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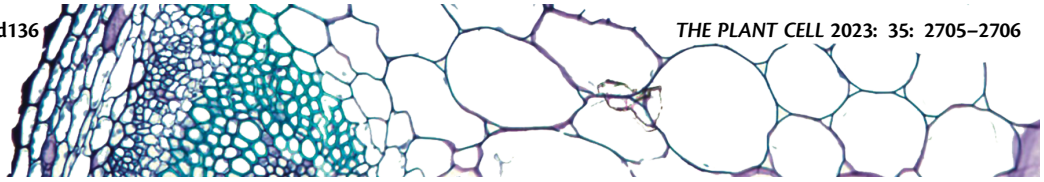

Vein density in C4 and C3 leaves: One step closer to understanding the molecular genetic basis

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In Brief

Climate change is leading to hotter and drier weather patterns in many regions of the world. Under hot and dry conditions, C4 plants are superior to C3 plants in carbon fixation due to their biochemical and anatomical adaptations that concentrate CO_2 at the site of fixation and minimize water loss. A high leaf vein density was a critical step in the origin of C4 from C3 and is crucial to supporting C4 photosynthesis (Kumar and Kellogg 2019). It has been a long-sought objective to engineer C4 features into C3 crops, such as rice, to increase their adaptability and productivity in a changing climate. A major bottleneck has been a lack of knowledge of the determinants of dense venation in C4 leaves and the difficulty in mimicking them in C3 plants.

In this issue, **Qiming Liu and colleagues (Liu et al. 2023)** delineate a developmental pathway and genetic circuit that regulate vein density in C3 and C4 leaves. The authors started with SHORTROOT/SCARECROW (SHR/SCR) genes, which are implicated in C4 development (Schlüter and Weber 2020). To study the role of SHR in vascular differentiation, the authors overexpressed *SHR* orthologs in C3 rice and the C4 grass *Setaria viridis*. Both *OsSHR1* and *OsSHR2* overexpression in rice and *SvSHR1* and *SvSHR2* overexpression in *S. viridis* resulted in fewer minor veins and increased mesophyll (M) cell number between adjacent minor veins compared with the respective wild-type (WT) genotypes. Interestingly, *osshr1* and *osshr2* single mutants displayed increased minor vein initiation and reduced M cell differentiation. The double mutant (*osshr1 osshr2*) plants also showed a significant increase in the total number of minor veins compared with WT. The *svshr1* and *svshr2* mutants showed an increase in minor vein number, with a disruption of uniform parallel venation. These findings strongly indicate that *SHR1* and *SHR2* from rice and *S. viridis* play a role in M cell and minor vein formation during leaf development.

The authors found out that *OsSHR1* and *OsSHR2* are strongly expressed in the vasculature. The authors showed that OsSHR1 and OsSHR2 specifically interact with INDETERMINATE DOMAIN (IDD) transcription factors OsIDD12 and OsIDD13, which also interact with each other. Whereas single gene knock-out alleles of *OsIDD12* and *OsIDD13* did not show any significant differences in features, double mutants (*osidd12 osidd13*) were short with wide leaves and had decreased M cell numbers and increased vein numbers.

Because the PIN-FORMED (PIN) auxin efflux protein family has long been associated with vascular differentiation in grasses (O'Connor et al. 2017), the authors wondered if the reduced M cell number and enhanced vein number in *osidd12 osidd13* are due to altered expression of *OsPIN* gene family members. Interestingly, they found a canonical IDD binding site (TTTGTCGCTTT) in the third intron of *OsPIN5c* gene among 12 PIN family members in rice. Moreover, *PIN5c* expression was enhanced in the *osshr1 osshr2* double mutants, indicating *PIN5c* as a potential binding target of IDD. DNA pull-down, electrophoretic mobility shift, and ChIP-quantitative PCR assays indicated that OsIDD12 and OsIDD13 directly bind with the IDD motif in the third exon of *OsPIN5c*. Furthermore, when *OsPIN5c* was overexpressed with its native promoter, the overexpressed (OX) lines displayed increased minor and major vein numbers and reduced M cell numbers between adjacent minor veins compared with WT. Interestingly, the flag leaves of

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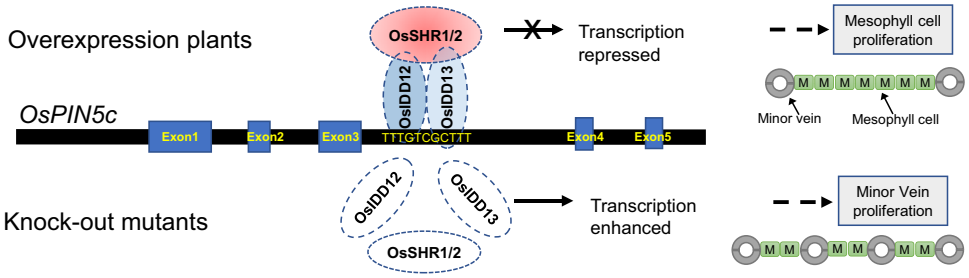

Figure. A proposed working model for minor vein differentiation in rice mediated by *OsPIN5c*, OsSHR1/2, and OsIDD12/13. Overexpression of OsSHR1/2 proteins (overexpression plants) promotes the binding of OsIDD12/13 to the "TTTGTCGCTTT" motif in intron 3 of *PIN5c*. The binding represses *PIN5c* expression and stimulates mesophyll cell differentiation. In contrast, loss of function of *OsSHR1/2* or *OsIDD12/13* (knockout mutants) relieves the repression of *PIN5c*. Higher expression of *PIN5c* results in the differentiation of more minor veins. Adapted and modified from Liu et al. (2023), Figure 12.

OsPIN5c OX lines occasionally showed intermediate veins like those of C4 grasses. These results indicate that the effects of IDD12 and IDD13 are mediated, at least partially, by the interaction with *OsPIN5c*.

To investigate the nature of regulation, the authors analyzed *OsPIN5c* expression in rice leaf primordia (P1–P5) isolated through laser capture microdissection. In *SHR* OX lines, the expression of *OsPIN5c* was reduced by approximately 50%. In contrast, the *OsPIN5c* expression was significantly increased in *shr1 shr2* and *idd12 idd13* double mutants. Several lines of evidence supported the hypothesis that *OsPIN5c* repression by SHR depends on the binding of IDD12 and IDD13 to the intron 3 of *OsPIN5c* (see Fig.).

Liu and coworkers thus provide a missing link between the interplay of SHR and IDD transcription factors and the regulation of auxin flux in determining the venation pattern in grass leaves. The key role of *OsPIN5c* revealed in the study may facilitate the installation of the C4 vascular chassis in C3 crops to support C4 biochemistry. Because *shr* and *idd* knockout mutants exhibit abnormal growth and agronomic phenotypes, native promoter-driven PIN5c overexpression along with weaker alleles of *IDD* genes could potentially represent a good strategy to support C4 biochemistry in C3 for better agronomical features in a warmer and drier climate.

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