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 ‘Pricilla’. These two cultivars were MR to apple blotch through phenotyping. However, SSR markers were detected, but RAPD markers were not and therefore 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.

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 (Filajdić 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 ‘Huauci’. 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
| No. | USDA inventory | Cultivar          | 2011 | 2012 | Detached leaf inoculated evaluation | Resistance to fire blight |
|-----|----------------|-------------------|------|------|-----------------------------------|--------------------------|
| 1   | PI 589855      | Akin R            | 0    | 0    | R                                 | 5                        |
| 2   | PI 589883      | Arkansas Black    | 0    | 0.3  | R                                 | 5                        |
| 3   | PI 589852      | Arkansas          | 4.2  | S    | 4.3                               | 5                        |
| 4   | PI 589141      | Autumn Arctic     | 2.3  | S    | 0.6                               | 5                        |
| 5   | PI 589884      | Baldwin R         | 1.5  | MR   | S                                 | 5                        |
| 6   | PI 589429      | Barry R           | 0.2  |      | 0.6                               | R                        |
| 7   | PI 589885      | Beacon R          | 1.7  | MR   | 2.4                               | R                        |
| 8   | PI 589953      | Barkley Rome      | 4.7  | S    | 4.5                               | S                        |
| 9   | PI 589120      | Benoni R          | 0    |      | 0.2                               | R                        |
| 10  | PI 589886      | Beverly Hills R   | 0.4  | R    | 0.6                               | 4                        |
| 11  | PI 588790      | Bisbee S          | 3.4  | R    | 0.8                               | 1                        |
| 12  | PI 589178      | Black Ben Davis R | 1.5  | MR   | 1.5                               | MR                       |
| 13  | PI 589973      | Black Gilliflower | 0    | R    | 0                                 | 5                        |
| 14  | PI 589976      | Black Oxford R    | 0.6  | R    | 0.8                               | R                        |
| 15  | PI 589888      | Blaxtayman R      | 0.3  | R    | 0.6                               | 5                        |
| 16  | PI 589889      | Britemac R        | 0    | R    | 0                                 | 1                        |
| 17  | PI 589961      | Boller McIntosh R | 0.4  | R    | 1.1                               | MR                       |
| 18  | PI 589946      | Bonum R           | 0    | R    | 0                                 | 5                        |
| 19  | PI 588835      | Burgundy R        | 0.2  | R    | 0.5                               | 5                        |
| 20  | PI 589075      | Cleopatra MR      | 4.4  | S    | 4.7                               | S                        |
| 21  | PI 589348      | Charlotte S       | 3.8  | S    | 3.6                               | S                        |
| 22  | PI 589071      | Chautauqua R      | 0    | R    | 0                                 | R                        |
| 23  | PI 589890      | Chehalis S        | 2.8  | S    | 3.2                               | S                        |
| 24  | PI 588803      | Chestnut Crab R   | 0.4  | R    | 0.5                               | R                        |
| 25  | PI 392306      | Crimson Gold R    | 0    | R    | 0                                 | 3                        |
| 26  | PI 589853      | Clear Gold R      | 0.7  | R    | 0.8                               | R                        |
| 27  | PI 589191      | Cathay S          | 2.1  | S    | 2.5                               | S                        |
| 28  | PI 589160      | C.P. Close R      | 0.3  | R    | 0.3                               | R                        |
| 29  | PI 589330      | Close R           | 0    | R    | 0                                 | 3                        |
| 30  | PI 589188      | Coombs Wealthy R | 0.9  | R    | 0.6                               | R                        |
| 31  | PI 588848      | Cortland R        | 0.6  | R    | 0.3                               | R                        |
| 32  | PI 589215      | Crandall R        | 0.2  | R    | 0.5                               | R                        |
| 33  | PI 589196      | Crow Egg R        | 0    | R    | 0                                 | 5                        |
| 34  | PI 589223      | Dakota R          | 0.1  | R    | 1.3                               | MR                       |
| 35  | PI 590183      | Dayton R          | 0    | R    | 0                                 | R                        |
| 36  | PI 589885      | Deacon Jones R    | 3.7  | S    | 3.5                               | S                        |
| 37  | PI 590147      | Delavine R        | 4.6  | S    | 4.3                               | S                        |
| 38  | PI 588797      | Delcon R          | 0.4  | R    | 0.7                               | R                        |
| 39  | PI 589015      | Early Harvest R   | 0    | R    | 0                                 | 5                        |
| 40  | PI 613816      | Empire S          | 2.2  | S    | 2.7                               | S                        |
| 41  | PI 589023      | Early McIntosh R  | 0    | R    | 0                                 | 5                        |
| 42  | PI 590148      | Erickson R        | 0.3  | R    | 0.6                               | 3                        |
| 43  | PI 588785      | Fall Rustet S     | 2.7  | S    | 2.5                               | S                        |
| 44  | PI 588954      | Fireside S        | 4.3  | S    | 4.4                               | S                        |
| 45  | PI 589047      | Farmer Spy R      | 0.6  | R    | 0.5                               | 5                        |
| 46  | PI 589182      | Frostproof R      | 0    | R    | 0                                 | R                        |
| 47  | PI 589034      | Gallia Beauty R   | 0.9  | R    | 0.5                               | 5                        |
| 48  | PI 589176      | Garrison R        | 0.7  | R    | 3.3                               | S                        |
| 49  | PI 589078      | Gideon R          | 0.4  | R    | 0.2                               | S                        |
| 50  | PI 589040      | Gloria Mundi R    | 3.6  | S    | 4.1                               | S                        |
| 51  | PI 588792      | Hawaii R          | 0.3  | R    | 0.7                               | R                        |
| 52  | PI 589911      | Hibernal R        | 0.6  | R    | 0.6                               | R                        |
| 53  | PI 589839      | Honeygold MR      | 2.4  | S    | 2.5                               | S                        |
| 54  | PI 588946      | Honeycrisp R      | 0.1  | R    | 0                                 | R                        |
| 55  | PI 589446      | Idajon R          | 0.8  | R    | 0.4                               | R                        |
| 56  | PI 588940      | Jonared S         | 4.6  | S    | 4.9                               | 5                        |
| 57  | PI 589002      | Jonagram R        | 0.5  | R    | 0.3                               | S                        |
| 58  | PI 590185      | Jonathan R        | 3.8  | S    | 3.7                               | S                        |
| 59  | PI 589296      | Jones Favorite R  | 3.8  | S    | 3.2                               | S                        |
| 60  | PI 589842      | June Wealthy R    | 0.3  | R    | 0.7                               | R                        |
| 61  | PI 589249      | Katherine R       | 0.4  | R    | 0.7                               | R                        |
| 62  | PI 589894      | Kendall R         | 0    | R    | 0                                 | 5                        |
| 63  | PI 588833      | King R            | 0.8  | R    | 0.5                               | R                        |
| 64  | PI 589156      | King David R      | 0.4  | R    | 0.6                               | R                        |
| 65  | PI 590150      | Kingjon R         | 0    | R    | 0                                 | R                        |
| 66  | PI 589843      | Liberty R         | 0.2  | R    | 0.3                               | R                        |
| 67  | PI 588787      | Late Strawberry R | 0.4  | R    | 0.7                               | R                        |
| 68  | PI 589063      | Lord Seedling R   | 0.7  | R    | 0.5                               | R                        |
| 69  | PI 589298      | Lovell R          | 0    | R    | 0                                 | 3                        |
| 70  | PI 619161      | Lyons R           | 0.5  | R    | 0.8                               | S                        |

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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 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 severity (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; 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

| No. | USDA inventory | Cultivar               | Field survey | Mean score | Detached leaf inoculated evaluation | Field survey | Mean score | Detached leaf inoculated evaluation | Resistance to fire blight |
|-----|----------------|------------------------|--------------|------------|------------------------------------|--------------|------------|------------------------------------|--------------------------|
| 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        | R                                  | 4                        |
| 74  | PI 589362      | Melrose                 | R            | 2.5        | S                                  | S            | 3.3        | S                                  | 5                        |
| 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          | 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                                  | S            | 3          | S                                  | 3                        |
| 80  | PI 589431      | Orley                   | 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      | Pimai                   | 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                                  | S            | 4.6        | S                                  | 1                        |
| 87  | PI 589229      | Pumpkin Swee            | 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                                  | R1           | 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        | R                                  | S            | 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.
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 spectrophotometer (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
apple (Li et al., 2011). Each 20-μL PCR system followed Li et al. (2011) and used the following primer pairs: forward-CCCAAGCAATATA-GTGAATCTCAA; reverse-TTCATCTCCT-GCTGCAAATAAC. 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 589593      | Barkley Rome        | Y   |      | 4                         |
| 9   | PI 589120      | Benoni              | Y   |      | 5                         |
| 10  | PI 588790      | Bisbee              | Y   |      | 1                         |
| 11  | PI 589178      | Black Ben Davis     | Y   |      | 2                         |
| 12  | PI 157052      | Black Oxford        | Y   |      | 4                         |
| 13  | PI 589889      | Britmac             | Y   |      | 1                         |
| 14  | PI 588946      | Bonum               | Y   |      | 5                         |
| 15  | PI 589348      | Charlotte           | Y   |      | 4                         |
| 16  | PI 58803       | Chestnut Crab       | Y   |      | 2                         |
| 17  | PI 392306      | Crimson Gold        | Y   |      | 3                         |
| 18  | PI 589191      | Cathay              | Y   |      | 5                         |
| 19  | PI 589160      | C.P. Close          | Y   |      | 2                         |
| 20  | PI 589330      | Close               | Y   |      | 3                         |
| 21  | PI 589188      | Coombs Wealthy      | Y   |      | 5                         |
| 22  | PI 588848      | Cortland            | Y   |      | 3                         |
| 23  | PI 589196      | Crow Egg            | Y   |      | 5                         |
| 24  | PI 589051      | Dayton              |      | Y    | 1                         |
| 25  | PI 589047      | Delawine            | Y   |      | 3                         |
| 26  | PI 589015      | Early Harvest        | Y   |      | 5                         |
| 27  | PI 613816      | Empire              | Y   |      | 2                         |
| 28  | PI 588785      | Fall Russet         | Y   |      | 1                         |
| 29  | PI 589594      | Fireside            | Y   |      | No Record                 |
| 30  | PI 589078      | Gideon              | Y   |      | 3                         |
| 31  | PI 589040      | Gloria Mundi        | Y   |      | 5                         |
| 32  | PI 588792      | Hawaii              | Y   |      | 2                         |
| 33  | PI 588940      | Jonared             | Y   |      | 5                         |
| 34  | PI 58833       | King                | Y   |      | 5                         |
| 35  | PI 589063      | Lord Seedling       | Y   |      | 4                         |
| 36  | PI 588955      | Macoun              |      | Y    | 4                         |
| 37  | PI 589362      | Melrose             | Y   |      | 4                         |
| 38  | PI 589077      | Mother              | Y   |      | 4                         |
| 39  | PI 589145      | Mildew Immune Seedling | Y   |      | 1                         |
| 40  | PI 588782      | Northern Spy        | Y   |      | 3                         |
| 41  | PI 589431      | Orley               | Y   |      | 1                         |
| 42  | PI 588841      | Priam               | Y   |      | 5                         |
| 43  | PI 588982      | Puregold            | Y   |      | 1                         |
| 44  | PI 589212      | Pacific Gold        | Y   |      | 1                         |
| 45  | PI 589229      | Pumpkin Sweet       | Y   |      | 1                         |
| 46  | PI 589965      | Priscilla           | Y   |      | 1                         |
| 47  | PI 589067      | Redgold             | Y   |      | 4                         |
| 48  | PI 589845      | Smith Jonathan      | Y   |      | 2                         |
| 49  | PI 589855      | Sweet Delicious      | Y   |      | 4                         |
| 50  | PI 589888      | 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 Vbj were first identified from the crab apple M. baccata jackii (Gaygax 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 makers. 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.

Literature Cited

Abe, K., H. Iwanami, N. Kotoda, and S. Moriya. 2010. Evaluation of apple genotypes and Malus species for resistance to Alternaria blotch caused by Alternaria alternata apple pathotype using detached-leaf method. Plant Breed. 129(2):208–218.

Aldwinckle, H., P. Forsline, H. Gustafson, and S. Hokanson. 1997. Evaluation of apple scab resistance of Malus sieversii populations from Central Asia. HortScience 32:440.

Brown, S. 2012. Apple. In: M. Badenes and D. Byrne (eds.). Fruit Breeding. Handbook of Plant Breeding, vol. 8. Springer, Boston, MA.

Bus, V.G., H.C. Bassett, D. Bowatte, D. Chagné, C.A. Ranatunga, D. Ulluwisewa, and S.E. Gardiner. 2010. Genome mapping of an apple scab, a powdery mildew and a woolly apple aphid resistance gene from open-pollinated Mildew Immune Selection. Tree Genet. Genomes 6(3):477–487.

Filajdi, N. and T. Sutton. 1995. Overwintering of Alternaria mali, the causal agent of Alternaria blotch of apple. Plant Dis 79:695–698.

Filajdi, N. and T. Sutton. 1991. Identification and distribution of Alternaria mali on apples in North Carolina and susceptibility of different...
varieties of apples to Alternaria blotch. Plant Dis. 75(10):1045–1048.

Fischer, C. and K. Richter. 1998. Results on fire blight resistance in the Pillnitz apple breeding programme. Paper presented at the VIII International Workshop on Fire Blight 489.

Fischer, R. and R. Hain. 1994. Plant disease resistance resulting from the expression of foreign phytoalexins. Curr. Opin. Biotechnol. 5(2):125–130.

Forte, A., A. Ignatov, V. Ponomarenko, D. Dorokhov, and N. Savelyev. 2002. Phylogeny of the Malus (apple tree) species, inferred from the morphological traits and molecular DNA analysis. Russ. J. Genet. 38(10):1150–1161.

Francini, A. and L. Sebastiani. 2013. Phenolic compounds in apple (Malus x domestica Borkh.): Compounds characterization and stability during postharvest and after processing. Antioxidants 2(3):181–193.

Gygax, M., L. Gianfranceschi, R. Liebhard, M. Kellerhals, C. Gessler, and A. Patocchi. 2004. Molecular markers linked to the apple scab resistance gene Vbj derived from Malus baccata jackii. Theor. Appl. Genet. 109(8):1702–1709.

Kellerhals, M. and B. Furrer. 1994. Approaches for breeding apples with durable disease resistance. Euphytica 77(1-2):31–35.

Khan, M.A., B. Duffy, C. Gessler, and A. Patocchi. 2006. QTL mapping of the fire blight resistance in apple. Mol. Breed. 17(4):299–306.

Khan, M.A., C.E. Durel, B. Duffy, D. Drouet, M. Kellerhals, C. Gessler, and A. Patocchi. 2007. Development of molecular markers linked to the ‘Fiesta’ linkage group 7 major QTL for fire blight resistance and their application for marker-assisted selection. Genome 50(6):568–577.

Lee, D. and G. Lee. 1972. Studies on causal agents, overwintering of organisms and control of Alternaria leaf spot of apple. J. Korean Soc. Hort. Sci 11:41–47.

Lee, D.H., S.W. Lee, K.H. Choi, D.A. Kim, and J.Y. Uhm. 2006. Survey on the occurrence of apple diseases in Korea from 1992 to 2000. Plant Pathol. J. 22(4):375.

Lee, T.G., R. Sjekasteband, N. Memda, L.A. Mueller, and S.F. Hutton. 2018. Molecular markers to select for the j-2-mediated jointless pedicel in tomato. HortScience 53:153–158.

Li, Y., L. Zhang, Z. Zhang, P. Cong, and Z.M. Cheng. 2011. A simple sequence repeat marker linked to the susceptibility of apple to Alternaria blotch caused by Alternaria alternata apple pathotype. J. Amer. Soc. Hort. Sci. 136:109–115.

Peace, C.P. 2017. DNA-informed breeding of rosaceous crops: Promises, progress and prospects. Hort. Res. 4:17006.

Poczai, P., I. Varga, M. Laos, A. Cseh, N. Bell, J.P. Valkonen, and J. Hyvönen. 2013. Advances in gene targeted and functional markers: A review. Plant Methods 9(1):6.

Saito, K. and K. Takeda. 1984. Genetic analysis of resistance to Alternaria blotch (Alternaria mali Roberts) in apple (studies on the breeding of the apple). Japan. J. Breed. 34:197–209.

Shehata, A.I., H.A. Al-Ghetar, and A.A. Al-Homaidan. 2009. Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. Saudi J. Biol. Sci. 16(2):57–62.

Soleimani, M.J. and M. Esmailzadeh. 2007. First report of Alternaria mali causing apple leaf blotch disease in Iran. Australas. Plant Dis. Notes 2(1):57–58.

Szewe-McFadden, A., S. Kresovich, S. Bliek, S. Mitchell, and J. McFerson. 1996. Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated Brassica species. Theor. Appl. Genet. 93(4):534–538.

Tsuge, T., Y. Harimoto, K. Hanada, Y. Akagi, M. Kodama, K. Akimitsu, and M. Yamamoto. 2016. Evolution of pathogenicity controlled by small, dispensable chromosomes in Alternaria alternata pathogens. Physiol. Mol. Plant Pathol. 95:27–31.

Yang, H.Y., S.S. Korban, J. Krüger, and H. Schmidt. 1997. A randomly amplified polymorphic DNA (RAPD) marker tightly linked to the scab-resistance gene Vf in apple. J. Amer. Soc. Hort. Sci. 122:47–52.

Wöhner, T. and O.F. Emeriewen. 2018. Apple blotch disease (Marssonina coronaria (Ellis & Davis) Davis)–review and research prospects. Eur. J. Plant Pathol. 153:657–669.

Zhu, L., W. Ni, S. Liu, B. Cai, H. Xing, and S. Wang. 2017. Transcriptomics analysis of apple leaves in response to Alternaria alternata apple pathotype infection. Front. Plant Sci. 8:22.