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Single Nucleotide Polymorphisms in the *Allene Oxide Synthase 2* Gene Are Associated With Field Resistance to Late Blight in Populations of Tetraploid Potato Cultivars

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ABSTRACT

The oomycete *Phytophthora infestans* causes late blight, the most relevant disease of potato (*Solanum tuberosum*) worldwide. Field resistance to late blight is a complex trait. When potatoes are cultivated under long day conditions in temperate climates, this resistance is correlated with late plant maturity, an undesirable characteristic. Identification of natural gene variation underlying late blight resistance not compromised by late maturity will facilitate the selection of resistant cultivars and give new insight in the mechanisms controlling quantitative pathogen resistance. We tested 24 candidate loci for association with field resistance to late blight and plant maturity in a population of 184 tetraploid potato individuals. The individuals were genotyped for 230 single nucleotide polymorphisms (SNPs) and 166 microsatellite alleles. For association analysis we used a mixed model, taking into account population structure, kinship, allele substitution and interaction effects of the marker alleles at a locus with four allele doses. Nine SNPs were associated with maturity corrected resistance ($P < 0.001$), which collectively explained 50% of the genetic variance of this trait. A major association was found at the *StAOS2* locus encoding allene oxide synthase 2, a key enzyme in the biosynthesis of jasmonates, plant hormones that function in defense signaling. This finding supports *StAOS2* as being one of the factors controlling natural variation of pathogen resistance.

THE oomycete *Phytophthora infestans* ("invasive plant destroyer") causes the late blight disease of potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*). Without chemical control, epidemics of *P. infestans* can lead to complete yield loss. The first outbreak of late blight in Europe in the 19th century was responsible for the infamous "Irish famine" (Salaman 1985). Today, potato ranks fourth among the world's most important crops after wheat, rice, and corn, and late blight remains a major threat to potato cultivation. The extensive use of pesticides in crop protection increases production costs, is damaging to the environment, and contributes to the emergence of resistant *P. infestans* isolates (Duncan 1999; Kamoun and Smart 2005).

Genetic resistance to *P. infestans* has been identified in wild, tuber-bearing Solanum species native to Mexico and Central and South America, which are closely related to the cultivated potato (Ross 1986; Hawkes 1990). Resistance factors were introgressed into cultivars by sexual hybridization with wild species and backcrossing to adapted germplasm. Resistance is expressed as local necrotic lesions around the infection sites [hypersensitive resistance, (HR)] or as retardation of the disease progression in infected leaves and tubers when compared to susceptible genotypes (quantitative or field resistance). The phenotypic classification in one or the other resistance type is not always clear cut.

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The HR type of resistance is triggered by single, dominant genes (*R* genes) and confers resistance to specific races of *P. infestans*, whereas field resistance is a complex trait controlled by multiple genes and environmental factors. Genetic analysis suggests that the defense mechanisms underlying both resistance phenotypes are similar. *R* genes as well as genes with sequence similarity to *R* genes, defense signaling genes as well as defense response genes, may play a role in quantitative resistance to *P. infestans* (Leonards-Schippers *et al.* 1992, 1994; Trognitz *et al.* 2002; Stewart *et al.* 2003; Pajerowska *et al.* 2005; Tan *et al.* 2008). Introgression of *R* genes from the wild species *Solanum demissum* into cultivated potato failed to equip cultivars with durable resistance in the field, because the HR type of resistance was soon overcome by new, virulent *P. infestans* races. Quantitative resistance to late blight holds the promise to be more durable, as it is conferred by multiple genes and is considered race unspecific (Ross 1986; Wastie 1991). However, under the long day conditions in the growing seasons in central and northern Europe, this type of resistance is correlated with late plant maturity, an undesirable characteristic due to its incompatibility with agricultural practice (Wastie 1991; Firman and Allen 2007). Plant maturity is a complex developmental trait that circumscribes the life cycle duration of an annual plant such as potato, starting with tuber sprouting and shoot emergence, continuing with tuber initiation and growth, flowering and ending with tuber maturation and leaf senescence. In their original habitats in Latin America, tuber-bearing Solanum species are adapted to short photoperiod lengths. In temperate regions of the northern and southern hemisphere, the life cycle duration of *S. tuberosum* genotypes varies from 3 to 6 months, which reflects the degree of adaptation to long photoperiods. Well-adapted genotypes set tubers irrespective of day length and mature early, whereas nonadapted genotypes require short days to induce tuberization and mature very late (Haverkort 2007). Correlations between pathogen resistance and other physiological or developmental parameters have been described for other plant–pathogen interactions (Vanderplank 1984). In potato, the correlation between quantitative resistance and late plant maturity complicates the phenotypic selection of late blight-resistant cultivars with acceptable early maturity type, as both characters cannot be reliably separated and assessed early in the selection process. DNA-based markers diagnostic for field resistance to late blight that is not compromised by late maturity, are therefore highly desirable. Such markers will facilitate the development of new, superior potato varieties, which combine improved field resistance to late blight with the good quality of other agronomic characteristics such as maturity type, yield, and culinary or processing quality.

Molecular mapping has led to the identification of a large number of loci for qualitative and/or quantitative resistance to late blight and other pathogens in potato (reviewed in Gebhardt and Valkonen 2001; Simko *et al.* 2007, see also the SOLanaceae function map for resistance at http://www.gabipd.org/database/maps.shtml in the Potato Maps and More database; Meyer *et al.* 2005). Few studies have analyzed quantitative trait loci (QTL) for resistance and plant maturity in the same mapping population (Collins *et al.* 1999; Oberhagemann *et al.* 1999; Visker *et al.* 2003; Bormann *et al.* 2004; Bradshaw *et al.* 2004; Sliwka *et al.* 2007). These experiments showed that many QTL for resistance to late blight colocalize with QTL for plant maturity, which explains the phenotypic correlation observed between these traits. But none of the genetic analyses resolved the question whether late plant maturity and resistance to late blight are pleiotropic effects of the same genes or effects of physically closely linked but unrelated genes. However, some QTL exist, where the variation of resistance to late blight cannot be fully explained by the variation of plant maturity, suggesting the existence of genetic factors, which control quantitative resistance to late blight independent of plant maturity.

The cultivated potato is autotetraploid with complex tetrasomic inheritance, which complicates genetic analyses (Luo *et al.* 2001). The generation of inbred lines is prevented by the number of selfing generations required in polyploids combined with severe inbreeding depression after few selfings. In breeding programs, genetic diversity is generated by intercrossing multiple, heterozygous parents that are often closely related by descent (Love 1999; Gebhardt *et al.* 2004). To facilitate genetic analysis, tetraploidy can be reduced to the diploid level, at which the plants are self-incompatible (Ross 1986). Segregating F_1 families for linkage studies are obtained by crossing partially heterozygous genotypes.

QTL mapping in such experimental, mostly diploid sibling populations resulted in numerous DNA-based markers linked to QTL for late blight resistance (Gebhardt and Valkonen 2001; Simko *et al.* 2007) (http://www.gabipd.org/database/maps.shtml). A wide-ranging diagnostic value for field resistance to late blight in populations of tetraploid varieties and breeding clones related by descent has been demonstrated for few DNA-based markers. The first ones identified were from a genomic region on potato chromosome V harboring major QTL for late blight resistance and plant maturity, which colocalize with the *R1* gene for qualitative, race-specific resistance to late blight (Leonards-Schippers *et al.* 1994; Collins *et al.* 1999; Oberhagemann *et al.* 1999; Visker *et al.* 2003; Bradshaw *et al.* 2004). Markers derived from the *R1* resistance gene itself (Ballvora *et al.* 2002) and markers tightly linked to *R1* were associated with late blight resistance and plant maturity evaluated in a historical collection of 400 potato varieties and breeding clones (Gebhardt *et al.* 2004). Two markers of unknown map position sharing sequence similarity with known *R* genes were associated with late blight resistance in a population of 123 potato

varieties (Malosetti *et al.* 2007). Both association studies did not separate the effects of resistance and plant maturity.

The most valuable diagnostic DNA-based markers are those derived from polymorphisms in the genes causal for a trait of interest, as such markers are in complete linkage disequilibrium with the quantitative trait alleles. With one exception (Pajerowska-Mukhtar *et al.* 2008), plant genes and their allelic variants, which control quantitative pathogen resistance, have not been identified. Candidates for underlying quantitative resistance loci (QRL) in plants, based on their function in pathogenesis and defense, are all genes that have a role in pathogen recognition, defense signaling, and defense response. A fraction of these genes has been mapped in potato (Leister *et al.* 1996; Rickert *et al.* 2003; Castillo Ruiz *et al.* 2005; Pajerowska *et al.* 2005) and a number of those are located in chromosome segments harboring genes for resistance to late blight and other pathogens (SOLanaceae function map for pathogen resistance at http://www.gabipd.org/database/maps.shtml).

On the basis of the current version of the potato function map for pathogen resistance comprising *R* genes, QRL, and candidate genes, we tested DNA polymorphisms at 24 loci in state-of-the-art breeding populations of tetraploid potato cultivars for association with field resistance to late blight not compromised by late plant maturity. Population structure and familial relatedness were considered in the association model. We report the identification of single nucleotide polymorphisms (SNPs) associated with maturity corrected resistance to late blight. A major association was found for SNPs at the *StAOS2* locus, which encodes a key enzyme in the biosynthesis of the defense signaling molecule jasmonic acid.

MATERIALS AND METHODS

Plant material: Ninety-six tetraploid, heterozygous genotypes were sampled, each from the breeding programs of Böhm-Nordkartoffel-Agrarproduktion OHG (Ebstorf, Germany) (population BNA) and SAKA-Pflanzenzucht GbR (Windeby, Germany) (population SKP). The genotypes were selected (i) to represent a broad range of breeding materials with respect to field resistance to late blight after excluding very late-maturing genotypes and (ii) to comprise no full sibs. Eight genotypes were included in both populations.

Collection of phenotypic data: The BNA population was evaluated for field resistance to late blight in Ebstorf (Germany) during 3 years from 2004 to 2006. Rows of 12 plants per genotype were planted in May in two replications. Plots were inoculated 5–6 weeks after planting with a mixture of two complex field isolates of *P. infestans* (obtained from the Biologische Bundesanstalt, Braunschweig, Germany) that were able to overcome the *R1–R11* late blight resistance genes of *Solanum demissum.* In 2005 and 2006, natural infection was observed shortly before artificial inoculation took place. Disease progress was monitored every 3–4 days, using a 1–9 scale (1, 0% infection; 9, >90%). The SKP population (12 plants per genotype, two replications) was evaluated for late blight resistance in Windeby (Germany) from 2004 to 2006 as described (Bormann *et al.* 2004). In short, infection of the experimental plots was started by inoculating infector rows with mixtures of two complex isolates of *P. infestans.* Disease progress was monitored similarly as for the BNA population. For both populations, relative area under the disease-progress curves (rAUDPC) (Fry 1978) were calculated from the 9–13 phenotypic evaluations available for each year–location combination. Plant maturity (PM) was evaluated in uninfected plants grown under standard phytosanitary regimes for multiplication, in comparison with standard potato varieties of known maturity type, using a 1–9 scale (1, very late and 9, very early maturing). Very late variety was Kuras (1). Late and mid-late varieties were Donella (2), Producent (2), Amado (3), Oktan (3), Sibu (3), Bonanza (4), Jelly (4), Panda (4), Saturna (4), and Ponto (5). Mid-early varieties were: Agria (5), Albatros (5), Laura (5), Nicola (5), Red Fantasy (5), Satina (5), Adretta (6), Lady Claire (6), Quarta (6), Sommergold (6), and Solara (6). Early varieties were Cilena (7), Karlena (7), Marabel (7), and Tomensa (7). Very early varieties were: Berber (8), Bellaprima (8), Velox (8), Premiere (8), Presto (8), Christa (9), Leyla (9), and Solist (9). Maturity corrected resistance (MCR) was calculated according to Bormann *et al.* (2004). A regression curve of rAUDPC against maturity was computed. For each rAUDPC measurement, the vertical distance to the regression curve was then calculated, which resulted in negative values for more resistant plants and positive values for more susceptible plants.

Collection of genotypic data: Total genomic DNA was isolated from freeze-dried leaf tissue (Bormann *et al.* 2004). Simple sequence repeat (SSR) markers were PCR amplified from genomic DNA of tetraploid, heterozygous individuals using primers and conditions reported in Milbourne *et al.* (1998) (STM**** markers in Figure 2) and Feingold *et al.* (2005) (StI*** markers in Figure 2). SSR alleles were size separated on Spreadex gels (Elchrom Scientific, CH-6330 Cham, Switzerland) according to the supplier's instructions. At 21 SSR loci the dosage of the SSR alleles from 0 (allele absent) to 4 (allele homozygous or quadruplex) was estimated by the number of different alleles present in each individual (between 1 and 4) and by their relative band intensities. A single band was scored as one allele with dosage 4; two bands with equal intensity as two alleles, each with dosage 2; two bands with unequal intensity as two alleles, one with dosage 3 and the other with dosage 1; three bands, one with higher intensity than the other two as three alleles, one with dosage 2 and the other two with dosage 1; four bands as four alleles each with dosage 1 (Gebhardt 2007). In 10 cases, where SSR allele dosage was not scorable due to limited experimental resolution, polymorphic SSR alleles were scored as present (1) or absent (0). Doubtful scores were declared as missing values. Twenty-four candidate loci were selected on the basis of (i) colocalization with known QTL for resistance to *P. infestans* or other pathogens, (ii) close linkage with *R* genes or *R* gene-like (RGL) sequences, and/or (iii) coding for a gene with known function in pathogen resistance (functional candidate gene). Twenty-one loci were analyzed for SNPs. Amplicons were generated from genomic DNA of tetraploid, heterozygous individuals with locus-specific primers (supplemental Table 1). The amplicons were purified with ExoSAP-IT (USB, Cleveland) and custom sequenced at the core facility for DNA analysis of the Max Planck Institute for Plant Breeding Research. The dideoxy chain-termination sequencing method was employed using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit and an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, Weiterstadt, Germany). SNPs were detected by sequence alignments and visual examination of the sequence trace files for overlapping

base-calling peaks. The biallelic SNPs were assigned in each tetraploid individual to one of five allelic states (two homozygous and three heterozygous). The SNP allele dosage in heterozygous individuals (1:3, 2:2, or 3:1) was estimated from the height ratio of the overlapping base-calling peaks, manually and using the data acquisition and analysis software DAx (Van Mierlo Software Consultancy, Eindhoven, The Netherlands). Insertion/deletion polymorphisms (indels) were detected in the sequence trace files of the amplicons by sequence overlaps starting at specific nucleotide positions. Length, nucleotide composition, and allele dosage of indels were retrieved by manual inspection of individuals homozygous for one or the other indel allele of the nucleotide sequence and of the height ratio of overlapping base-calling peaks. The genes *R1*, *R3a*, and *R3b* were analyzed by allele-specific amplification, scoring presence or absence of the amplicon without considering allele dosage. *R1* was tested according to Gebhardt *et al.* (2004). *R3a* and *R3b* were tested as described (Huang 2005; Huang *et al.* 2005), using as positive and negative controls cultivars (cv.) Escort and Bintje, respectively, and modifying the PCR conditions. For *R3a* and *R3b* we used 28 touch-down cycles from annealing temperature (T_a) 68°–54° (0.5° per cycle) followed by 12 cycles at 56° and from T_a 60°–50° (0.35° per cycle) followed by 12 cycles at 50°, respectively.

Statistical analyses: The fact that eight clones were in common between the BNA and SKP populations was used to perform a joint data analysis from both locations, Ebstorf and Windeby. Each year–location combination was treated as an environment and entry means for each environment were used for the statistical analyses.

Phenotypic data analyses: The phenotypic data were analyzed on the basis of the statistical model

$$y_{ij} = \mu + g_i + l_j + e_{ij},$$

where y_{ij} is the entry mean for the ith clone in the jth environment, μ is an intercept term, g_i is the genetic effect of the ith clone, l_j is the effect of the jth environment, and e_{ij} is the residual. Due to the fact that the environments comprised two purposefully selected locations, the environmental effects l_j were regarded as fixed.

For calculation of adjusted entry means, g_i was considered as fixed. Over all environments, an adjusted entry mean M_i was calculated for each of the 184 clones as $M_i = \hat{\mu} + \hat{g}_i$, where $\hat{\mu}$ and $\hat{g}_i$ denote the generalized least square estimates of μ and g_i, respectively.

For estimation of variance components, g_i was considered as random. Heritability on an entry mean basis was calculated as $h^2 = \sigma_g^2/(\sigma_g^2 + \bar{w}/2)$, where σ_g^2 is the genetic variance and $\bar{w}$ the mean variance of a difference between two adjusted entry means (Holland *et al.* 2003).

Analysis of population structure: The Rogers' distance (RD) was calculated according to Rogers (1972). Associations among the 184 clones were revealed with principal coordinate analysis (Gower 1966) on the basis of RD estimates between pairs of clones. These analyses were performed on the basis of the 31 SSRs using the software PLABSOFT (Maurer *et al.* 2008), which is implemented as an extension of the statistical software R (R Development Core Team 2004).

Population structure matrix **Q** was calculated on the basis of the 31 SSRs by software STRUCTURE (Pritchard *et al.* 2000), which gives for each clone under consideration the probability of membership in each of $z + 1$ subpopulations. In our investigations, the set of 184 clones was analyzed by setting z from 0 to 19 in each of five repetitions. For each run of STRUCTURE, the burn-in time as well as the iteration number for the Markov Chain Monte Carlo algorithm were set to 100,000, following the suggestion of Whitt and Buckler (2003). In accordance with Remington *et al.* (2001), the run with the highest log likelihood and the lowest number of $z + 1$ subpopulations was used to assign clones to subpopulations. The $z + 1$ columns of the **Q** matrix add up to 1 and, thus, only the first z columns were used as **S** matrix.

A resampling strategy was applied to examine whether the number of SSR markers used to estimate population structure matrix **Q** in our study was sufficient. In each of 25 replications m SSR markers were randomly drawn without replacement from the set of 31 markers. On the basis of these m markers, the population structure of the 184 potato clones was examined using STRUCTURE as mentioned above. We set m to 25, 20, 15, and 10.

Linkage disequilibrium: A chi-square test was applied to test for linkage disequilibrium (LD) between pairs of SNP markers. The test was based on genotype classes observed for the SNP markers. To overcome the multiple test problem, the Bonferroni–Holm procedure (Holm 1979) was applied to detect marker pairs with significant ($\alpha = 0.05$) LD.

Association analyses: The results of Stich *et al.* (2008) suggested that the empirical type I error rate of two-step approaches for association mapping is only slightly higher than that of the corresponding one-step approaches. Therefore, we used the adjusted entry means, which were calculated in the first step across all environments, for association mapping in the second step. The QK method described in Yu *et al.* (2006) was used for detection of marker–phenotype associations:

$$M_i = \mu + \alpha + h_1 + h_2 + h_3 + \sum\nolimits_{u=1}^{z} S_{iu} v_u + \breve{g}_i + e_i,$$

where α are the effects of allele substitution; h_1, h_2, and h_3 the bi-, tri, and tetragenic effects, respectively (Gallais 2003); v_u the effect of the uth column of the population structure matrix **S**; $\breve{g}_i$ the residual genetic effect of the ith clone; and e_i the residual. Except for $\breve{g}_i$, all effects were regarded as fixed. The variance of the random effects was assumed to be $\mathrm{Var}(\breve{g}) = 2\mathbf{K}\sigma_{\breve{g}}^2$ and $\mathrm{Var}(e) = \mathbf{R}\sigma_e^2$, where **K** is a 184 × 184 matrix of kinship coefficients that define the degree of genetic covariance between all pairs of clones, $\sigma_{\breve{g}}^2$ is the residual genetic variance, and σ_e^2 the residual, both estimated by restricted maximum likelihood. **R** is a 184 × 184 matrix in which the off-diagonal elements were 0 and the diagonal elements were reciprocals of the number of phenotypic observations underlying each adjusted entry mean. In accordance with Yu *et al.* (2006), the kinship matrix **K** was calculated on the basis of the SSR marker loci using software package SPAGeDi (Hardy and Vekemans 2002), where negative kinship values between clones are set to 0. Stich and Melchinger (2008) compared various association mapping methods on the basis of the data set of this study. The results suggested that the association mapping model used in this study is one of the most appropriate models with respect to the adherence to the nominal α level and the power for detection of marker–phenotype associations. For the allele specific amplicons of the *R* genes *R1*, *R3a*, and *R3b* the allele dosage could not be scored, and therewith bi-, tri-, and tetragenic effects cannot be estimated. We modified for these markers the model decribed above for detection of marker–phenotype associations in such a way that it comprised no bi-, tri-, and tetragenic effects.

Previous studies suggested that it is promising to use in the context of marker-assisted selection a significance level other than for gene identification (*e.g.*, Bernardo and Yu 2007). Therefore, two different significance levels were examined. For each of the markers significantly ($P < 0.01$ or $P < 0.001$) associated with rAUDPC, PM, or MCR, we calculated the proportion of the genotypic variance explained by the allele substitution effect ($\rho 1$) as well as by the bi-, tri-, and tetragenic

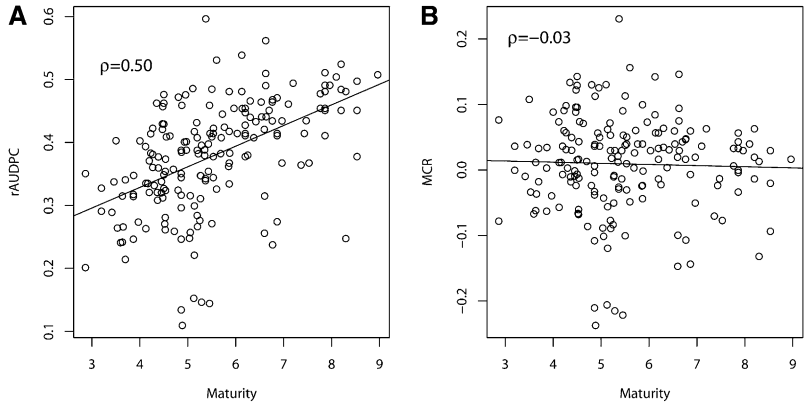

FIGURE 1.—Correlation between the adjusted entry means *M* for plant maturity and rAUDPC or MCR. Plant maturity scores (1–9, 9 being very early maturing) of 184 potato genotypes are plotted *vs.* (A) relative area under disease progress curve (rAUDPC) and (B) maturity corrected resistance (MCR). ρ is the correlation coefficient. Very late maturing genotypes (scores 1 and 2) were not present in the populations.

interaction effects (ρ2). The bi-, tri-, and tetragenic interaction effects correspond to the interaction effects of the heterozygous genotypes *1222*, *1122*, and *1112*, respectively, where *1* and *2* are alternative alleles.

All mixed-model calculations were performed with ASReml release 2.0 (GILMOUR *et al.* 2006).

Genetic mapping of SSRs: The diploid population F1840 was used for mapping, where detailed linkage maps have been constructed for all chromosomes on the basis of restriction fragment length polymorphism (RFLP) markers (GEBHARDT *et al.* 2003) (http://www.gabipd.org/database/maps.shtml). SSR markers were amplified from DNA of the parents and 92 F_1 individuals of the F1840 population and size separated as described above. Segregating SSR alleles were scored as present or absent and tested for linkage with RFLP markers of known map position. Genetic distance in centimorgan between linked marker loci was estimated as described by RITTER *et al.* (1990), using the software package MAPRF (E. Ritter, NEIKER, Vitoria, Spain).

RESULTS

One hundred ninety-two tetraploid potato clones were evaluated from 2004 to 2006 in replicated field trials for resistance to late blight and plant maturity. Eight clones were in common between both populations. The association mapping population consisted therefore of 184 different genotypes. The phenotypic and genotypic data obtained for these 184 genotypes were used to analyze population structure and to identify associations between DNA polymorphisms and the relative area under disease progress curve (rAUDPC), plant maturity (PM), and maturity corrected resistance (MCR).

Phenotypic analysis: rAUDPC was clearly correlated with plant maturity (Figure 1A), whereas MCR did not show significant correlation with plant maturity (Figure 1B). Negative values of MCR indicate increased resistance (BORMANN *et al.* 2004). Genotypes with negative means for MCR were distributed over all maturity groups (Figure 1B), indicating that factors for resistance to late blight independent of plant maturity were present in the population. The phenotypic characteristics of the 184 genotypes are summarized in Table 1. Heritability was high for all three traits. Furthermore, the residuals were normally distributed for all three traits.

Population structure: One hundred sixty-six SSR alleles were scored at 31 loci (between 2 and 10 alleles per locus) distributed on all 12 potato chromosomes (Figure 2). Genetic mapping of the SSR markers in the experimental mapping population F1840 (GEBHARDT *et al.* 2003) (http://www.gabipd.org/database/maps.shtml) was consistent with previously identified chromosomal locations (MILBOURNE *et al.* 1998; FEINGOLD *et al.* 2005). Principal component analysis using the SSR genotypic data showed that the BNA and SKP genotypes were intermixed (data not shown), indicating that the BNA and SKP populations were sampled from similar germplasm pools. Analysis of population structure on the basis of the SSR genotypic data revealed a complex structure. The log likelihood increased with the number of given subpopulations $z + 1$ and reached a maximum between $z + 1 = 15$ and $z + 1 = 17$ (Figure 3). $z + 1 = 15$ was chosen for the association model. To test whether the number of SSR loci analyzed was sufficient to detect population structure, the analysis was repeated with varying the number of SSR loci between 10 and 31. Using 25 SSR loci rather than 31 loci resulted in similar $z + 1$ values (Figure 4), indicating that 31 loci were sufficient to identify population structure.

Association analysis: Two hundred thirty biallelic SNP markers and five indels were scored in the amplicon

TABLE 1

First and second degree statistics for 184 potato genotypes for rAUDPC, PM, and MCR

Parameter	rAUDPC	PM	MCR
M_i	−0.02–0.52	3.4–9.5	−0.24–0.23
σ^2_g	$7.82e^{-2}$	1.55	$3.37e^{-3}$
σ^2_e	$3.19e^{-3}$	0.30	$2.03e^{-3}$
h^2	0.77	0.94	0.69

M_i, adjusted entry means calculated over all locations; σ^2_g and σ^2_e, genotypic and error variance, respectively; h^2, heritability on an entry mean basis; rAUDPC, relative area under disease progress curve; PM, plant maturity; MCR, maturity corrected resistance.

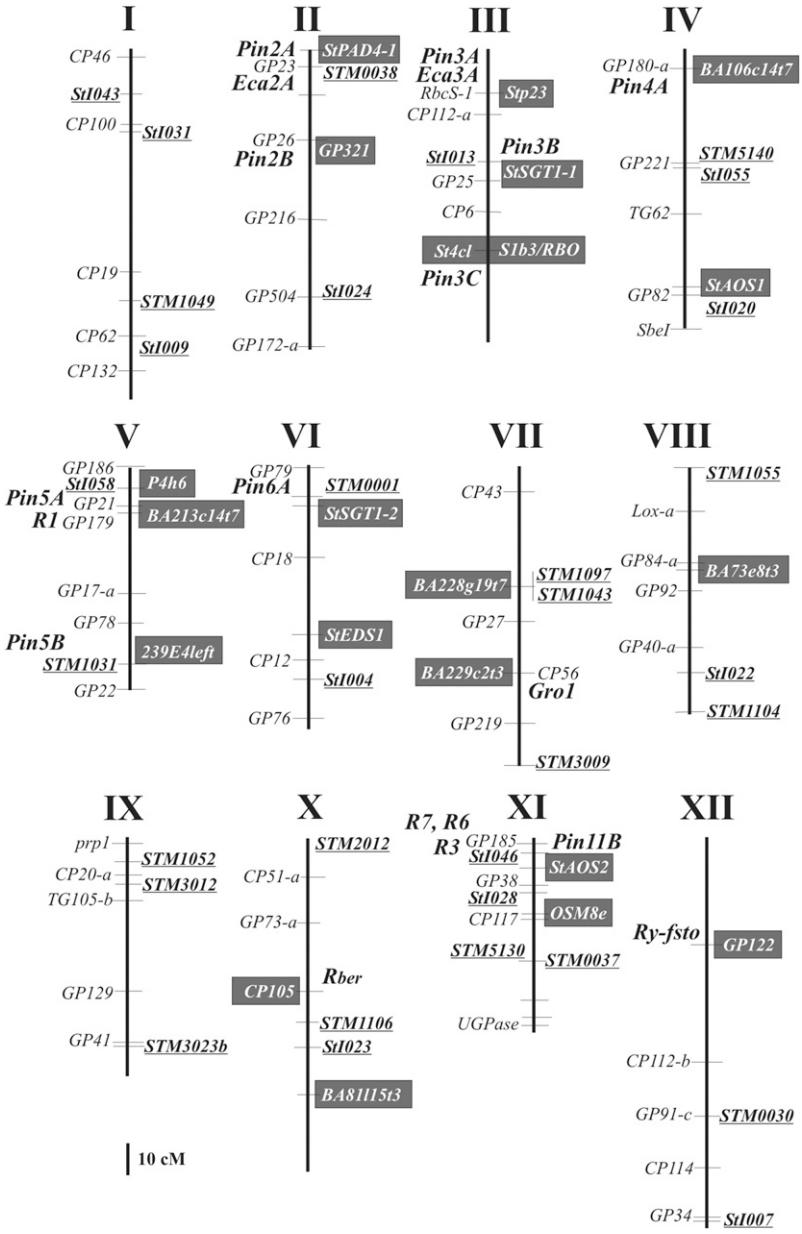

FIGURE 2.—Map positions of SSR and candidate loci on the 12 potato linkage groups of the diploid mapping population F1840 (GEBHARDT *et al.* 2003). SSR loci are in boldface type and underlined. Candidate loci analyzed for SNPs are shown in shaded rectangles. The remaining loci shown are a subset of RFLP markers mapped in the F1840 population. Late blight QRL and some *R* genes mapped in different genetic backgrounds are included at approximate positions. The full map including references is available at http://www.gabipd.org/database/maps.shtml.

sequences derived from 21 candidate loci in 184 individuals (Table 2, Figure 2, supplemental Table 1, sequences and SNPs at http://www.gabipd.org/database/maps.shtml). Also the allele dosage, between zero and four copies per locus in a tetraploid individual, was assessed. The percentage of intralocus pairs of polymorphisms in significant ($\alpha = 0.05$) LD was 48.4%. In contrast, the percentage of interlocus pairs of polymorphisms in significant LD was 1.5% (supplemental Figure 1).

SNPs and indels were tested individually for association with rAUDPC, PM, and MCR using a mixed model, which takes into account population structure, kinship, and additive as well as interaction effects (MATERIALS AND METHODS). The allele-specific amplicons for the *R* genes *R1*, *R3a*, and *R3b* were tested for association on the basis of a model neglecting interaction effects. Forty SNPs and three indels at 13 loci were significantly ($P < 0.01$) associated with at least one trait (supplemental Table 2). In contrast, none of the allele-specific amplicons for the *R* genes *R1*, *R3a*, and *R3b* showed significant association with any trait. Due to the heterozygocity of the individuals, potato SNP data are unphased and do not allow the direct inference of haplotypes from amplicon sequences. As shown by the LD plot (supplemental Figure 1), many SNPs within the same amplicon were in LD with each other. The SNPs that tested significant for association within the same amplicon were therefore not independent. The effects of single SNP alleles were mostly additive but interaction effects were also found. Nineteen SNPs and one indel at 7 loci were associated with rAUDPC. The additive effects of

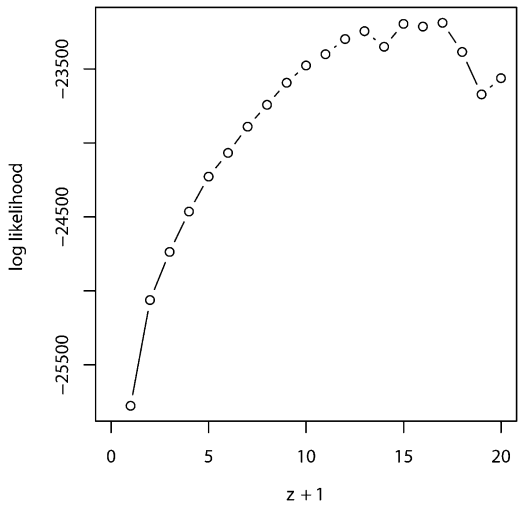

FIGURE 3.—Plot of the Schwarz Bayesian criterion of the data *vs.* the number of postulated subpopulations $z + 1$. $z + 1 = 15$ was chosen for the association model.

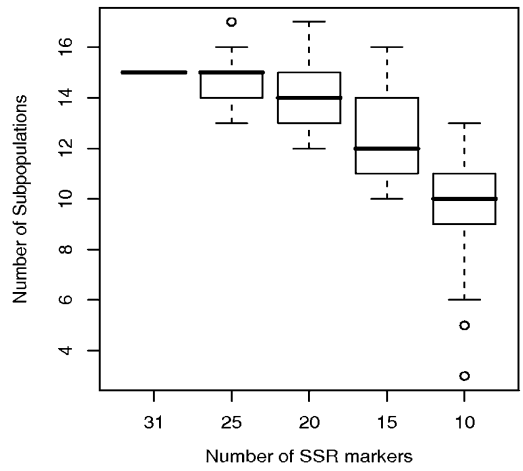

FIGURE 4.—Plot of the observed number of subpopulations $z + 1$ *vs.* the number of examined SSR loci.

these markers simultaneously explained 55%, whereas the di-, tri-, and tetragenic interactions of the markers explained only 1% of the genetic variance. Regarding PM, an only partially overlapping set of 23 SNPs and two indels at 11 loci were associated. The additive effects of these markers simultaneously explained 28%, where the di-, tri-, and tetragenic interactions of the markers explained 5% of the genetic variance. Most importantly, 16 SNPs at 6 loci were associated with MCR, where the additive and the di-, tri-, and tetragenic interaction effects explained 48% and 3% of the genetic variance, respectively. At the more stringent significance threshold of $P < 0.001$, 20 SNPs and one indel at 10 loci were associated with rAUDPC, PM, or MCR (Table 3). Two SNPs at the *StAOS2* locus were associated with the largest effect on resistance. StAOS2_snp691and StAOS2_snp692 explained 39% and 43% of the genotypic variance of rAUDPC, 31% and 36% of the genotypic variance of MCR, but only 13% and 14% of the genotypic variance of plant maturity, respectively (Table 3). The allele with the nucleotide combination "*AC*" at StAOS2_snp691 and StAOS2_snp692 is associated with increased resistance to late blight (decreased means of rAUDPC and MCR), whereas the allele with the combination "*GG*" is associated with susceptibility (Figure 5). Individuals with the *AC* allele are, on average, slightly later maturing than individuals with the *GG* allele. The effect on late blight resistance and plant maturity depends on the allele dosage, as the allele substitution effects are significant whereas the interaction effects are not significant. Susceptibility to late blight increases with increasing dosage of *G* alleles at both SNP markers StAOS2_snp691 and StAOS2_snp692, whereas plant maturity shifts to earlier maturation (Figure 5). Most resistant to late blight are genotypes homozygous for the *AC* haplotype (*AAAA/CCCC*).

DISCUSSION

In this article we report the first diagnostic SNP markers, which explain nearly half the genetic variation of the potato field resistance to late blight that is not compromised by late plant maturity. Our data offer significant progress toward improving the field resistance of new potato cultivars to this important disease by marker-assisted breeding. Available passport descriptors for potato varieties include scores for field resistance of foliage and tubers to late blight and maturity type (http://www.europotato.org). However the resistance scores are not corrected for the maturity effect. Heretofore QTL mapping studies, which discriminated between resistance and plant maturity, analyzed F_1 families descended from two heterozygous, diploid, or tetraploid parents (VISKER *et al.* 2003; BORMANN *et al.* 2004; BRADSHAW *et al.* 2004), thus limiting the diagnostic power of QTL-linked markers to specific genotypes and their progeny. In this study, we analyzed populations of tetraploid, heterozygous genotypes, which originated from multiple crosses among different parents and represent state-of-the-art potato breeding materials. Very late maturing genotypes were excluded from the populations to facilitate the identification of markers diagnostic for late blight resistance not compromised by late maturity. The populations were evaluated in replicated field trials separately for resistance to late blight and plant maturity, which allowed the correction of resistance for the maturity effect. The marker–trait associations identified in our study are expected to have diagnostic power for most potato breeding programs in temperate climates and aid in the early identification of individuals carrying specific alleles for maturity corrected resistance to late blight.

Polyploid, outcrossing plant species such as *S. tuberosum* provide a challenge for population genetics approaches. The full genotypic information requires the inclusion of the allele dosage, and the inference of

TABLE 2

Locus name, description, and chromosome location for the 24 loci tested for association with rAUDPC, PM, and MCR

Locus	Locus description[a]	Chr. no.
StPAD4-1	Defense signaling gene homologous to Arabidopsis *PAD4* (Pajerowska *et al.* 2005), linked to late blight QRL *Pin2A*	II
GP321	Sequence tagged site (STS) linked to late blight QRL *Pin2B* (Oberhagemann *et al.* 1999; Sliwka *et al.* 2007)	II
Stp23	Gene encoding L-type starch phosphorylase, linked to resistance "hot spot" including late blight QRL *Pin3A*	III
StSGT1-1	Defense signaling gene homologous to Arabidopsis *SGT1-1* (Pajerowska *et al.* 2005), linked to late blight QRL *Pin3B*	III
S1b3/RBO	Expressed sequence tag (EST) encoding a candidate gene (defense response, strong homology to respiratory burst oxidase (Gebhardt *et al.* 2003), linked to late blight QRL *Pin3C*	III
St4cl	Defense response gene encoding 4-coumarate: CoA ligase, linked to late blight QRL *Pin3C*	III
BA106c14t7	End of a BAC insertion containing a member of the *Gro1* resistance gene family (Paal *et al.* 2004), linked to a resistance hot spot including late blight QRL *Pin4A*	IV
StAOS1	Defense signaling gene homologous to Arabidopsis *AOS* (Pajerowska *et al.* 2005)	IV
P4h6	EST encoding a gene with nucleotide binding (NB) and leucine-rich repeat (LRR) domains linked to a resistance hot spot including late blight QRL *Pin5A*	V
BA213c14t7	End of a BAC insertion containing the *R1* resistance gene (Ballvora *et al.* 2002, 2007) linked to a resistance hot spot including late blight QRL *Pin5A*	V
R1	*R1* gene conferring hypersensitive resistance to late blight (Ballvora *et al.* 2002)	V
239E4left	STS marker linked to the *H1* nematode resistance gene (Bakker *et al.* 2004) and late blight QRL *PinVB*	V
StSGT1-2	Defense signaling gene homologous to Arabidopsis *SGT1-2* (Pajerowska *et al.* 2005), linked to a resistance hot spot including late blight QRL *PinVIA*	VI
StEDS1	Defense signaling gene homologous to Arabidopsis *EDS1* (Pajerowska *et al.* 2005)	VI
BA228g19t7	End of a BAC insertion containing resistance gene-like sequences (Rickert *et al.* 2003)	VII
BA229c2t3	End of a BAC insertion physically linked to the *Gro1* resistance gene family	VII
BA73e8t3	End of a BAC insertion containing resistance gene-like sequences (Rickert *et al.* 2003)	VIII
CP105	EST marker linked to the R_{ber} (Ewing *et al.* 2000) gene for resistance to late blight	X
BA81l15t3	End of a BAC insertion containing resistance gene-like sequences (Rickert *et al.* 2003)	X
StAOS2	Defense signaling gene, functional homolog of Arabidopsis *AOS* (Pajerowska *et al.* 2005; Pajerowska-Mukhtar *et al.* 2008) linked to a resistance hot spot including late blight QRL *Pin11B*	XI
R3a	*R3a* gene conferring hypersensitive resistance to late blight (Huang *et al.* 2005) linked to a resistance hot spot including late blight QRL *Pin11B*	XI
R3b	Member of the *R3* resistance gene family (Huang 2005), linked to a resistance hot spot including late blight QRL *Pin11B*	XI
OSM8e	Defense response gene, osmotin like (Castillo Ruiz *et al.* 2005)	XI
GP122	STS marker linked to the $Ry\text{-}f_{sto}$ resistance gene (Flis *et al.* 2005)	XII

[a] Approximate locations of QRL and *R* genes are shown in Figure 2. For further information and references see the Solanaceae function map for resistance at http://www.gabipd.org/database/maps.shtml.

haplotypes from the SNP data is not as straightforward as when dealing with inbreeding plant species. Identifying the associations between SNPs and the quantitative traits reported here required the application of a mixed model, which included in addition to population structure and kinship the allele substitution effect as well as bi-, tri-, and tetragenic interaction effects of the SNP alleles. The importance of considering the allele dosage in the association model is demonstrated by the allele substitution effects of the most significant SNP markers StAOS2_snp691 and StAOS2_snp692 on rAUDPC, PM, and MCR. This model may be useful for association mapping not only in potato but also in other polyploid plant species such as sugarcane or alfalfa.

The length of potato genetic maps is on the order of 10 M (Gebhardt *et al.* 1991) and the physical genome size is on the order of 800–900 Mbp (Arumuganathan and Earle 1991). When scoring 238 markers at 24 loci, only a minute portion of the potato genome (~14 kbp) was actually tagged. This was nevertheless sufficient to detect associations between SNP markers at 13 loci and rAUDPC, MCR, and PM, which collectively explained 56, 51, and 33% of the genetic variance, respectively, considering both additive and interaction effects. This high "hit rate" may have several reasons. First, the *a priori* selection of loci for the association study on the basis of knowledge of the positions on potato genetic maps of resistance factors and candidate genes targeted the

TABLE 3

SNP markers associated with rAUDPC, PM, or MCR ($P < 0.001$)

Locus	Chr. no.	Allele *1/2*	Trait	*a*	*P*-value *a*	ρ1	$h_1/h_2/h_3$	*P*-value *h*	ρ2
BA106c14t7_SNP300	IV	*T/G*	rAUDPC	$2.3e^{-3}/0$	$7.6e^{-1}$	0.0^{NS}	$6.9e^{-2}/-3.2e^{-2}/6.2e^{-3}$	$3.0e^{-4}$	9.3
StAOS2_SNP678	XI	*A/G*	rAUDPC	$-3.2e^{-2}/0$	$6.4e^{-5}$	8.6	$5.3e^{-2}/7.8e^{-2}/3.5e^{-2}$	$4.6e^{-2}$	2.8^{NS}
StAOS2_SNP681	XI	*T/A*	rAUDPC	$-3.1e^{-2}/0$	$6.8e^{-5}$	8.6	$5.3e^{-2}/7.4e^{-2}/3.5e^{-2}$	$5.4e^{-2}$	2.6^{NS}
StAOS2_SNP691	XI	*A/G*	rAUDPC	$-5.3e^{-2}/0$	$5.1e^{-20}$	39.3	$2.7e^{-2}/5.3e^{-2}/2.3e^{-2}$	$2.2e^{-2}$	2.8^{NS}
StAOS2_SNP692	XI	*C/G*	rAUDPC	$-5.3e^{-2}/0$	$1.4e^{-22}$	43.5	$3.4e^{-3}/-1.8e^{-2}/-5.1e^{-2}$	$2.0e^{-1}$	2.4^{NS}
StAOS2_SNP727	XI	*C/T*	rAUDPC	$-3.3e^{-2}/0$	$2.8e^{-5}$	9.5	$2.6e^{-2}/8.5e^{-2}/3.4e^{-2}$	$5.1e^{-1}$	3.6^{NS}
StAOS2_SNP744	XI	*A/G*	rAUDPC	$-4.0e^{-2}/0$	$2.2e^{-10}$	21.0	NA		
StAOS2_SNP774	XI	*C/T*	rAUDPC	$-3.3e^{-2}/0$	$2.4e^{-5}$	9.6	$3.1e^{-2}/9.2e^{-2}/3.0e^{-2}$	$1.5e^{-2}$	4.0^{NS}
StAOS2_SNP879	XI	*A/G*	rAUDPC	$-3.1e^{-2}/0$	$7.4e^{-6}$	10.9	$4.7e^{-2}/5.5e^{-2}/3.0e^{-2}$	$1.4e^{-1}$	1.4^{NS}
StAOS2_SNP900	XI	*A/T*	rAUDPC	$-2.8e^{-2}/0$	$4.3e^{-5}$	9.0	$1.4e^{-2}/4.4e^{-2}/2.3e^{-2}$	$2.5e^{-1}$	0.6^{NS}
StAOS2_SNP954	XI	*C/T*	rAUDPC	$-3.1e^{-2}/0$	$5.2e^{-5}$	8.8	$5.2e^{-2}/7.2e^{-2}/3.3e^{-2}$	$6.1e^{-2}$	2.4^{NS}
Simultaneous fit						47.7			0.1
StSGT1-1_SNP169	III	*T/C*	PM	$-2.7e^{-1}/0$	$5.9e^{-7}$	12.5	NA		
S1b3_SNP175	III	*A/G*	PM	$-2.5e^{-1}/0$	$1.1e^{-6}$	10.8	NA		
P4H6_SNP248	V	*G/C*	PM	$4e^{-3}/0$	$1.9e^{-1}$	0.9^{NS}	$6.7e^{-1}/8.0e^{-1}/1.0$	$2.9e^{-4}$	5.8
BA213c14t7_SNP457	V	*T/G*	PM	$-2.3e^{-1}/0$	$7.5e^{-6}$	11.2	NA		
239E4left_INDEL211	V	*T/N* [a]	PM	$-2.3e^{-1}/0$	$6.8e^{-4}$	7.5	NA		
239E4left_SNP133	V	*G/A*	PM	$-2.5e^{-1}/0$	$8.8e^{-4}$	7.2	$1.6e^{-1}/-2.7e^{-1}/-3.1e^{-1}$	$4.3e^{-1}$	0^{NS}
StAOS2_SNP691	XI	*A/G*	PM	$-3.7e^{-1}/0$	$2.3e^{-5}$	13.2	$-3.0e^{-3}/2.6e^{-1}/9.6e^{-2}$	$7.1e^{-1}$	0.0^{NS}
StAOS2_SNP692	XI	*C/G*	PM	$-3.6e^{-1}/0$	$1.5e^{-5}$	13.7	$-3.8e^{-1}/-3.7e^{-1}/-3.1e^{-1}$	$5.4e^{-1}$	0.0^{NS}
Osm8e_SNP873	XI	*C/T*	PM	$-2.4e^{-1}/0$	$1.8e^{-1}$	1.1^{NS}	$-9.3e^{-1}/-3.1e^{-1}/6.9e^{-2}$	$2.2e^{-4}$	6.2
GP122_SNP231	XII	*T/A*	PM	$-6.7e^{-1}/0$	$1.8e^{-4}$	5.4	NA		
GP122_SNP440	XII	*G/T*	PM	$-7.2e^{-1}/0$	$1.9e^{-4}$	5.2	NA		
Simultaneous fit						23.4			4.8
BA106c14t7_SNP300	IV	*T/G*	MCR	$-3.0e^{-3}/0$	$6.7e^{-1}$	0.0^{NS}	$3.6e^{-2}/-3.7e^{-2}/-8.0e^{-3}$	$3.2e^{-3}$	10.6
StAOS1_SNP803	IV	*A/G*	MCR	$-2.6e^{-2}/0$	$6.4e^{-4}$	7.0	NA		
StAOS2_SNP691	XI	*A/G*	MCR	$-3.3e^{-2}/0$	$1.9e^{-13}$	30.9	$2.6e^{-2}/4.0e^{-2}/2.1e^{-2}$	$2.8e^{-2}$	2.8^{NS}
StAOS2_SNP692	XI	*C/G*	MCR	$-3.4e^{-2}/0$	$7.2e^{-16}$	36.1	$1.9e^{-2}/1.0e^{-3}/-1.9e^{-2}$	$1.7e^{-1}$	0.9^{NS}
StAOS2_SNP744	XI	*A/G*	MCR	$-2.3e^{-2}/0$	$6.5e^{-7}$	14.8	NA		
StAOS2_SNP774	XI	*C/T*	MCR	$-1.9e^{-2}/0$	$8.5e^{-4}$	6.8	$4.3e^{-2}/7.0e^{-2}/2.9e^{-2}$	$9.7e^{-3}$	5.4
StAOS2_SNP879	XI	*A/G*	MCR	$-2.1e^{-2}/0$	$6.7e^{-5}$	9.8	$5.4e^{-2}/5.5e^{-2}/2.7e^{-2}$	$2.4e^{-2}$	4.1^{NS}
StAOS2_SNP900	XI	*A/T*	MCR	$-2.1e^{-2}/0$	$2.5e^{-5}$	11.0	$2.7e^{-2}/5.2e^{-2}/1.8e^{-2}$	$2.2e^{-2}$	4.1^{NS}
StAOS2_SNP954	XI	*C/T*	MCR	$-1.9e^{-2}/0$	$9.8e^{-4}$	6.7	$4.0e^{-2}/6.4e^{-2}/2.9e^{-2}$	$1.4e^{-2}$	4.9^{NS}
Simultaneous fit						47.2			2.7

a, allele substitution effect, *i.e.*, the effect of replacing one copy of allele 2 by allele 1. ρ1, proportion of the genotypic variance explained by the allele substitution effects *a*. h_1, h_2, and h_3 are the bi-, tri-, and tetragenic interaction effects (the interaction effects of the heterozygous genotypes 1222, 1122, and 1112), respectively, at the corresponding marker locus. ρ2 is the proportion of the genotypic variance explained by h_1, h_2, and h_3. NA, not applicable: interaction effects could not be estimated, because both homozygous genotype classes did not occur in the data set; NS, not significant.

[a] The indel consisted of a single nucleotide insertion/deletion.

genotyping effort to genomic regions that are indeed relevant for field resistance to late blight (Oberhagemann *et al.* 1999; Bormann *et al.* 2004). Second, in breeding materials such as used in this study, large haplotype blocks exist due to a limited number of meiotic generations separating the individuals in the populations. This facilitates the detection of indirect associations due to LD between SNP markers and trait alleles. Available evidence suggests that LD in tetraploid potato varieties and breeding clones can extend over several centimorgan (Simko *et al.* 2006; Li *et al.* 2008). This is also indicated by the fact that loci showing associations with rAUDPC and/or MCR (*BA106c14t7*, *BA213c14t7*, *BA228g19t7*, *GP122*) did not encode candidate genes themselves but were physically linked to candidate genes such as *R* genes or *R* gene-like sequences (Rickert *et al.* 2003; Flis *et al.* 2005). Third, numerous genes controlling field resistance to late blight and maturity may be widely distributed in the potato genome, such that any set of markers chosen will show some associations. The available QTL maps for resistance to late blight and plant maturity do not support this possibility but they also cannot exclude it. Whole genome association studies as performed in maize (Belo *et al.* 2008) are required to resolve this issue.

Associations with highly significant effects on rAUDPC and MCR were detected by two neighboring SNPs at the *StAOS2* locus on potato chromosome XI. The same two SNPs were also associated with plant maturity. The fact that there was no phenotypic correlation between

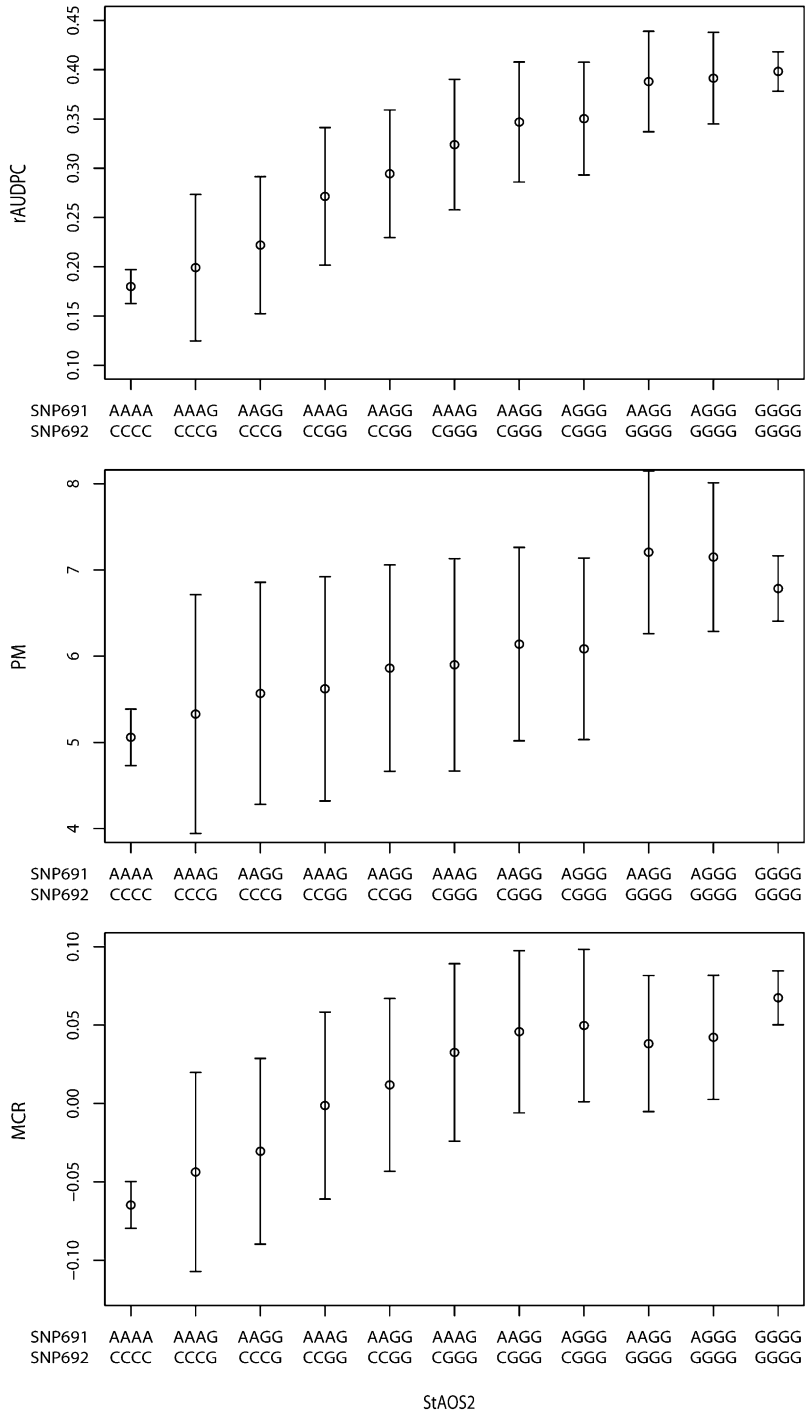

FIGURE 5.—Plot of the expected phenotypic mean values and their standard errors for the genotypes observed at *StAOS2_SNP691* and *StAOS2_SNP692*. Due to LD between the two adjacent SNPs, only 11 of 25 possible genotypes were observed in the population. Twenty-four and 7 individuals were scored homozygous for the *AC* and *GG* allele, respectively. The expected phenotypic mean values were calculated from the allele substitution effects as well as the bi-, tri-, and tetragenic interaction effects, which were calculated on the basis of the mixed-model described in MATERIALS AND METHODS comprising both markers simultaneously.

PM and MCR reduces the incidence of markers being associated with both traits but does not completely preclude it. It is therefore possible that markers associated with MCR also have effects on maturity. However, the overlap between the markers associated with PM and MCR was small (Table 3, supplemental Table 2). Moreover, StAOS2_snp691 and StAOS2_snp692 each explained 13 and 14% of the genetic variance of plant maturity but 31 and 36% of the genetic variance of MCR. The genotype class homozygous for the SNP alleles *A* and *C* associated with resistance scored on average 5 for maturity (Figure 5), which corresponds to maturity class "mid-early." Molecular variants of *StAOS2* are therefore the first shown to have general diagnostic potential for field resistance to late blight in the mid-early maturity class. The residual effect on plant maturity may either be pleiotropic or result from one or more physically linked gene(s). In the latter case, phenotypic selection for earliness among homozygous *AAAA/CCCC* individuals will enrich recombinants between resistance alleles at the *StAOS2* locus and "lateness" alleles at linked loci for plant maturity. Future availability of an annotated

whole genome sequence of potato (Zhu *et al.* 2008) (www.potatogenome.net) will facilitate further research on this topic.

Some of the SNPs tagging major QTL for resistance to late blight and plant maturity on potato chromosome V identified in previous linkage studies (Collins *et al.* 1999; Oberhagemann *et al.* 1999; Visker *et al.* 2003), showed highly significant associations with PM, minor associations with rAUDPC, but no association with MCR. The genetic variance of PM explained in the population studied here was much smaller than in the previous QTL linkage studies. This is likely the consequence of preselecting the genetic material against very late maturing genotypes, which excluded alleles with large effects in the QTL region of potato chromosome V. This preselection may also be the reason for the finding that in the present association experiment, the *R1* locus was not associated with any of the traits. This contrasts the previous findings in a historical collection of potato cultivars, where *R1* was associated with late blight resistance not corrected for the plant maturity effect (Gebhardt *et al.* 2004).

The strong association between several SNPs at the *StAOS2* locus and field resistance to late blight as reported here, in combination with previous functional analysis of natural *StAOS2* variants (Pajerowska-Mukhtar *et al.* 2008) support the model of *StAOS2* being indeed one of the causal genes that control quantitative disease resistance in potato by natural variation. *StAOS2* was selected for the association experiment as a positional and functional candidate gene for QRL against late blight (Oberhagemann *et al.* 1999) and black leg/tuber soft rot caused by *Erwinia carotovora* ssp *atroseptica* (Zimnoch-Guzowska *et al.* 2000) on the short arm of potato chromosome XI (Pajerowska *et al.* 2005). On the same chromosome arm map, distal to the *StAOS2* locus, several *R* genes for late blight resistance encoded by a large cluster of nucleotide binding–leucine rich repeat (NB–LRR)-type genes, one of which is the functional *R3a* resistance gene (Huang *et al.* 2005). Members of this known gene family as well as other linked, yet unknown genes are also candidates for underlying the QRL on this chromosome arm. The two members of the *R3* resistance gene family tested in our experiment, *R3a* and *R3b*, were however not associated with rAUDPC, MCR, or PM. At present, physical distances, gene content, and LD decay are unknown for this region of the potato genome. The available genetic data do not permit the distinction between direct and indirect associations or a combination of both. Increasing the genetic resolution by association analysis in larger populations with a large number of markers tagging the whole chromosome arm (Ducrocq *et al.* 2008) may resolve this ambiguity.

In any case, functional analysis of natural variants of the genes that are associated with late blight resistance is required. *StAOS2* encodes allene oxide synthase 2, a key enzyme in the biosynthesis of the defense signaling molecule jasmonic acid (JA) (Liechti and Farmer 2002), which plays a role in quantitative resistance to late blight. This was recently shown by dsRNAi-mediated silencing of *StAOS2* in the diploid potato genotype G87 (Oberhagemann *et al.* 1999), which compromised the quantitative late blight resistance expressed in G87 (Pajerowska-Mukhtar *et al.* 2008). Quantitative complementation analysis of five functional *StAOS2* alleles in *aos* mutant background of *Arabidopsis thaliana* demonstrated that *StAOS2* alleles linked to increased resistance in potato (alleles *StAOS2-1* and *StAOS2-6*) complemented *aos* mutant phenotypes of Arabidopsis better than alleles linked to increased susceptibility (alleles *StAOS2-7* and *StAOS2-8*). Better complementation phenotypes correlated with higher levels of JA and the precursor OPDA (Pajerowska-Mukhtar *et al.* 2008). Some SNP positions and alleles differed, however, between the five fully sequence-characterized *StAOS2* alleles and the 14 *StAOS2* SNPs scored in the tetraploid breeding populations of this study. This indicated that the *StAOS2* alleles present in the tetraploid germplasm were not all the same as the *StAOS2* alleles described in Pajerowska-Mukhtar *et al.* (2008), which originated from diploid, more exotic germplasm generated in prebreeding programs (Oberhagemann *et al.* 1999; Zimnoch-Guzowska *et al.* 2000). In fact, the variation of resistance explained by *StAOS2* alleles in the experimental, diploid QTL mapping populations was with 10–14% (Pajerowska-Mukhtar *et al.* 2008), much smaller than the variance explained in the association experiment reported here (39–43% for rAUDPC; 31–36% for MCR).

Three of the 10 SNPs in the *StAOS2* amplicon that were associated with field resistance to late blight (StAOS2_snp691, StAOS2_snp692, and StAOS2_snp727) cause two nonconservative amino acid changes. The two most strongly associated SNPs, StAOS2_snp691 and StAOS2_snp692, encode threonine (haplotype *AC*), glycine (haplotype *GG*), or serine (haplotype *AG*) at amino acid position 231. The previously described *StAOS2-8* allele represents a fourth haplotype "*AT*" and codes for isoleucine at position 231 (Pajerowska-Mukhtar *et al.* 2008). The *AT* haplotype was not detected in the tetraploid breeding population analyzed here. It was isolated from a novel, diploid source for resistance to *E. carotovora* ssp. *atroseptica* (Zimnoch-Guzowska *et al.* 2000), which has not yet been introgressed in the tetraploid gene pool. Thus, this high variability at amino acid position 231 in the *St*AOS2 protein may have functional relevance, for example as target site for post-translational modifications. Which amino acid changes in the *St*AOS2 holoenzyme may be relevant to its function and the mechanism by which these changes cause differential effects on jasmonate levels remains to be elucidated.

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