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REVIEW

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# Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance

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**Abstract** *Alternaria solani* causes diseases on foliage (early blight), basal stems of seedlings (collar rot), stems of adult plants (stem lesions), and fruits (fruit rot) of tomato. Early blight is the most destructive of these diseases and hence receives considerable attention in breeding. For over 60 years, breeding for early blight resistance has been practiced, but the development of cultivars with high levels of resistance has been hampered by the lack of sources of strong resistance in the cultivated tomato and by the quantitative expression and polygenic inheritance of the resistance. In some accessions of wild species, high levels of early blight resistance have been found, but breeding lines still have unfavorable horticultural traits from the donor parent. Recently, the first linkage maps with loci controlling early blight resistance have been developed based on interspecific crosses. These maps may facilitate marker-assisted selection. This overview presents the current knowledge about the *A. solani*–tomato complex with respect to its biology, genetics, and breeding.

**Key words** Early blight · Tomato · *Alternaria solani* · Resistance · Genetics · Breeding

## Introduction

Early blight is the major disease symptom caused by the fungus *Alternaria solani* (Ellis & Martin) Sorauer. This disease, which in severe cases can lead to complete defoliation, is most damaging on tomato [*Solanum lycopersicum* L. (Peralta et al. 2005, syn. *Lycopersicon esculentum* Mill.)] in regions with heavy rainfall, high humidity, and fairly high temperatures (24°–29°C). Epidemics can also occur in semiarid climates where frequent and prolonged nightly dews occur (Rotem and Reichert 1964). Apart from the leaf symptoms that are known as early blight (EB), *A. solani* can cause less economically important symptoms on tomato, including collar rot (basal stem lesions at the seedling stage), stem lesions on the adult plant, and fruit rot (Walker 1952). Yield losses up to 79% from EB damage have been reported from Canada, India, the United States, and Nigeria (Basu 1974b; Datar and Mayee 1981; Sherf and MacNab 1986; Gwary and Nahunnaro 1998). Collar rot can cause seedling losses of 20% to 40% in the field (Sherf and MacNab 1986).

Control measures for these diseases include a 3- to 5-year crop rotation, routine applications of fungicides, and the use of disease-free transplants (Madden et al. 1978; Sherf and MacNab 1986). Fungicide treatments are generally the most effective control measures, but are not economically feasible in all areas of the world and may not be effective under weather conditions favorable for epidemics (Herriot et al. 1986). Resistant cultivars are potentially the most economical control measure because they can extend the intervals between fungicide sprays while maintaining control of the disease (Madden et al. 1978; Shtienberg et al. 1995; Keinath et al. 1996).

Progress in breeding for EB resistance has been limited by the lack of effective resistance genes in cultivated tomato (Vakalounakis 1983; Poysa and Tu 1996; Banerjee et al. 1998; Vloutoglou 1999) and by the quantitative expression and polygenic inheritance of the resistance (Barksdale and Stoner 1977; Maiero et al. 1989; Nash and Gardner 1988a; Maiero et al. 1990a; Thirthamallappa and Lohithaswa 2000). Sources for EB resistance have been identified in wild relatives of tomato. Some of these have been utilized through traditional breeding approaches, but an increased level of resistance is negatively correlated with earliness (Nash and Gardner 1988a; Maiero et al. 1989; Foolad and Lin 2001; Foolad et al. 2002a) and yield (Barrat and Richards 1944). The most resistant breeding lines and hybrid cultivars with acceptable horticultural characteristics that are currently available have moderate resistance to EB and mature slightly later (Gardner 1988; Gardner and Shoemaker 1999; Gardner 2000). Hence, resistant cultivars with better horticultural traits are still needed.

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Classical quantitative genetic analyses have provided estimates of the number of quantitative trait loci (QTLs) for EB resistance, of the average gene action and of the heritabilities from which the prospects for progress in breeding programs based on phenotypic selection can be estimated (Nash and Gardner 1988a; Maiero et al. 1990a, b). However, with such studies, the effects of individual genes and their locations on the tomato genome cannot be determined. More recent genetic studies on EB resistance have been directed to the use of molecular marker maps for mapping and characterizing the QTLs that determine the resistance (Foolad et al. 2002b; Zhang et al. 2003; Chaerani et al. submitted). Markers closely linked to QTLs can be used to select individual plants with the most desirable QTLs. By fine mapping, we can also resolve whether the unfavorable traits associated with EB resistance are due to pleiotropic effects of resistance genes or to closely linked genes. If they are linked, marker-based selection might facilitate breaking of the linkage.

In this article we review the literature pertaining to aspects of resistance to EB, and, to a lesser extent, also to collar rot and stem lesions. The article describes:

- The biology of *A. solani* and the symptoms caused by the fungus, and methods for selecting resistance to EB and collar rot.
- The known sources of resistance followed by classical genetic studies of EB, resistance to collar rot and stem lesions, and the genetic interrelationship among the diseases.
- The mapping of resistance genes.
- Physiological aspects affecting EB resistance and characterization of EB resistance.
- Perspectives for EB resistance breeding.

## The pathogen

Since the first description by Ellis and Martin in 1882 (cited in Sherf and MacNab 1986), *Alternaria solani*, previously known as *A. porri* f. sp. *solani* (Neergaard 1945), has been the object of intensive studies (Strandberg 1992; Rotem 1994). *A. solani* belongs to the Fungi Imperfecti (Deuteromycotina) in the class Hyphomycetes and order Hyphales (Agrios 2005). An ascomycete fungus, *Pleospora solani*, has been claimed by Esquivel (1984) as the teleomorph stage of *A. solani*, but this has not been confirmed by others. *A. solani* belongs to the large-spored group, characterized by separate conidia borne singly on simple conidiophore, within the genus *Alternaria* (Neergaard 1945). The conidia of *A. solani* are muriform and beaked (Neergaard 1945; Ellis and Gibson 1975). Like other members of the genus *Alternaria*, *A. solani* has transverse and longitudinal septate conidia, multinucleate cells, and dark-colored (melanized) cells (Rotem 1994). The melanins protect against adverse environmental conditions including resistance to microbes and hydrolytic enzymes (Rotem 1994).

### Disease cycle

Under free moisture or near-saturated humidity at a wide range of temperatures (8°–32°C), conidia germinate to produce one or more germ tubes. These subsequently penetrate the host epidermal cells directly by means of appressoria or they enter through stomata or wounds by hyphal growth (Sherf and MacNab 1986; Perez and Martinez 1999; Agrios 2005; Fig. 1). Penetration can occur

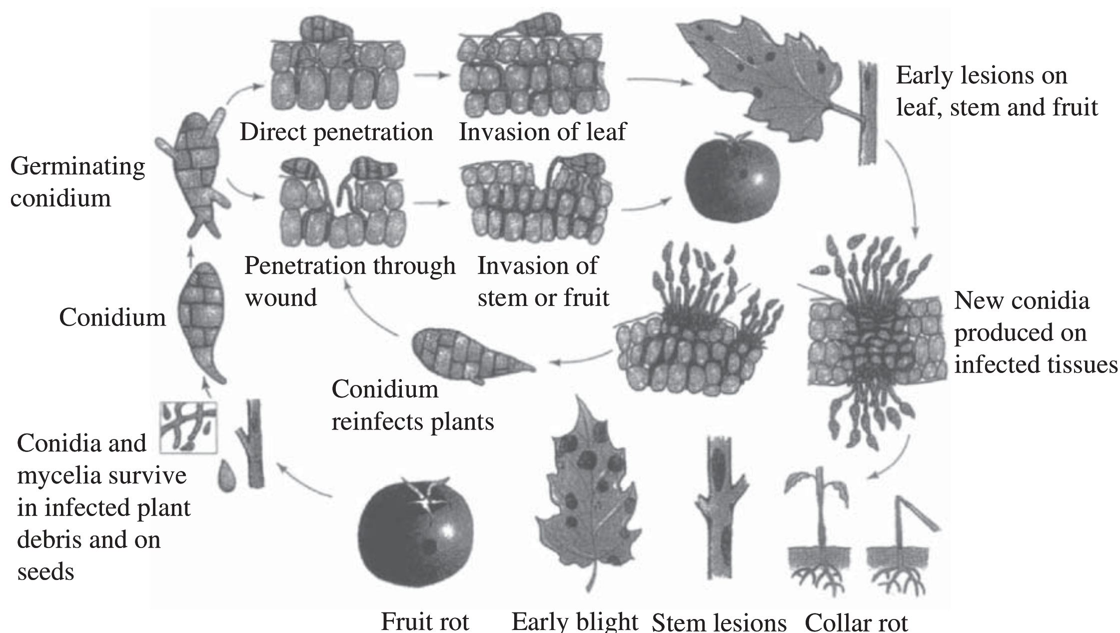


**Fig. 1.** Infection process, development and symptoms of diseases caused by *Alternaria solani*. Adapted from Agrios (2005, p 455), with permission from Elsevier

at temperatures between 10° and 25°C (Sherf and MacNab 1986). Host colonization is facilitated by enzymes (cellulases, pectin methyl galacturonase) that degrade the host cell wall and by a number of toxins that kill host cells and enable the pathogen to derive nutrients from the dead cells (Rotem 1994). Lesions become visible 2–3 days after infection, and spore production occurs 3–5 days later (Sherf and MacNab 1986). This relatively short disease cycle allows for polycyclic infection (Sherf and MacNab 1986). The fungus survives between crops as mycelia or conidia in soil, plant debris, and seed (Sherf and MacNab 1986; Fig. 1). Chlamydospores can also serve as survival structures (Basu 1974a; Patterson 1991). Therefore, the life cycle of *A. solani* includes soil- and seed- as well as air-borne stages, making the pathogen difficult to control by means of rotation and sanitation. The main hosts of *A. solani* are solanaceous crops including tomato, potato, eggplant, and pepper (Neergaard 1945; Ellis and Gibson 1975).

## Toxin production

Eleven toxins have been identified in culture filtrates of *A. solani* (Montemurro and Visconti 1992). Among these, alternaric acid and solanapyrone A, B, and C are able to induce necrotic symptoms similar to EB symptoms (Montemurro and Visconti 1992). Alternaric acid is one of the major metabolites in the filtrates (Brian et al. 1952) and is probably the main metabolite responsible for the development of necrotic and chlorotic symptoms (Pound and Stahmann 1951). Alternaric acid is already present in dormant spores and is produced and released by germinating spores (Langsdorf et al. 1990). Alternaric acid is not phytotoxic when sprayed alone on tomato leaves, but it enhances the infection process and the development of necrotic symptoms when added to spore suspensions of *A. solani* (Langsdorf et al. 1990). Another factor in *A. solani* spores was required for infection. This substance, referred to as S1, is nontoxic and is present in a water-soluble fraction from chloroform extracts of spore-germination fluid. This factor allowed the spores of a nonpathogenic strain of *A. alternata* to cause necrotic symptoms on tomato and potato (Langsdorf et al. 1990).

## Variability among isolates

Although *A. solani* appears to have only a nonsexual life cycle, it has a relatively high variation in morphology in vivo and in vitro, physiology, genetic makeup, and pathogenicity among isolates (Bonde 1929; Wellman 1943; Neergaard 1945; Henning and Alexander 1959; Rotem 1966; Weir and Huff 1998; Martinez et al. 2004; van der Waals et al. 2004). Bonde (1929) and Neergaard (1945) classified *A. solani* into conidial, mycelial, and intermediate types of isolates. Pathogenic differences were found among isolates originating from different germ tube tips from the same conidium (Stall 1958).

A high genetic diversity was detected among isolates of *A. solani* originating from the United States, South Africa, Cuba, Brazil, Turkey, Greece, Canada, China and Russia based on vegetative compatibility groups (VCG, van der Waals et al. 2004) and molecular markers [isozymes, random amplified polymorphic DNA markers (RAPDs), random amplified microsatellite markers (RAMs), and amplified fragment length polymorphisms (AFLPs); Petrunak and Christ 1992; Weir and Huff 1998; Martinez et al. 2004; van der Waals et al. 2004]. In studies where isolates from several countries were compared using VCG assays, RAMs (van der Waals et al. 2004), or RAPD markers (Weir and Huff 1998), *A. solani* isolates cluster according to country, indicating some degree of genetic isolation. In contrast, isolates from the same country are not distinctly separated by geographical origin (Petrunak and Christ 1992; Weir and Huff 1998; Martinez et al. 2004; van der Waals et al. 2004). This can be ascribed to short- or medium-distance dispersal of the airborne spores and movement of plant material within the countries (Weir and Huff 1998; van der Waals et al. 2004). In many cases, isolates originating from tomato and potato clustered according to their hosts based on RAPD (Weir and Huff 1998) and AFLP markers (Martinez et al. 2004), suggesting host specialization. Organ specificity was reported to occur among Bulgarian isolates by Stancheva (1990), but has not been described by other authors. Associations of molecular markers with variability in physiology, morphology, and virulence are not known.

So far, conclusive evidence for the existence of physiological races is lacking. Physiological races are defined based on differential host specificity (Mehrotra and Areja 1990; Schlegel 2003). Therefore, the report of the presence of physiological races of *A. solani* (Bonde 1929) is not correct according to the current definition because it described them in terms of variability in physiological, morphological, and ecological characters in in vitro culture. Henning and Alexander (1959) characterized isolates on tomato and related species with quantitative variation in resistance. Some of these isolates, which showed cultural differences, appeared to be host specific, but the pattern of infection was not consistent between experiments. This was attributed to heterogeneity of the host lines and the unstable nature of the isolate cultures (Henning and Alexander 1959). Similarly, Castro et al. (2000) could not demonstrate consistent host-specific reactions of isolates.

Heterokaryosis could be the driving force for genetic variation in *A. solani* (Stall 1958). Heterokaryosis is the occurrence of genetically different nuclei in the same cells. This can be the result of hyphal anastomosis, a process observed in *A. solani* (Stall and Alexander 1957; Stall 1958). After establishment of heterokaryosis, this state may be maintained or lost during further cell divisions. Also nuclear migration is possible through septal pores between cells of conidia, conidiophores, mycelia, and cells connecting these structures, allowing dissociation of unlike nuclei leading to homokaryosis, and, conversely, also to the reestablishment of heterokaryosis (Stall 1958). Therefore, even isolates obtained from single conidia and hyphal tips are genetically unstable. In their studies, Stall and Alexander

(1957) observed frequent occurrence of anastomoses but failed to obtain heterokaryosis as indicated by the absence of segregation of cultural types.

The ability of *A. solani* to maintain high genetic variability allows it to react quickly to changing environments. For example, a recent study demonstrated that isolates in the midwestern United States have become less sensitive to a fungicide resulting in significant losses of disease in glasshouse cultures (Pasche et al. 2004). The high genetic diversity and high degree of gene flow within countries could break down genetic resistance in the host; this possibility has been advanced as one of the reasons for the absence of potato cultivars with complete resistance to *A. solani* in South Africa (van der Waals et al. 2004).

## Disease symptoms

All aboveground parts of plants can be infected by *Alternaria solani*, and various names have been given for the different symptoms, which often leads to confusion (Sherf and MacNab 1986). In this article, we refer to symptoms on foliage as early blight (EB), to symptoms on fruits as fruit rot, to symptoms on stems of seedlings as collar rot, and those on stems of adult plants as stem lesions (Walker 1952).

The first symptoms of EB are small, dark, necrotic lesions that usually appear on the older leaves and spread upward as the plants become older (Sherf and MacNab 1986). As lesions enlarge, they commonly have concentric rings with a target-like appearance, and they are often surrounded by a yellowing zone. In severe epidemics, *A. solani* can cause premature defoliation, which weakens the plants and exposes the fruit to injury from sunscald (Sherf and MacNab 1986).

Large, dark, and sunken lesions may appear on the stems of seedlings at the ground line, causing partial girdling known as collar rot (Sherf and MacNab 1986). Seedlings are weakened and can die when the stem is completely girdled by the lesion. On the main stem and side branches of adult plants, the fungus causes small, dark, slightly sunken areas that enlarge to form dark brown, elongated spots, which occasionally have concentric rings like those on the leaves. These spots are scattered along the stem and branches (Walker 1952). Some authors make no distinction between collar rot and stem lesions (Gardner 1990). In older literature, collar rot and stem lesions are sometimes referred to as stem cankers (Barksdale and Stoner 1977), a term that is currently reserved for the disease caused by *A. alternata* (Sherf and MacNab 1986).

On green or ripe fruits, dark, velvety, sunken spots may occur at the stem end. These spots occasionally develop from mycelia extending from stem lesions and can reach a considerable size and also develop distinct concentric markings (Sherf and MacNab 1986). Semi-ripe fruits are more susceptible than ripe ones (Mehta et al. 1975). Heavily infected fruits frequently drop before they mature. On susceptible genotypes, the calyx and blossom may also be infected (Pandey et al. 2003).

## Screening methods

Reliable and repeatable techniques for large-scale screening are necessary to identify host plant resistance. Techniques have been developed for EB and collar rot resistance screening under field, glasshouse, and laboratory conditions. In the laboratory, both fungal inocula (spores and mycelia) and fungal toxins have been used in screening for resistance.

### Inoculum production

*Alternaria solani* can be artificially grown in various culture media, but it does not readily sporulate in vitro. Spore production requires special conditions such as mycelial wounding or the transfer of culture pieces onto a minimal medium or filter paper followed by exposure to harsh conditions (Lukens and Horsfall 1968; Barksdale 1969; Shahin and Shepard 1979). Efficient sporulation can be induced by exposing cultures under diurnal light in a partially opened culture dish, after removal of aerial mycelia (Barksdale 1969). For maintenance of a wild-type culture, Barksdale (1969) suggested mass transfer of sections of culture that have "normal appearing areas" because variants in culture are often obtained even though the culture was started from single spores. When spores derived from cultures are difficult to obtain, mixed inocula of spores and mycelia from dried, infected leaves are sometimes used in field experiments (Thirthamallappa and Lohithaswa 2000).

### Field screening

In field tests, large populations can be assessed under normal growing conditions during the whole life cycle of the plants. Artificial inoculation by (repeated) spraying of inoculum and/or the use of spreader rows is required to enhance natural infection and to obtain uniform disease pressure. Prior to inoculation, it is often necessary to prevent or eradicate foliar diseases with scheduled fungicide sprays (Nash and Gardner 1988a).

EB severity in the field is assessed in terms of percentage defoliation and the average fraction of necrotic leaf area on the plant (Horsfall and Barrat 1945). Symptoms on the upper leaves can be disregarded because the necrotic areas on these leaves are less than 2% of the total damage during the growing season (Basu 1974b). Therefore, counting the number of leaves with 75%–100% necrosis in the lower half of the plants (Basu 1974b) or estimating the percentage of necrotic area in the middle third of the plant canopy (Christ 1991) are reliable indicators for EB severity.

EB epidemics initially progress slowly but accelerate as plants mature, resulting in a typical sigmoidal disease progress curve (Nash and Gardner 1988b). Occasionally the disease curve is bimodal, which could be due to the emergence of new healthy leaves after the first cycle of infection (Pandey et al. 2003). Therefore, a once-only evaluation can

underestimate or overestimate the actual level of resistance of a particular host, and field assessments must be based on several observations over time that are used to calculate the area under the disease progress curve (AUDPC). The AUDPC integrates the host, pathogen, and environmental effects during the epidemic (Pandey et al. 2003).

In spite of their advantages, field tests also have their problems: they are slow, labor intensive, highly affected by the presence of other pathogens, not suitable for evaluation of single plants in a large-scale experiment, and they are sensitive to environmental conditions that are difficult to control.

## Glasshouse screening

Assays in a glasshouse or controlled-environment chamber with seedlings or small plants provide uniform, favorable, repeatable environmental conditions and permit several cycles of screening per year, thus offering more reliable results. Glasshouse and field test results correspond well (Banerjee et al. 1998; Foolad et al. 2000). Glasshouse or controlled-environment chamber evaluations of young plants were mainly used for preliminary selection of material resistant to *A. solani* from large germplasm collections (Barksdale 1969; Vakalounakis 1983; Poysa and Tu 1996; Vloutoglou 1999) and to study the inheritance of resistance to collar rot. Glasshouse evaluation of EB resistance is rarely performed for genetic studies (Chaerani et al. submitted).

The current glasshouse screening methods for *A. solani* resistance are based on the method established by Barksdale (1969). Generally, seedlings are spray-inoculated with spores at an age of 4–6 weeks (Barksdale 1969; Marcinkowska 1982; Nash and Gardner 1988b; Banerjee et al. 1998; Vloutoglou 1999; Foolad et al. 2000). Leaves can be injured prior to spraying by rubbing the leaf between thumb and forefingers (Poysa and Tu 1996). Plants are incubated for 24 h under 100% relative humidity (RH) followed by 12–16 h of 100% RH during the night for 5–7 days in a mist chamber, mimicking the repeated nightly dew in nature. During the day, plants are exposed to ambient RH to allow the development of disease symptoms. A leaf wetness period of at least 4 h after inoculation was required for infection (Moore 1942; Vloutoglou and Kalogerakis 2000). Increasing this period up to 24 h induced progressively higher EB severity, but longer periods of humidity did not increase severity further (Vloutoglou 1999).

EB severity is usually estimated 7 days after spray inoculation as the percentage necrotic area on leaves that were present at the time of inoculation (leaves emerging after inoculation are not affected, Barksdale 1969; Vloutoglou 1999). In the case of a low incidence of necrotic spots, EB severity is expressed as the number of lesions (Barksdale 1969).

Disease severity can be determined more precisely and objectively by measuring lesion sizes when the inoculum is applied as single drops on leaflets (Nash and Gardner 1988b; Chaerani et al. in press).

Glasshouse tests have also been used for assessing resistance to collar rot and stem lesions (Gardner 1990; Maiero et al. 1990b). The basal stem of seedlings is sprayed with spores and covered with soil (Maiero et al. 1990b) or seedlings are placed in a humidity chamber (Gardner 1990). Collar rot is usually rated in three to five symptom grades (Reynard and Andrus 1945; Gardner 1990; Maeiro et al. 1990b). Screening for collar rot and stem lesions in the glasshouse is fast and can be used instead of field screening for EB resistance, provided that the resistance to these disease symptoms is closely associated with EB resistance in the materials used, such as in C1943 and derived lines (Gardner 1990).

Glasshouse tests have the advantages over field tests that conditions are more reproducible, the duration of the test is shorter, and, especially after droplet inoculation, the test is more objective and precise data can be obtained. However, conditions in the glasshouse cannot be fully controlled, and some genotypes are not well adapted to glasshouse conditions.

## Laboratory assays

Locke (1948) used detached leaflet assays for evaluation of EB resistance as a means to circumvent the influence of growth habit, which may affect the reaction of plants in the field or glasshouse. The method involved the application of inoculum droplets on either punctured (Locke 1948) or nonpunctured (Foolad et al. 2000), young, fully expanded leaflets. Locke (1948) claimed the method to be reliable; Lynch et al. (1991) and Foolad et al. (2000), however, concluded that detached leaflet assays did not correlate well with field and glasshouse screenings. These results might imply that a whole plant is required for the expression of EB resistance, which is known to be influenced by physiological characters of the plant such as earliness, determinism (Nash and Gardner 1988a; Maiero et al. 1989; Foolad and Lin 2001; Foolad et al. 2002a), and potential yield (Barrat and Richards 1944), as well as by plant age and nutritional status (Rotem 1994).

To circumvent the problem of apparent resistance in late-maturing cultivars, Bussey and Stevenson (1991) induced early senescence in juvenile potato leaf tissue by floating excised disks on a solution containing auxins [1-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D)]. A very late-maturing cultivar that was highly resistant in the field was more susceptible when tested using the leaf disk assay, suggesting that the assay may be less influenced by cultivar maturity than the field test (Bussey and Stevenson 1991). The results of the other tested cultivars agreed with those obtained in the field (Bussey and Stevenson 1991).

Laboratory assays on detached leaflets therefore show promise for studying particular aspects of resistance and for eliminating confounding influences of whole-plant physiology. However, these methods need to be carefully adapted to the research question in hand and cannot be relied on as a replacement for field or glasshouse tests.

### Toxin assays

Several authors reported that culture filtrate of *A. solani* could be used to distinguish EB-resistant genotypes from susceptible genotypes, at least in progenies of some sources of resistance (Lodha 1977; Stancheva 1988; Maiero et al. 1991). Genotypes with collar rot resistance were more tolerant to culture filtrate than those with only EB resistance (Maiero et al. 1991). In contrast, Lynch et al. (1991) found that the result of culture filtrate assays using detached leaflets did not correspond with the result from glasshouse or field tests.

Darakov (1995) proposed a new approach of selecting EB resistance by means of gametophytic selection in the presence of an unidentified toxin obtained from culture filtrate of *A. solani*. Pollen tube elongation correlated well with the level of EB resistance of the mother plant. Female gametophytes were selected by treating styles of emasculated flowers with drops of toxin, and, after pollination, collecting seeds from plants that yielded the most seeds. After two rounds of selection with toxin, selected plants with enhanced seed-bearing capacity were assessed in the field for EB resistance. Plant selections from toxin-treated plants had enhanced EB resistance compared with those derived from plants selected with a water treatment.

Laboratory assays using *A. solani* toxins can help to elucidate specific aspects of the pathogenesis process. However, the effects of *A. solani* toxins do not seem to correlate with the pathogenicity of isolates and do not have a role in the establishment of the pathogen in the host (Langsdorf et al. 1990). This is in contrast to toxins produced by formae speciales of *Alternaria alternata,* which do elicit most symptoms of the disease on susceptible plants and have the same differential host specificity as the fungal isolates and can therefore be used reliably to screen for resistance (Gilchrist and Grogan 1975).

### Sources of resistance

In the cultivated tomato, high levels of resistance to EB are rare. Two old breeding lines, 71B2 and C1943, probably bred from *Solanum lycopersicum* sources, have been described as highly and moderately resistant to EB, respectively (Table 1). Some moderately resistant hybrids and breeding lines have been developed from these sources, such as "Plum Dandy," NC EBR-5 and -6 (71B2), "Mountain Supreme," and NC-EBR-2 (C1943). Poysa and Tu (1996) identified only 11 moderately resistant lines from more than 500 tomato cultivars and breeding lines for EB resistance.

Some accessions of the wild species *Solanum habrochaites* (syn. *Lycopersicon hirsutum*), *Solanum peruvianum* (syn. *Lycopersicon peruvianum*), and *Solanum pimpinellifolium* (syn. *Lycopersicon pimpinellifolium*) are resistant to EB (Table 1). Success in incorporating resistance is limited because most breeding lines, e.g., NC EBR-1, NC EBR-2 (Gardner 1988), NC EBR-4 (Gardner and Shoemaker 1999), and HRC90.303 and HRC91.341 (Poysa and Tu 1996) are still late maturing, indeterminate, and relatively low yielding. These lines are derived from *L. hirsutum* accessions.

A high level of collar rot resistance has been found in the cultivated tomato such as in the old cultivar Devon Surprise and breeding line C1943. Additionally, Stancheva et al. (1991) reported sources of resistance to collar rot and stem lesions in several wild species (Table 1).

## Classical studies of genetics of resistance

Most genetic studies on the inheritance of EB resistance using different sources of resistance (*Solanum lycopersicum*, *Solanum habrochaites*, and *Solanum pimpinellifolium*) arrived at the same conclusion that the resistance is a quantitative trait that is controlled polygenically (Table 2). The estimated minimum number of controlling factors is two (Barksdale 1977) or three (Nash and Gardner 1988a). Analysis using quantitative genetic methods (generation mean analysis and scaling tests) and several sources of resistance (C1943, NC EBR-2, IHR 1939, and IHR 1816) revealed additive and dominant genetic control with the presence of epistatic effects (Maiero et al. 1990a; Nash and Gardner 1988a; Thirthamallappa and Lohithaswa 2000).

The EB resistance genes in C1943 and 71B2 are recessive and not allelic (Barksdale and Stoner 1977; Maiero et al. 1989). However, in crosses of these two resistance sources with another susceptible genotype, the F1 hybrids were intermediate, indicating additive genetic control or partial dominance (Maiero et al. 1989). Recessive genes have been reported in *S. lycopersicum* 83602029 (Stancheva 1991), IHR1939, and IHR1816 by Thirthamallappa and Lohithaswa (2000). Partially dominant inheritance has been found in *S. pimpinellifolium* and *S. habrochaites* (Martin and Hepperly 1987).

The line 87B187, derived from *S. habrochaites* PI 390662, shared common resistance genes with NC EBR-2, although this line was developed via C1943 from a *S. lycopersicum* source (Maiero et al. 1990a). Also, Thirthamallappa and Lohithaswa (2000) reported independent genes in IHR 1939 (*S. pimpinellifolium* L4394) and IHR 1816 (derived from NC EBR-1, developed from *S. habrochaites* PI 126445).

In contrast to the studies just described, one study reported a monogenic, dominant inheritance in *S. habrochaites* PI 134417 (Datar and Lonkar 1985). Their conclusion is arguable because a highly resistant F1 does not necessarily indicate complete dominance of EB resistance as was observed by Foolad and Lin (2001). The resistance phenotypes in the F2 population derived from *S. habrochaites* PI 134417 were grouped into resistant, intermediate, and susceptible, and a 3:1 segregation was observed, leading to the conclusion of monogenic inheritance (Datar and Lonkar 1985). However, EB resistance is a quantitatively expressed character, and the assignment of three phenotypic classes is therefore arbitrary and may have led by chance to the 3:1 segregation (Foolad and Lin 2001).

**Table 1.** Genetic sources of resistance to early blight, collar rot, and stem lesion

| Original source | Resistant line or variety | Test(s) used to confirm resistance | References |
|---|---|---|---|
| Early blight resistance | | | |
| *Solanum lycopersicum* (syn. *Lycopersicon esculentum*)[a] | | | |
| Unknown source | C1943 | F | Barksdale 1971 |
| 68B134 | 71B2 | F | Barksdale 1969 |
| Syn. *Lycopersicon esculentum* f. sp. *cerasiforme*[b] PI 406758 | – | F | Martin and Hepperly 1987 |
| C1943 | NC EBR-2 | F, G | Gardner 1988 |
| Unknown accessions | HRC90.145, HRC 90.158, HRC 90.159 | G | Poysa and Tu 1996 |
| NC EBR-1 | NC EBR-4 | F | Gardner and Shoemaker 1999 |
| NC EBR-1 | IHR1816 | F | Thirthamallappa and Lohithaswa 2000 |
| NC EBR-1 and -2 | NC EBR-3 | F | Gardner and Shoemaker 1999 |
| NC EBR-3 and -4 | Mountain Supreme | F | Gardner and Shoemaker 1999 |
| NC EBR-5 and -6 | Plum Dandy | F | Gardner 2000 |
| 71B2 | NC EBR-5 | F | Gardner 2000 |
| 71B2 | NC EBR-6 | F | Gardner 2000 |
| *Solanum habrochaites* (syn. *Lycopersicon hirsutum*)[a] | | | |
| PI 127827 | – | L | Locke 1949 |
| PI 390514, PI 390662 | – | F | Martin and Hepperly 1987 |
| PI 126445 | NC EBR-1 | F | Gardner 1988 |
| PI 1390662 | 87B187 | F | Maiero et al. 1990a |
| B 6013 | H-7, H-22, H-25 | F | Kallo and Banerjee 1993 |
| Unknown accessions | HRC90.303, HRC 91.279, HRC 91.341 | G | Poysa and Tu 1996 |
| LA2100, LA2124, LA2204 | – | G | Poysa and Tu 1996 |
| PI 126445 | NC39E | F | Foolad et al. 2002a |
| *Solanum peruvianum* (*Lycopersicon peruvianum*)[a] | | | |
| PE33 | – | G | Poysa and Tu 1996 |
| *Solanum pimpinellifolium* (syn. *Lycopersicon pimpinellifolium*)[a] | | | |
| PI 365912, PI 390519 | – | F | Martin and Hepperly 1987 |
| A 1921 | P-1 | F | Kallo and Banerjee 1993 |
| L4394 (IHR1939) | – | F | Thirthamallappa and Lohithaswa 2000 |
| Collar rot resistance | | | |
| Unknown source | Devon Surprise | F | Reynard and Andrus 1945 |
| Unknown source | C1943 | G | Maiero et al. 1990b |
| *Solanum pimpinellifolium* (syn. *Lycopersicon racemigerum*)[b] 87610005 | – | ? | Stancheva et al. 1991 |
| *Solanum lycopersicum* (syn. *Lycopersicon humboldtii*)[b] 87610003 | – | ? | Stancheva et al. 1991 |
| *Solanum chilense* (syn. *Lycopersicon chilense*)[a] 87610011 | – | ? | Stancheva et al. 1991 |
| Stem lesion resistance | | | |
| *Solanum lycopersicum* 83602029 | – | ? | Stancheva et al. 1991 |
| *Solanum cheesmaniae* (syn. *Lycopersicon cheesmanii* f. *typicum*)[b] 15 | – | ? | Stancheva et al. 1991 |
| *Solanum neorickii* (syn. *Lycopersicon minutum*)[b] 87610006 | – | ? | Stancheva et al. 1991 |

F, Field; G, greenhouse; L, laboratory
[a] Peralta et al. (2005)
[b] Peralta, Knapp, and Spooner (personal communication)

There have been only a few genetic studies published on resistance to the other disease symptoms caused by *Alternaria solani*, a fact that may be caused by the less damaging effect of these two disease symptoms (Table 2). One study on collar rot resistance reported a monogenic inheritance (Reynard and Andrus 1945), whereas Maiero et al. (1990b) showed quantitative expression of resistance. Analysis by Maiero et al. (1990b) using a joint scaling test showed that both additive and dominance effects controlled collar rot resistance in C1943 and NC EBR-2 sources, although the dominance effect of susceptibility appeared to be more important.

Only one study on stem lesion resistance has been published that reported that the resistance is a quantitative trait controlled by dominant genes in *S. lycopersicum* source (Stancheva 1991; Table 2). Both additive and dominant genetic components conferred resistance, which was complicated by epistatic effects.

Fruit rot has escaped attention in genetic studies even though it may cause substantial direct losses (Datar and Mayee 1981). Resistance to fruit rot may be controlled independently from EB resistance because fruit rot incidence is not necessarily associated with EB severity (Barksdale 1971).

Little is known about the genetic relationships among the resistances to EB, collar rot, and stem lesions. Maiero et al. (1990b) postulated that the collar rot resistance gene in C1943 and its derived line, NC EBR-2, is one of the genes

**Table 2.** Classical genetic studies of early blight, collar rot, and stem lesion resistance in tomato

| Resistant parent[a] | Population type | Tests | Analysis method | Genetic control | Reference |
|---|---|---|---|---|---|
| Early blight | | | | | |
| *Solanum lycopersicum* 71B2 | F1 | F | Diallel, midparent–hybrid comparison | Recessive polygenic | Maiero et al. (1989) |
| *Solanum lycopersicum* C1943 | F1 | F | Diallel, midparent–hybrid comparison | Recessive polygenic | Maiero et al. (1989) |
| *Solanum lycopersicum* C1943 | F1, F2, BC1, BC2 | F | Diallel, midparent–hybrid comparison, generation means, joint scaling tests | Recessive polygenic with additive and epistatic (dom × dom) effects | Maiero et al. (1990a) |
| *Solanum lycopersicum* NCEBR-1 | F1, F2, BC1, BC2 | F | Generation means, joint scaling tests | At least 3 genes with additive, dominance, and epistatic (add × add, add × dom, dom × dom) effects | Nash and Gardner (1988b) |
| *Solanum lycopersicum* NCEBR-2 | F1 | F | Diallel, midparent–hybrid comparison | Polygenic, partial dominant | Maiero et al. (1990a) |
| *Solanum lycopersicum* 87B187 | F1 | F | Diallel, midparent–hybrid comparison | Polygenic, partial dominant | Maiero et al. (1990) |
| *Solanum lycopersicum* 83602029 | F1, F2, BC1, BC2 | ? | Diallel, generation means | Quantitative, dominant genes with additive, dominance, and epistatic effects | Stancheva (1991) |
| IHR 1816 (= *Solanum lycopersicum* NCEBR-1) | F1, F2, BC1, BC2 | F | Joint scaling tests | Recessive polygenic with additive and epistatic (add × dom) effects at seedling stage; with additive, dominance and epistatic (add × add) effects at adult stage | Thirthamallappa and Lohithaswa (2000) |
| IHR 1939 (= *Solanum pimpinellifolium* L4394) | F1, F2, BC1, BC2 | F | Joint scaling tests | Recessive polygenic with additive and epistatic (add × dom) effects at seedling stage; with additive, dominance and epistatic (add × add) effects at adult stage | Thirthamallappa and Lohithaswa (2000) |
| *Solanum lycopersicum* NC39E | F2, F3 | F | Midparent-segregating population means comparison | Polygenic, partial dominant | Foolad et al. (2002a) |
| Collar rot | | | | | |
| *Solanum lycopersicum* C1943, NCEBR-2 | F1, F2, BC1, BC2 | G | Diallel, midparent–hybrid comparison, generation means, joint scaling tests | Recessive polygenic with additive and dominant effects | Maiero et al. (1990b) |
| Stem lesion | | | | | |
| *Solanum lycopersicum* 83602029 | F1, F2, BC1, BC2 | ? | Generation means | Recessive polygenic with additive, dominance, and epistatic effects | Stancheva (1991) |

[a] New nomenclature based on Peralta et al. (2005); see Table 1 for synonyms

that confer EB resistance or is closely linked with EB resistance genes because these lines have both EB and collar rot resistance.

In addition, stem lesion resistance may be independent of EB resistance. Barksdale and Stoner (1973, 1977), based on field observations but unsupported by a genetic analysis, assumed that stem lesion resistance segregated independently from EB resistance. Recently, several QTLs that affected both EB severity and stem lesions have been reported (Chaerani et al. submitted).

Heritability of EB resistance has been estimated in crosses involving *S. habrochaites* PI 126445 (Foolad and Lin 2001) and derived lines NC EBR-1 and NC39E (Nash and Gardner 1988a; Foolad et al. 2002a). Depending on the calculation method, heritability estimates were low to moderate in two crosses involving NC EBR-1 (Nash and Gardner 1988a). Based on parent–offspring (PO) regression, narrow-sense heritability ($h^2$) for AUDPC was estimated as 0.26 and 0.38 (Nash and Gardner 1988a). Higher $h^2$ estimates were obtained from a cross with *S. habrochaites* PI126445 (0.70, Foolad and Lin 2001) and from a cross with *S. lycopersicum* NC39E (0.65, Foolad et al. 2002a), also based on PO regression.

Those studies showed that additive genetic components play a small to moderate role in the quantitative expression of resistance. The low to moderate heritability estimates indicate that progress based on phenotypic evaluations only is likely to be slow. Furthermore, these classical genetic studies give general indications on the likely progress in selection of resistant material but do not provide information on the effects of individual resistance genes and their location on the tomato genome.

## Mapping resistance genes

Given the low to moderate heritability estimates, a marker-aided selection approach is potentially useful to accelerate the transfer of EB resistance genes into new tomato cultivars. Foolad et al. (2002b) were the first to map QTLs for EB resistance. They used backcross progenies of a cross between *Solanum habrochaites* PI 126445 and a susceptible tomato line. Mapping was done in the BC1 generation and validated in the BC1S. Fourteen QTLs were identified, which together explained 57% of the total phenotypic variation. For all QTLs, the positive allele originated from the resistant parent. In a subsequent study, Zhang et al. (2003) used a selective genotyping approach on a different part of the same BC1 population. Seven QTLs were detected, including one previously mapped major and three minor QTLs. One of the QTLs in this study inherited the resistance allele from the susceptible parent.

Chaerani et al. (submitted) identified six QTLs for EB resistance in F2 and F3 populations from a cross between the resistant *Solanum arcanum* LA2157 and a susceptible tomato. Different environments and phenotypic scoring methods were used in this study, in contrast to the previous mapping studies, which used one type of environment and disease measure. In addition, resistance to stem lesions was also assessed in the F3 population. Interestingly, EB QTLs detected in the F2 population were not always detected in the F3 population, and vice versa. This indicates the presence of environment-specific or plant age-specific QTLs. Three QTL regions for stem lesion resistance coincided with EB resistance QTLs, which allows simultaneous selection for resistance to both types of disease symptoms. The explained phenotypic variation per EB resistance QTL, 7% to 16%, was in the same range as that of Foolad et al. (2002b). One QTL for stem lesion resistance, however, had a large effect, explaining 31% of the total variation. For two of the six QTLs, the susceptible parent contributed the resistance alleles. Several of the QTLs found in the cross of *S. habrochaites* PI 126445 (Foolad et al. 2002b; Zhang et al. 2003) overlapped with those found in the *S. arcanum* LA1257.

Although many EB resistance QTLs have been identified, many of them have relatively small effects. Not all QTLs need to be incorporated to achieve a significant increase in resistance. Foolad et al. (2002b) and Zhang et al. (2003) recommended a combination of four to six QTLs, which explained more than 40% of total phenotypic variation for use in marker-assisted breeding, and Chaerani et al. (submitted) suggested two QTLs that had prominent effects under different environments and gave both EB and stem lesion resistance. It still needs to be determined, however, whether the level of resistance contributed by these QTLs would be of sufficient practical importance. The EB mapping studies have not yet reached the stage at which QTLs can be mapped precisely enough to be included in a breeding program.

## Association of early blight resistance with plant maturity, potential yield, and determinism

The strong correlation between EB resistance and late maturity, low yield, and indeterminate plant type (Nash and Gardner 1988; Foolad and Lin 2001, Foolad et al. 2002a, b) has limited the development of lines or cultivars with a high level of resistance. The QTL study of Foolad et al. (2002b) just described aimed to identify QTLs for resistance without altering the agronomic traits. Therefore, they removed plants with poor characteristics from their population before attempting to map the QTLs. However, none of the plants in the ensuing generations had a resistance level equal to that of the donor parent or the F1 hybrid (Foolad et al. 2002b).

The association of late maturity with EB resistance has also been documented for potato EB (e.g., Johanson and Thurston 1990). As in tomato, it is not yet clear whether this correlation is caused by closely linked genes or by pleiotropic effects of genes. A mapping study for EB and maturity in potato identified five EB resistance QTLs, explaining 62% of the total phenotypic variation for resistance (Zhang 2004). Three of these five QTLs explained 98% of the total phenotypic variation for maturity. The other two EB resistance QTLs, which did not have an effect on (foliage) maturity, explained 33% of the total phenotypic variation for resistance (Zhang 2004). In potato therefore about half the genotypic variation for EB resistance is also linked to maturity; still, this may be due to either close linkage or to pleiotropic effects. A very similar situation occurs in the potato–late blight (*Phytophthora infestans*) interaction (Visker et al. 2003).

Even on susceptible plants, the younger, topmost leaves are usually free of EB symptoms, while the older, lower leaves may be necrotized by the fungus (Johanson and Thurston 1990). Attempts have been made to clarify the physiological mechanisms for this apparent resistance in young tissues and plants. Low sugar content has been suggested as the cause of higher EB susceptibility in older or weakened leaves and plants (Rotem 1994): late in the season, leaves of maturing plants might be susceptible due to translocation of sugars to the ripening fruit. An in vitro study by Sands and Lukens (1974) provided indirect evidence that abundant glucose in the medium inhibited the production of cell-degrading enzymes by *Alternaria solani*. The low sugar content hypothesis might explain the increased susceptibility of physiologically old plants or those that have a high fruit to foliage ratio (Barrat and Richards 1944). Another explanation of the relative resistance of young tissues is that the concentrations of three glycoalkaloids (solanine, chaconine, and solanidine), which are capable of inhibiting growth of *A. solani* in vitro, are higher in young tomato leaves, and then steadily decline as leaves and plant mature (Sinden et al. 1972).

The higher resistance of late-maturing cultivars can similarly be explained in terms of sugar and alkaloid contents. Late-maturing cultivars generally have an indeterminate, vine-type growth habit and continue producing new foliage

(Johanson and Thurston 1990). In contrast, early maturing types have a determinate growth habit and do not continue producing new foliage throughout the season. Therefore, late-maturing cultivars might appear resistant compared with the early maturing types just because fruit initiation is delayed and more young leaves are present throughout the season.

If physiological mechanisms are the only cause of EB resistance, then it might be impossible to find recombinants with a high resistance level and highly desirable horticultural characteristics in a segregating population. In that case, tomato breeders can only expect to obtain acceptable EB resistance levels in varieties with midseason or late season maturity. However, variation in the resistance of potato occurs between cultivars of the same maturity class, indicating that differences in resistance are not always or exclusively an artifact of maturity effects (Holley et al. 1983; Christ 1991). So far, EB resistance screening in tomato, in contrast to potato, has not taken into account maturity classes or yield potential (Douglas and Pavek 1972).

## Characterization of resistance

Several epidemiological parameters have been identified in *Alternaria solani*–tomato and *A. solani*–potato interactions, including infection efficiency (IE), lesion expansion rate (LER), latency period (LP), incubation period (IP), sporulation rate (SR), and sporulation capacity (SC). Tomato lines with a higher level of resistance typically had a lower IE, slower LER, slower SR, and lower SC, but LP did not differ significantly compared with susceptible lines (O'Leary and Shoemaker 1983). IP was most important in determining cultivar ranking in potato; resistant cultivars had a longer IP (Pelletier and Fry 1989). SC was found to correspond linearly with lesion size (Pelletier and Fry 1989; Johnson and Teng 1990).

Secondary plant metabolites correlated to EB resistance include a higher total phenolic content (tannin, flavonol, and phenol) in leaves and stems of EB-resistant varieties (Bhatia et al. 1972). The tannin content in all varieties fluctuated as the plant matured but reached a maximum content by the 14th week in leaves and by the 10th week in stems. In addition, the fruits of resistant varieties contained a higher amount of phenolic compounds than those of susceptible varieties (Bhatia et al. 1972). The constitutive expression of phenols, which are thought to function as preformed inhibitors, has been associated with nonhost resistance (Nicholson and Hammerschmidt 1992).

At the cellular level, events during the infection by *A. solani* involve general defense responses that are also found in other plant–pathogen interactions involving quantitative resistance. These responses are basically similar to those after hypersensitive responses in monogenic resistance, but they are expressed more slowly and at a lower level (Agrios 2005). In EB-resistant lines, a stronger, more rapid induction of the pathogenesis-related (PR) proteins chitinase and β-1,3-glucanase (Lawrence et al. 1996, 2000), peroxidase (PO, Fernandez et al. 1996), and phenyalalanine ammonium lyase (PAL; Solorzano et al. 1996) were observed during the early infection process compared with those in susceptible lines (Lawrence et al. 1996, 2000). Chitinases and glucanases probably slow fungal ingress in the plant as indicated by their inhibition of the in vitro growth of *A. solani* (Lawrence et al. 1996). Enzyme preparations from resistant lines also induced the in vitro release of elicitors of the hypersensitive response (HR) from *A. solani*, whereas enzymes from susceptible lines did not (Lawrence et al. 2000).

PO is involved in the production of reactive oxygen species, which are directly toxic to the pathogen or indirectly reduce the spread of the pathogen by increasing the cross-linkage and lignification of the plant cell walls (Hammond-Kosack and Jones 1996). PAL is the key enzyme in the synthesis of the secondary, endogenous signaling molecule salicylic acid (SA), which in turn activates the expression of a variety of PR genes (Mauch-Mani and Slusarenko 1996).

Polyphenol oxidase (PPO) F is systemically upregulated in response to *A. solani* infection and is detected in leaves of upper nodes but not in lower nodes (Thipyapong and Steffens 1997). This induction pattern coincides with the observation of temporary resistance of young leaves to *A. solani* infection (Johanson and Thurston 1990). PPOs catalyze the oxidation of phenols to quinines, reactive molecules that induce cell death and barriers to secondary infection (Thipyapong and Steffens 1997). PPO F is induced within lesions but not around the lesions during early infection and necrotic lesion development. Other defense-related responses to infection with *A. solani* involve the elevated expression of the PR-1B gene after exogenous application of SA on tomato roots (Spletzer and Enyedi 1999), the PR-1-like protein after leaf treatment of tomato with arachidonic acid (Coquoz et al. 1995), and the sequential expression of two ACC synthase genes (*ST-ACS4* and *ST-ACS5*) in potato (Schlagnhaufer et al. 1997).

The biological effects of the genes underlying the identified EB-resistance QTLs remain unclear. A candidate gene approach, either using genes involved in the pathogen recognition process [resistance genes (R genes) or R gene analogs (RGAs), Foolad et al. 2002b] or those involved in the defense response process [defense response genes (DR genes), Faris et al. 1999] as molecular markers for QTL analysis, is potentially useful for analyzing EB resistance. Because resistance to *A. solani* does not seem to be race specific and is not mediated by genes with a major effect, R genes are unlikely to be involved in this resistance. Therefore, DR genes are more likely candidate genes for the QTLs involved in EB resistance. Faris et al. (1999) provided a convincing example. They mapped DR genes on a wheat linkage map where QTLs for several diseases had previously been identified. These DR genes were shown to be more significantly associated with disease resistance and explained more of the phenotypic variation than did the original markers used for QTL detection. Mapping at a higher resolution is also needed, however, before establishing any functional relationship.

## Concluding remarks

A wealth of information on the tomato–*Alternaria solani* interaction is available. However, some important aspects need further attention.

No conclusive evidence is available so far concerning the existence of physiological races. This should be studied using homozygous tester lines and isolates that are as homogeneous as possible.

The strong association of negative horticultural traits with the expression of EB resistance seems to be a general rule, for which no conclusive genetic explanation has yet been offered. Meanwhile, breeders should be aware that selection for resistance will only produce useful results if the plant material is comparable in terms of earliness and yield.

QTLs for EB resistance have been identified in populations from interspecific crosses. Before these can be used in a marker-assisted breeding program, fine mapping is needed to avoid introgressing large parts of donor genome along with the resistance gene. Also, before QTLs are used in a breeding program, their pleiotropic effects on other traits should be investigated.

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