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: *OsWOX10* and *OsPLTs* regulates crown root formation

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ABSTRACT

Rice adventitious/crown roots developing from non-root tissues shape up the root architecture. Mechanisms underlying initiation and subsequent outgrowth of CR primordia (CRP) remain under explored. Here, we provide genome-wide dynamics of gene expression patterns and stage-specific transcriptional signatures at distinct developmental stages of CRP formation. Our analyses reveal that early regulated transcription of potential epigenetic modifiers, 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 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 mainly composed of shoot-borne post-embryonic 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 primary root (PR), whereas rice LRs originate from endodermal and pericycle cells located opposite to the protophloem (Rebouillat et al. 2009; Lavenus et al. 2013; Bellini et al. 2014). Similarly, the pericycle cells at xylem pole of the hypocotyl give rise to ARs in *Arabidopsis* 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, there also exists diverged root-type and species-specific regulatory mechanisms (Kitomi et al. 2011a, Orman-Ligeza et al. 2013; Meng et al., 2019).

The establishment of founder cells for rice crown root primordia (CRP) requires an induction phase for cell cycle reactivation in a localized domain of the innermost ground tissues of stem base to produce initial cells for CRP (Itoh et al., 2005; Guan et al., 2015). These initial cells, originated from shoot tissues, then divide and their daughter cells 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 their expression was restricted to the quiescent center (QC) cells, and the endodermis layer, respectively (Kamiya et al. 2003a; 2003b). *ADVENTITIOUS ROOTLESS 1* (*ARL1*)/*CROWN ROOTLESS 1* (*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*, *CROWN ROOTLESS5* (*OsCRL5*), and *WUSCHEL-RELATED HOMEOBOX 11* (*OsWOX11*), *CYTOKININ RESPONSE REGULATOR 2* (*OsRR2*) and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3* (*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 hormonal signaling pathways and transcription factors (TFs) is instrumental for root development (Lavenus et al. 2013; Lakehal and Bellini, 2019, Meng et al., 2019). Auxin maxima activates the signaling that initiate program for root founder cell specification and also ensures entire post-embryonic root development (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). Auxin signaling activates expression of TFs, 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 network operational during CRP specification and differentiation in rice. Our genome-wide LCM-seq based transcriptome analysis revealed a genetic blueprint for the localized developmental reprogramming during the trans-differentiation process and demonstrated spatio-temporal regulated expression patterns of a set of transcription factors during CRP initiation and outgrowth. Our detailed functional analysis of stem cell promoting factors, *OsWOX10* and *OsPLTs* genes reveal previously unrecognized role of *OsWOX10* and *OsPLT* genes in controlling CRP development. *OsWOX10* activates the expression of *OsPLT* genes in this process. Interestingly, *OsPLTs* can rescue lateral root primordia outgrowth defects in *Arabidopsis plt* mutants. Taken together, our studies reveal a novel regulatory network operating during rice crown root development and uncover a key regulatory module, *OsWOX10-OsPLTs* instructing the root architecture in rice.

RESULTS

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

To dissect out the determinants of cell fate specification and to generate high resolution temporal gene expression map of rice CRP at their progressive developmental stages, we performed LCM coupled with RNA sequencing (LCM-seq) to profile transcripts from CRP during their initiation and outgrowth. During the initiation stage, cell identity of CRP initials is established, which

eventually produce initials for epidermis-endodermis, root cap, and central stele (Figure 1A-1C). Subsequently, CRP progress to outgrowth stage, where tissue patterning leads to the fundamental tissue organization (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 competence 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. In order to uncover common patterns of gene expression, fuzzy c-means clustering was performed on the transcriptomic data, as studied earlier (Harrop et al., 2016) and eight clear cluster cores with distinct expression patterns were observed (Figure 1H). Further, GO enrichment analysis of these clusters provided association of expression patterns with biological processes. Clusters 1 and 2 genes, expressed in both the stages are mainly enriched with GO terms related to DNA replication and gene expression, cluster 3 genes, specifically induced during CRP initiation, are associated with cell cycle and division processes, whereas hormonal signaling genes are enriched with cluster 4 genes which are induced during CRP outgrowth (Supplemental Figure 1), indicating an order of events to set developmental program.

The differential gene expression analysis revealed that the expression of 2429 genes (1213 induced and 1216 repressed) during CRP initiation, and 1294 genes (786 up-regulated and 508 down-regulated) during CRP outgrowth, was specifically de-regulated, as compared to control tissues (Figure 1I; Supplemental Dataset 1-2). However, a total of 3744 (1619 induced and 2125 repressed) genes were commonly regulated during CRP initiation and outgrowth stages (Figure 1I; Supplemental Dataset 2). 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). We further observed that the activated genes during CRP development showed a higher expression in the actively dividing meristematic zone than the differentiation zone, whereas the CRP repressed genes showed opposite pattern between meristematic and differentiation zone of emerged roots (Figure 1J and 1K). Thus, our data show that a dynamic transcriptional reprogramming is instrumental for the cell fate change, cell division and differentiation.

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 differentially expressed genes (DEGs). The initiating CRP were exclusively enriched with genes regulating hormonal levels, transcription pre-initiation, RNA processing, cell cycle, cell division, and organ development 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 such as, 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-mediated pre-transcriptional gene regulation, are largely induced during CRP initiation (Figure 3A; Supplemental Table 1).

Stage-specific Signatures of Transcriptional Regulators During CRP Development

Transcription factors (TFs) are master regulators of cell fate determination and we observed that the expression of 191 TFs (83 induced and 108 repressed) was exclusively altered during CRP initiation, whereas 96 TFs (41 up-regulated and 55 down-regulated) were exclusively de-regulated during CRP outgrowth (Figure 3B; Supplemental Dataset 4). Furthermore, differentially expressed TFs display similar pattern as DEGs, in different zones of emerged roots (Figure 3C and 3D).

Auxin-induced TFs such as *OsCRL1*, *OsCRL5*, *OsERF3* and *OsRR2* promote CRP initiation (Inukai et al., 2005; Kitomi et al., 2011a; Zhao et al., 2015). Including *OsCRL1* and *OsERF3*, 83 TFs were specifically activated during CRP initiation, of which 15 TFs, including *OsWOX10*, *OsCRL1*, *OsERF3*, *OsHOX12*, *OsMFS1*, *OsHOX1*, *OsSta2* were auxin inducible, whereas remaining that includes *FREEZY PANICLE* (*FZP*), CRL1-like *OsCRL1L2*, *OsLBD37/ASL39*, *QHB*, *ROC4*, *OsRR24* and *OsHB34* were not regulated by auxin signaling (Supplemental Figure 2A; Supplemental Dataset 5). Similarly, 6 of 41 TFs, specifically induced during CRP outgrowth,

involving *OsAP2/EREBP127*, *OsNAC10*, *HSFB4A* and *OsRR5*, were auxin inducible (Supplemental Figure 2B; 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 2C; Supplemental Dataset 5). Thus, the study identified stage-specific, auxin-dependent and auxin-independent transcriptional regulators of CRP initiation and outgrowth.

Regulatory Modules Controlling Root Meristem Establishment and 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 are reduced in the outgrowing CRP (Figure 3F and 3G; Supplemental Dataset 3), suggesting that a mechanism with conserved regulators might be involved in establishing functional SCN in the developing CRP.

OsCRL1 is a master and specific 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 3A-3C; Supplemental Dataset 6). As *OsCRL1* is specific regulator of CR development, the study

reveals distinct root-type specific *OsCRL1*-dependent regulatory modules during CRP initiation and outgrowth.

Progressive activation of Auxin Signaling During CRP Initiation and Outgrowth

Generating local auxin maxima, an early event to activate auxin signaling in the localized domains to trigger post-embryonic root developmental programs, involved coordinated auxin biosynthesis, homeostasis and distribution (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, of which the expression of *OsYUC4, 7*, and *9* was progressively increased, but *OsYUC1* was reduced as CRP progress from initiation to outgrowth stage (Figure 4A; Supplemental Dataset 7). 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). However, 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), suggesting stage-specific YUC-GH3-PIN modules for initiating auxin signaling.

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 but only 3 genes were induced when CRP progress from initiation to outgrowth stage (Supplemental Dataset 7). This suggests that selective and progressive transcriptional repression of negative regulators of auxin signaling. Further, we observed 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 activation of auxin signaling by monitoring auxin response during rice CRP development. Cross sections of stem base containing developing CRP of transgenic rice lines expressing DR5-erYFP construct was hybridized with anti-sense RNA probes and anti-GFP antibodies. 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 4A). 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 4B). All these observations together suggest that auxin signaling is activated at the onset of CRP program initiation and progressively generates a robust signaling during CRP outgrowth.

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). *OsERF3* and *OsRR24* were expressed 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 Promotes 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 related rice WOX gene, *OsWOX10* whose expression was exclusively activated during CRP initiation (Figure 6A; Lian et al., 2014). Importantly, the expression of *OsWOX10* is strongly induced by auxin signaling (Figure 4E; Neogy et al., 2019). Our detailed temporal and spatial expression pattern analysis of *OsWOX10* transcript localization demonstrated that *OsWOX10* transcription is specifically activated in the founder cells of CRP, prior to their establishment that also coincided with auxin maxima (Figure 4B and 6B) and continue to express in the initiating and outgrowing CRP (Figure 6C and 6D). These observations suggest that auxin signaling activates *OsWOX10* expression at the onset of CRP specification.

To investigate function of *OsWOX10* during CR development, transgenic rice lines capable of down-regulating *OsWOX10* expression, were generated. For this, an inverted repeat RNA-interference (RNAi) construct was expressed under maize ubiquitin promoter using estradiol-inducible XVE system (p*Ubi::XVE:dsRNAiOsWOX10*). Estradiol-induced down-regulation of *OsWOX10* resulted in a significant reduction in the root number, when regenerated plantlets were transferred to the root induction media (Figure 6E; Supplemental Figure 5A), suggesting that expression of *OsWOX10* is required for root induction. In contrast, ectopic over-expression of *OsWOX10* under maize ubiquitin promoter in transgenic rice caused extensive root formation (Figure 6F-6I). In fact, regenerated rice plantlets initiated robust rooting even in the shoot induction media (Figure 6G) and also developed significantly higher number of roots in the root induction media (Figure 6I). The partial down-regulation and strong over-expression of *OsWOX10* was confirmed by qRT-PCR analysis in these lines (Supplemental Figure 5B and 5C). These

observations suggest that *OsWOX10* is necessary and sufficient for rice adventitious root development.

OsWOX10, an Upstream Regulator of CRP Development and its Function is Conserved in *Arabidopsis*

Our functional analysis of *OsWOX10* in rice suggested that it regulates CRP initiation and also ensures their outgrowth. We, therefore, next, investigated the genes regulated by *OsWOX10* during CRP initiation and outgrowth. In rice, *OsERF3* and *OsCRL1* regulate CRP initiation, whereas *Arabidopsis PLT* genes functions during LR primordia outgrowth (Inukai et al., 2005; Zhao et al., 2015; Du and Scheres, 2017). Consistently, the expression of *OsERF3* and *OsCRL1* and their related genes (i.e. *OsMFS1* and *OsCRL1L2*, respectively) is exclusively induced during CRP initiation and rice PLT genes were strongly expressed during CRP development, in our LCM-seq data. We quantified expression levels of *OsERF3, OsMFS1, OsCRL1, OsCRL1L2* and *OsPLT* genes in *OsWOX10* over-expression lines. We observed the expression of *OsERF3* and *OsCRL1,* and *OsPLTs* (*OsPLT2, OsPLT3* and *OsPLT4*) was induced upon *OsWOX10* over-expression, as compared to control. However, the expression of *OsCRL1L2* and *OsMFS1,* was not regulated by *OsWOX10* (Figure 6J; Supplemental Figure 5D), suggesting that *OsWOX10* functions upstream of *OsERF3, OsCRL1* and *OsPLTs*.

Arabidopsis WOX11/12 regulates expression of *LATERAL ORGAN BOUNDARIES DOMAIN29* (*LBD29*), a putative homolog of *OsCRL1*, suggesting conservation of regulatory mechanism during adventitious root development between rice and *Arabidopsis.* Therefore, we next studied if molecular function of *OsWOX10* is also conserved across plant species. When *OsWOX10* was ectopically over-expressed in *Arabidopsis,* using *Arabidopsis Ubiquitin 10* promoter (Siligato et al., 2016), in wild-type as well in *wox11* and *wox12* single and double mutant background where endogenous *WOX11/12* genes are not functional, *OsWOX10* is sufficient to promote adventitious root formation from the root-hypocotyl junction (Figure 6K; Supplemental Figure 5E). These data suggest conserved role of *OsWOX10* in promoting adventitious root formation.

PLETHORA Genes Are Required for Adventitious Root Development in Rice

The expression of few rice *PLETHORA* (*PLT*) genes were regulated by *OsWOX10* and their expression was activated during CRP development, 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) but their function was unknown. However, *OsPLT8* (*OsCRL5*) function during CR initiation (Kitomi et al., 2011b). We, therefore, analysed temporal and spatial expression pattern of *OsPLT7,* a close relative of *OsPLT8,* during CRP development. *OsPLT7* expression was activated very early at the 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, loss-of-function transgenic rice lines were generated for *OsPLT1* and *OsPLT4.* For this, an inducible RNAi construct was generated by fusing gene-specific fragment of *OsPLT1* and *OsPLT4* and was expressed under maize ubiquitin promoter using estrogen-inducible XVE system (Miki et al., 2005; Siligato et al., 2016). In the transgenic rice lines (p*Ubi::XVE:dsRNAiOsPLT1/4*), where either of the genes were partially down-regulated, the CR development was compromised (Figure 7E-7I). Upon *OsPLT1* down-regulation, growth of CRs were reduced, but no significant effect was seen on PR (Figure 7E and 7F). However, *OsPLT4* down-regulation affects growth of both, PR and CRs (Figure 7G and 7H). These together suggest that *OsPLT1* and *OsPLT4* are required for establishment of proper root architecture in rice.

Root Outgrowth Promoting Function of *OsPLTs* is Conserved in *Arabidopsis*

In *Arabidopsis, PLTs* regulate lateral root outgrowth (Du and Scheres, 2017). We next asked if function of *OsPLTs* is conserved in *Arabidopsis*. Towards this we delivered the regulators of shoot-borne crown root primordia (CRP), the rice *OsPLTs,* in the transcription domain of *Arabidopsis* lateral root primordia (LRP) of *plt3;plt5-2;plt7* triple mutant which were defective LRP outgrowth (Figure 7J2). Strikingly, the lateral root outgrowth defect in the *plt3;plt5-2;plt7* triple mutant was rescued by *OsPLT1* when it was expressed in *Arabidopsis PLT3* domain (Figure 7J3-7J4). Similarly, *OsPLT2* expression under *Arabidopsis PLT5* promoter (*plt 3;5;7; AtPLT5::OsPLT2-YFP*) also rescued lateral root outgrowth in the triple mutant wherein upon reconstitution, lateral root formation resembles the wildtype (Supplemental Figure 6; 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 6; Hofhuis et al.,2013). This suggest that *PLT*-like genes have acquired species-specific expression

domain while the function of proteins is conserved i.e., to promote the outgrowth of root primordia irrespective of their developmental origin.

DISCUSSION

The process of CRP development begins with an induction phase where innermost ground tissues of rice stem base re-enter 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 initiation and outgrowth but also reveals regulatory role of key cell fate determinants at different stages of CRP development. Importantly, our functional study reveals conserved role of rice WOX and PLT genes in controlling the root architecture across the plant species and bring out a novel regulatory module during rice root development.

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 to regulate the 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, their phylogenetic relationship and co-expression pattern in our LCM-seq analysis, OsIAA13/30-OsARF16/19-OsWOX10, OsCRL1 could be a possible regulatory module functional during CRP initiation and OsIAA11/23/30-OsARF8/22-OsDH1 could function 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 of the 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 that occurs during cell fate change associated with 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 LRP outgrowth defects in *Arabidopsis plt3plt5plt7* mutants, 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 the 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 network 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 are 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 the 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 7).

METHODS

Plant Material, Treatment and Laser Capture Microdissection

Oryza sativa var. IR-64 seed germination, growth, auxin treatment and sample collection were performed 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 using Mfuzz (Kumar and Futschik, 2007). The gene count table was made homoscedastic using the variance stabilizing transformation function from DESeq2 (Love et al., 2014). The biological replicates for each stage were collapsed using the geometric mean of the two values. 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 Principal Component Analysis (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). Cluster-wise gene list (log2 fold enrichment ≥1; $p < 0.05$) was used to perform GO enrichment analysis using monocot PLAZA 4.5 workbench (Bel et. al., 2018).

Differential Gene Expression and Gene Ontology Analysis

The count matrix was used as input for differential expression analysis using DESeq2. 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). For auxin responsiveness, genes with p-value less than 0.05 and the $\log_2$ fold-change ≥ 0.9 were considered as auxin inducible. 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 ΔΔCt 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 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 the plasmid pHm-DR5ev-erYFP_nosT2 as 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 inverted repeat RNAi hairpin loop and was cloned in a vector expressing estradiol-inducible 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 fused together to generate a double dsRNAi*OsPLT1/4* and was expressed under maize Ubiquitin promoter driving expression of estradiol-inducible XVE. 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 (Radhakrishnan et al., 2020). 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.

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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.

Bel, M.V., Diels, T., Vancaester, E., Kreft, L., Botzki, A., de Peer, Y.V., Coppens, F., and Vandepoele, K. (2018) PLAZA 4.0: an integrative resource for functional, evolutionary and comparative plant genomics. Nucleic Acids Res., **46,** D1190–D1196.

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.

Miki, D., Itoh, R., and Shimamoto, K. (2005) RNA Silencing of Single and Multiple Members in a Gene Family of Rice. Plant Physiol **138,** 1903-1913.

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.

Siligato, R., Wang, X., Yadav, S.R., Lehesranta, S., Ma, G., Ursache, R., Sevilem, I., Zhang, J., Gorte, M., Prasad, K., Wrzaczek, M., Heidstra, R., Murphy, A., Scheres, B., and Mähönen, A.P. (2016) MultiSite Gateway-Compatible Cell Type-Specific Gene-Inducible System for Plants. Plant Physiol **170,** 627-641.

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 to 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.

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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 is 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 plant (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. Site of CRP and developing CRP are highlighted with black arrowhead. 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: Heat-map showing mostly distinct GO terms enrichment in in different clusters. Log2 fold change ≥ 1 with $p<0.05$ parameter was considered, and p-value was used to generate the heatmap.

Supplemental Figure 2: 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 3: 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 4: (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 5: 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. (E) Expression of *OsWOX10* in 10d old *Arabidopsis wox11* and *wox12* mutants.

Supplemental Figure 6: 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 7: Schematic representation and genetic regulators during CRP initiation and outgrowth.

Supplemental Dataset 1: List of all genes de-regulated during CRP initiation and outgrowth

Supplemental Dataset 2: List of genes commonly and specifically de-regulated during CRP initiation and outgrowth

Supplemental Dataset 3: List of all TFs de-regulated during CRP initiation and outgrowth (log

Supplemental Dataset 4: List of TFs specifically de-regulated during CRP initiation and outgrowth

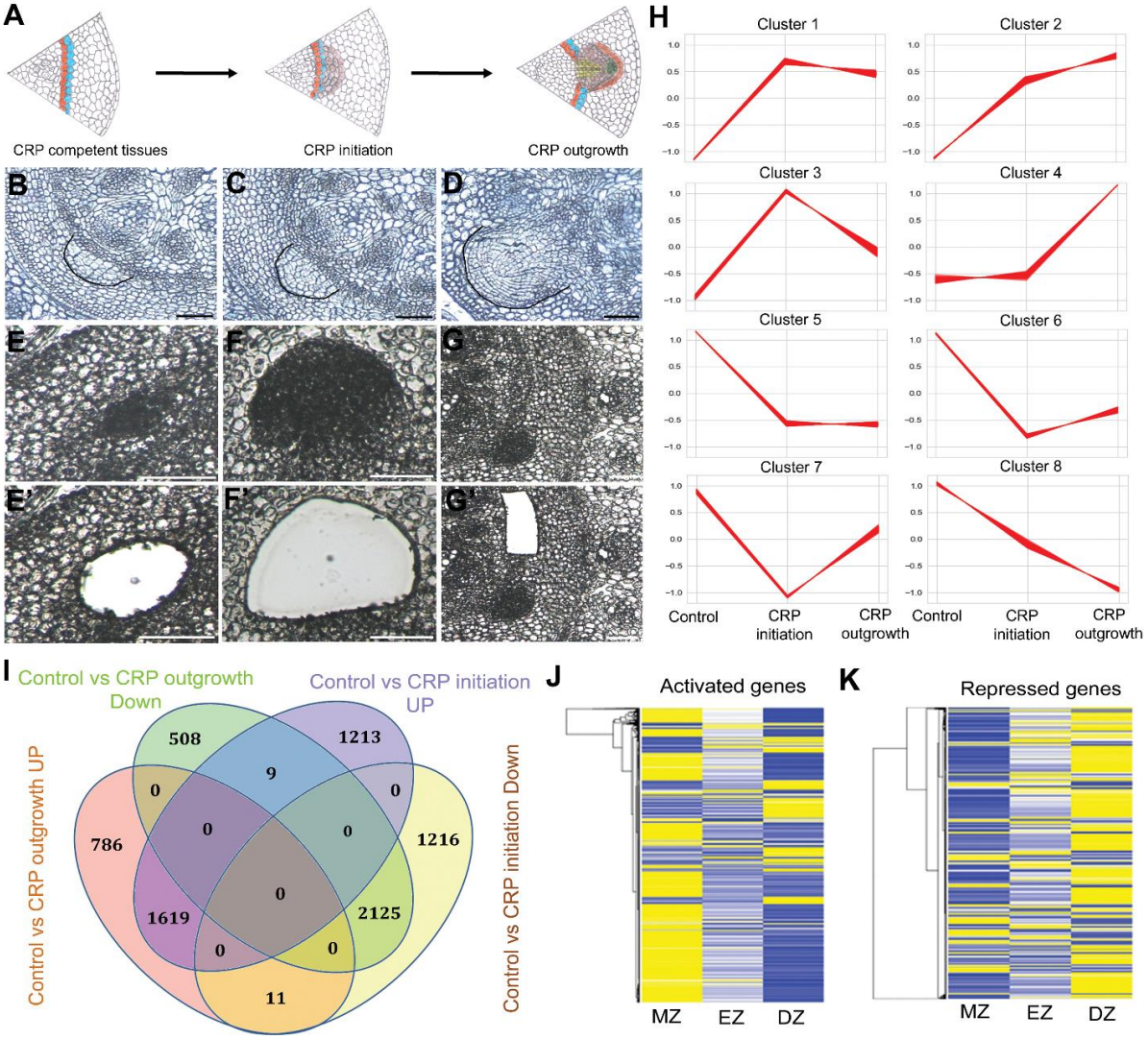
Supplemental Dataset 5: List of auxin-responsive TFs commonly and specifically de-regulated during CRP initiation and outgrowth

Supplemental Dataset 6: List of *OsCRL1*-regulated genes de-regulated during CRP initiation and outgrowth

Supplemental Dataset 7: List of auxin signaling genes de-regulated during CRP initiation and outgrowth

Supplemental Table 1: List of putative epigenetic regulators during CRP initiation and outgrowth

Supplemental Table 2: List of primers used in this study



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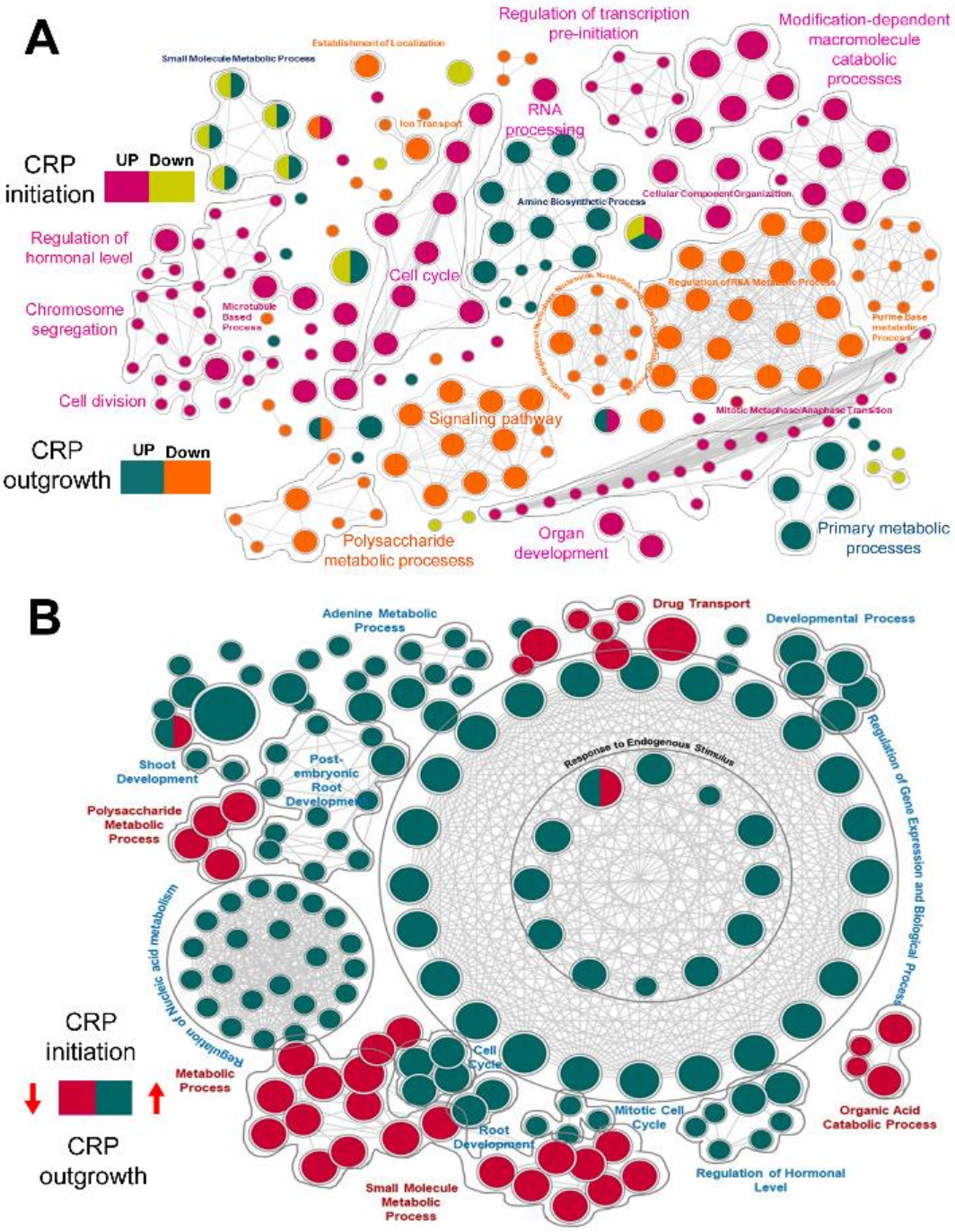

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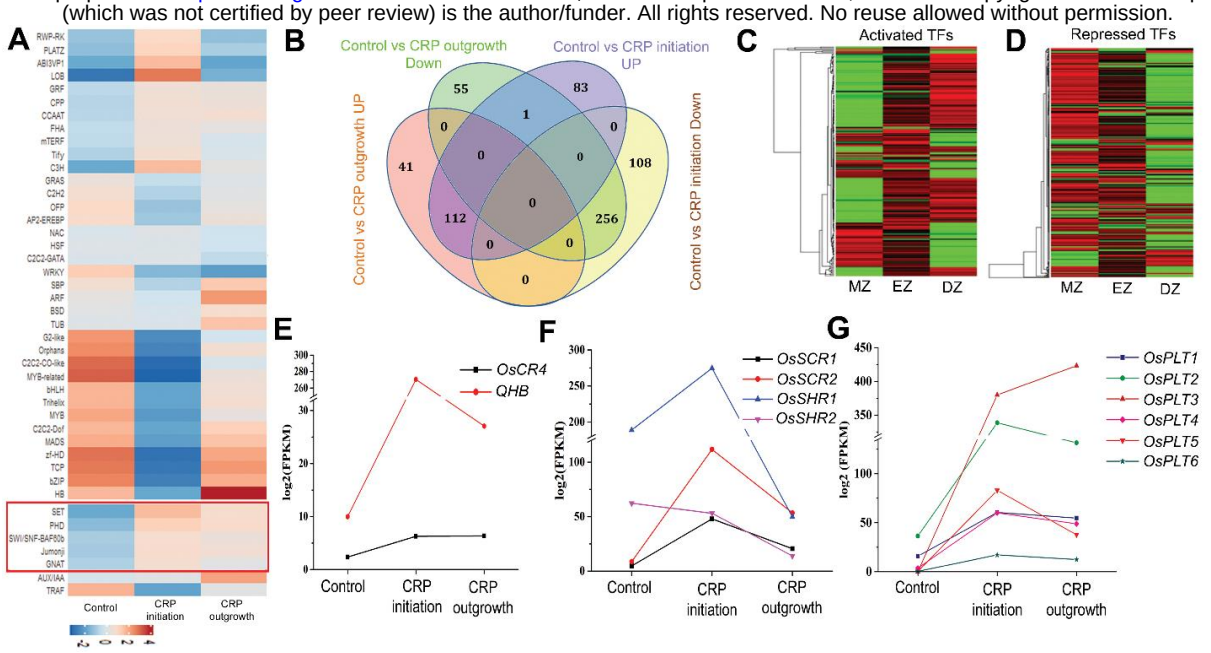

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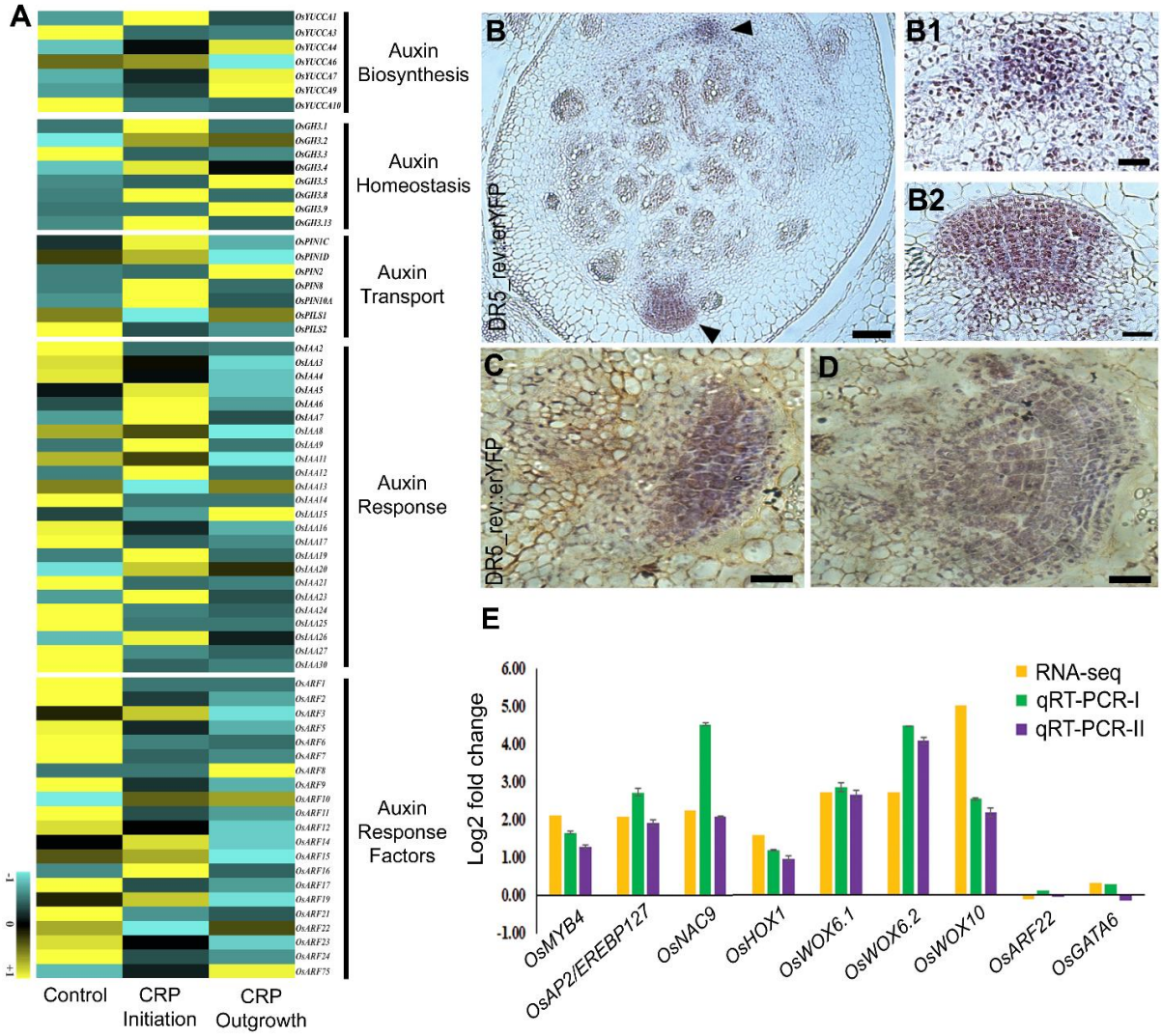

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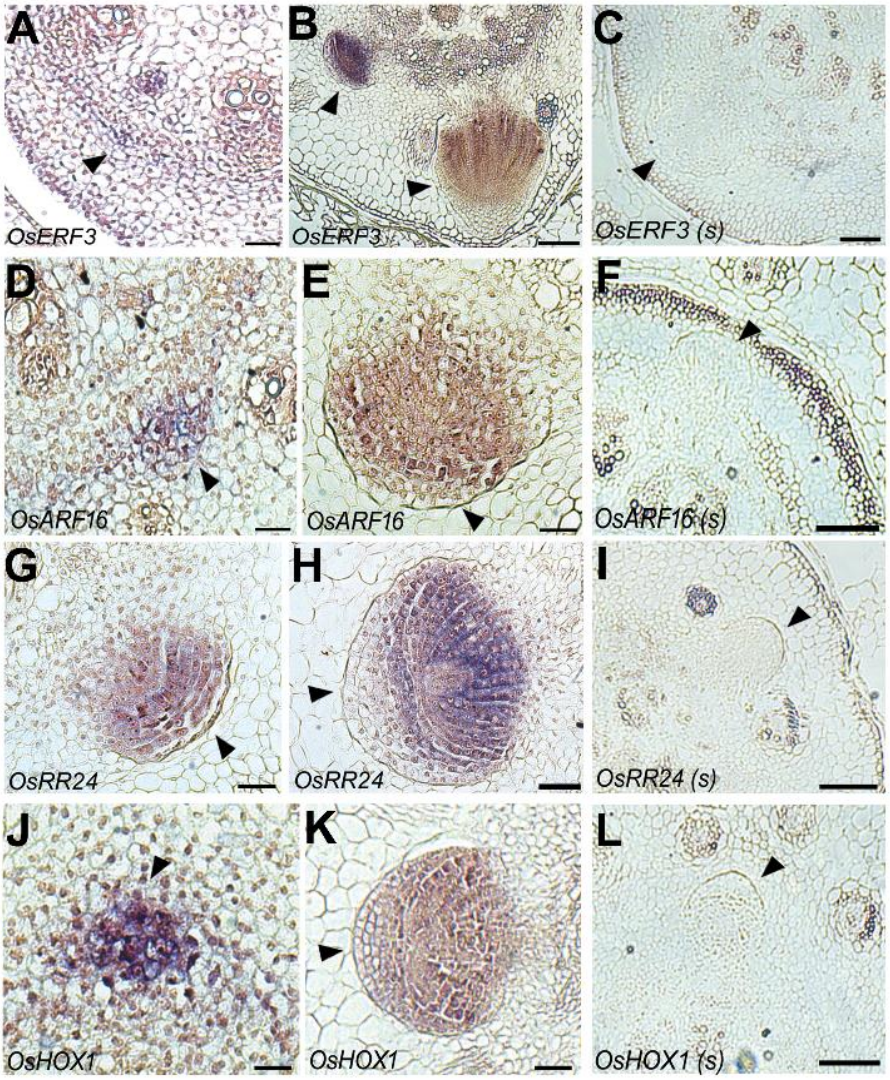

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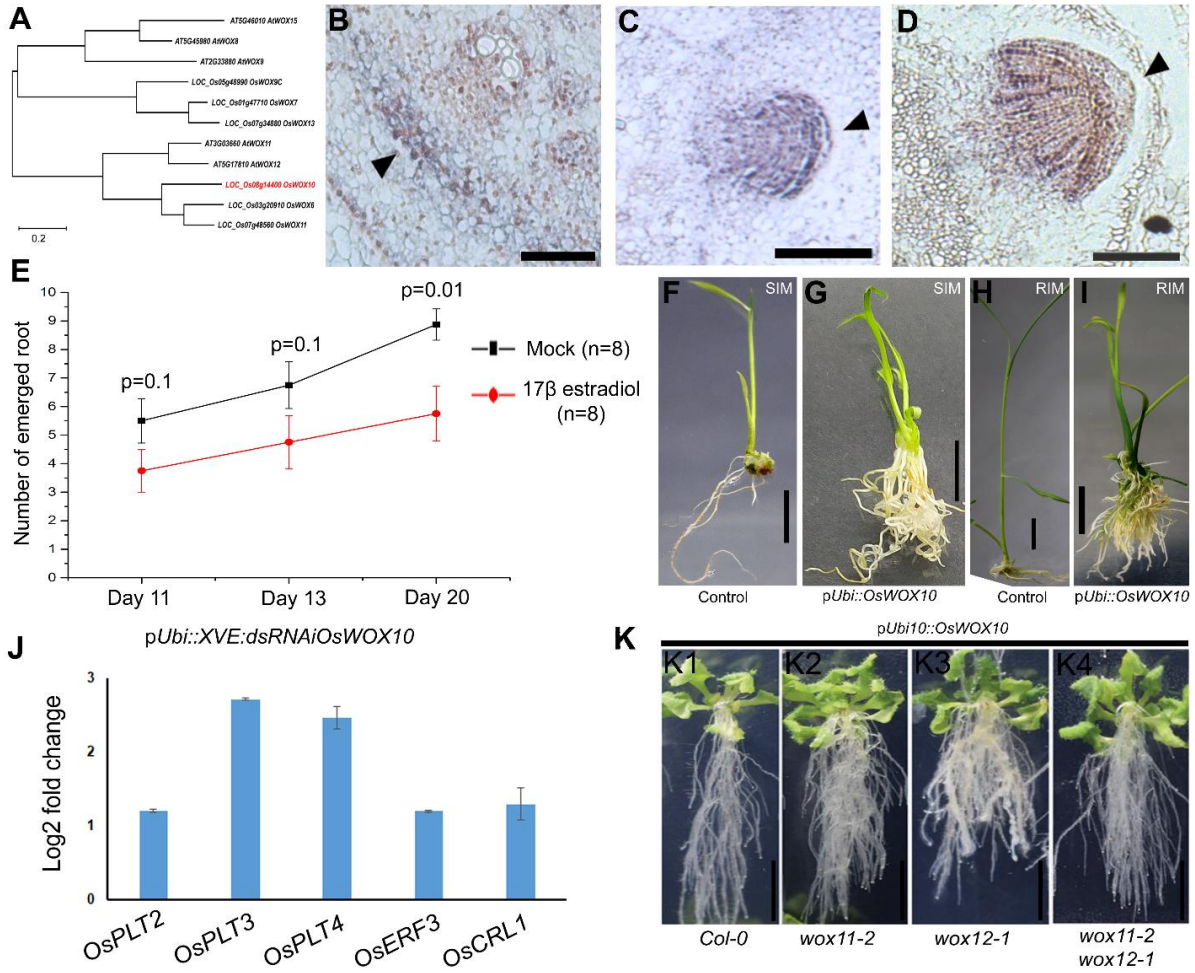

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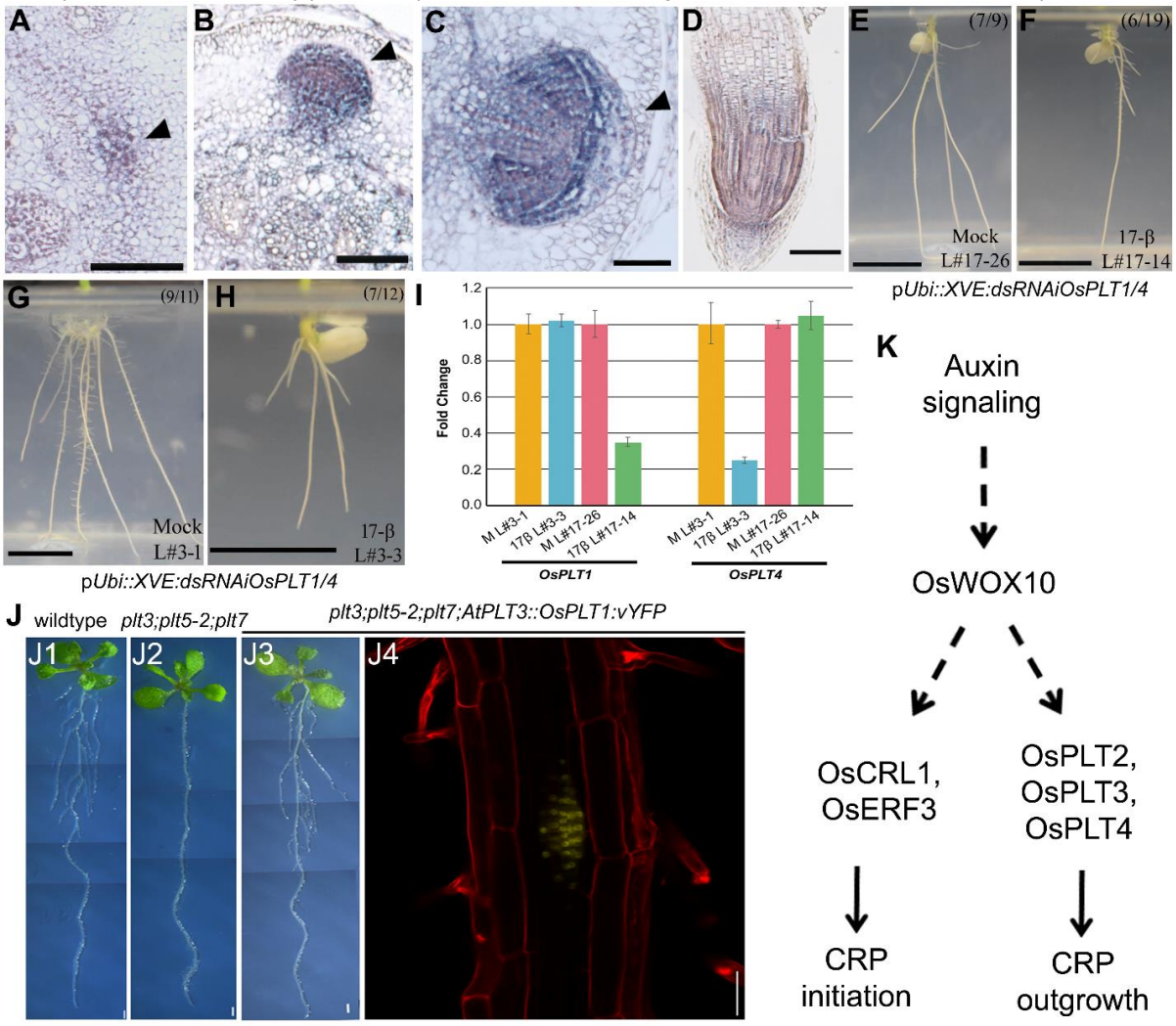

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