Enhancing Resistance to Pestalotia Through Gene Editing and Somaclonal Variation

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Summary

This study was conducted to identify genes and improved strawberries resistant to *Neopestalotiopsis* sp. causing Pestalotia leaf spot and fruit rot using CRISPR gene editing. We also generated somaclonal variant strawberries (somaclones) of 'Florida Brilliance' and 'Florida Medallion' using the tissue culture technique developed with previous support from FSGA support. These plants will be tested against *Neopestalotiopsis* sp. under both greenhouse and field conditions in the upcoming strawberry season.

Background

Two breeding techniques were applied to improved strawberry varieties to Pestalotia – CRISPR gene editing (GE) and somaclonal variation (SV).

GE is a powerful tool for modifying DNA with high precision. The technique allows to change or correct specific genes at precise locations to improve breeding characteristics such as disease resistance and fruit quality.

SV occurs naturally during the tissue culture process and can result in a wide range of genetic changes. This variation is less controlled compared to gene editing, which involves precise and targeted modifications to specific genes. The regulatory requirements for SV may be less stringent compared to those for gene editing. This can simplify the development and commercialization process for new cultivars.

Traditional breeding methods can be time-consuming. GE and SV allow for the rapid generation of new plant lines, potentially accelerating the development of new resistant cultivars to Pestalotia.

Methods

The high-quality genome information of 'Florida Brilliance' was used to identify genes associated with Pestalotia resistance. Two genes, named '*Resistance Neopestalotiopsis 1* (*RNp1*)' and '*Resistance Neopestalotiopsis 2* (*RNp2*)', were identified for CRISPR gene editing in 'Florida Brilliance'. Additionally, approximately 400 somaclonal variant plants (about 200 each of 'Florida Medallion' and 'Florida Brilliance' somaclones) were developed. These somaclones were tested for resistance to Pestalotia in the GCREC field plots.

Results

Improved Resistance to Pestalotia in 'Florida Brilliance' by Gene Editing

Runner propagated 'Florida Brilliance' plants were grown in a greenhouse for two months. Four isolates of *Neopestalotiopsis* sp. were grown on potato agar media for two weeks and used for inoculation at a spore concentration of $2x10^4$. The control and *RNp1*/*RNp2* edited plants of 'Florida Brilliance' were inoculated in a growth chamber, and disease symptoms were determined 10 days post-inoculation. As shown in Figure 1, when both *RNp1* and *RNp2* genes were edited (Brilliance-*RNp1* and *RNp2*), the plants showed greater resistance to *Neopestalotiopsis* sp. compared to the control plants.

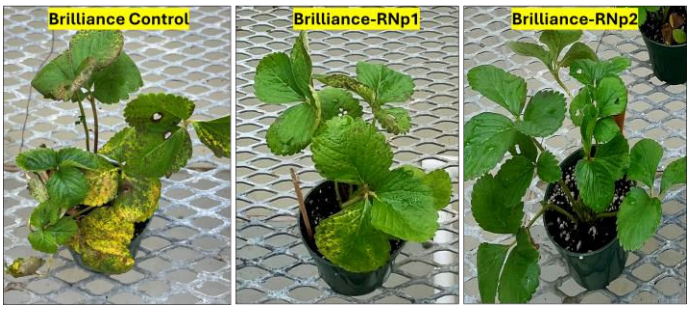

Figure 1. Inoculation of *Neopestalotiopsis* sp. in 'Florida Brilliance' and the two gene edited plants 'Florida Brilliance-*RNp1*' and 'Florida Brilliance-*RNp2*'.

Improved Resistance to Pestalotia in 'Florida Brilliance' and 'Florida Medallion' by Somaclonal Variation

Somaclonal variation is a breeding method that leverages natural genetic variation induced by tissue culture, rather than through hybridization. This technique can complement conventional strawberry breeding methods and is useful in developing new cultivars with novel traits. Plant regeneration through somaclonal variation is relatively fast and allows for easy screening of target traits, such as disease resistance, without the need for traditional crossbreeding.

Approximately 400 somaclones of 'Florida Brilliance' and 'Florida Medallion' were developed and tested for resistance to *Neopestalotiopsis* in the GCREC field. Most of the somaclones were susceptible, which is expected given the high susceptibility of 'Florida Brilliance' and 'Florida Medallion' to the pathogen. However, we identified several somaclones that exhibited increased tolerance or even high resistance (Table 1). The level of resistance in these somaclones was comparable to that of the resistant check plants. These promising somaclones are currently undergoing clonal multiplication and will be prepared for field trials in the next season to further evaluate their resistance under field conditions.

Table 1. Screening of 'Florida Brilliance' and 'Florida Medallion' somaclones for *Neopestalotiopsis* resistance in the GCREC field.

STRAWBERRY VARIETIES	PHENOTYPE	DISEASE SEVERITY (AUDPC)
FL22.98-21	Resistant	11
FL20.49-122	Resistant	27
FL17.76-93	Resistant	359
FL17.76-92	Resistant	287
FLORIDA MEDALLION	Susceptible	857
FLORIDA 127 'SWEET SENSATION'	Susceptible	832
FLORIDA BRILLIANCE	Susceptible	1104
FLORIDA BEAUTY	Susceptible	1061
BRILLIANCE SV-552	Resistant	125
BRILLIANCE SV-514	Resistant	338
MEDALLION SV-506	Resistant	124
MEDALLION SV-512	Resistant	338

AUDPC = Area Under the Disease Progress Curve.

Takeaways

- From this study, two genes, *RNp1* and *RNp2*, associated with resistance to *Neopestalotiopsis* sp. were discovered.
- These genes were edited in 'Florida Medallion', 'Florida Brilliance', Encore™ 'FL 20.34-183' and Ember™ 'FL 20.80-4' for resistance to Pestalotia. Gene edited lines will be tested in the coming strawberry season 2024-2025.
- Four resistant somaclones from 'Florida Brilliance' and 'Florida Medallion' have been developed. Field evaluation of these somaclones will be performed next year.

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