Plant-Microbe Interfaces: Genetic mapping and genomic resequencing identify a lectin receptor-like kinase as a regulator of *Populus-Laccaria bicolor* interaction

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Abstract

Populus **species, as keystone members of boreal and temperate ecosystems, interact with a wide variety of microbes. The *Populus-Laccaria bicolor* system has emerged as an excellent system of choice for studying plant-ectomycorrhizal interactions aided by the availability of both *Populus* and *Laccaria* reference genomes and genetic tools. Modes of action and molecular mechanisms underlying ectomycorrhizal interactions are poorly understood. Active recruitment and acceptance of mycorrhization have been proposed to occur in a species-specific manner and *L. bicolor* has been found to preferentially colonize *P. trichocarpa* over *P. deltoides*. We therefore hypothesized the existence of distinct genetic loci that are present in *P. trichocarpa* but absent in *P. deltoides* and harbor high-fidelity recognition mechanisms for *L. bicolor*. Using genetic mapping and re-sequencing, we identified a whole-gene deletion event in *Populus* that was associated with a decrease in colonization by the ectomycorrhizal fungus *L. bicolor*. This locus contains a gene encoding a lectin receptor-like kinase, designated as PtLecRLK1. The role of PtLecRLK1 mediating mycorrhization was validated via heterologous expression in a non-host species for *L. bicolor*, *Arabidopsis*, conveying the ability in the transgenic plants to accept interstitial hyphal growth and Hartig net-like structure formation by *L. bicolor*. Expression and metabolomics analyses indicated that plant defense-related genes and metabolites were down-regulated in *Arabidopsis* plants expressing *PtLecRLK1*. These results uncover an important molecular step in the establishment of symbiotic plant-fungal associations and provide a molecular target for rational design of mycorrhizal symbiosis in economically important crops to enhance water and nutrient acquisition in marginal lands.**

QTL mapping

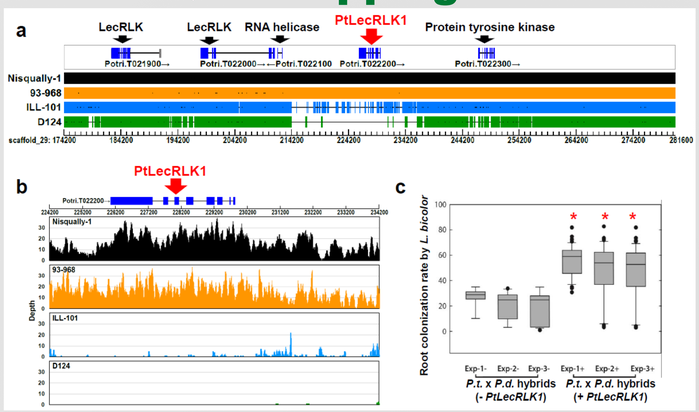

Figure 1. Identification of *PtLecRLK1* as a *Populus-Laccaria* QTL. (a) A genomic deletion event harboring *PtLecRLK1* locus. The genomic region containing *PtLecRLK1* locus is present in *P. trichocarpa* genotypes (Nisqually-1 and 93-968) but absent in *P. deltoides* (ILL-101 and D124). (b) A close view, represented by sequence depth, of the genomic region around *PtLecRLK1*. (c) Root colonization rate by *L. bicolor* evaluated among *P. trichocarpa* (*P.t.*) × *P. deltoides* (*P.d.*) hybrids with (-) or without deletion (+) of the *PtLecRLK1* locus.

Transgenic plant

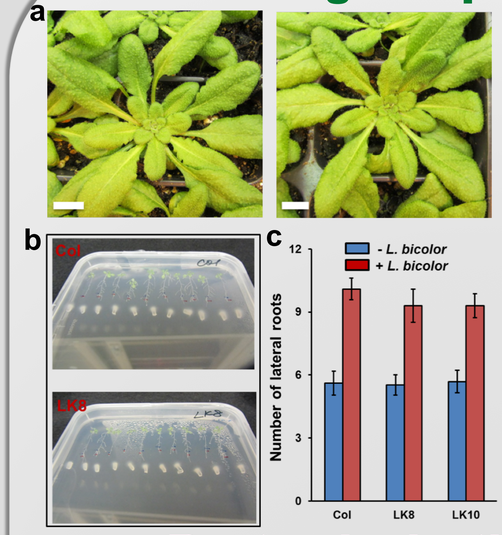

Figure 3. Arabidopsis transgenic plants expressing *PtLecRLK1*. (a) Plant morphology of wild type (left) and transgenic plant. (b) Root morphology of *35S:PtLecRLK1* transgenic seedlings with *L. bicolor* co-cultivation. (c) Lateral root formation with *L. bicolor* co-cultivation. Numbers of lateral roots were counted six days after *L. bicolor* co-cultivation. Shown are mean values of a minimum of 30 seedlings ± s.e. Col, Col-0 wild type. LK8, *35S:PtLecRLK1* transgenic line #8. LK10, *35S:PtLecRLK1* transgenic line #10.

Root colonization

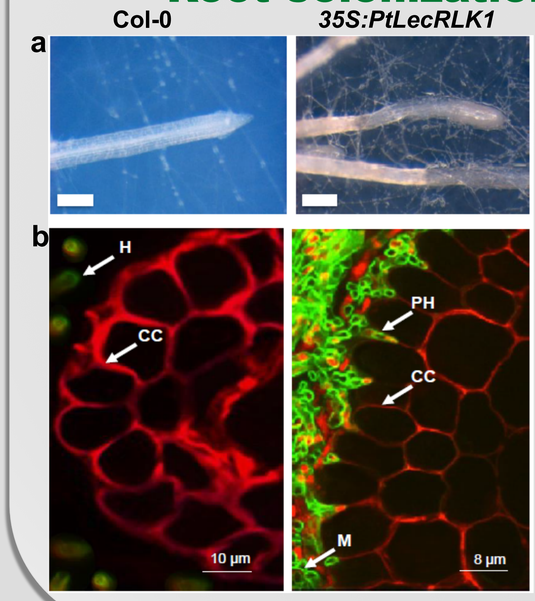

Figure 4. Co-cultures of Arabidopsis transgenics expressing *PtLecRLK1* with *L. bicolor*. (a) Root of wild type plant Col-0 (left) and *35S:PtLecRLK1* transgenic plant (right) co-cultivated with *L. bicolor*. Scale bar, 2mm. (b) Microscopic observation of a transversal section of a root of wild type plant Col-0 (left) and *35S:PtLecRLK1* transgenic plant (right) co-cultivated with *L. bicolor*. Propidium iodide (red) was used to stain root cell walls and UVitex 2B (green) was used to stain fungal cell walls. H, hyphae. CC, cortical root cell. PH, penetrating hyphae. M, mycelium.

Transcriptomics analysis

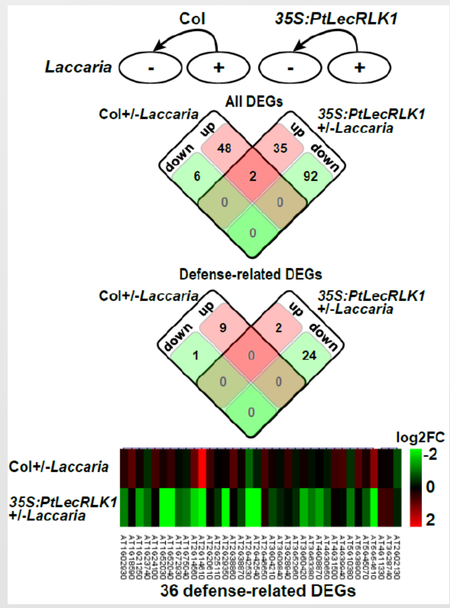

Figure 5. Transcriptomics analysis of defense-related genes in the wild-type Col-0 and *35S:PtLecRLK1* transgenic plants co-cultivated with *L. bicolor*. DEG, differentially expressed genes ($p<0.05$). Note that *L. bicolor* inoculation resulted in upregulation of nine defense-related genes in Col-0 but only two in the *PtLecRLK1* transgenic lines. More importantly, *L. bicolor* inoculation resulted in downregulation of 24 defense-related genes in *35S:PtLecRLK1* transgenic plants (vs one in Col-0).

Conclusions and future directions

- **We have identified a whole-gene deletion event in *Populus* that was associated with a decrease in colonization by the ectomycorrhizal fungus *L. bicolor*.**
- **We found that this locus contains a gene encoding a lectin receptor-like kinase, designated as PtLecRLK1.**
- **Down-regulation of genes and metabolites involved in plant defense is a mechanism underlying *L. bicolor* colonization.**
- **The function of *PtLecRLK1* in *L. bicolor* colonization can be further validated by generating overexpression lines in *P. deltoides background* and CRISPR/Cas9 knockout lines in *P. trichocarpa* background.**

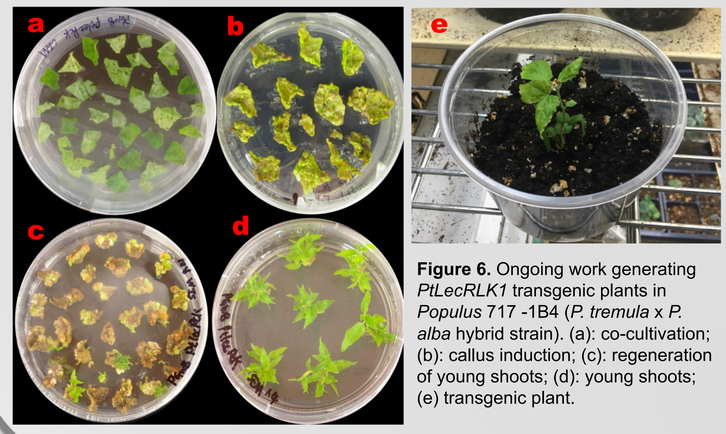

Figure 6. Ongoing work generating *PtLecRLK1* transgenic plants in *Populus* 717 -1B4 (*P. tremula* x *P. alba* hybrid strain). (a): co-cultivation; (b): callus induction; (c): regeneration of young shoots; (d): young shoots; (e) transgenic plant.

Subcellular localization

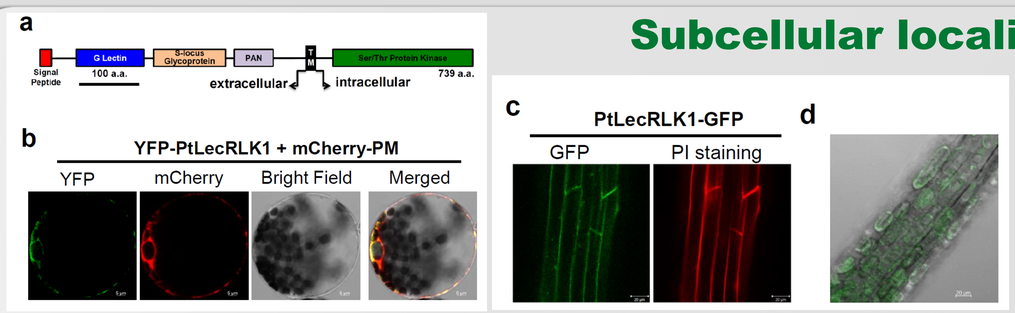

Figure 2. Subcellular localization of PtLecRLK1. **(a)** Domain structure of PtLecRLK1. **(b)** Subcellular localization analysis using the protoplast transient expression system. 35S:YFP-PtLecRLK1 was co-transfected with mCherry tagged plasma membrane marker (mCherry-PM). Scale bar, 5 μm. **(c)** Subcellular localization analysis using the *Arabidopsis 35S:PtLecRLK1-YFP* transgenic line. Propidium iodide (PI) was used to stain root cell walls (right). **(d)** Plasmolysis analysis of Arabidopsis *35S:PtLecRLK1-YFP* transgenic line.

References:

Labbé, J. *et al.* (2011) *Tree Genet. Genomes* 7: 61; Plett, J. M. *et al.* (2011) *Curr. Biol.* 21: 1197; Gottel, N. R. *et al.* (2011) *Appl. Environ. Microbiol.* 77: 5934; Yang, Y. et al. (2016) *BMC Genomics* 17: 699.

Acknowledgement:

This research was funded by the US DOE Office of Biological and Environmental Research, Genomic Science Program. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the US Department of Energy under Contract no. DEAC05-00OR22725.