From susceptibility to resistance against parasitic dodder (genus *Cuscuta*): What can we learn from a wild tomato introgression line population?

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Key message

Sexually compatible wild and cultivated tomato lines exhibit opposing defense reactions against parasitic giant dodder (*Cuscuta*) and offer novel approaches to study the genetic basis of resistance to parasitic plants.

Abstract

The parasitic flowering plant genus *Cuscuta* (dodder) attacks shoots of all plants within its range but the outcome of this attack depends on the response pattern of the potential hosts. Compatible (or susceptible) hosts cannot prevent that their tissue is being penetrated and their vascular vessels are being tapped into, while incompatible (or resistant) hosts can stop the infection at an early stage, preventing the penetration of the haustoria and starving the parasite to death. Susceptibility and resistance can occur even in closely related species, offering the possibility to identify the responsible genes using molecular genetic tools. In this work, such a ménage à trois, consisting of the giant dodder *C. reflexa*, the resistant host *Solanum lycopersicum* M82 (processing tomato) and the susceptible host *Solanum pennellii* (wild desert tomato), was characterized. Grafting of a susceptible *S. pennellii* scion onto the root stock of resistant *S. lycopersicum* showed that resistance could not be induced in *S. pennellii*. A screening of the *S. pennellii* introgression line population, furthermore, revealed that the local hypersensitive reaction typical for the M82 parent line was conserved in most lines, while a few lines exhibited either susceptibility, hyper-resistance or cell death that extended considerably beyond the infection site.

Introduction

Within the plant kingdom, parasitic flowering plants have found a special niche. They parasitize on other plant species and supply themselves with inorganic and organic nutrients as well as water from the hosts. The morphological changes that accompanied the evolutionary adaptation to a parasitic lifestyle differ a lot in their extent (Westwood *et al.* 2012; Smith *et al.* 2013), and reflect the fact that parasitism has arisen 12-13 times independently in flowering plants (Barkman *et al.* 2007). However, one common and important invention that has occurred in all parasitic plants was the development of a specialized organ, termed "haustorium", with which the physical and physiological connection to the host is established. In contrast to its fungal counterparts, the parasitic plant haustorium is a multicellular, complex structure whose development has been studied in some detail using histological, immunological and, more recently, molecular methods (Vaughn 2003; Lee 2009; Hong *et al.* 2011; Yang *et al.* 2013; Pielach *et al.* in press, Olsen *et al.* manuscript submitted).

The infection of host plants by parasitically living plants represents a particularly intriguing case within plant pathogenesis, for two reasons: First, from a standpoint of phylogenetic relationship, parasitic plants are much closer related to their hosts than any other parasite group. The dicotyledonous orders Lamiales and Solanales stand out as being particularly rich in parasitic plants (Westwood *et al.* 2010). They may seek their hosts not only among other plant lineages but often within the same order. Hosts, which are attacked by a parasite that is so closely related, are thus confronted with the problem that the attacker ostensibly possesses the same surface architecture and cytochemical building blocks and is thus much harder to identify than other pathogens. The second challenge concerns the parasite, which must gain access to the host vasculature to secure survival, which in turn requires degradation of the host's cuticle and cell walls (Mayer 2006). The parasite must, however, accomplish the deployment of degrading enzymes without compromising the integrity of its own tissue. Recently, it was shown that the degree and form of pectin methyl esterification may contribute to the protection of the parasitic tissue (Johnsen *et al.* manuscript submitted), but many questions still remain open.

Cuscuta is a cosmopolitan parasitic genus that encompasses 150 to 200 species (McNeal *et al.* 2007). The genus consists exclusively of shoot parasites with a varying degree of metabolic dependence on their hosts (van der Kooij *et al.* 2000). *Cuscuta* spp. lacks proper leaves and

roots, presumably because these organs became unnecessary in their ecological niche as parasites, and mostly consists of branching stems that grow in a rotating fashion until they locate a potential host, either by a tactile stimulus (Tada *et al.* 1996) or by sensing host-borne volatile signals (Runyon *et al.* 2006). After winding around the host stem, *Cuscuta* spp. attach themselves firmly to the surface by secreting glue-like substances (mainly de-esterified pectins) and start developing haustorial primordia (Vaughn 2002; Lee 2008). The host spectrum varies between species but is often very broad and includes herbaceous plants, shrubs and trees (Costea *et al.* 2005).

The order Solanales that contains not only the parasite *Cuscuta* but also frequently infected hosts like *Solanum lycopersicum* (tomato), has started to diversify only about 36 million years ago (Goldberg *et al.* 2010). In comparison, the last common ancestor of fungi, animals and plants is presumed to be 800 million years old. Domesticated tomato is a compatible host to many *Cuscuta* species (Goldwasser *et al.* 2001) but exhibits resistance, for example, towards the giant dodder, *C. reflexa*. Studies focusing on this interaction have provided evidence that tomato is able to react with a local hypersensitive reaction (HR) that manifests itself in the deposition of an impenetrable layer enriched in phenolic and aliphatic compounds, pectins and cell wall proteins (Ihl *et al.* 1988; Sahm *et al.* 1995). This layer effectively inhibits the penetration of the haustorium, resulting in starvation of *C. reflexa.* Although the key resistance proteins that are, for example, involved in the perception of the parasite still remain unknown, some tomato proteins were identified that accumulate at the sites of attempted infection. Among them are two aquaporin genes and several cell wall proteins or cell wall modifying proteins (Albert *et al.* 2008). Interestingly, mutants with defects in the jasmonic acid, brassinosteroid and ethylene pathways and plants overexpressing salicylate hydroxylase showed the same resistance reaction as wild-type plants, indicating that the response to *C. reflexa* differs from common pathways to wound response or systemic acquired resistance (SAR) (Albert *et al.* 2008).

Tomato has a breeding history of more than 200 years, resulting in a plethora of cultivated varieties and several wild species who were confirmed to be the ancestors of todays domesticated cultivars. In a pilot experiment, we screened different cultivated and wild tomato lines for their response to *Cuscuta reflexa*. We found that the wild green-fruited desert tomato species *Solanum pennellii*, a distant relative of *S. lycopersicum*, was susceptible to the attack by *C. reflexa*, allowing the haustoria to penetrate the cortex and connect to its vascular tissue. In contrast, *S. lycopersicum* cultivar M82 showed the same hypersensitive reaction

reported previously for other domesticated tomatoes and did not sustain growth of *C. reflexa*. Grafting experiments showed that this difference persisted also when *S. pennellii* and *S. lycopersicum* shared vascular connections, excluding the possibility that a mobile defense signal is produced and can confer systemic acquired resistance.

S. pennellii is sexually compatible with *S. lycopersicum* and produces fertile offspring which has been exploited to generate a comprehensive set of introgression lines (ILs) (Eshed and Zamir 1995), each containing a well-defined chromosomal segment of the *S. pennellii* genome in the *S. lycopersicum* M82 background. We screened 48 ILs for response to *C. reflexa* and found major differences in the reactions of these lines to parasitic attack. Here, we present these results and discuss the power of such a genetic approach and how it may enable us decipher the molecular responses in the host upon parasitic plant infection.

Material and Methods

Plant material and infection

Cuscuta reflexa was maintained at the Phytotron of the University of Tromsø, Norway, on the compatible host *Pelargonium zonale* as described earlier (van der Kooij *et al.* 2000). Tomato seeds were obtained from the C.M. Rick Tomato Genetics Resource Center (TGRC), Dept. of Plant Sciences, University of California, Davis, USA. Five seeds per tomato line were germinated and grown on soil supplemented with Perlite for 6 weeks before being infected. For infection, five to six *Cuscuta* stem tips with a length of 15 to 20 cm were put with their cut face into water-filled tubes which were either placed into the pots containing the tomato plants or attached to the stem half way up the height of the plant. This ensured that *Cuscuta* had access to both the primary shoot and younger side shoots and petioles. Attachment and infection was scored by eye and with a digital camera in weekly intervals after infection. All infections were repeated at least once at a later time point with an independent set of plants (but from the same seed batch). Photographs were taken with a digital camera or with a stereomicroscope (Zeiss StereoLumar V12) equipped with a digital camera before infection sites were examined microscopically.

Microscopy and immunohistolabeling

Infection sites were either sectioned with a vibratome without prior fixing or they were fixed for 2 hours at 4°C after trimming into approximately 1mm thick pieces. The fixation medium consisted of a mixture of 1% glutaraldehyde and 1% formaldehyde in PEM buffer (50 mM

piperazine-N,N'-bis[2-ethane-sulfonic acid] (PIPES), 5 mM ethylene glycol bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 5 mM MgSO4, pH 6.9). Following three 5-minute steps of washing in phosphate-buffered saline (PBS), samples were dehydrated in a gradual ethanol series (30, 50, 70, 90 and 100%), one hour per step at 4°C. Subsequently, the material was incubated in a dilution series of London Resin White (Agar Scientific, medium grade) in 100% EtOH (30, 50, 70, 90, 100, 100 and 100 %), 1-2 hours per step and at least one of the 100% liquid resin steps lasting a minimum of 24 hours. Incubation in resin was always carried out in the cold, preferably on a shaker on ice. Fully infiltrated samples were polymerized in BEEM capsules at 60 degrees for 24-48 hours.

Semi-thin sections (0.5–1 µm) were cut with a histodiamond knife (Diatome), mounted on water drops on poly-L-lysine-coated slides and dried on a hot plate at 60°C. Samples for anatomical observations were subsequentlystained with a solution of 0.2% Toluidine Blue O and 1% borax in distilled water for approximately 2 minutes. These samples were dried and observed. Immunolabelling was performed with monoclonal antibodies against arabinogalactan proteins (LM2, JIM8, JIM13), xyloglucans (LM15, LM25) and pectic homogalacturonans (LM19 and LM20) (PlantProbes, Leeds, UK). To eliminate non-specific binding, the sections were first incubated for 30 minutes in a blocking solution of 3% milk protein in PBS. Washed sections (3 × 5 min) were subsequently incubated for 1.5 h in the primary antibodies diluted 1:10 in the blocking solution, washed and incubated for further 1.5 h in the secondary antibody conjugated to AlexaFluor fluorochrome diluted 1:100. Immunolabelled samples were counterstained with Toluidine Blue O. Samples were then mounted in a glycerol-based anti-fade mounting medium (Agar Scientific AF1) and observed using a Leica Leitz DMRBE microscope equipped with a Leica DFC420 camera or a StereoLumar V12 stereo microscope (Zeiss, Germany).

Tube-grafting of tomato plants

The primary shoot of young seedlings (4 weeks) of the rootstock (e.g. *S. lycopersicum* M82) were cut at an angle of 45 degrees at a hight of 8-10 cm. A scion of similar stem diameter at the cut face (also at a 45 degree angle) was taken from the shoot stock (e.g. *S. pennellii*) and both interfaces were held together with a tightly fitting silicon tube and some string.

Results and discussion

S. pennellii is a compatible host to *C. reflexa*

A study conducted with five *Cuscuta* species and a selection of six domesticated and wild tomato varieties showed that *C. reflexa* could only be sustained on *S. pennellii* but did not infect M82 (Fig.1) or any other tested cultivars of *S. lycopersicum*. Generally, haustorium development is initiated by site-specific cell elongation causing swelling of the proximal half of the parasite stem facing the host (Vaughn 2002). Adhesive pectic substances are secreted from club-shaped epidermal cells of the parasite to glue the parasite onto the host and allow the endophytic part of the haustorium to penetrate the host tissue (Vaughn 2002; 2003; Lee 2007). We found that in contrast to the preferred compatible host of *C. reflexa*, *Pelargonium zonale*, where swelling usually started about 1-3 days after twining of the parasite around the stem or petioles of its host, the onset of swelling was entirely unpredictable on *S. pennellii* and could vary from one day to more than a week. Infection of compatible hosts proceeds when the rapidly growing haustorium, whose development is initiated in parallel with the formation of the attachment ring ("appressorium" or "upper haustorium"), breaks through the surface of the parasitic stem and forces its way into the host stem. When reaching host xylem or phloem elements, searching hyphae differentiate according to their respective function, i.e. uptake of water from the xylem or sugars and other organic compounds from the phloem (Vaughn 2006; Lee 2009). In *Cuscuta*, telltale signs of the establishment of a feeding connection are protruding side shoots from the infection site and a restoration of apical tip growth which typically stagnates during the previous stages (Birschwilks *et al.* 2006). Both was observed when *C. reflexa* was grown on *S. pennellii*. Stems and petioles were infected with similar success (Fig. 1). Microscopical analyses of the infection sites confirmed the penetration of the host cortex by the haustorium (Fig. 2). In some cases, the host parenchyma cells around the haustorium appeared enlarged, but such changes to host cell morphology were minor. A considerable part of the interface between host cells and distal parts of haustoria was formed by feeding hyphae (Fig. 2).

While *C. reflexa* attacked the incompatible *S. lycopersicum* M82 and the other domesticated tomato cultivars in the same way by twining and swelling, the signs indicating increased resource availability upon establishing a feeding connection were missing in the incompatible pairing and the stems of the parasite started to wither and die after 1-2 weeks. Stem surfaces of M82 showed local lesions with brown discolorations at the sites of infection after about a week (Fig. 1d). Cross sections through the infection sites showed that haustoria were formed

and had reached almost the same size as in the compatible interaction with *S. pennellii* but were only able to penetrate the epidermal tissue before being deflected by the formation of impenetrable wound tissue in the host (Fig. 2 and 3). These haustoria developed no vascular bridges, while displaying lesions and necrotic interfacial cells.

The wound tissue of the host can be divided into two morphologically distinct layers (Fig. 2e and f, Fig. 3a). The outermost layer was derived from collenchyma and consisted of cells with swollen, non-lignified walls while the underlying wound tissue layer is composed of hypertrophied cells derived from the cortex and displays autofluorescence in the blue channel (Fig. 3b). Negative staining of lignin with Weisner reagent (data not shown) suggests that this autofluorescence is attributed to a suberin or non-lignin type of phenolic component such as tannins. The latter is plausible considering the browning of this tissue (Fig. 2). Sahm *et al.* (1995) described a phenolic and aliphatic compound-rich reaction tissue from *S. lycopersicum*. These substances may have a direct cytotoxic effects on the haustorium or/and reinforce the natural barrier represented by the cell wall. The cell wall swellings in the outer wound tissue layer were composed mainly of esterified pectins while the non-swollen primary walls contained xyloglucan and arabinogalactan proteins (AGPs) in addition to pectins (Fig. 3c–d). It is worth noting that this is the first record of AGP presence in defence-related structures in *Cuscuta*-host interactions, in contrast with a previously described AGP which was shown to promote attachment of the parasite to the host (Albert *et al*. 2006). To our best knowledge, the type of cell wall swelling seen in M82 has not been previously described in wound tissue. The structure of swellings resembles somewhat that seen in ripe kiwi fruit tissue (Hallett *et al.* 1992) and is very similar to cell wall structures occasionally seen in haustoria and non-parasitic metahaustoria of *Rhinanthus minor* (Pielach 2013). The loosening and shedding of cells of the outer wound tissue layer suggests that its function might be to prevent the parasite from adhering to the host surface by flaking off upon attempted penetration, as seen in figure 2e and f.

Resistance against *C. reflexa* is not a systemically acquired trait

Local programmed cell death as a response to pathogen infection can induce defense responses in the rest of the plant. This phenomenon, known as systemic acquired resistance (SAR), is an immune mechanism in plants. While the initial trigger is local, a number of mobile signals are known to mediate the synthesis of the defense hormone salicylic acid (SA) in tissues distant from the infection site and protect the plant from secondary infections for a period of weeks or months (see recent review by Fu and Dong (2013)). The first molecule

connected to SAR was in fact reported for tomato and was called systemin (Schaller and Ryan 1996).

To test whether resistance in *S. pennellii* can be induced by a mobile signal, we performed grafting experiments in which the wild tomato was grafted onto the root stock of M82 in such a way that a side shoot of this resistant species was retained besides the graft. The result was a chimeric plant that consisted of both M82 and S. pennellii, was supported by the same root system and had vascular connections at the grafting site. A reciprocal graft with *S. pennellii* as root stock failed (see Fig. 4) for unknown reasons and was hence not investigated.

Both, the M82 shoot and the *S. pennellii* shoot were infected with *C. reflexa* and infection sites monitored externally. Both plants revealed the reaction that was typically seen also with normal (non-grafted) individuals (Fig. 4). In M82, visible necrosis was seen after one week and the parasite died, while *S. pennellii* stems were infected and the parasite continued to grow (Fig.4). The parasite was subsequently removed completely and both parts of the plant were re-infected with *C. reflexa* two weeks later. Again, we observed the same pattern of reaction in both M82 and *S. pennellii*.

Albert *et al.* (2008) mentioned that transgenic tomato plants overexpressing the enzyme salicylate hydroxylase and mutants in several hormone pathways were still able to produce the same resistance reaction compared to wild-type plants and conclude that not all common signaling pathways related to systemic acquired resistance are important for the successful defense of tomato against *C. reflexa.* Considering our own results, these observations may also corroborate the hypothesis that an attack does not create any mobile triggers that prime other parts of the plant for defense, suggesting that the response is strictly governed by local processes. Alternatively, it is possible that *S. pennellii* is lacking some essential compounds that prevent it from initiating a local cell death. Further investigations involve, among others, an analysis of the transcriptomic response to *C. reflexa* infection in *S. pennellii* grafted onto M82 are under way.

Resistance, susceptibility and "hyper resistance" occur among the lines of the *S. pennellii* introgression line population

Despite the disparate phenotypic differences of *S. pennellii* and M82, both produce fertile progeny when crossed and this has been exploited to create a set of introgression lines (ILs) between these two species (Eshed and Zamir 1995). The ILs are nearly isogenic to each other and to the cultivated parent *S. lycopersicum* M82 and differ only with respect to the sequence on the introgressed segments. Together, the entire original population of 76 lines covers the

entire genome counting twelve chromosomes. These lines have exposed thousands of quantitative trait loci affecting plant adaptation, morphology, yield, metabolism and gene expression (Lippman *et al.* 2007). They have also been screened successfully for resistance to late blight caused by *Phytophthora infestans* (Smart *et al.* 2007).

We obtained 48 lines of this collection in addition to the two parental lines (Tab. 1) and a second M82 line from the C.M. Rick Tomato Genome Resource Center (http://tgrc.ucdavis.edu/), and screened this collection twice. Four lines showed poor or no germination or inconsistent results and were not evaluated. A regular visual inspection of the progression of the infections revealed that six lines seemed to support growth of *C. reflexa.* Necrotic lesions, if visible at all, appeared later than on the resistant M82 parent line. This group thus resembled the phenotype of *S. pennellii* and is referred to as "group I". Cross sections of infection sites from these ILs showed that most of the haustoria had penetrated the cortex and reached the vascular tissue (Tab. 1, Fig. 5). Despite this success in penetration, the infected tissue showed beginning signs of a defense reaction that manifested itself in enlarged cells at in the cortex around the infection site (Fig. 5e). We also occasionally observed that the haustoria were deflected, where obviously this process had proceeded faster. In the vast majority of lines (group II), the visible defense reactions were faster but local, leading to withering and death of *C. reflexa* within 1-2 weeks (Tab. 1 and Fig. 5). This response was similar to that of M82. Interestingly, six lines belonging to group III showed more extensive cell death that exceeded the immediate area around the infection site (Tab. 1 and Fig. 5), which was a phenotype differing from those of the parents.

With the recent launching of an extensive set of 285 sub-ILs and other recombinant germplasm collections (Alseekh *et al.* 2013), an optimal setting for the dissection of the genetic and molecular basis of the different response patterns to infection by *C. reflexa* is available.

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Tables

Table 1: Overview over reactions of the *S. pennellii* ILs. The asterisk (*) marks the parental lines of the ILs.

	attach-ment	*C. reflexa* growth supported?	defence reaction	penetration of cortex	host/parasite interaction	Group
S. pennellii (LA0716)*	yes	yes	none	always	compatible	I
S. lycop. M82 (LA3475)*	yes	no	local lesions	never	incompatible	II
S. lycop. M82 (LA3488)	yes	no	local lesions	never	incompatible	II
IL1-1 (LA4028)	yes	no	local lesions	not examined	incompatible	II
IL1-2 (LA4031)	yes	no	local lesions	not examined	incompatible	II
IL1-3 (LA4032)	yes	Yes	minor lesions	often, not always	compatible	I
IL1-4 (LA4033)	yes	Yes	minor lesions	often, not always	compatible	I
IL2-1 (LA4035)	yes	no	extensive lesions	not examined	incompatible	III
IL2-2 (LA4037)	yes	no	extensive lesions	not examined	incompatible	III
IL2-3 (LA4038)	yes	Yes	minor lesions	often, not always	compatible	I
IL2-4 (LA4039)	yes	Yes	minor lesions	often, not always	compatible	I
IL2-5 (LA4040)	yes	Yes	minor lesions	often, not always	compatible	I
IL3-1 (LA4043)	yes	never	local lesions	not examined	incompatible	II
IL3-4 (LA4046)	yes	no	extensive lesions	not examined	incompatible	III
IL3-5 (LA4047)	yes	no	local lesions	not examined	incompatible	II
IL4-1 (LA4048)	yes	no	local lesions	not examined	incompatible	II
IL4-2 (LA4050)	yes	no	local lesions	not examined	incompatible	II
IL4-3 (LA4051)	yes	no	local lesions	not examined	incompatible	II
IL4-4 (LA4053)	yes	no	local lesions	not examined	incompatible	II
IL5-1 (LA4054)	yes	no	local lesions	not examined	incompatible	II
IL5-2 (LA4055)	yes	no	local lesions	not examined	incompatible	II
IL5-3 (LA4056)	yes	no	extensive lesions	not examined	incompatible	III
IL5-4 (LA4057)	yes	No	local lesions	not examined	incompatible	II
IL6-2 (LA4060)	yes	Yes	minor lesions	often, not always	compatible	I
IL6-3 (LA4062)	yes	no	extensive lesions	not examined	incompatible	III
IL6-4 (LA4063)	yes	no	local lesions	not examined	incompatible	II
IL7-1 (LA4064)	yes	no	local lesions	not examined	incompatible	II
IL7-2 (LA4065)	yes	no	local lesions	not examined	incompatible	II
IL7-3 (LA4066)	yes	no	local lesions	not examined	incompatible	II
IL7-4 (LA4067)	yes	no	local lesions	not examined	incompatible	II
IL7-5 (LA4069)	yes	no	local lesions	not examined	incompatible	II
IL8-1 (LA4071)	yes	no	local lesions	not examined	incompatible	II
IL8-2 (LA4074)	yes	no	local lesions	not examined	incompatible	II
IL8-3 (LA4076)	yes	no	local lesions	not examined	incompatible	II
IL9-1 (LA4078)	yes	no	local lesions	not examined	incompatible	II
IL9-2 (LA4081)	yes	no	local lesions	not examined	incompatible	II
IL9-3 (LA4084)	yes	no	local lesions	not examined	incompatible	II
IL10-1 (LA4087)	yes	no	local lesions	not examined	incompatible	II
IL10-2 (LA4089)	yes	no	extensive lesions	not examined	incompatible	III
IL10-3 (LA4091)	yes	no	local lesions	not examined	incompatible	II
IL11-1 (LA4092)	yes	no	local lesions	not examined	incompatible	II
IL11-2 (LA4093)	yes	no	local lesions	not examined	incompatible	II
IL11-4 (LA4095)	yes	no	local lesions	not examined	incompatible	II
IL12-1 (LA4097)	yes	no	local lesions	not examined	incompatible	II
IL12-3 (LA4100)	yes	no	local lesions	not examined	incompatible	II
IL12-4 (LA4102)	yes	no	local lesions	not examined	incompatible	II
IL12-4-1 (LA4103)	yes	no	local lesions	not examined	incompatible	II

Figure legends

Figure 1: Morphology of compatible and incompatible interactions of *Cuscuta reflexa*. **A)** Photograph of several shoots of *C. reflexa* tightly winding around the stem of compatible host *Solanum pennellii*. **B)** Close-up picture of the contact area. No signs of hypersensitive reaction can be seen on the stem surface at sites of attachment [←]. **C)** Infection of a petiole of S. pennellii. The insert shows a close-up of the boxed area. **D**) *C. reflexa* twining around the incompatible *S. lycopersicum* M82. **E)** Contact area between *C. reflexa* and *S. lycopersicum* M82. Necrotic lesions are visible at the sites of parasite attachment [←]. **F**) Infection of a petiole of M82. Scalebars in (B) and (E) represent 1 mm.

Figure 2: Anatomy of infection sites from the compatible interaction between *C. reflexa* and *S. pennellii* and incompatible interaction between *C. reflexa* and *S. lycopersicum*. **A)** Dark field overview image of a vibratome cross section showing the parasite **[P]** on top and the compatible host **[cH]** below. Growth direction of the haustorium is indicated by a thin arrow [←]. **B)** A semi-thin section taken from the infection area, stained with Toluidine Blue O. The tissues of the parasite are shaded. The haustorium has penetrated host stem and reached the vasculature. Histological changes in the host are limited to crushed cells [⬅] at the very point of entry. A zone of collapsed cells **[cz]** which is part of normal haustorial development can be seen. **C)** Close-up of the boxed area in (B). Parasite cells are shaded. Searching hyphae **[*]** of *Cuscuta* are tightly associated with host cells without effecting visible damage to the tissue. **D)** Dark field micrograph showing a vibratome section of a *C. reflexa* haustorium on a petiole of the incompatible host **[iH]** *S.lycopersicum* M82. The haustorium has not penetrated the cortex and browning can be observed in the host and the parasite at the interface. A layer of wound tissue **[wt]** has developed in response to attempted penetration. **E)** Light micrograph showing a toluidine blue O stained semi-thin section with the haustorium of the parasite **[P]** failing to protrude into the incompatible host **[iH]**. Parasitic tissue displays lesions [◄]. Wound tissue consists of a peeled off outer layer **[wt1]** and an impenetrable inner layer **[wt2]**. **F)** Close-up of the boxed area in (E) showing the interface between haustorial tip and wound tissue. Some necrotic interfacial cells **[n]** are present in the haustorium.

Figure 3: Anatomy and cell wall composition of *S. lycopersicum* M82 wound tissue. **A)** The point of entry [→] is isolated by a layer of wound tissue by a clear redifferentiation zone [◄]. Redifferentiation leads to a formation of an outer wound tissue layer **[wt1]** from collenchyma **[col.]** and an inner wound tissue layer **[wt2]** from the cortex **[cor.]**. **B**) The inner layer of the wound tissue is characterized by hypertrophied cells with autofluorescent cell walls, indicating impregnation with phenolics or adcrustation with suberin. The outer layer is not autofluorescent and displays an unusual form of "spongy" cell wall modifications composed of pectins (**C)**, but not xyloglucan or AGPs which are additionally detected in the non-swollen primary walls (**D** and **E** respectively).

Figure 4: Infection response in grafted plants. **A)** Left plant: *S. lycopersicum* M82 grafted onto the main shoot of *S. pennellii* (root stock). A side shoot of *S. pennellii* was retained. Right plant: *S. pennellii* grafted onto the main shoot of *S. lycopersicum* M82 (root stock). A side shoot of M82 was retained. *S.l. = S. lycopersicum* M82; *S.p. = S. pennellii*. The picture was taken two weeks after grafting (plant age: 6 weeks). **B)** 10 week-old *S. pennellii* graft one week after infection with *C. reflexa*. 10 cm-long shoot tips of *C. reflexa* were removed from maintenance cultures and placed into water-filled 2 ml plastic tubes attached to stems of *S. pennellii* (shoot stock) or *S. lycopersicum* M82 (side shoot of the root stock). Attachment and infection happened in *S. pennellii* **(C)**, while M82 reacted with hypersensitive reaction **(D)**.

Figure 5: Interactions of *C. reflexa* with *S. pennellii* introgression lines. **A-E)** Pictures show sites of attachment for group I-ILs (see Table 1) that show no or slow formation of minor lesions. **A-C)** Digital photographs of ILs2-3 to 2-5; **D)** Stereomicroscopic image of IL1-4; **E)** Dark field image of a cross section through an infection site of IL1-4. **F-H)** Digital photographs of sites of attachment for group II-ILs (see Table 1) that show similar local lesion formation as M82. **I,J)** Digital photographs of sites of attachment for group III-ILs (see Table 1) that show lesions extending far beyond the attachment site.

Figure 1

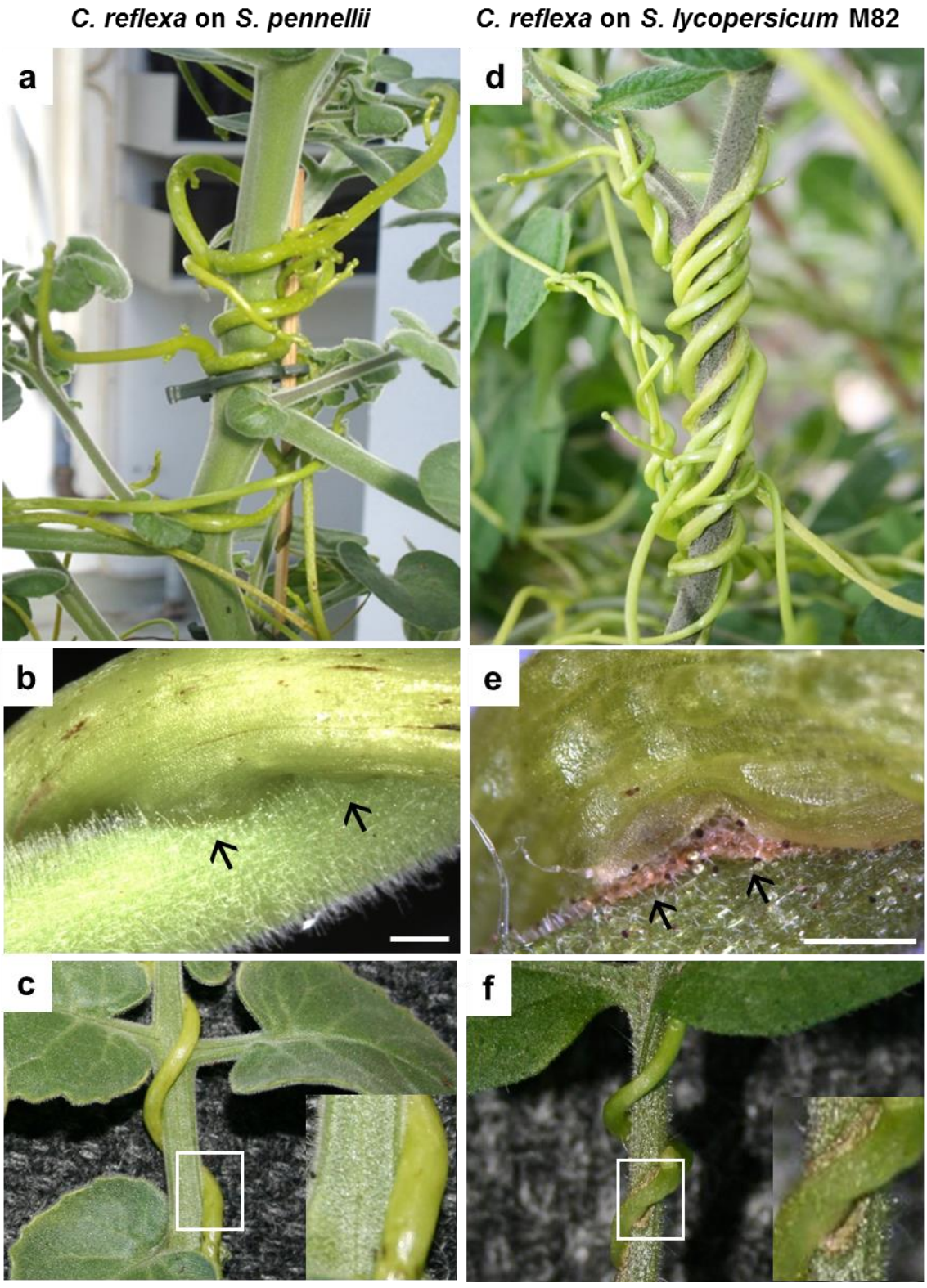
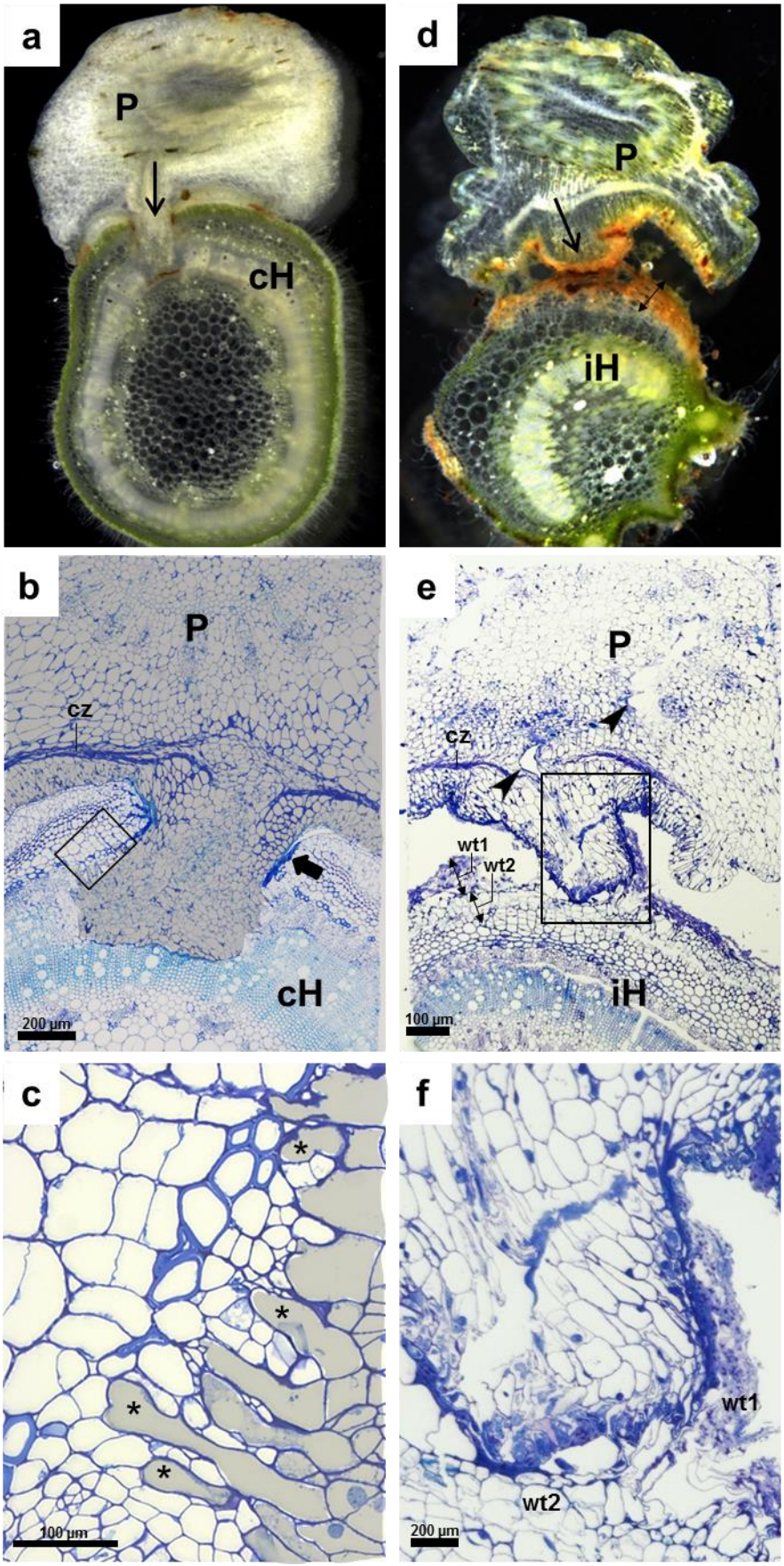

C. reflexa on S. pennellii
C. reflexa on S. lycopersicum M82
a
b
c
d
e
f

Figure 2


a
P
cH
d
P
iH
b
P
cz
cH
200 µm
e
P
cz
wt1
wt2
iH
100 µm
c
*
*
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*
100 µm
f
wt1
wt2
200 µm

Figure 3

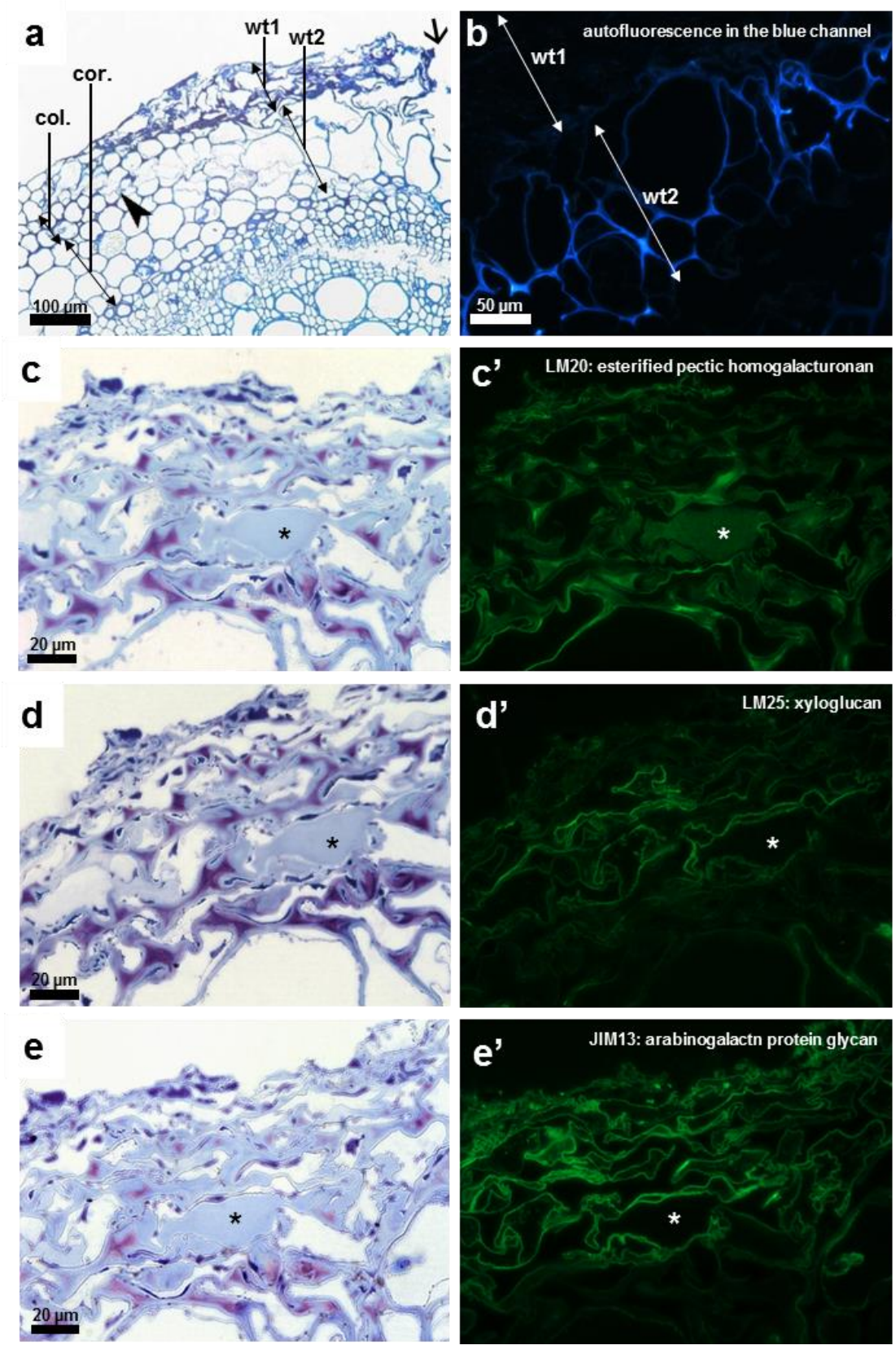

a
wt1
wt2
cor.
col.
100 µm
b
autofluorescence in the blue channel
wt1
wt2
50 µm
c
20 µm
c'
LM20: esterified pectic homogalacturonan
d
20 µm
d'
LM25: xyloglucan
e
20 µm
e'
JIM13: arabinogalactn protein glycan

Figure 4

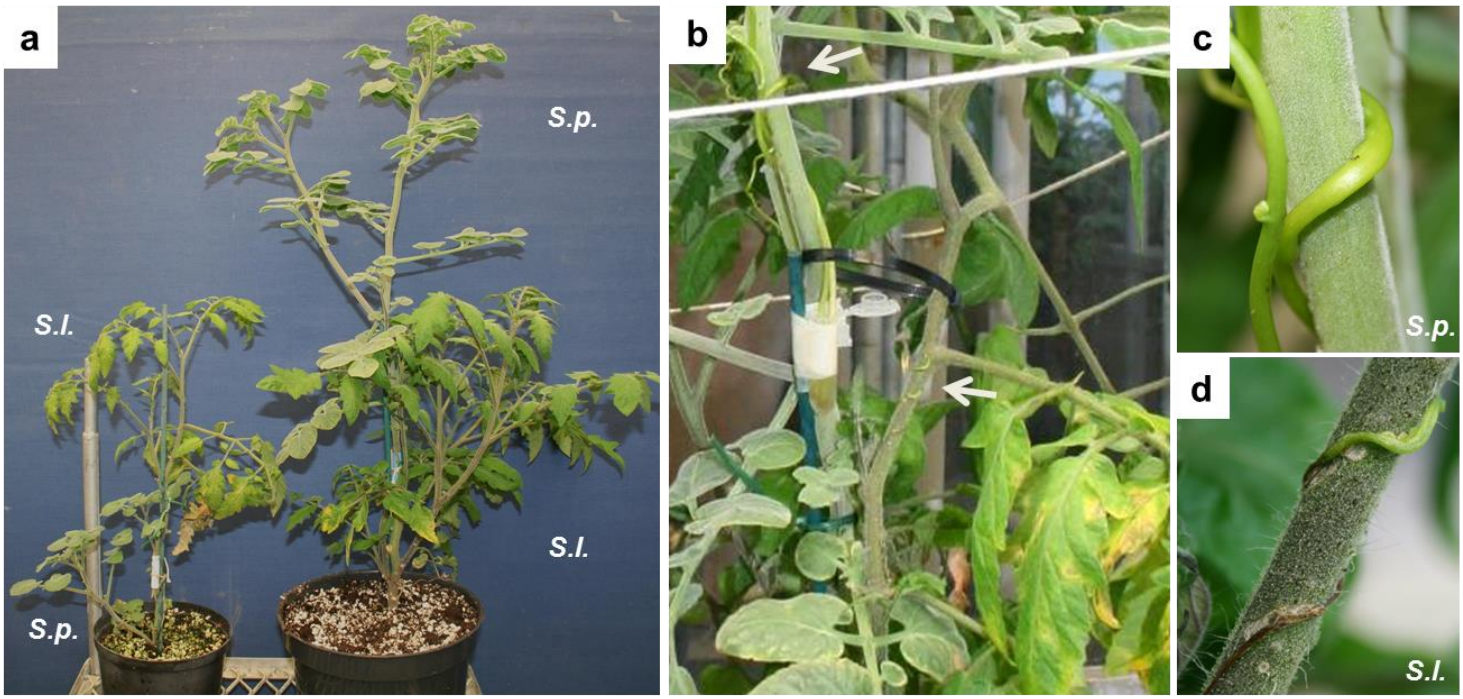
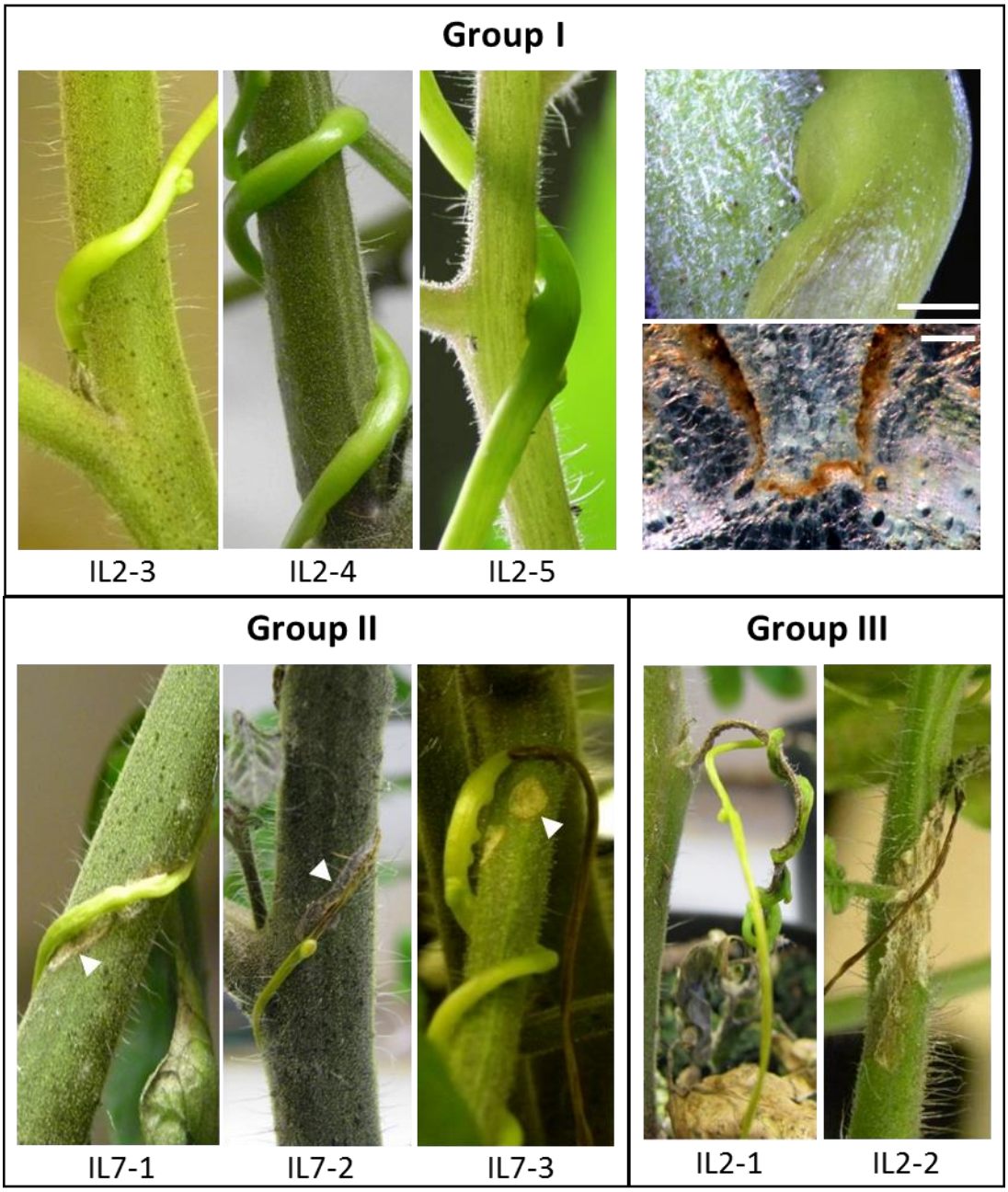


Figure 5


Group I
IL2-3
IL2-4
IL2-5
Group II
IL7-1
IL7-2
IL7-3
Group III
IL2-1
IL2-2