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Meet the Student-Author

Isabella Vaughn

Isabella with her Department Head, Dr. Wayne Mackay, accepting the 2023 Outstanding Horticulture Undergraduate award, received at the Horticulture Entrepreneurship Lecture Series Event

I am from Farmington, Arkansas. I will be graduating from the University of Arkansas in the Spring of 2023 with a degree in Horticulture and minors in Agriculture Communications and Agriculture Leadership. While at the University of Arkansas, I have learned practical skills and research in Horticulture. I have been working for the Fruit Breeding Program, under the direction of Dr. Margaret Worthington, since my sophomore year, during which I have had hands-on experience with research and networking opportunities. I attended the Southern Region American Society for Horticultural Science conference and competed in horticulture judging. This summer, I will attend the American Society for Horticultural Science conference and compete in an undergraduate oral research presentation competition. Senior year, I received the outstanding senior in Horticulture award and the Vail-Watts award. I served as a Bumpers College Ambassador for one year. I want to thank Dr. Margaret Worthington for her help on this project and her guidance throughout my collegiate career. I also want to thank Dr. John Clark, Carmen Johns, Lacy Nelson, Alexander Silva Cordoba, Dr. Jackie Lee, the fruit breeding team at the Fruit Research Substation, and the Horticulture Department for their support through countless hours of guidance. Lastly, I want to thank my husband and parents for always supporting my endeavors and being there to help with everything.

Research at a Glance

- Primocane-fruiting is a recessively inherited trait, which means developing a genetic marker could accelerate the breeding process.
- PF2 KASP is the first diagnostic molecular marker associated with a phenotypic trait (primocane-fruiting) that yields potential for economic importance in blackberries.
- A primocane-fruiting molecular marker would be effective for blackberry and raspberry breeders to effectively breed a crop that will work in various climates.

Validation of a Diagnostic Marker for Primocane-Fruiting in Blackberry

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Abstract

Typical blackberries (*Rubus* subgenus *Rubus*) have perennial crowns and roots and biennial canes. The first-year canes (primocanes) are usually vegetative, while second-year canes (floricanes) produce fruit. Primocane-fruiting blackberries produce fruit on first-year canes and are desirable to growers because they potentially allow for a longer harvest season in temperate regions and enable production in tropical areas where no natural chill hours are accumulated. The development of molecular markers for desirable traits can potentially increase efficiency in blackberry breeding. However, to date, there are no diagnostic molecular markers for economically important traits in blackberries. Primocane-fruiting is recessively inherited, and tetraploid blackberries must have four copies of the primocane allele for the trait to be expressed. A single locus strongly associated with primocane fruiting was recently identified on chromosome Ra03, and a new KASP marker (PF2) was developed within this locus. In this study, we validated the performance of the new PF2 marker in a seedling population (Population 1937) segregating for primocane-fruiting at the University of Arkansas System Division of Agriculture's Fruit Breeding Program. In 2022, 170 seedlings in the population were evaluated for primocane-fruiting. Of these seedlings, 68 were floricane-fruiting, 86 were primocane-fruiting, and 16 could not be phenotyped due to poor plant growth. The PF2 marker correctly predicted the phenotype for 146 of 154 progeny that were scored in the 1937 population. Some of the inconsistencies between the marker prediction and the observed phenotypes could have been due to weak plants shaded out by neighbors or human error.

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¶ Margaret Worthington, the faculty mentor, is an Associate Professor in the Department of Horticulture.

Introduction

Blackberries (*Rubus* subgenus *Rubus*) and raspberries (*Rubus idaeus*) are distinctive among fruit crops because they have a perennial root system and crown and biennial canes. Typically, the first-year canes (primocanes) are vegetative, and the second-year canes (floricanes) bear fruit (Clark, 2008). Primocane-fruiting (PF) is a valuable trait that has the potential to extend the harvest season because primocanes typically flower later in the growing season than floricanes and do not need to accumulate chill hours prior to flowering. The University of Arkansas System Division of Agriculture's (UADA) Fruit Breeding Program released 'Prime-Jan®' and 'Prime-Jim®,' the first PF blackberry cultivars, in 2004 (Clark et al., 2005). Since then, many other PF cultivars have been released by the UADA Fruit Breeding Program and other public and private programs. Potential advantages of the PF trait include late-season fruiting period on primocanes from late summer to fall; potential to schedule production based on primocane management; potential of two crops on the same plant in the same year (floricane crop first, then primocane crop); reduced cost of pruning by mowing canes (primocane crop only); avoidance of winter injury; avoidance of rosette/double blossom disease; eliminated need for chilling hours (for primocane crop only); and production of blackberries in new geographic areas (Clark, 2008).

In blackberries, all PF cultivars are derived from the wild PF *R. argutus* accession 'Hillquist' (Clark and Finn, 2011). PF is recessively inherited, and all four copies of the PF allele must be present for the trait to be expressed in autotetraploid blackberries (Lopez-Medina et al., 2000). PF is an important commercial trait in blackberries and raspberries; however, a diagnostic marker has not been developed yet in either species. Developing a genetic marker for PF could accelerate the breeding process by allowing breeders to design crosses more effectively, cull seedling populations to keep only PF seedlings, and discard floricane-fruiting (FF) seedlings.

Genetic research in blackberries has been delayed relative to other economically important fruit crops because of challenges including polyploidy and multisomic inheritance (Foster et al., 2019). Fortunately, new tools for genetic research in polyploids (Bourke et al., 2018) and genomic resources for blackberries (Bruna et al., 2023) have recently enabled molecular research in this crop. A low-density microsatellite marker-based genetic map of a segregating breeding population created in 2013 suggested that the PF locus was located on linkage group 7 (Castro et al., 2013). However, a recent genome-wide association study conducted with UADA Fruit Breeding germplasm indicated that PF was controlled by a major-effect locus on chromosome Ra03. Based on these findings, the UADA Fruit Breeding team developed a Kompetitive Allele Specific PCR (KASP) marker for primocane-fruiting (PF2).

The goal of this research was to validate the performance of the PF2 marker in a biparental breeding population (Population 1937) segregating for PF. Specifically, our objectives were to (1) determine if the progeny of Population 1937 fit the expected 1:1 segregation ratio for PF and (2) determine if the PF2 marker is linked to the PF trait in this population.

Materials and Methods

The population chosen for this study (Population 1937) was derived from a cross between 'A-2506T', a floricane-fruiting blackberry selection with three primocane alleles, and 'APF-409T', a PF blackberry selection. One hundred seventy progeny from the 1937 blackberry seedling population were planted in the spring of 2020 at the Uni-versity of Arkansas System Division of Agriculture's Fruit Research Station in Clarksville.

Each blackberry seedling in the 1937 population was planted at 45.72-cm spacing. To separate each individual plant, the selected primocanes from each plant were traced up from a crown and then given a tag number (1 through 170). This blackberry planting was untipped and was irrigated as needed using overhead sprinklers. The population was phenotyped for PF three times during the growing season, on 16 June 2022, 6 July 2022, and 1 August 2022. The population was phenotyped multiple times because the specific timing of primocane flowering varied from plant to plant due to various developmental and climatic factors that could trigger PF expression. PF was scored as a binary trait depending on the presence or absence of primocane flowers or fruit on the selected canes. Plants that were less than 1 m tall were scored as unknown phenotypes because the expression of the PF trait can be affected by weak plants or excessive shading by neighboring plants.

Young leaf tissue from primocane leaflets less than 1 cm long was collected from each parent and progeny primocane that was tagged on 16 June 2022, transported back to the Department of Horticulture, Fayetteville, in coolers, and stored in a freezer (-20 °C). DNA was extracted following a modified CTAB protocol (Porebski et al., 1997). Genomic DNA from the 1937 population seedlings and parents was then sent to LGC (LGC Genomics, Beverly, Mass., USA) to be genotyped with the PF2 marker. KASP marker reactions were performed as described by Varanasi et al. (2022). Fluorescence signals provided from each sample were converted to tetraploid dosage scores and used to create a cluster plot in the ggplot2 R package (Wickham, 2016).

Statistical analysis was performed with the Chi-squared test to determine if the progeny of population 1937 fit the 1:1 FF:PF segregation ratio expected for under normal random

chromosome assortment or the 13:15 FF:PF segregation ratio expected for random chromatid assortment in polyploid species that form multivalents joined together at the centromere during meiosis (Allard, 1960).

Results and Discussion

Of the 170 progeny in population 1937, 86 were scored as PF, 68 as FF, and 16 as unknown. By performing a Chi-square test, we determined that the null hypothesis could not be rejected and the population fit the expected segregation ratios for both random chromosome assortment (1:1 expected, $\chi^2 = 2.104$, $P = 0.147$) and random chromatid assortment (13:15 expected, $\chi^2 = 0.320$, $P = 0.571$) scenarios (Table 1). Similar results were obtained by Lopez-Medina et al. (2000), who found that many of the 36 populations studied fit expected segregation ratios for both random chromosome assortment and random chromatid assortment and that random chromatid assortment was a better fit than random chromosome assortment for 17 out of 36 populations.

When HEX and FAM fluorescence data were converted to tetraploid dosage scores, 6 progeny were scored as CCTT, 75 progeny as CTTT, and 89 progeny as TTTT (Table 2, Fig. 1). The female parent (A-2506T) was CTTT and the male parent (APF-409T) was TTTT. Thus, the T allele was determined to be the recessive PF allele, and the C allele was the dominant FF allele. For standard random chromosome segregation, the expected ratio of genotypes would be 0 CCTT: 85 CTTT: 85 TTTT, while the expected segregation ratio for random chromatid assortment would be 6.07 CCTT (1/28): 72.85 CTTT (12/28): 91.07 TTTT (15/28). It was impossible to conduct the Chi-squared test for random chromosome assortment with the PF2 marker data because one of our three genotype classes had no expected progeny under this scenario. However, our data fit the expected segregation ratio for random chromatid assortment almost perfectly ($\chi^2 = 0.111$, $P = 0.946$) (Table 2). The presence of progeny with two C alleles (CCTT) can only be explained by possible pollen contamination, accidental self-pollination of the female parent, or double reduction. Double reduction is the transmission of sister chromatids on the same gamete. It is only observed in poly-ploid species under random chromatid segregation and for loci that are unlinked to the centromere (Allard, 1960). Previous phenotypic data on 36 biparental populations suggested that double reduction may occur in tetraploid blackberry (Lopez-Medina et al., 2000). Furthermore, evidence for random chromatid assortment and double reduction has also been found in autopolyploid potato (Bourke et al., 2015) and yellow cress (*Rorippa* spp.) (Stift et al., 2008) using molecular marker data. It should be possible to confirm whether these six CCTT seedlings are actually true seedlings of the A-2506T x APF-409T cross by genotyping with more molecular markers in the future.

The PF2 marker incorrectly predicted the phenotype for only 8 of the 154 progeny that were assigned a score as PF or FF in 2022 (Fig. 1). Five progeny that had a CTTT genotype were scored as PF, and three progeny with a TTTT genotype were scored as FF. It is possible that the

Table 1. The 1937 population was scored as primocane-fruiting (PF), floricane-fruiting (FF), or unknown for the 170 progenies. The Chi-square (χ^2) test was performed to determine the population fit the expected segregation rations for both random chromosome assortment and random chromatid assortment.

				Random chromosome assortment			Random chromatid assortment		
Population	Parental genotypes	No. FF progenies	No. PF progenies	Expected ratio	χ^2	*P*	Expected ratio	χ^2	*P*
A-2506 x APF-409T	Aaaa:aaaa	68	86	1:1	2.104	0.147	13:15	0.320	0.571

Table 2. HEX and FAM fluorescence data were converted to tetraploid dosage scores. The data fit for expected segregation ratio for random chromatid assortment.

					Random chromatid assortment		
Population	Parental genotypes	Number CCTT	Number CTTT	Number TTTT	Expected ratio	χ^2	*P*
A-2506 x APF-409T	CTTT:TTTT	6	75	89	1:12	0.111	0.946

χ^2 = chi-square.

inconsistencies between the PF2 marker and phenotype data could be due to true recombination between the PF gene and the PF2 marker or mistakes in plant tissue collection or phenotype scoring because of the close plant spacing of the population. These 8 seedlings with inconsistencies between PF2 genotypes and phenotypes and the 16 seedlings that were not assigned a PF phenotype in 2022 because of small plant stature will be phenotyped and genotyped again in 2023 to validate 2022 data. The phenotypes that did not match the predictive marker will be rescored to validate 2022 data.

Conclusions

We identified a single locus strongly associated with PF on chromosome Ra03 and developed a new diagnostic KASP marker (PF2) based on a single nucleotide polymorphism (SNP) marker within the PF locus. This is the first diagnostic molecular marker associated with a phenotypic trait of economic importance to be developed in blackberries. The phenotypic and genotypic segregation ratios observed in the progeny of the 1937 validation population used in this study best fit expected ratios for random chromatid assortment. The double reduction landscape in blackberries should be further investigated by developing a high-resolution linkage map. The utility of the PF2 KASP marker for predicting PF phenotypes was demonstrated in this validation population. Further research should be conducted to investigate inconsistencies between the marker prediction and observed phenotypes and validate the performance of the PF2 marker in diverse breeding germplasm.

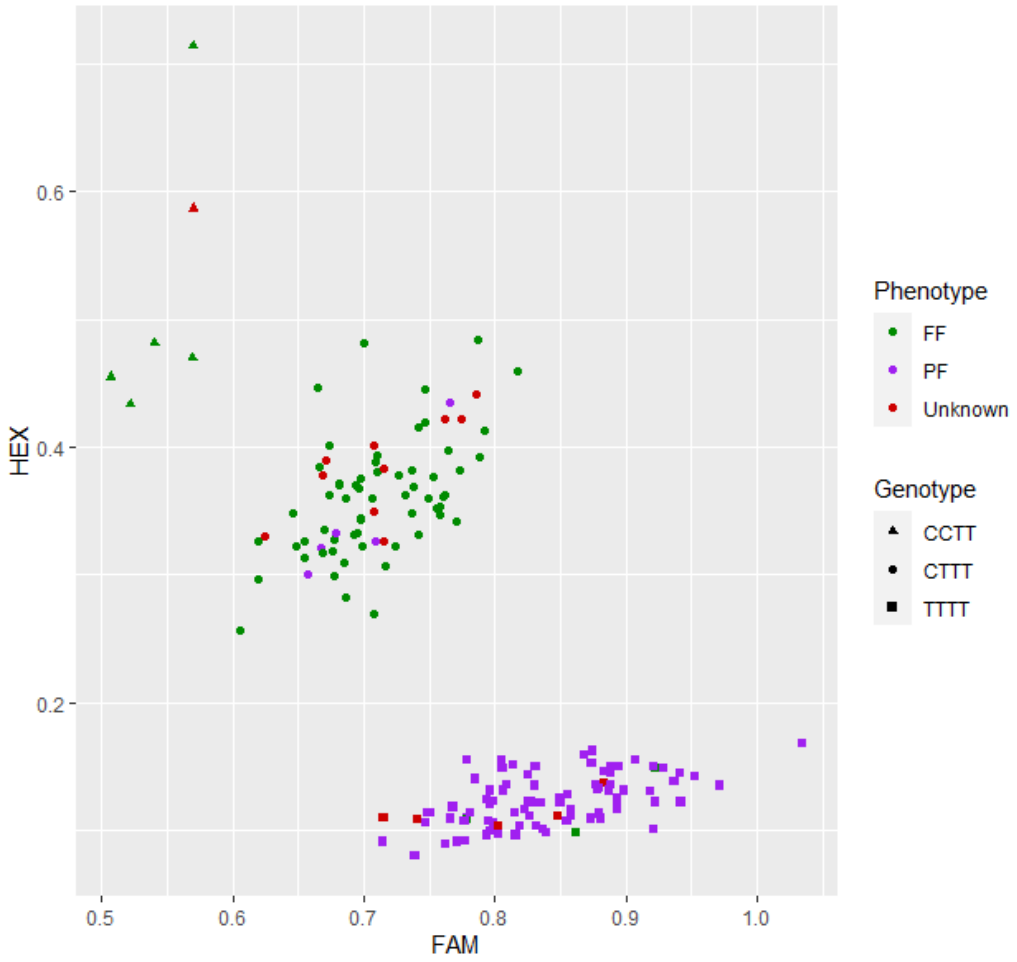

Fig. 1. The HEX and FAM fluorescence data for each sample genotyped with the PF2 marker was converted to tetraploid dosages, and the phenotypes were color coded. The marker incorrectly predicted the phenotype for only eight of the 154 progeny that were assigned a primocane-fruiting (PF) or floricane-fruiting (FF) score.

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