivity D1000 Kit (Agilent Technologies). A total of six RNA sequencing libraries were sequenced on Nova Seq 6000 Platform (Illumina).

Sequence Alignment and Gene Cluster Analysis

The sequenced paired-end reads were mapped to a reference genome (MSU release 7) using STAR (Dobin et al. 2013) in two-pass mode. A gene count matrix was generated using the quant mode-

GeneCounts with rows corresponding to individual genes and columns corresponding to samples. For common expression pattern analysis, fuzzy c-means clustering was performed on the data. The gene count table thus generated was made homoskedastic using the variance stabilizing transformation function from DESeq2 (Love et al., 2014) Both biological replicates for each stage were collapsed using the geometric mean of the two values (Kumar and Futschik, 2007). After running fuzzy c-means, a membership cutoff of 0.5 was used for assigning genes to individual clusters. The number of clusters was empirically determined by studying the PCA plots, minimum cluster centroid distance, and normalized expression plots, with the number of clusters varying from 2 to 25 as in (Harrop et al., 2016).

Differential Gene Expression and Gene Ontology Analysis

The count matrix was used as input for differential expression analysis using DESeq2 (Love et al., 2014). Genes with an adjusted p-value (or q-value) less than 0.05 and the $\log_2$ fold-change ≥ 1 or ≤ -1 were considered differentially expressed genes (DEGs). Gene expression in different root zones was analyzed using CoNekT database (Proost and Mutwil, 2018; https://conekt.sbs.ntu.edu.sg/heatmap/) and heatmap was generated using tool Heatmapper (Babicki et al. 2016; http://heatmapper.ca/expression/). GO enrichment analysis was performed using BiNGO plug-in of Cytoscape (version 3.3.0) with P-value ≤ 0.05. GO enrichment of each condition was further used to make a comparative enrichment map via Cytoscape.

RNA Extraction and Real-Time PCR

Total RNAs was extracted from crown tissues, and seedlings using RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) followed by elimination of DNA using on-column DNase (Qiagen) according to manufacturer's protocol. The cDNA synthesis and qRT-PCR was performed as described earlier (Neogy et al., 2019) using iScript cDNA synthesis kit and iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, India). Rice *UBQ5*-normalized $\Delta\Delta$Ct was used to calculate $\log_2$ fold change. A list of primers is provided as Supplemental Table 2.

RNA-RNA *in situ* Hybridization

For preparing anti-sense DIG-UTP-labeled riboprobes, 150 bp of *OsERF3*, 186 bp of *OsARF16*, and 170 bp of *OsHOX1* gene-specific fragments were cloned in *p*Bluescript SK+ (anti-sense),

linearized with *EcoRI* and transcribed with T7 RNA Polymerase (Sigma-Aldrich). Their sense clones, linearized with *HindIII* was used to generate sense probes. For *OsRR24*, 193 bp fragment cloned in pBS SK+ (anti-sense), *EcoRI/*T7 RNA polymerase and *HindIII/*T3 RNA polymerase (NEB) generated antisense and sense probes respectively. For YFP anti-sense probe, 609 bp of YFP fragment cloned in pBS SK+ (anti-sense), *EcoRI/*T7 RNA polymerase was used which was hydrolyzed to about 100 bp before use. For *OsWOX10*, 69 bp fragment cloned in pBS SK+ (sense), *HindIII/*T3 RNA polymerase was used to generate anti-sense probes. For *OsPLT7*, 1073 bp fragment cloned in pBS SK+ (anti-sense), *NruI/*T7 RNA polymerase generated anti-sense probes. Hybridization and detection was performed on cross-sections, as described by Neogy et al., (2019). The list of primers used are given in supplemental table 2.

Immunohistochemistry

For immunohistochemistry, tissues were treated for antigen retrieval in antigen retrieval buffer (10mM Tris, 1mM EDTA, pH- 9.0). Slides were blocked with 1% BSA in 1XTBST and incubated with anti-GFP primary antibody in 1:500 dilutions (Rockland Immunochemicals, cat. no. 600-301-215S-Anti GFP mouse monoclonal antibody) for 10-12 hours. Slides were washed with 1xTBST and was incubated with HRP tagged secondary antibody in 1:3000 dilutions. Colour detection was done using 3, 3′-Diaminobenzidine (Sigma-Aldrich) as a substrate. Sections were counterstained with Hematoxylin, dehydrated with graded ethanol, cleared with xylene and mounted using DPX.

Plasmid Construction and Generating Transgenic Lines

For generating DR5ev_erYFP transgenic rice lines, the DR5ev_erYFP-nosT (1.4kb) was PCR amplified using on the plasmid pHm-DR5ev-erYFP_nosT2 template (gift from Ari Pekka Mahonen's lab at Helsinki University, Finland) and cloned into pCAMBIA1390 vector. For *OsWOX10* down-regulation construct, a 526 bp gene-specific fragment was used to generate RNAi loop and was cloned in a vector expressing XVE under maize Ubiquitin promoter. For ectopic over-expression, full length CDS of *OsWOX10* was cloned under maize Ubiquitin promoter. For *OsPLT* RNAi construct, gene-specific fragments of *OsPLT1* (979 bp) and *OsPLT4* (398 bp) were combined together to generate a double dsRNAi*OsPLT1/4* and was expressed under maize Ubiquitin promoter. These constructs were mobilized to *Agrobacterium tumefaciens* LBA4404 and used to raise transgenic rice lines as described by Toki et al. (2006) and Neogy et al. (2019).

The constructs for *Arabidopsis* transformation were cloned using Multisite gateway recombination cloning system (Invitrogen) using pCAMBIA 1300 destination vector. For *OsWOX10* over-expression in *Arabidopsis*, full-length cDNA was cloned under *Ubiquitin 10* promoter. *OsPLT1 (LOC_Os04g55970.2)* was amplified from genomic DNA extracted from *Oryza sativa* leaf tissues. The *OsPLT1* gene was cloned under Arabidopsis *PLT3* promoter (7.7Kb) and tagged with v*YFP*. Similarly, *OsPLT2 (LOC_Os06g44750.1)* gene tagged with *vYFP* and driven by Arabidopsis *PLT5* promoter (5.0Kb) was cloned. These constructs were introduced into C58 Agrobacterium by electroporation and transformed into *Arabidopsis plt3; plt5-2; plt7* mutant plants by floral dip method (Clough and Bent, 1998).

Rice phenotyping

For studying phenotype of *OsWOX10* down-regulation on root regeneration, healthy regenerated shoot with no roots were transferred from shoot regeneration media supplemented with 50 mg/L hygromycin B to root induction media containing 50 mg/L hygromycin B, DMSO (mock) or 10 µM 17ß-estradiol. The number of regenerated roots were counted on the 11, 13 and 20 days post-induction. Similarly, roots of regenerated plants were cut and transferred to fresh root induction media. The number of regenerated roots were counted on the 8, 10 and 20 days after transfer. For down-regulation analysis by qRT-PCR, samples were collected after 24 h treatment with 10 µM 17ß-estradiol.

Author Contributions and Acknowledgments

T.G. performed experiments of LCM, RNA *in situ* hybridization and OsWOX10 function in rice. Z.S. performed experiments for auxin responses and functions of *OsPLT1* and *OsPLT4* in rice. A.K.D., R.S.S and M.J. performed RNA sequencing data analysis. M.Y. with T.G. contributed for function of *OsWOX10* in *Arabidopsis.* V.V. studied function of rice *PLTs* in *Arabidopsis.* D.C. contributed in LCM experiments. M.J., K.P. and S.R.Y. designed experiments, analyzed data and wrote the manuscript.

S.R.Y. acknowledges financial support from Department of Biotechnology (DBT), Government of India (grant # BT/PR13488/BPA/118/105/2015). Tata Innovation Fellowship to M.J. from the Department of Biotechnology, Government of India, is acknowledged. K.P. acknowledges grants from the DBT (grant# BT/PR12394/AGIII/103/891/2014) and DST-SERB (grant#

EMR/2017/002503/PS), Government of India and also IISER-TVM for infrastructure and financial support. Indian Institute of Technology, Roorkee (IIT Roorkee) is acknowledged to provide infrastructure support to S.R.Y. and fellowships to T. G. and Z. S. Fellowships to A.K.D. from Indian Council of Medical Research (ICMR) and to V.V. from Council of Scientific and Industrial Research (CSIR) are gratefully acknowledged. We thank Dr. Deepak Sharma, IIT Roorkee and Bencos Research Solutions Pvt. Ltd., Bangalore, India for their inputs in RNA-seq data analysis. Secondary antibodies for immunohistochemistry was gifted by Dr. Prabhat Kumar Mandal, IIT Roorkee, India. Dr. Lin Xu, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China is acknowledged for kindly providing *Arabidopsis wox11-2, wox12-1* and *wox11-2 wox12-1* seeds. Ashish Kumar and Sonia Choudhary are acknowledged for their support in growing plants.

REFERENCES

Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C., Nussaume, L., Noh, Y.S., Amasino, R., and Scheres, B. (2004). The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. Cell **119,** 109-120.

Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J.R., Maciejewski, A., and Wishart, D.S. (2016) Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res., **44,** W147–W153.

Bellini, C., Pacurar, D.I., and Perrone, I. (2014). Adventitious Roots and Lateral Roots: Similarities and Differences. Annual Review of Plant Biology **65,** 639-666.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G., and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell **115,** 591-602.

Chen, L., Tong, J., Xiao, L., Ruan, Y., Liu, J., Zeng, M., Huang, H., Wang, J.-W., and Xu, L. (2016). YUCCA-mediated auxin biogenesis is required for cell fate transition occurring during de novo root organogenesis in Arabidopsis. Journal of Experimental Botany **67,** 4273-4284.

Clough, S.J. and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J **16,** 735-743.

Coudert, Y., Bès, M., Van Anh Le, T., Pré, M., Guiderdoni, E., and Gantet, P. (2011). Transcript profiling of crown rootless1 mutant stem base reveals new elements associated with crown root development in rice. BMC Genomics **12,** 387.

Coudert, Y., Le, V.A., Adam, H., Bès, M., Vignols, F., Jouannic, S., Guiderdoni, E., and Gantet, P. (2015). Identification of CROWN ROOTLESS1-regulated genes in rice reveals specific and conserved elements of postembryonic root formation. New Phytol **206,** 243-254.

De Rybel, B., Vassileva, V., Parizot, B., Demeulenaere, M., Grunewald, W., Audenaert, D., Van Campenhout, J., Overvoorde, P., Jansen, L., Vanneste, S., Möller, B., Wilson, M., Holman, T., Van Isterdael, G., Brunoud, G., Vuylsteke, M., Vernoux, T., De Veylder, L., Inzé, D., Weijers, D., Bennett, M.J., and Beeckman, T. (2010). A novel aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. Curr Biol **20,** 1697-1706.

Ding, Z., and Friml, J. (2010). Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proceedings of the National Academy of Sciences of the United States of America **107,** 12046-12051.

Dinneny, J.R., and Benfey, P.N. (2008). Plant stem cell niches: standing the test of time. Cell **132,** 553-557.

Du, Y., and Scheres, B. (2017). PLETHORA transcription factors orchestrate de novo organ patterning during Arabidopsis lateral root outgrowth. Proceedings of the National Academy of Sciences **114,** 11709-11714.

Dubrovsky, J.G., Sauer, M., Napsucialy-Mendivil, S., Ivanchenko, M.G., Friml, J., Shishkova, S., Celenza, J., and Benková, E. (2008). Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. Proceedings of the National Academy of Sciences **105,** 8790-8794.

Fukaki, H., Taniguchi, N., and Tasaka, M. (2006). PICKLE is required for SOLITARY-ROOT/IAA14-mediated repression of ARF7 and ARF19 activity during Arabidopsis lateral root initiation. The Plant journal : for cell and molecular biology **48,** 380-389.

Guan, L., Murphy, A.S., Peer, W.A., Gan, L., Li, Y., and Cheng, Z.-M. (2015). Physiological and Molecular Regulation of Adventitious Root Formation. Critical Reviews in Plant Sciences **34,** 506-521.

Guo, T., Wang, D., Fang, J., Zhao, J., Yuan, S., Xiao, L., and Li, X. (2019). Mutations in the Rice OsCHR4 Gene, Encoding a CHD3 Family Chromatin Remodeler, Induce Narrow and Rolled Leaves with Increased Cuticular Wax. Int J Mol Sci **20,** 2567.

Harrop, T.W., Ud Din, I., Gregis, V., Osnato, M., Jouannic, S., Adam, H., and Kater, M.M. (2016). Gene expression profiling of reproductive meristem types in early rice inflorescences by laser microdissection. Plant J **86,** 75-88.

He, C., Chen, X., Huang, H., and Xu, L. (2012). Reprogramming of H3K27me3 is critical for acquisition of pluripotency from cultured Arabidopsis tissues. PLoS Genet **8,** e1002911.

Hofhuis, H., Laskowski, M., Du, Y., Prasad, K., Grigg, S., Pinon, V. and Scheres, B. (2013) Phyllotaxis and rhizotaxis in Arabidopsis are modified by three PLETHORA transcription factors. Current Biology **23,** 956-962.

Hu, X., and Xu, L. (2016). Transcription Factors WOX11/12 Directly Activate WOX5/7 to Promote Root Primordia Initiation and Organogenesis. Plant Physiol **172,** 2363-2373.

Inukai, Y., Miwa, M., Nagato, Y., Kitano, H., and Yamauchi, A. (2001). Characterization of rice mutants deficient in the formation of crown roots. Breeding Science 51, 123-129.

Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Shibata, Y., Gomi, K., Umemura, I., Hasegawa, Y., Ashikari, M., Kitano, H., and Matsuoka, M. (2005). Crown rootless1, which is essential for crown root formation in rice, is a target of an AUXIN RESPONSE FACTOR in auxin signaling. Plant Cell **17,** 1387-1396.

Itoh, J., Nonomura, K., Ikeda, K., Yamaki, S., Inukai, Y., Yamagishi, H., Kitano, H., and Nagato, Y. (2005). Rice plant development: from zygote to spikelet. Plant Cell Physiol **46,** 23-47.

Jain, M., Kaur, N., Garg, R., Thakur, J.K., Tyagi, A.K., and Khurana, J.P. (2006). Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (Oryza sativa). Funct Integr Genomics **6,** 47-59.

Jun, N., Gaohang, W., Zhenxing, Z., Huanhuan, Z., Yunrong, W., and Ping, W. (2011). OsIAA23-mediated auxin signaling defines postembryonic maintenance of QC in rice. Plant J **68,** 433-442.

Kamiya, N., Itoh, J., Morikami, A., Nagato, Y., and Matsuoka, M. (2003a). The SCARECROW gene's role in asymmetric cell divisions in rice plants. Plant J **36,** 45-54.

Kamiya, N., Nagasaki, H., Morikami, A., Sato, Y., and Matsuoka, M. (2003b). Isolation and characterization of a rice WUSCHEL-type homeobox gene that is specifically expressed in the central cells of a quiescent center in the root apical meristem. Plant J **35,** 429-441.

Kang, N.Y., Lee, H.W., and Kim, J. (2013). The AP2/EREBP gene PUCHI Co-Acts with LBD16/ASL18 and LBD18/ASL20 downstream of ARF7 and ARF19 to regulate lateral root development in Arabidopsis. Plant Cell Physiol **54,** 1326-1334.

Kitomi, Y., Kitano, H., and Inukai, Y. (2011a). Molecular mechanism of crown root initiation and the different mechanisms between crown root and radicle in rice. Plant Signal Behav **6,** 1270-1278.

Kitomi, Y., Ogawa, A., Kitano, H., and Inukai, Y. (2008). CRL4 regulates crown root formation through auxin transport in rice. Plant Root **2**.

Kitomi, Y., Inahashi, H., Takehisa, H., Sato, Y., and Inukai, Y. (2012). OsIAA13-mediated auxin signaling is involved in lateral root initiation in rice. Plant Sci **190,** 116-122.

Kitomi, Y., Ito, H., Hobo, T., Aya, K., Kitano, H., and Inukai, Y. (2011b). The auxin responsive AP2/ERF transcription factor CROWN ROOTLESS5 is involved in crown root initiation in rice through the induction of OsRR1, a type-A response regulator of cytokinin signaling. Plant J **67,** 472-484.

Kornet, N., and Scheres, B. (2009). Members of the GCN5 histone acetyltransferase complex regulate PLETHORA-mediated root stem cell niche maintenance and transit amplifying cell proliferation in Arabidopsis. Plant Cell **21,** 1070-1079.

Kumar, L., and Futschik, M. (2007) Mfuzz: A software package for soft clustering of microarray data. Bioinformation **2,** 5–7.

Lafos, M., Kroll, P., Hohenstatt, M.L., Thorpe, F.L., Clarenz, O., and Schubert, D. (2011). Dynamic regulation of H3K27 trimethylation during Arabidopsis differentiation. PLoS Genet **7,** e1002040.

Lakehal, A., and Bellini, C. (2019). Control of adventitious root formation: insights into synergistic and antagonistic hormonal interactions. Physiol Plant **165,** 90-100.

Lavarenne, J., Gonin, M., Guyomarc'h, S., Rouster, J., Champion, A., Sallaud, C., Laplaze, L., Gantet, P., and Lucas, M. (2019). Inference of the gene regulatory network acting downstream of CROWN ROOTLESS 1 in rice reveals a regulatory cascade linking genes involved in auxin signaling, crown root initiation, and root meristem specification and maintenance. Plant J **100,** 954-968.

Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., Fukaki, H., Beeckman, T., Bennett, M., and Laplaze, L. (2013). Lateral root development in Arabidopsis: fifty shades of auxin. Trends Plant Sci **18,** 450-458.

Li, P., and Xue, H. (2011). Structural characterization and expression pattern analysis of the rice PLT gene family. Acta Biochim Biophys Sin (Shanghai) **43,** 688-697.

Li, Y., Zhu, J., Wu, L., Shao, Y., Wu, Y., and Mao, C. (2019). Functional Divergence of PIN1 Paralogous Genes in Rice. Plant Cell Physiol **60,** 2720-2732.

Lian, G., Ding, Z., Wang, Q., Zhang, D., and Xu, J. (2014). Origins and Evolution of WUSCHEL-Related Homeobox Protein Family in Plant Kingdom. The Scientific World Journal **2014,** 534140.

Liu, H., Wang, S., Yu, X., Yu, J., He, X., Zhang, S., Shou, H., and Wu, P. (2005). ARL1, a LOB-domain protein required for adventitious root formation in rice. Plant J 43, 47-56.

Liu, J., Sheng, L., Xu, Y., Li, J., Yang, Z., Huang, H., and Xu, L. (2014). WOX11 and WOX12 Are Involved in the First-Step Cell Fate Transition during de Novo Root Organogenesis in Arabidopsis. The Plant Cell **26,** 1081-1093.

Liu, S., Wang, J., Wang, L., Wang, X., Xue, Y., Wu, P., and Shou, H. (2009). Adventitious root formation in rice requires OsGNOM1 and is mediated by the OsPINs family. Cell Res **19,** 1110-1119.

Love, M.I., Huber, W., and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology **15,** 550.

Mao, C., He, J., Liu, L., Deng, Q., Yao, X., Liu, C., Qiao, Y., Li, P., and Ming, F. (2020). OsNAC2 integrates auxin and cytokinin pathways to modulate rice root development. Plant Biotechnology Journal **18,** 429-442.

Mateo-Bonmatí, E., Casanova-Sáez, R., and Ljung, K. (2019). Epigenetic Regulation of Auxin Homeostasis. Biomolecules **9**.

Meng, F., Xiang, D., Zhu, J., Li, Y., and Mao, C. (2019). Molecular Mechanisms of Root Development in Rice. Rice **12,** 1.

Napsucialy-Mendivil, S., Alvarez-Venegas, R., Shishkova, S., and Dubrovsky, J.G. (2014). Arabidopsis homolog of trithorax1 (ATX1) is required for cell production, patterning, and morphogenesis in root development. Journal of experimental botany **65,** 6373-6384.

Neogy, A., Garg, T., Kumar, A., Dwivedi, A.K., Singh, H., Singh, U., Singh, Z., Prasad, K., Jain, M., and Yadav, S.R. (2019). Genome-Wide Transcript Profiling Reveals an Auxin-Responsive Transcription Factor, OsAP2/ERF-40, Promoting Rice Adventitious Root Development. Plant Cell Physiol **60,** 2343-2355.

Ojolo, S.P., Cao, S., Priyadarshani, S.V.G.N., Li, W., Yan, M., Aslam, M., Zhao, H., and Qin, Y. (2018). Regulation of Plant Growth and Development: A Review From a Chromatin Remodeling Perspective. Frontiers in Plant Science **9**.

Orman-Ligeza, B., Parizot, B., Gantet, P.P., Beeckman, T., Bennett, M.J., and Draye, X. (2013). Post-embryonic root organogenesis in cereals: branching out from model plants. Trends Plant Sci **18,** 459-467.

Proost, S., and Mutwil, M. (2018). CoNekT: an open-source framework for comparative genomic and transcriptomic network analyses. Nucleic Acids Res **46,** W133-w140.

Radhakrishnan, D., Shanmukhan, A.P., Kareem, A., Aiyaz, M., Varapparambathu, V., Toms, A., Kerstens, M., Valsakumar, D., Landge, A.N., Shaji, A., Mathew, M.K., Sawchuk, M.G., Scarpella, E., Krizek, B.A., Efroni, I., Mähönen, A.P., Willemsen, V., Scheres, B., and Prasad. K. (2020) A coherent feed-forward loop drives vascular regeneration in damaged aerial organs of plants growing in a normal developmental context. Development 147, dev185710. doi:10.1242/dev.185710.

Rebouillat, J., Dievart, A., Verdeil, J.L., Escoute, J., Giese, G., Breitler, J.C., Gantet, P., Espeout, S., Guiderdoni, E., and Périn, C. (2009). Molecular Genetics of Rice Root Development. Rice **2,** 15-34.

Sabatini, S., Heidstra, R., Wildwater, M., and Scheres, B. (2003). SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem. Genes Dev **17,** 354-358.

Sang, Y., Silva-Ortega, C.O., Wu, S., Yamaguchi, N., Wu, M.F., Pfluger, J., Gillmor, C.S., Gallagher, K.L., and Wagner, D. (2012). Mutations in two non-canonical Arabidopsis SWI2/SNF2 chromatin remodeling ATPases cause embryogenesis and stem cell maintenance defects. Plant J **72,** 1000-1014.

Servet, C., Conde e Silva, N., and Zhou, D.X. (2010). Histone acetyltransferase AtGCN5/HAG1 is a versatile regulator of developmental and inducible gene expression in Arabidopsis. Mol Plant **3,** 670-677.

Shao, Y., Zhou, H.Z., Wu, Y., Zhang, H., Lin, J., Jiang, X., He, Q., Zhu, J., Li, Y., Yu, H., and Mao, C. (2019). OsSPL3, an SBP-Domain Protein, Regulates Crown Root Development in Rice. Plant Cell **31,** 1257-1275.

Singh, S., Singh, A., Singh, A., Yadav, S., Bajaj, I., Kumar, S., Jain, A., and Sarkar, A.K. (2020). Role of chromatin modification and remodeling in stem cell regulation and meristem maintenance in Arabidopsis. Journal of experimental botany **71,** 778-792.

Stahl, Y., Wink, R.H., Ingram, G.C., and Simon, R. (2009). A signaling module controlling the stem cell niche in Arabidopsis root meristems. Current biology : CB **19,** 909-914.

Stahl, Y., Grabowski, S., Bleckmann, A., Kühnemuth, R., Weidtkamp-Peters, S., Pinto, K.G., Kirschner, G.K., Schmid, J.B., Wink, R.H., Hülsewede, A., Felekyan, S., Seidel, C.A., and Simon, R. (2013). Moderation of Arabidopsis root stemness by CLAVATA1 and ARABIDOPSIS CRINKLY4 receptor kinase complexes. Curr Biol **23,** 362-371.

Takatsuka, H., and Umeda, M. (2015). Epigenetic Control of Cell Division and Cell Differentiation in the Root Apex. Front Plant Sci **6,** 1178.

Toki, S., Hara, N., Ono, K., Onodera, H., Tagiri, A., Oka, S. and Tanaka, H. (2006). Early infection of scutellum tissue with Agrobacterium allows high-speed transformation of rice. The Plant Journal, **47**, 969-976.

Wang, D., Pei, K., Fu, Y., Sun, Z., Li, S., Liu, H., Tang, K., Han, B., and Tao, Y. (2007). Genome-wide analysis of the auxin response factors (ARF) gene family in rice (Oryza sativa). Gene **394,** 13-24.

Wang, Y., Wang, D., Gan, T., Liu, L., Long, W., Wang, Y., Niu, M., Li, X., Zheng, M., Jiang, L., and Wan, J. (2016). CRL6, a member of the CHD protein family, is required for crown root development in rice. Plant Physiol Biochem **105,** 185-194.

Yadav, S.R., Bishopp, A., and Helariutta, Y. (2010). Plant Development: Early Events in Lateral Root Initiation. Current Biology **20,** R843-R845.

Yadav, S.R., Khanday, I., Majhi, B.B., Veluthambi, K., and Vijayraghavan, U. (2011). Auxin-Responsive OsMGH3, a Common Downstream Target of OsMADS1 and OsMADS6, Controls Rice Floret Fertility. Plant and Cell Physiology **52,** 2123-2135.

Yamamuro, C., Zhu, J.-K., and Yang, Z. (2016). Epigenetic Modifications and Plant Hormone Action. Molecular Plant **9,** 57-70.

Yang, J., Yuan, Z., Meng, Q., Huang, G., Périn, C., Bureau, C., Meunier, A.C., Ingouff, M., Bennett, M.J., Liang, W., and Zhang, D. **(2017)** Dynamic Regulation of Auxin Response during Rice Development Revealed by Newly Established Hormone Biosensor Markers. Frontiers in Plant Science **8,** 256.

Zhang, T., Li, R., Xing, J., Yan, L., Wang, R., and Zhao, Y. (2018). The YUCCA-Auxin-WOX11 Module Controls Crown Root Development in Rice. Frontiers in Plant Science **9,** 523.

Zhang, Y., Jiao, Y., Liu, Z., and Zhu, Y.-X. (2015). ROW1 maintains quiescent centre identity by confining WOX5 expression to specific cells. Nature Communications **6,** 6003.

Zhao, Y., Hu, Y., Dai, M., Huang, L., and Zhou, D.X. (2009). The WUSCHEL-related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. Plant Cell **21,** 736-748.

Zhao, Y., Cheng, S., Song, Y., Huang, Y., Zhou, S., Liu, X., and Zhou, D.X. (2015). The Interaction between Rice ERF3 and WOX11 Promotes Crown Root Development by Regulating Gene Expression Involved in Cytokinin Signaling. Plant Cell **27,** 2469-2483.

Zhou, S., Jiang, W., Long, F., Cheng, S., Yang, W., Zhao, Y., and Zhou, D.X. (2017). Rice Homeodomain Protein WOX11 Recruits a Histone Acetyltransferase Complex to Establish Programs of Cell Proliferation of Crown Root Meristem. Plant Cell **29,** 1088-1104.

Figure Legends

Figure 1: Laser capture microdissection-RNA sequencing (LCM-seq) analysis of developing crown root primordia (CRP). (A) Schematic diagram showing CRP competent tissues and developing CRP at the stage of initiation and outgrowth. (B-C) Cross sections of rice stem base with CRP at initiation (B, C) and outgrowth stage (D). (E-G') LCM of CRP during their initiation (E, E') and outgrowth (F, F'). Innermost ground tissues prior CRP initiation were collected as control (G, G'). (E)-(G) are before and (E')-(G') after LCM. (H) Patterns of gene expression during CRP initiation and outgrowth. (I) Venn diagram showing common and unique differentially expressed genes (DEGs) during CRP initiation and outgrowth. (J-K) Expression pattern of DEGs in different zones of emerged roots. (MZ, meristematic zone; EZ, elongation zone; DZ, differentiation zone). Bars= 50µm.

Figure 2: Gene ontology analysis of DEGs. (A) GO terms associated with genes specifically de-regulated during CRP initiation and outgrowth. (B) GO analysis of DEGs when CRP progress from initiation to outgrowth stage.

Figure 3: Differentially regulated transcriptional regulators in developing CRP. (A) Geneset enrichment analysis of TFs. Gene families in red box are known to involved in epigenetic regulation. (B) Venn diagram showing common and unique differentially expressed TFs during CRP initiation and outgrowth. (C-D) Expression pattern of de-regulated TFs in different zones of emerged roots. (MZ, meristematic zone; EZ, elongation zone; DZ, differentiation zone). (E-G) Expression pattern of genes involved in establishing and positioning stem cell niche (SCN) during CRP initiation and outgrowth.

Figure 4: Dynamic expression pattern of auxin signaling genes. (A) Heatmap showing expression pattern of auxin biosynthesis (YUCCA), homeostasis (GH3), transport (PIN and PIN-like) and auxin signaling genes (IAA and ARF). (B-D) Spatially regulated auxin response analyzed of DR5rev::erYFP expression pattern during CRP development in rice. RNA *in situ* hybridization using anti-sense RNA probes (B-B2) and immuno-histochemistry using anti-GFP antibody (C-D) were performed. (G) Validation of auxin responsiveness of selected TFs differentially expressed during CRP development using qRT-PCR. Bars=100µm in (B) and 25µm in (B1-D).

Figure 5: Temporal and spatial expression pattern of selected TFs activated during CRP development. (A-C) AP2-domain containing *OsERF3*, a regulator of CRP initiation. (D-F) Auxin response factor, *OsARF16.* (G-I) Type-B cytokinin response regulator, *OsRR24.* (J-L) Homeobox containing TF, *OsHOX1.* (C, F, I and L) Developing CRPs probed with DIG-labeled sense RNA probes of respective genes. Rest all are probed with DIG-labeled anti-sense RNA probes. Bars= 25 µm in (A), (B), (D), (G), (H), and (J) and 100 µm in (C), (E), (F), (I), (K), and (L).

Figure 6: Functional studies of *OsWOX10* during root development. (A) Phylogenetic analysis of WOX members of intermediate clade show that *OsWOX10* is closely related to *Arabidopsis WOX11/12.* (B-D) Onset and tissue-specific expression pattern of *OsWOX10* during rice CRP development. *OsWOX10* expression is initiated in the CR founder cell (B) and later expression is specific to developing CRP. (E) Root numbers are significantly reduced 20 days after root induction when *OsWOX10* is down-regulated upon estradiol treatment in pUbi-dsRNAi*OsWOX10* lines. (F-I) Consequence of ectopic over-expression of *OsWOX10* during rice plantlet regeneration. Extensive rooting is seen in shooting (G) and rooting (I) media as compared to control (F) and (H), respectively. (J) Induced expression level of *OsPLT3, OsPLT3, OsPLT4, OsERF3* and *OsCRL1* in pUbi-OsWOX10 seedling as compared to wild-type seedling. (K) Extensive rooting as a consequence of ectopic overexpression of *OsWOX10* in wild-type *Arabidopsis* (K1), *wox11-2* (K2), *wox12-1* (K3), and *wox11 wox12* double mutant (K4) background. Bars= 5 µm in (B), 100 µm in (C), 25 µm in (D), 1 cm in (F)-(I) and (K1)-(K4).

Figure 7: Functional studies of rice *PLETHORA* genes during post-embryonic root development. (A-D) Onset and tissue-specific expression pattern of *OsPLT7* during rice CRP development. *OsPLT7* expression begins with CRP establishment (A) and continue to express in the developing CRP (B, C) and restricted to the root meristem in emerged CR (D). (E-F) Phenotypes of inducible down-regulation of *OsPLT1* upon estradiol treatment (F) as compared to mock treated plant (E).

CR development was reduced. (G-H) Consequence of down-regulation of *OsPLT4* in rice (H) using inducible dsRNAi construct results reduced root growth and development as compared to control (G). (I) Partial down-regulation of *OsPLT1* and *OsPLT4* was confirmed by qRT-PCR. (J) Stereo images of 8-dpg wildtype (J1), *plt3;plt5-2;plt7* defective in lateral root primordia outgrowth (J2), and *plt3;plt5-2;plt7;AtPLT3::OsPLT1:vYFP* (J3). The rescue of lateral root formation in *plt3;plt5-2;plt7* mutant reconstituted with *AtPLT3::OsPLT1:vYFP* (*plt3;plt5-2;plt7;AtPLT3::OsPLT1:vYFP*). Confocal image showing expression of *OsPLT1:vYFP* in the lateral root primordia of *plt3;plt5-2;plt7;AtPLT3::OsPLT1:vYFP* (J4). Red colour in (J4) represents propidium iodide staining. (K) Model depicting that auxin inducible expression of *OsWOX10* in the incipient CRP promotes CRP initiation by activating *OsERF3* and *OsCRL1* and CRP outgrowth via activating expression of *OsPLT* genes. Bars= 100 µm in (A)-(C), 200 µm in (D), 1 cm in (E)-(H), 1 mm in (J1-J3) and 50µm in (J4).

Supplementary Data

Supplemental Figure 1: Expression pattern of auxin inducible transcription factors, activated exclusively during CRP initiation (A and A1), specifically during CRP outgrowth (B and B1), and in both the stages (C and C1).

Supplemental Figure 2: Dynamic expression pattern of *OsCRL1*-activated genes, exclusively induced during CRP initiation (A), specifically during outgrowth (B), and induced in both the stages (C).

Supplemental Figure 3: (A) RNA *in situ* hybridization using anti-sense YFP riboprobes on wild-type plant. (B) Expression pattern of transcription factors during CRP development, selected for validation of their auxin responsiveness by qRT-PCR.

Supplemental Figure 4: Functional characterization of *OsWOX10*. (A) Root numbers are significantly reduced on 20 days after root induction in pUbi-dsRNAi*OsWOX10* lines upon estradiol treatment in rooting media. (B, C) Partial down-regulation (B) and over-expression (C) of *OsWOX10* in pUbi-dsRNAi*OsWOX10* and pUbi-OsWOX10 lines, respectively. (D) Unaltered expression levels of *OsCRL1L2* and *OsMFS1* in pUbi-OsWOX10 lines.

Supplemental Figure 5: Expression of *OsPLT2* in the lateral root primordia of *Arabidopsis plt3plt5plt7* triple mutant can promote outgrowth of these arrested primordia. (A) wild-type, (B) *plt3plt5plt7* triple mutants, (C) *plt3plt5plt7* triple mutants complemented by AtPLT5::OsPLT2-vYFP and, (D) LRP specific expression of AtPLT5::OsPLT2-vYFP in *plt3plt5plt7* triple mutants.

Supplemental Figure 6: Schematic representation and genetic regulators during CRP initiation and outgrowth.

Supplemental Dataset 1: List of all genes de-regulated during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Dataset 2: List of genes commonly and specifically de-regulated during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Dataset 3: List of all TFs de-regulated during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Dataset 4: List of TFs specifically de-regulated during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Dataset 5: List of auxin-responsive TFs commonly and specifically de-regulated during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Dataset 6: List of *OsCRL1*-regulated genes de-regulated during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Dataset 7: List of auxin signaling genes de-regulated during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Table 1: List of putative epigenetic regulators during CRP initiation and outgrowth (log FC ≤ - 1.0; q<0.05)

Supplemental Table 2: List of primers used in this study

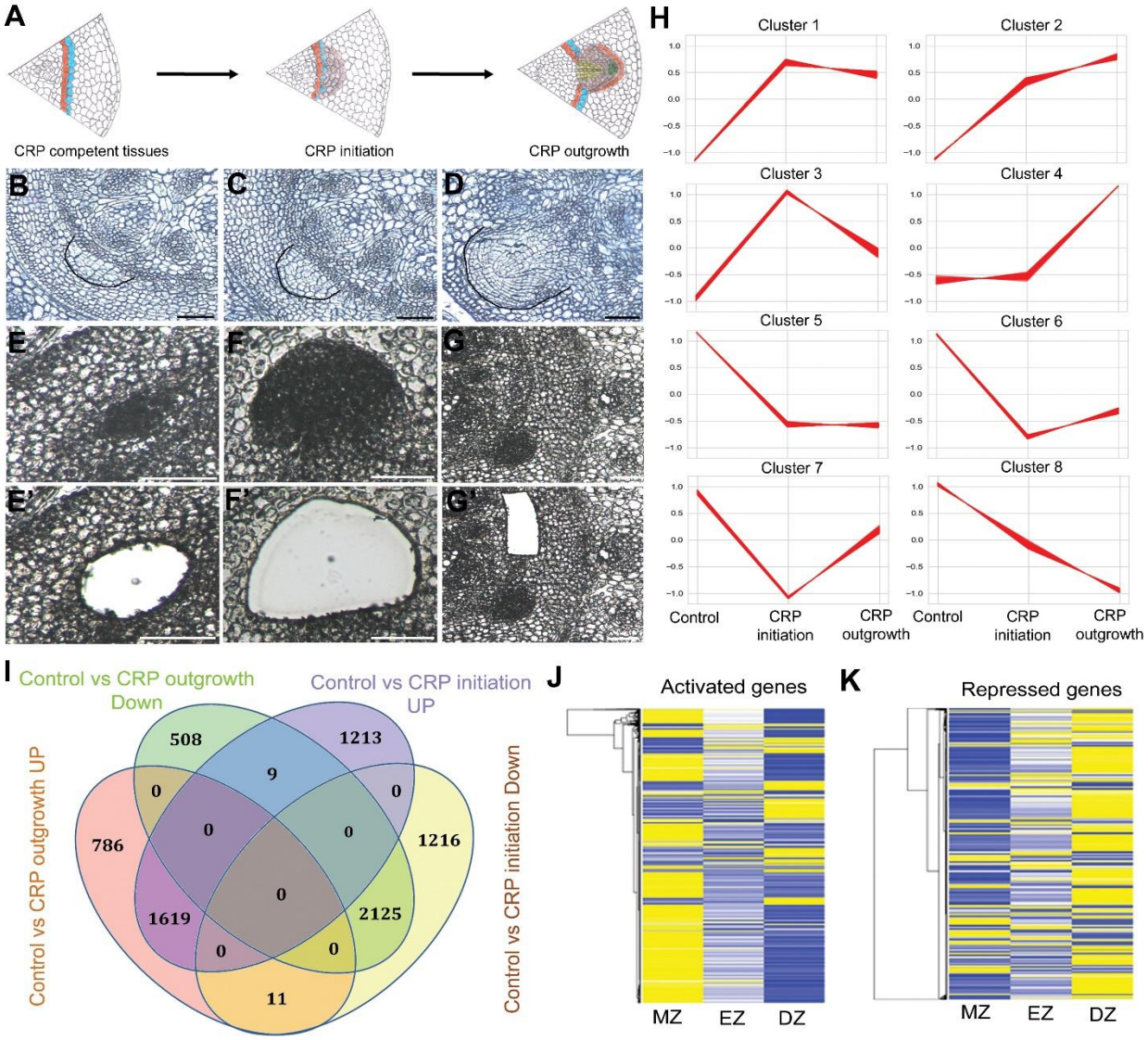

Figure 1: Laser capture microdissection-RNA sequencing (LCM-seq) analysis of developing crown root primordia (CRP). (A) Schematic diagram showing CRP competent tissues and developing CRP at the stage of initiation and outgrowth. (B-C) Cross sections of rice stem base with CRP at initiation (B, C) and outgrowth stage (D). (E-G') LCM of CRP during their initiation (E, E') and outgrowth (F, F'). Innermost ground tissues prior CRP initiation were collected as control (G, G'). (E)-(G) are before and (E')-(G') after LCM. (H) Patterns of gene expression during CRP initiation and outgrowth. (I) Venn diagram showing common and unique differentially expressed genes (DEGs) during CRP initiation and outgrowth. (J-K) Expression pattern of DEGs in different zones of emerged roots. (MZ, meristematic zone; EZ, elongation zone; DZ, differentiation zone). Bars= 50µm.

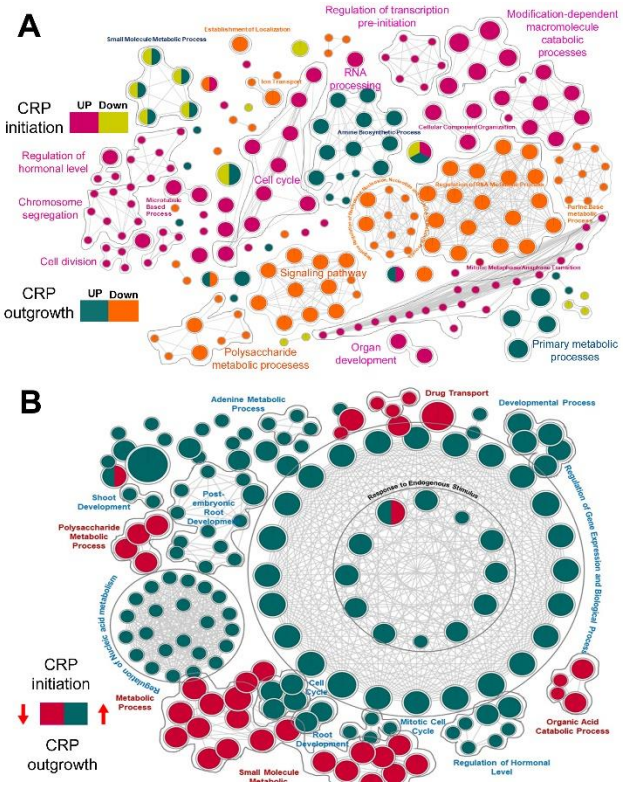

Figure 2: Gene ontology analysis of DEGs. (A) GO terms associated with genes specifically de-regulated during CRP initiation and outgrowth. (B) GO analysis of DEGs when CRP progress from initiation to outgrowth stage.

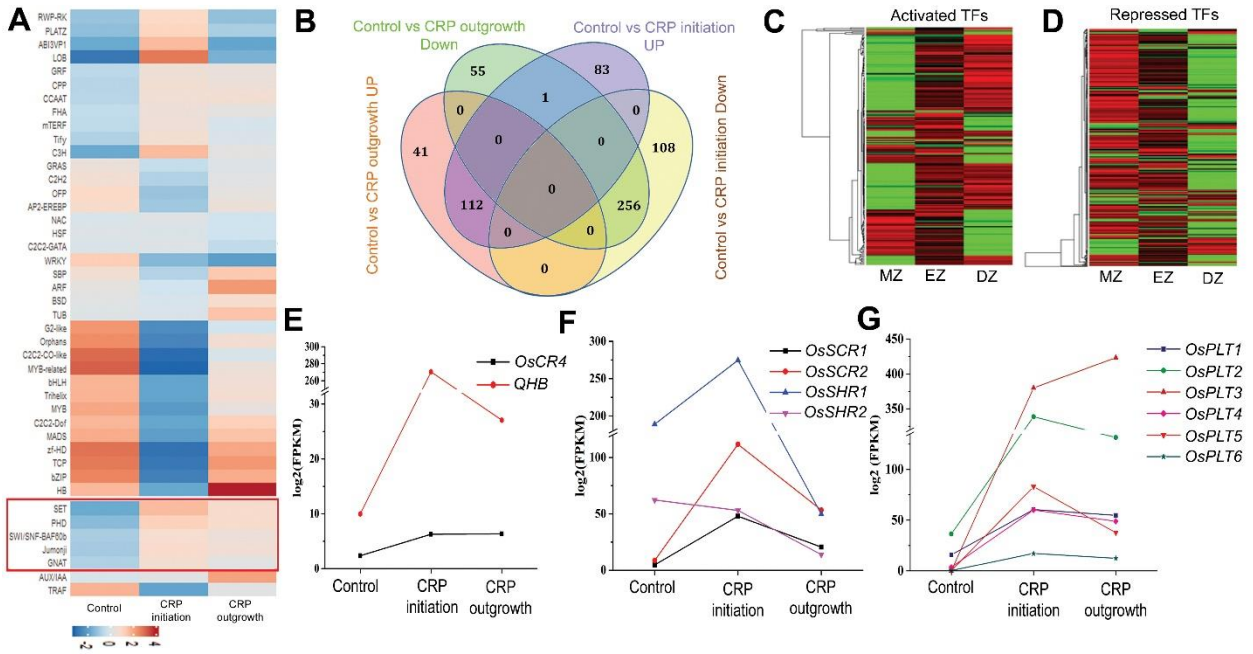

Figure 3: Differentially regulated transcriptional regulators in developing CRP. (A) Geneset enrichment analysis of TFs. Gene families in red box are known to involved in epigenetic regulation. (B) Venn diagram showing common and unique differentially expressed TFs during CRP initiation and outgrowth. (C-D) Expression pattern of de-regulated TFs in different zones of emerged roots. (MZ, meristematic zone; EZ, elongation zone; DZ, differentiation zone). (E-G) Expression pattern of genes involved in establishing and positioning stem cell niche (SCN) during CRP initiation and outgrowth.

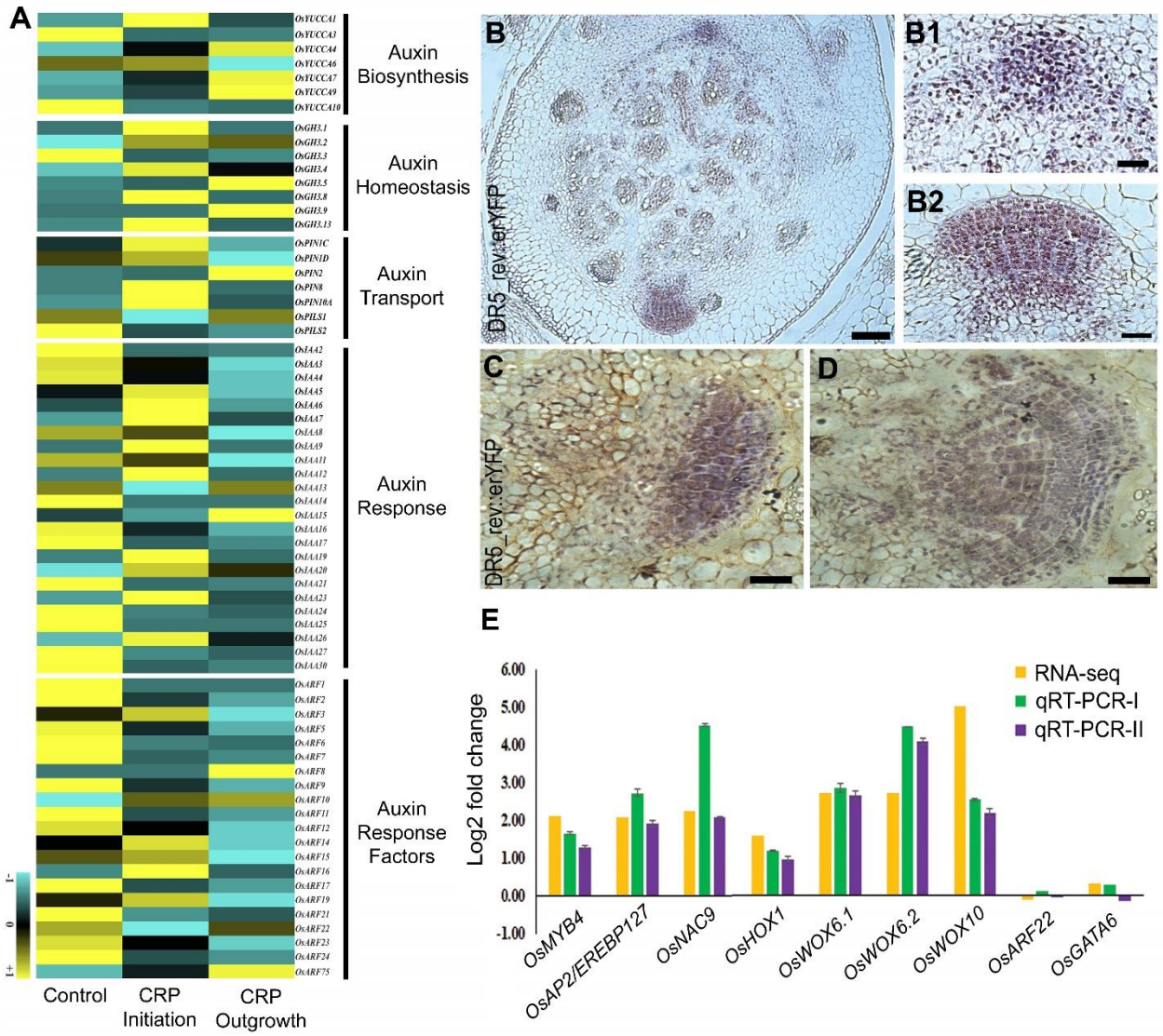

Figure 4: Dynamic expression pattern of auxin signaling genes. (A) Heatmap showing expression pattern of auxin biosynthesis (YUCCA), homeostasis (GH3), transport (PIN and PIN-like) and auxin signaling genes (IAA and ARF). (B-D) Spatially regulated auxin response analyzed of DR5rev::erYFP expression pattern during CRP development in rice. RNA *in situ* hybridization using anti-sense RNA probes (B-B2) and immuno-histochemistry using anti-GFP antibody (C-D) were performed. (G) Validation of auxin responsiveness of selected TFs differentially expressed during CRP development using qRT-PCR. Bars=100µm in (B) and 25µm in (B1-D).

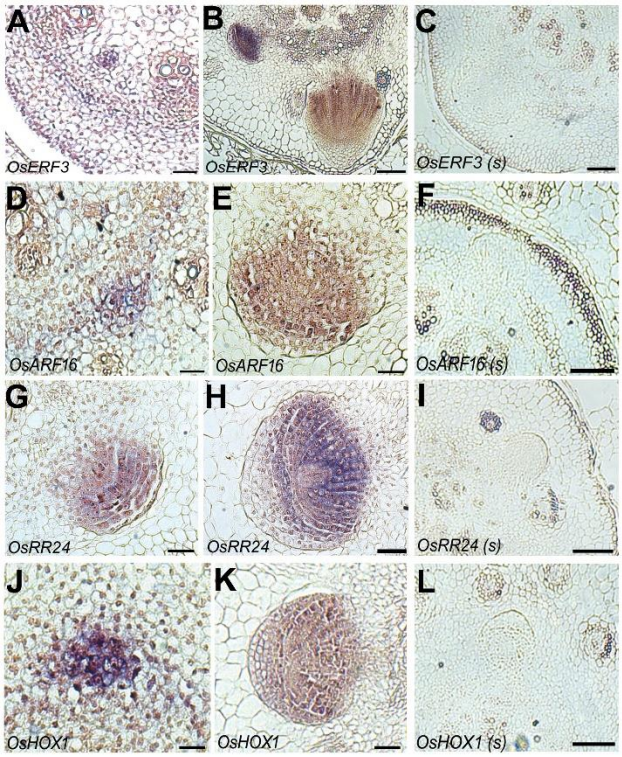

Figure 5: Temporal and spatial expression pattern of selected TFs activated during CRP development. (A-C) AP2-domain containing *OsERF3*, a regulator of CRP initiation. (D-F) Auxin response factor, *OsARF16.* (G-I) Type-B cytokinin response regulator, *OsRR24.* (J-L) Homeobox containing TF, *OsHOX1*. (C, F, I and L) Developing CRPs probed with DIG-labeled sense RNA probes of respective genes. Rest all are probed with DIG-labeled anti-sense RNA probes. Bars= 25 µm in (A), (B), (D), (G), (H), and (J) and 100 µm in (C), (E), (F), (I), (K), and (L).

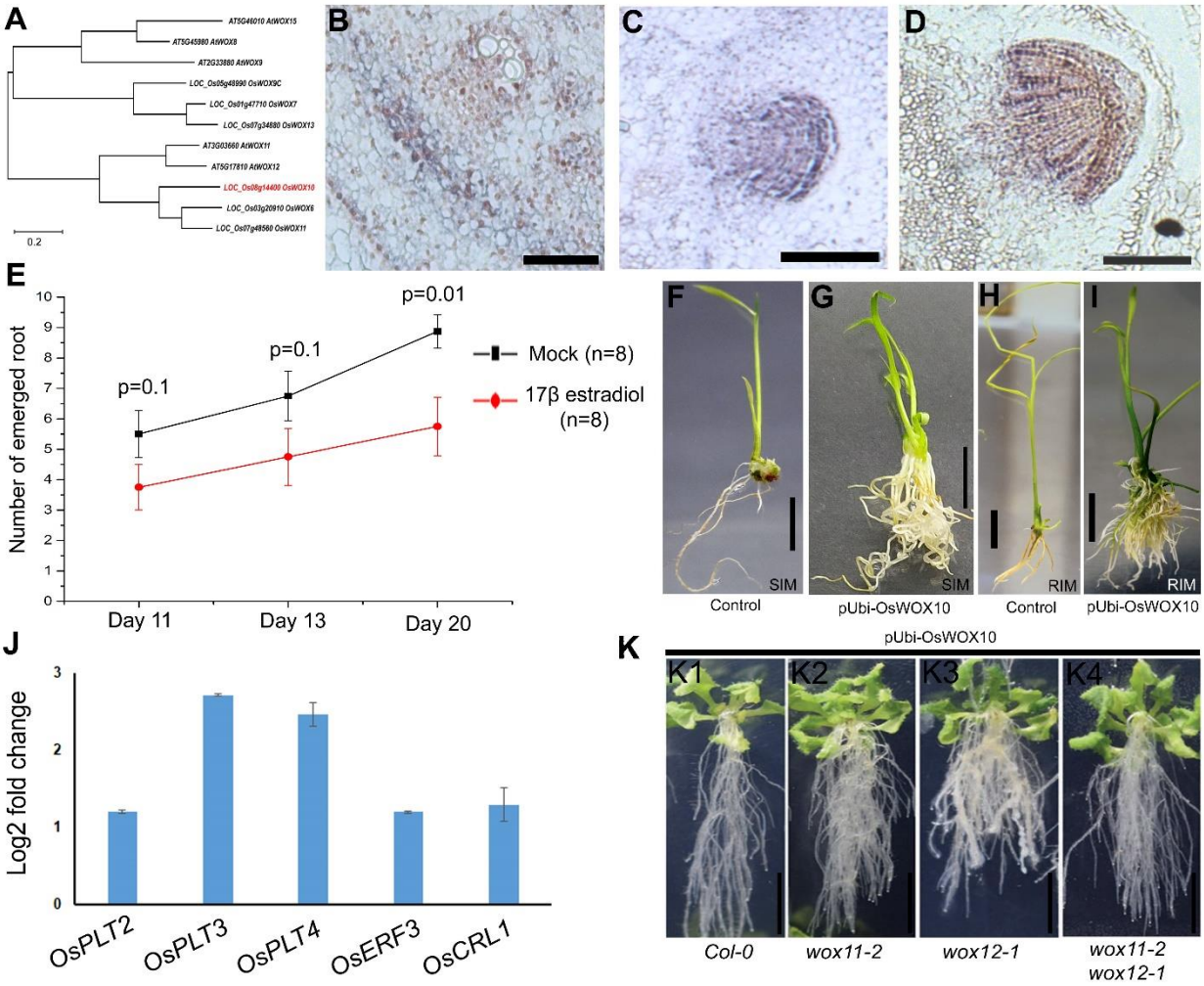

Figure 6: Functional studies of *OsWOX10* during root development. (A) Phylogenetic analysis of WOX members of intermediate clade show that *OsWOX10* is closely related to *Arabidopsis WOX11/12*. (B-D) Onset and tissue-specific expression pattern of *OsWOX10* during rice CRP development. *OsWOX10* expression is initiated in the CR founder cell (B) and later expression is specific to developing CRP. (E) Root numbers are significantly reduced 20 days after root induction when *OsWOX10* is down-regulated upon estradiol treatment in pUbi-dsRNAi*OsWOX10* lines. (F-I) Consequence of ectopic over-expression of *OsWOX10* during rice plantlet regeneration. Extensive rooting is seen in shooting (G) and rooting (I) media as compared to control (F) and (H), respectively. (J) Induced expression level of *OsPLT3*, *OsPLT3*, *OsPLT4*, *OsERF3* and *OsCRL1* in pUbi-OsWOX10 seedling as compared to wild-type seedling. (K) Extensive rooting as a consequence of ectopic overexpression of *OsWOX10* in wild-type *Arabidopsis* (K1), *wox11-2* (K2), *wox12-1* (K3), and *wox11 wox12* double mutant (K4) background. Bars= 5 µm in (B), 100 µm in (C), 25 µm in (D), 1 cm in (F)-(I) and (K1)-(K4).

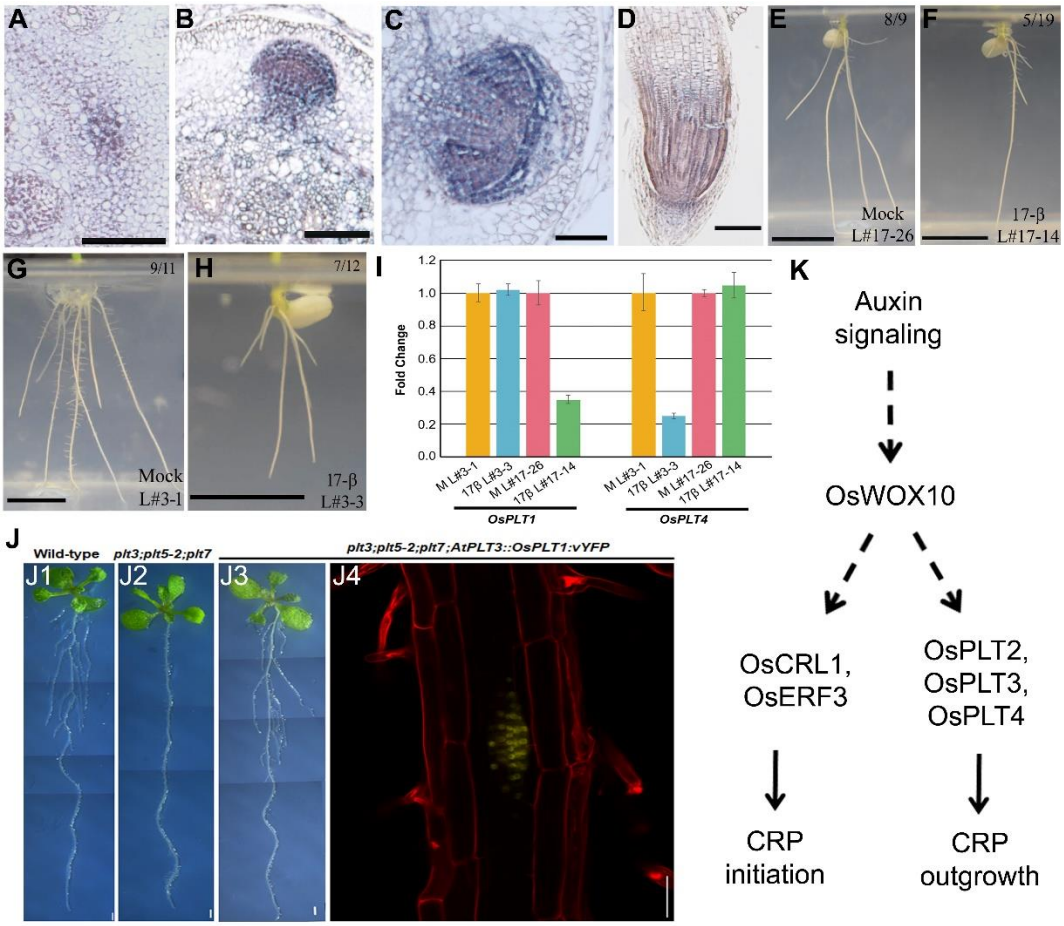

Figure 7: Functional studies of rice *PLETHORA* genes during post-embryonic root development. (A-D) Onset and tissue-specific expression pattern of *OsPLT7* during rice CRP development. *OsPLT7* expression begins with CRP establishment (A) and continue to express in the developing CRP (B, C) and restricted to the root meristem in emerged CR (D). (E-F) Phenotypes of inducible down-regulation of *OsPLT1* upon estradiol treatment (F) as compared to mock treated plant (E). CR development was reduced. (G-H) Consequence of down-regulation of *OsPLT4* in rice (H) using inducible dsRNAi construct results reduced root growth and development as compared to control (G). (I) Partial down-regulation of *OsPLT1* and *OsPLT4* was confirmed by qRT-PCR. (J) Stereo images of 8-dpg wildtype (J1), *plt3;plt5-2;plt7* defective in lateral root primordia outgrowth (J2), and *plt3;plt5-2;plt7;AtPLT3::OsPLT1:vYFP* (J3). The rescue of lateral root formation in *plt3;plt5-2;plt7* mutant reconstituted with *AtPLT3::OsPLT1:vYFP* (*plt3;plt5-2;plt7;AtPLT3::OsPLT1:vYFP*). Confocal image showing expression of *OsPLT1:vYFP* in the lateral root primordia of *plt3;plt5-2;plt7;AtPLT3::OsPLT1:vYFP* (J4). Red colour in (J4) represents propidium iodide staining. (K) Model depicting that auxin inducible expression of *OsWOX10* in the incipient CRP promotes CRP initiation by activating *OsERF3* and *OsCRL1* and CRP outgrowth via activating expression of *OsPLT* genes. Bars= 100 µm in (A)-(C), 200 µm in (D), 1 cm in (E)-(H), 1 mm in (J1-J3) and 50µm in (J4).