

DNA Marker-Assisted Seedling Selection Program in Strawberry

Seonghee Lee

Summary

Developing new strawberry varieties with high levels of disease resistance and superior fruit quality is essential for successful strawberry production. We have developed new DNA markers for resistance to *Pestalotia*, as well as for fruit flavor and sweetness, which have been integrated into our marker-assisted seedling selection (MASS) program. This year, approximately 70,000 seedlings were screened using 14 DNA markers for traits such as disease resistance, flowering, and fruit flavor. About 16,000 of these seedlings were selected for further field evaluations and potential new variety development.

Background

DNA markers have been successfully applied for improving strawberry varieties through marker-assisted breeding (MAB) and marker-assisted seedling selection (MASS). For example, in 2015, when the marker was initially employed for selecting fruity aroma flavor, we screened approximately 20,000 seedlings and subsequently narrowed down the selection to 12,000 for field evaluation. From 2021 to 2023, the University of Florida (UF) strawberry breeding program subsequently increased the intensity of seedling selection and number of DNA markers for disease resistance, flavor, and fruit quality. In 2021, the program retained 18,625 seedlings out of 73,500 for further field evaluation. In 2022, we screened approximately 80,000 and selected 15,425 seedlings by DNA markers. In 2023, due to disease incidence of *Pestalotia* in the greenhouse, a significant number of our seedlings perished, leading to the screening of only 42,201 seedlings. However, we managed to select

approximately 16,000 seedlings using 12 different DNA markers for further field evaluation. MASS not only enhances the improvement of strawberry varieties but also accelerates the efficiency of the breeding program, increasing the likelihood of developing superior-quality strawberries within a shorter time frame.

Methods

To develop new DNA markers for resistance to charcoal rot (*Macrophomina phaseolina*), *Pestalotia* (*Neopestalotiopsis* sp.) and for flavor traits related to sweetness, we compared field phenotype data with DNA information from breeding germplasm and fruit volatile data. These DNA markers were then used to select parent plants for crossing. A total of 110 crossing families were generated between December 2023 and February 2024. The seeds were germinated in a growth room, and the seedlings were grown in a greenhouse at GCREC for MASS.

MASS was conducted at our lab from the last week of April to the last week of May, lasting for a month. The selected seedlings were then packed and shipped to the summer nursery in the first week of June.

Results

Strawberry DNA markers available for disease resistance, flavor, and fruit quality

A collection of fourteen DNA markers have been effectively employed for MASS within the UF strawberry breeding program (Table 1). The resistance to charcoal rot is conferred by three QTLs, designated as *FaRMp1*, *FaRMp2*, and *FaRMp3* with variable effects against the pathogen. The marker for

FaRmp2 and *FaRmp3* were developed. The marker-assisted pyramiding of *FaRmp1*, *FaRmp2*, and *FaRmp3* can be achieved for durable resistance to charcoal rot disease in strawberry. Additionally, markers for new resistance genes (*RNp1* and *RNp2*) for *Pestalotia* were developed for use in MASS. Markers for caramel-like sweet flavor and soluble solid content were also created.

Table 1. DNA markers available for the marker-assisted breeding in strawberry.

	Trait	DNA Marker	Chromosome
Phytophthora crown rot	<i>Phytophthora cactorum</i>	<i>FaRpc2-H3</i>	7B
	<i>Phytophthora cactorum</i>	<i>FaRpc2-H2</i>	7D
Anthracnose fruit rot	<i>Colletotrichum acutatum</i>	<i>FaRCa1</i>	6B
Colletotrichum crown rot	<i>Colletotrichum gloeosporioides</i>	<i>FaRCg1</i>	6B
Disease resistance	Charcoal rot	<i>Macrophomena phaseolina</i>	<i>FaRmp1/FaRmp2/FaRmp3</i> 2A
	<i>Neopestalotiopsis</i> sp.	<i>FaRNp1/FaRNp2</i>	6B/7A
Bacterial angular leaf spot	<i>Xanthomonas fragariae</i>	<i>FaRXf1</i>	6C
Fusarium wilt	<i>Fusarium oxysporum</i>	<i>FaFW1</i>	2B
	f. sp. <i>fragariae</i>		
Fruit quality	Fruity peach-like aroma	γ -Decalactone	<i>FaFAD1</i> 3B
	White fruit	White fruit color	<i>FaMYB10</i> 1B
	Caramel-like sweet flavor	Mesifurane	<i>FaOMT</i> 7D
		Furaneol	<i>FaFUR</i> 1C
Sweetness	Solid soluble content	<i>FaSSC1</i>	3B
		<i>FaSSC2</i>	6A
Flowering	Flower	Perpetual flowering	<i>FaPFRU</i> 4A

Efforts of Marker-Assisted Seedling Selection for Strawberry Improvement

In this year's MASS, approximately 42,000 seedlings from 111 crosses were screened using 13 different DNA markers for traits such as flavor, flowering, fruit color, and multiple disease resistance (Figure 2). About 16,000 seedlings with the target breeding characteristics were selected for clonal propagation in a summer nursery.

We initially expected to have 100,000 seedlings, but due to unexpected germination issues, we achieved a lower number, with 45,611 seedlings transplanted (47% of the original target). Despite the reduced number of seedlings, we were able to select the planned number of seedlings for this coming breeding season because of the increased number of marker combinations available (Figure 2).

All selected plants will be planted at the beginning of October and tested in the fields during the 2024-2025 breeding season.

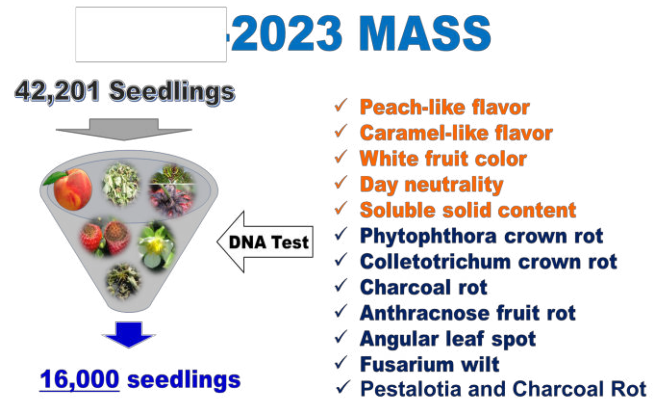


Figure 2. Summary of marker-assisted seedling selection conducted in the season of 2023.

Takeaways

- New DNA markers were developed for resistance to *Pestalotia* and charcoal rot, as well as for caramel-like sweet flavor and soluble solid content.
- A total of 13 DNA markers were used in the marker-assisted seedling selection in 2023, resulting in 16,000 seedlings being retained for clonal multiplication in the summer nursery.
- These plants will be planted for field evaluation in the upcoming 2024-2025 strawberry season.

Contact

Dr. Seonghee Lee
 UF/IFAS Gulf Coast Research and Education Center
 P: 813.633.4151
 E: seonghee105@ufl.edu
<https://gcrec.ifas.ufl.edu/gcrec-facultystaff-directory/seonghee-lee/>