Plant Cell Advance Publication. Published on October 22, 2020, doi:10.1105/tpc.20.00884

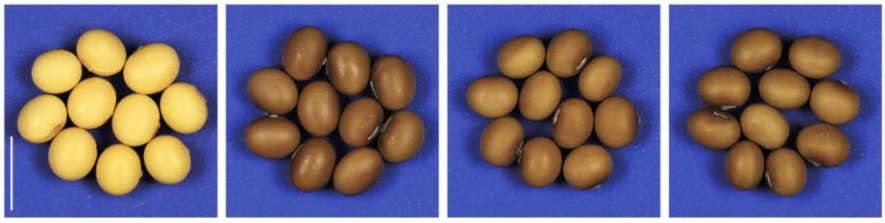
IN BRIEF

Slice and Dice: DCL2 Mediates the Production of 22-nt siRNAs That Influence Trait Variation in Soybean

In plants, small interfering RNAs (siRNAs) serve as key regulators of gene expression. While 24-nucleotide (nt) siRNAs are produced by DCL3 and mediate transcriptional silencing of transposons and pericentromeric chromatin through RdDM (Borges and Martienssen, 2015), 22-nt siRNAs are processed by DCL2 and participate in transgene silencing and viral defense (Wang et al., 2018; Parent et al., 2015). However, since 22-nt siRNAs are relatively rare in wild type Arabidopsis (Henderson et al., 2006), and the loss of DCL2 doesn't produce obvious development defects (Henderson et al., 2006; Wang et al., 2018), their role is still largely unknown. By contrast, crops such as soybean (*Glycine max*) exhibit abundant 22-nt siRNAs and are ideal systems to study their biogenesis, targeting, and functions in genome activity.

In a new study, Jinbu Jia and co-authors combine genome editing, sRNA-seq, and transcriptomics to investigate the functions of DCL2-dependent 22-nt siRNAs in soybean (Jia et al., 2020). The authors used CRISPR-Cas9 genome editing to simultaneously generate loss-of-function mutants of the two *GmDCL2* genes in soybean, and performed sRNA-seq on a selection of tissues. The analysis revealed a substantial decrease in 22-nt siRNAs abundance throughout the genome in *Gmdcl2,* but also a drastic reduction of secondary 21-nt siRNAs derived from the 22-nt siRNAs. By investigating the distribution of these DCL2-dependent siRNAs in wild-type soybean, the authors identify that a large majority of 22-nt siRNAs are overlapping with transposable elements (TEs), suggesting a possible role in silencing. In the *Gmdcl2* mutant, the production of such TE-associated 22-nt siRNAs is drastically reduced, but this does not correlate with local reduction in DNA methylation, nor accumulation of TE transcripts. Next, the authors examined the structural features of GmDCL2 substrates. The transcriptomic approach reveals that DCL2 preferentially processes PolII-transcribed long inverted repeats to generate 22-nt siRNAs.

The most dramatic developmental phenotype resulting from *GmDCL2* loss-of-function is a darkening of the seed coat, from yellow in wild-type soybean to dark brown in *Gmdcl2s* (**Figure**). Seed color in soybean is dictated by the activity of Chalcone Synthase (CHS)-encoding genes that enable flavonoid biosynthesis that results in dark brown seed coat. The yellow seed coat phenotype is associated to the abundant production of siRNAs from a particular *CHS* gene cluster, with the siRNAs targeting and silencing other *CHS* genes *in trans*. With a higher genomic resolution provided by a *de novo* assembly of the CHS cluster, Jinbu Jia and colleagues propose that the *CHS* cluster generating siRNAs can form a long inverted repeat transcript that is possibly processed by DCL2. This hypothesis is further supported by the accumulation of DCL2-dependent 22-nt siRNAs at this *CHS* cluster, which disappear in *Gmdcl2s*. In the absence of *CHS trans*-targeting siRNAs, other *CHS* genes accumulate transcripts at higher level compared to wild type, and flavonoid accumulation results in dark seed coat.



Loss-of-Function Mutations in GmDCL2 Results in Darkening of the Seed Coat. CRISPR-Cas9-engineered frame-shift mutations in *GmDCL2a* and *GmDCL2b* triggers overaccumulation of *CHS* transcripts and darkening of the seeds coat in the Tianlong1 cultivars (*Adapted from Jia et al. [2020], Figure 1.*)

Further from facilitating trait diversification, DCL2-dependent 22-nt siRNAs are also involved in translational repression and stress adaptation (Wu et al., 2020). The high abundance of 22-nt siRNAs in crops suggests more unexplored functions in regulating genome activity in plants. This study revealed that GmDCL2s play important roles in the regulation of natural traits by generating endogenous 22-nt siRNAs and provided a new strategy for improving the appearance quality of soybean seeds.

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Plant Cell; originally published online October 22, 2020;
DOI 10.1105/tpc.20.00884

This information is current as of March 7, 2021

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