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Linkage disequilibrium mapping of a *Verticillium dahliae* resistance quantitative trait locus in tetraploid potato (*Solanum tuberosum*) through a candidate gene approach

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Abstract We have used the linkage disequilibrium mapping method to test for an association between a candidate gene marker and resistance to *Verticillium dahliae* in tetraploid potato. A probe derived from the tomato *Verticillium* resistance gene (*Ve1*) identified homologous sequences (*StVe1*) in potato, which in a diploid population map to chromosome 9, in a position analogous to that of the tomato resistance gene. When a molecular marker closely linked (1.5 cM) to the homologues was used as a candidate gene marker on 137 tetraploid potato genotypes (mostly North American cultivars), the association between the marker and resistance was confirmed ($P<0.001$). The amount of phenotypic variation in resistance explained by the allele of the STM1051 marker was greater than 10% and 25% in two subpopulations that were inferred from coancestry data matrix. Cloning of homologues from the highly resistant potato cv. Reddale indicates that the resistance quantitative trait locus (QTL) comprises at least an eleven-member family, encoding plant-specific leucine-rich repeat proteins highly similar to the tomato *Ve* genes. The sequence analysis shows that all homologues are uninterrupted open reading frames and thus represent putative functional resistance genes. This is the first time that the linkage disequilibrium method has been used to find an association between a resistance gene and a candidate gene marker in tetraploid potato. We have shown that it is possible to map QTL directly on already available potato cultivars, without developing a new mapping population.

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Introduction

The genetic components of continuous phenotypic variation are generally multiple loci with varying degrees of effect. To detect these quantitative trait loci (QTLs), techniques that can identify modest effects are required. Currently, the most common method for mapping plant genes involves generating a population derived from a single cross to estimate recombination frequencies between the marker loci and the gene of interest. However, a bi-parental cross samples only a small fraction of the possible alleles in the population from which the parents originated. Moreover, the genetic background and QTL effects observed in bi-parental crosses are often not representative of those encountered in elite cultivars (Jannink et al. 2001), since QTL mapping is usually not performed on breeding populations. For example, in potato, most of the molecular mapping is performed on diploid populations partly or completely originating from wild species (Simko 2002), though cultivated potato (*Solanum tuberosum* L.) is a tetraploid species ($2n=4x=48$). Nevertheless, using diploid accessions and bi-parental crosses is vital in potato genetic mapping and a linkage map construction, and many valuable genes were detected by these means (Gebhardt and Valkonen 2001).

In human genetics, where controlled genetic experiments are not feasible, different analysis methods were developed. Linkage disequilibrium (LD) based methods (association mapping) effectively incorporate the effect of many past generations of recombination (Jorde 2000) into a single analysis. Because no mapping population needs to be created for the study, the linkage test can be performed relatively quickly and inexpensively. Also, the power to accurately detect loci of interest is greatly improved, due to the high resolution of this approach (Buckler and Thornsberry 2002). In linkage disequilibrium mapping, a whole genome may be scanned to identify regions that are associated with a particular phenotype, or alleles at a few selected candidate genes may be tested for association with a phenotype (Rafalski 2002a, 2002b).

These methodologies have only recently been used in plant genetics to identify the association of a marker (or candidate gene) locus with developmental traits (Hansen et al. 2001; Remington et al. 2001).

Verticillium wilt (also known as potato early dying disease) is caused predominantly by two closely related soilborne fungi: *Verticillium dahliae* (Kleb) and *Verticillium albo-atrum* (Reinke & Berthold). This common vascular disease is a limiting production factor for all commonly grown potato cultivars. Although Verticillium wilt resistance in cultivated tetraploid potato has been previously examined (Jansky 2000), the genetic basis of resistance is still not known. In tomato, the *Ve* gene located on a short arm of chromosome 9 is implicated in race-specific resistance to infection by *Verticillium* species (Diwan et al. 1999). Recent positional cloning of the gene identified not one, but two closely linked inverted genes (*Ve1*, *Ve2*) that independently confer resistance to the same pathogen. When the tomato *Ve1* or *Ve2* gene is expressed functionally in potato, resistance is observed in otherwise susceptible plants (Kawchuk et al. 2001). This observation suggests that all necessary components of the resistance response are present and functional in the related host genus. Earlier comparative genome analysis showed that the tomato and potato genomes are highly collinear and differ by only five paracentric inversions (Tanksley et al. 1992). The similarity of the two genomes allows for the possibility to map conserved resistance genes in these closely related *Solanaceous* species.

The objective of the present study was to use a candidate gene mapping approach combined with LD mapping and genetic mapping to analyze an association between the tomato *Ve* resistance genes and quantitative variation in resistance to *V. dahliae* observed in tetraploid potato cultivars. We tested for the association in four steps. First, candidate gene for potato resistance to *V. dahliae*—the tomato *Ve1* gene—was selected. Second, potato homologues of the tomato *Ve1* gene were placed on (diploid) potato molecular map. Third, a molecular marker closely linked with the *Ve1* gene homologues was used to screen tetraploid genotypes LD and the marker/resistance association was calculated. Fourth, similarity between the tomato *Ve1* gene and its potato homologues was estimated from sequence data analysis.

Materials and methods

Plant material

The diploid potato population BD410, consisting of 256 individuals, was derived from a cross between two parental clones from an intermating population of *Solanum phureja* and *Solanum stenotomum* (Haynes and Christ 1999). A subset of 132 clones was chosen randomly from the initial population and this subset, together with the two parental clones BD142-1 and BD172-1, was used for RFLP analysis and linkage-map construction.

To test for an association between the tomato *Ve* resistance genes and the Verticillium wilt resistance phenotype of various potato pedigrees, a set of 139 potato cultivars and advanced breeding lines was evaluated in this study. These cultivars represent most of the North American breeding pool and the major commercial cultivars currently grown for fresh market and processing (Table 1).

Disease screening

Inoculum was prepared from a combination of *V. dahliae* race 1 cultures grown for 30 days on potato dextrose agar plates or Czapek-Dox broth. Distilled water was used to dilute the resulting slurry to a concentration of 5×10^6 conidia per ml. Pre-sprouted potato minitubers produced from healthy in vitro plantlets from the greenhouse were planted into a pathogen-free Jiffy Mix Plus (Jiffy Products of America Inc., Batavia, Ill.) in 15-cm pots. The pots were placed on greenhouse benches in a randomized complete block design consisting of four replicates (one pot per replicate). Only one stem per tuber was allowed to grow; the rest were removed. During the experiment, the greenhouse temperatures fluctuated between 20° and 28°, and daylight was about 14 h a day. Two weeks after planting, the plants were inoculated by adding 10 ml of spore suspension to each of three holes made 3 cm from the base of the stem and penetrating into the soil through the root zone. Reaction of the plants was rated 4 weeks after inoculation on a scale from 1 to 5 according to Hunter et al. (1968): (1) no disease

Table 1 Tetraploid potatoes used for the linkage disequilibrium mapping. Division into subpopulations is based on degree of relatedness estimated from coancestry matrix (as described in "Materials and methods")

Subpopulation A	Subpopulation B	Subpopulation C	Unknown pedigree
AC Novachip, Allegany, Alturas, Amey, Atlantic, B0169-56, B0172-22, B0718-3, B0766-3, Belchip, CalRose, Campbell 11, Canoga, Castile, Chieftain, Chipbelle, Chippewa, Harley Blackwell, IdaRose, Islander, Katahdin, Keuka Gold, MaineChip, Monona, NemaRus, NorDonna, Norland, Norwis, Ontario, Penobscot, Pike, Raritan, Red LaSoda, Red Pontiac, Reddale, Redsen, Rideau, Rosa, Sebago, Shepody, Simcoe, Spartan Pearl, Suncrisp, Sunrise, USDA41956, USDA X927-3, Viking, Wauseon, Yukon Gold	AC Belmont, Arran Consul, B1145-2, B1240-1, Belrus, Bounty, Desiree, Donna, Dorita, Elba, Eva, Green Mountain, Haig, Hampton, Jacqueline Lee, Kanona, Krantz, LaChipper, NorChip, NorValley, Patrones, Red Warba, Steuben	Acadia Russet, Alamo, Anoka, Arenac, B0692-4, B0767-2, BakeKing, Bannock Russet, Belle Isle, Buckskin, Butte, CalWhite, Caribe, Centennial Russet, Cherokee, Coastal Russet, Delta Gold, Denali, Early Gem, Early Ohio, Early Rose, Eide Russet, Erik, Frontier Russet, Fundy, Garnet Chili, Gemchip, Gem Russet, Goldrush, Grand Falls, HiLite Russet, Hudson, Irish Cobbler, Ivory Crisp, Jemseg, Kennebec, LaBelle, Langlade, Lemhi Russet, Lenape, Marygold, Merrimack, ND860-2, Nooksack, Norgold Russet, Norking Russet, Plymouth, Prestile, Pungo, Ranger Russet, Red Cloud, Redskin, Rhinered, Russet Burbank, Russet Legend, Russet Norkotah, Russette, Saco, Seminole, Snowden, Superior, Teton, Triumph, Umatilla Russet, USDA X96-56	Banana, Cherry Red

Fig. 1 Plant reaction to inoculation with *Verticillium dahliae* race 1. From *left to right*: rating of 1 - no disease symptoms, rating of 3 - moderate wilting involving more than one-half of the plant, and rating of 5 - plant dead from wilt

symptoms, (2) slight wilting and unilateral discoloration of lower leaves, (3) moderate wilting involving more than one-half of the plant, (4) severe wilting involving more than one-half of the plant, and (5) plant dead from wilt (Fig. 1). Each plant was rated separately and mean values from four replications were subjected to statistical analyses.

RFLP analysis

Approximately 10 μg of total genomic DNA from potato leaf tissue of the two parents (BD142-1 and BD172-1) was digested separately with five different restriction enzymes (*Hin*dIII, *Eco*RI, *Dra*I, *Xba*I, and *Taq*I) to prepare Southern-blot survey filters. The filters were hybridized with 140 RFLP probes derived from random genomic DNA or cDNA clones of potato and tomato (provided by Dr. C. Gebhardt and Dr. S.D. Tanksley). Twelve other RFLP markers were developed from resistance gene analogs belonging to the NBS-LRR group (Simko, unpublished). In addition, a cloned fragment (845 bp) from the tomato *Ve1* resistance gene was also used as a probe (*Ve1-845*) for hybridization analysis. All probes were labeled with α-P^{32} dCTP using the random hexamer procedure (Oligolabelling Kit, Amersham Pharmacia Biotech, Buckinghamshire, UK). The hybridizations were carried out, following a standard protocol, at 65° for 24 h (Sambrook et al. 1989). RFLP probes that showed polymorphism between parents were used to screen the BD410 population of 132 clones and to construct a molecular linkage map.

SSR assays

Genomic DNA was extracted from in vitro plantlets using a commercially available kit (GenElute Plant Genomic DNA Miniprep Kit Sigma-Aldrich, St. Louis, Mo.). The 10 μl of SSR-PCR reaction mixture consisted of 25 ng genomic DNA as a template, 1×PCR buffer, 0.25 U of *Taq* polymerase, 0.25 μM of forward and reverse primers, and 200 μM of dNTPs. The cycling conditions were: 94° for 2 min, followed by 30 cycles of 94° for 30 s, annealing temperature for 30 s, 72° for 30 s, followed by 72° for 10 min. The SSR primers and PCR annealing temperatures were as in Milbourne et al. (1998). The amplified products were separated on 6% PAGE gel run with 1×TBE buffer and stained with ethidium bromide. In addition, microsatellite lengths were determined on selected genotypes using Beckman Coulter CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, Calif.). PCR amplification conditions were same as described before; only the reverse primer was labeled with blue (D4) fluorescent tag (Invitrogen Corporation, Carlsbad, Calif.).

Ve1 homologues detection

The *Ve1* gene specific primer pair was used in PCR amplification of the homologues from resistant cv. Reddale. All PCR conditions and sequences of the primer pair were as in Kawchuk et al. (2001). The amplified products were separated on 1% agarose gel run with 1×TBE buffer and stained with ethidium bromide. PCR products of expected size were extracted, cloned with the TOPO TA cloning kit (Invitrogen Corporation, Carlsbad, Calif.), and sequenced on an ABI 3100 automatedgenetic analyzer (Applied Biosystems, Foster City, Calif.). The deduced amino-acid sequences were aligned using the Clustal X version 1.81 comparison program (Thompson et al. 1997) and their similarity was calculated. Phylogenetic analysis was conducted using the computer software MEGA version 2.1 (Kumar et al. 2001).

Linkage map construction and statistical analysis

The genetic linkage map was constructed using the computer software JoinMap version 3.0 (Stam 1993). The critical value of LOD 3.0 was set as a threshold to assign marker loci to the linkage group. The Kosambi estimation method was used to convert recombination frequencies to map distances in centiMorgans (cM). The molecular map was drawn with Mapchart version 2.1 (Voorrips 2002).

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. Genotypes without known pedigree were excluded from further data analysis. The degree of relatedness of any two individuals was estimated with Pedigree Viewer version 5.0 software (Kinghorn and Kinghorn 2002). The resulting coancestry matrix was then subjected to cluster analysis by Ward's minimum variance clustering method. To evaluate population structure, SAS version 8.02 procedures CLUSTER and MODECLUS were used (SAS 1989). Optimal number of subpopulations was estimated based on a consensus among the cubic clustering criterion (CCC), pseudo F statistic, and pseudo t^2 statistic.

LD between the marker and QTL was detected as a significant difference between phenotypic measures of resistance in two allelic states of the marker. Two testing methods were used to assess marker/phenotype association: t-test and a permutation-based approach that is robust with respect to the trait distribution (Churchill and Doerge 1994). Ten thousand permutations were computed to determine significance of observed P values. The amount of phenotypic variance explained by the tested marker was determined from regression analysis. Independence of allele distribution among subpopulations was tested by a χ^2 test with the appropriate degrees of freedom.

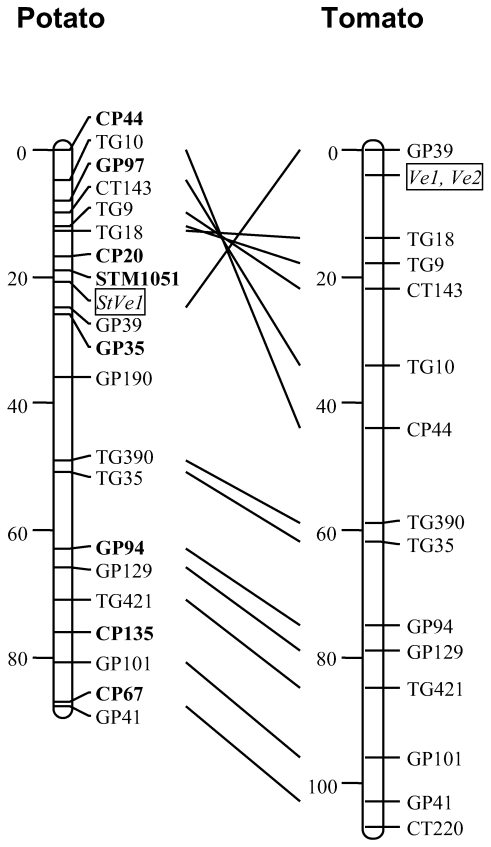

Fig. 2 Comparison of the potato and tomato molecular linkage maps of chromosome 9. Markers segregating in the BD410 population (*bold*) were placed onto the consensus molecular map (Gebhardt et al. 1991; Tanksley et al. 1992). Position of the *Ve1* and *Ve2* gene is estimated from Diwan et al. (1999); marker locations on tomato map are from Tanksley et al. (1992). Map distances are in centiMorgans. The tomato *Ve* genes and *StVe1* potato homologues are *boxed*. *Solid lines* link corresponding markers on two maps (note the paracentric inversion)

Results

Mapping *Ve1* gene homologue on diploid potato molecular map

The tomato *Ve1-845* probe identified three polymorphic and five monomorphic restriction fragments between the two potato parental DNAs when digested with the restriction enzyme *Dra*I. All three polymorphic fragments inherited from the BD142-1 parent segregated as alleles of one locus (*StVe1*) on chromosome 9. Segregation analysis of the diploid progeny located *StVe1* 1.5 cM from the SSR marker STM1051. Comparison of the potato and tomato molecular maps (Fig. 2) suggested that the *StVe1* locus is located in the position analogous to the tomato *Ve* genes (Diwan et al. 1999). Subsequently, the STM1051 marker that is closely linked to the *StVe1* locus was used as a "candidate gene marker" to test for linkage disequilibrium with resistant phenotypes observed in tetraploid cultivars.

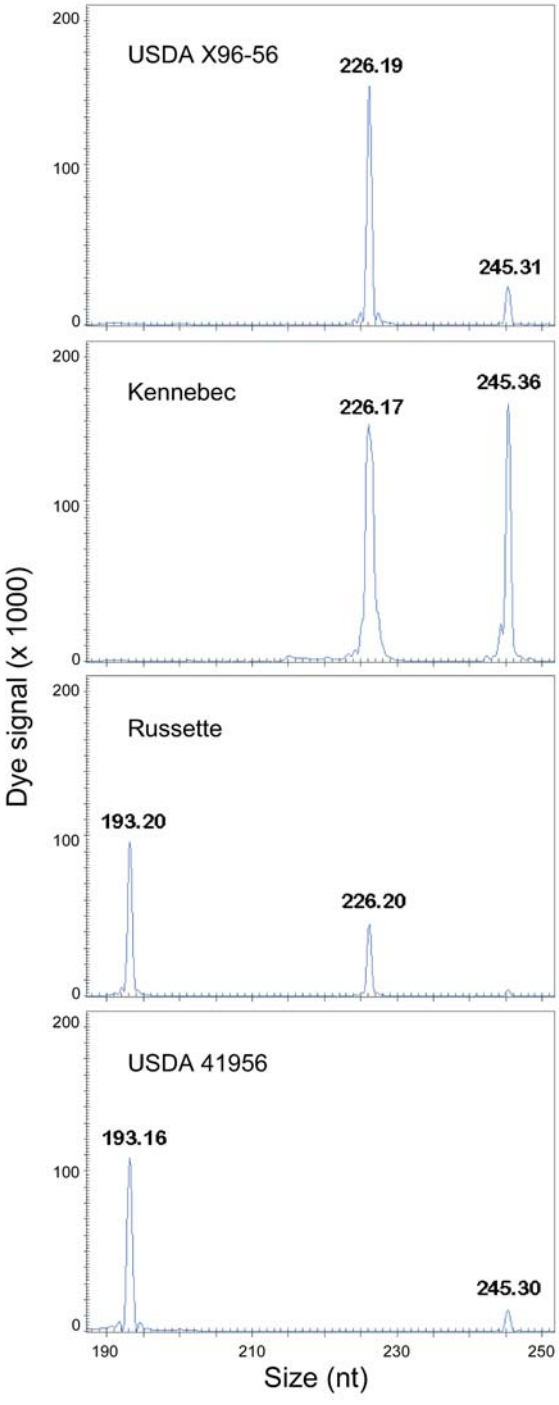

Fig. 3 Segregation of the STM1051 microsatellite marker alleles in tetraploid potato. USDA X96-56 and Kennebec are highly susceptible while Russette and USDA 41956 are genotypes resistant to *Verticillium dahliae*

Association mapping of candidate gene marker at tetraploid level

At least three different alleles (193-, 226-, and 245-bp long) were polymorphic for the marker locus STM1051 at the tetraploid level (Fig. 3). The 193-bp allele at marker

Table 2 Association of the allele *STM1051-193* with the *Verticillium dahliae* resistance QTL in potato

Population[a]	Population size	Frequency of allele detection[b]	Estimated allele frequency [c]	Allele effect[d]	*t*-test[e]	Permutation test[f]	Variance[g]
ABC	137	0.796	0.328	1.31±0.211	<0.001	<0.001	20.9
A	49	0.898	0.435	1.20±0.510	0.015	0.016	12.4
B	23	0.870	0.400	0.87±0.412	0.194	0.209	7.9
AB	72	0.889	0.423	1.09±0.339	0.005	0.007	10.8
C	65	0.692	0.255	1.33±0.280	<0.001	<0.001	25.3

[a] Population *ABC* is a whole tetraploid population used for the association test; *A*, *B*, and *C*' denote individual subpopulations; *AB* is the pooled subpopulation consisting of *A* and *B*
[b] Frequency of the allele *STM1051-193* detection among genotypes in the population
[c] Estimated frequency of the allele *STM1051-193* assuming Hardy-Weinberg equilibrium in tetraploid population
[d] Effect of the allele on resistance was computed as difference between mean values detected on genotypes without and with the allele. Positive value indicates better resistance in presence of the allele. Standard error on the effect was calculated as in Long et al. (1998)
[e] Significance of the allele effect when tested with *t*-test
[f] Significance of the allele effect when estimated with 10,000 permutations
[g] Total phenotypic variance explained by the allele *STM1051-193* as estimated from regression analysis

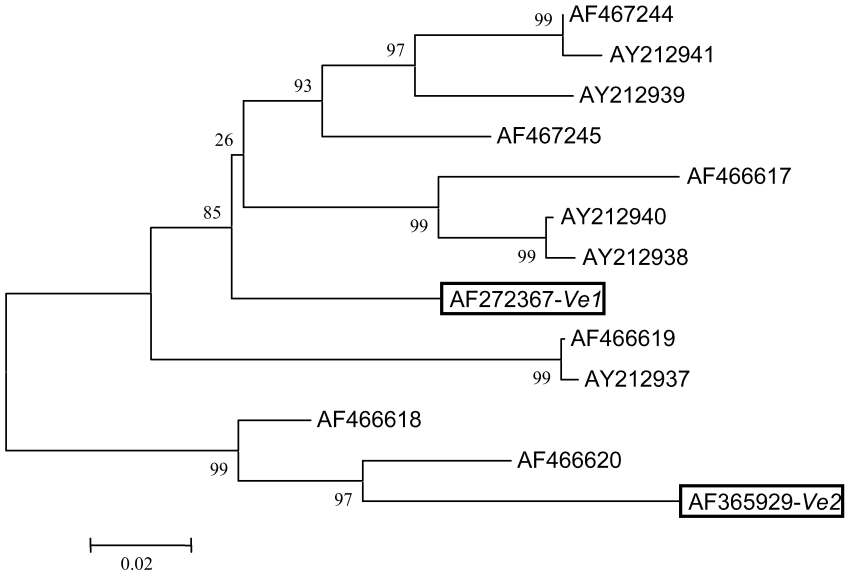

Fig. 4 Dendogram generated from potato homologues of the tomato *Ve1* gene. The neighbor-joining algorithm was applied to the deduced amino-acid sequence alignment. Bootstrap values from 1,000 cycles of resampling are given at the branching point. GenBank nucleotide accession numbers are used to identify each gene or homologue. Tomato *Ve1* and *Ve2* genes are *boxed*

locus STM1051 (allele *STM1051-193*) was absent in about 20% of the genotypes. The absence of the allele was strongly associated ($P<0.001$) with high susceptibility to Verticillium wilt. This association was supported also by the permutation test ($P<0.001$). However, mapping in a diverse population can lead to spurious marker-gene associations due to different allele frequencies in subpopulations (Pritchard et al. 2000b). Therefore, data in the coancestry matrix were submitted to clustering analyses to estimate an optimal number of subpopulations for the data. The consensus among clustering statistics yielded three subpopulations (A, B, and C) consisting of 49, 23, and 65 individuals, respectively (Tables 1 and 2). Testing for independence ($P=0.017$) suggested significant differences in the frequency of the *STM1051-193* allele detection among the subpopulations. Additional statistical analyses revealed that data from subpopulations A and B could be pooled, since the allele frequency detection in those two groups was not significantly different ($P>0.900$). A new independence test of two subpopulations AB versus C confirmed the previously detected difference ($P=0.008$) in allele frequency detection. Resistance data from two subpopulations (pooled AB and C) were thus recalculated to examine whether the association found in the whole population (ABC) can also be detected in separate analyses. (For comparison, results from individual A and B subpopulations are shown in Table 2.) In all cases, the presence of the *STM1051-193* allele was related to better Verticillium wilt resistance. On average, genotypes with the *STM1051-193* allele in subpopulation AB had a Verticillium wilt rating of 2.60 versus genotypes without the allele with rating of 3.69. In subpopulation C the difference was even higher (2.75 vs 4.08). These differences in resistance were highly significant (subpopulation AB $P<0.01$; subpopulation C $P<0.001$) when tested with both statistical tests. From all the analyses done, only the separate test of subpopulation B yielded a non-significant *P* value of 0.194 (or 0.209 by permutation test), though the pattern of association was in the same direction as in other tests. However

it is possible, that the higher *P* value in this subpopulation resulted from a relatively small sample size. The amount of phenotypic variation in resistance explained by the allele *STM1051-193* was greater than 10% and 25% in subpopulations AB and C, respectively.

Cloning of *Ve1* gene homologues

The amplification obtained using the *Ve1* primer pair on resistant potato cv. Reddale genomic DNA resulted in a seemingly single band with expected product size. However, the band contained a mixture of DNA fragments, as detected by electrophoretic analysis of the restriction enzyme digest. The sequence data from cloned fragments corresponded with at least 11 different *StVe1* homologues (GenBank accessions accession nos. AF466617-AF466620, AF467244-AF467245, and AY212937-AY212941). The deduced amino acid sequences of the homologues shared high similarity with tomato *Ve1* (GenBank accession no. AF272367) and *Ve2* (GenBank accession no. AF365929) resistance genes. Overall amino-acid similarity among the potato *StVe1* homologues ranged between 76.2% to 99.6%, while their similarity to the tomato *Ve1* and *Ve2* genes was at 82.9–90.8% and 74.2–90.8%, respectively (Fig. 4). Sequence analysis of the 11 homologues indicated that all of them were uninterrupted open reading frames similar to the tomato *Ve* genes and thus represent putative functional resistance genes. Four *StVe1* homologues with highest and lowest similarity to the *Ve1* and *Ve2* genes, were converted into RFLP probes, and were used to screen the diploid BD410 population. All of the probes, plus a probe developed from the *Ve2* gene, showed a banding pattern identical to that of the *Ve1-845* RFLP probe and cosegregated on chromosome 9.

Discussion

Linkage disequilibrium analysis is extensively used for identifying genes in human genetics (Jorde 2000), but only recently has this approach found its application in plant genetics. The key advantages of association tests include their speed, because no mapping population needs to be created, and high resolution. The primary obstacle to successful association studies in plants is the population structure, particularly the presence of subgroups with an unequal distribution of alleles. In such populations, false-positive associations can be detected between a marker and a phenotype, even if the marker is not physically linked to the locus responsible for the phenotypic variation (Pritchard et al. 2000b; Buckler and Thornsberry 2002). Thus, a crucial first step in LD mapping is to define a set of subpopulations. Pritchard et al. (2000a, 2000b) developed effective methods that control for population structure in association tests. The approach uses a genetic similarity matrix that is estimated from molecular markers data. This method was successfully implemented on a maize population consisting of three subpopulations (Remington et al. 2001; Thornsberry et al. 2001). When compared, the model-based and origin-based subpopulations were in agreement for 86% of the lines (Remington et al. 2001). We have used an alternative approach to assess the genetic similarity matrix from comprehensive pedigree data. Earlier studies demonstrated that in most instances, similarity in marker banding pattern reflects potato pedigree relationship (Hosaka et al. 1994; Demeke et al. 1996), though exceptions to this general trend do occur (Demeke et al. 1996). A significant difference in frequency of the *STM1051-193* allele detection in AB (0.886) and C (0.692) inferred subpopulations suggests that the pedigree based method effectively divided the mapping population. Permutation test with two subpopulations indicates that it is rather unlikely (P=0.0054) to get this big (or bigger) difference in the allele frequency detection by chance alone. LD tests that take into consideration presence of subpopulations greatly reduce the number of false positive associations (Thornsberry et al. 2001) and thus lead to more reliable results.

Hunter et al. (1968) investigated inheritance of Verticillium resistance in tetraploid potatoes. They reported that the resistance is heritable, and their results indicate that a genetic mechanism of resistance is based predominantly on additive genes. We have used their original data to estimate a narrow-sense heritability (h^2) of the resistance. Regressing of progeny means onto mid-parent value yielded h^2 of 0.39. Our present results show that the *STM1051-193* allele explained 25.3% of the total phenotypic variance in subpopulation C. If inheritance of resistance in this subpopulation is similar to that reported above, the QTL near STM1051 marker explains approximately 65% of the additive genetic effect. This is relatively high proportion indicating presence of a major effect locus that could be potentially used for marker-assisted selection.

The STM1051 microsatellite contains $(TAT)_4TTT(TAT)_7$ motif (Milbourne et al. 1998) from potato invertase invGE gene (GenBank no. AJ133765) (Maddison et al. 1999). Though allele *STM1051-193* itself is not a part of the resistance QTL, we have tested whether varying levels of resistance among resistant cultivars can be explained by different copy number of this allele; or in other words, whether resistance is dose-dependent. When highly resistant potato cultivars Reddale and Russette were crossed to susceptible cv. Cherokee, the allele segregated in approximately 1:1 (Reddale) and 5:1 (Russette) ratios (Simko, unpublished results). Since cv. Cherokee does not have the allele (nulliplex allele status), in tetrasomic inheritance those segregation ratios indicate simplex and duplex allele status of Reddale and Russette, respectively. Thus, it appears that even a single *STM1051-193* allele can be indicative of very high resistance in tetraploid potato, and that differences among resistant cultivars can not simply be explained by the allele copy number.

The extent of linkage disequilibrium in potato is unknown, thus it is difficult to estimate physical distance

between the STM1051 marker and Verticillium resistance QTL. Generally, LD is affected by many factors, including population history and the frequency of recombination in the examined genome segment (Rafalski 2002a). Cultivated potato is an autotetraploid species, with a very narrow genetic base. Most of the potato cultivars grown in the North America are strongly inter-related, originating from relatively few introductions (Mendoza and Haynes 1974). The high genetic similarity of potato cultivars was confirmed with RFLP, RAPD, and SSR molecular markers (Powell et al. 1991; Demeke et al. 1996; Provan et al. 1996). In other cultivated plant species, where population bottlenecks have occurred, LD could be extensive. In sugarcane and sugar beet, LD extending for several cM was found in RFLP and AFLP marker studies (Jannoo et al. 1999; Kraft et al. 2000). Similarly, data available for soybean, which also has a very narrow genetic base, show that LD decays at distances of 2.0–2.5 cM which is roughly equivalent to 1.0–1.5 mbp (Zhu et al. 2003). Thus it is likely that most of the North American potato cultivars are comprised of a relatively small number of haplotypes and, as a result, probably extensive LD. In such a case, the LD between the allele *STM1051-193* and Verticillium resistance QTL could be detected at a distance of several cM.

In tomato, the *Ve* locus mapped to the short arm of chromosome 9, which has been suggested to be syntenous (except for a paracentric inversion) to the potato chromosome 9. Probe *Ve1-845*, derived from the tomato *Ve1* gene, identified homologous sequences, which map as alleles of one locus on potato chromosome 9. This locus is comprised of at least an eleven-member family, encoding plant-specific, leucine-rich repeat proteins highly similar to the tomato *Ve* proteins. The SSR molecular marker closely linked with these *Ve1* homologues at the diploid level also demonstrated linkage disequilibrium with Verticillium resistant phenotypes on the tetraploid level. Association of the *Ve1* homologous potato gene family and the Verticillium wilt resistance phenotype located on chromosome 9 suggests that a member of this family might encode the Verticillium resistance specificity. Also, the fact that all of the *StVe1* homologues from the highly resistant cv. Reddale are uninterrupted open reading frames highly similar to the tomato *Ve* genes indicates that they may represent functional genes. Of course, cosegregation and similarity itself does not yet demonstrate a gene function. Many resistance gene analogues with (so far) unknown function were previously detected in clusters near potato resistance genes (Leister et al. 1996). However, our results from population mapping (Fig. 2), linkage disequilibrium mapping (Table 2), and sequence analysis (Fig. 4) indicate that the potato *StVe1* homologues represent a gene family that has retained high homology to the tomato Verticillium resistance genes and potentially could represent an ortholog of the tomato *Ve* genes. If true, this would be the first example of Verticillium resistance genes that retain the same biological function in different species of *Solanum* genera.

Our adoption of a linkage disequilibrium strategy combined with a candidate gene approach for the identification of molecular marker linked to QTL for Verticillium wilt resistance in tetraploid potatoes was effective, and the SSR associated with the resistance QTL was identified. We have shown that it is possible to map a QTL directly on already available potato cultivars, without developing a new mapping population. This is a novel approach for tetraploid potato, where most of the QTL mapping populations to-date are developed from crosses between diploid wild species and/or dihaploids derived from cultivated potato (Simko 2002). Such populations are very useful for detecting new genes (e.g., for resistance) not available in the present commercial genepool (Ewing et al. 2000); however, they have considerable limitations when developing and testing markers for marker-assisted selection (Niewöhner et al. 1995). Association mapping methods that use already existing cultivars may provide a highly suitable model for direct testing of candidate gene markers.

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