CRISPR Gene-Editing in Strawberry

Seonghee Lee

Summary

Recent plant breeding technology has been developed to precisely modify genes for traits of interest. This approach is known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gene-editing. This is not GMO technology and should not be over-regulated, as the final product matches what may be done by conventional breeding. This technology has been applied in many agronomic crops, and is poised to make contributions to strawberry. We anticipate that gene-editing can be used to bring strawberries to industry with improvements in disease resistance, fruit quality and other valuable attributes.

What is CRISPR gene-editing?

CRISPR-based gene-editing is a revolutionary scientific tool that is being rapidly utilized in many crops. This technology can modify a gene of interest without otherwise altering the DNA of an elite variety. For example, instead of breeding for many years to move a resistance gene from wild strawberry into elite germplasm, gene-editing allows direct correction of the genetic sequence in already-elite breeding selection or variety. This is particularly useful for cultivated strawberries because they are genetically complex, and it would be much easier to simply adjust a single trait than reshuffle the genetic deck and hope for a plant with all traits incorporated.

The UF/IFAS strawberry breeding program has identified several important genetic locations controlling disease resistance traits directly relevant to Florida growers. By using CRISPR technology these genes or gene variants can be moved into desirable breeding selections and can be further moved via conventional crossing in later generations. Importantly, we can utilize established DNA marker-assisted breeding tools to track the edited genes. Furthermore, in several years it is possible that the first-generation plants containing the edited genes will not require extensive regulation, so these resources will be extremely valuable in the long-term efforts of the UF/IFAS breeding program.

Does CRISPR gene-editing = GMO?

New advances in gene-editing provide a means to correct a single deficiency, if the gene that causes it is known. This is not GMO technology, and all indications suggest that gene-editing will not be over-regulated, as the final product matches what may be done by conventional breeding. Recently, USDA-ARS announced to the public that a new mushroom developed using CRISPR will not be regulated as a GMO in the U.S.A., and countries like Sweden have made similar proclaimations. Due to the complexities of deregulation and persistent social barriers to genetic engineering, the CRISPR approach has a much greater potential than 'GMO' technologies for developing new and superior strawberry cultivars.

Tissue culture optimization

To utilize the gene-editing technology for strawberry, we first need to develop and optimize a tissue culture and transformation system for UF breeding parents. To identify the optimal conditions for regeneration for Sweet Sensation® 'Florida127', 'Florida Beauty' and FL 13.26-134, explants were grown on a range of media with varying compositions of plant growth regulators. About one inch of petiole or srunners from the leaf-end or shoot-end, respectively, were collected from greenhouse grown

plants and used for the tissue culture process (Fig. 1). Optimal conditions were identified for all three UF accessions. It takes about 14 weeks for Sweet Sensation® 'Florida127' to develop from tissue culture to plantlet.

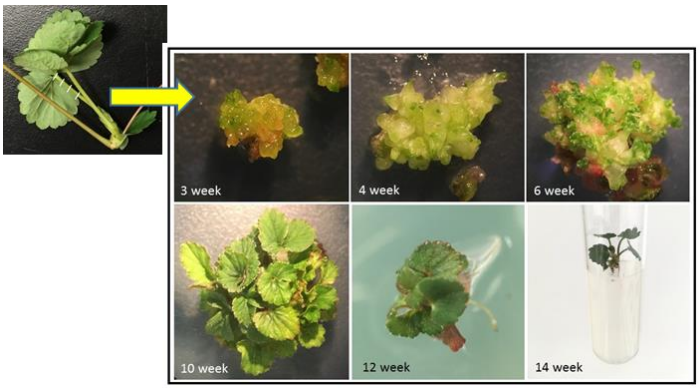

Figure 1. Progression of shoot regeneration of Sweet Sensation® through the process of tissue culture in a lab.

Foreign DNA-free gene-editing system

Some transformation systems result in foreign bacterial DNA sequences being inserted into the plant. In contrast, we are using a particle bombardment method in which DNA-coated metal particles delivered using a gene gun, and there is no need for foreign DNA insertion. Later the gene-edited tissues will be regenerated to mature plants using the tissue culture protocol mentioned above. Because this technique is foregin DNA-free, plants developed from this method should be exempt from current 'GMO' regulations.

Creating Sweet Sensation® resistant to Phythophthora crown rot

Gene-editing works by providing the plant with a set of genetic instructions that can make the prescribed genetic change. We are currently cloning important disease resistance genes for Phytophthora crown rot resistance in octoploid strawberry. The identified genes necessary for disease resistance can be added to important advanced selections (FL 13.26-134) and/or cultivars (Sweet Sensation®). Fig. 2 shows a basic overview of the procedure for gene-editing of Sweet Sensation® tissue.

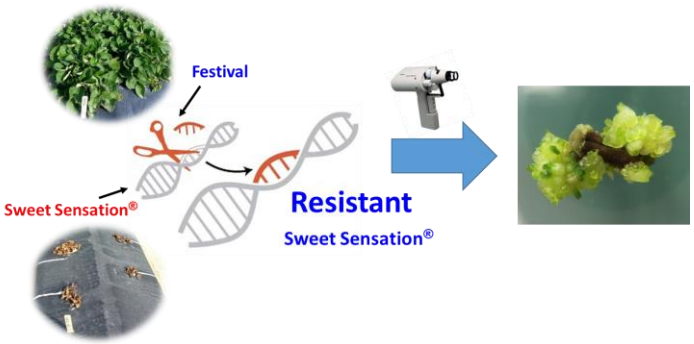

Figure 2. General procedure of CRISPR gene-editing to develop Sweet Sensation® resistant to Phytophthora crown rot.

Adding resistance genes to UF breeding parents and/or varieties by CRISPR gene-editing should be routine once the methods are fully developed. Evaluations of the edited traits will be performed in concert with crosses to integrate the changes into other major varieties and advanced selections with conventional hybridization.

Contact

Dr. Seonghee Lee
UF/IFAS Gulf Coast Research and Education Center
P: 813.633.4151
E: seonghee105@ufl.edu