Breeding next generation Florida strawberries using targeted genome editing and cisgenesis technology

Seonghee Lee

Summary

To utilize the targeted genome editing in cultivated strawberry, we established methods for tissue culture and CRISPR gene-editing in three major cultivars; 'Florida Radiance', Sweet Sensation® 'Florida 127' and 'Florida Brilliance'. A gene gun was used for delivery of CRISPR constructs to strawberry cells. This is a critical step for DNA-free gene-editing. Furthermore, we successfully developed a protocol for somaclonal variation. This is an alternate approach to conventional breeding that can effectively introduce desirable characteristics to new cultivars. Somaclones developed from Sweet Sensation® 'Florida 127' and 'Florida Radiance' have been evaluated in the field for fruit quality, and will be tested for Phytophthora crown rot resistance.

Methods and Results

Identifying candidate genes for the resistance to Phytophthora and Colletotrichum crown rot resistance.

Three major cultivars such as 'Florida Radiance', Sweet Sensation® 'Florida 127' and 'Florida Brilliance' are susceptible to both crown rot pathogens, *P. cactorum* and *C. gloeosporioides*. Using CRISPR, we can improve cultivars for resistance to these diseases. Firstly, it is important to find the genes that need to be targeted. In this project, we investigated the genes involved in the susceptibility to crown rot diseases. If we knockout the susceptibility gene, strawberry plants should be more resistant to the pathogens.

Strawberry cultivars shown in Table 1 were infected by each crown rot pathogen separately. Tissue samples were collected 72 hours after pathogen inoculation. DNA sequencing was performed for all samples and data were analyzed. We discovered 37 genes that are highly expressed only in susceptible cultivars after pathogen infections. Among the genes, we selected one gene "the disease susceptibility protein LOV1" for CRISPR targeted gene-editing. This LOV1 gene is known to confer disease susceptibility in response to fungal pathogens in other crops.

Table 1. Strawberry cultivars used for gene expression and DNA sequencing for crown rot resistance.

Pathogen	Cultivar	Phenotype
Colletotrichum gloeosporioides	Festival Elyana	Susceptible Resistant
Phytophthora cactorum	Sweet Sensation Elyana	Susceptible Resistant

Transformation method for CRISPR-mediated gene targeting in strawberry

CRISPR complexes were directly delivered into strawberry embryogenic cells on gold particles using a helium gene gun. This is not Agrobacterium-mediated transformation, which is the most widely used technique for the production of transgenic plants. We coated the CRISPR complex on gold particles and delivered into strawberry embryogenic cells following the conditions described in Table 2. As shown in Figure 1, a gene gun fires small CRISPR-coated gold particles directly to tissues, and a successful transformation results in the expression of the green fluorescent protein. To test whether our

strawberry CRISPR system works, we knocked out the "Phytoene Desaturase (PDS)" gene involved in chlorophyll synthesis. In Figure 1C, several shoots developed that were white. This indicates that CRISPR precisely knocked out the chlorophyll gene in 'Florida Brilliance'.

Table 2. Optimization of particle bombardment conditions for the system of CRISPR delivery.

- Particle size: 1.0 or 1.6 mm
- Burst pressure: 650 psi
- Vacuum pressure: 28 in.-Hg
- Target distance: 9 cm
- Number of shots: 2
- Amount of Gold: 0.1 mg/shot
- Tethering reagent: protamine
- Amount of DNA: 200 ng/shot
- Age of tissue culture: 12 days

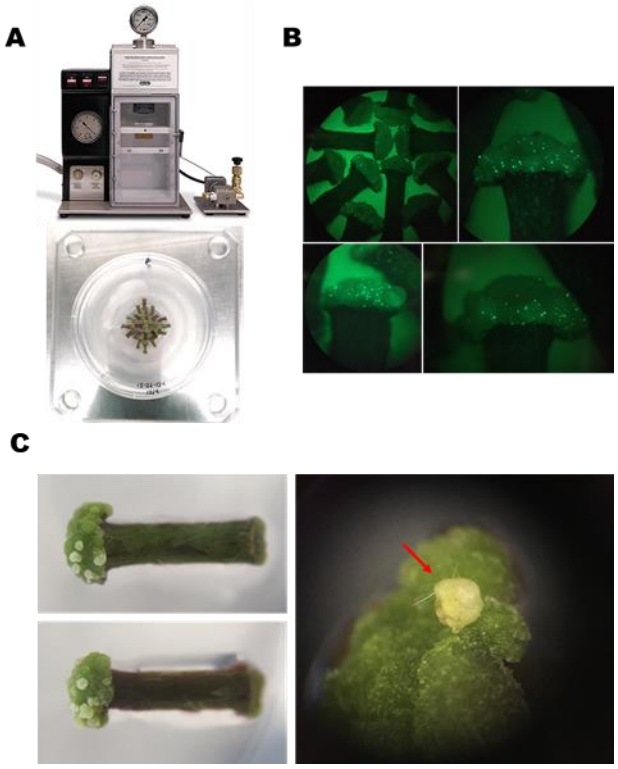

Figure 1. CRISPR system delivered by particle bombardment using a gene gun (A). Determination of transformation efficiency with the green fluorescent protein, which serves as a good indicator of successful transformation (B). Shoot development after the PDS gene knock-out using CRISPR.

Somaclonal variation to improve plant shape, fruit quality and disease resistance.

Somatic variation is the natural variation seen in plants that have been generated through plant tissue culture. The variation occurrs because of genetic mutation developed by in vitro conditions or by plant growth hormones in media. This technique has been used for many other horticultural crops and cultivar development. In this project, we developed 450 somaclones from Sweet Sensation® 'Florida 127' and 150 from 'Florida Radiance'. Figure 2 shows the development of somaclones from Sweet Sensation® 'Florida 127' via tissue culture. In the 2018-2019 season, 250 somaclones from Sweet Sensation® 'Florida 127' were planted at GCREC, and examined for plant shape and fruit quality (Figure 2). We identified several somaclones that are smaller and more compact, open and erect but less vigorous than the original Sweet Sensation® 'Florida 127'. These somaclones will be propagated and tested again next season. We are also planning to test other somaclones from Sweet Sensation® 'Florida 127' and 'Florida Radiance' for resistance to Phytophthora crown rot.

Figure 2. Somaclonal variation in Sweet Sensation® 'Florida 127'. Tissue culture (A); Propagated somaclones in greenhouse (B); Field evaluation (C).

Contact

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