The Strawberry DNA Testing Handbook

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Abstract. The availability of strawberry (*Fragaria × ananassa*) genomic resources has increased dramatically in recent years. Some of these resources are readily applicable to strawberry breeding programs for use in DNA-informed breeding. Information about these tests and how to interpret them is dispersed through numerous manuscripts or in the laboratories that use them routinely. To assist breeders in identifying tests available to their breeding program and in implementing them in their program, a compendium of strawberry DNA tests was created. This compendium is available for download from the Genome Database for Rosaceae (<https://www.rosaceae.org/organism/Fragaria/x-ananassa?pane=resource-4>). This resource will be updated continually as old tests are modified and new tests are created.

The development of new strawberry (*Fragaria × ananassa*) cultivars with improved flowering, fruit quality, and disease resistance is an ultimate goal for fruit breeders worldwide. Selective breeding has resulted in tremendous crop improvement, but even greater progress can be achieved with the use of new technologies, such as molecular markers (Peace, 2017). As genome sequences and modern genetic resources for fruit crops

have become available, it is critical that these resources be used to develop practical tools for crop improvement. DNA tests are tools that help breeders predict traits of interest in parents and offspring, and how they will be inherited in future generations.

The RosBREED project (<https://www.rosbreed.org>), funded by U.S. Department of Agriculture (USDA)-Specialty Crop Research Initiative, focused on leveraging genetic information to improve eight rosaceous crops: apple, blackberry, peach, pear, rose, strawberry, sweet cherry, and tart cherry. For strawberry, these improvement efforts included the development of genomic resources, the discovery of quantitative trait loci (QTL), and the facilitation of DNA-informed breeding (DIB, also known as marker-assisted breeding). DNA-informed breeding helps breeders select potential cultivars while reducing the need to maintain plants long term and perform phenotypic trials for traits that are expensive or difficult to measure (Peace, 2017; van Geest, 2017). The DIB process involves extracting DNA from parents or offspring and running DNA tests that have been associated with desirable traits. The DNA tests implemented in DIB may be for a closely linked rare allele or a functional mutation within the causal gene for a trait, as exemplified in the work by Perrotte et al. (2016) and Zorrilla-Fontanesi

et al. (2012). Ideally, the DNA tests are high throughput, accurate, low cost, and user friendly. These factors are especially important when a large number of samples need to be genotyped with limited resources and time. When implemented in a breeding program, DIB can enhance the efficiency, accuracy, and pace of breeding while reducing the cost. Implementing DIB enables breeders to identify and exploit the genetic potential in their programs and use their resources effectively.

The first RosBREED project, “RosBREED: Enabling Marker-assisted Breeding in Rosaceae” (Iezzoni et al., 2010), was initiated in 2009 and identified many important QTL for flowering, fruit quality, and disease resistance traits. The first 90K Axiom® single nucleotide polymorphism (SNP) array (Bassil et al., 2015), developed as part of the first RosBREED project, has been used as a high-throughput genotyping tool for QTL discovery in numerous strawberry programs worldwide (Anciro et al., 2018; Nellist et al., 2019; Roach et al., 2016; Verma et al., 2017). The second RosBREED project, “RosBREED: Combining Disease Resistance and Horticultural Quality in New Rosaceous Cultivars” (Iezzoni et al., 2016, 2017), built on the initial work by identifying disease-resistant QTL and combining them with horticultural quality QTL. This was accomplished through the development and use of many DNA tests. Moreover, the genomic resources developed by the RosBREED projects also facilitated the development of many DNA tests by the international community. This article describes briefly the development of an online compendium of DNA markers for DIB called the Strawberry DNA Testing Handbook, created with RosBREED support for the benefit of strawberry breeders and geneticists worldwide.

Rationale

In recent years, a number of chromosome regions (loci) in strawberry have been identified that control flowering, fruit quality, and disease resistance traits in strawberry. DNA tests have been developed for many of these loci in octoploid strawberry. Many breeding programs have adopted DIB as part of their breeding process by either running the tests internally or outsourcing the work to companies providing diagnostic testing services. The initial optimization process, as well as the performance and interpretation of DNA tests, often require a skill set that might not be readily available in all laboratories (Peace, 2017). As such, we created the Strawberry DNA Testing Handbook as an easy-to-use resource. The purpose of the handbook is to distill information from primary literature related to the DNA tests, provide a starting point for test optimization, and assist in the interpretation of tests for informed breeding decisions. Overall, we hope this resource can serve as a training tool and troubleshooting guide for the implementation of existing DNA tests in strawberry breeding programs. To ensure availability, the handbook has been

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published at the Genome Database for Rosaceae (Jung et al., 2019) and can be downloaded as a Microsoft Word document (<https://www.rosaceae.org/organism/Fragaria/x-ananassa?pane=resource-4>). As new DNA tests are published or old tests are modified or replaced, the authors will continually update the handbook to maintain its relevance and usefulness to the strawberry breeding community.

Technical Information

In the Strawberry DNA Testing Handbook, tests are organized by trait (e.g., fruit quality and disease resistance), the gene/locus being tested, and the test being used. Numerous SNP-based high-resolution melting markers are available for both horticultural quality and disease resistance traits (Table 1). Electrophoresis-based tests are also listed for breeding programs without access to a real-time thermocycler (Table 1). Each test in the Strawberry DNA Testing Handbook is described in the same format: 1) background information, 2) technical details, 3) test interpretation, and 4) additional notes. The background information describes the trait and gene/QTL with which the test is associated, as well as the primary literature describing the test development and validation. Suggested reaction mixtures, primer sequences, and polymerase chain reaction protocols provided as a starting point for test optimization and troubleshooting are presented in the technical section. A description of how to interpret the test, positive and negative control cultivars (Table 2), and a picture of positive and negative results are shown in the interpretation section. Many of the strawberry cultivars that can serve as positive and negative controls for the tests are part of the USDA-Agriculture Research Service National Clonal Germplasm Repository (NCGR) *Fragaria* collection in Corvallis, OR. Leaves or runners of plants are available for distribution through the U.S. National Plant Germplasm System (<https://npgsweb.ars-grin.gov/gringlobal/search.aspx?>). The final section consists of additional notes and caveats surrounding the test. These notes consist of items such as known false-positive or false-negative strawberry cultivars, pathogen virulence information, or tips to help in optimization or multiplexing. To get the most out of each test, users should read the entire protocol before use to identify any caveats related to their testing needs.

Future Strawberry Community Involvement

As mentioned previously, the handbook will be updated as new tests are developed and validated. As such, there is much potential for future community involvement to maintain the usefulness of the handbook. The recent publication of the *F. xananassa* genome (Edger et al., 2019) will likely spur the development of new DNA tests. Moreover, breeding programs are likely to have

Table 1. DNA tests in cultivated strawberry, including traits and the corresponding genes/quantitative trait loci (QTL), tests, platforms used for the tests, marker types, and citations.

Trait	Gene/QTL	Test	Platform/marker type	Reference	
				Locus	DNA test
Flowering					
Perpetual flowering	<i>FaPFRU</i>	Bx215 SSR	Capillary electrophoresis/SSR	Gaston et al. (2013), Verma et al. (2017)	Perrotte et al. (2016), Salinas et al. (2017)
Perpetual flowering	<i>FaPFRU</i>	UFDNHRM01	HRM	Gaston et al. (2013), Verma et al. (2017)	Unpublished
Fruit quality					
γ-Decalactone	<i>FaFAD1</i>	qFaFAD1	Agarose electrophoresis	Sánchez-Sevilla et al. (2014), Chambers et al. (2014)	Chambers et al. (2014)
γ-Decalactone	<i>FaFAD1</i>	UFGDHRM5	HRM	Sánchez-Sevilla et al. (2014), Chambers et al. (2014)	Noh et al. (2017)
γ-Decalactone	<i>FaFAD1</i>	UFGDHRM01	HRM	Sánchez-Sevilla et al. (2014), Chambers et al. (2014)	Unpublished
Mesifurane	<i>FaOMT</i>	FaOMTSI/NO	Agarose electrophoresis/SCAR	Zorrilla-Fontanesi et al. (2012)	Zorrilla-Fontanesi et al. (2012)
Disease resistance					
<i>Phytophthora fragariae</i> var. <i>fragariae</i>	<i>Rp1</i>	SCAR-R1 ^a	Agarose electrophoresis, capillary electrophoresis/RAPD, SCAR, SSR	van de Weg (1997)	Haymes et al. (1997, 2000), Rugienius et al. (2006), Sasnauskas et al. (2007), Matthey (2013)
<i>P. cactorum</i>	<i>FaRPe2-H2</i>	UFPe2HRM01	HRM	Mangandi et al. (2017)	Unpublished
<i>P. cactorum</i>	<i>FaRPe2-H2</i>	UFPe2HRM03	HRM	Mangandi et al. (2017)	Unpublished
<i>P. cactorum</i>	<i>FaRPe2-H3</i>	UFPe2HRM02	HRM	Mangandi et al. (2017)	Unpublished
<i>P. cactorum</i>	<i>FaRPe2-H3</i>	UFPe2HRM04	HRM	Mangandi et al. (2017)	Unpublished
<i>P. cactorum</i>	<i>FaRPe2-H3</i>	RPCKASPH3	HRM/KASP	Mangandi et al. (2017)	Unpublished
<i>Xanthomonas fragariae</i>	<i>FaRxf1</i>	HRM6D_33.083	HRM	Roach et al. (2016)	Noh et al. (2018)
<i>X. fragariae</i>	<i>FaRxf1</i>	UFxf1HRM01	HRM	Roach et al. (2016)	Roach et al. (2016)
<i>X. fragariae</i>	<i>FaRxf1</i>	UFxf1HRM02	HRM	Roach et al. (2016)	Unpublished
<i>Colletotrichum acutatum</i>	<i>FaRcal</i>	UFcalHRM01	HRM	Salinas et al. (2018)	Unpublished
<i>C. acutatum</i> pathogenicity group 2	<i>Rca2</i>	Rca2_240 ^a	Agarose electrophoresis/SCAR	Denoyes-Rothan et al. (2005)	Lerceteanu-Köhler et al. (2005)
<i>C. gloeosporioides</i>	<i>FaRcgl</i>	UFcglHRM01	HRM	Ancito et al. (2018)	Unpublished

^aMarkers not yet included in the handbook, because they were not validated with our DNA testing accessions. They will be added as validation is finished. HRM = high-resolution melting; PFRU = perpetual flowering and runnering; FAD1 = delta-12 fatty acid desaturase; OMT = O-methyltransferase.

Table 2. Strawberry varieties found in the U.S. National Strawberry Collection at the National Clonal Germplasm Repository in Corvallis, OR, that can serve as positive (+) and negative (–) controls for some of the genes/quantitative trait loci presented in the DNA test handbook.

Name	Accession	FaPFRU	FaFAD1	FaOMT	FaRPe2-H2	FaRPe2-H3	FaRXf1	FaRCa1	FaRCg1
Seascape	PI 660779	+	–	–	–	+	–	–	–
Selva	PI 551814	+	–	–	–	+	–	–	–
Tribute	PI 551953	+	+	–	–	+	–	+	–
Camarosa	PI 670238	–	–	+ Het	–	+	–	–	–
Charm	PI 664911	–	–	–	–	+	–	–	–
Honeoye	PI 551588	–	+	–	–	+	–	+	–
Jewel	PI 551927	–	–	+ Het	–	+	–	–	–
Strawberry Festival	PI 664337	–	+	+ Het	–	+	–	–	–
Sweet Charlie	PI 664317	–	+	+ Het	+	+	–	+	–
Deutsch Evern	PI 551626	–	–	–	+	–	–	+	+
Hood	PI 551502	–	–	–	–	+	–	+	–
Earliglow	PI 551394	+ ²	+	+ Het	–	+	–	+	–
Hecker	PI 551490	+	–	–	–	+	–	+	–
Aromas	PI 684680	+	+	–	–	+	–	–	–
Mara des Bois	PI 687353	+	–	+	+	–	–	+	+
US 4808	PI 637937	–	+	–	–	–	+	–	–
US 4809	PI 637938	–	+	–	–	–	+	–	–

²Earliglow does not have the day neutral phenotype, but is positive for the marker.

Het = heterozygous marker genotype. Empty cells in FaOMT mean that the accessions were not tested.

regional positive and negative control cultivars they will discover as part of the testing process. Some of these cultivars might be publicly available as part of NCGR collection. To facilitate continued use of the handbook, we encourage feedback about use of these tests as well as information about new tests and potential control cultivars that the strawberry breeding and testing community can provide.

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