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Review

Tomato as a model plant for plant-pathogen interactions

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Abstract Tomato has been a good model plant to analyze plant-pathogen interactions and its prospects for the future are promising. An international consortium named International Solanaceae Genomics Project (SOL) is proceeding with whole genome sequencing of tomato. In order to be relevant in the post-genomic era, accumulation of information on tomato-pathogen interactions is important. In this review, the following topics are addressed from the perspective of plant pathology: cultivars of tomato, wild species of tomato, disease-resistance in modern breeding of tomato, fungal, bacterial, and viral pathogens of tomato, known interactions between tomato and pathogens, fungicides and biocontrol agents applicable to tomato, and systemic resistance induced by microbes and by plant activators. Tomato is one of the most popular vegetables worldwide, however, its cultivation has been limited by an abundant attack by pathogens. In order to establish effective control methods to control them, analysis of tomato-pathogen interactions is also important.

Key words: Fungus, pesticide, plant activator, resistance, virulence.

In modern and basic plant science, *Arabidopsis thaliana* and *Lotus corniculatus* have been excellent model plants because each has a relatively small genome and is suitable for genome manipulation. Much information on plant-microbe interactions has been accumulated using these model plants and the genomic information obtained from them. However, additional models are desirable for comprehensive evaluation of plant-pathogen interactions. For example, the number of *Arabidopsis* pathogens is relatively small, e.g., *Perenospora parasitica*, *Botrytis cinerea*, *Fusarium oxysporum*, *Pseudomonas syringae* pvs. *maculicola* and *tomato*, *Xanthomonas campestris* pv. *campestris*, *Cucumber mosaic virus* (CMV), *Tomato mosaic virus* (ToMV), and *Turnip mosaic virus* (TuMV). Ascomycetes, the predominant pathogens of crops, are not well represented among known pathogens of *Arabidopsis*. In Japan there is the additional difficulty that regulatory approval from the Ministry of Agriculture, Forestry and Fisheries of the Japanese government is required before non-domestic pathogens of *Arabidopsis* can be imported for experimental use. Recently, Chinese cabbage (*Brassica napa* or *B. campestris* Pekinensis group), which is in the same family (cruciferae) as *Arabidopsis*, has been used as an alternative to *Arabidopsis*. Although many fungal, bacterial, viral, and protozoan diseases have been reported on Chinese cabbage, as a crop it is restricted to East Asian countries such as Japan, Korea, and China, and therefore of limited value as a general model.

Here, we suggest that solanaceous plants, especially tomato (*Lycopersicon esculentum*), have provided (and will continue to provide) excellent model systems to study plant-pathogen interactions (Meissner et al. 1997; Meissner et al. 2000; Emmanuel & Levy 2002). Tomato is one of the most popular vegetables worldwide. Its cultivation, however, has been limited by an abundance of diseases caused by fungi, bacteria, viruses, and nematodes. Jones et al. (1991) presented major diseases of tomato caused by 24 fungi, 7 bacteria, 10 viruses, 3 viroids, and multiple nematodes. In Japan, 41 fungi, 10 bacteria, 1 phytoplasma, 15 viruses, and 14 nematodes have been reported to be pathogens of tomato, most of which are distributed worldwide (The Phytopathological Society of Japan 2000).

Abbreviations: *AAL*, *Alternaria alternata* tomato pathotype; ASM, acibenzolar-*S*-methyl; CMV, *Cucumber mosaic virus*; DMI, dimethylation inhibitor; EBI, ergosterol biosynthesis inhibitor; EST, expressed sequence tag; *FOL*, *Fusarium oxysporum* f. sp. *lycopersici*; HST, host specific toxin; ISR, induced systemic resistance; I-PCR, inverse-PCR; ISR, induced systemic resistance; LRR, leucine rich repeat; MIT, Massachusetts Institute of Technology; MOA, mode of action; PBZ, probenazole; PCR, polymerase chain reaction; PR-protein, pathogenesis related-protein; REMI, restriction enzyme mediated integration; *R* gene, resistant gene; SA, salicylic acid; SAM, sphinganine-analog mycotoxin; SAR, systemic acquired resistance; TAIL-PCR, thermal asymmetric interlaced-PCR; TIGR, The Institute for Genomic Research; TGRC, Tomato Genetics Resource Center; ToMV, *Tomato mosaic virus*; VMA, validamycin A.

Table 1. Disease resistances in present tomato cultivars.

Disease	Pathogen	Resistance gene	Origin
Fungal diseases			
Alternaria cancer	*Alternaria alternata* tomato pathotype	*Asc*	*Lycopersicon esculentum*
Corky root	*Pyrenochaeta lycopersici*	*Py*	*L. hirsutum*
Crown and root rot	*Fusarium oxysporum* f. sp. *radicis-lycopersici*	*Frl*	*L. peruvianum*
Early blight	*Alternaria solani*	[1]	*L. hirsutum*
Late blight	*Phytophthora infestans*	*Ph*	?
Leaf mold	*Fulvia fulva*	*Cf*	*L. peruvianum*
Leaf spot	*Stemphylium lycopersici*	*Sm*	*L. pimpinellifolium*
Powdery mildew	*Leveillula taurica*	*Lv*	
	Oidium neolycopersici	*Ol*	*L. hirsutum*
Wilt	*Fusarium oxysporum* f. sp. *lycopersici* race 1	*I*	*L. pimpinellifolium*
	Fusarium oxysporum f. sp. *lycopersici* race 2	*I2*	*L. pimpinellifolium*
	Fusarium oxysporum f. sp. *lycopersici* race 3	*I3*	*L. pennellii*
Verticillium wilt	*Verticilliium dahliae*	*Ve*	*L. esculentum*
Bacterial disease			
Bacterial speck	*Pseudomonas syringae* pv. tomato	*Pto*	*L. pimpinellifolium*
Viral diseases			
Mosaic	*Tomato mosaic virus* (ToMV)	*Tm-1*, *Tm-2*, *Tm2a*	*L. hirsutum*, *L. peruvianum*
Spotted wilt	*Tomato spotted wilt virus* (TSWV)	*Sw*	*L. peruvianum*
Yellow leaf curl	*Tomato yellow leaf curl virus* (TYLCV)	*Ty-1*,*Ty-2*	*L. peruvianum*, *L. hirsutum*
Nematode disease			
Root knot	*Meloidogyne arenaria*	*Ma*	*L. peruvianum*
	Meloidogyne incognita	*Mi*	*L. peruvianum*
	Meloidogyne javanica	*Mj*	*L. peruvianum*

[1] quantitative trait loci (QTLs) was identified by Foolad et al. (2002).

This diversity of pathogens emphasizes the importance of the tomato pathosystem as a favorable model for studying plant-pathogen interactions. A good deal of progress toward understanding the interactions between tomato and the leaf mold fungus, *Fulvia fulva*, has been reported by Dutch scientists (Joosten & de Wit 1999). There has also been substantial investigation of interactions between tomato and other pathogens, such as *Fusarium oxysporum*, *Alternaria alternata*, *Pseudomonas syringae*, and *Cucumber mosaic virus* (CMV) (Di Pietro et al. 2003).

The cause of tomato research has recently been advanced by an international consortium, including Kazusa DNA Research Institute (Kisarazu, Chiba, Japan), which is proceeding with whole genome sequencing of tomato cv. Moneymaker. In preparation for the post-tomato-genome era, Japanese plant pathologists are now building a research consortium[1] for tomato-pathogen interactions (Takahashi et al. 2005). Available resources are described below.

Cultivars and wild species

Wild species and breeding history

The origin of tomato is thought to be the Andean regions of Peru, Chile, and Ecuador. At present eight wild species (*L. cheesmanii*, *L. chilense*, *L. chmielewskii*, *L. hirsutum*, *L. parviflorum*, *L. pennellii*, *L. peruvianum*, and *L. pimpinellifolium*) have been reported from the area. Only *L. cheesmanii* is found in the Galápagos Islands, Ecuador. Because most of the wild species are relatively resistant against diseases, they have been used as a source of resistance genes in modern tomato breeding (Table 1). *L. peruvianum* and *L. chilense* collected from Chile are actually resistant against wilt, powdery mildew, and Alternaria stem canker (Okabe et al. 2005). There is a genebank of tomatoes including wild species in The C. M. Rick Tomato Genetics Resource Center (TGRC)[2] at UC Davis (Davis, CA, USA).

Although debatable, *L. esculentum* (ordinary tomato) probably developed from *L. pimpinellifolium* that originated in Peru and Ecuador. Probably, the first-stage of breeding occured in Mexico over a period of more than 1000 years, culminating in the 15th century. That is why tomatoes with great diversity in size, shape, and color can still be found in Mexico. Now *L. esculentum* var. *cerasiforme* (similar to cherry tomato) is a wild plant in the Southern part of Mexico and the local variety of *L. esculentum* (so-called 'jtomate creollo') is still cultivated in some rural areas of Mexico. In the 16th century, tomato was carried to Europe by Spanish explorers and after that modern breeding led to development of the tomato now cultivated.

[1] http://www.agri.tohoku.ac.jp/ppathol/tomato
[2] http://tgrc.ucdavis.edu/

Table 2. Pathogens which cause typical symptoms on tomato cv. Micro-Tom[1].

Pathogen	Disease	Major symptom
Fungi		
Basidiomycetes		
Athelia rolfsii	southern blight	necrosis; wilt
Ascomycetes		
Botrytis cinerea	gray mold	gray to brown necrotic lesion; soft rot
Oidium sp.	powdery mildew	white powder on the leaf
Sclerotinia sclerotiorum	sclerotinia rot (white rot)	water salked gray to brown necrotic lesion; soft rot
Chromista		
Phytophthora infestans	late blight	water-soaked pale green lesions; foliage becomes brown, shrivelled, and died
Bacteria		
Ralstonia solanacearum	bacteria wilt	wilt; necrotic lesion by leaf-infiltration
Agrobacterium tumefaciens	crown gall	crown gall
Pseudomonas syringae pv. *tomato*	bacterial speck	yellowing; no leaf spot
Viruses		
Tomato mosaic virus (ToMV)	mosaic	mild mosaic
Tomato aspermy virus (TAV)	mosaic	mosaic; systemic necrosis
Cucumber mosaic virus (CMV)	mosaic	systemic necrosis

[1] Takahashi et al. (2005).

Present cultivars

Previously, most commercial varieties of tomato were purebred, and it was easy to buy seeds from distributors. However, at present, most commercial tomato varieties are hybrid, and it is often difficult to buy seeds of purebred varieties such as Ponderosa, Moneymaker, and Rutgers, which are frequently used for research purposes. Although we can buy the seeds from special suppliers[3], it is not difficult for individual investigators to produce the seeds for their own purposes because tomato usually self pollinates (Rick 1987).

Cultivars resistant to many fungal, bacterial, viral, and nematode diseases have been developed. Table 1 shows the major disease resistance traits that tomato cultivars carry. Most of these traits have been introduced from wild tomato species by crossing with *L. esculentum.*

cv. Micro-tom

Micro-tom (Scott & Harbaugh 1989) is a dwarf cultivar of *L. esculentum* that yields mature fruits within about 2 months after sowing. Micro-tom has potential as a model representative of tomato and other solanaceous plants because it is easily transformed by the Agrobacterium-mediated technique, and can be manipulated by enhancer trapping, gene trapping, or knockout systems based on the Ac/Ds transposable elements and T-DNA (Shibata 2005; Takahashi et al. 2005). Moreover, the group in Kazusa DNA Research Institute is now preparing full-length cDNA libraries from leaves, fruits, and roots of Micro-Tom for EST sequencing and subsequently full-length sequencing of selected clones (Shibata 2005).

Micro-tom is susceptible to at least four fungal species, one chromista species, two bacterial species, and two viruses (Table 2). The details and the methods for inoculation are described in Takahashi et al. (2005).

Fungal pathogens

Fungi are eukaryotic microbes with genomes smaller (ca. 40 Mb) than plants and animals. Fungi are composed of mycelia, spores, sclerotia, and fruiting bodies and most can grow on agar- or broth-media. They secrete enzymes to degrade substrates into small molecules and absorb them into cells. They have potential for both asexual and sexual lifecycles but asexual (clonal) reproduction through mycelial elongation or conidial (asexually produced spore) formation usually predominants. During asexual reproduction, fungi carry single or multiple haplophase nuclei in their cells. Protoplasts generated from mycelia or germinating conidia of fungi may contain sole nucleus. Protoplasts of many fungi are readily transformed using standard vectors and methods, often involving treatment of protoplasts with polyethylene glycol. Homologous recombination involving double crossover events between chromosomal DNA and vector DNA frequently occurs, allowing targeted gene disruption. Restriction enzyme mediated integration (REMI; Lu et al, 1994; Kawabe et al. 2004) and Agribacterium mediated integration (Takahara et al. 2004) are frequently used to generate random insertion of plasmid DNA into fungal genome. Since most fungal nuclei are haploid, primary transformants immediately

[3] cv. Ponderosa is available from Noguchi Seeds, 8-6 Nakamachi, Hannou, Saitama, Japan (http://noguchiseed.com/) in Japan; cvs. Moneymaker and Rutgers can be obtained from Baker Creek Heirloom Seed, 2278 Baker Creek Rd. Mansfield, MO 65704, USA (http://www.rareseeds.com/).

show phenotypic changes caused by transformation, an advantage of using fungi for functional gene analysis. The gene disrupted by plasmid integration by REMI or Agrobacterium mediated transformation in the genome of the mutant is easily rescued by thermal asymmetric interlaced (TAIL)-PCR (Liu and Whittier 1995; Arie et al. 1998) or inverse (I)-PCR. Commonly used selective agents include hygromycin B, geneticin (neomycin, Sigma, st. Louis, MO, USA), bialaphos (Meiji Seika Kaisha, Tokyo, Japan), phleomycin (InvivoGen, San Diego, CA, USA), and blasticidin S (Kaken Pharmaceutical, Tokyo, Japan); the corresponding genes for resistance are *hph* (hygromycin B phosphotransferase; Gritz & Davies 1983; Cullen et al. 1987; Turgeon et al. 1987), *NPTII* (neomycin phosphotransferase II; Lang-Hinrichs et al. 1990), *bar* (phosphinothricin-N-acetyltransferase; Strauch et al. 1988), *ble* (13.7 kDa phleomycin binding protein; Rohe et al. 1996), and *BSD* (blasticidin S deaminase; Kimura et al. 1994). Reporters available for fungi include β-galactosidase (GUS; Couteaudier et al. 1993) and fluorescent proteins such as GFP and DsRed (Vanden Wymelenberg et al. 1997; Mikkelsen et al. 2003). Constitutively expressed promoters often used in fungal vectors are those of p*trpC* from *Aspergillus nidulans* (tryptophan biosynthesis from chorismate; Mullaney et al. 1985; Cullen et al. 1987; Couteaudier et al. 1993), p*gpd* (glyceraldehydes-3-phosphate dehydrogenase; Punt et al. 1990; Choi and Nuss 1990; Couteaudier et al. 1993; Mikkelsen et al. 2003) from *A. nidulans*, and p*TEF* (transformation elongation factor; Vanden Wymelenberg et al. 1997) from *Aureobasidium pullulans.*

To illustrate typical fungal tomato diseases, four species have been chosen and described below, although there are other important fungal pathogens of tomato such as *Alternaria solani*, *Athelia rolfsii*, *Corynespora cassiicola*, *Oidium* spp., *Sclerotinia sclerotiorum*, *Septoria lycoperesici*, *Pyrenochaeta lycopersici*, and *Verticillium dahliae*; these also have been intensively studied.

Alternaria alternata tomato pathotype

Alternaria stem canker caused by *Alternaria alternata* tomato pathotype (*AAL*) (synonym *A. alternata* f. sp. *lycopersici*, synonym *A. arborescens*) was first reported in San Diego, USA in 1960 and was later found worldwide. Symptoms with dark brown to black cankers occur on foliage including leaves, petioles, and stems. *AAL* produces the host-specific (HST) AAL-toxins, a family of polyketides. In contrast to interactions conforming to the gene-for-gene concept, described later, the toxin molecule is extremely selective for susceptible tomato cultivars such as First (Takii Seed, Kyoto, Japan) and causes severe necrosis on stems and leaves, whereas resistant cultivars are tolerant and show no symptoms. In *AAL*-tomato interactions, a major factor in pathogenicity is the production of AAL-toxins (Akamatsu et al. 1997; Brandwagt et al. 2000).

AAL-toxins and the fumonisins of *Fusarium verticillioides* (synonym *F. moniliforme*, teleomorph *G. moniliformis*, synonym *G. fujikuroi* mating population A) are sphinganine-analog mycotoxins (SAMs), which are toxic to certain plant species and mammalian cells (Gilchrist et al. 1995; Yamagishi et al. 2006). AAL-toxins cause apoptosis in susceptible tomato cells and mammalian cells by inhibiting ceramide biosynthesis (Gilchrist et al. 1995; Wang et al. 1996; Spassieva et al. 2002).

Insensitivity to AAL-toxins and resistance to *AAL* are both mediated by the *Alternaria* stem canker (*Asc*) locus in tomato genome (Van der Biezen et al. 1995). The gene *Asc-1* is homologous to the yeast longevity assurance gene *LAG1* (Brandwagt et al. 2000; Brandwagt et al. 2002). Sensitivity to AAL-toxin is associated with disfunction of *Asc-1*. Insensitivity to the toxin and resistance to the pathogen are due to a normally functioning *Asc-1* mediated-salvage pathway for ceramide-depleted plant cells (Brandwagt et al. 2000). Most tomato plants, including commercial cultivars, have an intact *Asc-1* gene, making them resistant to the pathogen and the toxin. Amomg wild species, only *L. cheesmanii* (found in the Galápagos Islands) is susceptible to the pathogen and the toxin.

*Botrytis cinerea (*teleomorph, *Botryotinia fuckeliana)*

This ascomycetous fungus is the causal agent of gray mold disease on not only tomato but also many plant species. The fungus first induces gray to brown water-soaked necrotic lesions, which enlarge rapidly, affecting the leaflet, petiole, stem, and sometimes the whole plant; symptoms may include soft rot (Cotoras & Silva 2005). The method of inoculation of tomato with *B. cinerea* is described in Takahashi et al. (2005). Pectic enzymes produced by the fungus are involved in the development of soft rot. Although the fungus was first thought to be mitosporic (asexual), it was later found to reproduce sexually if a microconidial (spermatial) suspension was poured over the sclerotia (Fukumori et al. 2004). Recently, genetic transformation of the species has been reported (Nakajima et al. 2001).

*Fulvia fulva (*synonym, *Cladosporium fulvum)*

This asexual ascomycetous fungus causes leaf mold disease, primarily under greenhouse conditions. The fungus colonizes the intercellular spaces in the leaves, causing yellowish spots with unclear margins. On abaxial surfaces of infected leaves, an olive green to gray mold appears. Severe infection causes foliar blight

(Jones et al. 1991).

The interaction between *Fulvia fulva* races and tomato varieties, described by Joosten & de Wit (1999), fits the gene-for gene concept (Flor 1971): for each gene in the pathogen that confers avirulence there is a corresponding gene in the host that confers resistance. The first fungal avirulence gene (*Avr9*) was identified in this fungus. Now several avirulence genes including *Avr2*, *Avr4*, and *Avr9*, and their corresponding proteins, have been identified in *F. fulva. Avr9* is expressed only in plant tissues, and translated into proproteins of 32–34 amino acids (aa); the mature AVR9 peptide of 28 aa is produced by proteolysis. AVR9 contains three antiparallel strands that form a compact β-sheet region and two solvent-exposed loops bearing hydrophobic residues that are essential for the necrosis-inducing activity (Joosten & de Wit 1999).

Fusarium oxysporum

Fusarium oxysporum is an asexual ascomycetous fungus that causes severe soilborne vascular wilt diseases of many crops (Booth 1971). The fungus invades epidermal tissues of the root, extends to the vascular bundles, produces mycelia and/or spores in the vessels, and kills plants by plugging the vessels and/or by producing toxic chemicals (Beckman 1987).

F. oxysporum f. sp. *lycopersici* (*FOL*) is a form of the species that infects tomato (Booth 1971). Three physiological races (designated 1, 2, and 3 in order of discovery), are distinguished by their specific pathogenicity to different tomato cultivars. Single race-specific genes conferring resistance to *FOL* have been identified in wild *Lycopersicon* spp. and introgressed into commercial tomato cultivars (Table 1; Sela-Buurlage et al. 2001). The *I*, *I2*, and *I3* loci confer resistance to race 1, race 2, and race 3, respectively, and relationships between the races and tomato cultivars follow the gene-for-gene pattern (Table 3). Standard cultivars can be used to determine races of *FOL* are listed in Table 3. The method of inoculation of tomato with *FOL* is described in Takahashi et al. (2005).

Sequencing the genome of *FOL* race 2 is underway by a consortium managed by Cereal Disease laboratory, USDA-ARS (St. Paul, MN, USA).

F. oxysporum f. sp. *radicis-lycopersici* is an another form of the species which causes crown and root rot on tomato (Jarvis and Shoomaker 1978).

Oomycetous pathogen

Phytophthora infestans

Although this pathogen was previously understood to be a member of Mastigomycotina, Eumycota in the Kingdom of Fungi, it is now recognized as a member of the Oomycota in The Kingdom Chromista. Late blight disease caused by this pathogen resulted in the notorious Irish potato famine in 1845–46; it is still one of the most devastating diseases of potato, as well as tomato (Agrios 2005). In the late 1980s and early 1990s, strains resistant to metalaxyl, the most effective fungicide against the pathogen, emerged and late blight is a significant present-day menace to agriculture (Money 2001). Agrios (2005) has predicted this disease is one of the most likely to cause severe losses in the future.

The pathogen causes a severe foliar blight under cool moist conditions. *P. infestans* can be maintained on agar medium and the zoosporangia, or conidia, produced in culture are suitable for artificial inoculation of tomato plants (Takahashi et al. 2005).

Considerable number of information about epidemiology, co-evolution with host plants, race diversity, phylogeny, genetic linkage map, and sexual reproduction has been accumulated on the pathogen (Erwin and Ribeiro 1996), and molecular tools such as

Table 3. Relationships between tomato cultivars and the races of wilt pathogen based on the gene-for-gene concept.

Tomato		*Fusarium oxysporum* f. sp. *lycopersici*		
Genotype	Cultivar	race 1 *Avr1 Avr2 Avr3*	race 2 *avr1 Avr2 Avr3*	race 3 *avr1 avr2 Avr3*
i i2 i3	Ponderosa[3] Sekaiichi[3]	S[2]	S	S
I i2 i3	Okitsu No. 1[3] Momotaro (Takii)[4] Odoriko (Sakata)[4]	R	S	S
I I2 i3	Walter[3] Myrock (Sakata)[4]	R	R	S
I I2 I3	Block (Sakata)[4] Protect 3 (Takii)[4]	R	R	R

[1] Genotype of avirulence genes based on gene-for-gene concept (Flor 1971).
[2] S indicates the host susceptible interaction; R indicates the host resistant interaction.
[3] Purebred. [4] Hybrid.

[4] http://staff.vbi.vt.edu/estap/

expressed sequence tag (EST) libraries are available from EST Analysis Pipeline[4] (ESTAP, Virginia Bioinfomatics Institute, Blacksburg, VA, USA). In addition, analyses of the mitochondrial and nuclear genomes are being pursued by a group that includes scientists from North Carolina State University (Raleigh, NC, USA), The Institute for Genomic Research (TIGR[5]; Rockville, MD, USA), and Broad Institute at MIT (Cambridge, MA, USA). The method of inoculation of tomato with the pathogen is described in Takahashi et al. (2005).

Bacterial pathogens

Ralstonia solanacearum

This soilborne bacterium, previous *Pseudomonas solanacearum*, causes a serious systemic wilt disease on tomato. Initial symptoms appear as flaccidity in younger leaves and expand to a systemic wilt in several days under favorable conditions (at relatively high temperature). Vascular tissues in diseased tomato become water-soaked brown, and when the stem is cut crosswise and put in water, white viscous ooze (stream of bacterial cells) exudes from the vascular bundles (Jones et al. 1991). The method of inoculation of tomato with *R. solanacearum* is described in Takahashi et al. (2005).

Clavibacter michiganensis subsp. michiganensis

Canker caused by this bacterium, previously *Corynebacterium michiganense*, is one of the most important diseases of tomato worldwide. Bacteria overseason in plant debris in soil and are spread by splashing water. The disease is less prevalent in fields with plastic cover, which reduces rain-water dispersal. Down-turned leaves with marginal necrosis initially appear on infected tomato, and are immediately followed by systemic wilt. Vascular tissues in diseased tomato show brown to reddish brown symptoms. On infected fruit surfaces, small spots with raised brown centers surrounded by an opaque white halo develop, and the fruits have a scabby appearance (Jones et al. 1991).

Viral and viroid pathogens

The viruses and viroids reported from tomato are listed in Jones et al. (1991). *Tomato mosaic virus* (ToMV; previously Tobacco mosaic virus-T), *Cucumber mosaic virus* (CMV), and *Tomato yellow leaf curl virus* (TYLCV) have been characterized at the molecular level (Nishiguchi et al. 1978; Nishiguchi et al. 1985; Nishiguchi et al. 1990; Takahashi et al. 2005; Yoneyama et al. 2006). The method of inoculation of tomato with ToMV, TAV and CMV is described in Takahashi et al. (2005).

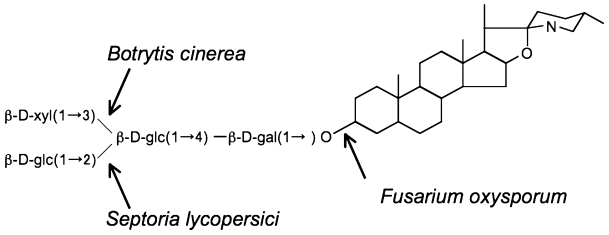

Figure 1. α-tomatine and the cleavage sites for tomatinases from plant pathogenic fungi of tomato. *Fusarium oxysporum*, the wilt fungus, *Septoria lycopersici*, the septoria leaf spot fungus, and *Botrytis cinerea*, the gray mold fungus.

Plant viruses on tomato are transmitted mainly by insect vectors, such as aphids, whiteflies and thrips. Therefore, control of the disease is often achieved using insecticides. Transgenic tomatoes expressing CMV coat protein display excellent field resistance to CMV infections (Fuchs et al. 1993).

Tomato-pathogen interactions

Plant-pathogen interactions can be explained by two stages. The first includes interactions between general constitutive plant defense mechanisms and virulence factors produced by the pathogen aimed at destroying the defense. Second, following initial recognition, the plant induces acquired resistance while the pathogen tries to escape from the resistance.

General defense

A plant's general defense consists of physical and chemical factors. Physical defenses include cutins, which are hardy polymers covering plant external surfaces, pectins that exist in cell walls and middle lamellae to effect adherence between cells, and cell walls, which protect plant cells from external harm. Examples of general chemical defenses are the phytoanticipins such as saponins. Tomato has an antifungal saponin tomatine (Figure 1).

General pathogenicity-related factors and genes in tomato pathogens

Pathogens of tomato have to nullify the general defenses that tomato originally carries before they establish in tomato. Physical defenses such as cutins, pectins, and cell walls can generally be degraded by cutinases, pectinases, xylanases, and cellulases produced by pathogens, respectively. For example, *FOL* secretes pectinases and xylanases. Genes encoding pectinases or xylanases such as *pg1*, *pg5*, *pgx4*, *xyl2*, and *xyl3* have been cloned from *FOL* (Arie et al. 1998; Di Pietro and Roncero 1996; Di Pietro and Roncero 1998; García-

[5] http://www.tigr.org/tdb/e2k1/pima1/index.shtml

Maceira et al. 2000; García-Maceira et al. 2001; Gómez-Gómez et al. 2001; Gómez-Gómez et al. 2002; Ruiz-Roldán et al. 1999). Although pectinases are supposed to be responsible for virulence, disruption of each gene has not eliminated pathogenicity. Probably pectinases are encoded by more than one gene, and lack of a particular pectinase is compensated by the pectinases encoded by other genes (Agrios 2005; Kawabe et al. 2004). A chemical defense, tomatine, is enzymatically detoxified by *Botrytis cinerea*, *Septoria lycopersici*, and *FOL* (Figure 1; Roldán-Arjona et al. 1999; Ito et al. 2004; Agrios 2005).

From *FOL*, pathogenicity-related genes such as *FPD1* (Kawabe et al. 2004) have been reported. From *AAL*, a cluster of the genes responsible for biosynthesis of AAL-toxin has been identified (Yamagishi et al. 2006). *R. solanacearum* has as many as 35 genes homologous to type IV pili genes, which may be involved in cell-to-cell aggregation (Kang et al. 2002).

Cultivar-race specific interactions

According to the gene-for-gene concept, *avr* genes make a pathogen avirulent. A pathogen carrying *avr* is unable to induce disease on a specific variety of the host plant because the *avr* product warns the plant of the presence and impending attack by the pathogen. The host then mobilizes its defenses and blocks infection by the pathogen (Agrios 2005). Avirulence genomic loci and/or genes were determined in tomato pathogens such as *F. fulva* (Joosten & de Wit 1999), *FOL* (Mes et al. 1999; Teunissen et al. 2002; Rep et al. 2004), and *Pseudomonas syringae* pv. *tomato* (Hanekamp et al. 1997).

Plant genes for resistance to pathogens carrying the corresponding *avr* genes are called resistance (*R*) genes. Generally, products of *R* genes contain a domain rich in the amino acid leucine (leucine rich repeat, LRR), which may function as a receptor for *avr* products. Tomato genes *Cf2*, *Cf4*, *Cf5*, and *Cf9*, which confer resistance to *F. fulva* races 2, 4, 5, and 9, (carrying avirulence genes *avr2*, *avr4*, *avr5*, and *avr9*, respectively) have been isolated (Joosten & de Wit 1999; Agrios 2005).

Behavior of pathogens in tomato tissues

Behavior of the pathogens on/in tomato tissues has been visualized by immunofluorescence using pathogen-specific antibodies (Arie et al. 1995) and using fluorescence protein-expressing pathogen (Nahalkova & Fatehi 2003). Detection of pathogens from tomato tissues for diagnostic purposes has been achieved by immunological methods (Arie et al. 1995) and PCR. Recently, Hirano & Arie (2006) reported primer sets for specific detection of the three races in *FOL* and *F. oxysporum* f. sp. *radicis-lycopersicon* in tomato tissues by PCR. The primer sets and the PCR conditions were also effective for DNA extracted from the tomato rhizospherical soil (Yoshioka et al.: unpublished data).

Tomato-pathogen co-evolution

Phylogenetic studies have been performed on several tomato pathogens including *P. infestans* (Fry et al. 1992), *FOL*, and *Verticillium dahliae.*

The Toluca Valley in Mexico is the only place where sexual reproduction of *P. infestans* has been observed, and much of the genetic diversity in *P. infestans* is found there. Elsewhere, including Ireland, Ecuador, UK, and US, most of the isolates from diseased potatoes and tomatoes are derived from a strain (US-1) that reproduces asexually. Hence, plant pathologists originally thought that Mexico is the center of origin for *P. infestans*, and that strain US-1 is a direct descendant of *P. infestans* responsible for the Irish potato famine and subsequent epidemics (Money 2001). Ristaino et al. (2001) amplified fragments of genomic DNA extracted from herbarium specimens of infected potato and tomato leaves, which were collected from Ireland during the famine. Sequencing analysis of the fragments proved that the samples were infected with *P. infestans*, but showed that the causal strain of the Irish famine was not US-1. This casts doubt on the idea that the pathogen spread from Mexico. Ristaino et al. proposed that Mexico is a center of diversity for *P. infestans*, but the center of diversity may not coincide with its center of origin; perhaps the center of origin for *P. infestans* is in South America, the ancestral home of solanum.

Gale et al. (2003) collected 121 isolates of *FOL* race 3 in the US. Phylogenetic study showed that molecular diversity among the isolates was highest in Manatee County, FL, suggesting that Manatee County is the center of origin of race 3. Kawabe et al. (2005) performed a phylogenetic analysis based on sequences of the ribosomal DNA intergenic spacer (IGS) region using a worldwide collection of *F. oxysporum* isolates, and found three evolutionary lineages (A1, A2, and A3) of *FOL* among the isolates. They found that each lineage consisted of isolates mainly belonging to a single or closely related vegetative compatibility group (VCG) and having a single mating type (MAT). Race 1 and race 2 isolates belonged to the A1 or A2 lineages, and race 3 of A2 or A3 lineages. They found no correlation between race and lineage. However, for isolates from Japan, race 1 (with one exception), race 2, and race 3 isolates are members of the A2, A1, and A3 lineages, respectively. They concluded that the races could have evolved independently in each lineage, and in Japan, the present races were likely to have been introduced independently, maybe through seeds, after they evolved in other locations.

Table 4. Chemical fungicides registered for tomato in Japan[1].

Fungicide	Chemical group[2]	Targeted disease	Mode of action (MOA)	Target site[3]
azoxystrobin	strobilurin	gray mold, leaf mold	inhibition of respiration	complex III: Qo site of cytochrome$_{bc1}$
benomyl	carbamate	gray mold, leaf mold, sclerotium rot, wilt	inhibition of mitosis and cell division	β-tubulin assembly
boscalid	anilide	gray mold, sclerotium rot, leaf mold	inhibition of respiration	complex II: succinate dehydrogenase
captan	polyhaloalkylthio	late blight, damping-off, leaf mold	multi-site inhibition	multi-site
chlorothalonil (TPN)	organochlorine	early blight, late blight, leaf mold	multi-site inhibition	multi-site
copper sulfate	copper	late blight early blight	multi-site inhibition	multi-site
copper hydroxide	copper	early blight, 'kappan' disease (*Cylindrosporium*), late blight, leaf mold, leaf spot	multi-site inhibition	multi-site
cyazofamid	imidazole	late blight	inhibition of respiration	complex III: Qi site of cytochrome$_{bc1}$
dazomet	others	bacterial wilt, corky root, damping-off (*Rhizoctonia*), root-knot, wilt	unknown	unknown
DBECD	others	gray mold, leaf mold, powdery mildew	unknown	unknown
diethofencarb	carbamate	gray mold	inhibition of mitosis and cell devision	β-tubulin assembly
difenoconazole	triazole	leaf mold	inhibition of sterol biosynthesis in membranes	C14-dimethylase
dimetomorph	morpholine	late blight	inhibition of glucan and cell wall synthesis	cell wall synthesis
fenarimol	pyrimidine	leaf mold	inhibition of sterol biosynthesis in membranes	C14-dimethylase
fludioxonil	pyrrole	damping-off (*Rhizoctonia*), gray mold	inhibition of osmotic signal transduction	MAP-kinase
flutolanil	anilide	damping-off (*Rhizoctonia*)	inhibition of respiration	complex II: succinate dehydrogenase
iminoctadine-albesilate	aliphatic	gray mold, leaf mold	multi-site inhibition	multi-site
iprodione	imidazole	early blight, gray mold, leaf spot	inhibition of lipids and membrane synthesis	NADH cytochromec reductase in lipid peeroxidation
mancozeb	dithiocarbamate	early blight, late blight, leaf mold	multi-site inhibition	multi-site
mepanipyrim	pyrimidine	gray mold	inhibition of protein synthesis or secretion	methionine synthesis
mepronil	anilide	damping-off (*Rhizoctonia*)	inhibition of respiration	complex II: succinate dehydrogenase
metalaxyl	anilide	late blight	inhibition of nucleic acids synthesis	RNA polymerase I
polycarbamate	dithiocarbamate	early blight, late blight, leaf mold	unknown	unknown
polyoxin D	antibiotic	early blight, gray mold, leaf mold	inhibition of glucan and cell wall synthesis	chitin synthase
potassium hydrogen carbonate	inorganic	powdery mildew, leaf mold	unknown	unknown
procymidone	heterocycle	gray mold	inhibition of lipids and membrane synthesis	NADH cytochrome c reductase in lipid peroxidation
tetraconazole	triazole	leaf mold	inhibition of sterol biosynthesis in membranes	C14-dimethylase
thiophanate-methyl	thioallophanateester	gray mold, leaf mold, sclerotium rot	inhibition of mitosis and cell devision	β-tubulin assembly
tolclophos-methyl	organophosphorus	damping-off (*Rhizoctonia*)	inhibition of lipids and membrane synthesis	lipid peroxidation
triflumizole	imidazole	leaf mold	inhibition of sterol biosynthesis in membranes	C14-dimethylase
triforine	others	leaf mold	inhibition of sterol biosynthesis in membranes	C14-dimethylase
validamycin A	antibiotic	damping-off (*Rhizoctonia*)	inhibition of glucose synthesis, plant activator	trehalase, upper SA in SAR

[1] The chemicals registered for tomato and 'mini-tomato' in Japan are listed.
[2] The chemical group classification followed Sato & Miyamoto (2003).
[3] Target site includes proposed.

Fungicides

Chemical fungicides

Many chemicals for control of tomato diseases have been registered in Japan (Table 4). The mode of action (MOA) suggested for each chemical is also shown in Table 4. Most of these chemicals are fungicidal or fungistatic. Difenoconazole, fenarimol, tetraconazole, triflumizole, and triforine inhibit the biosynthesis of ergosterol, which is an important component of the fungal cell membrane, by inhibiting the C14-demethylase (Brent 1995; de Waard 1996). They are called dimethylation inhibitors (DMI) or ergosterol biosynthesis inhibitors (EBI). Azoxystrobin is a derivative of strobilurin produced by *Strobilurus tenacellus*, which inhibits respiration by binding with the quinone 'outside' (Qo) site of cytochrome$_{bc1}$ in complex III, and is called a Qo-inhibitor (QoI; Bartlett et al. 2002; Sauter et al. 1999; Ypema & Gold, 1999). Cyazofamid inhibits respiration by binding with the quinone 'inside' (Qi) site of cytochrome$_{bc1}$ in complex III, and is called a Qi-inhibitor (QiI). Mepanypyrim does not have much fungicidal activity but it affects the fungal cell membrane and reduces secretion of cell wall degrading enzymes such as pectinases, which are thought to be virulence factors (Nagata et al. 2004). These chemicals can be valuable tools to investigate interactions between tomato and pathogens.

Biological fungicides and biocontrol agents

Several biological fungicides composed of *Pseudomonas fluorescence* and *Bacillus subtilis* are registered for tomato diseases in Japan. A *B. subtilis*-fungicide named 'Bot-killer' (Idemitsu Kosan, Tokyo, Japan) is commonly used in tomato production to prevent gray mold disease caused by *Botrytis cinerea*. Its MOA against *B. cinerea* is thought to be competition for space. A soil-formulation of *P. fluorescence* named 'Cell-nae-genki' (Taki Chemical, Kakogawa, Japan) is used to protect tomato plants from bacterial wilt caused by *R. solanacearum* and crown and root rot caused by *F. oxysporum* f. sp. *radicis-lycopersici*. Tomato plants treated with 'Cell-nae-genki' acquire resistance against the diseases.

There are several reports on application of non-pathogenic *F. oxysporum* isolates against soilborne tomato wilt diseases caused by *FOL* or *Verticillium dahliae* (Amemiya et al. 1989; Fuchs et al. 1997). Non-pathogenic *F. oxysporum* competes with the pathogens for space on tomato or induces resistance in tomato and suppresses disease development. Soil mycoflora in tomato field is discussed by Abdul Wahid et al. (1997).

Pythium oligandrum controls tomato crown and root rot. Benhamou et al. (1997) found that *P. oligandrum* not only acts as a mycoparasite but also triggers induction of defense-related reactions in tomato roots when challenged with the pathogen *F. oxysporum* f. sp. *radicis-lycopersici*. Takenaka et al. (2006) suggested that the cell wall protein elicitor of *P. oligandrum* induces resistance against *R. solanacearum* in tomato through the jasmonate-mediated signaling pathway.

Application of attenuated virus is an option for biological control of viral diseases. Many studies have been done using attenuated ToMV to control mosaic disease of tomato (Kiho & Nishiguchi 1984). At present, tomato nursery seedlings pre-inoculated with the attenuated CMV which carries satellite RNA are commercially sold by Nippon Del Monte (Tokyo, Japan) in Japan. The 'vaccinated' tomato plants are resistant against CMV in the field because of cross-protection or interference.

Induced resistance and plant activators

The plant recognizes the pathogen and activates structural and biochemical defenses to protect itself. This phenomenon is called induced or acquired resistance. Usually elicitors, such as carbohydrate, glycoproteins, proteins, peptides, and toxins produced by the pathogens, are recognized by receptors probably on the cell membrane. Systemic acquired resistance (SAR) is a concept in which systemic resistance against pathogens is activated by increased levels of salicylic acid (SA) and pathogenesis related-proteins (PR-proteins) after primary infection with a necrotizing pathogen (Ryals et al. 1996; Sticher et al. 1997; Bostock et al. 2001; Agrios 2005). In other words, SA and PR-proteins are the markers of SAR. Other concepts of induced resistance, such as induced systemic resistance (ISR) via jasmonic acid and ethylene as signal molecules, have also been proposed (van Loon et al. 1998; Siddiqui & Shaukat 2004).

To test if SAR or ISR is involved in induced resistance, experiments are now being done with the following mutants of tomato: *NahG*-Moneymaker (John Innes Center, Norwich, UK), which is a transformant of cv. Moneymaker carrying the salicylate hydroxylase gene *NahG* from *Pseudomonas putida* and cannot accumulate SA (Brading et al. 2000), *jai1*-Micro-Tom, which is a jasmonic acid-insensitive mutant of cv. Micro-Tom (Li et al. 2004), and *Nr*-Rutgers, which is an ethylene insensitive mutant of cv. Rutgers (Lanahan et al. 1994); these plants are currently available.

Recently, plant activators such as probenazole (PBZ; Meiji Seika Kaisha, Tokyo, Japan), acibenzolar-*S*-methyl (ASM; Syngenta Japan, Tokyo, Japan), thiadinil (TDL; Nihon Nohyaku, Tokyo, Japan), and harpin (EDEN Bioscience, Bothell, WA, USA), all of which induce SAR in plants, have received much attention because they control a wide range of diseases and their efficacy is of long duration. Several of these plant activators have been reported to induce SAR in tomato. Only harpin, or

Messenger, is the plant activator registered on tomato in the US.

Miyazawa et al. (1998) reported that in tomato tissues treated with 4-hydroxybenzoic hydrazide, salicylic hydrazide, or 2-furoic acid by root-dipping, a peroxydase was induced, and the treated plants were resistant against wilt caused by *FOL*.

Benhamou & Bélanger (1998) reported that treatment of tomato with ASM by foliar spraying was effective against crown and root rot caused by *F. oxysporum* f. sp. *radicis-lycopersici*. They observed that in the root of ASM-treated tomato, pathogen growth was restricted to the epidermis and the outer cortex and fungal ingress was halted by the formation of callose-enriched wall appositions at site of fungal penetration.

Validamycin A (VMA; Sumitomo Chemical, Tokyo, Japan) is an aminoglucoside produced by *Streptomyces hygroscopicus.* Although VMA does not kill or inhibit the growth of *FOL*, a foliar spray of VMA controls soilborne wilt of tomato caused by *FOL* (Ishikawa et al. 2005). The treatment was also effective against bacterial wilt, a soilborne disease caused by *R. solanacearum*, and foliar diseases such as powdery mildew caused by *Oidium* sp. and late blight caused by *P. infestans.* They found several days time lag between VMA-treatment and emergence of the control effect, and that control efficacy lasted 20–60 days. Moreover, in VMA-treated tomato, accumulation of SA and high transcription of genes encoding PR-proteins were observed. They concluded that VMA is a plant activator that induces SAR in treated tomato tissues. Foliar spraying with plant activators could be an especially good method to control soilborne diseases.

Tomato is now, and will continue to be, one of the most popular vegetables worldwide. Diseases caused by pathogens described in this review are major constraints on tomato production. Modern breeding has produced new cultivars resistant to the diseases, however, emergence of new races of pathogens often invalidates the resistance in a short period. A variety of fungicides effective against tomato diseases have been developed, however, occurrence of fungicide-resistant strains often nullifies the fungicide. We frequently are caught in these vicious cycles between resistant cultivars vs. new virulent races or novel fungicides vs. fungicide-resistant strains. This suggests that tomato-pathogen interactions still require much investigation to be fully understood. In order to produce healthy tomatoes indefinitely, further analyses of the interaction between tomato and its pathogens are needed; the results may have general relevance to plant-pathogen interactions.

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