CRISPR Gene Editing in Strawberry[1]

Seonghee Lee, Cheolmin Yoo, Kevin Folta, and Vance M. Whitaker[2]

Recent technology has been developed to precisely engineer genes for traits of interest. This approach is known as "CRISPR" gene editing. Gene editing is distinct from other forms of genetic engineering, such as transgenic technology, often colloquially referred to as "GMO". With gene editing, the final product can match that obtained by conventional plant breeding, but in a much shorter timeframe. CRISPR has been applied in many agronomic crops and is poised to make contributions in strawberry. We anticipate that, over the next decade, CRISPR and other gene editing techniques will be used to rapidly develop elite strawberry varieties with improved disease resistance, fruit quality, and other valuable attributes.

What is CRISPR gene editing?

One of the great disappointments in the pursuit of improved varieties is the discovery of a new advanced selection that would be valued by the industry except for one critical flaw. Gene editing technology can be used to almost surgically adjust the gene or genes behind that deleterious trait. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is one form of gene editing that can be used to precisely modify a gene of interest without otherwise compromising the favorable traits of an elite variety [Rani et al. 2016, Bortesi and Fischer 2015]. For example, instead of breeding for many years to move a disease resistance gene from a wild strawberry into a modern strawberry, gene editing allows a direct introduction of the genetic information. Think of it as a "cut and paste" mechanism. This is particularly useful for cultivated strawberries because they are genetically complex, making conventional breeding difficult. The UF/IFAS strawberry breeding program has identified several important gene regions controlling disease resistance traits that are directly relevant to Florida growers. By using CRISPR technology, these genes or gene variants can be moved into desirable genetic backgrounds that can be further moved via conventional crossing in later generations. We can utilize established DNA marker-assisted breeding tools to track the edited genes in subsequent generations, adding to the speed of new variety development. Based on policy discussions, it is possible that the first-generation plants containing the edited genes will not require extensive regulation, and these tools will be extremely valuable in the long-term efforts of every strawberry breeding program.

Does CRISPR gene editing = transgenic technology?

"Transgenic technology" refers to the transfer of a genetic material from one species to another. CRISPR, on the other hand, can be used to precisely change DNA sequence, switching it from one naturally occurring variant to another naturally occurring variant. Using this new technique, we can cut a strawberry's genome at a desired location so that existing genes can be removed or added. Early indications suggest that gene editing should be regulated like conventionally bred crops, as the final product can simply match what may be done by conventional breeding. Recently, the USDA announced that it would not regulate

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2. Seonghee Lee, assistant professor; Cheolmin Yoo, biological scientist; Kevin Folta, professor; and Vance M. Whitaker, associate professor; Horticultural Sciences Department, UF/IFAS Gulf Coast Research and Education Center; UF/IFAS Extension, Gainesville, FL 32611.

a new mushroom developed using CRISPR [Waltz 2016]. Countries like Sweden and Argentina have made similar proclamations, indicating that the finished CRISPR-edited varieties do not fall under certain regulations because they do not contain "foreign" DNA.

Development of a CRISPR System in Strawberry

While the finished varieties do not contain DNA from other organisms, the process introduces genetic information that orchestrates the desired genetic change. The first step is to develop and optimize a tissue culture and transformation system (protocols to introduce foreign DNA to new plants) for UF strawberry lines, so that new plants can be regenerated from cells containing introduced DNA. However, just as each cultivar has different traits and qualities, they also behave differently with respect to introduction of new genes.

The recent cultivar Sweet Sensation® 'Florida127' and advanced selection 'Florida Brilliance' (FL 13.26-134) were used for tissue culture optimization. As shown in Figure 1, callus induction was tested with different strawberry tissues, and embryogenic callus growth was most vigorous on stolons (runners) and petioles. To identify the optimal conditions for shoot and root regeneration for Sweet Sensation® 'Florida127' and 'Florida Brilliance' (FL 13.26-134), explants were grown on a range of media with varying compositions of plant growth regulators.

Figure 1. Test for callus induction with different tissues of Sweet Sensation® 'Florida127'. Leaf tissues, meristem tissues from crowns, stolons, and petioles were harvested from greenhouse-grown plants that were six to eight months old.
Credits: Cheolmin Yoo

About one inch of petiole or stolon from the leaf-end or shoot-end, respectively, were collected from greenhouse grown plants and used for the tissue culture process. Optimal conditions for tissue culture medium, nutrient, and hormone were tested for the UF accessions. The runner (stolon) produced calli more vigorously than petiole segments. It takes about 14 weeks for Sweet Sensation® 'Florida127' and 'Florida Brilliance' (FL 13.26-134) to develop from embryogenic callus to young plantlet in rooting media (Figure 2). 'Florida Brilliance' (FL 13.26-134) produced more regenerated plants than Sweet Sensation® 'Florida127'.

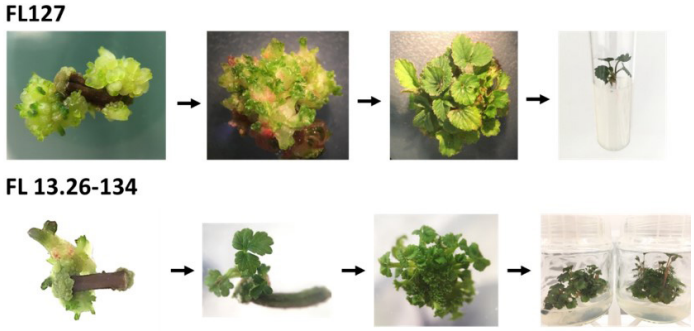

Figure 2. Progression of shoot and root regeneration for Sweet Sensation® 'Florida127' and 'Florida Brilliance' (FL 13.26-134). First image from left: shoots and embryos are generated from the callus of the explants; second image: expanded shoots; third image: elongated shoot clusters; fourth image: plantlets in rooting media.
Credits: Cheolmin Yoo

No "Foreign" DNA Sequences

Once the genetically engineered gene product is ready for CRISPR gene editing, transformation, where the new genetic material is delivered to a single strawberry cell, is the first step in the genetic engineering process. For DNA delivery, two major transformation methods, such as *Agrobacterium tumefaciens*-mediated or biolistic (gene gun)-mediated transformation, are widely used for CRISPR gene editing. *Agrobacterium tumefaciens* is a widespread, naturally occurring soil bacterium that causes crown gall in many plant species and has the ability to introduce new genetic material into plant cells [Gelvin 2003]. This bacterium works as a natural genetic engineer and is used in labs for plant transformation. Gene-edited plants using *Agrobacterium*-mediated transformation will contain foreign bacterial DNA sequences. It is not an easy process to remove the bacterium-derived DNA sequences through breeding.

In contrast, we are currently using a biolistic particle bombardment method in which DNA-coated metal particles are delivered to the plant cells using a "gene gun" (Figure 3). This method can be applied to a wide range of cell and tissue types, and there is no need for bacterial DNA insertion. Later, the gene edited tissues can be regenerated to mature plants using the tissue culture protocol outlined above.

Recently, the development of gene editing using protoplasts and regeneration of plants from protoplasts has been demonstrated in other plant species [Woo et al. 206; Kanchiswamy 2016; Dutt et al. 2015]. This method is known as a completely "DNA-free" gene editing system. Briefly, the protein/genetic material complex for gene editing will be assembled in vitro and the complex mixed with strawberry protoplast isolated from embryogenic calli and polyethylene glycol, which allows direct transfer by endocytosis into protoplasts. The gene edited with protoplasts is cultured (cell suspension culture) into calli, and mature plants can be regenerated using the tissue culture protocol outlined above. We are currently developing this cell suspension culture protocol at UF.

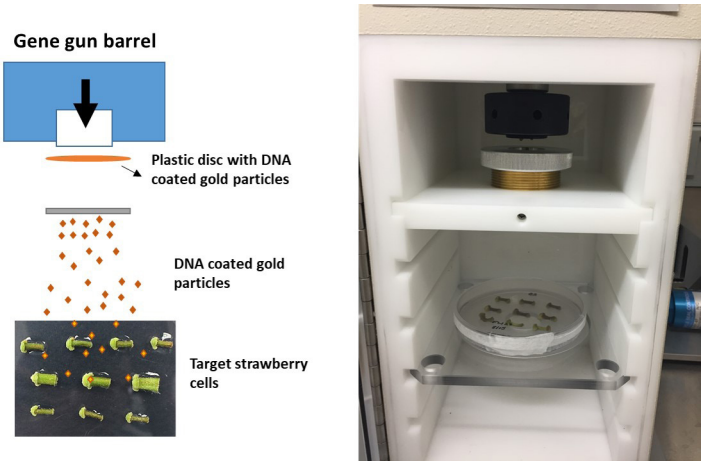

Figure 3. General procedure of transformation using the metal particle bombardment system.
Credits: UF/IFAS

Gene Editing for Disease Resistance

The UF strawberry breeding program provides a direct commercialization path for disease-resistant varieties to reach Florida growers. New varieties with better resistance will bring increased profitability to the Florida strawberry industry. The Florida Strawberry Growers Association estimates that diseases cost the Florida industry at least $15 million each year. In the last two years, the UF strawberry breeding program has identified regions of strawberry chromosomes that control resistance to bacterial angular leaf spot [Roach et al. 2016] and Phytophthora crown rot [Mangandi et al. 2017], with more to come for other diseases such as Colletotrichum crown rot, charcoal rot, and anthracnose fruit rot. Efforts are ongoing to narrow these chromosomal regions down and identify the exact gene sequences that provide these disease resistances, with Phytophthora resistance as the first priority. Our goal is to add Phytophthora resistance to 'Florida Brilliance' (FL 13.26-134) and Sweet Sensation® 'Florida127,' which are currently highly susceptible to this disease. Evaluations of the gene-edited lines will be performed in concert with crosses to integrate the changes into other major varieties and advanced selections with conventional hybridization.

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