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Review

Cutting the Gordian knot: taking a stab at corky root rot of tomato

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Abstract Corky root rot (CRR) is an escalating plant disease of tomato (*Solanum esculentum*), caused by a soil-borne fungus, *Pyrenochaeta lycopersici*. During the last two decades there have almost been no progress in the understanding of the molecular mechanisms promoting infection and plant susceptibility. As there are no CRR-resistant lines of cultivated tomato on the market and no other known means for plant protection, a deeper molecular knowledge about the infection process is urgently needed. We have therefore outlined an efficient strategy to search for corky root rot-resistance genes in wild tomato. In addition, we are investigating the genetic determinants for infection and virulence of the fungal pathogen, *P. lycopersici*. In this review we summarize the quite limited molecular knowledge about the pathogen and the disease, and discuss the possibilities to overcome previous technical obstacles in this new era of molecular biology.

Key words: Plant immunity, plant resistance, *Pyrenochaeta lycopersici*, tomato.

Everyone loves tomatoes...

Tomato (*Solanum lycopersicum*) is a member of the *Solanaceae* family, together with other important commercial crop plants such as tobacco, pepper, potato and eggplant. Next to potato, tomato is the second most important vegetable crop in the world. It is native from South America but is now grown globally. In 2005, 125 million tons of tomatoes were grown worldwide, China being the largest producer accounting for about one-fourth of the global output, followed by the United States and Turkey (FAOSTAT Database, 2005). There are hundreds of different tomato cultivars on the market carrying different traits and qualities. Generally, home-garden tomatoes are most commonly bred for flavor to the exclusion of all other features, while commercial varieties are bred for factors such as consistent size and shape, suitability for mechanized picking and shipping, and for disease and pest resistance. The latter traits are of great importance as there are numerous viruses, fungi, bacteria and insects that can attack tomato, cause disease and thereby lower production yield (www.ipm.ucdavis.edu/2007; FAOSTAT Database, 2005). During the last decade, substantial research efforts have led to a new level of understanding about immune mechanisms in tomato and other plants, and to the identification of specific resistance genes that can protect plants against pathogenic microbes (Chisholm 2006; Jones and Dangl 2006). There are several commercial tomato cultivars available providing resistance against a number of different pests such as Tobacco mosaic virus, *Pseudomonas syringae* and different *Fusarium* species, among others. However, producers are still defenseless against some diseases for which there are no resistant plant varieties, and for which there is very little knowledge about infection and disease mechanisms.

Pyrenochaeta lycopersici and Corky Root Rot, the cause and concern

Corky root rot is a growing concern for tomato growers all over the world, including major producers such as China, USA, Italy, and Japan. Yield loss up to 70–75% has been reported for certain years (Campbell 1982). Until now, CCR has mainly been an economically detrimental disease for organic tomato producers. However, after the banning of soil fumigation with Bromomethane (Methyl bromide) due to global restrictions against the use of ozone-depleting substances (UNEP 2000), keeping CRR at bay has rapidly become a major problem also for conventional tomato producers (Campbell 1982) (www.ipm.ucdavis.edu/2007). No other efficient method to control CRR has been discovered so far.

CRR is a soil-borne disease, causing progressive deterioration of the entire root system, constricting uptake of water and nutrients. As a consequence, plants get stunted and productivity is correspondingly reduced (Goode-

nough and Maw 1973). Progression of the disease in soil culture is slow and plants do generally not show clear symptoms until 2–3 months after infection. Initially, infected roots will develop necrotic lesions that will spread along the entire root system. Small roots disintegrate and larger roots grow thick and dark and become highly suberized. In the late stages of infection the root bark will fall off and expose the vascular bundles (Last and Ebben 1966; Pohronezny and Volin 1991). CRR infection usually does not kill plants, but severely reduces fruit yield. If infected soil is left untreated (that is, not fumigated), an increasing infection pressure will build up and reach its maximum after 5-6 years, causing correspondingly increased root damage (Forsberg et al. 1999).

Today, there is no adequate way to control the disease. However three main strategies are used; improved nursing practice, chemical pesticides and the use of semi-resistant plant varieties. Using husbandry techniques for large-scale cultivation is generally not applicable as it is labor-intensive (expensive), and not reliable. Soil fumigation with methyl bromide has frequently been used but is now restricted worldwide (UNEP 2000). Therefore, using genetically resistant plant varieties is currently the most sustainable and effective control strategy against *P. lycopersici*. However, the few available varieties claimed to confer resistance against CRR have proved inadequate and do not provide reliable plant protection (www.ipm.ucdavis.edu/2007) (Fiume and Fiume 2003).

Pyrenochaeta lycopersici a pathogen with predicaments

Corky root rot is caused by *Pyrenochaeta lycopersici*, a soil-living filamentous fungus of the Ascomycete clade found in temperate zones worldwide (Termohlen 1962; Gerlach and Schneider 1964; Schneider and Holliday 1966). Several different strains have been reported, and their temperature optima for infection vary according to the original source of each isolate. Generally, it ranges between 15–20°C for temperate climate zones and between 26–30°C for Mediterranean strains (Schneider and Holliday 1966). Quite unusually, *P. lycopersici* can alternate hosts. More than just on tomato, it is also found on the roots of many of our common crop species, such as pepper, eggplant, melon, cucumber and lettuce, but without causing disease symptoms as dramatic as the ones observed in tomato (Grove and Campbell 1987; Shishkoff and Campbell 1990; Infantino et al. 2000).

Pyrenochaeta is an alternative biotroph that can grow saprophytically on artificial media, but will spread on living host tissue in natural environment (Shishkoff 1992). Without available living host tissue, the fungus can still survive as microsclerotia, a kind of resting spores that are very resistant to draught and temperature changes (White and Scott 1973). These can stay inactive for very long periods of time, —spores that have been dormant for up to 15 years were reported to be viable and infectious (Grove and Campbell 1987; Forsberg et al. 1999). The fungus cannot grow on dead root material but will survive (Ball 1979). At the end of growing-season when plants are removed, pieces of dead root tissue and bark containing the fungi will remain in the soil. Careless handling of infected plants, soil and equipment is thereby a major cause of spreading disease (Ball 1979). However, from a more practical aspect, *P. lycopersici* can safely be maintained on dead root tissue for laboratory storage (Infantino A, personal communication).

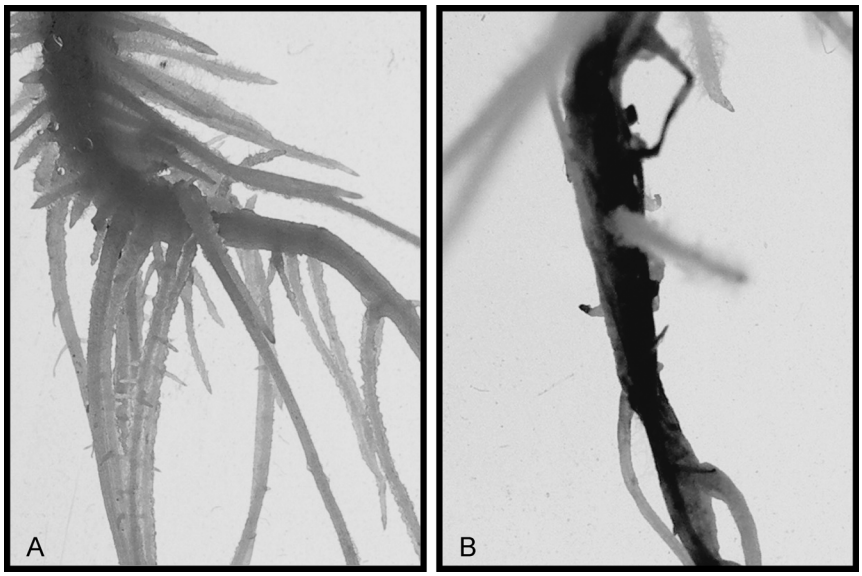

Figure 1. Corky root rot symptoms on tomato root grown in hydroponic culture. Un-infected root of Money Maker grown in hydroponic culture. (A) Root of Money Maker infected with *P. lycopersici* 4 days post infection. (B) Disease development is fast and the plant will not survive.

Disease resistance, a difficult deed

For most other important plant diseases, the strong demand for pathogen resistant crop species has stimulated plant breeders to introgress naturally occurring resistance genes from wild relatives into plant varieties more suited for commercial large-scale production. This has also been attempted for Corky root rot. In the case of tomato, the wild relatives *Solanum peruvianum*, *S. hirsutum* and *S. glandulosum* have shown inheritable resistance towards CRR and have been used as sources for CRR-resistance genes in different breeding programs (Szteyn 1962; Smith and Proctor 1965; Hogenboom 1970; Latterot 1983). The resistance of *S. hirsutum* is reported to be mediated by a single dominant gene (Smith and Proctor 1965). CRR-resistance from *S. glandulosum* is "controlled by one gene with a very low degree of dominance", which "when heterozygous the expression of this gene is highly influenced by the environment." (Hogenboom 1970). In our lab we have also identified a cultivar of *S. pimpinellifolium* that shows a high degree of resistance (Ekengren S and Hamiduzzaman MM, unpublished data). Volin and Macmillan set out to determine if resistance to *P. lycopersici* is also present in cultivated tomato, and whether this resistance could be inherited. They concluded that gene-mediated CRR resistance is present in *S. esculentum* as a polygenic trait, controlled by at least 4–8 genes. In agreement with other studies, they also deduced resistance as weak, variable and highly influenced by environmental factors (Volin and McMillan JR 1977).

Fifteen years ago, H. Latterot succeded in breeding two fresh market tomato varieties (Moboglan and Mogeor) carrying a recessive CRR resistance gene denoted *py-1*. The *py-1* gene was introgressed into *S. esculentum* from *S. peruvianum* (Latterot 1983). As *py1* was identified from near-isogenic lines (NILs) of tomato only differing in their relative corky root rot resistance, it could be mapped using RAPD and RFLP marker analysis. By this means, Doganlar et al. could locate the gene to the tip of the short arm of the third chromosome and generate PCR markers that can be used to track inheritance/presence of *py1* in large populations of tomato (Doganlar et al. 1998). Initially this report gave renewed hope for tomato breeders, however, as *py1* on its own turned out to be insufficient for reliable disease protection, the work to find other means and/or genes to confer robust CRR-resistance must be pursued (Fiume and Fiume 2003).

Overall, the unusually high difficulty to identify and transfer CRR-resistance genes into commercially suitable tomato cultivars has two reasons. Infection and growth of *P. lycopersici* in soil is very slow and variable, and in addition, plants respond very differently to infection when grown in greenhouse as compared to the field (Jones et al. 1989). Altogether, it has been almost impossible to perform efficient and reliable breeding programs, which is clearly demonstrated by the lack of reliable CRR-resistant varieties.

As summarized above, several obstacles, mainly depending on the nature of the fungal pathogen, have limited progress towards the establishment of resistant tomato lines using conventional methods. In our search for plant resistance genes, —or for other ways to control fungal spread, we therefore have to make use of other techniques. In the following section we outline current strategies as well as future visions for how to achieve this goal.

Previous hampers, current strategies and future possibilities

As mentioned in the previous section, one of the practical obstacles involved in screening for CRR-resistance genes, has been the slow and erratic infection of the plant roots in soil. Several different techniques for plant infection have been reported, however, most of them have not been easy, fast and/or reproducible enough to support any large-scale genetic screens (Fiume and Fiume 2003; Polley 1985; Jones et al. 1989; Clerjeau and Conus 1973; Clerjeau and Latterot 1976; Infantino et al. 2003). Therefore, we have worked out an alternative soil-free infection strategy using a hydroponic cultivation system, thereby simplifying the infection procedure and scoring of disease (Cabanero F. unpublished data). With this as a starting point, we have now commenced our molecular work to isolate and identify the key determinants important for fungal virulence and for plant disease resistance. Most plant pathogens secrete virulence factors and effectors into the apoplastic space of the host plant during the infection process (Kamoun 2006; Paper et al. 2007). Reciprocally, plant cells respond to infection by extra-cellular secretion of defense peptides and proteins (Oh et al. 2005; Paper et al. 2007). For the purpose of speed and efficiency, we have decided to take a selective approach in our gene hunting, using the yeast-trap system (YTS), a method designed to isolate secreted gene products (Lee et al. 2006). As we will isolate proteins secreted *in planta* upon fungal infection we anticipate to identify both putative fungal effectors and secreted plant immune proteins.

Excluding all intracellular plant proteins brings an obvious limitation to our approach, as many plant resistance genes are known to be located in the cytoplasm (van Ooijen et al. 2007). However, microbial effectors very often target key plant defense proteins, and accumulating evidences suggest that biotrophic fungi are suppressing induction of plant defense responses and are in-

ducing specific host genes for the establishment of biotrophy (Schulze-Lefert P and Panstruga 2003). In that respect, our initial findings can act as a basis for additional screens designed to identify important intracellular plant immune molecules.

A general problem when performing larger screens, is the abundance of information gained, and as a consequence, the high proportion of data retrieved that have no, or very low level of significance (Li et al. 2006). It is therefore important to have fast and efficient assays to validate the biological importance of the genes/proteins identified (Ahmed 2006; Cristoni and Bernardi 2004).

Unlike in *Arabidopsis*, large sets of lines with specific T-DNA-tagged gene mutations are not available in tomato, but, we can specifically target plant gene expression using virus induced gene silencing (VIGS), a fast and potent method to quickly associate gene sequence with function (Burch-Smith et al. 2004). VIGS has proven to be a very useful tool to dissect resistance responses in many other plant/pathogen interactions (Ekengren et al. 2003; Jin et al. 2002; Liu et al. 2004; Gabriels et al. 2007). The tobacco rattle virus (TRV) based vector developed by Liu et al. has previously been used for silencing of root tissue and should therefore work well in our studies (Liu et al. 2002; Ryu et al. 2004). By this means we can quickly target the putative defense genes derived from the yeast trap system, and assay for alterations in resistance towards *P. lycopersici*.

For fungal genes we aim to perform targeted gene replacement (or RNAi) and investigate alterations of fungal virulence. However, one initial challenge is to prove that *P. lycopersici* is genetically tractable. One problem is that it grows slowly and very few strains sporulate under laboratory conditions (Infantino et al. 2003). It can however be transformed (Clergeot P-H, unpublished data; A. Infantino, personal communication), and we are currently trying to improve our protocol to perform insertion mutagenesis, either using enzyme mediated integration or T-DNA insertion.

So far, we have performed basal analysis of the plant/pathogen interaction. In our preliminary studies, resistance of tomato to *P. lycopersici* correlates with the ability of the plant to respond rapidly by salicylic acid (SA)-mediated PR-gene expression and by lignification of the cells attacked by the fungus (Hamiduzzaman MM., unpublished results). Blocking plant SA-synthesis and/or perception seems to be a common and effective way for plant pathogens to interfere with plant immunity (DebRoy et al. 2004; Loake and Grant 2007). It would be highly interesting to investigate further if this also is a strategy for *P. lycopersici*. As SA is a common defense molecule this could explain how it so successfully can infect multiple plant species.

As reviewed in this article it exists a pressing need for molecular information concerning the interactions between *P. lycopersici* and its host, tomato. Today, there are almost unlimited possibilities to investigate any specific biological phenomenon including complex interactions between organisms. We are therefore ascertained to soon have established the systems needed to be able resolve the genetic basis for disease and disease resistance in this plant/pathogen interaction.

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