

DNA Marker-Assisted Seedling Selection Program to Improve Strawberry Varieties

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Summary

Developing new strawberry varieties with strong disease resistance and superior fruit quality is essential for successful strawberry production. For nearly 10 years, DNA marker development and testing for parental and seedling selection has been a critical component of the UF strawberry breeding program, accelerating the release of superior varieties. Multiple DNA markers have been developed for resistance and fruit quality traits. In this report, new DNA markers were developed for Pestalotia resistance as well as for fruit flavor and sweetness, and these have been integrated into our marker-assisted seedling selection (MASS) program. In the 2025 MASS cycle, approximately 80,000 seedlings were screened using 13 DNA markers for traits including disease resistance, flowering, and fruit flavor. About 16,000 seedlings were selected for further field evaluations and potential advancement toward new variety development

Background

DNA markers have been successfully applied in the UF strawberry breeding program through marker-assisted breeding (MAB) and marker-assisted seedling selection (MASS). In 2015, when DNA markers were first employed for fruity aroma selection, approximately 20,000 seedlings were screened, and 12,000 were advanced for field evaluation. Since then, the program has steadily expanded the use of DNA markers to target disease resistance, flavor, and fruit quality traits, while also increasing the intensity of seedling selection.

From 2021 to 2023, marker use significantly accelerated the breeding pipeline. In 2021, 73,500 seedlings were screened, and 18,625 were retained for field evaluation. In 2022, approximately 80,000 seedlings were screened, with 15,425 selected based on DNA markers. In 2023, disease pressure from Pestalotia in the greenhouse reduced the total seedling population to 42,201; however, 16,000 seedlings were still advanced for field evaluation using 12 different DNA markers. In 2024, the program returned to larger-scale seedling production, screening approximately 85,000 seedlings with 13 DNA markers targeting disease resistance, fruit quality, and flowering time. Of these, 17,200 seedlings were selected for further evaluation. In 2025, about 80,000 seedlings were screened using 14 DNA markers, including new markers for Pestalotia resistance, flavor, and sweetness. About 16,000 seedlings were selected for field trials and potential advancement toward new UF varieties.

Overall, the MASS approach has consistently improved the precision and efficiency of the UF strawberry breeding program. By integrating DNA markers into seedling selection, the program is accelerating the development of superior strawberry cultivars with enhanced fruit quality, disease resistance, ultimately benefiting growers and consumers alike.

Methods

To develop new DNA markers for multiple disease resistance and flavors related to sweetness, field phenotype data were compared with DNA sequence information from breeding germplasm as well as fruit flavor data. For flavor traits, ripe fruit samples were

harvested from replicated field trials and evaluated for soluble solids content (SSC, Brix) and titratable acidity (TA). In addition, volatile compounds were analyzed using headspace solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC-MS). Key volatiles associated with fruity and caramel-like aroma, including furaneol and mesifurane, were quantified and used for marker–trait association analysis.

DNA markers were applied to select parent plants for controlled crosses. Between December 2024 and February 2025, about 110 crossing families were generated. Seeds from these families were germinated in a controlled growth room, and the seedlings were subsequently transferred to the greenhouse at GCREC for marker-assisted seedling selection (MASS). MASS was conducted over a four-week period, from the last week of April through the last week of May. DNA markers were applied to screen seedlings for disease resistance and fruit quality, and the selected seedlings were packed and shipped to the summer nursery during the first week of June for field evaluation and further breeding advancement.

Results

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

Thirteen DNA markers have been effectively used for marker-assisted seedling selection (MASS) (Table 1). For the 2025 MASS cycle, some markers are not yet available and will need to be developed in future funding period. For example, resistance to charcoal rot (*Macrophomina* crown rot) is conferred by three genes as Mp1, Mp2, and Mp3, each with variable effects against the pathogen. Currently, markers for Mp1 has been developed, while marker development for Mp2 and Mp3 is ongoing. A new marker for resistance *Neopestalotiopsis*, Np2, was developed and used in MASS in 2025. For fruit quality, markers linked to caramel-like flavor, fruity aroma, sweetness, and soluble solids content were also applied in selection.

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

	Trait	DNA Marker	MASS in 2025
Disease resistance	Phytophthora crown rot	Pc2-H2, Pc2-H3	Yes
	Anthraxnose	Ca1	Yes
	Colletotrichum crown rot	Cg1	No
	Macrophomina crown rot	Mp1, Mp2, Mp3	Mp1
	Bacterial angular leaf spot	Xf1	Yes
	Fusarium wilt	FW1, FW2, FW3, FW4, FW5	FW1
	Neopestalotiopsis	Np1, Np2	Np2
	Fruity aroma	FAD1	Yes
	White fruit	MYB10	Yes
	Caramel-like	OMT	Yes
Fruit quality	Sweet	FUR	Yes
	Solid soluble content	SSC1, SSC2	Yes
	Perpetual flowering	PFERU	Yes

Efforts of Marker-Assisted Seedling Selection for Strawberry Improvement

In this year’s MASS cycle, approximately 80,000 seedlings were screened using 13 different DNA markers targeting flavor, flowering, fruit color, and multiple disease resistance. About 16,000 seedlings with target breeding characteristics were selected for clonal propagation in a summer nursery. All selected plants will be planted in early October and evaluated in the field during the 2025–2026 breeding cycle.

Takeaways

- Fourteen DNA markers are now available for disease resistance, fruit flavor, and fruit quality, with new markers for *Neopestalotiopsis* resistance and sweetness.
- Efficient selection: About 80,000 seedlings from over 100 crosses were screened with 13 markers, selecting to about 16,000 seedlings for summer nursery.
- Breeding impact: MASS continues to accelerate the development of superior strawberry cultivars by combining multiple markers, enhancing multiple disease resistance and improved fruit quality.

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