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Infection process and wheat response to Fusarium head blight caused by *Fusarium graminearum*

Abbas Kheiri · Sayed Ali Moosawi Jorf · Ali Malihipour

Abstract Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most important diseases of wheat in humid and warm areas. It reduces yield and seed quality significantly. Knowledge on fungal infection of wheat spikelets is essential in effective control of FHB disease. Adult plants of the cultivar FALAT and the line ERN92–4 were inoculated at anthesis stage by injection of 10μl spore suspension at concentration of 5×10^4 per ml in medial spikelets using point inoculation method. The number of infected spikelets was counted 7, 14, 21 and 28 days post inoculation (dpi). At 4, 8, 24, 48, 72, 84 and 120 h post inoculation (hpi), parts of infected spikelets were observed with light microscopy and TEM. Disease severity (percentage of infected spikelets per spike) in line ERN92–4 significantly differed from the cultivar FALAT at 14 dpi and 21 dpi. Hyphal colonization and germination of spores in line ERN92–4 spikelets were more than in FALAT spikelets. Light microscopy and TEM observations indicated that mycelium penetrated into the cells through the stoma and spread to other cells via cell wall or plasmodesmata. The fungus exhibited biotrophic and necrotrophic life styles at 24–84 and 120 hpi, respectively. Accumulation of lignin displayed a different pattern during the activation of plant defense responses, and showed brownish sites on the infected florets at 72 and 120 hpi. From 72 hpi, lignin accumulation was observed in both the examined cultivar and line, and elevated lignin accumulation reached by 120 hpi in both treatments. With the exception of the initial stages of infection of florets, defense response of none of the both genotypes was effective in the suppression of disease progress, as, *F. graminearum* is a necrotrophic fungus; however, lignin and O_2^- accumulations were increased at 4 to 72 hpi.

Abbreviations

FHB	Fusarium head blight
HR	Hypersensitive reaction
POX	Peroxidase
O_2^-	Superoxide radicals
ERWYT-N-92-4 or ERN92–4	Elite Regional Wheat Yield Trials-North-92-4
DPI	Days post inoculation
HPI	Hours post inoculation
TEM	Transmission electron microscopy
RT	Room temperature
JA	Jasmonic acid

A. Kheiri (✉) · S. A. Moosawi Jorf (✉)
Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
e-mail: abbas.kheiri@modares.ac.ir
e-mail: moosawijorf@modares.ac.ir

A. Malihipour
Cereal Research Department, Seed & Plant Improvement Institute (SPII), AREEO, Karaj, Iran

Introduction

Fusarium graminearum Schwabe (*Gibberella zeae* (Schwein.) Petch.) is the predominant species causing Fusarium head blight (FHB) in many countries (Clear and Abramson 1986; Schroeder and Christensen 1963; Sutton 1982; Wang et al. 1982; Wiese 1987). The pathogen also is associated with stalk and ear rot of corn and may cause a root rot of cereals too (McMullen et al. 1997). It is a devastating disease of wheat (*Triticum aestivum* L) and other small grains in humid and semi-humid areas worldwide. This disease can completely destroy the crop production of a potentially high-yielding crop within few weeks (McMullen et al. 1997).

The airborne spores released from the crop residues are deposited on or inside wheat florets where they germinate and initiate infection. The pathogen penetrates host tissues 36–48 hpi (Kang and Buchenauer 2000). The affected organs are anther (Pugh et al. 1933), lemma and tip of the ovary (Kang and Buchenauer 2000; Wanjiru et al. 2002), and glume and rachis (Schroeder and Christensen 1963). The hypha of *F. graminearum* invades the host tissues predominantly by direct penetration and through the stomata (Kang and Buchenauer 2000; Schroeder and Christensen 1963). Pathogen propagates into the spikelet cells and degrades them (Kang and Buchenauer 2002; Kang and Buchenauer 2000; Pugh et al. 1933; Schroeder and Christensen 1963). The fungus infects other spikelets internally through vascular bundles of the rachilla and rachis (Leonard and Bushnell 2003). Dark-brown, water-soaked spots appear on the glumes of infected florets which become entirely blighted.

Host–pathogen interactions, such as infection and colonization processes, have been studied in FHB (Gueldener et al. 2006; Jansen et al. 2005; Pritsch et al. 2001), but there is only limited available information on lignin accumulation tracing in host tissues. Most studies however relate to expression of the host defense-associated genes (Gueldener et al. 2006; Kruger et al. 2002; Pritsch et al. 2000; Pritsch et al. 2001). Resistance to FHB in wheat involves active and passive mechanisms (Mesterhazy 1995). Passive mechanisms mostly exploit the primary barriers including cell as the first barrier and its reinforcement with lignin deposition (Osbourn 2001). The local protection against pathogen invasion could also be achieved through hypersensitive reaction (HR), strengthening of invaded tissue by lignin deposition, production of phytoalexins and induction of

expression of defense related genes (Osbourn 2001). Lignin deposition could be triggered by abiotic stresses and pathogen attack (Vance et al. 1980). Besides other commonly used methods, the host defense response could also be determined by detecting and measuring of the amount of lignin accumulations in plant tissues.

Several enzymes viz. β-1,3-glucanase, chitinase, superoxide dismutase, catalase, phenylalanine ammonia lyase, peroxidase (POX), and oxidase have been related to FHB resistance in wheat (Bai and Shaner 2004). POX is involved in the synthesis of lignin and induces the reinforcement of cell walls during plant-pathogen interactions (Chittoor et al. 1999). On the other hand, POX can produce O_2^- by reduction of H_2O_2 in plant tissues (Arora et al. 2002; Hiraga et al. 2001; Mittler 2002). Thus, the amount of POX can be indirectly determined by measurement of O_2^- and lignin accumulation.

The foliar fungicide application provides little protection at anthesis (Mesterhazy et al. 2003). Moreover, resistant cultivars, soil tillage, crop rotation and removing the residues alone are not enough to keep the disease in low levels of intensity (Bai and Shaner 2004). Knowledge on fungal infection of wheat spikelets would be essential in adapting effective control measures against FHB disease.

Detailed studies directly aiming the infection process in plant tissues especially in compatible pathosystems are scarce. Thus, the aim of this research was mainly to elucidate the infection process of *F. graminearum* in spikelets of wheat cultivar and line. In addition, the host resistance response was assessed through the detection and measurement of lignin accumulation in tissues.

Materials and methods

Materials

Causal agent of FHB (*F. graminearum*) and seeds of cultivar FALAT and line ERWYT-N-92-4 or ERN92–4 were provided by the Seed and Plant Improvement Institute, Karaj, Iran. The fungus was subcultured on Potato Sucrose Agar (PSA).

Preparation of *F. graminearum* inoculum

Straw debris of wheat (2.5 g) and distilled water (120 ml) were added to 250-ml flasks and autoclaved successively for 2 days. Fungus plugs from an actively growing culture were aseptically

placed in flasks; the flasks were shaken vigorously on rotary shaker for 4–7 days at 25 °C. The culture was passed through a cheese cloth. Conidia were counted using a haemocytometer (Zeiss, Germany) and their number was adjusted to 5×10^4 per ml by gradual addition of sterile distilled water.

Greenhouse trials

The greenhouse experiments to investigate the infection progress of *F. graminearum* on FALAT and ERN92–4 were conducted at the Seed and Plant Improvement Institute, Karaj, Iran in 2015. As spikes reached 50% anthesis, they were inoculated by injecting a 10 µl droplet of conidial suspension (5×10^4 macroconidia/ml) into the floret in a spikelet positioned 1/3 from the top of the spike using a micropipette. Each treatment was comprised of 18 spikes. Distilled water was used as the control treatment. The number of infected spikelets was counted 7, 14, 21 and 28 days post inoculation (dpi).

Processing of infected spikelet tissue samples for fungus detection and inspection of lignin and O_2^- accumulation

Spikelet fragments (approximately 5–7 mm^2) having visible FHB symptoms were collected at 4, 8, 24, 48, 72, 84 and 120 hpi, placed in plastic vials containing 20 ml glutaraldehyde 4% (Merck, Germany) at pH 7–7.4 and stored for 24 h at 4 °C.

Processing of spikelet samples for light microscopic studies

The entire specimens were rinsed twice with distilled water and subsequently fixed in osmium tetraoxide 1% (Sigma Aldrich, Germany) for 1 h, washed with distilled water, dehydrated in technical grade ethanol 95% series up to 100%, and embedded in xylene/paraffin mixtures. All chemicals were sourced from Merck (Germany) except where stated. Blocks containing spikelet fragments were cut in longitudinal or cross sections (7–10 µm) using rotary microtome model RM2235 (Leica, Germany), stained with 1% safranin and 1% methylene blue for 15 and 3 mins, respectively and observed under a light microscopy (Olympus, model BX51, Germany).

The number of germinated spores on spikelet tissues and infected parts of spikelets (anther, palea, lemma, glume, rachis, first and second floret) treated with fungus was counted by light microscopy, after 8 and 4, 8, 24, 48, 72, 84 and 120 hpi, respectively.

Processing of spikelet samples for transmission electron microscopy (TEM) studies

For TEM observations, the prepared fragments were rinsed twice by 0.1 M cacodylate buffers (Sigma Aldrich, Germany) and fixed with osmium tetraoxide 1% for 1 h at 20 °C. Afterwards, they were rinsed with 0.1 M cacodylate buffer, dehydrated in a graded ethanol series for 10 mins, embedded in Epon resin 812 (Shell Chemicals, USA), cut with diamond knife (Reichert, model Omus 3, Austria), collected in Formvar coated nickel grids (Sigma Aldrich, Germany), contrasted in uranylacetate and Lead citrate mix (both from Sigma Aldrich, Germany), washed and examined using a TEM (Zeiss, model EM-900, Germany) at 60 kV.

Processing of spikelet samples for accumulation of lignin and O_2^- detection

For studying of defense responses triggered in the wheat spikelet, another trial similar to the previous trial was conducted. In order to estimate the lignin accumulation, samples were stained with safranin 1% for 15 mins and observed under light microscope and for observation of O_2^- accumulation in spikelet tissues, they were stained with safranin 1% and methylene blue 1% for 15 and 3 mins, respectively, placed at room temperature (RT) for 2 days in darkness, conserved in ethanol 95% (Adam et al. 1989; Agurell 2012; Bielski et al. 1980; Sono 1988), twice cleaned in sterilized water and observed under the light microscopy.

Estimation of vessel fungal infection area, lignification and O_2^- accumulations

Microscopic images were processed with the Digimizer v.4.5.2®(Medcalc software 2015) to determine the vessel fungal infection area, lignification and blue spotting (O_2^- accumulations) of tissues. The percentages of vessel fungal infection, lignin and O_2^- accumulations were assessed by calculating the quotient of area of vessel fungal infection and vessel, lignified tissue and tissue, blue spots and tissue.
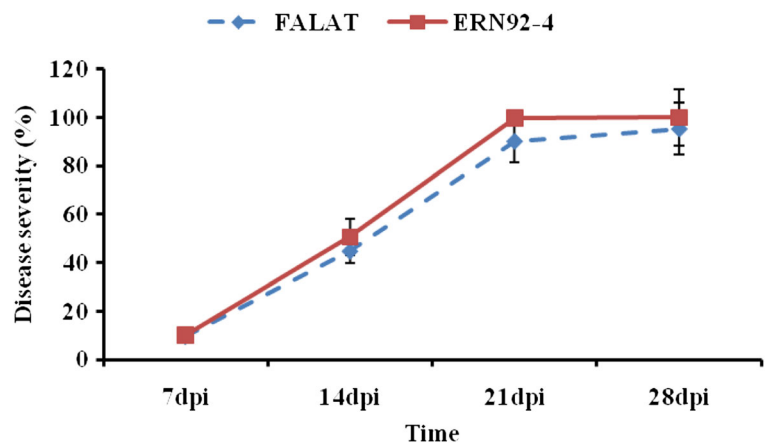
Statistical analyses

Statistical analyses were done using the MSTAT-C (Michigan University, USA) software. Results were expressed as the mean values ± standard deviation and compared by Duncan's multiple range test (DMRT). Differences at $p < 0.01$ were considered to be significant. Each treatment consisted of three replicates.

Results

Disease severity assessment

Disease symptoms such as bleached spikelets were observed on ears of the both the examined cultivar and line. These symptoms appeared on spikelets at 5 dpi. At 7 dpi, FHB severity was similar in both cultivar and line; and partially discolored ears were seen in both treatments. At 7 dpi, almost the whole rachis of the line ERN92–4 was bleached, indicating a high susceptibility to *F. graminearum*. At 21 dpi, ERN92–4 presented a high level of bleached ears. Disease severity in both cultivar and line did not change significantly from 21 dpi to 28 dpi. Disease severity in line ERN92–4 differed significantly from that in FALAT at 14 dpi and 21 dpi (Fig. 1). On the 14th and 21st dpi, the percentage of disease severity recorded for ERN92–4, and FALAT was 50.6% ± 7.5 and 99.8% ± 0.2%, and 44.9% ± 5.2 and 90.2% ± 8.7%, respectively (Fig. 1).

Detection of pathogen growth in plant tissues and cells by light microscopy and TEM

Comparison of pathogen growth in spikelet tissues of wheat cultivar FALAT and line ERN92–4

The infection progress of *F. graminearum* in ERN92–4 spikelets slightly differed from that in FALAT. At 8 hpi, more germinated spores were observed in ERN92–4 spikelets compared to that in FALAT spikelets (Figs 2 and 3). The number of germinated spores in FALAT and ERN92–4 spikelets treated with *F. graminearum* was 4 ± 2.3 and 6.5 ± 3.5, respectively (Fig. 3). The hyphal colonization in ERN92–4 spikelets was somewhat faster than that in FALAT spikelets. At 84 hpi, five parts (anther, palea, lemma, glume and rachis) of ERN92–4 spikelets were infected as well as FALAT spikelets. At 120 hpi, the number of infected parts of ERN92–4 spikelets increased faster than that in FALAT spikelets (Fig. 4). At this time point, the number of infected parts of FALAT and ERN92–4 spikelets treated with *F. graminearum* was 6 ± 1 and 6.5 ± 1, respectively (Fig. 4).

The comparison of vessels of healthy and infected rachis tissues is presented in Fig. 5. Infected rachis tissues (Fig. 5b, e), showed accumulation of methylene blue (bluish colored, arrowheads). The accumulation of blue color indicates the *F. graminearum* hyphae have infected rachis tissues. Blue color is due to pathogen absorbing methylene blue dye. The presence of *F. graminearum* hyphae in the rachis vascular tissues of ERN92–4 was more intense than those in FALAT plants (Figs. 5c, f and 6). The fungal infection percentage of vessel in FALAT and ERN92–4 rachis tissues treated with *F. graminearum*
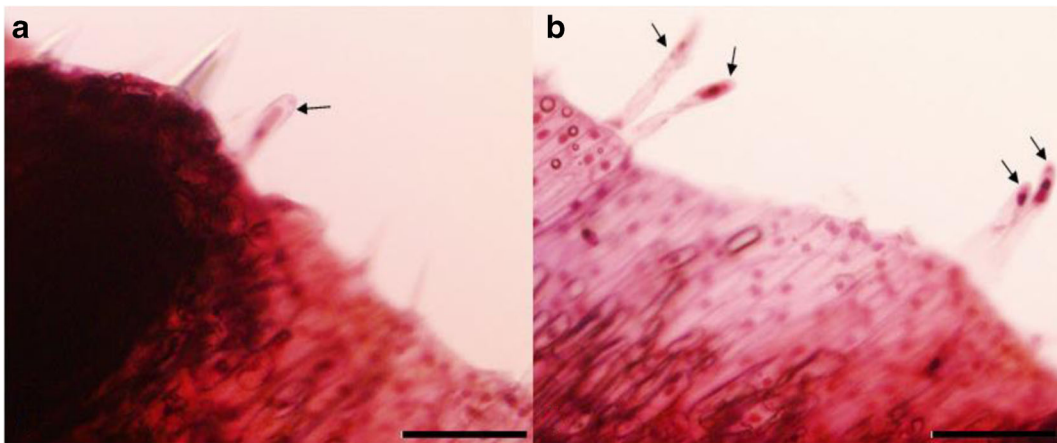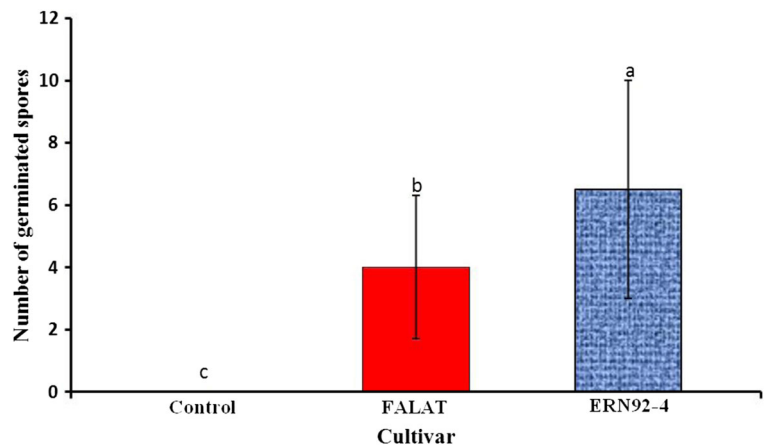
Fig. 1 The percentage of disease severity of FHB on FALAT and ERN92–4 at different times. Bar shows mean ± standard deviation

Fig. 2 Light microscopic images of germinated conidia of *F. graminearum* on the anther. **a** Anther of wheat cultivar FALAT covered with conidia and **b** Anther of wheat line ERN92–4 covered with conidia at 8 hpi. (Scale bars =100 μm)

was $19.6\% \pm 7.9$ and $31.1\% \pm 12.5\%$, respectively (Fig. 6). In the FALAT and ERN92–4 rachis tissues, some vascular tissues were completely occupied by *F. graminearum* hyphae (Fig. 5b, e). A greater amount of *F. graminearum* hyphae was observed in the vascular tissues of ERN92–4 (Fig. 5f).

Penetration and distribution of fungal hyphae in plant tissue and cells

At 24 hpi, fungal hyphae were observed to have penetrated into the parenchyma tissues via the stomata openings (Fig. 7a). At 48 hpi, hyphae grew intra- and intercellularly within spikelet tissue; however, the distribution of the fungal hyphae in the spikelet was not uniform (Fig. 7b). Also, the results indicated that mycelium spread to other cells through cell wall or plasmodesmata (Fig. 7c, d).

Spikelet cell responses to the fungal growth

Reaction of the spikelet cells before fungal penetration

At 24 hpi, no plasmolysis was observed in plant cells. There were no observed differences in arrangements of cells and organelle structure between inoculated and non-inoculated tissue. Infected cells were not plasmolysed after contact with fungus and there was no evidence of organelles alteration (Fig. 8a). At 48 hpi, the plasma membrane of plant cells smoothly invaginated around the penetration site of hypha (Fig. 8b).

Reactions during inter- and intracellular penetration

At 72 hpi, at the penetration site of the fungus into the epidermal cell the hypha was constricted (Fig. 8c) and during subsequent fungal penetration into the plant cell
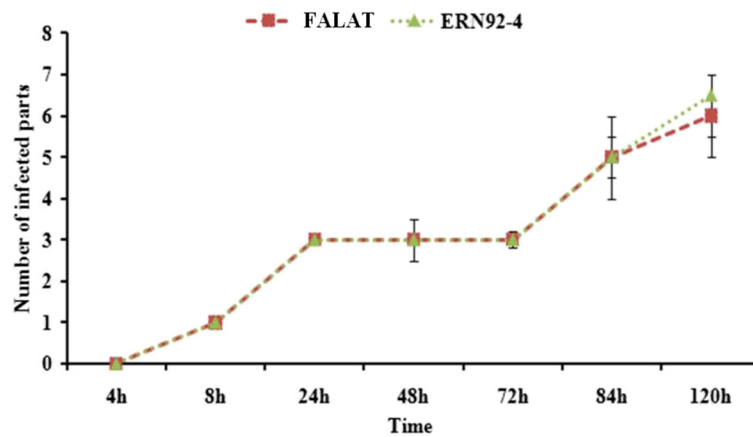
Fig. 3 The number of germinated spores of *F. graminearum* in FALAT, ERN92–4 and non-treated treatment (control) at 8 hpi. Different letters show significant levels according to Duncan's multiple range test ($p < 0.01$). Vertical bars represent the standard deviation

Fig. 4 The numbers of infected parts of FALAT and ERN92–4 spikelets inoculated with *F. graminearum* at different times (hpi). 1:Anther, 2:Anther, palea, 3:Anther, palea, lemma, 4: Anther, palea, lemma, glume, 5: Anther, palea, lemma, glume, rachis, 6: Anther, palea, lemma, glume, rachis, second floret, 7: Anther, palea, lemma, glume, rachis, first and second floret. Bar shows mean ± standard deviation
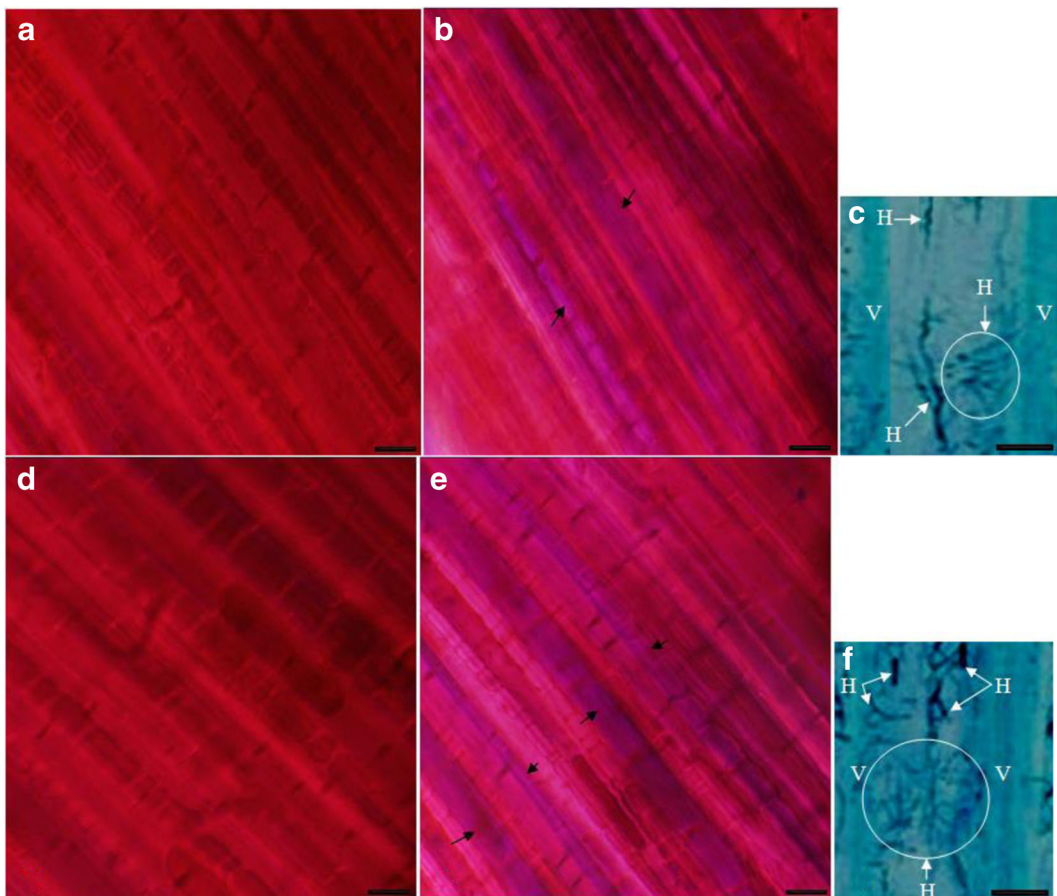

Fig. 5 Photomicrographs of rachis tissues infected with *F. graminearum*. **a-c** Rachis vascular tissues of wheat cultivar FALAT. a. Control. b. Infected rachis tissues with *F. graminearum* have been marked as blue color. c. Covered with fungal hyphae. d-f. Rachis vascular tissues of wheat line ERN92–4. **d** Control. e. Infected rachis tissues with *F. graminearum* have been marked as blue color. f. Covered with fungal hyphae. (Scale bars =10 μm) V = Vessel, H = Hyphae
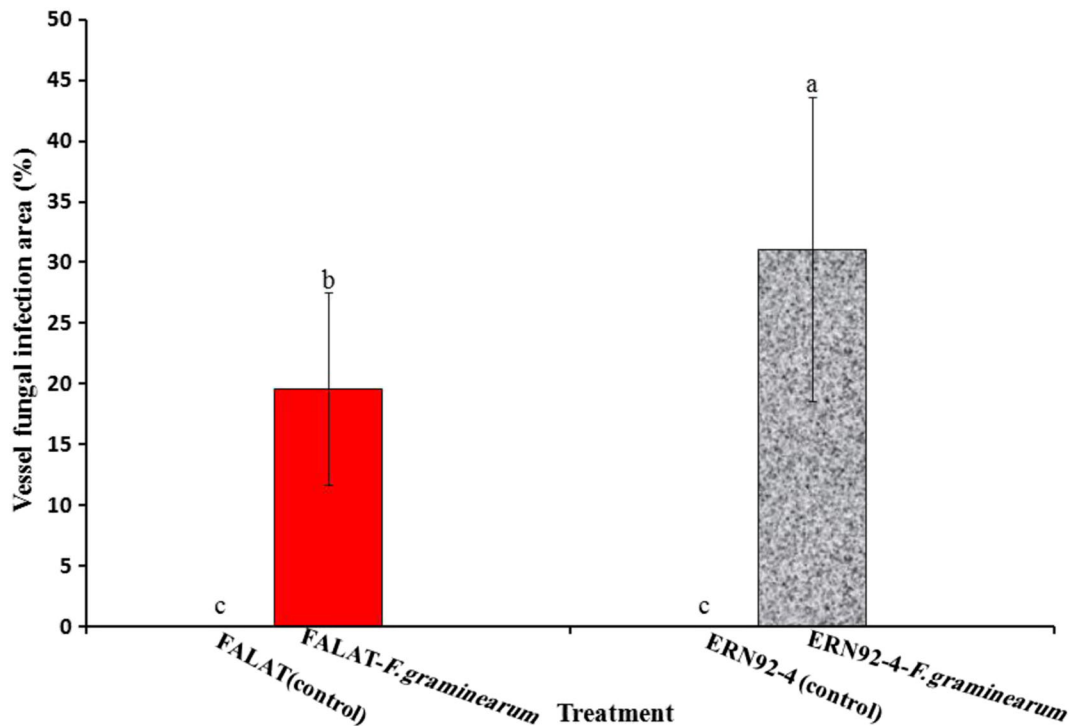
Fig. 6 The percentage of vessel fungal infection area in FALAT and ERN92–4 rachis tissues inoculated with *F. graminearum* and non-inoculated (control). Different letters show significant levels according to Duncan's multiple range test ($p < 0.01$). Vertical bars represent the standard deviation

the invaginated plant plasma membrane surrounded the hypha evenly (Fig. 8e). Neither plant cell nor fungal cell showed any sign of degeneration (Fig. 8d, e). At 84 hpi, fungal hyphae extensively colonized the cells both vertically and horizontally and no organelles of plant cell showed degeneration symptoms (Fig. 8f, g). At 120 hpi, fungal hyphae expanded in the cells and organelles of plant cell started to degenerate (Fig. 8h). The death of plant cells was associated with increased fungal colonization at 120 hpi (Fig. 8h).

Defense response of spikelet tissues

Detection of lignin accumulation using safranin staining

The microscopic observation of spikelet tissue stained with 1% safranin revealed a lignin accumulation on the tissue especially around the site of infection. The presence of lignin accumulation was detected by brown spots, these brown marks were connected together occasionally (Fig. 9). Accumulation of lignin displayed a different pattern during the activation of plant defense

systems. From 72 hpi, the lignin accumulation was shown in both cultivar and line, and the elevated lignin accumulation appeared at 120 hpi in both treatments (Fig. 10). At120 hpi, the percentage of lignin accumulation in FALAT and ERN92–4 tissues treated with *F. graminearum* was $12.8\% \pm 3.7$ and $16.5\% \pm 6.8\%$, respectively (Fig. 10). At 72 and 120 hpi, the degree of tissue lignification in FALAT and ERN92–4 tissues treated with *F. graminearum* was higher than it in uninoculated ERN92–4 and FALAT, respectively (Fig. 10).

Detection of O_2^- accumulations using methylene blue 1% staining

O_2^- accumulations in the palea tissue were found as blue spotting in the compatible interaction of FALAT-*F. graminearum* (Fig. 11). From 4 hpi, O_2^- accumulation was observed in wheat cultivar FALAT, and the elevated O_2^- accumulation was traced at 72 hpi (Fig. 12). From 4 to 72 hpi, lignin and O_2^- accumulations were increased (Fig. 12). At 72 hpi, the percentage of O_2^- and lignin accumulation in *F. graminearum* treated tissues was $25.2\% \pm 12.2$ and $5.8\% \pm 2.1\%$, respectively (Fig. 12).
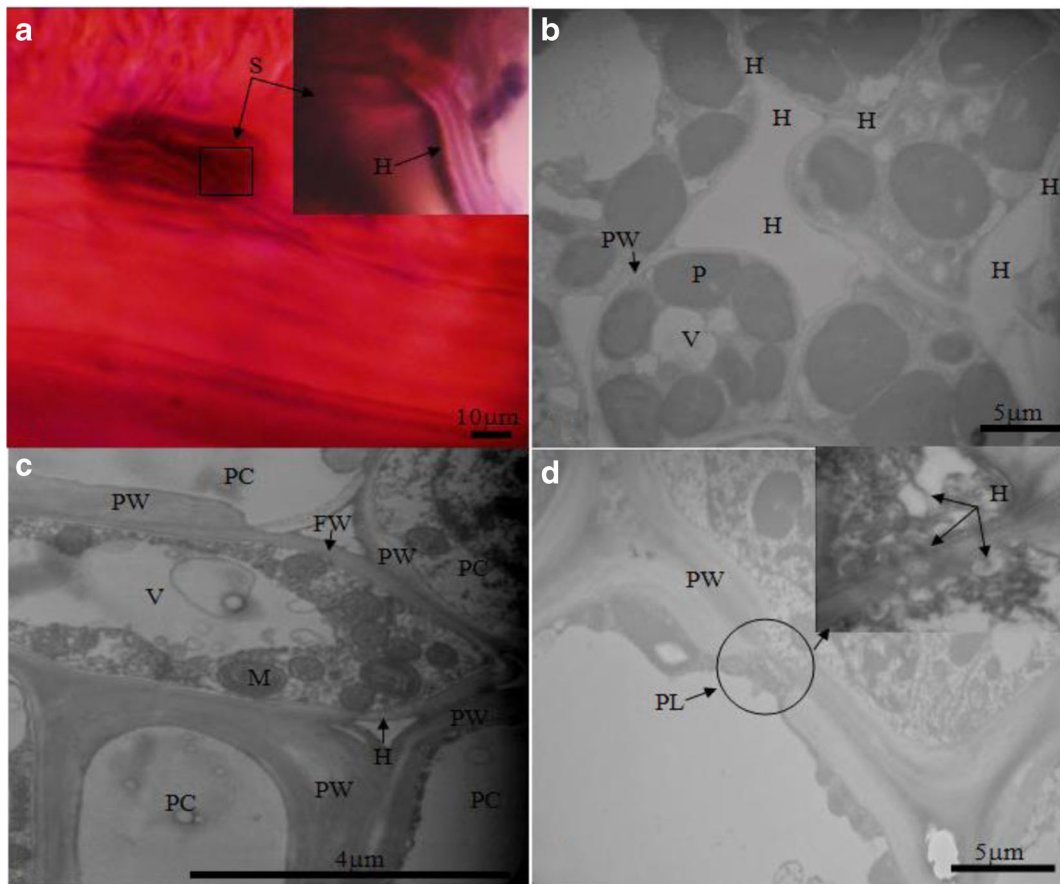
Fig. 7 TEM and light micrographs of *F. graminearum* mycelial growth and penetration into the cells. **a** Penetration of fungus through stoma observed at 24 hpi. **b** Intra- and intercellular growing of hyphae. **c-d** Penetration of fungal hyphae into cells via pits in the cell wall (c) and plasmodesmata (d). H = Hypha, S = Stoma, p = Chloroplast, V = Vacoule, PW = Plant cell wall, M = Mitochondria, PC = Plant cell, FW = Fungal cell wall, PL = Plasmodesmata

Discussion

Genetic resistance mechanisms against pathogens in plants are categorized either as monogenic or polygenic (Agrios 2005). Wheat cultivar FALAT and the line ERN92–4 were susceptible to *F. graminearum*. Generally, this may result by the R genes from cultivar FALAT and the line ERN92–4 lose the target in *F. graminearum* that cause the compatible interaction. Moreover, *F. graminearum* spores easily germinate on the anther of the line ERN92–4. Presumably, some substances in the anther can provide nutrition or stimulate the germination. Strange et al. (Strange et al. 1974) reported presence of two major components (choline chloride and betaine hydrochloride) in anthers and wheat germ that could stimulate *Fusarium* growth in vitro. Using a strain of *F. graminearum* Miller et al. (Miller et al. 2004) inoculated resistant and susceptible wheat cultivars, and

observed hyphae of the pathogen inside the floret at the point of inoculation with a particular affinity to the pollen and anthers of both cultivars. In this study, the more rapid and intense symptoms on the ear and more intense progress of *F. graminearum* in rachis of ERN92–4 in comparison to FALAT, indicated that ERN92–4 has very little resistance to the invasion by this pathogen. In general, ERN92–4 lacks effective resistance to *F. graminearum*, both passively and actively.

Fig. 8 The infection process of *F. graminearum* in the spikelet ▶ cells. **a** No plasmolysis is observed in plant cells. **b** Invagination of plasma membrane of plant cell around the penetration site of hypha **c** Constriction of the hypha at the penetration site. **d-e** The hypha is embedded within the host apoplast. Cells of neither organism show any sign of degeneration. **f-g** View of the infected plant cell. **h** Clonization of plant cells by fungal hyphae. H = Hypha, M = Mitochondria, p = Chloroplast, V = Vacuole, PW = Plant cell wall, PM = Plant plasma membrane, FW = Fungal cell wall, N = Nucleus, NC = Nucleolus, CD = Cell death
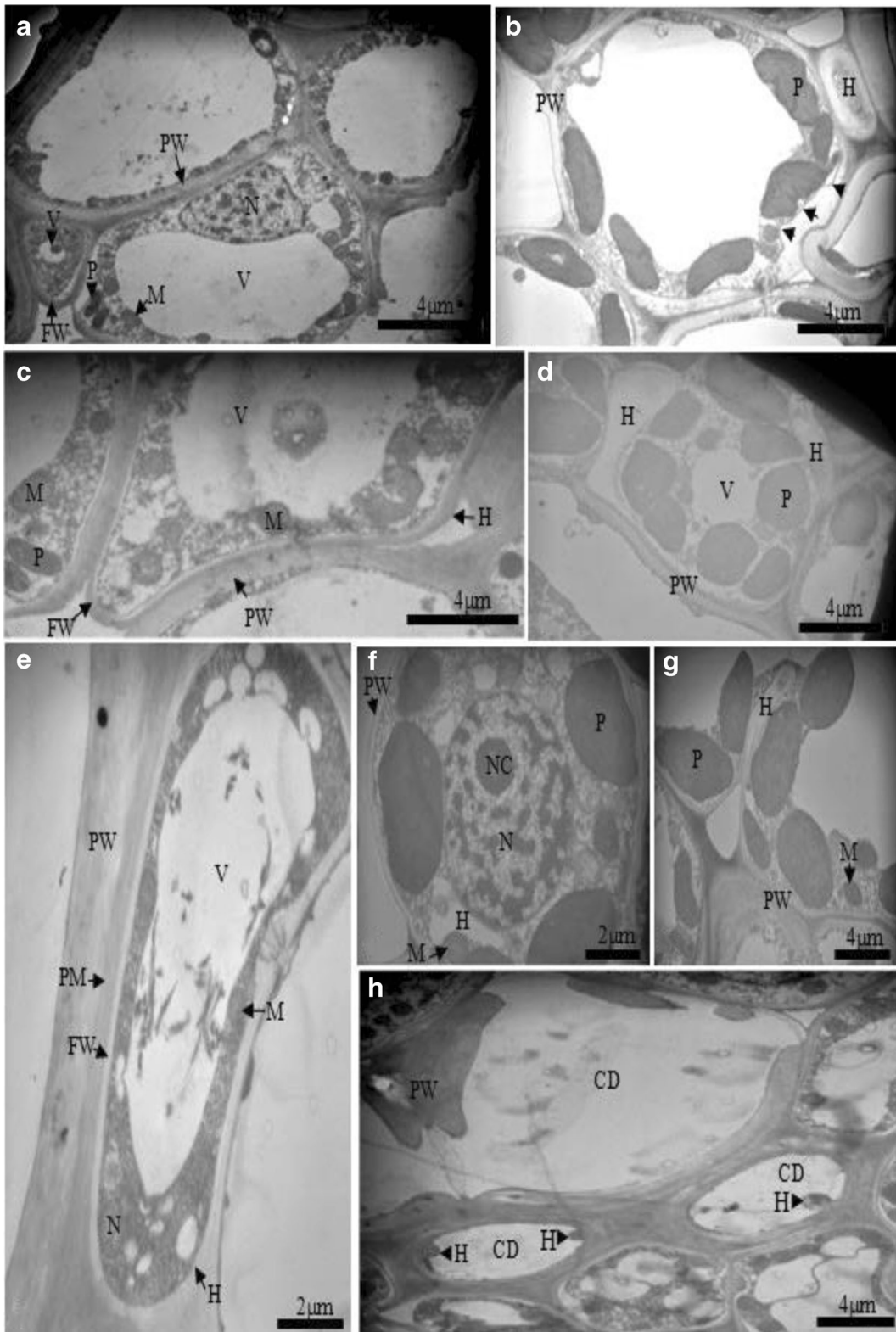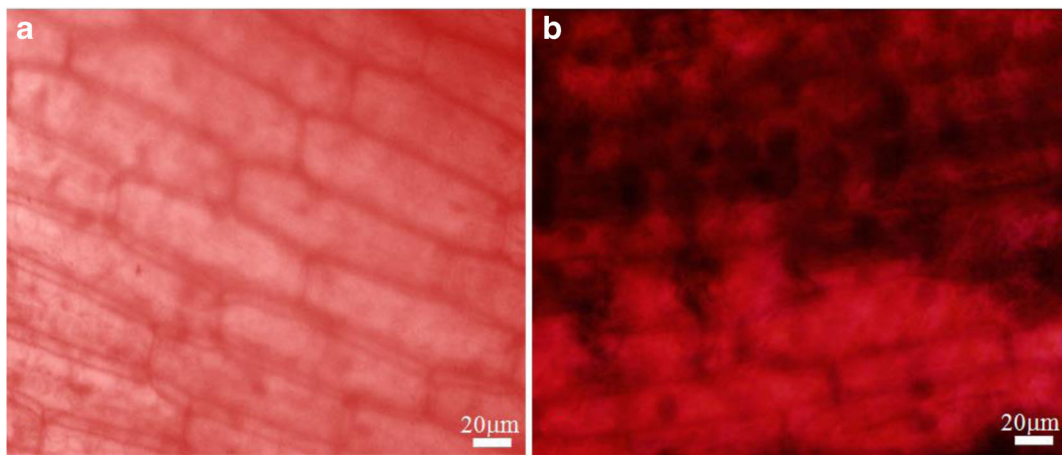
Fig. 9 Microscopic views of lignin accumulations on the spikelet tissues of wheat cultivar FALAT. **a** Non-inoculated spikelet of FALAT stained with safranin. **b** Lignin traces on the spikelet from FALAT-*F. graminearum* interaction

Furthermore, this susceptibility has been associated with physical features like the long and thin ear, noticeable space between spikelets, plant height or thickness of the epidermis. These physical traits increase the possibility of *F. graminearum* invasion and spread.

Although Najaphy et al. (Najaphy et al. 2006) considered the cultivar FALAT as susceptible to FHB, earlier studies indicate the spikelet traits place the cultivar in type II resistance grouping of Schroeder and Christensen (Schroeder and Christensen 1963). Furthermore, this type of resistance could be associated with passive resistance.

Adjusting anthesis time could also help the plant to escape from pathogen. This implies the postponed anthesis helps plant to avoid massive spore or conidia invasion in comparison to ERN92–4.

The rachilla connects spikelet to rachis and stem. It plays a key role in the systemic pathogen progress in plant. The vessels inside the rachis are linked to those in the rachilla and stem, which form a path for the fungal spread into the plant. A great amount of hyphae of *F. graminearum* was observed in the xylem vessels of rachis of both FALAT and especially ERN92–4. Studies
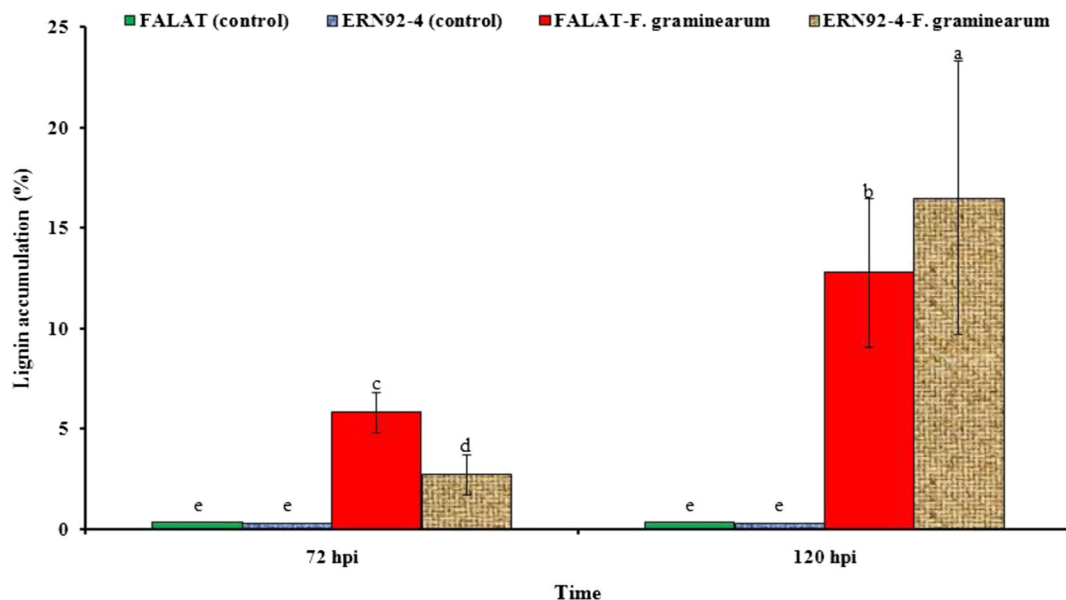

Fig. 10 The percentage of lignin accumulation in FALAT and ERN92–4 spikelet tissues inoculated with *F. graminearum* and non-inoculated (control) at different times (hpi). Different letters

show significant levels according to Duncan's multiple range test ($p < 0.01$). Vertical bars represent the standard deviation
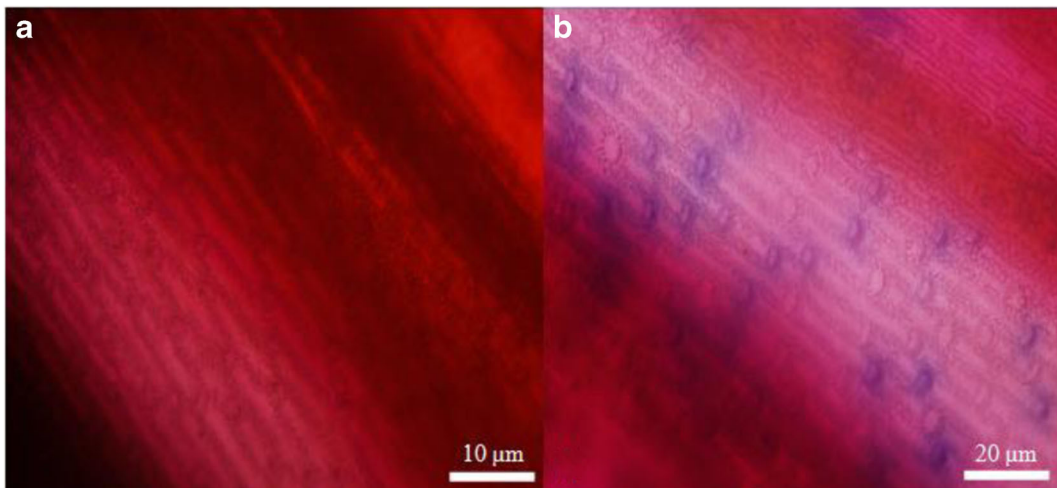
Fig. 11 Microscopic views of O_2^- accumulations on the palea of wheat cultivar FALAT. **a** Non-inoculated palea of FALAT stained with methylene blue. **b** O_2^- traces on the palea from FALAT-*F. graminearum* interaction

have shown that the host resistance to vascular pathogens may be linked to the thickening of the cell walls and production of the fungal toxins. Hall et al. (Hall et al. 2011) reported that thickness of the xylem cell walls of a cotton cultivar with moderate resistance to *F. oxysporum* f. sp. *vasinfectum*, is more than that of susceptible cultivars. Bishop and Cooper (Bishop and

Cooper 1983) reported that infection of susceptible tomato cultivars by *F. oxysporum* f. sp. *lycopersici* and of susceptible pea cultivars by *F. oxysporum* f. sp. *pisi* is favored after degradation of the xylem cell walls by fungal hypha. Jansen et al. (Jansen et al. 2005) also reported that deoxynivalenol (DON) toxin acts as a virulence factor and causes tissue necrosis in wheat,
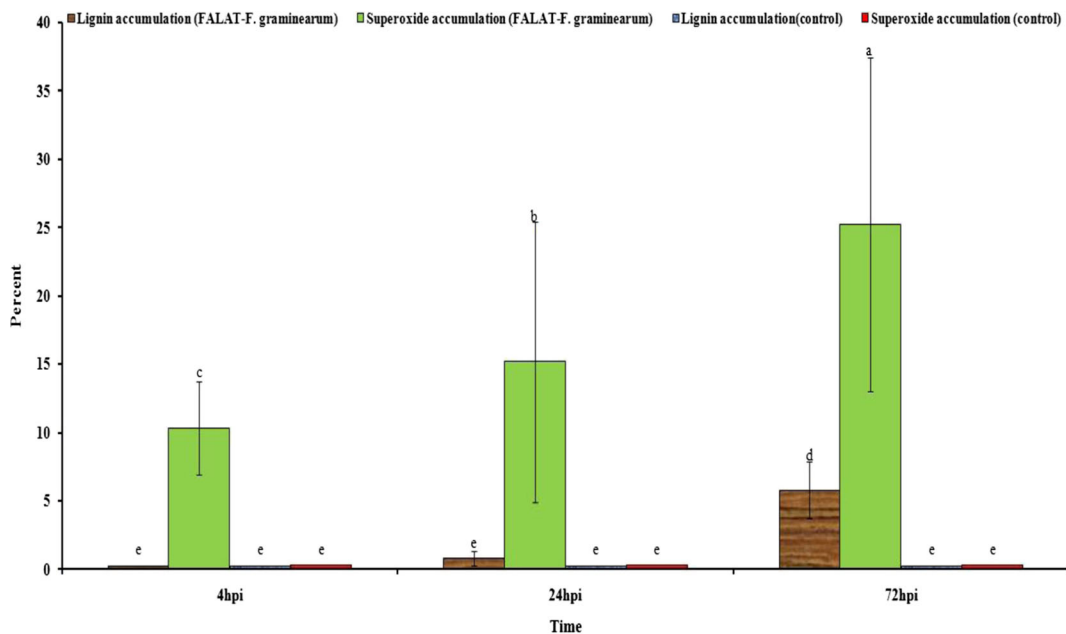

Fig. 12 The percentage of blue spots (O_2^- accumulation indicator) and lignin accumulation in FALAT spikelet tissues inoculated with *F. graminearum* and non- inoculated (control) at different times (hpi). Different letters show significant levels according to Duncan's multiple range test ($p < 0.01$). Vertical bars represent the standard deviation

while Maier et al. (Maier et al. 2006) showed that development of the fungus in barley tissues was limited by the lack of toxin.

Microscopic study of the *F. graminearum* infection process in spikelet cells did not show necrotrophic lifestyle at the initial stages of infection which was therefore, symptomless (Brown et al. 2010). The plant plasma membrane surrounded fungal cell wall and showed no evidence for degradation during intracellular growth of *Fusarium* hyphae in the initial stages of infection. One strategy of some fungi to successfully establish and spread in host tissues is to live epiphytically without causing symptoms (a non-parasitic lifestyle) (Clement and Parry 1998). Once the host cell death is initiated, this was found to be accompanied by intracellular colonization and necrotrophic feeding of dead host cells. The necrotrophic stage is generally associated with an increased fungal colonization, subsequent cell death and necrosis. Thus, *F. graminearum* can be classified as a hemibiotrophic pathogen, but with a remark that *F. graminearum* probably displays a unique lifestyle which is different from other described hemibiotrophic lifestyles (Kazan et al. 2012).

Lignin plays an important role in host plant resistance against pathogen attack (Bhuiyan et al. 2009). Lignification is an important mechanism in the defense response. Lignification in the cell wall inhibits the development of pathogens by several mechanisms: First, increasing the mechanical resistance of the host plant cell wall, Second, reducing the susceptibility of cell wall to extracellular enzymes, Third, limiting the diffusion of pathogenic compounds and the fourth, inhibiting the growth of pathogens by the presence of lignin precursors in the form of phenolic compounds which are toxic to pathogens (Kuc 1983).

The process of lignin formation is closely related to the POX activity (Hasegawa et al. 2005). POX is one of the enzymes associated with the lignin biosynthesis and high POX activity triggers the formation of lignin. The older the plant, the harder the spikelet and the more the lignin synthesis will be available. During anthesis, this condition led to the increasing of defense response of plant against pathogen attack. *F. graminearum* has a biotrophic and necrotrophic lifestyle during the initial and later stages of infection of florets, respectively. In contrast to the initial stages of infection of florets, the increasing of lignin leads to development and growth of pathogen in during later infection stages in which the pathogen systemically colonizes infected tissues and/or spreads into non-infected floret parts.

The plant defense response as lignin as well as O_2^- accumulations increased in the interaction FALAT-*F. graminearum* in course of time. This implies that O_2^- induces cell death and prevents pathogen growth in the initial stages of infection. O_2^- is considered to be a cell death-inducing signal in some plant-pathogen interactions which is related to the production of jasmonic acid (JA). JA is a signal compound which may induce O_2^- under stress and pathogen development (Overmyer et al. 2003; Turner et al. 2002). Thus, the increasing of O_2^- accumulation which occurred in the interaction FALAT-*F. graminearum* may be due to serious cell death and induce pathogen growth in the later stages of infection same as lignin does.

Conclusion

In this study, FHB disease assessment was made at 7, 14, 21 and 28 dpi. The results indicated that the best recording time of disease severity was at 21 dpi. This study also suggested that wheat line ERN92–4 had higher percentage disease severity compared to FALAT. As the result, the line ERN92–4 is recommended as a sensitive or susceptible check when evaluating wheat lines and cultivars for their tolerance to FHB disease or when checking fungicides for FHB. It was however concluded that the spread of *F. graminearum* in the spikelet was effectively influenced by the type of interaction.

F. graminearum exhibits a biotrophic lifestyle during the initial stages of infection of florets. During biotrophic colonization, no specialised feeding structures were observed within living host cells. Therefore, it is assumed that *F. graminearum* hyphae initially absorb nutrients from extracellular exudates.

HR was present in spikelet at 72hpi and, characterized by lignin accumulation as a defense response of wheat spikelet to *F. graminearum* infection. With the exception of the initial stages of infection of florets, defense response of cultivar FALAT and line ERN92–4 was not effective against the pathogen growth, since *F. graminearum* is a necrotrophic fungus.

Ultramicrotomy, TEM and *F. graminearum* and seeds of cultivar FALAT and line ERN92-4, respectively.

Compliance with ethical standards

Conflict of interest All authors are fully aware of this submission and have declared that no competing interests exist.

Human and animal participants This study did not involve any human and/or animal participants.

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