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Innovation at Work: Dogwood Breeding at Rutgers University

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Summary

At Rutgers University, we are continuing a tradition of innovation as we adopt advanced genetic tools and analyses in our dogwood breeding program. In this paper, we present preliminary results of two studies. The first is a genetic diversity study of 181 *Cornus florida*, *C. kousa*, and interspecific hybrids using the ddRADseq technique. We found that the pink-bracted *C. florida* formed a distinct clade separate from white-bracted trees and were more genetically similar than expected. For *C. kousa*, the accessions separated clearly into two different subspecies groups based on country of origin: ssp. *chinensis* from China and ssp. *kousa* from Korea and Japan. We verified eight previously described ssp. *chinensis* cultivars and found 13 additional cultivars that were previously unknown to be ssp. *chinensis*. We also found 17 cultivars that were genetically intermediate between the two subspecies, indicating they are subspecies hybrids. For both *C. kousa* and *C. florida*, there were also several cases of cultivars that are phenotypically and genetically indistinguishable, representing potential mix-ups in the nursery trade. Our data suggests these cultivars are clones that have been sold under different names in the industry. The largest group of such cultivars contains *C. kousa* 'Satomi', 'Rosabella', 'Schmred' Heart Throb®, 'Hanros' Radiant

Rose®, and 'Grist Mill Pink'. The second study is a Quantitative Trait Loci (QTL) mapping study of *C. florida* to identify regions of the genome associated with powdery mildew (PM) resistance and tolerance that could be used in breeding. Based on 196 full-sibling seedlings of Rutgers H4AR15P25 (PM resistant) x Rutgers H4AR15P28 (PM susceptible), we discovered a QTL on Chromosome 3. This QTL was found to be statistically significant, but explains only 7.8% of the variation in the seedling population.

INTRODUCTION

The Rutgers dogwood breeding program has been innovative since its inception in the 1960s under Dr. Elwin Orton. Dr. Orton pioneered interspecific crosses between the three main species of big-bracted dogwoods, *Cornus florida, C. kousa,* and *C. nuttallii.* Over the course of his career, Dr. Orton released 14 dogwood cultivars (Molnar and Capik, 2013).

Figure 1. *Cornus florida* var. *rubra* 'Rutnam' Red Beauty® dogwood.

Today, the program under the direction of Dr. Tom Molnar is focused on breeding powdery mildew (PM) resistant *C. florida* and unique dark pink-bracted *C. kousa* (Molnar, 2018). To support the program, we are using advanced genetic tools to better understand the genetic makeup, pedigrees, and relationships of our breeding selections and cultivars in the industry. We are also using these tools to understand the genetic basis of powdery mildew resistance and tolerance in our *C. florida* breeding program to help more effectively and efficiently breed cultivars with resistance to this disease.

CORNUS FLORIDA AND *CORNUS KOUSA* GENETIC DIVERSITY STUDY

We embarked on a genetic diversity study of 181 *C. florida, C. kousa,* and interspecific hybrid accessions. Our study focused mostly on cultivars but also included breeding selections and wild-collected plants. We were interested in answering questions such as: how genetically diverse are dogwood cultivars that are being sold today and in the past? How are these cultivars related to each other and to plants in the wild? Have different subspecies of *C. kousa* been used in breeding and are important ornamental characteristics like pink bract color and variegation specific to certain subspecies or genetic groups? How genetically diverse are the plants in the Rutgers University breeding program? Have our selection efforts narrowed genetic diversity?

Abbreviated methods

We used a technique called double digest restriction-site associated DNA sequencing (ddRADSeq) (Poland et al., 2012) to genotype the plants. The ddRADSeq technique yields thousands more markers than older techniques like SSR markers (simple sequence repeats) and DAF (DNA amplification fingerprinting) that have been used in

previous dogwood genetic diversity studies. For the analysis, we used 13,274 markers for *C. florida* and 7,978 markers for *C. kousa*. We are analyzing the data using GBS-SNP-CROP, STRUCTURE, and R programs (Melo et al., 2016; Pritchard et al., 2000; R Core Team, 2020).

Main takeaways so far

For *C. florida*, our data shows that var. *rubra* (pink-bracted) cultivars form a genetic grouping that is distinct from the white bracted plants. This means that they are more genetically similar to each other than expected. This knowledge will be useful as we breed for cultivars with dark-pink bracts and powdery mildew disease resistance while striving to maintain a high level of genetic diversity.

Table 1. *Cornus kousa* cultivar subspecies assignment based on a ddRadSeq genetic diversity study

***Cornus kousa* cultivars**		
ssp. *chinensis*	**ssp. Hybrids**	**ssp. *kousa***
‘Autumn Rose’	‘Gay Head'	‘Akatsuki'
‘Big Apple’[a]	‘Girard's dwarf'	‘Benifuji'
‘Blue Shadow’[a]	‘KN 144-2' Rosy Tea-cups®	‘Elizabeth Lustgarten'
‘Brotzman Dwarf’[a]	‘Madame Butterfly'	‘Eva'
‘China Girl’	‘Moonbeam'	‘Fascination'
‘Flowertime’[a]	‘National'	‘Gold Star'
‘Galzam’ Galilean®	‘Par Four'	‘Grist Mill Pink’
‘Greensleeves’	‘Primrose Cloak'	‘Hanros’ Radiant Rose®
‘Highland’[a]	‘Rochester'	‘Kristen Lipka's Variegated Weeper'
‘Little Poncho’[a]	‘Rutpink' Scarlet Fire®	‘Lemon Ripple'
‘MADI-11’ Mandarin Jewel®	‘Snowbird'	‘Little Beauty'
‘Milky Way’	‘Snowboy'	‘Rosabella'
‘Ohkan'	‘Southern Cross'	‘Satomi'
‘Pam’s Mountain Bouquet’[a]	‘Square Dance'	‘Silver Cup'
‘Samzan’ Samaritan®	‘Summer Fun'	‘Schmred' Heart Throb®
‘Snow Tower’[a]	‘Teddy Scout'	‘Summer Games'
‘Snowy Peak’[a]	‘White Ball'	‘Summer Majesty'
‘Temple Jewel’[a]		‘Summer Stars'
‘Trinity Star’[a]		‘Tsukuba no mine'
‘Triple Crown'		‘Weaver's Weeping’
‘Tri-Splendor’		
‘Wolfeyes’[a]		

[a]Denotes a cultivar that was previously unclassified as ssp. *chinensis*

For *C. kousa*, the accessions were found to clearly group by subspecies origin. Plants collected in China (ssp. *chinensis*) were distinct from plants collected in Japan or South Korea (ssp. *kousa*). Cultivars were identified as ssp. *chinensis*, ssp. *kousa*, or as hybrids of the two subspecies based on their similarity with the wild collected plants of known origins (Table 1). We verified eight previously described ssp. *chinensis* cultivars (Cappiello and Shadow, 2005) and found 13 additional cultivars that were previously unclassified as ssp. *chinensis*. Some authors have written that *C. kousa* ssp. *chinensis* is the superior ornamental form of the species, describing increased vigor, earlier flowering, larger bracts, and excellent fall color (Cappiello and Shadow, 2005; Rehder, 1927). However, it appears this judgement may have been based on a small pool of cultivars and should be revisited with a larger breadth of ssp. *chinensis* accessions.

Most of Rutgers' *C. kousa* breeding selections are ssp. hybrids and form three distinct groups (pink or white bracted) that are relatively closely related. This finding agrees with our breeding program's pedigree information dating back to the 1960s.

Our results also showed that for both C. *kousa* and *C. florida*, variegated plants have arisen spontaneously in different genetic backgrounds and in distantly related plants.

However, all four weeping *C. kousa* cultivars ('Elizabeth Lustgarten', 'Kristin Lipka's Variegated Weeper', 'Lustgarten Weeping', and 'Weaver's Weeping') are closely related to each other, suggesting that the weeping trait has arisen once in the cultivated material and comes from a single source. Additionally, the genetic similarity of 'Kristin Lipka's Variegated Weeper' to 'Lustgarten Weeping' is evidence that 'Lustgarten Weeping' was the source of the original 'Kristin Lipka's Variegated Weeper' sport.

For both species, there are several instances of cultivars that are phenotypically indistinguishable (look the same) that were also found to be genetically identical, suggesting that they are the same plant being propagated under different names (Figure 2).

Figure 2. Four cultivars in our study that are phenotypically and genetically indistinguishable and are likely clones. Photos were taken in the Rutgers dogwood trial in New Brunswick, New Jersey on June 9th, 2019.

Notably, the *C. kousa* 'Satomi' (aka 'Miss Satomi'), 'Rosabella', 'Schmred' Heart Throb®, 'Hanros' Radiant Rose®, and 'Grist Mill Pink' accessions that we included in the study had identical genetic profiles, confirming previous research that these plants represent the same clone (Cappiello and Shadow, 2005; Trigiano et al., 2004). Historical and genetic evidence points to 'Satomi' as the original cultivar of this group.

Currently we are adding more accessions to expand the study and confirm results. These insights will be useful to plant breeders, arboreta, and the industry, as most modern cultivars and popular historic cultivars are represented.

INVESTIGATING PM RESISTANCE IN *CORNUS FLORIDA*

Powdery mildew (PM) caused by *Erysiphe pulchra* is one of the most problematic diseases of *C. florida*. If left untreated in the nursery, PM can decrease growth in stem caliper by 80% and height by 50% in one growing season (Windham et al., 1999). Growers have relied on expensive biweekly fungicide applications since it became a widespread problem in the 1990s (Li et al., 2009). In the landscape PM is rarely treated, but in mature trees it can decrease flowering and overall aesthetic value.

Breeding for resistance has been recognized as the ideal strategy for controlling PM (Li et al., 2009). However, resistance is very rare in natural populations, estimated at 0.1% (Windham and Witte, 1998).

One selection in the Rutgers University dogwood breeding program, H4AR15P25, shows excellent resistance to PM (Molnar, 2018). We are using a technique called QTL mapping to find the regions of DNA where resistance genes may be located. The goal was to learn more about this source of resistance so that we can more effectively use it in our breeding program.

Figure 3. Dogwood powdery mildew

Abbreviated methods

A graphic outline of the methods is presented in Figure 4.

Briefly, to find resistance QTL we crossed the PM resistant tree, H4AR15P25, by a susceptible tree and obtained 196 seedlings for testing. We grew the seedlings in the greenhouse and rated the PM severity in summer 2019, about 1.5 years after sowing. We rated the plants using a 0-100% categorical severity scale (0, 1, 5, 10, 20, 30, …100). We genotyped the parents and 196 progeny using the ddRADSeq technique and analyzed the raw genetic data with Stacks and JoinMap (Catchen et al., 2011; Van Ooijen, 2006). For the final step of the analysis, we combined the genetic and phenotypic data using the MQM mapping method of MapQTL 6 (Van Ooijen, 2006) to find DNA regions associated with PM resistance.

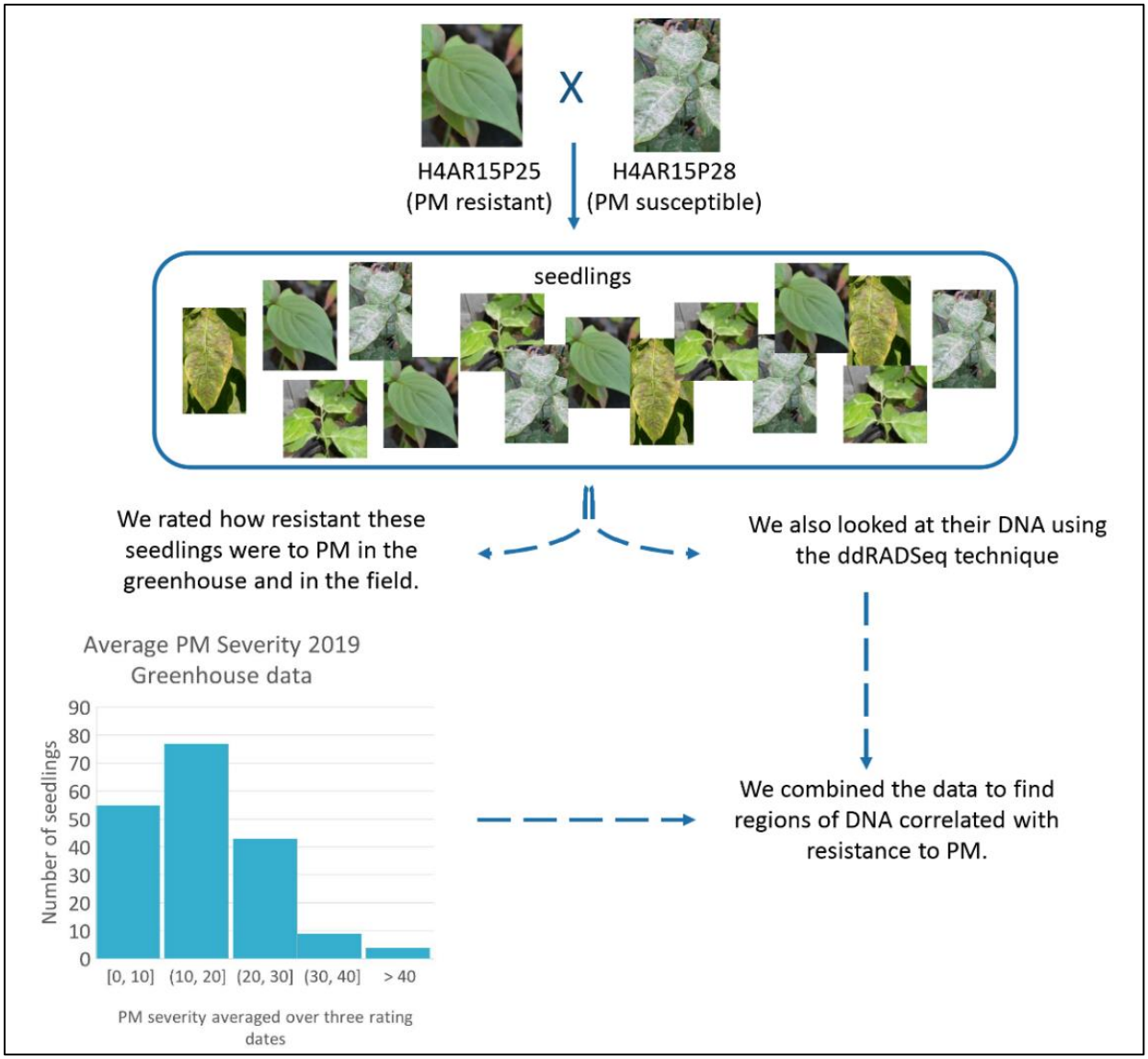

Figure 4. Graphic describing QTL mapping study workflow.

Main takeaways so far

We found one QTL (DNA region) on chromosome 3 associated with PM resistance. It explains 7.8% of the variation we see in the population. This is relatively small, and when taken with the continuous distribution of the PM severity data, suggests that resistance in this seedling population is controlled by many genes with small effects instead of one major gene. Thus, it will be more challenging to utilize this form of resistance in our breeding program; however, we can be more confident that this form of resistance won't break down as fast as when working with a single resistance gene.

The QTL in our study is on a different chromosome than previously discovered QTL (Parikh et al., 2017). These QTL could possibly be stacked to enhance PM disease resistance and breeding efforts are now in progress to combine different, unrelated sources of resistance and tolerance into new breeding populations.

Currently, we are adding to the QTL study by analyzing the results for a related mapping population with 84 individuals—the resistant H4AR15P25 crossed with a different susceptible parent.

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