

E. A. van der Biezen · H. J. J. Nijkamp · J. Hille

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

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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 M_2 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 phytotoxins, 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 phytotoxins 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 phytotoxins 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 (*Asc1¹*) to AAL-toxins, and the *Asc1²* allele from *L. pennellii* that confers high insensitivity (Van der

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E. A. van der Biezen¹ (✉) · H. J. J. Nijkamp · J. Hille
Department of Genetics, Institute for Molecular Biological
Sciences (IMBW), BioCentrum Amsterdam, Vrije Universiteit,
De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Present address:

¹ Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich,
NR4 7HH, UK

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 M_2 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 F_2 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 emasculating and pollination techniques. Heterozygotes (*Asc/asc*) were made by reciprocal crosses between the susceptible (*asc/asc*) and resistant (*Asc/Asc*) isolines; the F_1 progenies were verified for heterozygosity by segregation analysis of *Asc* in the F_2 . 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°–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 isolate (*asc/asc*) were submerged in freshly prepared 60 mM ethyl methanesulphonate ($C_2H_5OSO_2CH_3$) solutions (EMS, Eastman Kodak Company, USA) buffered in 10 mM $NaHPO_4$ (pH 5.7) for 24 h on a gyratory shaker in the dark at 22°C. M_1 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 M_2 seeds that were harvested from individual fruits of the first two clusters and then analysed per fruit separately. The viability of the M_1 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 M_1 , M_2 and M_3 generations and was expressed as the percentage of M_2 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% $NaClO$ (w/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. Toxin 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 (10^6 spores ml^{-1}) and mycelium directly on the wounds, or by spraying the plants with fungal spores (10^6 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

Total number of lines	Mutant phenotype	Number of mutant lines	Mutant frequency (%) ^a
340	Lethal seedling/albino	23	6.8
	Turgor incompetent seedling	2	0.6
	3-4 cotyledons	12	3.5
	Chlorotic cotyledons	24	7.1
	Dark green cotyledons	9	2.6
	Deformed cotyledons	19	5.6
	High anthocyanin in cotyledons	11	3.2
	Necrotic cotyledons	5	1.5
	Yellow cotyledons/leaves	64	18.8
	No anthocyanin in hypocotyl	8	2.4
	No apical meristem	11	3.2
	Small habitus/dwarf	29	8.5
	Deformed leaves	1	0.3
	581	Insensitive to AAL-toxins	44

^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 ($n \approx 50$) for insensitivity to 100-times diluted cell-free culture-filtrate (CFCF; approximately 0.2 μM 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.

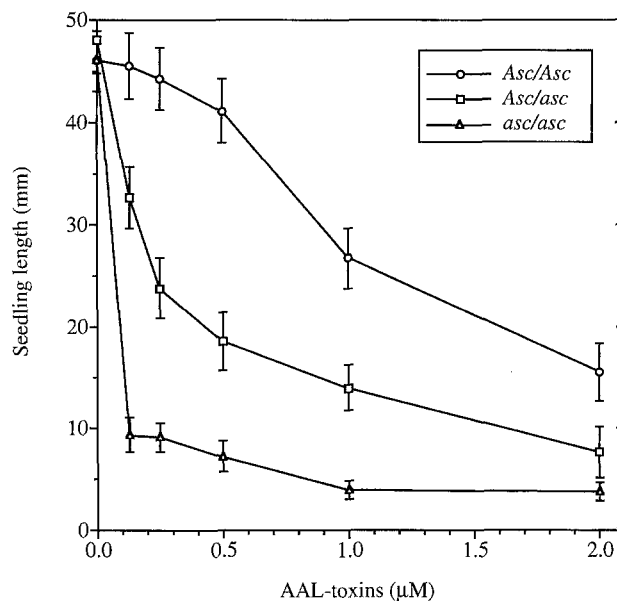


Fig. 1 AAL-toxin-induced inhibition of tomato seedling development. The mean lengths (mm) of seedlings ($n \approx 60$) of the three *Asc* isolines were taken 8 days after germination in the presence of different AAL-toxin concentrations

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 μM 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 ($n \approx 100$). 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-

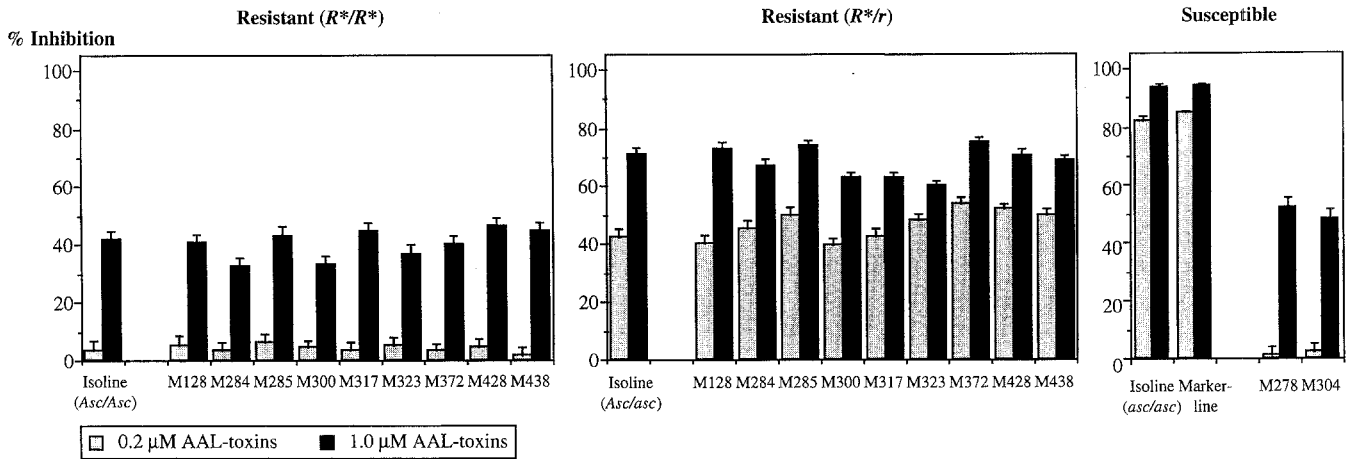


Fig. 2 AAL-toxin-induced inhibition of seedling development (%). Inhibition was measured as the percentage reduction in the lengths of EMS-induced resistant (homozygous R^*/R^* or heterozygous R^*/r) and susceptible tomato seedlings ($n \approx 60$) germinated in the pres-

ence 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

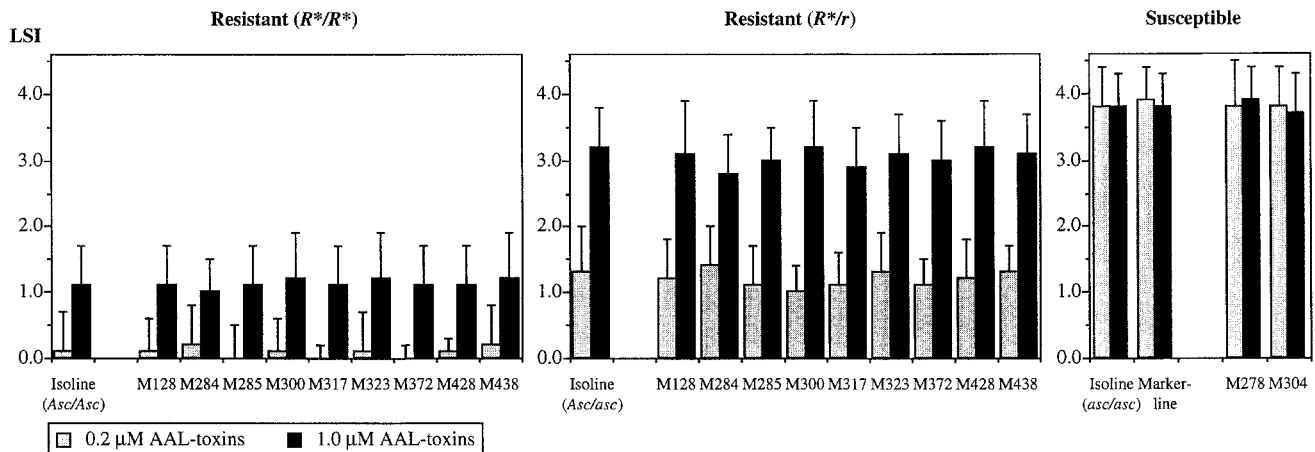


Fig. 3 AAL-toxin-induced leaf necrosis (LSI). Sensitivity to AAL-toxins of EMS-induced resistant (homozygous R^*/R^* or heterozygous R^*/r) and susceptible tomato lines was monitored by leaf bioassays (6 plants per line, three to five leaflets per dish in triplicate), and the percentage necrosis classified according to the Leaf Sensi-

tivity 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

zygous (R^*/R^*) or heterozygous (R^*/r) for induced resistance were as comparably insensitive to AAL-toxins as the *Asc/Asc* or *Asc/asc* isolines, respectively.

For more detailed examination, 9 M_3 lines were chosen, and siblings of each line were investigated for sensitivity to a range of AAL-toxin concentrations (0.01–2.0 μM) in seedling ($n \approx 60$) and leaf assays (6 plants). In addition, these lines were crossed to a susceptible markerline (*asc/asc*). An analysis of AAL-toxin sensitivity was performed on self progenies that were homozygous (R^*/R^*) or on F_1 progenies that were heterozygous (R^*/r) for induced resistance. The seedlings and leaves were generally as insensitive to the AAL-toxins as those of the resistant isolines (Figs. 2 and 3). Fungal spore inoculation assays showed that the AAL-toxin-insensitive R^* plants were resistant to *A. a. lycopersici* infection.

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 ($n \approx 60$) 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

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 F_2 populations

R^* lines	Total	Segregation data ^a				χ^2 ^b	Rec (%) ^c
		R^* - <i>Sf</i>	R^* - <i>sf</i>	<i>r</i> - <i>Sf</i>	<i>r</i> - <i>sf</i>		
<i>Asc</i> isolate ^d	447	300	36	39	72	130.1	18.7 ± 2.3
M128	228	155	19	17	37	70.6	17.6 ± 3.1
M284	249	170	19	22	38	68.2	18.6 ± 3.1
M285	222	150	15	22	35	63.5	18.7 ± 3.2
M300	239	162	23	17	37	66.1	18.9 ± 3.2
M323	245	158	16	23	48	103.7	16.7 ± 2.8
M372	221	151	15	18	37	74.8	16.5 ± 3.1
M428	232	152	21	23	36	56.7	21.2 ± 3.4
M438	214	143	16	21	34	59.7	19.3 ± 3.4

^a R^* =leaves insensitive, r =leaves sensitive to 0.2 μ M AAL-toxins

^b χ^2 for independent assortment; if $\chi^2 > 11.3$ then $P < 0.01$

^c Recombination percentages with standard error calculated using JOINMAP

^d Near-isogenic line (*Asc/Asc*)

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 F_1 plants were self-pollinated to produce F_2 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 F_1 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 F_2 plants would be expected that carry recombinations between the R^* genes and the *Asc* gene. An average of 1200 F_2 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 μ M 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 F_1 plants (*sAc*, *Ds*) were self-pollinated and outcrossed (OC_1) 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 F_2 and OC_1 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 transposon 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.

Discussion

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*-acyltransferase 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* isoline that was homozygous dominant (*Asc/Asc*); at the plant level, toxin-sensitivity was comparable with that of the susceptible isoline 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*-acyltransferase), 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).

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