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Histopathology of the infection on resistant and susceptible lentil accessions by two contrasting pathotypes of *Fusarium oxysporum* f.sp. *lentis*

Hamid Reza Pouralibaba^{1,2}, Alejandro Pérez-de-Luque³ & Diego Rubiales¹

¹ Institute for Sustainable Agriculture, CSIC, Avda. Menéndez Pidal s/n, 14004 Córdoba, Spain, email:

diego.rubiales@ias.csic.es

² Dryland Agricultural Research Institute of Iran, 119, Maragheh, Iran

³ IFAPA Centro Alameda Del Obispo, Avda. Menéndez Pidal s/n, 14004 Córdoba, Spain

Abstract:

Wilt disease of lentil caused by *Fusarium oxysporum* f.sp. *lentis* (*Fol*) is one of the most important diseases affecting lentil worldwide. Differential response of six lentil accessions with reported differences in the level of resistance to *Fol* was studied micro and macroscopically. Penetration took place through root epidermal cells without formation of any specific structure. Hyphae reached the stele within two days after inoculation (*dai*) and subsequently invaded xylem bundles having colonised the endodermis, vascular system and even vascular parenchyma phloem already by 4 *dai*. Resistance was observed as a quantitative trait in all studied accessions resulting from varying levels of xylem occlusion with gum-like substances and of degree of colonization observed only after 4 *dai*. An indication of a qualitative resistance was detected in accession BGE019696 inoculated with pathotype 1 as a fast secretion of phenolic compounds at 4 *dai*. Plasmolysis of cytoplasm, lignification and accumulation of phenolic compounds, gum-like substances and/or tyloses were observed from 15 to 30 *dai*. As a result of the various operative mechanisms, significantly lower numbers of propagules were recovered from roots by 15 *dai*, and a retardation of disease was measured as lower disease index by 30 *dai* in plants inoculated with pathotype 1, but not in those inoculated with pathotype 7.

Key words: Lentil, *Lens culinaris*, Fusarium wilt, *Fusarium oxysporum* f. sp. *lentis*, pathotype, histopathology, quantitative resistance

Introduction

Vascular wilt caused by *Fusarium oxysporum* W.L. Gordon (*Fol*) is an important soil-borne disease affecting lentil (*Lens culinaris* Medik) worldwide (Chen 2011). Wilt symptoms in the field include wilting of top leaves, stunting of plants, shrinking, and curling of leaves from the lower part of the plants that progressively move up to the stems of the infected plant. Plants finally become yellow and die. Although there are fungicides effective against *Fol* under controlled conditions, field applications are not always practical because of the cost and technical difficulty of incorporating chemicals into soil during the growing season (Taylor et al. 2007).

The most effective, economical and environmentally friendly method for controlling the disease is the use of resistant cultivars. A number of sources of resistance to *Fol* have been reported (Bayaa et al. 1997; Bejiga et al. 2001; Pouralibaba et al. 2015, 2016) but little is known about their inheritance or way of action. Resistance to other *formae specialis* of *Fusarium oxysporum* has been reported in other crops to operate at various stages of the infection process, preventing or retarding penetration or colonisation in epidermis, cortex or endodermis of the roots as mechanisms of extravascular resistance, or later in the xylem of roots and/or stem as mechanisms of vascular resistance (Conway and MacHardy 1978; Baayen et al. 1991; Jiménez-Fernández et al. 2013). A clear understanding of the defensive mechanisms underlying host resistance is essential to facilitate the breeding and development of *Fusarium* wilt resistance in lentil. Unfortunately, this has not been studied yet in lentil.

The aim of this work was to understand the histopathology of the infection process in several lentil accessions with different levels of resistance to *Fol* inoculated with two contrasting *Fol* pathotypes.

Materials and methods

Plant materials

Six lentil accessions were selected based on their different reactions to the disease from previous studies (Pouralibaba et al. 2015, 2016) (Table 1).

Table 1 Lentil accessions used in this study and their Disease Index (*DI*) to two pathotypes of *Fol* at 30 *dai*

Accession	Disease Index	
	Pathotype 1	Pathotype 7
ILL 4774	58.3	50.9
ILL 10241	14.7*	50.0
ILL 4360	17.1*	47.0
BGE019696	11.9*	41.4
BGE022526	12.0*	35.4
BGE032290	21.6*	26.9

*Differs significantly from the susceptible check ILL 4774 (One-sided Dunnet's Multiple comparison, $P < 0.05$)

Lentil seeds were surface-sterilized using 2% sodium hypochlorite for 4 min and rinsed twice in sterile distilled water for the next 4 min. The seeds were pre-germinated in Petri dishes placing the seeds on moistened filter papers. Seeds were kept in darkness for 48h in a cold chamber at $4^{\circ} \pm 1^{\circ}\text{C}$, and then placed for another 72-96 h in a growth chamber at $26 \pm 2^{\circ}\text{C}$. The germinated seeds were selected for further inoculation.

Fungal materials and inoculations

Fol isolates 7 and 20, collected on naturally infected lentil plants from Oshnaviyeh and Bilehsavar (North West of Iran) fields, and identified as pathotypes 1 and 7, respectively

(Pouralibaba et al. 2016) were used for inoculations. The isolates were stored as micro conidial suspensions in 150 mL vials containing 70 mL spore suspension: 30mL glycerol 98% at -80°C. The hyphal-sandwich root inoculation method (Xue et al. 1998) was used to inoculate seedlings of lentil plants. 10 mL of conidial suspension of *Fol* isolates, after de-freezing at room temperature, were placed on the surface of Petri dishes containing PDA medium and incubated at $26 \pm 2^{\circ}\text{C}$ under a 12 h light/12 h dark photoperiod for 6 days. After that, these solid PDA media were cut by half and the roots (4-8 cm length) of germinated seedlings were placed in between the two layers of the solid media containing fresh fungal colonies. The leaves were left outside the media. Control treatment was set by putting seedlings between two un-inoculated PDA layers. At 4 days after inoculation (*dai*), because of lack of enough space for seedlings in the Petri dishes, the inoculated plants were transferred to the pots (6×6×8 cm) containing sterilized perlite and were irrigated with full strength Hoagland's solution ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}:\text{KH}_2\text{PO}_4:\text{KNO}_3:\text{MgSO}_4 \cdot (5:1:5:2 \text{ mM}) : 7\text{H}_2\text{O}:\text{FeEDTA} (5\mu\text{M})$) (Hogland and Arnon 1958) and incubated in a growth chamber at $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ with 12 h light/12 h dark photoperiod at 28°C and 25°C respectively. The pots were left in the trays of 40×70×10 cm distinctly based on the inoculated isolate, and plants per every accession × pathotype combination were arranged following a completely randomized design. During the incubation period, plants were irrigated with tap water three times per week (each 6 L per tray). At 30 *dai* the inoculated plants were first visually assessed for symptoms and then processed for histological observations.

Sampling

Three plants per each accession × pathotype combination were harvested at 2, 4, 15 and 30 *dai* for root/stem microscopic studies. Additionally, three plants were used for pathogen colonization studies at 15 and 30 *dai*. At 2 and 4 *dai*; samples were harvested directly from Petri dishes of hyphal-agar sandwiches while for 15 and 30 *dai* studies, sampling was conducted from seedlings in the pots.

Microscopic studies

Samples harvested at 2, 4, 15 and 30 *dai* were fixed in (I) FAA (50% ethanol + 5% formaldehyde + 10% glacial acetic acid, in water) for 48 h. Series of ethanol (50, 80, 95, 100, 100% for 12 h each) were applied for dehydration and then transferred to an embedding solvent (Xylene; Panreac Quimica SA, Montcada i Reixac, Spain) through a xylene-ethanol series (30, 50, 80, 100, 100% for 12 h each). Consequently, paraffin was applied for saturation of the samples. A rotary microtome (RM 2245; Leica Microsystems) was used to get 7 µm-thick sections which were then attached to adhesive-treated microscope slides. Different dyes were applied to sections for staining: (i) 0.05% toluidine blue O (TBO) in PO₄ buffer (pH 5.5) for 5-10 min which was applied before removal of paraffin (Ruzin 1999). Phenolics, tannins, lignin and suberin were detected using this method (Bayeen et al. 1996; Bordallo et al. 2002; Mellersh et al. 2002; Crews et al. 2003). (ii) 0.05% ruthenium red in water for 5 min which can detect non-methyl-estrified pectins (Vallet et al. 1996). II) Karnovsky solution (paraformaldehyde 4% + glutaraldehyde 5% in 0.5 mg/ml calcium chloride and cacodylate buffer 0.025 M) was used for fixation of samples. In order to facilitate infiltration of the fixative, 6 slight vacuum series of 1 min each were applied. This solution was replaced by 0.025M cacodylate buffer after 48h. In order to prepare resin embedded samples (Historesin; Leica Microsystems GmbH), 12h immersion in a series of

50, 80 and 95% ethanol in distilled water was applied. Subsequently, the samples were immersed in 1:1 ethanol-infiltration solution (Historesin; Leica Microsystems GmbH) and finally the solution was replaced by pure infiltration solution and stored at 4°C in darkness. The samples were embedded in mould blocks containing 1:15 hardener: infiltration solution (Ruzin 1999; Pérez-de-Luque et al. 2007). A rotary microtome (RM 2245; Leica Microsystems) equipped with carbon–tungsten blades (TC-65; Leica Microsystems) was used to obtain semi-thin (2µm thickness) sections, which were then attached to microscope slides. The samples were stained with 0.05% toluidine blue O (TBO) in phosphate buffer (pH 5.5) during 1 min (Ruzin, 1999).

Sections were observed using a light microscope (Eclipse 50i; Nikon Instruments Inc., magnification ×100 to ×400) and photographed using a digital optical device (DS-Fi1; Nikon Instruments Inc.) connected to a computer through the control unit DS-U2 (Nikon Instruments Inc.). Inoculated plant samples were compared with non-inoculated control samples.

Fungal colonization assay and disease assessments

Fungal colonization was assayed following the method proposed by Conway and MacHardy (1978) with minor changes. Stems and roots were harvested at 15 *dai* and washed with tap water. Then all branches, leaves, lateral roots were removed and only two 10 cm-length segments of above and below the cotyledon node were kept. Subsequently, the segments were thinly sliced with a sterile razor blade and ground separately using a surface-sterilized ceramic pestle and mortar by adding 20 mL sterile distilled water. Finally, samples of the homogenized plant material were pipetted into sterile plastic Petri dishes and then molten PDA (46°C) was added. Carnamycin (30 mg/µL) was added to the medium in order to minimize bacterial growth. Colony counts were recorded five days after incubation

Disease response of inoculated plants was visually assessed at 30 *dai* using a 0-4 scale where 0=no symptoms; 1=leaf showing tiny yellowing in the border of the leaves; 2=leaf completely yellow; 3=wilted/curled/dried leaf, or defoliation; and 4=completely died back. In order to characterize all possible patterns of the disease in the plants, this score was applied on each leaf separately. Only leaves from the main stem were evaluated. To further facilitate the comparison of resistance between accessions, a disease index (*DI*) for the whole plant was applied using the formula: $DI = ((n_1 \times 0.25) + (n_2 \times 0.5) + (n_3 \times 0.75) / t) \times 100$, being n_1 , n_2 and n_3 the number of leaves showing symptoms type 1, 2 and 3, respectively; and t the total number of leaves, including the no symptomatic and fallen leaves due to the disease. Plants with no symptoms were considered as $DI=0$ and those that died $DI=100$ (Pouralibaba et al. 2015).

Statistical analysis

ANOVA was performed using STATISTIX 8.0 (Analytical software, Tallahassee, USA) based on completely randomized design (CRD). Comparison of means was conducted using One-sided Dunnet's Multiple comparison with a control test at $P < 0.05$. The values of number of pathogen colonies were transformed according to the formula $Y = \log (X+1.50)$. A minimum of ten samples from a pull collected from three replicates were used for each cytochemical assay.

Results

Histological observations

No basic differences were observed during the initial stages of *Fol* infection among lentil accessions at 2 *dai*. The infection process began with the development of a hyphal net around root surface, destroying external sheath and by attaching the hyphae around the root, including

root cap, apical meristem and sub-apical zones during the first two days after inoculation. The colonization of lateral roots was similar to that of the main roots. During this period, the external mucilaginous sheath of root surface was destroyed and its remnants were observed in the vicinity of hyphal net (Figs. 1B, 1C).

The hyphae penetrated directly into the epidermal cells and cortex without formation of any specific structure such as appressorium or penetration peg. Penetration was not limited to any specific site and it occurred either through the plane surface or the corner of the epidermal cell walls (Fig. 1D). In some cases, following heavy colonisation, the epidermis was completely destroyed (Fig. 1E). In infected roots, cortex was completely colonised by inter and intra cellular hyphae (Fig. 1F) and cells were plasmolysed. A great accumulation of hyphae was observed in the host epidermal cells and the neighbouring cortical cells. Such hyphae were either growing apoplastically in between the host cell walls or penetrated and elongated inside the cells while arranging mostly peripherally inside the cells. Colonisation from cell to cell by hyphae in the cortex took place through the cell wall. Firstly, hyphae tip is attached to the cell wall of the infected cell. Then, a thin hyphal portion penetrated cell walls and middle lamellae of both adjacent cells, and protruded against the inside of the new host cell (Fig. 2A). Development of hyphae inside the root after penetration and colonisation of the epidermis is followed a radial pattern towards the stele (Fig. 2B). Presence of inter-cellular and intra-cellular hyphae was abundant across the cortex within 2 *dai* in all combination of lentil accessions inoculated with both pathotypes.

Cells with condensed areas in the cytoplasm that intensely stained dark blue were observed in accession BGE019696 inoculated with pathotype 1 by 4 *dai* (Figs. 3A, 3B, 3C). Furthermore, in this combination a greenish colour was observed inside the cells or the

intercellular space, pointing towards the presence of phenolic compounds for this accession × pathotype interaction (Fig. 3D). This phenomenon was not observed in any other interactions in this sampling time. The most common event during the interactions of other lentil accessions with *Fol* pathotypes at this stage was hyphae penetration into the vascular system by trespassing the endodermis layer. Subsequently, xylem vessels were plenty occupied by mycelium (Fig. 3E), and finally whole root tissues were completely colonized by hyphae (Fig. 3F). Colonisation of xylem vessels by hyphae was observed in all combinations of lentil accessions with *Fol* pathotypes, except for accession BGE019696 inoculated with pathotype 1 *Fol* at this stage.

Although no symptoms were yet observed macroscopically by 15 *dai*, histological observations revealed heavy plant root colonisation in all accessions, both at the xylem, endodermis layer and vascular parenchyma . The pathogen continued growing and spreading into adjacent vessel elements through the xylem pits (Fig. 4A). Following staining with TBO, presence of various materials was detected. Purple and pinkish material pointed to carboxylated polysaccharides (Fig. 4B) whereas green and/or blue turquoise stained material indicated the presence of phenolic compounds (Fig. 4C). Presence of non-methyl-esterified pectin (carboxylated polysaccharides) was confirmed by staining with ruthenium red (Fig. 4D). These gum-like substances caused blockage of xylem vessels.

By 30 *dai* presence of tyloses secreted by paravascular parenchyma cells (Fig. 5A) and massive occlusion of xylem vessels with mucilage was the dominant state, either in the root (Fig. 5B) and stem (Figs. 5C, 5D) at both susceptible check ILL 4774 or all other lentil accessions inoculated with *Fol* pathotypes.

Fungal colonization assay

Colonization of the pathogen was quantitatively measured assessing fungal colonies recovered from infected tissue of roots and stems samples. ANOVA and comparison of means showed that significantly fewer colonies were recovered from roots but not from stems of the five resistant accessions compared to the check ILL 4774 when inoculated with pathotype 1. However, these numbers were not significantly different when inoculated with pathotype 7 (Table 2).

Table 2. Number of fungal propagules of *Fol* pathotypes harvested from different parts of the lentil accessions at 15 *dai*.

Accession	Pathotype 1		Pathotype 7	
	Root	Stem	Root	Stem
ILL 4774	11.0±6.24	0.0	4.3±6.65	2.0±1.73
ILL 10241	1.3±2.30*	0.3±0.57	missing	0.3±0.57
ILL 4360	0.0*	0.0	1.0±1.00	1.3±2.30
BGE019696	0.3±0.57*	0.0	2.7±4.61	0.0
BGE022526	0.0*	0.0	1.3±2.30	0.3±0.57
BGE032290	0.0*	0.0	3.3±2.88	0.3±0.57

*Differs significantly from the susceptible check ILL 4774 (One-sided Dunnet's Multiple comparison, $P<0.05$)

Macroscopical assessments

Response to *Fol* was quantitative with *DI* ranging from 0 to 75.0. ILL 4774 showed the highest disease index for both pathotypes. ANOVA of *DI* values yielded significant difference ($P<0.01$) for lentil accessions inoculated with *Fol* pathotype 1. Comparison of means for this combination grouped them into two distinct classes when comparing with most susceptible accession of ILL

4774, and all other lentil accessions were significantly more resistant than ILL 4774. No significant differences were observed among accessions for pathotype 7 (Table 1).

Discussion

Infection by *Fol* followed similar steps to those previously reported in other *F. oxysporum*-host pathosystems. Germinated spores attached to the root surface and hyphae, caused a degradation of epidermis and external tissue layers and penetration took place without formation of appressoria or appressoria-like structures as reported by Bhalla et al. (1992). This is in agreement with findings in other *F. oxysporum* ff. spp. (Nyval et al. 1972; Bishop and Cooper 1983; Rodríguez-Gálvez et al. 1995; Kroes et al. 1998; Zivirin et al. 2010; Li et al. 2011; Jiménez-Fernández et al. 2013). Colonization of epidermal cells and cortex continued by intra- and intercellular hyphal growth. Anatomical responses were similar in all studied accessions in the penetration and first stages of pathogen invasion into epidermal cells by 2 *dai*, followed by invading the vascular core at 4 *dai* which is in harmony with observations of Bhalla et al. (1992) and similar to the report for *F. oxysporum* f. sp. *melonis* by Zvirin et al. (2010). However, some notable differences were observed at 4 *dai* by presence of phenolic compounds (greenish staining, Fig. 3D) as observed in BGE01969×pathotype1. This was not observed in the sections of the same lentil accession inoculated with pathotype 7, where cortex and pith were quickly colonized by the pathogen hyphae at the same time span in the absence of phenolic compounds. This type of pre-xylem defence responses was well defined by Zvirin et al. (2010) and the role of phenolic compounds in defence mechanisms in *F. oxysporum*-host is well known (Panina et al. 2007).

It has been shown that the origin of extravascular resistance is mainly from stored phenolic compounds in the xylem tissue, which are released from storing cells and diffuse out of their vascular compartments into neighbouring cells (Beckman 2000). Nevertheless, this early response as extravascular resistance observed in BGE01969×pathotype 1 does not prevent xylem colonisation as seen at 15 *dai*, but certainly it results in a delay of disease development as seen by the reduced *DI* (Table 1). Pathogenic hyphae were observed in all parts of the root including epidermis, cortex and xylem bundles at 15 *dai* in every lentil accessions×*Fol* pathotype combinations, including BGE01969×pathotype 1, despite of absence of any symptoms of infection, similar to a report on *F. oxysporum* f. sp. *cubense* on bananas by Guo et al. (2015). At this stage, significant difference in the number of produced propagules in root system between susceptible and resistant accessions inoculated with pathotype 1 was observed, indicating presence of host-specificity in this combination. Different patterns of root colonization between resistant and susceptible cultivars of other legume - *F. oxysporum* ff. spp. pathosystems have been reported previously, such as pea - *F. oxysporum* f. sp. *pisi* (Elgersma et al. 1972; Charchar and Kraft 1989), bean - *F. oxysporum* f. sp. *phaseoli* (García-Sánchez et al. 2010) and chickpea - *F. oxysporum* f. sp. *ciceris* (Jiménez-Fernández et al. 2013). At the same time, the presence of carboxylated polysaccharides, phenolic compounds and gum-like substances in the root vascular system was detected using different staining methods. Such vessel-occluding gels could lead to the expression of wilt symptoms (Soni and Soni 2010) and/or fungal inhibition. Vascular gelation has been reported as a resistant mechanism to *F.oxysporum* ff. spp. (Charchar and Kraft, 1989). We observed accumulation of gum like substances in the resistant accessions but also in the susceptible ILL4774. An explanation might be that this accession, although being the most susceptible among the ones used by us here and in previous studies (Pouralibaba et al. 2016),

might still carry some levels of incomplete resistance, as in fact it was ranked as resistant before by Bayaa et al. (1997). Previous studies on *F. oxysporum* f. sp. *dianthi* on carnation (Bayeen et al. 1988; 1991; Baayen and Elgersma 1985).) and on *F. oxysporum* f. sp. *ciceris* on chickpea (Jiménez-Fernández et al. 2013) showed that cultivars varying in levels of resistance varied in the vascular gel localization capacity.

Vascular gelation was more frequent at 30 *dai* and was associated with invagination of the pits of paravascular paraenchyma cells forming tyloses, leading to wall off the affected vessels above the site of invasion, what is in agreement with Charchar and Kraft (1989) in *F. oxysporum* f. sp. *pisi*-pea pathosystem. Macroscopic observations showed clear differences in *DI* in some accessions (Table 1) although this was not associated with recovering pathogen propagules from root/stem segments at 30 *dai*.

Conclusion

The results obtained in this study indicated that the main courses of infection of lentil accessions with *Fol* pathotypes are: (i) attachment of hyphae to the root surface, penetration through epidermis and colonization of the cortex cells at 0-2 *dai*; (ii) hyphal penetration through endodermis layer and invasion of vascular tissues at 2-4 *dai*; (iii) colonization of all plant root tissues by the pathogen, including horizontal and lateral spreading, and presence of different gum-like substances inside the xylem vessels at 4-15 *dai*; and (iv) development of tylosis and accumulation of mucilage inside the vessels, colonization of the hypocotyls and stem, and finally phenotypic expression (yellowing and/or wilt) at 15-30 *dai*.

Resistance observed in studied accessions is mainly a quantitative nature which is identified as uniform xylem occlusion with gum-like substances or continuous degree of

colonization of all host accessions; although a qualitative resistance was identified in BGE01969 inoculated with *Fol* pathotype 1 as fast secretion of phenolic compounds in the cortex at 4 *dai* which could led to lower colonisation, (Table 2) and finally a lower *DI* (Table 1). Host specificity shown by different patterns of colonization of studied pathotypes across the lentil accessions confirmed our last report on host specificity observed by phenotypic measurements (Pouralibaba et al. 2016) even by histo-pathological methods. Based in these results, breeding strategies to introducing new disease resistant cultivars should consider different features of resistance including qualitative and quantitative aspects of the pathosystem.

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Figure 1: Toluidine Blue O (TBO) stained sections showing the penetration process and colonisation of epidermis and cortical cells.

1A: Longitudinal section of un-inoculated root from accession ILL 4774 used as a control at 2 days after inoculation (*dai*) of treated plants.

1B: Longitudinal section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 2 *dai*. Arrows indicate the presence of *Fol* hyphae attached to the root surface. Remnants of detached cuticle cells (asterisks) and plasmolysed cortex cells (arrow heads) can be observed.

1C: Longitudinal section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 2 *dai*. High magnification ($\times 100$) of (2), detached cuticle cells (asterisks) and hyphae (arrows).

1D: Longitudinal section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 2 *dai*. Penetration of hyphae (arrows) through middle lamella between adjacent cells or plane surface of individual cell directly without formation any specific structure.

1E: Longitudinal section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 2 *dai*. Huge amount of invaded hyphae in outer surface of the root and epidermal cells, destroying the epidermis (arrows), then massive invade of hyphae (arrowheads) into the cortex.

1F: Longitudinal section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 2 *dai*. Presence of hyphae growing inter (arrows) and intra cellularly (arrow head) in cortical tissue.

EP: Epidermis, CX: Cortex, EN: Endodermis, VB: Vessel bundles

Figure 2: TBO stained sections showing horizontal spread of pathogenic hyphae within root cells and tissues

2A: Longitudinal section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 2 *dai*. Pathogen transition from cell to cell by swelling of attached hyphae onto the cell wall, thinning, penetration (arrows) and swelling again in the next cell.

2B: Cross section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 2 *dai*. Radial colonisation of hyphae (arrows) from epidermis towards the stele.

EP: Epidermis, CX: Cortex, PR: Parenchyma, VB: Vessel bundles, VP: Vascular parenchyma, XL: Xylem

Figure 3: TBO stained sections showing vertical spread of pathogenic hyphae within root tissues and response of the host as secretion of phenolic compounds

3A: Longitudinal section of root from accession BGE019696 inoculated with *Fol* pathotype 1 at 4 *dai*. Details of condensed areas in the cytoplasm (arrow heads) and hyphae attached to the host surface (asterisks) or in the epidermis and cortex (arrows).

3B: Longitudinal section of root from accession BGE019696 inoculated with *Fol* pathotype 1 at 4 *dai*. Condensed areas (arrow heads) beside the xylem bundles where vascular tissue and cortex are free of infection.

3C: Longitudinal section of root from accession BGE019696 inoculated with *Fol* pathotype 1 at 4 *dai*. Details of high amount of pathogenic hyphae (asterisks) and of condensed cells (arrow heads) across the cortex to xylem where is free of infection

3D: Longitudinal section of root from accession BGE019696 inoculated with *Fol* pathotype 1 at 4 *dai*. Arrows showing granulized cytoplasm (arrow head) in the presence of hyphae (arrow). Inter and intra cellular accumulation of phenolic compounds shown by asterisks.

3E: Longitudinal section of root from accession ILL4774 inoculated with *Fol* pathotype 7 at 4 *dai*. Xylem vessel (arrow) occupied with mycelium (asterisks)

3F: Cross section of root from accession ILL 4360 inoculated with pathotype 1 at 4 *dai*. Cortex and stele occupied by pathogenic hyphae.

EP: Epidermis, CX: Cortex, XL: Xylem, VP: Vascular parenchyma

Figure 4: TBO (4A-C) and Ruthenium (4D) stained sections showing extension of pathogenic hyphae through vessels and occlusion of xylem with gum like substances

4A: Longitudinal section of root from accession ILL 4774 inoculated with *Fol* pathotype 7 at 15 *dai*. Details of transition of pathogenic hyphae (arrows) among neighbouring root xylem vessels via pits (arrow head).

4B: Cross section of root from accession ILL 4774 inoculated with *Fol* pathotype 1 at 15 *dai*. Vascular root tissue invaded by hyphae (asterisks) accumulated with pinkish materials

4C: Same combination where turquoise materials filled up vascular elements

4D: Same combination showing vascular tissue filled up by deep blue and red

XL: xylem

Figure 5: TBO stained sections showing tyloses formation and sealing off the xylem vessels with mucilage in the root and stem

5A: Longitudinal section of root from accession ILL4774 inoculated with *Fol* pathotype 7 at 30 *dai*. Protruding tyloses droplets (arrows) from paravascular parenchyma cells (arrow heads) into the vessel element via pits.

5B: Cross section of stem root from accession BGE019696 inoculated with *Fol* pathotype 7 at 30 *dai* showing sealing off the vascular system with mucilage

5C: Cross section of stem from accession ILL 10241 inoculated with *Fol* pathotype 7 at 30 *dai* showing sealing off the vascular system with mucilage

5D: Cross section of stem from accession ILL 10241 inoculated with *Fol* pathotype 7 at 30 *dai*. Higher magnification of 5C showing xylem completely filled up with mucilage

XL: Xylem