

Research finds key advances towards reducing the cost of plant improvement

November 9 2021



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Crop improvement often involves the transfer of genetic material from one organism to another to produce a valuable trait. Some major examples of crops with these so-called "transgenes" include disease-

resistant cotton and beta-carotene-enhanced golden rice. However, when foreign DNA is introduced into a host organism, a natural defensive response in plants is to repress or silence the expression of the unfamiliar genetic material. This "silencing," a process known to involve DNA methylation, is a multimillion-dollar problem in the global agricultural improvement industry.

Research spearheaded by Keith Slotkin, Ph.D., member, Donald Danforth Plant Science Center and associate professor, Division of Biological Sciences, University of Missouri Columbia, has established a new understanding as to how DNA methylation begins in the first place—in other words, how the silencing of new and foreign genetic material is triggered in plants. These findings, An siRNA-guided ARGONAUTE [protein](#) directs RNA Polymerase V to initiate DNA methylation, were recently published in the scientific journal *Nature Plants*.

As the culmination of four years of work by the Slotkin laboratory's graduate students, postdoctoral associates, and technicians, all of whom are represented as authors, their work has substantial implications for reducing the cost and effort that goes into producing transgenic crops. "Gene silencing is a key bottleneck that is inhibiting plant improvement... no matter what new trait a plant biologist works on, they are going to have to fight against the tidal wave of gene silencing," said Slotkin. Normally, breeders need to start with thousands of plants to identify the few that express, instead of repress, the trait of interest. By discovering how and why DNA methylation occurs, this work enables crop breeders to avoid the silencing of the trait from the outset. "One day we could start with three plants instead of thousands. All of the time and money that is usually put into producing a crop is slimmed down," said Slotkin.

Another major advancement of the work by Slotkin and his team was the

establishment of a new model as to how gene silencing is initiated. One conventional theory in the field proposes that a critical protein called RNA Polymerase V is present all across the genome and surveils different regions of genetic material to test for regions that need silencing. Once RNA Polymerase V identifies a gene region to silence, the DNA methylation process starts. However, in a breakthrough finding, the authors discovered that it is not the presence of RNA Polymerase V itself that triggers gene silencing, but rather the presence of small RNAs (which are critical for plant growth and development) that recruit RNA Polymerase V to the gene or transgene. "Our model is saying that small RNAs are driving RNA Polymerase V to the new location in the first place. If we get rid of the small RNA machinery, RNA Polymerase V doesn't know where to go," Slotkin described.

In addition, the methods used by the Slotkin team were just as valuable as their findings—the researchers overcame several significant technical hurdles in the process. As one example, gene silencing is typically studied as a cycle, rather than how it starts. Consequently, the researchers needed to do everything in the first generation of transgenic plants to watch the initiation of the process. "That's a huge challenge," Slotkin notes. "We plant thousands of seeds that may have integrated a transgene. Sometimes we only get five plants back because they did not transform well. This isn't enough, as we want a lot of tissue off of them in order to measure DNA methylation... and these experiments require biological replicates, so more tissue is needed, and the experiment needs to be done again." While it is a monumental amount of work to grow and regrow enough [plants](#) for such an experiment, "it is all worth it to be able to investigate the first generation of transgene silencing," remarked Slotkin.

The first author of the paper is Meredith Sigman, a former graduate student at the Danforth Center, and the last author of the paper is Andrea McCue, a former postdoctoral associate in the Slotkin lab. The entire

Slotkin research team continues to investigate the initiation of gene silencing. They explore fundamental questions regarding how DNA methylation is triggered and its potential contribution to overcoming obstacles in transgenic plant production. "We are at a crop research institute like the Danforth Center to make the plant improvement process easier for everyone," concluded Slotkin.

More information: Meredith J. Sigman et al, An siRNA-guided ARGONAUTE protein directs RNA polymerase V to initiate DNA methylation, *Nature Plants* (2021). [DOI: 10.1038/s41477-021-01008-7](https://doi.org/10.1038/s41477-021-01008-7)

Provided by Donald Danforth Plant Science Center

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