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Apical Stem Cell(s): Evolutionary Basis for 3D Body Plans in Land Plants

A functionally informed evolutionary framework for the study of LRR-RLKs during stem cell maintenance

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Abstract

Plants maintain populations of stem cells to generate new organs throughout the course of their lives. The pathways that regulate plant stem cell maintenance have garnered great interest over the past decades, as variation in these pathways contributes plant morphological diversity and can be harnessed for crop improvement. In order to facilitate cross-species comparisons of gene function and better understand how these stem cell regulatory pathways evolved, we undertook a functionally informed phylogenetic analysis of leucine-rich receptor-like kinases (LRR-RLK) and related proteins across diverse land plant model systems. Based on our phylogenetic analysis and on functional data, we propose a naming scheme for these stem cell signaling genes. We discovered evidence for frequent loss of protein domains in angiosperms but not in bryophytes. In addition, several clades of stem cell signaling genes are closely related to genes that function in immunity, although these distinct developmental and immune functions likely separated or after the divergence of lycophytes and angiosperms. Overall, the phylogenetic framework and evolutionary hypotheses we provide here will empower future research on cross-species comparisons of stem cell signaling pathways.

Keywords Leucine-rich receptor-like kinase · Stem cells · Phylogeny

Introduction

New plant tissues develop from reserves of stem cells called meristems that are found at the tips of roots and shoots and at the sites of vasculature formation. Maintenance of stable stem cell populations poses a challenge during development: if the stem cell population grows too large then development becomes disorganized, whereas under-proliferation of the stem cell pool can lead to meristem consumption and the termination of development. Signaling pathways dedicated to meristem maintenance are thus critical for maintaining indeterminate growth, a hallmark of plant development and a strategic source of morphological diversity.

Decades of research have revealed widespread functions for a suite of leucine-rich receptor-like kinases (LRR-RLK), receptor-like proteins (LRR-RLP), and pseudokinases in the regulation of plant meristem maintenance. These include the LRR-RLKs CLAVATA1 (CLV1) (Clark et al. 1997), PHLOEM INTERCALATED WITH XYLEM (PXY) (Fisher and Turner 2007), and RECEPTOR-LIKE PROTEIN KINASE 2/TOADSTOOL 2 (RPK2) (Kinoshita et al. 2010), the LRR-RLPs CLAVATA2 (CLV2) (Kayes and Clark 1998) and FASCIATED EAR 3 (FEA3) (Wu et al. 2016), the pseudokinase CORYNE (CRN) (Miwa et al. 2008), and the CLAVATA INSENSITIVE KINASE (CIK) co-receptors (Hu et al. 2018). Each of these cell-surface proteins are thought to act in signal perception and transduction that is elicited by mobile CLAVATA3/EMBRYO SURROUNDING REGION (CLE) peptide ligands.

LRR RLKs and related proteins have been studied predominately in the model plant *Arabidopsis thaliana*. While much progress has been made, we are still far from understanding the downstream signaling pathways or how these

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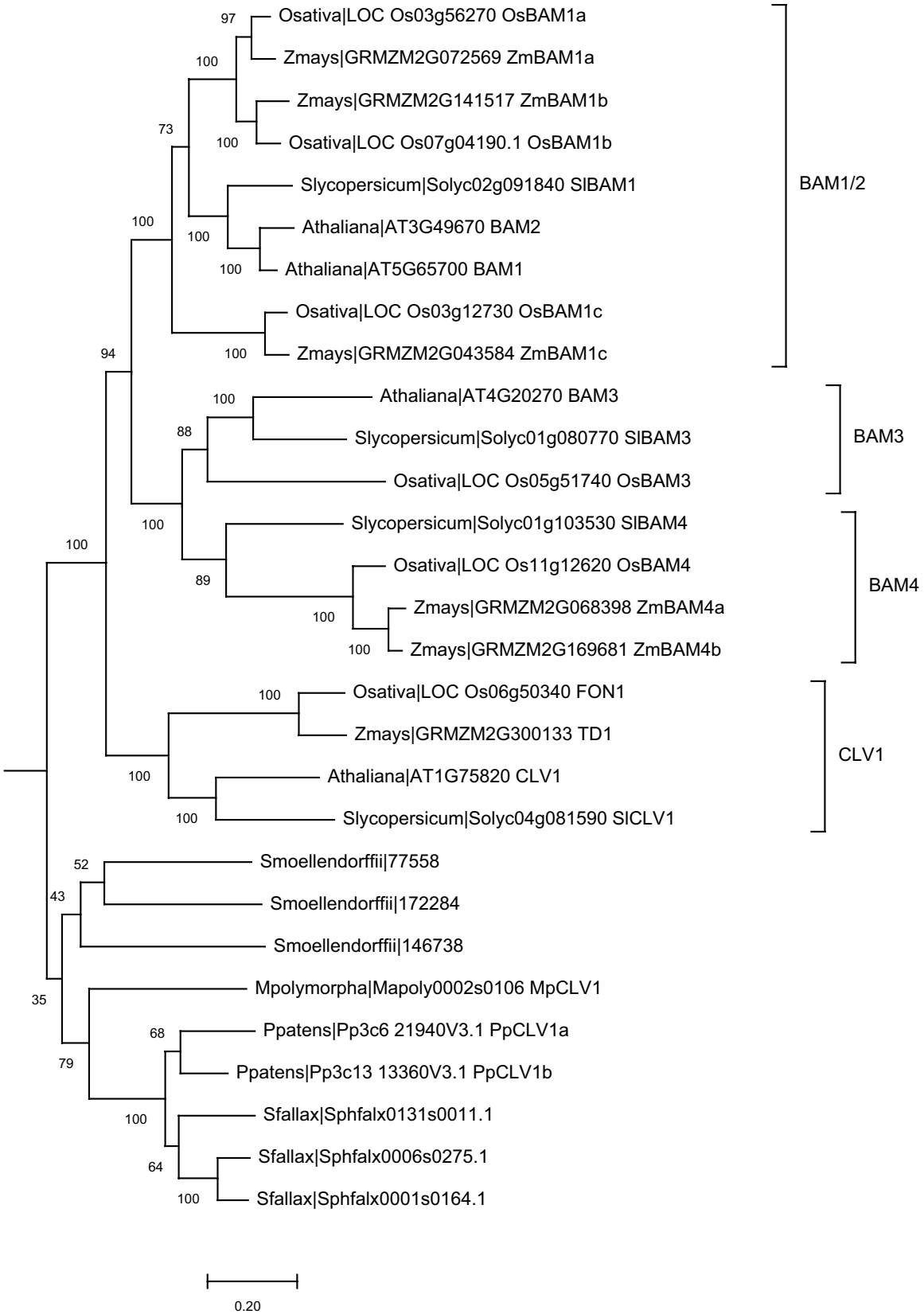

Fig. 1 Maximum Likelihood tree of CLV1/BAM LRR-RLKs based on full length peptide sequences. Subtree shown here is taken from a larger maximum likelihood tree; bootstrap support at the base of this tree was 100%. *CLAVATA1* and *BAM* genes are paralogs that evolved after the split between lycophytes and angiosperms, while Selaginella and bryophyte orthologs underwent lineage-specific duplications

signaling components are employed in different developmental contexts. More recently, research on crop and bryophyte species has revealed that many of these signaling components have conserved functions, and that these pathways can be altered for agronomic benefit (Bommert et al. 2013; Je et al. 2018; Rodríguez-Leal et al. 2017; Whitewoods et al. 2018). These results demonstrate the insights gleaned from and the benefits of studying stem cell maintenance in diverse model species.

This work promotes a strategy of using a framework of combined phylogenetic and functional data to facilitate future analyses of meristem regulatory LRR RLKs from diverse species. We assess how stem cell regulating LRR-RLKs and related proteins have evolved across several plant model organisms, namely Arabidopsis, Tomato, Maize, Rice, the moss *Physcomitrella patens* (Hedw.) Bruch & Schimp., and the liverwort *Marchantia polymorpha* L. We extend our analysis to include the lycophyte *Selaginella moellendorfii* Hieron. and the moss *Sphagnum fallax* H. Klinggr. in order to shed light on gene duplications associated with the evolution of vasculature, and to gain a broader understanding of bryophyte stem cell signaling. We also propose a cogent, functionally and phylogenetically based nomenclature for heretofore unannotated orthologs of these meristem signaling components (Table S1). Finally, we use our phylogenetic analysis to highlight trends and propose testable hypotheses about the evolution of stem cell signaling in land plants.

Materials and methods

Starting with *Arabidopsis thaliana* gene of interest (i.e. CLV1), except in the case of FEA3 where the maize ortholog was used, we performed pBLAST in Phytozome 11 against: *Arabidopsis thaliana* TAIR10, *Solanum lycopersicum* iTAG2.4, *Zea mays* Ensembl-18, *Oryza sativa* v7_JGI, *Selaginella moellendorfii* v1.0, *Physcomitrella Patens* v3.3, *Marchantia polymorpha* v3.1, and *Sphagnum fallax* v 0.5 proteomes. Peptide sequences for the top 250 blast hits were selected and then filtered so that only peptides encoded by primary transcripts remained.

We used the CIPRES portal to run mafft set to the slowest but most accurate mode (linsi) (Katoh 2005). We then trimmed multiple sequence alignments of positions high in gaps using trimal (Capella-Gutiérrez et al. 2009), removing any position comprised of over 50% gaps. Using these trimmed multiple sequence alignments, we then constructed phylogenetic trees using RaXML (Stamatakis 2014) set to the PROTCATDAYHOFF model with 1000 rapid bootstrap via the CIPRES server (Miller et al. 2010). We viewed trees with the highest likelihood score with bootstrap values represented on bipartitions using MEGAX. From these larger trees, we found the most basal bipartition with a support value over 90% that contained our gene family of interest and selected that subtree for representation here.

Trees were visualized and annotated in MEGAX or using ete3 (Huerta-Cepas et al. 2016). Alignments juxtaposed to trees were alignments only of the sequences referenced on that tree (not the whole set of 200) aligned using Muscle (Edgar 2004) visualized in Aliview.

Results

CLV1 and BAM: dynamic gene gain and loss

CLV1 encodes an LRR/RLK that regulates stem cell identity in the SAM by acting in a negative feedback loop wherein the homeobox transcription factor WUSCHEL (WUS), expressed in the middle of the shoot apical meristem (SAM), diffuses to overlying cells to activate the expression of the CLE peptide-encoding gene *CLAVATA3* (*CLV3*) (Schoof et al. 2000). CLV3 is secreted and diffuses back down to the middle of the meristem, where it acts through the CLV1 receptor to repress *WUS* expression, completing the negative feedback loop. Angiosperm genomes contain a suite of paralogs of *CLV1* called *BARELY ANY MERISTEM* (*BAM*). Our analysis of the *CLV1/BAM* clade of *LRR-RLK* genes suggests that Arabidopsis *BAM1* and *BAM2* were generated following a recent gene duplication (Fig. 1). We also detected a clade of *BAM* genes absent from the Arabidopsis genome that includes the recently characterized tomato gene *SlBAM4* (Rodriguez-Leal et al. 2019). Meanwhile, maize lacks a member of the *BAM3* clade, but contains two copies of *BAM4*. Overall, there is evidence for frequent gain and loss of *BAM* genes, while *CLV1* was maintained as a single copy in each angiosperm sampled.

Our analysis also shows that *CLV1* and *BAM* genes diverged after the separation of the lycophyte and flowering plant lineages (Fig. 1). Thus, lycophyte and bryophyte genes presented here are co-orthologous to the *CLV1* and *BAM* clades. Recent work demonstrated a conserved role for the moss genes *PpCLV1a* and *PpCLV1b* in inhibiting meristem identity and uncovered a previously undescribed function in the regulation of cell division plane orientation (Whitewoods et al. 2018). Intriguingly, a role for CLV1/BAM in the control of cell division plane orientation was also found to be conserved in Arabidopsis, wherein *clv1, bam1, bam2, bam3* quadruple mutations resulted in cell division plane defects in the root (Whitewoods et al. 2018). It is likely that historical challenges in generating higher order mutants had obscured the role of CLV1/BAM during cell division plane orientation in Arabidopsis. Moreover, these reverse genetic challenges had previously rendered cross-species comparisons of 'loss of clade' rather than 'loss of gene' function untenable.

However, with the advent of facile genome editing technologies and a wealth of genomic information, we can, informed by phylogenies, test and gain a general understanding of gene family function.

Many developmental functions for *BAM1* and *BAM2* have been demonstrated, including CLE perception and regulation of cell fate and periclinal divisions in root vasculature and in anther development, and buffering of CLE signaling in the SAM (Cui et al. 2018; DeYoung and Clark 2008; Hord et al. 2006; Qian et al. 2018; Shimizu et al. 2015). Despite these distinct roles for *CLV1* and *BAM1/BAM2*, *BAM1* also compensates for *clv1* loss of function in shoot meristems (Nimchuk et al. 2015). These data suggest that BAM1/BAM2 can perform the same biochemical function as CLV1, and that differences in mutant phenotypes between these related LRR-RLKs are due to differences in gene expression.

PXY: an ancient LRR-RLK recruited to vascular development

Within the broader LRR-RLK phylogeny, the clade containing *PHLOEM INTERCELATED WITH XYLEM* (*PXY*) is sister to the *CLV1* and *BAM* clade of receptor kinases (Liu et al. 2017). Like *CLV1*, *PXY* encodes a CLE receptor and regulates the activity of a *WUSCHEL-like HOMEOBOX* (*WOX)* gene, here *WOX4* in the stem cell niche comprising the vascular procambium (Etchells et al. 2013; Hirakawa et al. 2010). The PXY ligand is TDIF/CLE41, a different class of CLE from CLV3 (Goad et al. 2017). Whereas *PXY* is conserved across flowering plants and Selaginella, the moss genomes sampled here lack both *PXY* (Fig. 2) and TDIF orthologs (Whitewoods et al. 2018). However, the genome of the liverwort *Marchantia polymorpha* harbors a *PXY* ortholog, as well as TDIF peptide encoding gene, which together reduce cell proliferation near the apical notch of the thallus (Hirakawa et al. 2019). This topology and the functional characterization of TDIF signaling in Marchantia suggests that PXY function predates the evolution of vasculature, and that a function during vascular formation was co-opted later in land plant evolution.

CLAVATA2 and CORYNE: pieces of a whole

Conclusive evidence for protein-protein interactions among LRR-RLKs is scarce, owing to the inherent difficulties in studying low-abundance membrane-associated proteins. However, data supporting the formation of a CLV2:CRN complex is compelling (Bleckmann et al. 2010; Guo et al. 2011; Somssich et al. 2015). CLV2 possesses an LRR-ectodomain while CRN possesses a cytoplasmic domain but no ectodomain; it is attractive to think that together these two proteins constitute a complete LRR-RLK. However, the CRN cytoplasmic domain possesses a pseudokinase that is important for its function, although the mechanism is unclear. Like other LRR-RLK complexes that maintain stem cell populations in the SAM, *CLV2* and *CRN* have roles in diverse developmental processes including phloem development (Hazak et al. 2017). The function of *CLV2* and *CRN* appear to be conserved in grasses, as mutants of the maize *CLV2* ortholog *FASCIATED EAR 2* (*FEA2*) also develop enlarged and fasciated inflorescence meristems (Taguchi-Shiobara et al. 2001). In both models, the effects of *clv1* and *clv2/crn* loss of function are additive, suggesting that CLV1 and CV2/CRN comprise distinct CLE signaling pathways (Müller et al. 2008).

In our phylogenetic analysis, we find that *CLV2* exists as a single-copy gene in the four angiosperm genomes sampled, and we did not detect *CLV2* orthologs in *Selaginella moellendorffii* or bryophytes (Fig. S1). Further analysis, however, is limited by very low support values for relationships along the backbone of the phylogenetic tree, hindering our ability to draw further conclusions about the evolution of *CLV2* within land plants.

Similar to *CLV2*, each angiosperm genome assayed here possesses one ortholog of *CRN* (Fig. 3). In the case of *CRN* however, we were able to identify a well-supported sister clade containing the Arabidopsis receptor kinase gene *SUPPRESSOR OF BIR1* (*SOBIR1*). Unlike CRN, SOBIR1 possesses an extracellular domain with LRRs and has been described to function in immunity-induced and developmentally programmed cell death (Gao et al. 2009; Leslie et al. 2010). We identified orthologs of *SOBIR1* in Marchantia, Physcomitrella, and Selaginella; the Marchantia and Physcomitrella genes are predicted to encode proteins containing the longest extracellular domains of any in the clade (Fig. 3). Interestingly, the maize and rice orthologs of SOBIR1 have short extracellular domains, similar to CRN. These disparities raise questions as to whether these grass SOBIR1 orthologs functionally resemble *SOBIR1* or *CRN*, or whether they possess separate functions entirely. These phylogenetic data support a model wherein CRN is evolutionarily derived from a full-length LRR-RLK, raising the appealing hypothesis that *CLV2* is similarly derived from a gene encoding a full length LRR-RLK but lost its cytoplasmic domain. However, whereas conservation of the kinase domain enables phylogenetic analyses of *CRN*, discerning the evolution of *CLV2* will prove much more difficult given that the sequence is largely composed of repeat domains.

RPK1/RPK2: Structural changes and possible neofunctionalization

RPK2 acts downstream of CLE signaling in multiple developmental contexts. In the SAM, *RPK2* performs a similar function as *CLV1* and *CLV2*, but via a separate pathway (Kinoshita et al. 2010). During anther development, *RPK2* acts with *BAM1* and *BAM2* to coordinate cell division plane

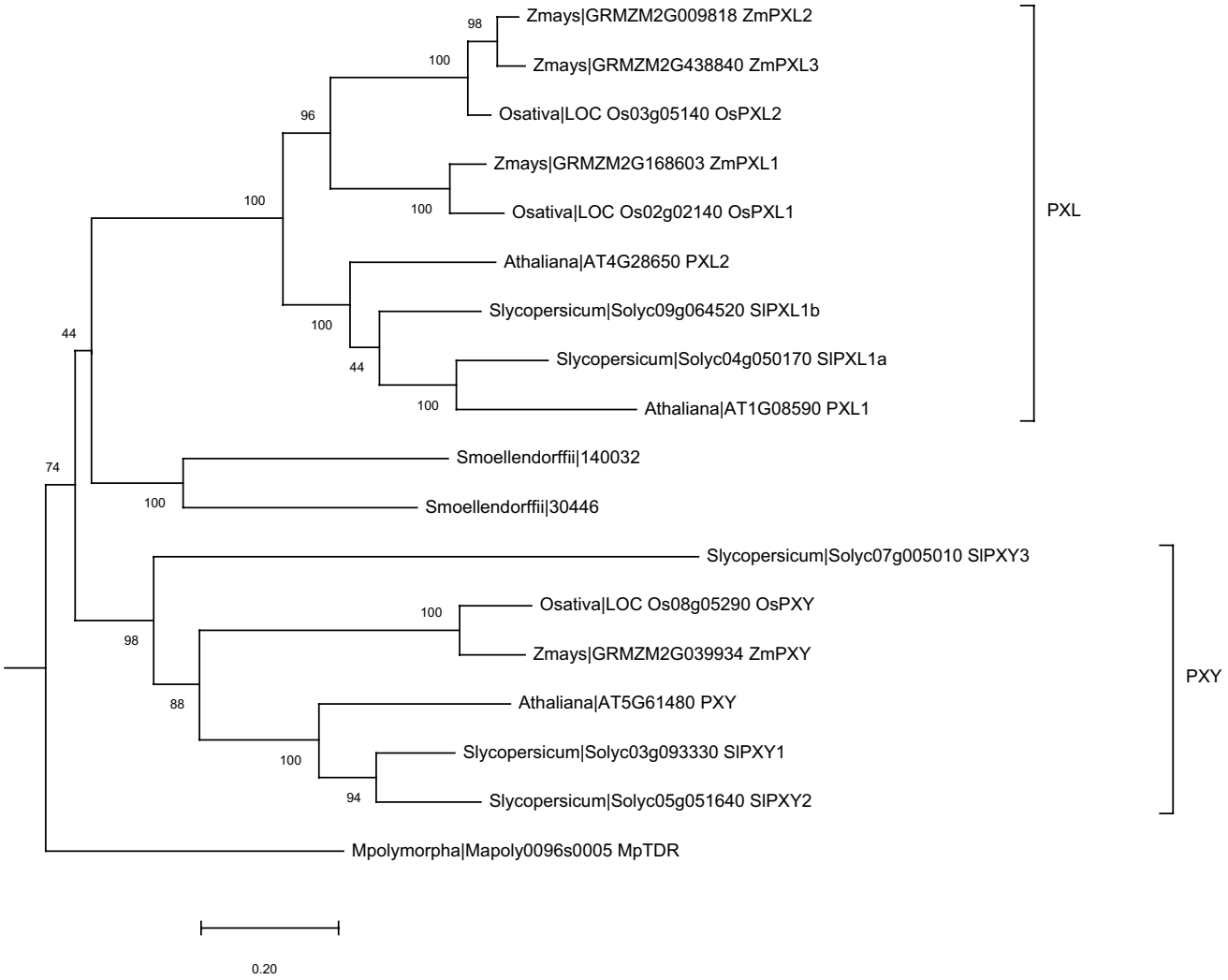

Fig. 2 Maximum Likelihood tree of PXY LRR-RLKs based on full length peptide sequences. Subtree shown here is taken from a larger maximum likelihood tree that also included the CLV1/BAM clade; bootstrap support at the base of this subtree was 100%. *PXY* is typically associated with vascular development, but the non-vascular liverwort *Marchantia polymorpha* possesses one *PXY* ortholog

orientation and cell identity (Cui et al. 2018; Mizuno et al. 2007). As a final example of overlapping function with CLV1/BAM type LRR-RLKs, RPK2 and BAM1 interact to inhibit cell proliferation in the root (Shimizu et al. 2015). Interestingly, and despite the repeated discovery of overlap between *RPK2* and *CLV1/BAM* genes, the Arabidopsis *RPK2* paralog *RPK1* appears to function in a completely distinct pathway. RPK1 is required for ABA response (Osakabe et al. 2005) and is essential for shoot regeneration (Motte et al. 2014). However, there is an exception to this separation of *RPK2* and *RPK1* pathways, as these genes appear to have redundant functions in the embryo where they are implicated in organizing auxin efflux carriers during embryonic patterning (Nodine et al. 2007). While this impact on auxin efflux is the most mechanistic description of RPK1 or RPK2 function, whether such changes in auxin transport could account for other *rpk1* or *rpk2* mutant phenotypes has not been explored.

As *RPK1* and *RPK2* have distinct functions in most developmental contexts, we used our phylogenetic analyses to determine whether *RPK2* or *RPK1* is more likely to carry out the ancestral *RPK1/RPK2* function, and whether one gene's activity is likely the result of neofunctionalization. Given that Selaginella, Physcomitrella, and Marchantia each have a single *RPK1/RPK2* homolog, tree topology alone provides little useful information toward answering this question (Fig. 4). Comparing the structures of the bryophyte and angiosperm *RPK1/RPK2* homologs (Fig. 4) reveals that *RPK1* and several angiosperm orthologs are truncated, with shorter extracellular domains, whereas *RPK2* resembles the ancestral, full-length form. Together with recent functional data from Physcomitrella showing that *PpRPK2* is a

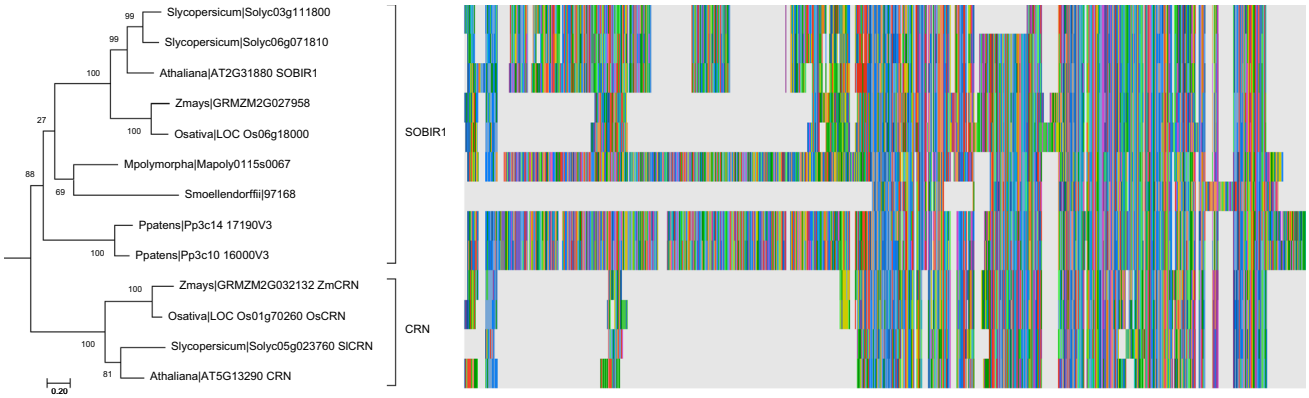

Fig. 3 Maximum Likelihood tree of the CRN pseudokinase and the related LRR-RLK SOBIR1 based on full length peptide sequences. Appended to the right is a realignment of the full-length peptide sequences from the genes represented in the tree. Subtree shown here is taken from a larger maximum likelihood tree; bootstrap support at the base of this subtree was 100%. CRN and SOBIR1 family members have distinct functions, but repeated domain loss has led to the convergent evolution of similar protein structures between the clades with truncated ectodomains

regulator of stem cell number and cell division plane similar to *RPK2* (Whitewoods et al. 2018), this evidence suggests that *RPK1's* role in ABA signaling might represent neofunctionalization of the ancestral *RPK1/RPK2* gene, concurrent with a loss of LRR domains.

Like *RPK1*, several other genes in this clade encode proteins that are truncated or appear to be missing internal LRR domains (Fig. 4). While one of these short-ectodomain variants includes the tomato gene most closely related to *RPK1*, in several cases the number of LRR domains is unrelated to the position of the gene on the tree. This suggests, as others have shown (Liu et al. 2017), that LRR domain number is highly dynamic. In the case of *RPK1/RPK2*, it would be interesting to see whether, as appears to be the case for moss and Arabidopsis *RPK2*, functional conservation can be predicted based on conserved LRR domain structure more than by relatedness as depicted by the gene tree (determined by sequence). In accordance with this hypothesis, we named short-ectodomain homologs of *RPK1/RPK2* as *RPK1 LIKE* and long-ectodomain homologs *RPK2 LIKE* (Table S1).

FEA3 and TMM: close relatives with distinctive ligands

The RLP-encoding gene *FEA3* was discovered in maize as a single copy gene regulating meristem size, akin to *CLV1* (Wu et al. 2016). FEA3 is hypothesized to binds to and transduce signals from the CLE peptide FON2-LIKE CLE PROTEIN 1 (FCP1). The discovery of *FEA3* led to the hypothesis that different LRR-RLKs contribute to the regulation of meristem size by controlling the expression of *WUS* in different meristematic domains. In this model, *FEA3* represses *ZmWUS1* in the center of the SAM toward the stem in response to FCP1, whereas in Arabidopsis CLV1

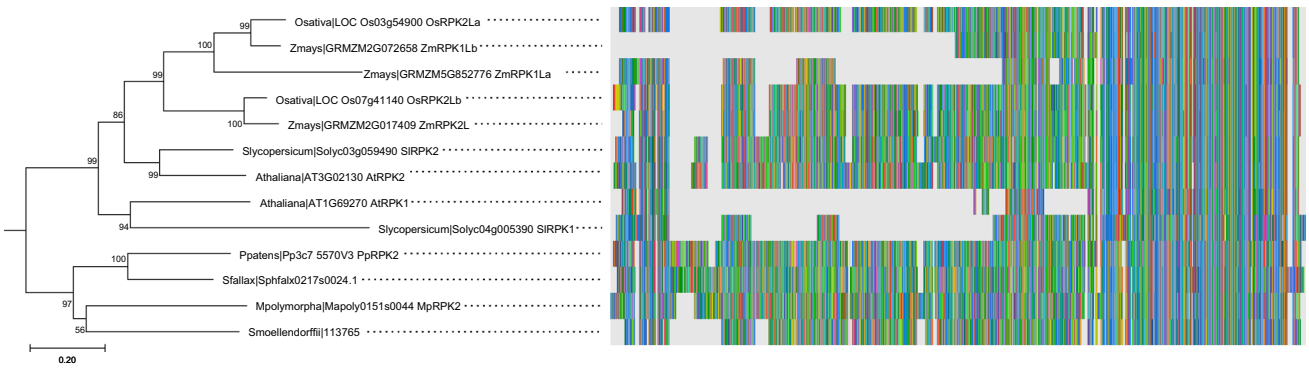

Fig. 4 Maximum Likelihood tree of the RPK1 and RPK2 LRR-RLKs based on full length peptide sequences. Subtree shown here is taken from a larger maximum likelihood tree; bootstrap support at the base of this subtree was 100%. Adjacent to the phylogeny are the realigned, full-length peptide sequences encoded by the genes depicted on this subtree. *AtRPK2* more closely resembles the bryophyte ortholog of *RPK1/RPK2*. *T*runcations of genes in this clade appear to be common derived features

responds to CLV3 to repress *WUS* nearer the apex of the shoot meristem. While FEA3 was originally hypothesized to regulate SAM size based on leaf-derived FCP1 (Wu et al. 2016), more recent data conflicts with the originally reported expression domains for *FCP1*, indicating that the model of FCP1-FEA3 activity should be revisited (Knauer et al. 2019).

Unexpectedly, our phylogenetic analysis revealed that the clade sister to *FEA3* contains the Arabidopsis gene *AT4G28560*, which is annotated as *ROP-INTERACTIVE CRIB MOTIF-CONTAINING PROTEIN 7* (*RIC7*) that contains a CRIB (Cdc42/Rac-interactive binding) domain (Fig. 5). This annotation led us to question the close position of *AT4G28560* to *FEA3* in our maximum likelihood analysis. After subjecting AT4G28560 to a conserved protein domain search, we found that this gene encodes a protein predicted to contain 9 LRR domains and no CRIB domain (Fig. S2). Additionally, the highest scoring pBLAST hits against the Arabidopsis thaliana genome are the Arabidopsis orthologs of *FEA3*, but not other *RIC* gene family members (pBLAST data not shown). Finally, another gene model, *AT4G28556*, is annotated as *RIC7* in the paper where RIC7 function was originally characterized (Jeon et al. 2008).Altogether, these results suggest that *AT4G28560* is currently misannotated as *RIC7*. Furthermore, the clade containing *AT4G28560* also contains one rice and one tomato gene, but no maize genes, suggesting that the maize ortholog may have been lost and that the current closest maize ortholog is *FEA3*.

We next tried to determine whether *FEA3* is conserved in bryophytes and Selaginella. Although the tree topology with the highest likelihood places a set of Selaginella and bryophyte genes sister to the clade containing *FEA3*, bootstrap support for these relationships are low (Fig. 5). It is thus difficult to tell conclusively whether the lycophyte and bryophyte clades are more closely related to *FEA3*, or to the gene family sister to *FEA3* containing the *ERECTA* (*ER*) co-receptor *TOO MANY MOUTHS* (*TMM*) (Lee et al. 2012). Given that *TMM* has a well-supported moss ortholog (*Pp3c3 3780V3.1*) separate from the putative bryophyte *FEA3* orthologs, we propose that the bryophyte clade sister to the *FEA3* clade likely comprises true orthologs of *FEA3*. Interestingly, while *Physcomitrella patens* contains a high-confidence ortholog of *TMM* with a demonstrated conserved function (Caine et al. 2016), the peat moss *Sphagnum fallax* and the liverwort *Marchantia polymorpha* do not. Thus, as neither Sphagnum nor Marchantia possess stomata and Physcomitrella does, the presence/absence of *TMM* tracks well with the evolution of stomata. These analyses suggest that TMM functions specifically in stomatagenesis as far back as the earliest land plants.

Unlike the *CRN* and *RPK1/RPK2* gene families, the RLPs comprising the FEA3 and TMM clades vary little in their protein length and overall domain structure, at least in the taxa sampled (Fig. S3). These data suggest that while dynamic LRR-domain gain and loss is common in many LRR-RLK gene families, it is not the case universally.

CIK: co-receptors at the crossroads of immune and stem cell signaling

CLAVATA insensitive kinases (CIKs) are recently discovered LRR-RLKs that act as co-receptors within diverse developmental contexts. CIKs form co-receptor complexes with many of the signaling proteins discussed above, including CLV1, BAM1/2, RPK2, CLV2, and CRN (Anne et al. 2018; Cui et al. 2018; Hu et al. 2018). CIK receptors are closely related to the NSP-INTERACTING KINASE (NIK) LRR-RLKs that function in plant immunity (Fontes et al. 2004; Zorzatto et al. 2015). Expansion and diversification of CIK and NIK receptors occurred following the speciation events that separated bryophytes and lycophytes from vascular plants (Fig. 6). We thus resolve a well-supported clade of bryophyte genes co-orthologous to all angiosperm CIK and NIK LRR-RLKs.

The CIK/NIK clade thus presents us with a family of receptors that function in both immunity and development. Expression of *NIK* genes in Arabidopsis under *CIK* promoters can complement *cik* mutant phenotype (Anne et al. 2018), indicating that while the function of these genes has diverged, the biochemical operations they can conduct have not. Immune and developmental pathways consistently exhibit substantial crosstalk, and how similar signaling pathways are parsed differently during development and immune response is an open question in plant biology. Given that CIK1/2 and NIK1/2 are such similar proteins with quite distinct functions, we propose that comparison of all *CIK/NIK* genes to their bryophyte orthologs will prove a fertile ground for experiments seeking to understand how subfunctionalization of LRR-RLKs occurs, how different receptor protein complexes evolve, and the crosstalk between immune and developmental signaling.

Discussion

Many of the LRR-RLKs discussed herein are have distinct functions across many tissues but are unified in their regulation of stem cell specification. Within a clade, the ability of various homologs to complement one another is widespread, despite differences in mutant phenotypes among these homologs. Often times these differences in loss-of-function phenotype are ascribed to variations in expression domain. However, given that a protein accumulates within a new domain, two distinct outcomes are possible. First, the protein can perform the same biochemical operation it did in its original domain (i.e. subfunctionalization); which can

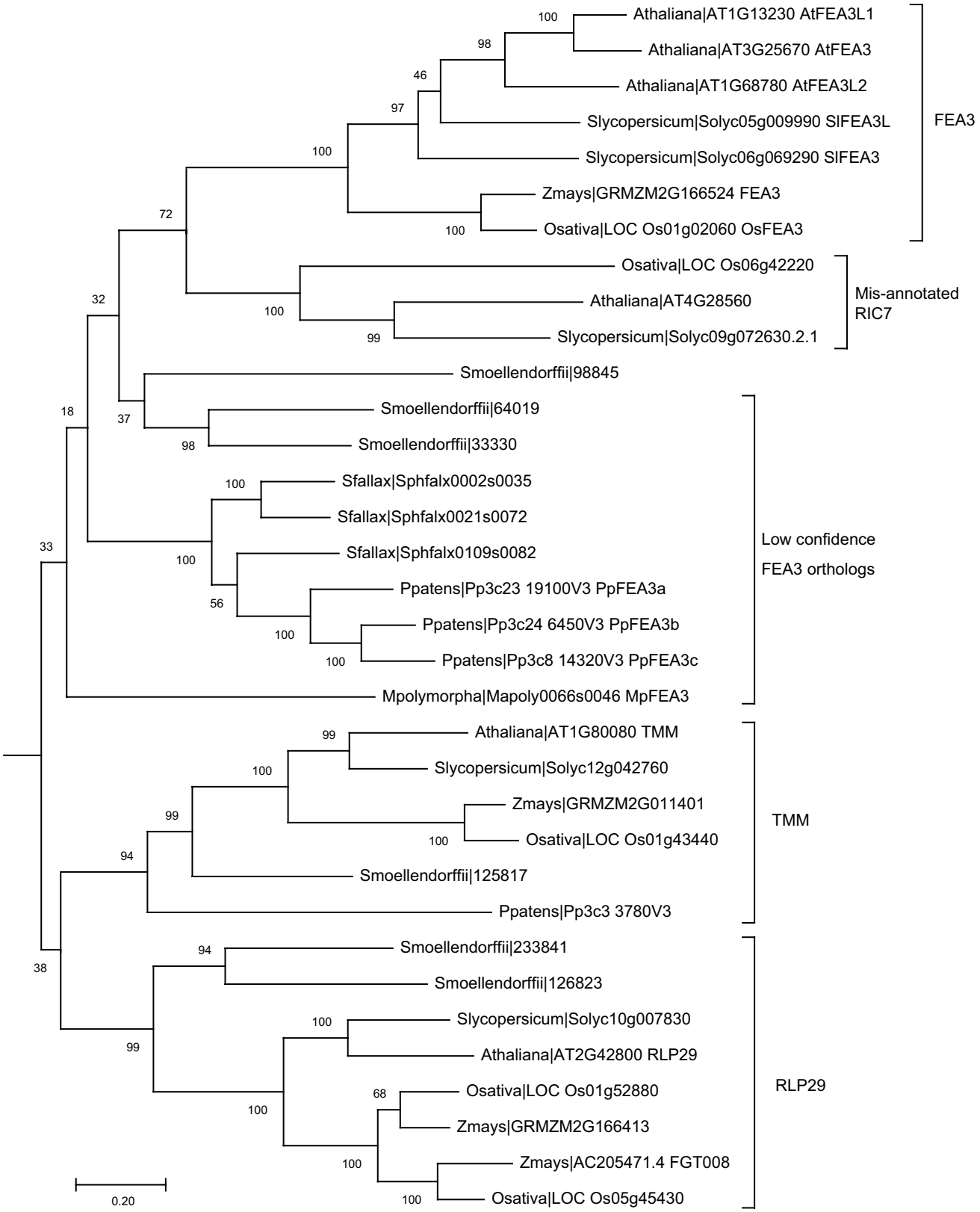

Fig. 5 Maximum Likelihood tree of the FEA3 and TMM LRR-RLPs based on full length peptide sequences. Subtree shown here is taken from a larger maximum likelihood tree; bootstrap support at the base of this subtree was 94%. Whereas FEA3 is a putative CLE receptor, TMM binds a distinct class of ligands. Support for placement of bryophyte orthologs of FEA3 is low

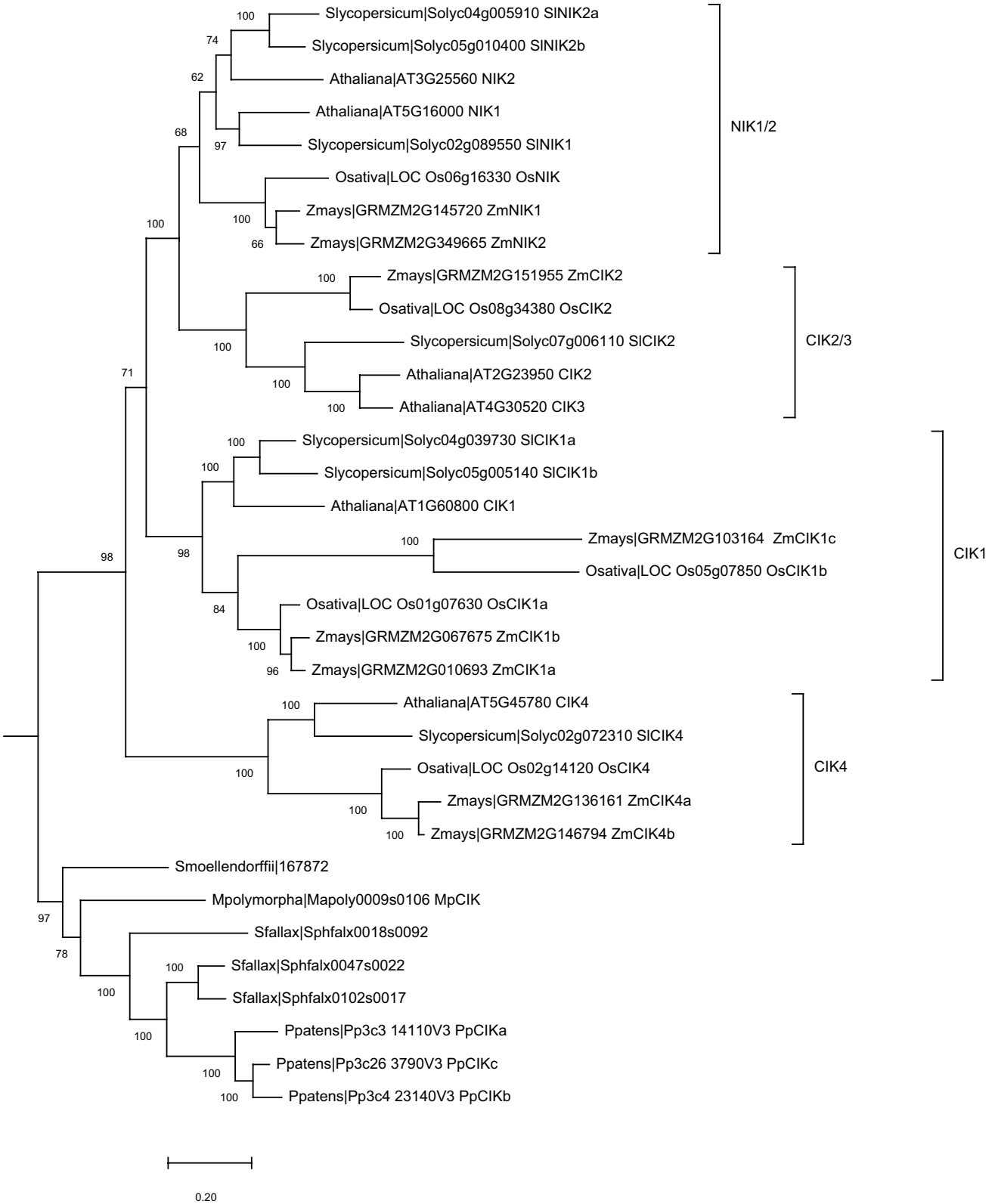

Fig. 6 Maximum Likelihood tree of CLAVATA insensitive kinases (CIKs) based on full length peptide sequences

lead to unexpected mutant phenotypes for the subfunctionalized paralog. For example, a mutation that reduces proliferation in leaf initial cells will have quite distinct developmental consequences from a mutation of a paralogous gene functioning in SAM stem cell proliferation even though both regulate the same process, i.e. proliferation. Second, the protein may evolve new biochemical functions in its new domain (i.e. neofunctionalization), which might involve new binding new partners, phosphorylating novel downstream targets, or binding to different ligands. Such neofunctionalization may stem from structural changes to the protein itself that arise after gene duplication or might be entirely dependent on novel interactions in the new context. Uncovering which of the above scenarios are operating during the diversification of the LRR-RLKs and related proteins will lead to a better understanding of stem cell maintenance pathways in land plants. Working within a functionally informed phylogenetic framework like the one provided here will expedite such studies.

Given the degree of redundancy, apparent promiscuity in complex formation, and diversity of downstream responses possible even from the same receptors (Je et al. 2018), understanding LRR-RLK function will require experiments with high spatial and temporal resolution. This pursuit will be aided by the advent of single-cell technologies; high-throughput experiments providing a set of plausible protein-protein interactions (Smakowska-Luzan et al. 2018) can be combined with single-cell RNAseq data to generate hypotheses about which receptors might be forming a complex in a given cell type during development.

Here we presented an evolutionary framework for analyses of signaling genes involved in stem cell maintenance. We identified orthologs of these signaling components in diverse plant model systems and propose a nomenclature for unannotated genes based on functional and phylogenetic (Table S1). We see, as has been previously reported (Liu et al. 2017), that LRR domain number is highly dynamic within some (*RPK1/RPK2*) but not all (*FEA3*) gene families. We identify bryophyte orthologs for most *LRR-RLK*-like genes examined, reaffirming that LRR-RLK gene families diversified early in land plant evolution. Interestingly, even in clades with frequent truncations in extracellular protein domains such as *CRN/SOBIR1* or *RPK1/RPK2*, bryophyte extracellular domains were always the longest and exhibited no evidence of domain loss. It will be interesting to see whether this is a general trend that extends beyond the taxa and gene families sampled here, and will require the assembly of a greater number of bryophyte genomes.

Within clades, we find evidence that the pseudokinase CRN evolved from a full-length LRR-RLK that is the likely ancestor of *CRN* and *SOBIR1*. In the case of *CRN* and *SOBIR1* as well for *CIK* and *NIK* genes, we find that many of these regulators of stem cell signaling are closely related to genes that function in plant immunity. Intriguingly, *cik* mutations can be complemented by *NIK* genes, which suggests that the context within which a protein functions is determined not only by cell type, but also by that the biotic and abiotic stimuli perceived by that cell. Moreover, a gene closely related to *FEA3* was identified to be misannotated, and we found that *FEA3* is closely related to the *ER* co-receptor encoding gene *TMM*. This relationship is interesting, as the TMM/ER complex binds a distinct class of ligands from FEA3, adding another level of promiscuity to these LRR-RLK gene families that will need to be untangled.

In this work we sought to provide a useful reference to facilitate research on stem cell signaling pathways in diverse model and crop species. As plant transformation and genome editing technologies improve, the number of systems available for functional genetic studies will expand, and analyses like the one conducted here will need to be replicated. Altogether, increasing the number of model systems and performing clade to clade rather than gene to gene comparisons will provide us with a deeper and more general understanding of plant stem cell signaling.

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