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Tissue-Specific siRNAs That Silence *CHS* Genes in Soybean

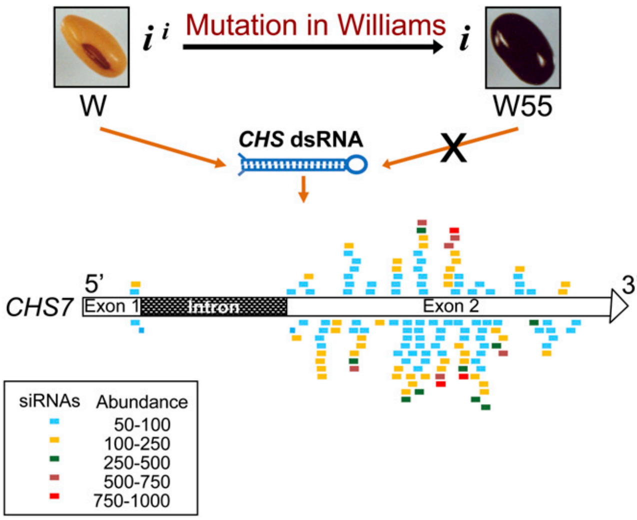
Chalcone synthase (CHS) is required for the biosynthesis of anthocyanin pigments that give color to various plant tissues, such as the flower and seed coat. The silencing of *CHS* genes produces a highly visible phenotype, lack of color in the seed coat or flower, that facilitated the discovery of gene silencing in eukaryotes (Napoli et al., 1990) and continues to provide a useful system for investigating the mechanistic basis and regulation of the phenomenon.

In soybean (*Glycine max*), two naturally occurring dominant alleles of the *I* locus (*I* and i^i) silence nine *CHS* genes to inhibit pigmentation in the seed coat, resulting in a colorless or light-yellow seed coat. By contrast, the homozygous recessive *i* allele allows for expression of *CHS* genes, resulting in pigment production and a dark brown or black color in the seed coat. Most cultivated soybean varieties have been selected for homozygous *I* or i^i alleles to mitigate undesirable effects of anthocyanin pigments on protein and oil extractions. These varieties continue to express *CHS* genes in other plant tissues, suggesting that there is tissue specificity to silencing in the seed coat. It has been presumed that silencing is due to the production of small interfering RNA (siRNA) at the *CHS* locus, which in *I* and i^i genotypes is a complex locus containing a series of duplicated and inverted *CHS* genes (Clough et al., 2004; Senda et al., 2004; Tuteja et al., 2004; Tuteja and Vodkin, 2008). However, the mechanistic basis and tissue specificity of this silencing have not been fully characterized.

In this issue, **Tuteja et al. (pages 3063–3077)** investigate the *CHS*-derived small RNA populations in several soybean varieties using RNA gel blotting and high-throughput sequencing of small RNAs. They show that *CHS* siRNAs accumulate in the yellow seed coats of plants carrying dominant *I* or i^i alleles but not in pigmented seed coats with a homozygous recessive *i* genotype. Furthermore, in the *I* and i^i genotypes, *CHS* siRNAs are restricted to the seed coat and absent from cotyledons of developing seed. Therefore, the dominant *I* and i^i alleles confer a highly tissue-specific accumulation of *CHS* siRNAs, leading to silencing of the genes only in the seed coat.

The authors show that the population of small RNAs in the seed coat of the yellow i^i genotype maps primarily to the coding regions (both sense and antisense strands) of *CHS* and is predominantly 21 nucleotides in length (see figure). They conclude that silencing from the complex *CHS* loci in soybean *I* and i^i varieties occurs because these loci contain clusters of *CHS* sequences in inverse orientation (some of which are deleted in the recessive *i* genotypes), which generate RNA transcripts that could fold and create aberrant double-stranded RNA (dsRNA). The latter are diced to generate primary siRNAs that target other *CHS* family member genes. The size and sequence distribution of the *CHS*-siRNA is consistent with the hypothesis that aberrant dsRNA are synthesized and processed by an RNA-dependent RNA polymerase, dicer-like, and argonaute-like effector complex that amplifies the silencing signal by cleavage of these dsRNAs into phased 21- to 22-nucleotide secondary siRNAs (Chapman and Carrington, 2007).

This naturally occurring instance of gene silencing is analogous in some ways to cosuppression of *CHS* in petunia, in which plants transformed with extra copies of *CHS* produce aberrant dsRNA that leads to silencing of the endogenous *CHS* genes in floral tissue. De Paoli et al. (2009) recently provided evidence that the induction of *CHS* siRNAs is causally related to the cosuppression of *CHS* in petunia that gives rise to loss of flower pigmentation. In petunia, there are at least six *CHS* genes (*CHS-A*, *-B*, *-D*, *-F*, *-J*, and *-H*), but only



CHS siRNAs in Soybean.

Primary and secondary *CHS* siRNAs from a seed coat small RNA population of the yellow seed Williams (i^i) variety aligning to the sense and antisense strands of the two exons of one of the nine *CHS* genes but none to the intron. The colored segments represent number of occurrence in the library and the location of their alignment. (*Adapted from Figures 5 and 7 of Tuteja et al. [2009].*)

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CHS-A and *CHS-J* are expressed in flower tissues (Koes et al., 1989). De Paoli et al. (2009) sequenced the small RNA from wild-type and cosuppressed transgenic petals and found that only the *CHS-A* sequence, and to a lesser extent *CHS-J*, matched a significant number of small RNAs, whereas the other *CHS* genes not expressed in flower tissues were not represented. Tissue specificity of gene silencing in the naturally occurring *I* and i^i soybean genotypes and in cosuppressed petunia are triggered by different mechanisms, but both appear to feed into a similar silencing pathway via generation of phased ~21-nucleotide siRNAs. Tuteja et al. speculate that tissue specificity in *I* and i^i genotypes is due to seed coat–specific biogenesis of *CHS* siRNAs from the inverted repeat *I* locus clusters, rather than a lack of signal amplification in other tissues. Future work will focus on the regulatory mechanism that determines the tissue-specific production and accumulation of *CHS* siRNAs in *I* and i^i soybean genotypes.

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