Mutations at the Asc locus of tomato confer resistance to the fungal pathogen Alternaria alternata f. sp. lycopersici
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Published in:
Theoretical and Applied Genetics

DOI:
10.1007/BF00221904

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1996

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Mutations at the Asc locus of tomato confer resistance
to the fungal pathogen Alternaria alternata f. sp. lycopersici

Abstract The fungal pathogen Alternaria alternata f. sp. lycopersici produces host-selective AAL-toxins that cause Alternaria stem canker in tomato. Susceptibility to the disease is based on the relative sensitivity of the host to the AAL-toxins and is controlled by the Asc locus on chromosome 3L. Chemical mutagenesis was employed to study the genetic basis of sensitivity to AAL-toxins and susceptibility to fungal infection. Following the treatment of seeds of a susceptible line with ethyl methanesulphonate (EMS), resistant M2 mutants were obtained. Most plants with induced resistances showed toxin-sensitivity responses that were comparable to those of resistant control lines carrying the Asc locus. In addition, genetic analysis of the mutagenised plants indicated that the mutations occurred at the Asc locus. Furthermore, novel mutants were identified that were insensitive to the AAL-toxins at the seedling stage but toxin-sensitive and susceptible to fungal infection at mature stages. No AAL-toxin-insensitive insertion mutants were identified following a transposon mutagenesis procedure. Molecular mechanisms involved in host defence against A. a. lycopersici are discussed.

Key words AAL-toxins • Asc locus • Alternaria stem canker disease • EMS and transposon mutagenesis • Susceptibility genes

Introduction

Necrotrophic fungal plant pathogens commonly secrete phytoxins, and depending on whether species other than the hosts of the pathogens are affected, the toxins are classified as host-selective or non-host-selective toxins. Host-selective toxins are the primary factors for pathogenicity and determine the specificity of plant-necrotrophic pathogen interactions at the molecular level (Walton and Panaccione 1993). Host sensitivity to various phytoxins is conferred by single genes that can be inherited in a dominant, semi-dominant, recessive or maternal manner (Yoder 1980). Plant genes that confer sensitivity to toxins often encode target enzymes involved in various processes (Mazur and Falco 1989; Walton and Panaccione 1993). Inhibition of these molecular targets by the toxins results in local cell death and establishes a nutritional source for the necrotrophs. The deleterious effects of the toxins are avoided if the host plants lack toxin uptake or metabolic conversion mechanisms, or following biochemical detoxification of the toxin (Meeley et al. 1992; Johal and Briggs 1993). Alternatively, pathogen ingress can be resisted if plants express toxin-insensitive forms of the molecular targets encoded by modified sensitivity genes. To search for resistance by field selection, crop breeders have traditionally used the natural variation within plant species. Also, resistance to pathogens and insensitivity to diverse phytoxins have frequently been induced by mutational procedures (Van den Bulk 1991; Jørgensen 1992; Feys et al. 1994).

The necrotrophic fungus Alternaria alternata f. sp. lycopersici produces AAL-toxins that function as chemical determinants of the Alternaria stem canker disease (reviewed by Van der Biezen et al. 1994a). The host range of the pathogen is confined to some tomato cultivars (Lycopersicon esculentum) and to the wild tomato, L. cheesmanii (Van der Biezen et al. 1995). Insensitivity to the host-selective AAL-toxins is expressed at the cellular level, in all tissues, and is controlled by the semi-dominant Alternaria stem canker (Asc) gene located on the long arm of tomato chromosome 3 (Van der Biezen et al. 1994b). Three alleles have been identified at the Asc locus: two alleles from tomato that confer sensitivity (asc1) and moderate insensitivity (Asc12) to AAL-toxins, and the Asc12 allele from L. pennellii that confers high insensitivity (Van der
Biezen et al. 1995). The AAL-toxins are thought to act on the sphingolipid biosynthesis pathway by inhibiting the enzyme sphinganine N-acyltransferase, which catalyses the conversion of sphinganine to ceramide. It has been suggested that the AAL-toxins are structural homologues of sphinganine that are erroneously recognised by this enzyme as substrate analogues (Merrill et al. 1993).

We previously hypothesised that resistance and susceptibility to A. a. lycopersici are based on the differential sensitivities to AAL-toxins conferred by different alleles at the Asc locus (Van der Biezen et al. 1993, 1994a). Here, we describe a genetic approach to test the possibility that mutagenesis of genes involved in sensitivity to AAL-toxins results in insensitivity to the toxins. Genes could potentially be mutated which encode products that interact directly with the AAL-toxins, or alternatively, that establish an AAL-toxin perception and transduction pathway leading to sensitivity. Chemically mutagenised M₂ seeds of an A. a. lycopersici-susceptible tomato line were screened for insensitivity to AAL-toxins. Toxin-insensitive mutants occurred at a high frequency, and it was shown that lost sensitivity to AAL-toxins coincided with resistance to A. a. lycopersici. In addition, mutants were obtained that showed differential sensitivity to the AAL-toxins during development. Genetic characterisation of independent mutations revealed that the induced resistances were conferred by single semi-dominant genes that mapped at the position of the Asc locus. Possible mechanisms for the interaction between tomato and A. a. lycopersici, determined by alleles at the Asc locus, are discussed.

**Materials and methods**

**Plant materials**

Near-isogenic lines (isolines) differing with respect to the Asc locus were obtained from D. Gilchrist, UC Davis, USA. The A. a. lycopersici-susceptible markerline (sy/sy, asc/asc, sf/sf) containing the phenotypic seedling markers sy (sunny: cotyledons bleached, leaves emerge yellow) and sf (solanifolia: leaves entire) and the resistant line with the sf marker (Asc/Asc, sf/sf) were selected from an F₂ derived from LA1182×LA1444 (obtained from C. Rick, Tomato Genetics Stock Center, UC Davis, USA). The Ds-containing ET90 line (cv ‘VF36’) was obtained from K. Theres, Universität zu Köln, Germany, and the Ac-containing TS101 line (cv ‘VF36’) was obtained from J. Yoder, UC Davis, USA.

**Genetic crosses and linkage analysis**

All controlled crosses were performed in the greenhouse using standard emasculation and pollination techniques. Heterozygotes (Asc/asc) were made by reciprocal crosses between the susceptible (asc/asc) and resistant (Asc/Asc) isolines; the F₁ progenies were verified for heterozygosity by segregation analysis of Asc in the F₂. For transposon mutagenesis of the asc allele, susceptible lines (asc/asc) were used. To that end, the non-autonomous Ds element located on chromosome 3L of the ET90 line (Ds/Ds, asc/asc) was transactivated by a stabilised, and thus immobile, Ac element (sAc) following reciprocal crosses with the TS101 line (sAc/sAc, asc/asc), and outcrosses were made with the markerline (sy/sy, asc/asc, sf/sf) as the staminate parent. Plants were grown under standard greenhouse conditions (20°C–25°C, 60% relative humidity, 5000 lux). Recombination values and map positions were calculated using the computer package JOINMAP (version 1.3) with a critical LOD score of 3.0 for linkage and 0.05 for mapping using Kosambi’s mapping function (Stam 1993).

**EMS mutagenesis**

Tomato seeds from the A. a. lycopersici-susceptible isoline (asc/asc) were submerged in freshly prepared 60 mM ethyl methanesulphonate (C₂H₅SO₂CH₃) solutions (EMS, Eastman Kodak Company, USA) buffered in 10 mM NaH₃PO₄ (pH 5.7) for 24 h on a gyratory shaker in the dark at 22°C. M₁ seeds were rinsed with tapwater prior to sowing in soil (63 seeds per tray). The resulting plants were transferred to pots and allowed to self-pollinate to produce M₂ seeds that were harvested from individual fruits of the first two clusters and then analysed per fruit separately. The viability of the M₁ seeds was measured by the germination delay and the germination percentage relative to that of untreated control plants. The frequency of random mutations was determined by scoring various visible phenotypes in the M₁, M₂, and M₃ generations and was expressed as the percentage of M₂ families segregating for mutations.

**Seedling and leaf bioassays**

Seedlings and leaflets were assayed for sensitivity to AAL-toxins following essentially identical procedures (Van der Biezen et al. 1995). Sterilised seeds [1% NaClO (v/v) for 20 min] or three to five leaflets from greenhouse-grown plants (two to three true leaf stage) were placed on filter papers that had been saturated with cell-free culture filtrates (CFCF) or purified AAL-toxin dilutions in 9-cm diameter petri dishes and left in a climate chamber under standard conditions (16 h, 3000 lux, 22°C). The bioassays were terminated after defined duration to avoid differences in symptoms as a function of time. Leaf necrosis development was scored after 3 days. Toxic severity ratings were determined visually by assessing the percentage of the leaflet lamina area with necrotic symptoms and were expressed in the Leaf Sensitivity Index (LSI) with 0=no necrosis, 1=1–25% necrosis, 2=26–50% necrosis, 3=51–75% necrosis, and 4=76–100% necrosis. Leaf bioassays were carried out in triplicate. The inhibition of seedling growth was determined after 8 days by measurement of the seedling lengths (hypocotyl plus root) relative to those germinated on water.

**Fungal infections, fungal culture filtrates and AAL-toxins**

All inoculations were carried out on plants that were grown in the greenhouse until the two to three true leaves stage either by cutting off the lowest leaves at the petiole base and placing a small amount of A. a. lycopersici spores (10⁷ spores ml⁻¹) and mycelium directly on the wounds, or by spraying the plants with fungal spores (10⁶ spores ml⁻¹) followed by incubation for 72 h at 100% humidity, 20°C and 3000 lux. Subsequently, the plants were grown under greenhouse conditions (20–25°C, 60% relative humidity, 5000 lux), and the final observations were made 15 days later when similarly inoculated control plants (asc/asc) had developed Alternaria stem canker symptoms. Fungal cultures and cell-free culture filtrates (CFCFs) were prepared, and AAL-toxins were purified and characterised as described previously (Van der Biezen et al. 1995).

**DNA analysis**

Southern blot hybridisations and preparation of radiolabeled DNA probes were performed as described previously (Van der Biezen et al. 1995).
Table 1 Mutant frequencies in the $M_2$ generation following EMS tomato seed treatment

<table>
<thead>
<tr>
<th>Total number of lines</th>
<th>Mutant phenotype</th>
<th>Number of mutant lines (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>Lethal seedling/albino</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Turgor incompetent seedling</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3-4 cotyledons</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Chlorotic cotyledons</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Dark green cotyledons</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Deformed cotyledons</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>High anthocyanin in cotyledons</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Necrotic cotyledons</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Yellow cotyledons/leaves</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>No anthocyanin in hypocotyl</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>No apical meristem</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Small habitus/dwarf</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Deformed leaves</td>
<td>1</td>
</tr>
<tr>
<td>581</td>
<td>Insensitive to AAL-toxins</td>
<td>44</td>
</tr>
</tbody>
</table>

a Mutant segregation 3:1 significant at $P<0.05$

Results

Recovery of resistant mutants

Following treatment with 60 mM EMS, 15435 tomato seeds from the susceptible isolate ($asc/asc$) were sown. Several parameters were used as references for the mutagenic effect of the EMS seed treatment. First, the low relative viability (47%) of the $M_1$ seeds, the large proportion of $M_1$ plants with severe deviant phenotypes (11.7% no apical meristem, 5.2% physiological deviant, 11.4% chimeric for chlorophyll deficiency) and the low frequency (11.6%) of $M_1$ plants that produced viable $M_2$ seeds showed the effectiveness of the mutagenesis procedure. Secondly, a large proportion of the $M_2$ families segregated for visible mutant phenotypes, of which 32% had more than one mutation (Table 1). The 100 untreated control plants developed normally, and no mutants were identified in self progenies.

The development of the seedlings in the presence of AAL-toxins was an excellent marker for the degree of sensitivity (Fig. 1). The simple assays were sensitive in discriminating the resistant ($Asc/Asc$) and susceptible ($asc/asc$) plants and, moreover, as a result of semi-dominance, the heterozygotes ($Asc/asc$) could be distinguished. The 581 $M_2$ families were tested in seedling assays ($n=50$) for insensitivity to 100-times diluted cell-free culture filtrate (CFCF; approximately 0.2 $\mu M$ AAL-toxins). Forty-four $M_1$ plants were identified that produced progenies that segregated (5–75%) for CFCF-insensitive $M_2$ seedlings (Table 1). As a result of chimerism, 14 of these $M_1$ plants also had one to two fruits with only toxin-sensitive seeds, and insensitivity was inherited in a non-Mendelian manner. Furthermore, a considerable proportion (22%) of the CFCF-insensitive $M_2$ progenies also segregated for visible mutants. No CFCF-insensitive seedlings were identified in the self progenies of the 100 untreated control plants.

Analysis of insensitivity to AAL-toxins

Three CFCF-insensitive $M_2$ seedlings of each of the 44 lines were transferred to soil, grown to mature plants and subjected to leaf assays with 0.2 $\mu M$ AAL-toxins. To study the inheritance and to determine the genotypes of the selected $M_2$ plants, we analyzed $M_3$ progenies for segregation of induced insensitivity to AAL-toxins ($R^*$) in seedling assays ($n=100$). Insensitivity to AAL-toxins segregated in a Mendelian manner as a single, semi-dominant character in the progeny of plants heterozygous for the induced resistance ($R^*/r$). The leaves of 39 $M_2$ lines homo-
Fig. 2 AAL-toxin-induced inhibition of seedling development (%). Inhibition was measured as the percentage reduction in the lengths of EMS-induced resistant (homozygous $R^*/R^*$ or heterozygous $R^*/r$) and susceptible tomato seedlings ($n=60$) germinated in the presence of AAL-toxins compared to those germinated on water. Near-isogenic lines (Asc isolines) and the markerline (sy/sy, asc/asc, sf/sf) were used as controls.

Fig. 3 AAL-toxin-induced leaf necrosis (LSI). Sensitivity to AAL-toxins of EMS-induced resistant (homozygous $R^*/R^*$ or heterozygous $R^*/r$) and susceptible tomato lines was monitored by leaf bioassays (6 plants per line, three to five leaflets per dish in triplicate), and the percentage necrosis classified according to the Leaf Sensitivity Index (LSI) with: 0=no necrosis, 1=1–25% necrosis, 2=26–50% necrosis, 3=51–75% necrosis, 4=76–100% necrosis. Near-isogenic lines (Asc isolines) and the markerline (sy/sy, asc/asc, sf/sf) were used as controls.

Remarkably, the leaves of 5 $M_2$ plants that had been selected as CFCF-insensitive seedlings, were as sensitive as those from the susceptible isoline (asc/asc). This unusual characteristic proved to be heritable, since their $M_3$ progenies were also insensitive as seedlings but had sensitive leaves. However, in contrast to the stable resistant lines, these plants did not segregate for insensitivity to AAL-toxins in a Mendelian manner. Twenty $M_3$ progeny plants of each mutant were self-pollinated, and the $M_4$ seedlings ($n=60$) analysed for insensitivity to AAL-toxins. All 5 lines showed similar non-Mendelian inheritance: out of the 20 $M_3$ plants that were analysed per line, only between 2 and 4 $M_3$ individuals per line produced AAL-toxin-insensitive $M_4$ seedlings. Self-pollination of these plants again resulted in the non-Mendelian inheritance of this phenotype to the $M_5$. Two lines (M278 and M304) were analysed...
in more detail (Figs. 2 and 3). During seedling development these mutants displayed similar levels of AAL-toxin insensitivity as resistant control seedlings (Asc/Asc), and at the plant level these mutants were as sensitive to AAL-toxins as susceptible control plants (asc/asc). These mutants, although selected on the basis of AAL-toxin insensitivity at the seedling level, were susceptible to fungal infection at the plant level, thereby reaffirming the correlation between fungal infection and sensitivity to AAL-toxins.

Genetic mapping and allelism tests of induced resistances

For genetic mapping and segregation analysis, 9 plants with stable induced resistances (R*/R*) were crossed with a chromosome 3 markerline (sy/sy, asc/asc, sf/sf). The resulting F1 plants were self-pollinated to produce F2 families that segregated for R* and for the phenotypic markers sy and sf as single Mendelian characters (P>0.01). In all of the progenies, R* was unlinked to sy but linked to sf (Table 2). Similar results were obtained in control crosses with the resistant isolate (Asc/Asc), indicating that the nine independently obtained induced resistance genes (R*) were closely linked to the Asc gene on chromosome 3L.

To further test whether the induced resistance genes (R*) were allelic to the Asc gene, we reciprocally crossed 5 R* lines (M128, M284, M300, M428, M438) with the resistant markerline (Asc/Asc, sf/sf), and the resulting F1 plants were self-pollinated. The sf marker was used to verify the success of the crosses. If the genes were not allelic but linked in repulsion, toxin-sensitive F2 plants would be expected that carry recombinations between the R* genes and the Asc gene. An average of 1200 F2 seeds from each combination was analysed for AAL-toxin sensitivity in seedling assays and 60 plants were grown in soil to verify the segregation of sf. No recombinant seedlings were found that were sensitive to 0.2 μM AAL-toxins, indicating that all five R* genes and the Asc gene are possibly allelic.

Transposon mutagenesis of susceptibility

To inactivate the asc allele by transposon mutagenesis with the Activator (Ac)/Dissociation (Ds) system from maize (Van der Biezen et al. 1994c), the Ds-containing ET90 transformant and the stabilised Ac (sAc)-carrying TS101 line were used (Knapp et al. 1994; Lassner et al. 1989). Both transposon containing T-DNAs had been transferred to the A. a. lycopersici susceptible and AAL-toxin-sensitive tomato cv ‘VF36’ (asc/asc). Integration of the restriction fragment length polymorphism (RFLP) mapping data of ET90 with that of the Asc locus (Van der Biezen et al. 1994, 1995) by the JOINMAP computer programme (Stam 1993) resulted in a calculated genetic distance of 9.6 cM between the Ds element and the Asc locus. Two hundred double-hemizygous F1 plants (sAc, Ds) were self-pollinated and outcrossed (OC1) to the susceptible markerline (sy/sy, asc/asc, sf/sf). Southern blot hybridisations showed that Ds was somatically active and that independently transposed Ds elements were germinally transmitted with a frequency of 7.5% (not shown). In total 135,000 F2 and OC1 progenies were screened for loss of sensitivity to CFCF by seedling assays, but no insensitive seedlings were obtained. The recovery of an insertion mutant depended on the conditions that insertional mutagenesis of the susceptibility allele, using the Ds element at the ET90 position, resulted in a viable mutant that was insensitive to AAL-toxins. Estimations that included the frequency of the germinal Ds transpositions and the strong preference (80%) for transposition to genetically linked sites (<10 cM) predicted a mutation rate in the range of 3.5×10^-5 (Van der Biezen et al. 1994c). Ac/Ds mutation frequencies in maize (Gierl and Saedler 1992) and tomato (Jones et al. 1994) were on average ten times higher, most likely as a result of the smaller genetic distances (4 cM) between the initial transposition positions and the target genes. On the basis of the mutation frequency and the size of the analysed population, it was calculated that a high probability (99%) of finding an insertion mutant had been reached.

Table 2 Segregation data and recombination (Rec) values of EMS-induced loci (R*) conferring insensitivity to AAL-toxins, and of the classical marker sf on the long arm of tomato chromosome 3 determined in F2 populations

<table>
<thead>
<tr>
<th>R* lines</th>
<th>Total</th>
<th>Segregation data</th>
<th>χ²</th>
<th>Rec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R*-Sf</td>
<td>R*-sf</td>
<td>r-Sf</td>
</tr>
<tr>
<td>Asc isolate</td>
<td>447</td>
<td>300</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td>M128</td>
<td>228</td>
<td>155</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>M284</td>
<td>249</td>
<td>170</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>M285</td>
<td>222</td>
<td>150</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>M300</td>
<td>239</td>
<td>162</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>M323</td>
<td>245</td>
<td>158</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>M372</td>
<td>221</td>
<td>151</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>M428</td>
<td>232</td>
<td>152</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>M438</td>
<td>214</td>
<td>143</td>
<td>16</td>
<td>21</td>
</tr>
</tbody>
</table>

Notes:
- R* = leaves insensitive, r = leaves sensitive to 0.2 μM AAL-toxins
- χ² for independent assortment; if χ² > 11.3 then P < 0.01
- Recombination percentages with standard error calculated using JOINMAP
- Near-isogenic line (Asc/Asc)
Mutagenesis was used to study the genetic basis of sensitivity to the host-selective AAL-toxins and susceptibility to the pathogenic fungus Alternaria alternata f. sp. lycopersici in tomato. To that end, EMS-mutagenised M₂ families were screened for insensitivity to AAL-toxins in seedling assays. Toxin-insensitive plants were obtained at a high frequency, and the majority of these plants were resistant to infection with fungal spores. Genetic mapping and allelic tests of nine stable inherited resistances indicated that single semi-dominant mutations occurred at the Asc locus on chromosome 3L. The mutagenesis procedure revealed no resistance loci at other genomic positions. Hence, no indications were obtained for the presence of additional genes that could take part in a hypothetical AAL-toxin transduction route. Small, but significant, variation in the AAL-toxin-induced inhibition of seedling development between R* lines was observed (Fig. 3), suggesting an allelic series of mutations with subtle differences in effect. These differences might possibly be confirmed by biochemical assays, e.g. measurements of the sphinganine N-acetyltransferase activity in the presence of AAL-toxins.

At a lower frequency, plants were identified that were insensitive to the AAL-toxins at the seedling stage but toxin-sensitive and susceptible to fungal infection at mature stages. During seedling development, toxin insensitivity was comparable to that of the resistant Asc isole that was homozygous dominant (Asc/Asc); at the plant level, toxin-insensitivity was comparable with that of the susceptible isole that was homozygous recessive (asc/asc). This novel AAL-toxin-sensitivity phenotype was inherited in a non-Mendelian fashion, which hampered genetic analysis. Consequently, the genotypes of these mutants were unknown and, also, it was not determined whether only the Asc locus was involved or whether other loci were mutated. The unusual phenomenon could be the consequence of reversible epigenetic mechanisms resulting in transient somatic insensitivity to AAL-toxins, e.g. (trans)inactivation of the asc allele by DNA methylation or modification of its chromatin structure (Matzke and Matzke 1993).

The alkylating mutagen EMS has been found to be highly effective in inducing point mutations, mainly those consisting of GC → AT transitions (Vogel and Nivard 1994). Hence, mutagenesis of the asc allele, controlling sensitivity to AAL-toxins, might comprise specific base pair changes that resulted in the semi-dominant mutant Asc alleles. In contrast to EMS mutagenesis, no toxin-insensitive insertion mutants were identified by transposon mutagenesis. Transposon insertions generally cause loss-of-function mutations which following insertion in the asc locus, might not have resulted in insensitivity to AAL-toxins: in plants that were hemizygous for a Ds insertion allele (Ds/−), sensitivity to AAL-toxins could still have been conferred by the asc allele on the homologue chromosome, and homozygotes (Ds/Ds) could have been lethal. This suggestion is in line with the hypotheses that (1) AAL-toxins cause cell death by inhibition of the asc-encoded target molecule, e.g. an essential enzyme, and (2) the semi-dominant EMS-induced point mutations resulted in Asc alleles coding for toxin-insensitive forms of the target molecule. Mutagenesis of plant genes encoding the molecular targets of phytotoxins (i.e. various herbicides) has been shown to cause single amino acid substitutions that modified the toxin binding site and resulted in toxin-insensitive forms of the target enzymes (e.g. Hirschberg and McIntosh 1983; Erickson et al. 1985; Yadav et al. 1986; Lee et al. 1988).

Accordingly, it is predicted that (1) EMS mutations at the toxin-insensitive Asc allele would result in sensitive asc alleles at a very low frequency, and (2) transposon mutagenesis of the Asc or the asc allele in heterozygous genotypes (Asc/asc) would result in viable AAL-toxin-sensitive or -insensitive insertion mutants, respectively. However, identification of these insertion mutants might be troublesome: the latter would be hard to detect phenotypically, and it cannot be excluded that transposon knockouts of either allele generate lethal haplo-insufficiencies. The lack of Ds insertion mutants could also be the consequence of transposition-intrinsic characteristics, e.g. the Asc locus could reside in a chromosomal location that is not readily accessible to Ds insertions from the ET90 position. The large number of AAL-toxin-sensitive seedlings that were screened for insensitivity indicated a high genetic stability of the asc allele. A discrepancy between EMS and transposon mutation frequencies has also been observed following mutagenesis of the Adh₁ locus of maize. EMS treatments of kernels resulted in “inexplicable and extraordinarily” high Adh₁ mutant recovery frequencies, whereas transposon-induced mutants were obtained at low frequencies (Freeling and Bennett 1985).

The AAL-toxin-insensitive Asc allele has traditionally been designated as the dominant allele because the heterozygotes (Asc/asc) resist fungal infection and, hence, resistance inherits in a dominant manner. However, in AAL-toxin assays the Asc locus clearly behaves as a semi-dominant trait and, moreover, intermediate insensitivity of heterozygotes (Asc/asc) can readily be overcome by higher AAL-toxin applications. Presumably, the heterozygous genotypes (Asc/asc) resist fungal infection because the host cells are insensitive to the low AAL-toxin levels produced during spore germination and fungal growth. If the two alleles of the Asc locus encode two functional forms of an essential enzyme (e.g. sphinganine N-acetyltransferase), one of which is inhibited by AAL-toxins, then the mode of inheritance would depend on whether the non-inhibited enzyme could compensate completely (insensitivity dominant), partially (insensitivity semi-dominant), or not (insensitivity recessive) for the inhibited enzyme in the heterozygote (Asc/asc). Accordingly, it is hypothesised that the Asc alleles encode different forms of the molecular target with differential inhibition by the AAL-toxins. It is anticipated that the molecular cloning of the Asc alleles will open the way to study the biochemical mechanisms underlying the allele-specificity of the tomato-A. a. lycopersici interaction. To that end, map-based cloning procedures are in progress (Van der Biezen et al. 1994a, 1995).
**Acknowledgements**  David Gilchrist is acknowledged for the generous gift of tomato seeds of the susceptible isoline; Klaus Therese and John Yoder are thanked for the transposon-containing lines. EvdB is grateful to the anonymous reviewer for making helpful comments on the manuscript, thanks everybody (!) that participated in the EMS experiment by sowing seeds, counting seedlings and taking care of the plants, and thanks Arnold van der Luit, Dick van Egmond, Gerrit van den Vijver and Ans Otten for producing tomato seeds within the transposon tagging program (under contract). This research was partly carried out in the framework of contract BIOT-900192 of the BRIDGE program of the European Community.

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