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ORIGINAL PAPER

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Mining data from potato pedigrees: tracking the origin of susceptibility and resistance to *Verticillium dahliae* in North American cultivars through molecular marker analysis

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Abstract Potato (*Solanum tuberosum* L.) cultivated in North America is an autotetraploid species with a narrow genetic base. Most of the popular commercial cultivars are susceptible to *Verticillium dahliae*, a fungal pathogen causing Verticillium wilt disease, though some cultivars with relatively high resistance also exist. We have used the available pedigree information to track the origin of susceptibility and resistance to Verticillium wilt present in cultivated potatoes. One hundred thirty-nine potato cultivars and breeding selections were analyzed for resistance to the pathogen and for the presence of the microsatellite marker allele *STM1051–193* that is closely linked to the resistance quantitative trait locus located on the short arm of chromosome 9. We detected an unusually high frequency of susceptible genotypes in the progeny descending from the breeding selection USDA X96–56. Molecular analysis revealed that USDA X96–56 does not have the *STM1051–193* allele. Most of the first-generation progeny of this breeding selection also lack the allele. On the other hand, pedigree analysis indicated that breeding selection USDA 41956 often transfers *V. dahliae* resistance to its progeny. Molecular analysis detected presence of (at least) three *STM1051–193* alleles in this breeding selection. These two genotypes (USDA X96–56 and USDA 41956) appear to have contributed greatly to the susceptibility or resistance, respectively, found in present commercial cultivars. Our results also indicate that the maturity class substantially affects the plant resistance response. In the intermediate to very late maturing class, the presence of the *STM1051–193* allele significantly increases the resistance. Early to very early potatoes are usually more susceptible to the disease regardless of the allelic status, though the pattern of the allele effect is always the same. The results indicate that the *STM1051–193* allele can be used for marker-assisted selection, but the potato maturity class also needs to be considered when making the final decision about the plant resistance level.

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Introduction

Verticillium dahliae (Kleb.) together with *Verticillium albo-atrum* (Reinke and Berth) are the primary causes of Verticillium wilt disease (also called potato early dying disease). This fungal soil-borne disease is a limiting factor in many potato-producing areas, and soil fumigation is often the only way to control it. Since soil fumigation may be restricted in the future due to its cost and the hazard it poses to the environment, the best long-term solution seems to be breeding and development of new resistant cultivars. However, the evaluation of potato germplasm and breeding material for resistance to *V. dahliae* under field conditions is a time-consuming and expensive process. Moreover, the disease symptoms may be confused with natural senescence, especially when the maturity of the genotype is not yet known. For this reason, marker-assisted selection appears to be a promising method that could considerably facilitate and accelerate the development of new resistant varieties. To develop a molecular marker that can be successfully used for marker-assisted selection, however, more information about the genetics of plant resistance and susceptibility in currently cultivated potato varieties is needed.

Most genetic studies indicate that resistance to Verticillium wilt is polygenic and complex (Jansky 2000), but the molecular basis of the resistance is still not well understood. Recently, a quantitative trait locus (QTL) associated with plant resistance was identified through linkage disequilibrium mapping on the short arm of chromosome 9 (Simko et al. 2003). This QTL is a part of the resistance-gene family closely linked to the microsatellite marker STM1051. Molecular analysis revealed that the absence of the *STM1051–193* allele is significantly associated with the highly susceptible phe-

notype in North American potato cultivars. The objective of the present work was (1) to identify genotypes in the pedigree of North American potato that are the probable source of high susceptibility and high resistance to the disease and are also known to commonly transfer the trait to their progeny, (2) to use molecular analysis to identify whether these individuals also have the anticipated *STM1051–193* allele and (3) to examine the relationship among the *STM1051–193* allele, the disease resistance level, and the genotype maturity.

Materials and methods

Pedigree information (up to 25 generations) was obtained from published data (Werner and Love 1996; Swiezynski et al. 1997), The Potato Association of America web page (http://www.ume.maine.edu/PAA/PVI.htm) and the records of the USDA Beltsville area potato breeders. The test of plant resistance against *V. dahliae* was performed on 139 potato cultivars and breeding selections as described previously by Simko et al. (2003). Briefly, 2-week-old greenhouse-grown potato plants were inoculated with 30 ml of suspension containing *V. dahliae* race 1 conidia at a concentration of 5×10^6 per ml. Four weeks after inoculation, the plant reaction to the pathogen was scored on a scale of 1–5: (1) no disease symptoms (highly resistant), (2) slight wilting and unilateral discoloration of lower leaves (resistant), (3) moderate wilting involving more than one-half of the plant (intermediate), (4) severe wilting involving more than one-half of the plant (susceptible) and (5) plant dead from wilt (highly susceptible). Concurrently, a second set of uninoculated plants was grown under the same conditions to observe the foliage maturity. This set was slightly smaller (122 available genotypes) and the maturity of the plants was estimated from vine senescence 6, 8, 10 and 12 weeks after planting. The overall foliage maturity was rated on the scale from 1 to 5: (1) very late maturity, (2) late maturity, (3) intermediate maturity, (4) early maturity and (5) very early maturity. There were a few cases for which genotypes were not available for the tests, but their resistance reaction or the maturity class was known from records of the USDA Beltsville area potato breeders. In such a case, the previously recorded data were converted to the current scale, based on comparison of genotypes present in both assessments.

Plant DNA extraction and the molecular analysis with the STM1051 microsatellite was described by Simko et al. (2003). Briefly, 10 µl of PCR reaction mixture consisted of 25 ng genomic DNA as a template, 1× PCR buffer, 0.25 U *Taq* polymerase, 0.25 μM forward and reverse primers and 200 μM dNTPs. The cycling conditions were 94° for 2 min, followed by 30 cycles of 94° for 30 s, 65.3° for 30 s, 72° for 30 s, followed by 72° for 10 min. The STM1051 primers were described in Milbourne et al. (1998). The amplified products were separated on a 6% PAGE gel run with 1× TBE buffer and stained with ethidium bromide. Alternatively, the microsatellite lengths were determined on selected genotypes using a Beckman Coulter CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, Calif.). PCR amplification conditions were the same as described above, except that the reverse primer was labeled with a blue (D4) fluorescent tag (Invitrogen, Carlsbad, Calif.).

Simple linear correlation and regression analyses were applied to calculate the relationship between plant maturity and resistance. A *t*-test was used to evaluate the effect of the allelic status on the resistance reaction within each plant maturity class. The amount of phenotypic variance explained by the allele or allele plus maturity model was determined from one-way ANOVA and ANCOVA, respectively. All statistical analyses were performed with SAS version 8.02 software (SAS 1989).

Results and discussion

To determine the origin of the *V. dahliae* susceptibility and resistance in potato, the pedigrees of North American cultivars were examined. We detected an unusually high frequency of susceptible genotypes in progeny descending from breeding selection USDA X96–56. Nine out of 11 first-generation progeny of USDA X96–56 have high (Avon, Caribe, Plymouth) or very high (Cherokee, Early Gem, Fundy, Kennebec, Onaway, Superior) susceptibility to *V. dahliae* (Fig. 1A). The other two cultivars in the first-generation progeny (Merrimack, Saco) show an intermediate reaction. Two crosses between half-siblings originating from USDA X96–56 (Kennebec×Merrimack, F47024×F52094) produced three cultivars (Alamo, Buckskin, Sable), which were susceptible and highly susceptible to the disease. Many other second-generation progeny of USDA X96–56 also show high (AC Belmont, Belleisle, Grand Falls) to very high (Anoka) susceptibility to *V. dahliae*. Molecular analysis revealed that USDA X96–56 does not carry the *STM1051–193* allele on chromosome 9, the absence of which is often associated with susceptibility to *V. dahliae* (Simko et al. 2003). Most of the first-generation progeny of USDA X96–56 that were analyzed molecularly also lack the allele. The only two exceptions are the cultivars Saco and Superior. We cannot determine with certainty the origin of disease susceptibility found in USDA X96–56, since not all of the ancestors of this breeding selection were available for analysis. USDA X96–56 originates from a cross between USDA 3895–13 and cultivar Earlaine (Fig. 1B). USDA 3895–13 is an open pollinated seedling originally developed in Germany using hybrids between *Solanum tuberosum* and *Solanum demissum* (Love 1999). Reaction of USDA 3895–13 to *V. dahliae* was not tested, however, many of the *S. demissum* accessions in the United States Potato Genebank (http://www.ars-grin.gov/ars/MidWest/NR6/srch.html) are highly susceptible to Verticillium wilt. The paternal parent of USDA X96–56, Earlaine, is also susceptible to Verticillium wilt. This cultivar originates from Irish Cobbler, which is possibly a clonal selection from Early Rose (Swiezynski et al. 1997). Both Irish Cobbler and Early Rose are highly susceptible to *V. dahliae* and do not carry the *STM1051–193* allele. Since Earlaine shares a high level of coancestry with Early Rose (coefficient of coancestry θ=0.336), it is likely that Earlaine also does not carry the *STM1051–193* allele. Another well-known first-generation progeny of Early Rose is Burbank, from which the russet clonal selection named Russet Burbank was made. Russet Burbank remains the most widely grown potato in North America. Russet Burbank does not carry the *STM1051–193* allele either, and is susceptible to *V. dahliae*, although less so than either Irish Cobbler or Early Rose.

It was previously observed that potato resistance to *V. dahliae* is often associated with the presence of the *STM1051–193* allele (Simko et al. 2003). Although resistance itself does not appear to be dose-dependent, the more alleles a genotype possesses, the more likely it is

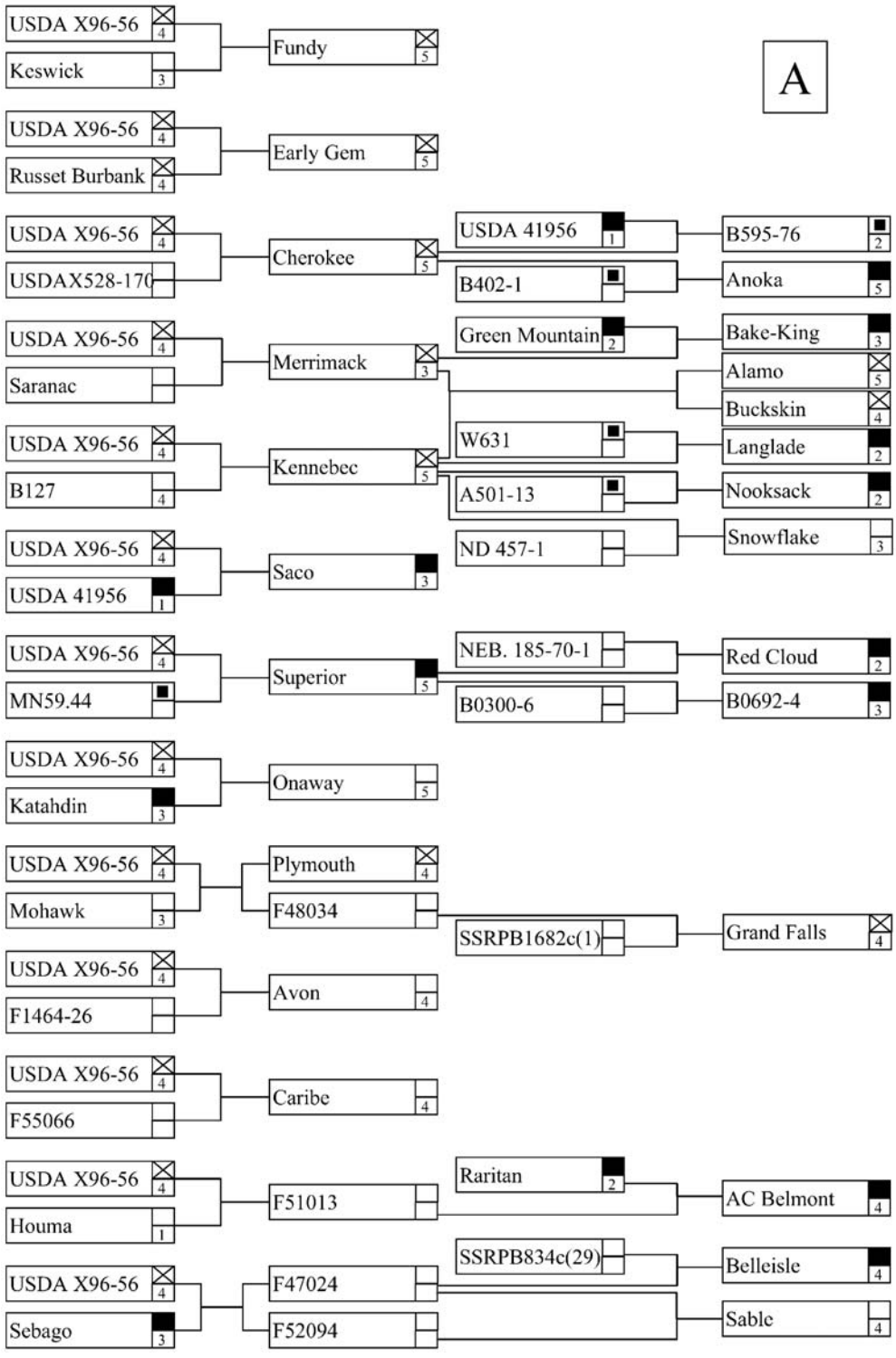
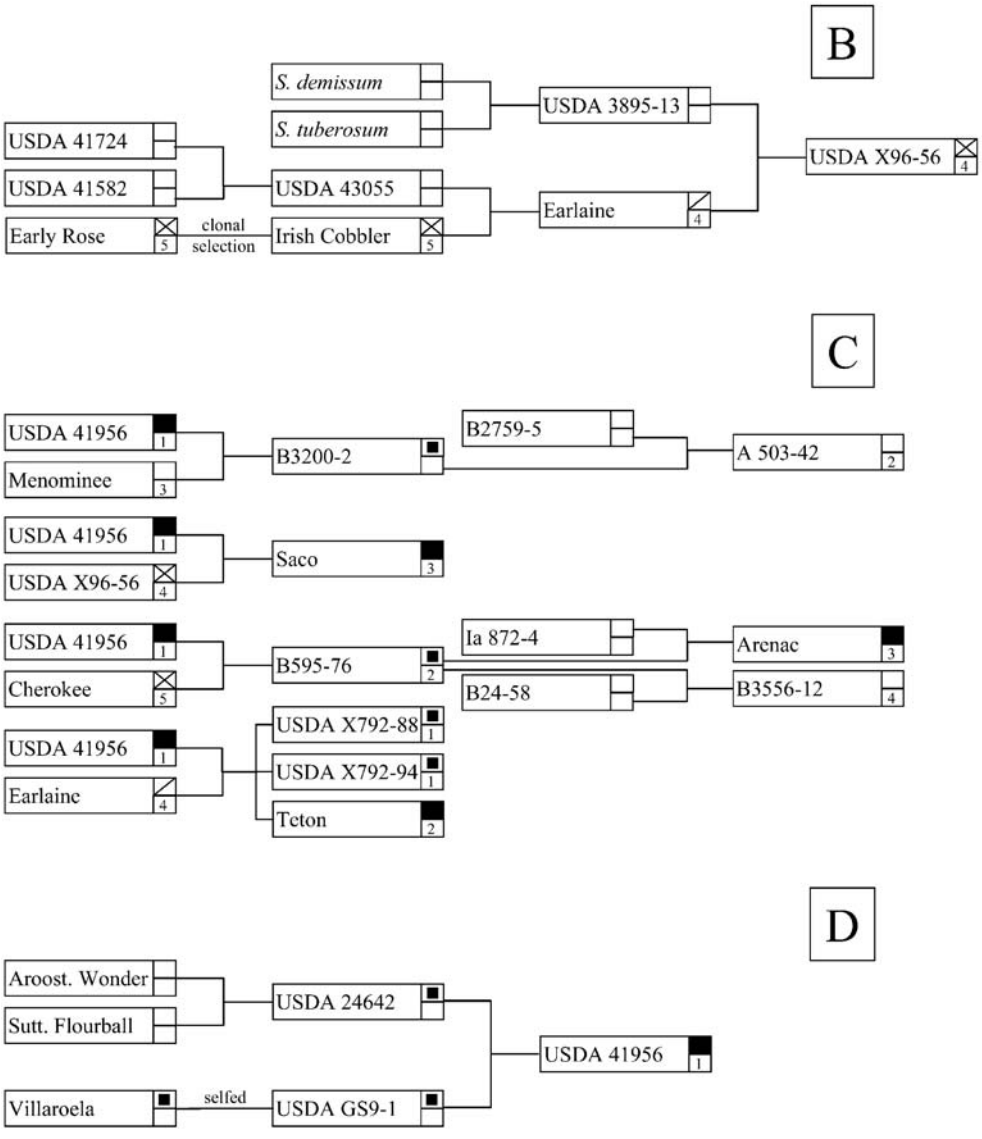


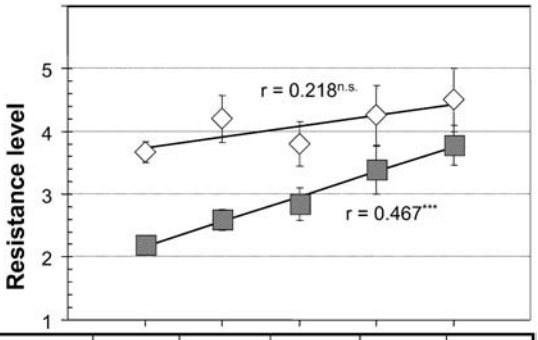
Fig. 1 Potato pedigree showing progeny (**A**, **C**) and ancestors (**B**, **D**) of USDA X96–56 (**A**, **B**) and USDA 41956 (**C**, **D**) breeding selections. Only the genotypes with known *Verticillium dahliae* resistance level are included in the progeny. The number after cultivar name or breeding selection identification denotes resistance level: (*1*) very resistant, (*2*) resistant, (*3*) intermediate, (*4*) susceptible, and (*5*) very susceptible. The *STM1051–193* allele status detected by molecular analysis is indicated as present (*filled square*) or absent (× *in square*). When a genotype was not analyzed, but the allele status can be deduced from known information, the allele presence (*closed square within open square*) and absence (*/ in square*) is also shown. Unknown allele status or unknown resistance level is symbolized by an empty field (*open square*)

to transfer those resistance-linked alleles to its progeny. If the allele is found only in a simplex state in one of the parents, half of the progeny on average will inherit the allele. If the allele is found in a duplex state and there is random allele pairing, at least one allele will be present in about five-sixths of the progeny. When the resistance allele is found in a triplex state, one or two alleles will be present in progeny, assuming chromosomal segregation. If the resistance allele is found in a quadriplex state, all of the progeny will have two resistant alleles. Potato pedigree analysis indicated that USDA 41956 has transferred *V. dahliae* resistance to its progeny (Fig. 1C). Our greenhouse test and laboratory analysis confirmed that USDA 41956 is indeed highly resistant to the disease and



possesses (at least) three *STM1051–193* alleles. Five first-generation progeny of this breeding selection were also tested for resistance to *V. dahliae*. Two of the progeny were highly resistant (USDA X792–88, USDA X792–94), two were resistant (B595–76, Teton) and one was in the intermediate class (Saco). However, it needs to be stressed that all of the above USDA 41956 progeny originate from crosses made with susceptible (Earlaine, USDA X96–56) and highly susceptible (Cherokee) genotypes lacking the *STM1051–193* allele. In other crosses these genotypes often tend to produce progeny with a high level of susceptibility. Ancestors of USDA 41956 were not available for disease screening and molecular analysis (Fig. 1D). Villaroela, which contributes half the alleles to USDA 41956, is a *S. tuberosum* ssp. *tuberosum* or ssp. *andigena* introduction from Chile (Plaisted and Hoopes 1989). Aroostook Wonder and Suttons Flourball are ancestral cultivars from 19th century US and UK breeding programs, respectively (Swiezynski et al. 1997). Their reaction to *V. dahliae* is unknown.

Verticillium wilt resistance can be to a large extent affected by maturity of a genotype, with early maturing potatoes generally showing higher susceptibility to the disease (Nachmias et al. 1990; Jansky and Rouse 2000). For that reason, 122 genotypes in which the *STM1051–193* allele status, the disease resistance level, and the maturity class were known, were re-analyzed to examine the relationship among the three characteristics. It was confirmed that maturity class plays an important role in determining the level of resistance, since a highly significant correlation (r=0.470, P<0.0001) was observed between the two traits (data not shown). When the *STM1051–193* allele status was included in the analysis, the correlation between resistance and maturity was still highly significant (r=0.467, P<0.0001) in the group of plants possessing the allele. The correlation coefficient was not significant (r=0.218, P=0.2734), however, in the



Maturity class	1	2	3	4	5
Difference in resistance level	1.47	1.61	0.96	0.86	0.72
p - value	0.005	<0.001	0.041	0.225	0.221

Fig. 2 Relationship among the *STM1051–193* allele status, the resistance level, and the maturity class. Presence of the allele is indicated by a *filled square*, genotypes without the allele are denoted by an *open diamond*. Only the mean resistance level and its standard error for each maturity class/allele status combination is plotted. Differences in the resistance levels are indicated in the lower part of the figure; significance of the difference within each maturity class was tested by a *t*-test. Foliage maturity is rated as (*1*) very late, (*2*) late, (*3*) intermediate, (*4*) early, and (*5*) very early. Rating for resistance is same as in Fig. 1; more information on the rating system is described in Materials and methods. Correlation coefficients were significant at P=0.0001 (***) or not significant at P=0.05 (*n.s.*)

group of genotypes without the *STM1051–193* allele. In the next step, we used a *t*-test to determine how the allele status affected the resistance reaction in each of the five maturity classes (Fig. 2). The presence of the allele was associated with better resistance in very late (P=0.005) and late maturing (P<0.001) potatoes, and to a lesser extent (P=0.041) in the intermediate maturity class. Neither the presence nor absence of the allele significantly affected the resistance level in early (P=0.225), and very early (P=0.221) maturing potatoes. These results imply a decreasing effect of the allele with earlier maturity. While in the intermediate to very late maturing class the presence of the allele significantly increased disease resistance, early and very early potatoes were usually more susceptible to *V. dahliae* regardless of the allele status. The estimated effect of the allele alone on total phenotypic variation of the trait was 22.7%; inclusion of the maturity class into the model increased the explained variation to 37.1%. Comparison of several molecular maps revealed that the *V. dahliae* resistance QTL (Simko et al. 2003) is not linked to any of the known plant maturity genes as mapped at the diploid level (van den Berg et al. 1996; Collins et al. 1999; Oberhagemann et al. 1999; Simko et al. 1999). Assuming that the same effect holds for tetraploid potatoes, the *STM1051–193* allele would not be associated with immature plant resistance. Immature plant resistance complicates selection and the clones with this type of (indirect) resistance should be eliminated from the breeding population (Jansky and Rouse 2000).

The *STM1051–193* allele frequency in North American cultivars was estimated to be 0.328 (Simko et al. 2003). This estimate indicates that only a small number of the tested genotypes are likely to possess all four of the *STM1051–193* alleles. Hypothetically (assuming Hardy-Weinberg equilibrium), out of the 139 molecularly tested genotypes, approximately 28.8 would be expected to be in nulliplex, 55.6 in simplex, 40.2 in duplex, 12.9 in triplex and only 1.5 in quadriplex allele status. Of course, presence of the *STM1051–193* allele itself does not guarantee high resistance to the disease. It does mean, however, that a potato with the allele is likely to be more resistant than a genotype without the allele in the mid- to later maturity classes. The *STM1051–193* allele can thus be used for marker-assisted selection and for screening germplasm when results from the Verticillium wilt resistance field test are not available. If the allele is absent in a particular genotype, it is rather likely that the genotype will be susceptible or highly susceptible to the disease. In our test of North American potato cultivars and breeding selections, we have never detected a highly resistant genotype in which the *STM1051–193* allele was absent. To test the effectiveness of the *STM1051–193* allele for marker-assisted selection, several tetraploid populations were developed and molecularly analyzed in our laboratory. Those populations are currently being evaluated for resistance to Verticillium wilt in field trials. If the field tests confirm our prediction, the *STM1051–193* allele will be used for marker-assisted selection in the Beltsville area USDA potato breeding program.

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