Evaluation of 110 Apple Cultivars for Resistance to Alternaria Blotch Caused by *Alternaria alternata* Apple Pathotype

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Abstract. Apple blotch caused by *Alternaria alternata* apple pathotype is a severe disease of apple (*Malus × domestica* Borkh) occurring throughout the world, especially in eastern Asia. Phenotypic and genetic information about resistance/susceptibility of apple germplasm to this disease will be extremely valuable for selecting and developing new disease resistant cultivars. In this study, 110 apple cultivars obtained from the USDA apple germplasm in Geneva, NY, were evaluated for their resistance/susceptibility to apple blotch by field surveys, and inoculation of detached leaves with a suspension of germinated conidia of *A. alternata* apple pathotype. Disease incidence were different among the cultivars and categorized into resistant (R), moderately resistant (MR), or susceptible (S). Two molecular markers, S428, a random amplified polymorphic DNA (RAPD) marker associated with disease resistance, and a simple sequence repeat (SSR or microsatellite) marker CH05g07, linked to susceptibility were used to correlate the phenotypes expressed in field surveys and laboratory inoculations. The detection using either the S428 marker or the CH05g07 marker in 50 common breeding cultivars was consistent with R or S traits except for ‘Bisbee’ and ‘Priscilla’. These two cultivars were MR to apple blotch through phenotyping. However, SSR markers were detected, but RAPD markers were not and therefore were considered susceptible. Combined with the record of resistance to fire blight from Germplasm Resources Information Network (GRIN), ‘Dayton’, ‘Mildew Immune Seedling’, ‘Puregold’, and ‘Pumpkin Sweet’ were highly resistant to both diseases and considered as the best choices of parents for stacking resistance to multiple diseases in breeding program.

Zong-Ming (Max) Cheng designed the research. Ying Li conducted the experiments in field and lab. Ying Li and Xiao-Li Hu wrote the manuscript. Ying Li, Xiao-Li Hu, Robert N. Trigiano, Herbert Aldwinckle, and Zong-Ming (Max) Cheng revised and proofread the manuscript. All authors have read and approved the final version of the manuscript.

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Apple (*Malus × domestica* Borkh.) is one of the most important temperate fruit trees. Improving fruit quality and enhancing the ability to resist multiple diseases remain the primary goals in apple breeding programs worldwide (Aldwinckle et al., 1997). Fireblight, induced by the Gram-negative enterobacterium *Erwinia amylovora*, infects primarily species in the Rosaceae and poses a great economic threat to apple production around the world (Khan et al., 2006). Apple blotch caused by *Alternaria alternata* is another severe disease of apple. It was first reported in the United States (Filajdic and Sutton, 1995; Tsuge et al., 2016). This pathogen occurs worldwide, especially in

eastern Asia, including Japan (Saito and Takeda, 1984; Szewc-McFadden et al., 1996) and Korea (Lee and Lee, 1972). The fungus infects leaves and fruits and causes 60% to 80% defoliation, as well as extensive premature fruit drop (Filajdić and Sutton, 1991). In China, where almost one-half of the world’s production of apples occurs, great economic losses have occurred (Brown, 2012).

Accurate characterization of resistance/susceptibility to apple blotch of existing cultivars is essential for breeding programs, patent protection, and nursery disease control. Selecting cultivars with high disease resistance as parents is the most important strategy for breeding new disease-resistant cultivars. Several molecular markers associated with disease resistance screening have been developed, including RFLP (restriction fragment length polymorphism), RAPDs, AFLPs (amplified fragment length polymorphism), and SSRs (or microsatellites) (Peace, 2017). RAPD primers target many anonymous sequences (Shehata et al., 2009) and may produce confusing, often uninterpretable results, although occasionally one of the amplicons may be linked to a specific trait. In contrast, SSRs target specific sequences and are highly reproducible, codominant markers, in which a single pair of polymerase chain reaction (PCR) primers flanking the repeated sequences produces polymorphic patterns among alleles and often can be associated with phenotypic traits (Peace, 2017), such as disease resistance. Molecular markers associated with independent resistance genes against the same pathogen can be used to estimate the possible relationship among various, apparently unrelated resistance sources as well.

Zhao et al. (2008) analyzed F₁ population of ‘Qinguan’ × ‘Fuji’ and identified a RAPD marker, S428, which was linked to resistance to apple blotch. Li et al. (2011) reported one SSR marker, CH05g07, that was related to susceptibility to apple blotch, by analyzing an F₁ population derived from a cross between ‘Golden Delicious’ and ‘Huacui’. The molecular markers mentioned earlier can be used to identify cultivars as resistant or susceptible to apple blotch. However, only one kind of molecular marker was used for screening in these two previous studies (Li et al., 2011; Zhao et al., 2008). We hypothesized that these two markers are linked to susceptibility and resistance loci in an independent population for a given single genotype, and therefore both markers could not coexist within an individual. Using two independent markers to evaluate a germplasm collection for specific traits should help to eliminate false-positive or false-negative individuals in the disease prescreening process. In this study, we used several methods to evaluate the resistance to *Alternaria alternata* of apple cultivars collected from U.S. Department of Agriculture (USDA) apple germplasm, including field survey, inoculation trial in lab and test by molecular markers. Our aim was to identify potential apple cultivars which could resist

Table 1. Evaluation for resistance levels to *Alternaria alternata* apple pathotype by detached-leaf method, evaluation of the disease incidence at field and fire blight resistance levels among 110 apple cultivated apple accessions.

No.	USDA inventory	Cultivar	2011			2012			Resistance to fire blight
			Field survey	Mean score	Detached leaf inoculated evaluation	Field survey	Mean score	Detached leaf inoculated evaluation	
1	PI 588958	Akin	R	0	R	R	0	R	5
2	PI 589883	Arkansas Black	R	0.3	R	R	0.2	R	5
3	PI 588952	Arkansas	R	4.2	S	S	4.3	S	5
4	PI 589141	Autumn Arctic	S	2.3	S	R	0.6	R	5
5	PI 589884	Baldwin	R	1.5	MR	S	2	S	5
6	PI 589429	Barry	R	0.2	R	R	0.6	R	5
7	PI 589885	Beacon	R	1.7	MR	R	2.4	S	5
8	PI 588953	Barkley Rome	S	4.7	S	S	4.5	S	4
9	PI 589120	Benoni	R	0	R	R	2.7	S	5
10	PI 589886	Beverly Hills	R	0.4	R	R	0.6	R	4
11	PI 588790	Bisbee	S	3.4	S	R	0.8	R	1
12	PI 589178	Black Ben Davis	MR	1.5	MR	MR	1.5	MR	2
13	PI 588973	Black Gilliflower	R	0	R	R	0	R	4
14	PI 157052	Black Oxford	R	0.6	R	R	0.8	R	4
15	PI 589888	Blaxtayan	R	0.3	R	R	0.6	R	5
16	PI 589889	Britemac	R	0	R	R	0	R	1
17	PI 588961	Boller McIntosh	R	0.4	R	R	1.1	MR	4
18	PI 588946	Bonum	R	0	R	R	0	R	5
19	PI 588835	Burgundy	R	0.2	R	R	0.5	R	5
20	PI 589075	Cleopatra	MR	4.4	S	S	4.7	S	5
21	PI 589348	Charlotte	S	3.8	S	S	3.6	S	4
22	PI 589071	Chautauqua	R	0	R	R	0	R	5
23	PI 589890	Chehalis	R	2.8	S	S	3.2	S	2
24	PI 588803	Chestnut Crab	R	0.4	R	R	0.5	R	2
25	PI 392306	Crimson Gold	R	0	R	R	0	R	3
26	PI 589853	Clear Gold	R	0.7	R	R	0.8	R	1
27	PI 589191	Cathay	S	2.1	S	R	2.5	S	5
28	PI 589160	C.P. Close	R	0.3	R	R	0.3	R	2
29	PI 589330	Close	R	0	R	R	0	R	3
30	PI 589188	Coombs Wealthy	R	0.9	R	R	0.6	R	5
31	PI 588848	Cortland	R	0.6	R	R	0.3	R	3
32	PI 589215	Crandall	R	0.2	R	R	0.5	R	4
33	PI 589196	Crow Egg	R	0	R	R	0	R	5
34	PI 589223	Dakota	R	0.1	R	R	1.3	MR	5
35	PI 590183	Dayton	R	0	R	R	0	R	1
36	PI 588985	Deacon Jones	R	3.7	S	S	3.5	S	4
37	PI 590147	Delawine	R	4.6	S	S	4.3	S	3
38	PI 588797	Delcon	R	0.4	R	R	0.7	R	2
39	PI 589015	Early Harvest	R	0	R	R	0	R	5
40	PI 613816	Empire	S	2.2	S	R	2.7	S	2
41	PI 589023	Early McIntosh	R	0	R	R	0	R	5
42	PI 590148	Erickson	R	0.3	R	R	0.6	R	3
43	PI 588785	Fall Russet	S	2.7	S	R	2.5	S	1
44	PI 588954	Fireside	R	4.3	S	S	4.4	S	No Record
45	PI 589047	Farmer Spy	R	0.6	R	R	0.5	R	5
46	PI 589182	Frostproof	R	0	R	R	0	R	3
47	PI 589034	Gallia Beauty	R	0.9	R	R	0.5	R	5
48	PI 589176	Garrison	R	0.7	R	R	3.3	S	5
49	PI 589078	Gideon	R	0.4	R	R	0.2	S	3
50	PI 589040	Gloria Mundi	R	3.6	S	R	4.1	S	5
51	PI 588792	Hawaii	R	0.3	R	R	0.7	R	2
52	PI 589911	Hibernal	R	0.6	R	R	0.6	R	2
53	PI 588939	Honeygold	MR	2.4	S	S	2.5	S	5
54	PI 265302	Honeycrisp	R	0.1	R	R	0	R	No Record
55	PI 589446	Idajon	R	0.8	R	R	0.4	R	4
56	PI 588940	Jonared	S	4.6	S	S	4.9	S	5
57	PI 589002	Jonagram	R	0.5	R	R	0.3	S	4
58	PI 590185	Jonathan	R	3.8	S	S	3.7	S	5
59	PI 589296	Jones Favorite	R	3.8	S	S	3.2	S	1
60	PI 588942	June Wealthy	R	0.3	R	R	0.7	R	5
61	PI 589249	Katherine	R	0.4	R	R	0.7	R	3
62	PI 589894	Kendall	R	0	R	R	0	R	2
63	PI 588833	King	R	0.8	R	R	0.5	R	5
64	PI 589156	King David	R	0.4	R	R	0.6	R	5
65	PI 590150	Kingjon	R	0	R	R	0	R	5
66	PI 588943	Liberty	R	0.2	R	R	0.3	R	2
67	PI 588878	Late Strawberry	R	0.4	R	R	0.7	R	4
68	PI 589063	Lord Seedling	R	0.7	R	R	0.5	R	4
69	PI 589298	Lovell	R	0	R	R	0	R	3
70	PI 619161	Lyons	R	0.5	R	R	0.8	S	5

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Table 1. (Continued) Evaluation for resistance levels to *Alternaria alternata* apple pathotype by detached-leaf method, evaluation of the disease incidence at field and fire blight resistance levels among 110 apple cultivated apple accessions.

No.	USDA inventory	Cultivar	2011			2012			Resistance to fire blight
			Field survey	Mean score	Detached leaf inoculated evaluation	Field survey	Mean score	Detached leaf inoculated evaluation	
71	PI 589895	Macoun	S	3.5	S	S	3.8	S	4
72	PI 589153	Magnolia Gold	R	0.6	R	R	0.5	R	5
73	PI 589531	Medina	R	0.2	R	R	0.5	S	4
74	PI 589362	Melrose	R	2.5	S	S	3.3	S	4
75	PI 588772	Minnehaha	R	0.8	R	R	0.3	R	4
76	PI 589077	Mother	R	0.4	R	R	0.7	R	4
77	PI 589145	Mildew Immune Seedling	R	0	R	R	0	R	1
78	PI 588810	Nugget	R	3.2	S	S	4	S	1
79	PI 588872	Northern Spy	S	4.3	S	R	3.9	S	3
80	PI 589431	Ortley	R	0.6	R	R	0.4	R	1
81	PI 589173	Ohlson	R	0.2	R	R	0.5	R	5
82	PI 588841	Priam	R	0.4	R	R	0.2	R	5
83	PI 127703	Prima	R	0	R	R	0	R	4
84	PI 588982	Puregold	R	0	R	R	0	R	1
85	PI 588980	Paulared	R	0.6	R	R	0.8	R	4
86	PI 589212	Pacific Gold	S	4.2	S	R	4.6	S	1
87	PI 589229	Pumpkin Sweet	R	0	R	R	0	R	1
88	PI 589965	Priscilla	R	1.6	MR	R	1.8	MR	1
89	PI 589899	Peek Pleasant	R	0	R	R	0	R	4
90	PI 589136	Quindell	R	0.6	R	R	0.9	R	4
91	PI 589265	Red Canada	R	0.5	R	R	0.5	R	5
92	PI 594111	Redfree	R	0.8	R	R	0.3	R	4
93	PI 589067	Redgold	S	2.4	S	R	2.7	R	4
94	PI 589901	Redwell	R	0.7	R	R	0.5	R	4
95	PI 589083	Saratoga	R	0	R	R	0	R	5
96	PI 589322	Sharon	R	3.5	S	S	3.8	S	2
97	PI 589845	Smith Jonathan	S	2.6	S	R	3.2	S	2
98	PI 589903	Smokehouse	R	0.4	R	R	0.6	R	2
99	PI 589307	Stafford	R	0.2	R	R	0.7	R	5
100	PI 589192	Starking	MR	2.8	S	R	3.5	S	3
101	PI 588975	Secor	R	0.8	R	R11	0.4	R	5
102	PI 589264	Sublett	R	0	R	R	0	R	3
103	PI 588955	Sweet Delicious	S	4.2	S	S	4.5	S	4
104	PI 589266	Tioga	R	0.3	R	R	0.6	R	3
105	PI 588805	Tolman Sweet	R	2.4	S	S	2.1	S	5
106	PI 588988	Wagener	S	2.8	S	R	2.3	S	5
107	PI 588788	Wealthy	MR	1.7	MR	S	2.6	S	2
108	PI 613887	White Winter Pearmain	MR	3.9	S	S	3.7	S	No Record
109	PI 588799	Winesap	R	1.5	MR	MR	1.8	MR	3
110	PI 589499	Purple Wave	R	0.9	R	R	0.9	R	1

Ratings for apple blotch: R = resistant; MR = moderately resistant; S = susceptibility. The information on fire blight was searched on the Germplasm Resources Information Network database (<http://www.ars-grin.gov>). Ratings for fire blight: 1 = very resistant, no visible necrosis; 2 = moderately resistant, only light rating; 3 = intermediate, light to medium rating; 4 = moderately susceptible, moderate to heavy rating; 5 = very susceptible, very heavy rating. USDA = U.S. Department of Agriculture.

two diseases alternaria blotch caused by *Alternaria alternata* and fire blight at the same time. It could enable us to improve efficiency during apple breeding.

Materials and Methods

Field disease incidence evaluation. According to previous records about disease severity, a survey of disease incidence was conducted from July to September in 2011 and 2012. One hundred and ten cultivars and two seedlings for each cultivar were selected from apple germplasm preservation orchard at the USDA Agricultural Research Service of Cornell University in Geneva, NY (Table 1). One hundred leaves of each seedling were randomly selected in four cardinal directions (south, north, east, and west). The severity of blotch was divided into three levels. Disease incidence = (Number of infected leaves/leaves) × 100%. A rating of 0% to 10% was

considered resistance (R), 10% to 30% was considered moderate resistance (MR), and 30% to 100% was considered susceptibility (S).

Preparation of inoculum. A single-spore isolate of *A. alternata* apple pathotype from the USDA repository was used and grown on potato dextrose agar medium for 7 d at 25 °C under light. Conidia were collected in 5 mL sterile, distilled water added to each plate and the concentration of spores adjusted to 1×10^5 conidia/mL.

Leaf collection and pathogen inoculation. Ten fully expanded, asymptomatic leaves (15–25 d old) from each seedling were harvested in 2011 and 2012, washed briefly (30 s) with sterile distilled water, and blotted dry with paper towels. A prepared conidial suspension was sprayed evenly onto the entire lower surface of leaves. Inoculated leaves were put on the trays containing wet filter paper, covered with plastic wrap to maintain high humidity, and then incubated

in growth chamber (26 °C, 12 h of light, 12 h of dark per 24 h). After 48 h, leaves were rated for disease resistance (Abe et al., 2010). The tests were repeated twice in each year. The back sides of leaves were sprayed with distilled water and served as negative controls.

Disease assessment. Disease assessment of detached leaves was performed 48 h post-inoculation. Symptoms were recorded, disease severity for each leaf scored, and the scores averaged for each cultivar. Infection levels of inoculated leaves were categorized into six groups developed by Abe et al. (2010), where: 0 = no visible symptoms; 1 = <1 mm in diameter small spots with pits, scattered on leaf surface; 2 = one or two small necrotic spots, <10% of leaf area with pit-type spots; 3 = 10% to 50% of the leaf area necrotic; 4 = >50% of necrosis of leaf area necrotic; and 5 = almost complete necrosis of leaf. Leaves with mean scores between 2 and 5 were considered S, whereas leaves scored

Fig. 1. Polymerase chain reaction results of marker CH05g07 screened among 50 apple cultivars using capillary electrophoresis. A01, B01, C01, D01, E01: DNA marker. A02-A12: No. 1, 2, 3, 4, 5, 6, 7, 8, 11, 18, 25; B02-B12: No. 14, 12, 16, 87, 30, 31, 44, 50, 27, 21, 29; C02-C12: No. 9, 40, 79, 56, 80, 71, 74, 63, 84, 51, 43; D02-D12: No. 86, 77, 93, 82, 97, 76, 24, 33, 103, 49, 106; E02-E07: No. 68, 88, 35, 28, 39, 37; E08-E12: the same volume of ddH₂O. Numbers correspond to cultivars listed in Table 1.

with means between 1 and 2 where considered MR. Leaves with mean scores between 0 and 1 were regarded as R.

Validation by SSR and RAPD markers. Fifty cultivars of apple were selected to validate resistance by markers according to

the following two criteria: 1) seedlings were healthy without other diseases and 2) seedlings where common varieties of a breeding program. DNA was isolated from young leaves without symptoms using the DNeasy Plant Mini Kit (Qiagen) and stored at -80°C

until further analyses. DNA concentrations were measured with a spectrofluorometer (Shimadzu, Kyoto, Japan) at the absorbance wavelengths of 260 and 280 nm.

The SSR molecular marker CH05g07 was linked to an apple blotch-susceptible gene of

Fig. 2. Polymerase chain reaction results of marker S428 screened among 50 apple cultivars. M: Marker; A01-A16: No. 4, 7, 8, 9, 77, 1, 2, 3, 30, 31, 93, 50, 76, 24, 84, 27; B01-B20: No. 40, 86, 79, 87, 71, 14, 56, 44, 11, 97, 16, 25, 74, 63, 80, 21, 28, 88, 39, 51; C01-C14: No. 6, 5, 37, 18, 35, 12, 103, 106, 49, 68, 82, 29, 43, 33. Numbers correspond to cultivars listed in Table 1.

apple (Li et al., 2011). Each 20- μ L PCR system followed Li et al. (2011) and used the following primer pairs: forward-CCCAAGCAATATAGTGAATCTCAA; reverse-TTCATCTCCTGCTGCAAATAAC. Amplicons were separated by capillary electrophoresis (eGene; Irvine, CA; www.egeneinc.com). RAPD molecular marker S428 linked to an apple blotch-resistant gene was amplified according to Zhao et al. (2008) and used the single primer ACC-TCAGCTC. Amplicons were separated by electrophoresis at 100 V on 1.5% agarose gel for 3 h and visualized with ethidium bromide.

Results

Differences in resistance levels among apple cultivars in the United States through field survey and inoculation test. Field survey and inoculation of detached leaves both showed marked variations in resistance to *A. alternata* apple pathotype among different varieties (Table 1). Fifty-four apple cultivars were categorized as R according to ratings from both field survey and inoculation of detached leaves. Twenty-two varieties, including ‘Dayton’, ‘Mildew Immune Seedling’, ‘Puregold’ and ‘Pumpkin Sweet’ showed no visible symptoms of disease on all inoculated leaves. In contrast, there were fourteen cultivars in the S category, including ‘Arkansas’, ‘Autumn Arctic’, ‘Cleopatra’, ‘Deacon Jones’, ‘Delawine’, ‘Fireside’, ‘Gloria Mundi’, ‘Honeygold’, ‘Jonared’, ‘Jonathan’,

‘Melrose’, ‘Nugget’, ‘Sharon’, and ‘White Winter Pearmain’. Some contradictory results with the inoculation test were found in varieties such as ‘Arkansas’, ‘Autumn Arctic’, ‘Baldwin’, ‘Beacon’, and ‘Benoni’, which were classified R in 2011 but S in 2012. To characterize disease resistance among cultivars more convincingly, we screened selected varieties using the RAPD and SSR molecular markers.

Identification of resistance to A. alternata apple pathotype tested through two molecular markers. Fifty cultivars were screened using the SSR (Fig. 1) and RAPD (Fig. 2) markers to amplify the specific amplicons that indicate susceptibility or resistance to apple blotch, respectively. Seventeen varieties showed presence of CH05g07-163 bp fragment that was SSR marker linked to apple blotch susceptibility. The results corresponded to the field survey and inoculation trial. However, two cultivars (Bisbee and Priscilla) that were evaluated as MR to apple blotch pathotype by field survey and detached leaf inoculation, also had the S fragment. The remaining cultivars had the S428-854 bp fragment, which was RAPD marker associated with resistance to apple blotch. This fragment was not amplified among 17 susceptible varieties or in ‘Bisbee’ and ‘Priscilla’. Therefore, we treated these two cultivars as S due to molecular marker analysis. Additionally, 31 cultivars were identified as apple blotch resistant (Table 2).

Discussion

Discovering and developing disease resistant varieties has been the primary goal for the majority of apple breeding programs around the world (Kellerhals and Furrer, 1994). Although using fungicide controls the incidence and spread of disease, it poses pollution threats to the environment (Zhu et al., 2017). The ultimate goal is to select disease-resistant cultivars (Soleimani and Esmailzadeh, 2007). Evaluation of disease resistance was achieved solely through field survey when molecular markers were not available (Forte et al., 2002). However, due to the influence of climate and human factors, the results were biased. Bus et al. (2010) stated that marker assisted selection (MAS) based on tracking specific amplicons or alleles of genomic or plastid DNA (Poczai et al., 2013) is a reliable method to accelerate apple breeding. MAS was applied to screen disease-resistant cultivars decades ago. For example, Yang et al. (1997) reported that *Vf* is a dominant gene linked to resistance to Apple scab.

As one of the severest diseases of apple around the world, apple blotch can induce about 70% of the young leaves to drop prematurely causing great economic loss (Lee et al., 2006). The need to select apple blotch-resistant cultivars is urgent. In the present study, we combined data from field surveys with inoculated detached leaf trials, which made it possible to assess the reliability

Table 2. Presence of two molecular markers among fifty common apple cultivars and reported resistance to fire blight.

No.	USDA inventory	Cultivar	SSR	RAPD	Resistance to fire blight
1	PI 588958	Akin	Y		5
2	PI 589883	Arkansas Black	Y		5
3	PI 588952	Arkansas	Y		5
4	PI 589141	Autumn arctic		Y	5
5	PI 589884	Baldwin		Y	5
6	PI 589429	Barry		Y	5
7	PI 589885	Beacon		Y	5
8	PI 588953	Barkley Rome		Y	4
9	PI 589120	Benoni		Y	5
11	PI 588790	Bisbee	Y		1
12	PI 589178	Black Ben Davis		Y	2
14	PI 157052	Black Oxford		Y	4
16	PI 589889	Britemac		Y	1
18	PI 588946	Bonum		Y	5
21	PI 589348	Charlotte	Y		4
24	PI 588803	Chestnut Crab	Y		2
25	PI 392306	Crimson Gold	Y		3
27	PI 589191	Cathay	Y		5
28	PI 589160	C.P. Close		Y	2
29	PI 589330	Close		Y	3
30	PI 589188	Coombs Wealthy		Y	5
31	PI 588848	Cortland		Y	3
33	PI 589196	Crow Egg	Y		5
35	PI 590183	Dayton		Y	1
37	PI 590147	Delawine		Y	3
39	PI 589015	Early Harvest		Y	5
40	PI 613816	Empire		Y	2
43	PI 588785	Fall Russet	Y		1
44	PI 588954	Fireside	Y		No Record
49	PI 589078	Gideon		Y	3
50	PI 589040	Gloria Mundi		Y	5
51	PI 588792	Hawaii		Y	2
56	PI 588940	Jonared		Y	5
63	PI 588833	King	Y		5
68	PI 589063	Lord Seedling		Y	4
71	PI 589895	Macoun	Y		4
74	PI 589362	Melrose		Y	4
76	PI 589077	Mother		Y	4
77	PI 589145	Mildew Immune Seedling		Y	1
79	PI 588872	Northern Spy	Y		3
80	PI 589431	Ortley	Y		1
82	PI 588841	Priam		Y	5
84	PI 588982	Puregold		Y	1
86	PI 589212	Pacific Gold	Y		1
87	PI 589229	Pumpkin Sweet		Y	1
88	PI 589965	Priscilla	Y		1
93	PI 589067	Redgold		Y	4
97	PI 589845	Smith Jonathan	Y		2
103	PI 588955	Sweet Delicious		Y	4
106	PI 588988	Wagener	Y		5

Ratings for fire blight: 1 = very resistant, no visible necrosis; 2 = moderately resistant, only light rating; 3 = intermediate, light to medium rating; 4 = moderately susceptible, moderate to heavy rating; 5 = very susceptible, very heavy rating. Y means the molecular marker was detected. USDA = U.S. Department of Agriculture.

of bioassay tests for predicting the level of resistance to apple blotch of various apple cultivars. Most cultivars were consistent for evaluation of resistance or susceptibility in this 2-year study. However, ‘Arkansas’, for example, was R in field survey but S in inoculation trial in 2011. This observation might be explained by in vitro conditions, which are more suitable for disease development (Wöhner and Emeriewen, 2018). For example, ‘Autumn Arctic’ was classified as S in 2011 but R in 2012. This might result from the improvement of tree vigor, which made the tree more resistant to pathogens. Assessment of traits made from only phenotypic observations was unstable and unconvincing. Many factors, such as rainfall, temperature, and humidity,

could affect the assessments. To reduce this limitation and produce more reliable results, we used two molecular markers: an SSR was linked to susceptibility, and an RAPD that was related to resistance to assess cultivars. These two molecular marker validated resistance to apple blotch in 50 cultivars. Two markers were also tested among cultivars reported to be resistant to fire blight. The results were consistent with attributes of resistance or susceptibility in the field survey and inoculation trials for all cultivars except for two, Bisbee and Priscilla, which were regarded as MR by detached leaf inoculation but appeared to be S via molecular marker tests. This could be possibly explained by the presence of epigenetic processes (Francini and

Sebastiani, 2013). Our hypothesis was correct in that these two markers are linked to susceptibility and resistance loci in an independent population, and both markers did not coexist within a single individual. The independent markers make it possible to identify resistance or susceptibility of apple blotch from two aspects at the same time, which would be helpful to make unbiased decisions.

An increasing number of molecular markers identify specific traits in apple. For instance, three RAPD markers linked to *Vbh* were first identified from the crab apple *M. baccata jackii* (Gygax et al., 2004). Two RAPD markers and one SSR marker were identified that were linked to fire-blight resistance (Khan et al., 2007). Under pressure from high labor costs and an increase in imported products from competing markets (Lee et al., 2018), breeders seek to create cultivars that are able to resist several diseases simultaneously (Fischer and Hain, 1994; Fischer and Richter, 1998). Generally, cultivars with several good traits in addition to disease resistance would be used as parental lines. In this study, efforts were made to identify cultivars that would be resistant to both fire blight and apple blotch by searching the record of fire blight-resistant cultivars from Germplasm Resources Information Network and screening apple blotch-resistant cultivars via field survey, laboratory inoculation, and two molecular markers. Some cultivars not only were resistant to fire blight but also exhibited resistance to *A. alternata* apple pathotype; these comprised four common varieties: ‘Dayton’, ‘Mildew Immune Seedling’, ‘Puregold’, and ‘Pumpkin Sweet’. These cultivars could be used as parents to pyramid disease-resistant traits in apple breeding program. In the future, more markers associated with various disease resistance traits will be used to shorten the time in breeding programs and offer more choices to cultivate multi-resistant varieties.

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