Development of a CRISPR gene editing system in UF strawberries

Seonghee Lee, Kevin Folta and Alan Chambers

Summary

A method for tissue culture and plant regeneration was established from two strawberry varieties, Sweet Sensation® 'Florida 127' and 'Florida Brilliance'. More than 80% of runner tissues successfully produced regenerated plants. The progression from explants to plants in soil takes about 12 to 15 weeks. We also developed a convenient technique to perform CRISPR gene editing in strawberries without introducing any foreign DNA. This biolistic particle bombardment method involves delivering CRISPR into strawberry cells on gold particles using a gene gun.

Methods

Optimizing tissue culture conditions for CRISPR

Runner and petiole segments were collected from four- to six-month-old greenhouse grown Sweet Sensation® 'Florida 127' and 'Florida Brilliance' plants. The tissues were surface-sterilized and placed on agar media containing mineral salts, vitamins, 3% of sucrose, and plant growth regulators. A total of 56 different media types were tested to determine the optimal condition for plant regeneration for Sweet Sensation® 'Florida 127' and 'Florida Brilliance'.

Developing a CRISPR gene editing system

For delivering CRISPR into strawberry cells, two methods were developed: Agrobacterium-mediated transformation and direct CRISPR transfer using the gene gun. For developing DNA-free gene editing methods, a method for strawberry protoplast isolation was developed using Sweet Sensation® 'Florida 127'.

Results

Optimizing tissue culture conditions for CRISPR

The optimal plant regeneration media for Sweet Sensation® 'Florida 127' and 'Florida Brilliance' were developed, following a previous protocol (Folta et al., 2006) with modifications. Explants from the both varieties successfully produced regenerated plants in the media found from this study (SRM25 for Sensation® 'Florida 127' and SRM11 for 'Florida Brilliance'). First shoot initials were visible after 3 weeks of culture (Fig. 1). After 5 weeks, shoot induction was apparent on runner explants. The expanded shoot clusters were transferred to hormone-free media for rooting in culture jars, developing into independent plantlets over 2 to 4 weeks. These plantlets were then moved to potting soil.

Figure 1. Regeneration of whole plants 'Florida Brilliance' from tissue cultures.

Developing a CRISPR gene editing system

To optimize the particle bombardment parameters for CRISPR delivery, we used green fluorescent protein (GFP) as an indicator of a direct transfer of DNA into strawberry tissues. Gold particles (0.6 µm) coated with GFP DNA were delivered into strawberry cells using a helium-powered 'gene gun'. Figure 2 shows that this 'DNA-free' CRISPR method works in strawberry.

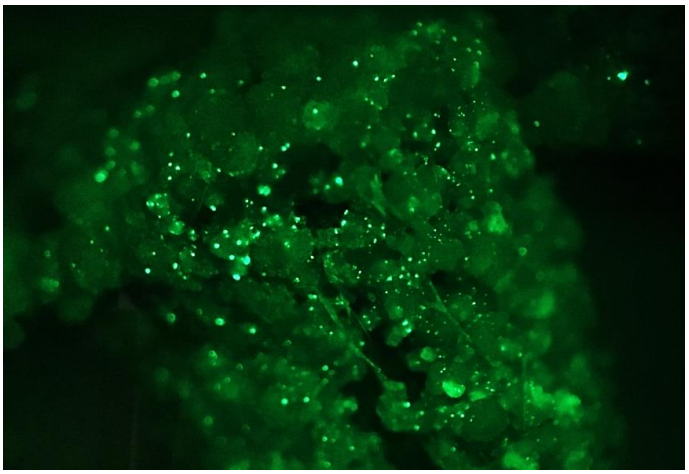

Figure 2. Transient expression of synthetic green fluorescent protein (GFP) in Sweet Sensation® strawberry. The GFP was delivered to cells using a hand-held "gene gun". When GFP is successfully delivered into cells, green fluorescence can be observed under a UV-microscope.

Recently, "DNA-free" CRISPR gene editing of protoplasts have been demonstrated in grape, apple and maize. We also successfully developed the protocol to isolate a high density of clean protoplasts from strawberry callus grown on media. We are currently working to optimize conditons for plant regeneration from protoplasts and/or CRISPR transformed protoplasts.

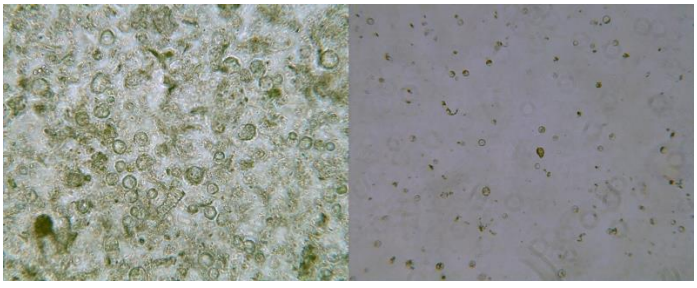

Figure 3. Strawberry protoplasts from callus: Isolated protoplasts pre-purification (left), cleaned and diluted protoplasts (right).

Since we already understand the sequence of a gene involved in chlorophyll synthesis called the "Phytoene Desaturase (PDS)" gene, we have used CRISPR to interfere with the function of this gene as a way to test whether our strawberry CRISPR system works. Figure 4 shows that CRISPR precisely knocked out the chlorophyll gene in Sweet Sensation®.

Figure 4. The lighter strawberry plants (Sweet Sensation®) have been edited using CRISPR to alter the phytoene desaturase (PDS) gene, which gives them a white color.

Contact

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