

DNA Marker and Gene Editing Technologies to Improve Strawberry Flavor and Disease Resistance

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Summary

New DNA markers for sweet flavor and charcoal rot resistance have been developed. These markers were applied to improve varieties through marker-assisted seedling selection. To enhance the development of *Neopestalotiopsis*-resistant varieties, we integrated alternative breeding techniques, such as somaclonal variation and gene editing that supplement traditional breeding methods.

DNA markers and genetic engineering tools to improve strawberry varieties

Strawberry DNA testing is available for enhancing fruity aroma, day-neutral flowering, fruit color, and resistance for multiple diseases. DNA markers allow breeders to select for desired traits at the genetic level, ensuring a higher degree of precision and efficiency in breeding efforts. Furthermore, marker-assisted selection (MAS) can significantly accelerate the breeding process. Recently, our breeding program has incorporated genetic engineering tools, such as somaclonal variation and CRISPR gene editing. This advanced approach, as an alternative to traditional strawberry breeding, can potentially speed up the development of improved strawberry varieties (Figure 1).

Methods

A high quality of 'Florida Brilliance' genome was used to identify chromosome regions and genes associated with fruit flavor and disease resistance. This identified DNA information was used to develop highly accurate DNA markers. To identify key aroma compounds, fruits collected from the population of

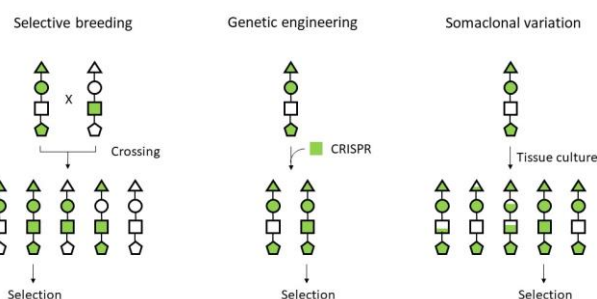


Figure 1. Each string of shapes represents a strawberry variety with different traits. Each shape represents a trait, such as fruit yield, flavor, disease resistance, sweetness, etc. Green color indicates a desirable trait. Partial coloring indicates a compromised desirability. (<https://edis.ifas.ufl.edu/publication/HS1448>)

400 varieties were evaluated using gas chromatography-mass spectrometry for volatile profiling analysis. In addition, the whole genome of each strawberry variety was analyzed using an automated DNA genotyping kit (Axiom™ Strawberry FanaSNP 50k Genotyping Array). To improve resistance to *Neopestalotiopsis*, about 150 somaclones were generated from both 'Florida Medallion' and 'Florida Brilliance'. Each somaclone was then challenged by the pathogen using a detached leaf assay. A candidate gene associated with the resistance to *Neopestalotiopsis* was identified for gene editing in strawberry.

Results

New DNA markers for sweet flavor, furaneol and mesifuran.

Around 30 volatile organic compounds (VOCs) were found to positively influence the perception of strawberry flavor and sweetness in consumer and expert panel studies. Furaneol and mesifurane are

two of the most important, characteristic flavor compounds contributing to a sweet, caramel like aroma. We discovered two chromosome regions (chromosome 1 and 7) associated with higher mesifurane and furaneol production, respectively. DNA markers were devised and applied to predict the concentration of these compounds, thus enabling the implementation of marker-assisted seedling selection. This DNA marker is over 95% accuracy for predicting the production of caramel like flavor.

Table 1. UF strawberry cultivars producing ‘Caramel-like’ flavor.

Cultivar	Average of Mesifurane	DNA Test
Florida Medallion	Low	Yes
WinterStar	Low	Yes
Florida Beauty	Medium	Yes
Florida Brilliance	Medium	Yes
Sweet Sensation ‘FL127’	Medium	Yes
Florida Elyana	Medium	Yes
Florida Radiance	Medium	Yes
Tresure	High	Yes
Winter Dawn	Low	Yes

Improving resistance to *Neopestalotiopsis*

Somaclonal variations that arise in vitro are similar to mutations that occur naturally in the field. Approximately 150 somaclones were developed from both ‘Florida Medallion’ and ‘Florida Brilliance’, respectively. After inoculation of three field isolates of *Neopestalotiopsis*, 19 somaclones from ‘Florida Medallion’ and 10 from ‘Florida Brilliance’ showed resistance. These selected somaclones are currently being propagated in the summer nursery for field testing during the 2023-24 season.

For the application of gene editing technique, the first step is to identify a gene associated with the desired breeding trait. In this project, we discovered a single gene, *OCP3* (overexpressor of cationic peroxidase 3), that is responsible for the susceptibility to *Neopestalotiopsis*. As shown in Figure 3, when *OCP3* gene was precisely mutated in ‘Florida Brilliance’ using the CRISPR gene editing system, we found that the plants significantly increased their level of resistance against *Neopestalotiopsis*. Currently, we are developing gene editing lines that target the *OCP3* gene in ‘Florida Medallion’ and ‘Florida Brilliance’ to enhance the resistance against *Neopestalotiopsis*.



Figure 2. Detached assay of somaclones of ‘Florida Medallion’. Some somaclones (arrow) are resistant against the pathogen, *Neopestalotiopsis*.

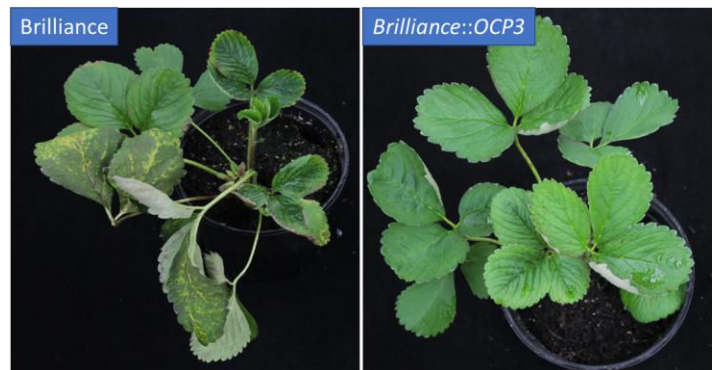


Figure 3. Inoculation of *Neopestalotiopsis* in ‘Florida Brilliance’ and *OCP3* mutated ‘Florida Brilliance’. An increased level of resistance was observed in the plants with mutation in the *OCP3* gene.

Takeaways

With the DNA marker technique, the newly discovered sweet caramel-like aroma will be effectively integrated into upcoming strawberry varieties. Breeding for *Neopestalotiopsis*-resistant varieties has been expedited using somaclonal variations and gene editing technologies.

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