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FULL RESEARCH PAPER

Cytological and immunocytochemical studies on responses of wheat spikes of the resistant Chinese cv. Sumai 3 and the susceptible cv. Xiaoyan 22 to infection by *Fusarium graminearum*

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Abstract Fusarium head blight (FHB), predominantly caused by *Fusarium graminearum* and *F. culmorum*, is one of the most destructive diseases of wheat, reducing grain yield and quality of kernels. In diseased kernels trichothecne mycotoxins accumulate, which are harmful to human and animal health. Pathogen development and host responses to infection by *F. graminearum* were investigated in wheat spikes of the resistant cv. Sumai 3 and susceptible cv. Xiaoyan 22 to infection by means of electron microscopy and immunogold labelling techniques. The infection process of the pathogen in wheat spikes was similar in both the resistant and susceptible cultivars, but the pathogen developed more slowly in the resistant cv. Sumai 3 compared to the susceptible cv. Xiaoyan 22, indicating that fungal spread was restricted to the spike tissues of the resistant cultivar. The formation of thick-layered appositions and papillae was essentially more pronounced in the infected host tissues of the resistant cultivar than in the susceptible one. ß-1,3-glucan was detected in the appositions and papillae. Immunogold labelling studies demonstrated that labelling densities for lignin, thionins and hydroxyproline-rich glycoproteins (HRGP) over the cell walls of the infected tissues of the susceptible wheat cv. Xiaoyan 22 only slightly increased whereas these compounds intensely accumulated in the host cell walls of the infected wheat spikes of the resistant cv. Sumai 3. The labelling densities for the two plant hydrolases, ß -1,3-glucanase and chitinase, increased slightly in the infected wheat spike tissues of the susceptible cv. Xiaoyan 22, whereas higher labelling densities of both enzymes were found in the infected wheat spikes from the resistant cv. Sumai 3. Immunogold labelling of the Fusarium toxin DON in the infected wheat spike tissues showed that labelling densities in spike tissues for DON in the resistant cv. Sumai 3 were significantly lower than those in the susceptible cv. Xiaoyan 22. The significance of the induced morphological (e.g. thick-layered wall appositions and papillae) and chemical defence constituents (e.g. ß -1,3-glucanase, chitinase, lignin, thionin and HRGP) in resistance to FHB as well as the possible role of DON as an aggressiveness factor in translation of transcripts of defence response genes and spreading of *F. graminearum* and *F. culmorum* are discussed.

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Abbreviations

FHB	Fusarium head blight
DON	deoxynivalenol
TBS	Tris-buffered saline
HRGP	hydroxyproline-rich glycoproteins
TEM	transmission electron microscopy

Introduction

Fusarium head blight (FHB) of wheat (*Triticum aestivum*), also known as scab or ear blight, is a serious fungal disease in humid and semi-humid wheat-growing regions throughout the world (McMullen et al. 1997). Epidemics of FHB not only result in significant yield losses and low quality of grains (Boyacioglu and Hettiarachchy 1995), but also contaminate grain with a variety of mycotoxins produced by *Fusarium* spp. (Snijders and Perkowski 1990). Mycotoxin contamination of cereal food and feed may be harmful to human and animal health (Desjardins and Hohn 1997).

FHB may be caused by 17 different *Fusarium* spp., the most common of which are *Fusarium graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *Microdochium nivale* (Parry et al. 1995). Worldwide, FHB is predominantly caused by *F. graminearum* (perfect state *Gibberella zeae*). Wheat heads are most susceptible to *F. graminearum* infection for a short time period from flowering to the soft dough stage of kernel development. More recently, we elucidated in detail the infection process and pathway of spread of *F. culmorum*, *F. graminearum*, *F. avenaceum* and *M. nivale* in wheat spikes by means of light microscopy and scanning and transmission electron microscopy, and found that these pathogens exhibited a similar behaviour regarding infection and colonization of wheat spikes (Kang and Buchenauer 2000a; Kang et al. 2004, 2005). Furthermore, using enzyme-gold and immuno-gold labelling techniques, it has been demonstrated that these pathogens produced cell wall degrading enzymes such as cellulases, xylanases and pectinases at early stages of infection in wheat spikes (Kang and Buchenauer 2000b; Wanyoike et al. 2002; Kang et al. 2004, 2005). In addition, immunogold labelling studies revealed a close relationship between the accumulation of Fusarium toxins in infected wheat spikes and the pathogenic changes in the host cells, colonization of the pathogen in the host tissues and symptom appearance (Kang and Buchenauer 1999).

Resistance of wheat to FHB is expressed as a quantitative or horizontal characteristic (Parry et al. 1995; Mesterházy 1995). Different types of active resistance mechanisms to FHB have been described: (1) resistance to initial infection (type I), (2) resistance to spreading of the pathogen within the host tissues (type II) (Schroeder and Christensen 1963), (3) the ability of the host to degrade deoxynivalenol (DON) (type III; Miller and Arnison 1986), (4) tolerance to mycotoxins (type IV; Wang and Miller 1988) and (5) resistance to kernel infection (type V; Mesterházy 1995).

The Chinese wheat cv. Sumai 3, highly resistant to FHB, presents an essential genetic source and has been widely used in the breeding of FHB resistant cultivars (Bai and Shaner 1994; Ban and Suenaga 2000). Two resistant genes in Sumai 3 have been identified expressing predominantly type II resistance (Van Ginkel et al. 1996). However, field observations suggest that the two resistance genes of Sumai 3 are associated also with type I resistance (Ban and Suenaga 2000). Substitution lines were used to evaluate the effect of individual Sumai 3 chromosomes on type II resistance to *F. graminearum* and type V resistance. Positive effects on type II FHB resistance were found on chromosomes 2B, 3B, 5A and 6B. Chromosomes 3B and 5A from Sumai 3 have the largest effect on resistance to disease spread and DON accumulation (Zhou et al. 2002; Snijders 2004).

Development of *F. graminearum* and transcript accumulation of different defence response genes were comparatively studied in *F. graminearum*-inoculated spikes of the resistant genotype Sumai 3 and the susceptible genotype Wheaton within the first 48–76 h after inoculation (hai) (Pritsch et al. 2000). During this infection period no significant differences between the resistant and the susceptible genotype in the infection process and the intracellular colonization of the parenchyma tissues were observed. Both genotypes were successfully colonized. The expression pattern of six defence response genes, POX (peroxidase), PR-1, PR-2 (ß-1,3-glucanase), PR-3 (chitinase), PR-4 and PR-5 (thaumatin-like protein), showed that these genes were induced in spray-inoculated heads of both the

susceptible cv. Wheaton and the resistant cv. Sumai 3. Transcripts began to accumulate 6–12 hai and reached highest amounts 36 to 48 hai. Only transcripts of PR-4 and PR-5 accumulated earlier and to a higher extent in Sumai 3 than in Wheaton.

However, induction of high levels of transcripts of defence response genes after infection of wheat spikes by *F. graminearum* or *F. culmorum* might not necessarily indicate also a high accumulation of their corresponding translation products. During penetration and colonization of spike tissues by both *Fusarium* spp., trichothecene mycotoxins including DON, the acetylated forms 3-ADON and 15-ADON, nivalenol, diacetoxyscirpenol and the T-2 and HT-2 toxins may be accumulated in high concentrations especially in spike tissues of susceptible genotypes (Mesterházy et al. 2005). The predominant toxin produced by *F. graminearum* is DON. DON and its trichothecne derivatives interfere with protein synthesis in eukaryotic cells and they might, thus, effectively inhibit translation of induced transcripts of defence response genes in cells of *F. graminearum*-infected spike tissues. This could possibly result in marked suppression of accumulation of post-infectional morphological and chemical defence factors. These assumptions agree with findings that DON represents an important aggressiveness factor of *F. culmorum* and *F. graminearum* and is a major determinant of fungal spread and FHB development in cereal species (Snijders 2004; Langevin et al. 2004).

In this paper, the response of spike tissues of the resistant wheat cv. Sumai 3 and the susceptible cv. Xiaoyan 22 to *F. graminearum* infection was studied by means of electron microscopy as well as immunogold labelling techniques. The following aspects in both cultivars were investigated: (a) infection process and colonization of different spike tissues, (b) accumulation of morphological defence components, (c) enrichment of lignin, ß-1,3-glucanase, chitinase, thionins and hydroxyproline-rich glycoproteins and (d) accumulation of DON.

Materials and methods

Plant and pathogen

The resistant wheat cv. Sumai 3 and the susceptible winter wheat cv. Xiaoyan 22 were used throughout the present experiments. The wheat plants were grown as described previously (Kang and Buchenauer 1999). When the plants were at the middle stage of flowering (growth stage (GS) 65; Zadoks et al. 1974), the wheat spikes were used for inoculation. An isolate of *F. graminearum* (No.8) from the Institute of Plant Pathology, Northwest A&F University was used for inoculation. Macroconidial suspension (1×10^5 conidia ml^{-1}) of the pathogen was prepared as earlier reported (Kang and Buchenauer 1999).

Inoculation of wheat spikes

The wheat spikes were inoculated using a pipette by injection of 10 µl of a macroconidial suspension into the floral cavity between the lemma and palea of the first floret on spikelets. Florets with an interval of two spikelets at only one side of the spike were inoculated. Control plants were treated with distilled water instead of the macroconidial suspension. Inoculated spikes were covered with a moistened plastic bag tied to the stalk to uphold a high relative humidity for 24 h.

Tissue processing for electron microscopy

Inoculated florets were taken 24 and 36 h as well as 2, 3, 4, 5, 6, 8, 10 and 12 days after inoculation (dai). Lemmas, ovaries, paleas and rachis from the florets were excised into pieces and fixed in 3% (v/v) glutaraldehyde in 50 mM phosphate buffer (pH 6.8) for 3–6 h at 4°C. Thereafter, samples were rinsed thoroughly with 50 mM phosphate buffer (pH 6.8) and post-fixed with 1% (w/v) osmium tetroxide in the same buffer for 2 h at 4°C. Samples were dehydrated in a graded ethanol series, embedded in LR White (TAAB Laboratories, Munich) and polymerized at 50°C for 2 days. Three replicate experiments were performed. For immunocytochemical localization of the Fusarium toxins deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-A-DON), the samples were processed as previously described by Kang and Buchenauer (1999).

Electron microscope observations

For electron microscopy, ultra-thin sections of the samples were cut with a diamond knife and collected on 200-mesh copper grids. After contrasting with

uranyl acetate and lead citrate, the grids were examined with a Zeiss-EM10 electron microscope at 80 kV.

Immunogold labelling for lignin, thionin, hydroxyproline-rich glycoprotein, β-1,3-glucan, β-1,3-glucanase and chitinase

The rabbit polyclonal antibody against lignin was kindly supplied by Dr. Ruel (Centre de Recherches sur les Macromolécules Végétales, Université Joseph Fourier, France). The rabbit polyclonal antisera against acidic β-1,3-glucanase and acidic chitinase from tobacco were obtained from Prof. Fritig (C.N.R.S., Institut de Biologie Moléculaire des Plantes, Strasbourg, France). The rabbit polyclonal antiserum against thionin from barley leaf was obtained from Dr. K. Apel (Institut für Pflanzenwissenschaften, Swiss Institute of Technology (ETH), Zürich, Switzerland). A mouse monoclonal antibody against hydroxyproline-rich glycoprotein (HPRG) from maize was obtained from Dr. Hood (Department of Biochemistry and Biophysics, Texas A&M University, USA). A mouse monoclonal antibody recognising β-1,3-glucan was purchased from Biosupplies Australia Pty. Ltd. (Parkville, Victoria, Australia). All these antibodies showed immunocytochemical labelling for corresponding compounds in wheat spike tissues (Kang and Buchenauer 2000a, 2002). The secondary antibodies (goat anti-rabbit IgG and goat anti-mouse IgG) coupled to 15 nm gold particles were purchased from British Biocell International Ltd. (Cardiff, UK).

Immunogold labelling was carried out as follows: (1) incubation of ultra-thin sections with blocking solution containing 1% (w/v) of bovine serum albumin (BSA) in Tris-buffered saline (TBS, 10 mM Tris–HCl, 150 mM NaCl, pH 7.4) for 20 min; (2) incubation of the sections with the primary antibody in the blocking solution (the anti-lignin antiserum was diluted at 1:600, the anti-thionin antiserum and the monoclonal anti-callose antibody were diluted at 1:100, the monoclonal anti-HRGP antibody, the anti-β-1,3-glucanase antiserum and anti-chitinase antiserum were diluted 1:200) for 2 h at room temperature; (3) washing in four 10 min baths in TBS; (4) incubation of the sections with the corresponding secondary antibody (all diluted 1:40 in TBS); (5) rinsing with TBS followed by a distilled water rinse. The labelling specificity was verified by replacing the primary antibody with the buffer. After immunogold labelling, the ultra-thin sections were contrasted with uranyl acetate and lead citrate before examination with the electron microscope.

Immunogold labelling for Fusarium toxin

The antiserum raised in rabbit against pure DON was kindly provided by Dr. E. Usleber (Institute for Hygiene and Technology, University Munich, Germany). The measurements of immunosorbent assays (ELISA) have indicated that DON-antiserum not only reacted with DON, but also strongly cross-reacted with 3-ADON and 15-ADON (Usleber et al. 1991). The immunogold labelling and the assessment for labelling specificity were carried out following the protocol outlined in Kang and Buchenauer (1999).

Quantification of labelling

The experiments were repeated three times. The labelling densities for lignin, thionin, HRGP, β-1,3-glucanase, chitinase and Fusarium toxins (DON, 3-ADON and 15-ADON) in the uninoculated and infected wheat spikes of different cultivars were compared by determining the number of gold particles μm^{-2} over specified areas on 10–15 microphotographs. The difference in the number of gold particles between uninoculated healthy and infected wheat spikes was statistically analysed by the paired *t*-test.

Results

Infection process and responses in the infected host tissues

Electron microscopic observations showed that the infection process and spreading pathway of *F. graminearum* in the wheat spikes were very similar between the resistant cv. Sumai 3 and the susceptible cv. Xiaoyan 22. The primary penetration of the host tissue by the pathogen occurred on the inner surfaces of the lemma, palea and on the top of the ovary between 36 and 48 hai. The pathogen invaded the host tissues predominantly by direct penetration with infection hyphae. Thereafter, the hyphae of the pathogen spread downward to the rachis by inter- and intracellular growth from the lemma, palea and ovary (Figs. 1a–d,

2a–d). However, time-course studies revealed a marked difference in the fungal colonisation in the wheat spikes between the susceptible and resistant cultivar. The pathogen reached the rachis of cv. Xiaoyan 22 at 4–5 dai and hyphae extended predominantly downward by inter- and intracellular growth in the rachis. A pronounced higher hyphal number was usually detected in the infected wheat spike tissues (*e.g.* lemmas, paleas, ovaries) of cv. Xiaoyan 22 as compared to the corresponding tissues in cv. Sumai 3. On the other hand, it took 8–10 days for the pathogen to reach the rachis in the resistant cv. Sumai 3. With regard to the susceptible cultivar, spreading of hyphae of *F. graminearum* in the rachis of the resistant cv. Sumai 3 was distinctly restricted.

An obvious difference of host response to the infection and spreading of the pathogen was detected between the resistant cultivar and susceptible cultivar. In cv. Xiaoyan 22, a thin layer of wall apposition or small vesicles and electron-dense material of different size and texture were very often observed in the periplasmic space between the host cell wall and the plasmalemma in the infected lemma, palea, ovary and rachis (Fig. 1a–d). On the other hand, one of the marked reactions in the infected tissues (e.g. lemma, palea, ovary and rachis) of cv. Sumai 3, was the pronounced formation of wall appositions and papillae in host cells both in proximity and distant to the fungal hyphae (Fig. 2a–d). The cell wall appositions varied distinctly in their appearance: from elongated depositions along a large portion of the host cell wall to hemispherical or dome like protuberances. Sometimes, electron-dense or fibrous material was detected on the hyphal surfaces or in the host intercellular

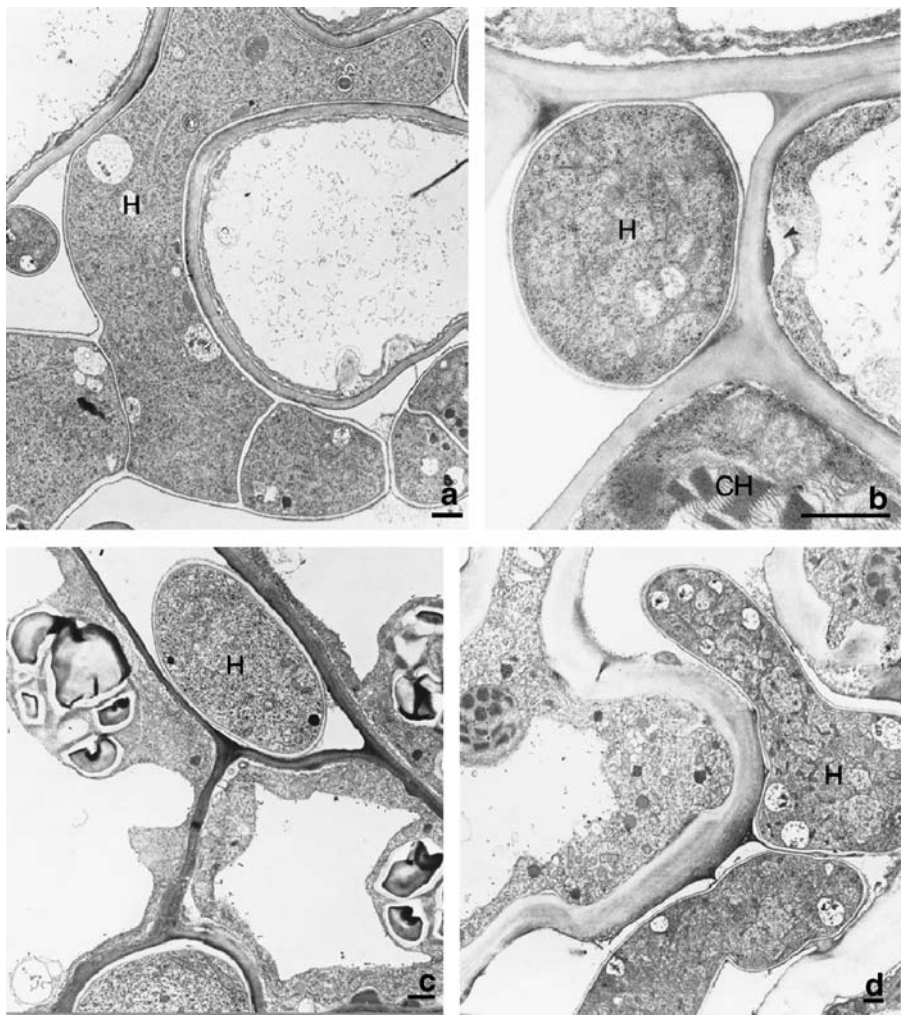

Fig. 1 Transmission electron micrographs of colonisation of the spike of the susceptible wheat cv. Xiaoyan 22 by *F. graminearum*. **a** Hyphae in the intercellular spaces between parenchyma cells in the lemma, 3 dai. **b** Infected lemma at a high magnification, 3 dai. Some electron – dense material of small size was deposited in the periplasmic spaces (*arrowhead*). The stroma in the chloroplast appeared loose in arrangement. **c** Hyphae extending intercellularly in the pericarp tissue of the ovary, 3 dai. Cell wall appositions of small size appeared between host cell wall and plasmalemma. **d** Hyphae spreading between cortical cells in the infected rachis, 5 dai. All *bars* = 1 μm. *H* hyphal cell, *CH* chloroplast

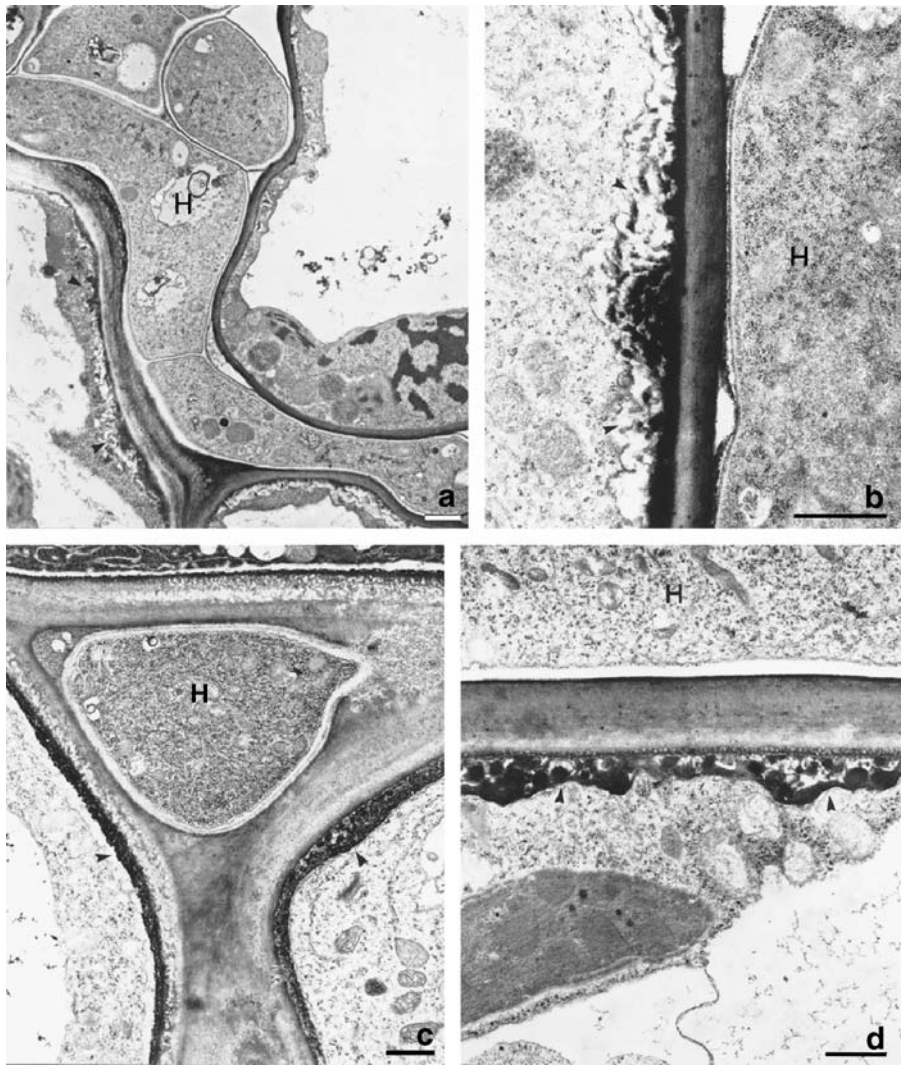

Fig. 2 Transmission electron micrographs of colonisation of the spike of the resistant wheat cv. Sumai 3 by *F. graminearum*. **a** Infected lemma, 3 dai. Hyphae extended intercellularly and pronounced cell wall appositions have been formed (*arrowheads*). **b** Hypha spreading between host cells in the lemma, 3 dai. A thick layer of wall appositions was formed between host cell wall and plasmalemma (*arrowheads*) and electron – dense material was detected on the hyphal surface. The host cell walls also attained a very dense appearance. **c** Hyphae extending intercellularly in the pericarp tissue of the ovary, 3 dai. Very thick layers of wall appositions were formed between host cell wall and plasmalemma (*arrowheads*). **d** Hyphae in intercellular space of the cortical tissue of the rachis, 8 dai. A thick layer of wall appositions was formed between host cell wall and plasmalemma (*arrowheads*) and host cell walls also appeared very dense. All *bars* = 1μm. H: hyphal cell

spaces (Fig. 2b,c). The host cells close to the hyphae also displayed a higher electron density than unaffected cells (Fig. 2a–d); this probably indicates the infiltration of the cell walls with newly synthesised material from the host cells.

Immunogold localization of lignin in healthy and infected wheat spikes

Following incubation of ultra-thin sections of different tissues from the uninoculated and infected wheat spikes with anti-lignin antiserum and the secondary antibody, a clear deposition of gold particles occurred over the cell walls of the different tissues whereas the host cytoplasm and the hyphal cells were free of labelling (Fig. 3a–c). While the labelling patterns and labelling densities of lignin in the uninoculated spike tissues of the two wheat cvs Xiaoyan 22 and Sumai 3 were very similar, the labelling densities in the infected tissues varied markedly between both cultivars. Compared to the uninoculated corresponding tissues, gold particles over the cell walls in the infected host tissues of cv. Sumai 3 increased considerably, whereas in cv. Xiaoyan 22, the labelling density over the cell walls in the infected tissues showed only a slight increase (Table 1, Fig. 3b,c). However, cell wall appositions formed in wheat cv. Sumai 3 were almost free of gold particles (Fig. 3c). For labelling specificity assessment, the incubation of sections with the secondary antibody alone yielded no labelling.

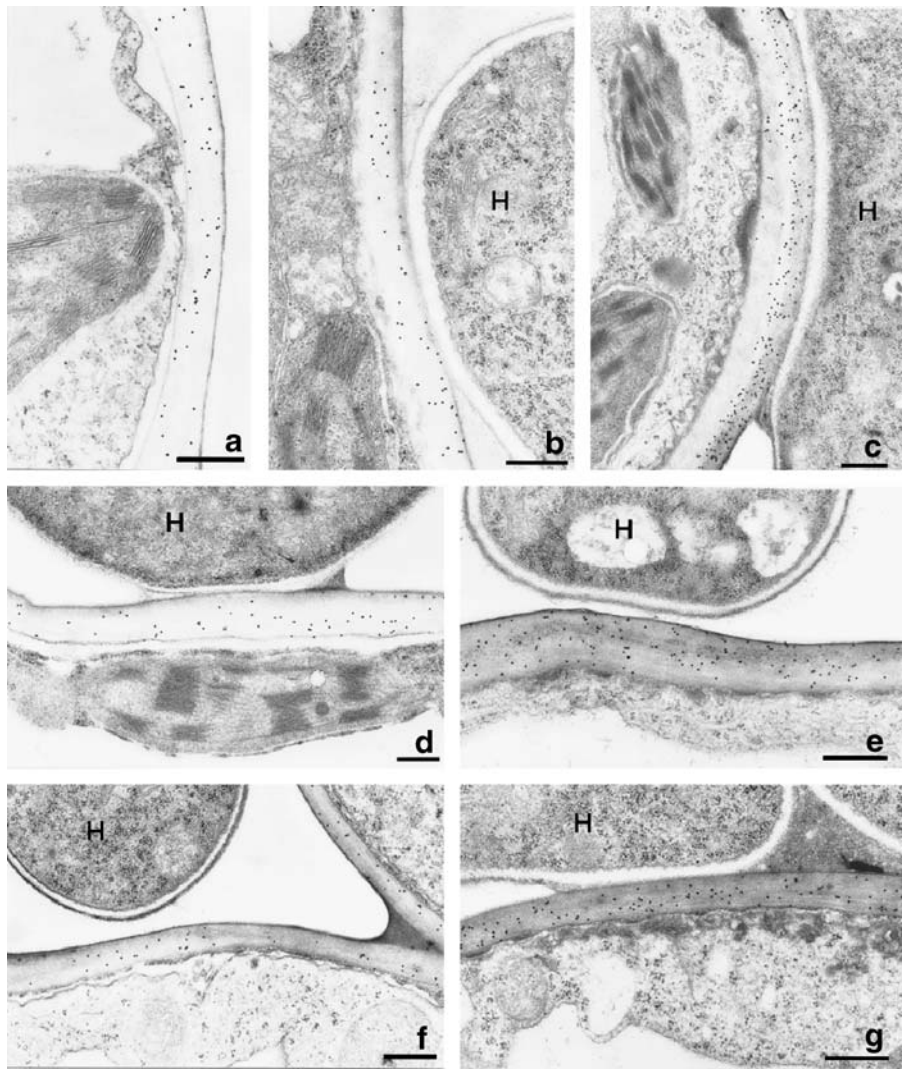

Fig. 3 **a**–**c** Immunogold localization of lignin in the uninoculated and infected wheat spikes from the resistant and susceptible cultivars. **a** Uninoculated lemma of the susceptible cv. Xiaoyan 22. Host cell wall showed labelling with gold particles, while host cytoplasm and organelles were free of labelling. **b** Infected lemma from the susceptible cv. Xiaoyan 22, 3 dai. Gold particles were distributed over the host cell walls, but not over the fungal wall. **c** Infected lemma from the resistant cv. Sumai 3, 3 dai. Host cell wall was labelled with a higher density of gold particles, while the hyphal wall was free of labelling. **d**, **e** Immunogold localization of thionin in infected wheat spikes from the resistant and susceptible cultivar. **d** Infected lemma from the susceptible cv. Xiaoyan 22, 3 dai. Gold particles were deposited over the host cell walls, but not over the host cytoplasm and the fungal wall. **e** Infected lemma from the resistant cv. Sumai 3, 3 dai. The host cell wall was labelled with a higher density of gold particles, while the hyphal wall was free of labelling. **f**, **g** Immunogold localization of hydroxyproline – rich glycoprotein in infected wheat spikes from the resistant and susceptible cultivar. **f** Infected lemma from the susceptible cv. Xiaoyan 22, 3 dai. Gold particles were distributed over the host cell walls, but not over the host cytoplasm or the fungal wall. **g** Infected lemma from the resistant cv. Sumai 3, 3 dai. The host cell wall was labelled with a high density of gold particles, while the hyphal wall was free of labelling. All *bars* = 0.5 μm. *H* hyphal cell

Immunogold localization of thionin and HRGP in healthy and infected wheat spikes

The labelling pattern and densities for thionin and HRGP in the cell walls of uninoculated spike tissues showed no difference between the resistant cv. Sumai 3 and the susceptible cv. Xiaoyan 22. In contrast, labelling densities for thionin and HRGP in the infected wheat spikes varied markedly between cultivars. In cv. Xiaoyan 22 a slight but not significant

Table 1 Labelling densities of lignin, thionin, HRGP, β-1, 3-glucanse, chitinase and DON over parenchyma cell walls in the lemma of uninoculated and *Fusarium graminearum*-infected wheat spikes of different cultivars 3 days after inoculation

	cv. Xiaoyan 22			cv. Sumai 3		
	Healthy	Infected		Healthy	Infected	
Lignin	26.82±3.56[a]	28.24±3.24	n[b]	27.46±3.62	44.82±4.62	s[c]
Thionin	24.36±2.82	25.84±3.16	n	25.64±3.28	40.68±4.28	s
HRGP	22.60±2.46	23.62±2.84	n	23.82±2.86	38.24±3.82	s
β-1,3-glucanse	12.26±2.25	13.60±2.46	n	13.46±2.26	34.66±3.26	s
Chitinase	11.48±2.34	12.32±2.16	n	12.62±2.42	38.20±3.88	s
DON	0.00	16.24±3.42		0.00	10.46	

Density of labelling was expressed by the number of gold particles μm^{-2}.

[a] Mean of values and ±standard deviation of the gold particles determined μm^{-2}. The mean densities for uninoculated healthy and infected wheat tissues were compared by the *t*-test, $P=0.05$.

[b] n=values in the same row not significantly different at $P=0.05$

[c] s=values in the same row significantly different at $P=0.05$

increase in labelling density for thionin and HRGP was found over the cell walls of the infected lemma and ovary (Fig. 3d,f; Table 1), whereas gold labellings for thionin and HRGP over the cell walls increased significantly in the infected host tissues of cv. Sumai 3 (Fig. 3e,g; Table 1). For labelling specificity assessment, the incubation of sections with the secondary antibody alone yielded no labelling.

Immunogold localization of β-1,3-glucanase and chitinase in healthy and infected wheat spikes

Both enzymes were localized mainly in the cell walls of different tissues including the lemma, ovary and rachis of the wheat spike, while the cytoplasm and organelles in these tissues showed almost no labelling (Fig. 4c). The accumulation of β-1,3-glucanase and chitinase in the infected wheat spikes differed distinctly between the resistant and susceptible wheat cultivar. In relation to the corresponding uninoculated tissues, the labelling densities for the two enzymes in the infected lemma, ovary and rachis of cv. Xiaoyan 22 increased slightly, whereas higher labelling densities of β-1,3-glucanase and chitinase were found in the different infected tissues of wheat spikes from cv. Sumai 3 (Fig. 4c–g; Table 1). Furthermore, the labelling of both enzymes also occurred over the cell walls of the hyphae in the infected wheat spike, but not over the hyphal cytoplasm. The hyphal cells in the infected tissue of cv. Sumai 3 usually showed more labelling on their walls than the hyphae in the susceptible cultivar, particularly at the region in contact with the host cell, (Fig. 4e,g). For labelling specificity assessment, the incubation of sections with the secondary antibody alone yielded no labelling.

Immunogold localization of callose in healthy and infected wheat spikes

Following incubation of the sections of the uninoculated healthy and infected wheat spikes with the monoclonal antibody against β-1,3-glucan and the secondary antibody, the labelling occurred over host tissues and the pathogen. In the uninoculated host tissue, only translucent areas of the sieve plate and plasmodesmata showed a dense labelling, while all cell walls of the wheat spike tissues were free of gold particles. In the infected wheat spikes of cv. Xiaoyan 22, a few gold particles were often detected in the periplasmic spaces between plasma membrane and cell wall of the host cells (Fig. 4a), while the wall appositions and papillae formed in Sumai 3 were labelled with a high density of gold particles (Fig. 4b). Usually, more gold particles were distributed over the translucent areas in the appositions while the electron-dense areas showed a lower density of gold particles. In the fungal cells, gold particles were detected over the hyphal walls, but not over the hyphal cytoplasm (Fig. 4a,b). In the labelled control, where the primary antibody was omitted, no labelling was observed over the sections.

Immunogold localization of mycotoxin DON in wheat spikes

Incubation of the infected host tissues with the antiserum against mycotoxin DON and secondary

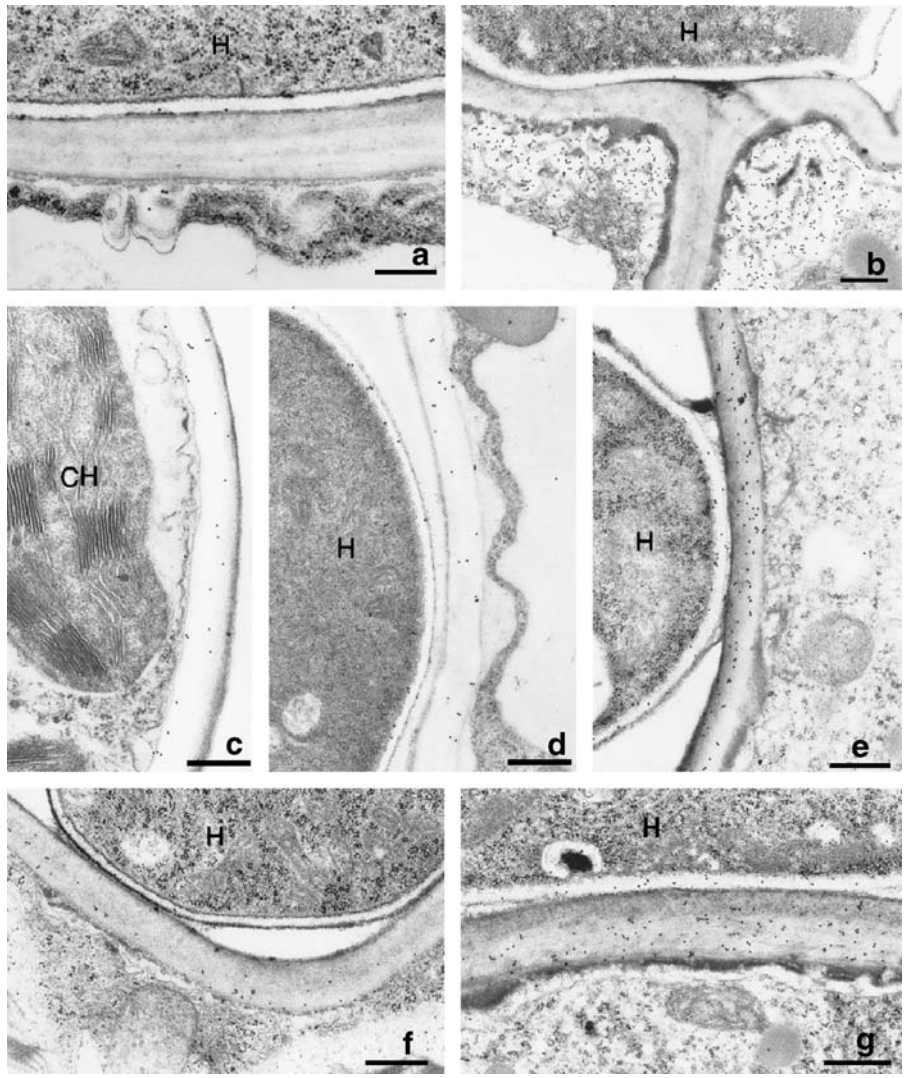

Fig. 4 **a**, **b** Immunogold localization of β-1, 3-glucan in infected wheat spikes from the resistant and susceptible cultivar. **a** Infected lemma from the susceptible cv. Xiaoyan 22, 3 dai. A few gold particles were detected in the periplasmic space (*arrowheads*) and on the fungal cell wall, while the host cell wall showed no labelling. **b** Infected lemma from the resistant cv. Sumai 3, 3 dai. The wall apposition was densely labelled with gold particles over the translucent areas in the apposition. **c**–**e** Immunogold localization of β -1,3-glucanase in the uninoculated and infected wheat spikes from the resistant and susceptible cultivar. **c** Parenchyma cell in the lemma from the uninoculated wheat spike of the susceptible cv. Xiaoyan 22. A low density of gold particles was localized over the cell wall while the cytoplasm and organelles are almost free of gold particles. **d** Infected lemma from the susceptible cv. Xiaoyan 22, 3 dai. A low density of gold particles was found over the plant cell walls and also over the hyphal cell wall. **e** Infected lemma from the resistant cv. Sumai 3, 3 dai. A high density of gold particles was detected over the plant cell walls; the hyphal cell wall also showed labelling. **f**, **g** Immunogold localization of chitinase in the infected wheat spikes from the resistant and susceptible cultivars. **f** Infected lemma from the susceptible cv. Xiaoyan 22, 3 dai. The plant cells were labelled with a low density of gold particles over the cell walls, but not over the cytoplasm and organelles. The hyphal cell wall showed a few gold particles. **g** Infected lemma from the resistant cv. Sumai 3, 3 dai. Numerous gold particles were deposited over the plant cell wall. Over hyphal cell wall in contact with the host cell gold particles were also detected. All *bars* = 0.5 μm. *H* hyphal cell, *CH* chloroplast

antibodies showed that in the host cells, gold particles were localized over the cell walls, cytoplasm, plasmalemma, as well as in chloroplasts and vacuoles, while mitochondria and Golgi bodies showed no labelling (Fig. 5a,b). In the hyphal cells, the toxin was localized in the cell walls, cytoplasm, mitochondria and vacuoles. However, quantification of gold particles over the specific areas in the host cells revealed

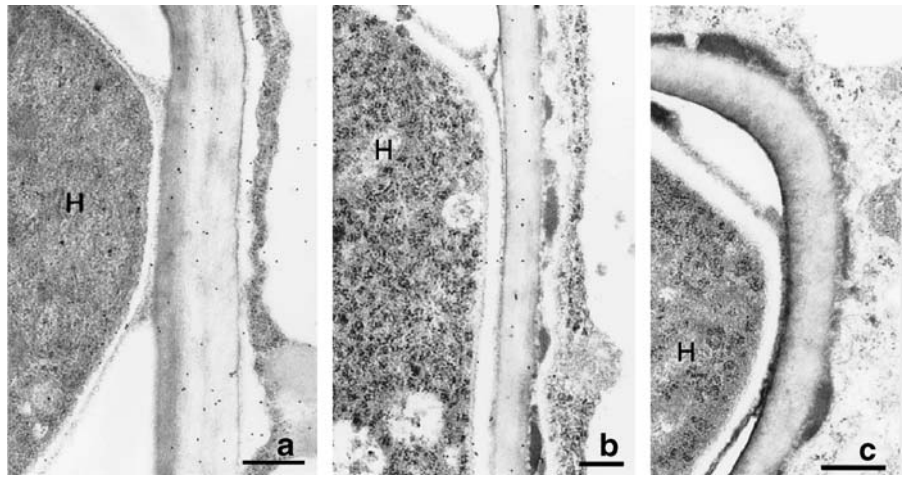

Fig. 5 Immunogold localization of Fusarium toxin DON in the infected lemma from the susceptible and resistant wheat cultivar. **a** Infected lemma from the susceptible cv. Xiaoyan 22, 3 dai. Gold labelling occurred over the host cell wall, cytoplasm and vacuole. The hyphal cell showed labelling over the wall and cytoplasm. **b** Infected lemma from the resistant cv. Sumai 3, 3 dai. A low density of gold particles was determined over the host cell wall, and only rarely over the cytoplasm and vacuole. Gold particles were detected over the hyphal cell wall and cytoplasm. **c** Control of immunocytochemical labelling for the *Fusarium* toxin DON. The infected lemma from the resistant cv. Sumai 3, 3 dai, incubated with DON antiserum which was pre-absorbed with pure DON. No labelling was found over the hyphal cell and host cell. All *bars* = 0.5 μm. *H* hyphal cell

that the labelling density of the toxin in the infected host tissue of cv. Xiaoyan 22 was usually higher than that in corresponding infected host tissues of cv. Sumai 3 (Fig. 5a,b, Table 1). A very low labelled was found in cytoplasm of cells in cv. Sumai 3 (Fig. 5b). For labelled control, incubation either with anti-DON antiserum, which had been pre-absorbed with antigen DON, or only with the secondary antibody yielded no labelling in the infected host tissue and in the hyphal cells (Fig. 5c).

Discussion

The electron microscopic studies demonstrated that the infection process and initial spread of *F. graminearum* in wheat spikes in cv. Sumai 3 proceeded in a manner similar to that in cv. Xiaoyan 22. Comparable results have been reported by Pritsch et al. (2000). They observed no major differences between the resistant genotype Sumai 3 and the susceptible genotype Wheaton during the early infection of wheat spikes by *F. graminearum*. However, by extending the incubation time our studies revealed that the distribution of the hyphae of the pathogen in wheat spikes at different intervals after inoculation showed significant differences in colonisation between the susceptible and the resistant wheat cultivar. The pathogen reached the rachis from the lemma and ovary of the inoculated wheat spikes earlier in cv. Xiaoyan 22 than in cv. Sumai 3, and more hyphae were usually detected in the spike tissues of cv. Xiaoyan 22 compared to corresponding tissues in cv. Sumai 3, indicating that the fungal spread in wheat spikes was restricted in the resistant cultivar. These findings are in agreement with an earlier report by Schroeder and Christensen (1963), who found that *F. graminearum* grew more slowly in wheat spikes of the resistant cvs Lee and Frontana as compared with the susceptible cv. Thatcher. They described the resistance to spread of the pathogen in the wheat spike as type II resistance. However, they did not find any anatomical or histological alterations associated with this type of resistance in wheat spikes. Our observations at the ultrastructural level, however, revealed that the susceptible and resistant wheat cultivar responded differently to infection and spread of *F. graminearum* in wheat spikes. In cv. Xiaoyan 22, the host cells reacted to the *Fusarium* infection only to a limited extent such as the formation of small local wall appositions in periplasmic spaces in the infected tissues. In contrast, the host cells in the infected spike tissues of cv. Sumai 3 showed strong active reactions to the invading pathogen, including extensive formation of very thick layers of wall appositions or papillae. The formation of the apposi-

tions or papillae in cv. Sumai 3 constitute an additional physical barrier, which may contribute to the restriction of pathogen attack and spread in the host tissues as well as to the impairment of the passage of small molecules and ions including Fusarium toxins into the host cells. Similar defence responses have been reported from other incompatible interactions of host plants and fungal pathogens (Enkerli et al. 1997; Aist 1976) and also occurred in *F. culmorum*-infected spike tissues of the resistant wheat cvs. Arina and Frontana as described by Kang and Buchenauer (2000a).

It has been demonstrated that β-1,3-glucan (callose) is one of the main components in cell wall appositions formed in the infected host cells (Aist 1976; Enkerli et al. 1997; Kang and Buchenauer 2000c). In *F. graminearum*-infected wheat spikes of cv. Xiaoyan 22, a very low density of callose deposition was detected in the periplasmic space in the host cells that were in close contact with the hyphae, whereas the appositions and papillae formed in cv. Sumai 3 showed dense labelling of callose. Similar differential accumulations of callose were detected in spike tissues of resistant and susceptible wheat genotypes infected by *F. culmorum* (Kang and Buchenauer 2000c). These results demonstrate that the resistance expressed in wheat spike tissue to FHB is associated with rapid deposition of callose in the infected tissues.

Changes in plant cell walls regarding defence responses include, for example, deposition of lignin-like material, callose, phenolic compounds and proteins such as thionins and HRGPs. The production and accumulation of these molecules are well-documented responses of many plants to attack by a variety of plant pathogenic agents (Epple et al. 1997; Benhamou et al. 1991; Kang and Buchenauer 2000c, 2003). The immunogold labelling of lignin, thionins and HRGPs in the *F. graminearum*-infected wheat spikes demonstrated that there is a marked difference between the resistant and susceptible wheat cultivars. Whereas in the infected tissues of cv. Xiaoyan 22 the contents of lignin, thionins and HRGPs in host cell walls increased only slightly, in cv. Sumai 3 these components accumulated to particularly high levels. These findings correspond to earlier reports of marked accumulation of lignin, thionins and HRGPs induced in resistant wheat cvs Arina and Frontana following infection by *F. culmorum* (Kang and Buchenauer 2000c, 2003). The pronounced induced synthesis of such compounds may play an essential part in the restriction in the spread of the pathogen in the wheat spike. Thus, cell wall modification might represent a major role in the resistance to FHB.

Moreover, the induced synthesis of two plant hydrolases, β-1,3-glucanase and chitinase, in the infected plants tissues, has been intensively studied for function in plant defence reactions in different fungal pathogen-host systems (Boller 1987; Kombrink et al. 1988; Wubben et al. 1992; Benhamou et al. 1990; Kang and Buchenauer 2003) because many pathogenic fungi contain as major structural components in their cell walls β-1,3-glucans and chitin, the substrates for these two enzymes (Wessels and Sietsma 1981). β-1,3-Glucanase and chitinase can degrade fungal wall components, resulting in growth inhibition of fungi in vitro (Arlorio et al. 1992) and, moreover, the breakdown products of fungal wall components, caused by the hydrolases, have been shown to act as elicitors of plant defence responses (Keen and Yoshikawa 1983). Studies on the subcellular localization of β-1,3-glucanase and chitinase showed that in the incompatible plant-pathogenic fungal interaction higher activities of both hydrolytic enzymes were detected compared with the compatible interactions (Benhamou et al. 1990; Wubben et al. 1992), indicating that β-1,3-glucanase and chitinase could play an important role in the active defence of plants against fungal pathogens. When compared to the corresponding uninoculated tissues, the labelling densities for the two enzymes in the infected wheat spike tissues of cv. Xiaoyan 22 increased only slightly, whereas higher labelling densities of β-1,3-glucanase and chitinase were found in the infected wheat spikes from cv. Sumai 3. This also resulted in higher labelling densities of both enzymes over the cell walls of the hyphae in the infected wheat spikes of cv. Sumai 3 compared to cv. Xiaoyan 22. Similar results were reported in *F. culmorum*-infected wheat spikes (Kang and Buchenauer 2002). Thus, the pronounced induction of β-1,3-glucanase and chitinase activities in wheat spikes of resistant cultivars infected by *Fusarium* spp. may be involved in the restricted spreading of the pathogen in the resistant host tissues.

The important role of trichothecenes as a virulence (pathogenicity) factor in FHB of wheat has been demonstrated by using transgenic strains of

F. graminearum lacking DON production and the wild-type DON-producing strains. While no differences in initial infection of wheat spikes were found, the DON-producing and DON-non-producing strains differed distinctly in subsequent colonization and spread in wheat spikes. The DON-non-producing strains of *F. graminearum* were markedly impaired in colonization of wheat spikelets and grain compared to trichothecene-producing strains (Desjardins et al. 1996). Furthermore, transgenic strains lacking DON production were not able to colonize the rachis of wheat spikes and disease spread was suppressed under greenhouse and field conditions (Proctor et al. 2002; Bai et al. 2002)

Our cytochemical studies indicated that DON, 3-ADON and 15-ADON are already produced by *F. culmorum* (Kang and Buchenauer 1999) and *F. graminearum* (Kang et al. 2004) when hyphae come in close contact with host tissues. However, DON concentrations at this initial stage were probably too low to interfere in the initial infection process. However, with extended incubation time (e.g. 72 hai) significant higher DON labelling densities were determined in the infected spike tissues of cv. Xiaoyan 22 compared to cv. Sumai 3. It might be suggested that the high DON concentration in cv. Xiaoyan 22 interferes in translation of transcripts of defence response genes, induced at early stages of infection. Studies of Pritsch et al. (2000) showed that transcripts of defence response genes were activated in *F. graminearum*-infected spike tissues both in the susceptible cv. Wheaton and the resistant cv. Sumai 3 at early infection stages.

The distinct lower DON labelling densities in cv. Sumai 3 plus the findings that Sumai 3 ribosomes are more tolerant than ribosomes of susceptible genotypes to DON (Miller and Ewen 1997) and that resistant genotypes are able to degrade DON (Miller and Arnison 1986) indicate that translation of the induced transcripts of defence genes will be hardly impaired in cv. Sumai 3. This results in pronounced accumulation of morphological and chemical defence constituents in infected spike tissues of Sumai 3 already at early incubation stages and the defence responses may continue with extended incubation time.

Pronounced differences in DON contents were determined in grain between susceptible and resistant wheat genotypes infected by *F. graminearum* and *F. culmorum*. While in grain of susceptible cultivars between 30 and 105 mg kg^{-1} DON were analyzed, in grain of cv. Sumai 3 only 2.05 mg kg^{-1} of toxin were determined (Mesterházy et al. 2005). The very low labelling density of DON in spike tissue agrees with the very low toxin accumulation in infected grain of cv. Sumai 3. DON produced in high concentrations in spikelets of susceptible cultivars is translocated as a water-soluble compound in the phloem of the rachis and found in tissues of *F. culmorum*-infected spikes not invaded by the fungus (Snijders and Krechting 1992; Kang and Buchenauer 1999). DON might, thus, cause predisposing effects in other parts of plants, especially the rachis, because studies with transgenic strains of *F. graminearum* lacking DON production were not able to spread in wheat spikes.

The lower DON levels in infected spikelet tissues of genotype Sumai 3 and the simultaneous pronounced defence responses indicate that DON might be translocated in the phloem to healthy spike tissues in lower concentrations which will not cause detrimental physiological effects. Following floret inoculations of spikes of cv. Sumai 3 with *F. graminearum* the fungus did not spread basipetally in the rachis and caused no head bleaching in the upper part of the spike (Langevin et al. 2004).

The accumulation of induced general morphological and chemical defence mechanisms at early stages after infection mediated by low DON concentrations may be related to resistance type II (resistance to spread of the pathogen in spike tissue), type III (degradation of DON) and type IV (tolerance to DON) expressed by cv. Sumai 3.

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References

Aist, J. R. (1976). Papillae and related wound plugs of plant cells. *Annual Review of Phytopathology*, *14*, 145–163.

Arlorio, M., Ludwig, A., Boller, T., & Bonfante, P. (1992). Inhibition of fungal growth by plant chitinases and β-1,3-glucanases: A morphological study. *Protoplasma*, *171*, 34–43.

Bai, G. H., Desjardins, A. E., & Plattner, R. D. (2002). Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia*, *153*, 91–98.

Bai, G. H., & Shaner, G. (1994). Scab of wheat: Prospects for control. *Plant Disease*, *78*, 760–766.

Ban, T., & Suenaga, K. (2000). Genetic analysis of resistance to Fusarium head blight caused by *Fusarium graminearum* in Chinese wheat cultivar Sumai 3 and Japanese cultivar Saikai 165. *Euphytica*, *113*, 87–99.

Benhamou, N., Joosten, M. H. A. J., & De Wit, P. J. G. M. (1990). Subcellular localization of chitinase and of its potential substrate in tomato root tissues infected by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Plant Physiology*, *92*, 1108–1120.

Benhamou, N., Mazau, D., Grenier, J., & Esquerré-Tugayé, M.-T. (1991). Time-course study of the accumulation of hydroxyproline-rich glycoproteins in root cells of susceptible and resistant tomato plants infected by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Planta*, *184*, 196–208.

Boller, T. (1987). Hydrolytic enzymes in plant disease resistance. In T. Kosuge, & E. W. Nester (Eds.) *Plant-microbe interactions. Molecular and genetic perspectives, vol 2* (pp. 385–413). New York: Macmillan.

Boyacioglu, D., & Hettiarachchy, N. S. (1995). Changes in some biochemical components of wheat grain that was infected with *Fusarium graminearum*. *Journal of Cereal Science*, *21*, 57–62.

Desjardins, A. E., & Hohn, T. M. (1997). Mycotoxins in plant pathogenesis. *Molecular Plant-Microbe Interactions*, *10*, 147–152.

Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., & Buechley, G., et al. (1996). Reduced virulence of trichothecene non-producing mutants of *Gibberella zeae* in wheat field tests. *Molecular Plant-Microbe Interactions*, *9*, 775–781.

Enkerli, K., Hahn, M. G., & Mims, C. W. (1997). Ultrastructure of compatible and incompatible interactions of soybean roots infected with the plant pathogenic oomycete *Phytophthora sojae*. *Canadian Journal of Botany*, *75*, 1493–1508.

Epple, P., Apel, K., & Bohlmann, H. (1997). Overexpression of an endogenous thionin enhances resistance of arabidopsis against *Fusarium oxysporum*. *Plant Cell*, *9*(4), 509–520.

Kang, Z., & Buchenauer, H. (1999). Immunocytochemical localization of Fusarium toxins in infected wheat spikes by *Fusarium culmorum*. *Physiological and Molecular Plant Pathology*, *55*, 275–288.

Kang, Z., & Buchenauer, H. (2000a). Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. *Mycological Research*, *104*, 1083–1093.

Kang, Z., & Buchenauer, H. (2000b). Ultrastructural and cytochemical studies on cellulose, xylan and pectin degradation in wheat spikes infected by *Fusarium culmorum*. *Journal of Phytopathology*, *148*, 263–275.

Kang, Z., & Buchenauer, H. (2000c). Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. *Physiological and Molecular Plant Pathology*, *57*, 255–268.

Kang, Z., & Buchenauer, H. (2002). Immunocytochemical localization of ß-1,3-glucanase and chitinase in *Fusarium culmorum*-infected wheat spikes. *Physiological and Molecular Plant Pathology*, *60*, 141–153.

Kang, Z., & Buchenauer, H. (2003). Immunocytochemical localization of cell wall-bound thionins and hydroxyproline-rich glycoproteins in *Fusarium culmorum*-infected wheat spikes. *Journal of Phytopathology*, *151*(3), 120–129.

Kang, Z., Huang, L., & Buchenauer, H. (2004). Ultrastructural and cytochemical studies on infection of wheat spikes by *Microdochium nivale*. *Journal of Plant Diseases and Protection*, *111*(4), 351–361.

Kang, Z., Zingen-Sell, I., & Buchenauer, H. (2005). Infection of wheat spikes by *Fusarium avenaceum* and alteration of cell wall components in the infected tissue. *European Journal of Plant Pathology*, *111*(1), 19–28.

Keen, N. T., & Yoshikawa, M. (1983). ß-1,3-Endoglucanases from soybean release elicitor-active carbohydrates from fungal cell walls. *Plant Physiology*, *71*, 460–465.

Kombrink, E., Schröder, M., & Hahlbrock, K. (1988). Several pathogenesis-related proteins in potato are 1,3-ß-glucanases and chitinases. *Proceedings of the National Academy of Sciences of the United States of America*, *85*, 782–786.

Langevin, F., Eudes, F., & Comeau, A. (2004). Effect of trichothecenes produced by *Fusarium graminearum* during Fusarium head blight development in six cereal species. *European Journal of Plant Pathology*, *110*, 735–746.

McMullen, M., Jones, R., & Gallenberg, D. (1997). Scab of wheat and barley: A reemerging disease of devastating impact. *Plant Disease*, *81*, 1340–1348.

Mesterházy, Á. (1995). Types and components of resistance against Fusarium head blight of wheat. *Plant Breeding*, *114*, 377–386.

Mesterházy, Á., Bartók, T., Kászonyi, G., Varga, M., Tóth, B., & Varga, J. (2005). Common resistance to different *Fusarium* spp. causing Fusarium head blight in wheat. *European Journal of Plant Pathology*, *112*, 267–281.

Miller, J. D., & Arnison, P. G. (1986). Degradation of deoxynivalenol by suspension cultures of the Fusarium head blight resistant wheat cultivar Frontana. *Canadian Journal of Plant Pathology*, *8*, 147–150.

Miller, J. D., & Ewen, M. A. (1997). Toxic effects of deoxynivalenol on ribosomes and tissues of the spring wheat cultivars Frontana and Casavant. *Natural Toxins*, *5*, 234–237.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals, a review. *Plant Pathology*, *44*, 207–238.

Pritsch, C., Muehlbauer, G. J., Bushnell, W. R., Somers, D. A., & Vance, C. P. (2000). Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. *Molecular Plant-Microbe Interactions*, *13*, 159–169.

Proctor, R. H., Desjardins, A. E., McCormick, S. P., Plattner, R. D., Alexander, N. J., & Brown, D. W. (2002). Genetic analysis of the role of trichothecene and fumonisin mycotoxins in the virulence of *Fusarium*. *European Journal of Plant Pathology*, *108*, 691–698.

Schroeder, H. W., & Christensen, J. J. (1963). Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. *Phytopathology*, *53*, 831–818.

Snijders, C. H. (2004). Resistance of wheat to Fusarium infection and trichothecene formation. *Toxicology Letter*, *153*, 37–46.

Snijders, C. H. A., & Krechting, C. F. (1992). Inhibition of deoxynivalenol translocation and fungal colonization in Fusarium head blight resistant wheat. *Canadian Journal Botany*, *70*, 1570–1576.

Snijders, C. H. A., & Perkowski, J. (1990). Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. *Phytopathology*, *80*, 566–570.

Usleber, E., Märtlbauer, E., Dietrich, R., & Terplan, G. (1991). Direct enzyme-linked immunosorbent assays for the detection of the 8-ketotrichothecene mycotoxins deoxynivalenol, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol, in buffer solutions. *Journal of Agricultural and Food Chemistry*, *39*, 2091–2095.

Van Ginkel, M., Van der Schaar, W., Zhuping, Y., & Rajaram, S. (1996). Inheritance of resistance to scab in two wheat cultivars from Brazil and China. *Plant Disease*, *80*, 863–867.

Wang, Y. Z., & Miller, J. D. (1988). Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to fusarium head blight resistance. *Journal of Phytopathology*, *122*, 118–125.

Wanyoike, M. W., Kang, Z., & Buchenauer, H. (2002). Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat heads. *European Journal of Plant Pathology*, *108*(1), 803–810.

Wessels, J. G. H., & Sietsma, J. H. (1981). Fungal cell walls: A survey. In W. Tanner, & F. A. Loewus (Eds.) *Plant carbohydrates II. Extracellular carbohydrates. Encyclopedia of plant physiology, new series vol. 13B* (pp. 352–395). Berlin, Germany: Springer Verlag.

Wubben, J., Joosten, M. H. A. J., Van Kan, J. A. L., & De Wit, P. J. G. M. (1992). Subcellular localization of plant chitinases and 1,3-β-gluanases in *Cladosporium fulvum* (syn. *Fulvia fulva*)-infected tomato leaves. *Physiological and Molecular Plant Pathology*, *41*, 23–32.

Zadoks, J. C., Chang, T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*, 415–421.

Zhou, W. C., Kolb, F. L., Bai, G. H., Domier, L. L., & Yao, J. B. (2002). Effect of individual Sumai 3 chromosomes on resistance to scab spread within spikes and deoxynivalenol accumulation with kernels in wheat. *Hereditas*, *137*(2), 81–89.