Applications of DNA Testing and Gene Editing to Improve Strawberry Fruit Quality and Disease Resistance

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Summary

In this period of research report, we summarized results of application of DNA markers and gene editing methods to improve fruit quality and disease resistance in strawberry. During this year marker-assisted seedling selection (MASS), approximately 94,000 seedlings were screened by various DNA markers for disease resistance, flowering, and fruit flavor. Appoximately 15,000 seedlings were selected for field evaluations and new variety development. In addition, we identified several somaclones and genes for the resistance to Neopestalotiopsis.

DNA tests for Florida strawberries

Strawberry DNA testing is available for fruit flavor, day-neutral flowering, fruit color (white strawberry), and multiple disease resistances (Phytophthora and Colletotrichum crown rot, charcoal rot, anthracnose fruit rot, Fusarium wilt, and bacterial angular leaf spot). Using the high-throughput DNA tests, we can accelerate the breeding process of new variety development for high levels of disease resistance with superior fruit quality.

Methods

To develop high-throughput and accurate DNA markers, we performed whole genome sequencing and assembly of Florida strawberry varieties and advanced selections. In additona, we will use other DNA sequence resources available in public database. This genome information is used to identify unique DNA sequences associated with disease resistance and fruit quality. To develop gene-specific DNA markers, we conducted gene expresson data analysis and RNA sequencing. A rapid DNA extraction method combined with high-throughput DNA markers was applided to screen large number of breeding samples (Fig. 1). DNA markers were applied for validating crossing parents. Small leaf punches are collected from seedlings, DNA rapidly extracted, and DNA tests run and scored. Only the seedlings with desirable trait combinations are retained, and the rest are discarded.

Figure 1. The procedure of high-throughput marker-assisted seedling selection (MASS) conducted in 2022. In this year breeding season, about 100 crossings were developed. Because of the disease of Neopestalotiopsis, all seedlings were maintained in a commercial greenhouse with free of the pathogen. In California summer nurseries, clonal propagations for approximately 15,000 seedlings are conducted for the field evaluaton.

Results

DNA markers for improving fruit quality and disease resistance

Currently, we have DNA markers for fruity aroma (Y-decalactone), day-neutrality, phytophthora crown rot, anthracnose fruit rot, white fruit color, bacterial angular leaf spot, charcoal rot, Collectrotrichum crown rot, and Fusarium wilt (Figure 2). These markers were successfully applied for 2021-2022 marker-assisted seedling selection.

To maximize the level of fruity aroma flavor, we need to obtain two copies of *FaFAD1* (the gene producing Y-decalactone) in a strawberry variety. Our DNA marker can identify which varieties have two copy genes for highest level of fruity aroma flavor. For example, the latest cultivar 'Florida Medallion' has two copies of *FaFAD1* and produce the maximum level of fruity aroma in fruits. The flavor DNA marker was used during the process of developing 'Flodia Medallion'. In addition, we are working on identifying genes and DNA markers for other important fruit flavor such as tropical flavor (pineapple and grape notes) and sweet flavor (mesifurane and esters).

The marker for day-neautrality was used selected seedlings from the cross 'Florida Beauty' (day-neutral) and other advanced selections (no day-neutral). Seedlings will be tested for their performance in the GCREC field in 2022-2023 breeding season. We also used the marker for white fruit to select seedlings from different crossings (Red and white fruit accessions). Thehse seedlings are validated for fruit color, marketing yield, and quality in the field.

For Phytophthora and Colletotrichum crown rot, we identified candidate genes for the resistance and developed gene-specific markers. Using the markers, it is possible to select resistant parents and seedligns against the pathogens without field phenotyping. Also, a gene conferring resistance to anthracnose fruit rot was identied to develop a functional gene-specific marker that can be used for the new resistance cultivar development via marker-assisted selection. In this year, we successfully developed new DNA markers for the resistance to charcoal rot. The markers were applied for selecting seedlings that contain resistance genes to charcoal rot in strawberry. Using the markers, we can more effectively and rapidly integrate the resistance gene into new varieties.

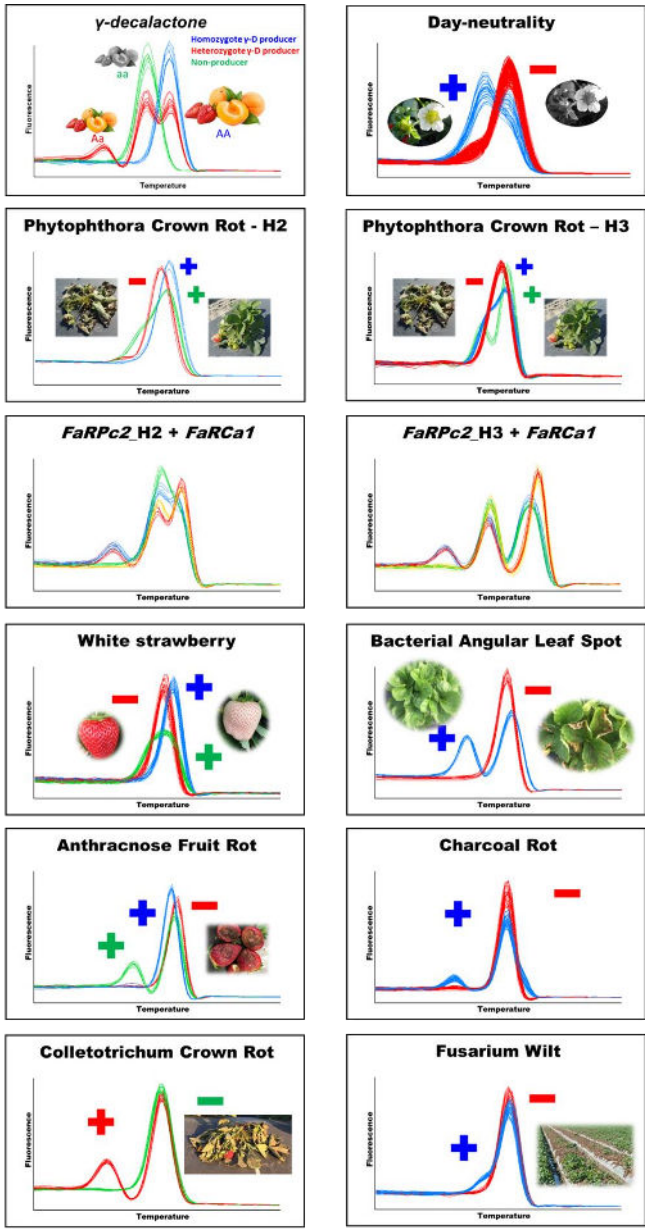

Figure 2. DNA markers for fruit quality and disease resistance available for marker-assisted seedling selection in strawberry. γ-decalactone: fruity aroma, Day-neutrality: flowering, FaRPc2-H2 and H3: Phytophthora crown rot, FaRCa1: Anthracnose fruit rot.

Marker-assisted seedling selections for new cultivar development

In this year MASS, we screened a total of 94,000 seedlings from 107 crossings using 10 differnet DNA markers for flavor, flowering, fruit color, and multiple disease resistance (Figure 3). About 15,000 seedlings containing target breeding characteristics were

retained for clonal propagation in a summer nuersery. All plants will be planted in the beginning of October and tested in the fields during the 2021-2022 breeding season.

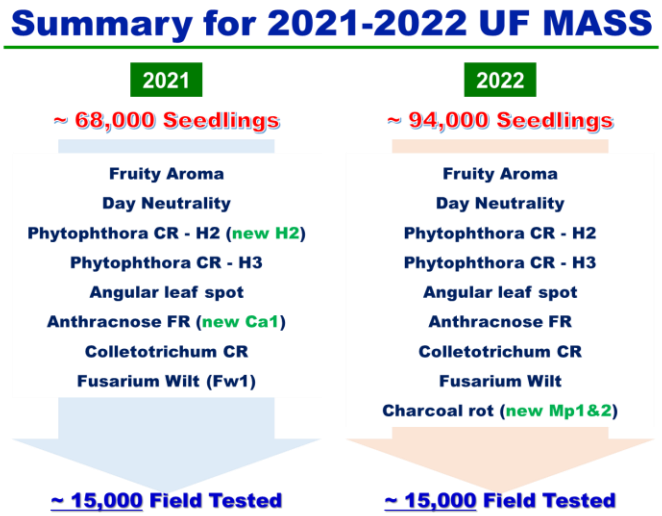

Figure 3. Summary of marker-assisted seedling selection conducted in seasons of 2021 and 2022.

Improving Resistance to Neopestalotiopsis Using Somacloanal Variations and Gene Editing

Somaclonal variation is a breeding method utilizing natural genetic variation induced by a tissue culture process instead of by hybridization. This technique can complement current conventional strawberry breeding methods and used in the development of new cultivars with novel traits. Plant regeneration through this method is relatively fast and easy to screen for the target traits such as disease resistance without crossings. We developed approximately 700 somaclones of 'Florida Brillance' and tested for the resistance to Neopestalotiopsis. As shown in Figure 4, most of the somaclones were dead because 'Florida Brillaince' is susceptible. However, some somaclones showed increased tolerance or highly resistant against the pathogen. The somaclones will be examined for the resistance in field conditions.

To apply CRISPR gene editing for the resistance to Neopestalotiopsis, we must identify target genes to edit. But there is no information available in strawberry. To identify genes, we used the Arabidopsis system which is a model plant for all dicots. Thus, genes identified from Arabidopsis can be applied for strawberry. As shown in Figure 5, we screened a number of Arabidopsis mutants after inoculation of *Neopestalotiopsis*, and found two genes (WRKY70 and OCP3) associated with the resistance against *Neopestalotiopsis* pathogen. We cloned the genes in 'Florida Brilliance' and developed constructs for CRISPR gene editing to develop resistance varieties against *Neopestalotiopsis*.

Figure 4. Screening 'Florida Brilliance' somaclones for Neopestalotiopsis resistance. All somaclones were inoculated with *Neopestalotiopsis* spores to select survived plants at the end of the screening period.

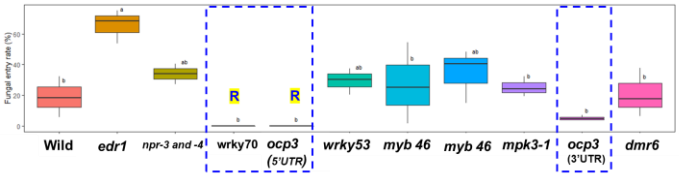

Figure 5. Representatives of disease phenotype in Arabidopsis mutants against *Neopestalotiopsis*. Plants with knock-out of WRKY70 and OCP3 genes are highly resistant to Neopestalotiopsis disease.

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