SCANNING ELECTRON MICROSCOPY OF FUSARIAUM CULMORUM-INFECTED KERNELS OF ANCIENT WHEAT SPECIES

D. Packa, T. Kulik and M. Hośćik

Abstract

Kernels of ancient hulled wheat species, Triticum monococcum, T. dicoccon, T. spelta, and threshable wheat species, T. polonicum, T. turanicum ('Kamut®'), were studied. Fusarium culmorum-infected kernels, classified as FDKs (Fusarium damaged kernels), were smaller, shrunken, wrinkled and lighter in colour than healthy kernels. The seed coats of the former were usually damaged, and white or pinkish-white mycelium formed on their surface. FDKs showed different fungal infection levels. Characteristic structural changes were noted in endosperm cells, including loosely arranged starch granules, a partial or even complete absence of the protein matrix enveloping starch granules, the disappearance of small starch granules and the presence of damaged large starch granules. The lowest degree of damage was observed in emmer and spelt kernels.

Key words: Fusarium culmorum, Triticum spp., infected kernels, SEM

Introduction

The ancient forms of wheat provide valuable breeding material due to their high quality and resistance traits. Under natural infection conditions, they are infected by Fusarium pathogens to a lower degree than common wheat, and smaller amounts of toxins accumulate in their grain (Suchowilska et al. 2010, Wiwart et al. 2011). Following artificial inoculation, symptoms typical of Fusarium infection can be observed on spikelets and kernels. The infection and colonization of host plants by necrotrophic pathogens of the genus Fusarium involves the production of extracellular hydrolytic enzymes and phytotoxic compounds. Fusarium culmorum, one of the causal agents of Fusarium head blight (FHB) in north-eastern Poland, is
capable of producing cell wall degrading enzymes—cellulases, xylanases and pectinases, as well as proteases and amylases that break down storage compounds in the endosperm of infected wheat kernels (Kang and Buchenauer 2000). The consequent damage to the endosperm may be observed using a scanning electron microscope (SEM). Damage to common wheat kernels, in particular endosperm cells, caused by *F. culmorum* is well documented in literature (Meyer et al. 1986, Jackowiak et al. 2005). A similar type of kernel damage occurs also in the endosperms of barley and wheat infected by *F. graminearum* (Nightingale et al. 1999, Schwarz 2003) and winter triticale infected by *F. culmorum* (Packa et al. 2008). A different type of damage is observed in oat endosperm cells which are composed of compound starch granules (Packa 2005). Having collected extensive experimental materials, we launched a study to investigate and document damage to the kernels of ancient hulled wheats—*T. monococcum*, *T. dicoccon*, *T. spelta* and naked, threshable wheats—*T. polonicum*, *T. turanicum* (‘Kamut®’), resulting from *F. culmorum* infection.

**Materials and methods**

The experimental plant materials comprised three spring hulled wheat species: *Triticum monococcum* (2n = 14), *T. dicoccon* (2n = 28) and *T. spelta* (2n = 42), and two spring threshable wheat species: *T. polonicum* (2n = 28) and *T. turanicum* (2n = 28). The hulled wheats *T. monococcum* (einkorn), *T. dicoccon* (emmer), *T. spelta* (spelt) and the threshable wheat *T. polonicum* (Polish wheat species) were represented by three accessions each. *Triticum turanicum* was represented by one cultivar, ‘Kamut®’ (King Tut’s wheat). The common wheat *T. aestivum* (2n = 42) was represented by two cultivars, ‘Parabola’ (group A, quality wheat) and ‘Frontana’ (used as a Fusarium head blight resistance source). The studied wheat species and accessions are presented in Table 1. Kernel samples were collected from a field experiment conducted at the Experimental Station of the University of Warmia and Mazury in Balcyny near Ostróda (53°36’N, 19°51’E). Single spikelets used as sowing materials were sown manually at 10 × 20 cm in plots of 6 m². No chemical plant protection was applied. The infection material was prepared from DON producing *F. culmorum* isolate originating from the author’s own collection. The *F. culmorum* isolate was grown at 22°C on PDA medium for 14 days. Inoculation was carried out by spraying an aqueous spore suspension of 5 × 10⁵ per 1 ml onto the heads at the full flowering stage (65 BBCH). Inoculation of all tested accessions was carried out from 28 June to 10 July 2008. The weather conditions during flowering, ripening and harvest of spring wheat in the growing season 2008 are shown in Figure 1. Inoculation was performed before the evening, twice within two consecutive days using knapsack sprayer with 2 × 600 per 1 ml of spore suspension on each plot. Non-inoculated plants served as control. The presence of *F. culmorum* in infected kernels was confirmed with the use of species-specific molecular markers (Kulik and Hościk, unpublished data). FDKs and control kernels showing no symptoms of infection were examined under a scanning microscope. The
Table 1

<table>
<thead>
<tr>
<th>Accession</th>
<th>Species</th>
<th>Accession No.</th>
<th>Origin</th>
</tr>
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<tr>
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<td>NCPGR</td>
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<td>K-15</td>
<td>Triticum monococcum</td>
<td>PI 584654</td>
<td>NGRL</td>
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<td>‘Terzino’</td>
<td>Triticum monococcum</td>
<td></td>
<td></td>
</tr>
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<td>Triticum dicoccon</td>
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</tr>
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<td>PL 20770</td>
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</tr>
<tr>
<td>‘Kamut®’</td>
<td>Triticum turanicum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Parabola’</td>
<td>Triticum aestivum</td>
<td>Cultivar</td>
<td>PBC</td>
</tr>
<tr>
<td>‘Frontana’</td>
<td>Triticum aestivum</td>
<td>Cultivar</td>
<td>PBAI</td>
</tr>
</tbody>
</table>

NCPGR – National Centre for Plant Genetic Resources (Radzików, Poland), NGRL – National Germplasm Resources Laboratory (Beltsville, USA), IPK – The Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany), PBC – Plant Breeding Company (Strzelce, Poland), PBAI – Plant Breeding and Acclimatization Institute (Radzików, Poland).

Fig. 1. Weather conditions during flowering, ripening and harvest of spring wheat in the growing season 2008 in Balcyny
cross-sections of kernels were analysed to determine the most common locations of mycelium (at the base and along the crease, in the seed coat, between the seed coat and the endosperm) and endosperm microstructure (Phot. 1).

Results

Fusarium culmorum-infected kernels, classified as FDKs (Fusarium damaged kernels), were smaller, shrunken, wrinkled and lighter in color than healthy kernels. The seed coats of the former were usually damaged, and white or pinkish-white mycelium formed on their surface (Photos. 2 and 3). The kernels of all studied accessions from the FDK fraction had deformed structure (Photos. 4 and 5). The F. culmorum mycelium was most frequently found at the base and along the crease, in the seed coat, between the seed coat and the endosperm (Phot. 6). FDKs showed different fungal infection levels, from the colonization of the seed coat to the invasion of endosperm cells. Characteristic structural changes were noted in endosperm cells, including loosely arranged starch granules, a partial or even complete absence of the protein matrix enveloping starch granules, the disappearance of
small starch granules and the presence of damaged large starch granules (Phot. 7 and 8). The lowest degree of damage was observed in emmer and spelt kernels.

Discussion

In cereal crops, FHB (Fusarium head blight) develops as a result of flower infection by pathogens of the genus *Fusarium*. The spikes in the flowering stage are at the highest risk of infection, but infection also occurs in the later stages of kernel development. Infections that occur during the flowering stage are most dangerous
to plants (Parry et al. 1995). Microscopic examinations of different cereal species supported the identification of *Fusarium* hyphae on the surface and inside kernels. In the FDKs of the studied wheats, hyphae were found over the entire surface of kernels, inside the seed coat, between the seed coat and the endosperm, and in the inner endosperm layers of severely infected kernels. Similar locations of *Fusarium* hyphae have been reported for other cereal species, including winter wheat (Srobar and Srobarova 1978, Srobarova 1987, Chelkowski et al. 1990), spring wheat (Jackowiak et al. 2005), winter triticale (Koczowska and Packa 1993, Packa et al. 2008) and oat (Packa 2005). SEM enabled to visualize endosperm ultrastructure in ancient wheat kernels which accumulate larger amounts of protein than common
wheat kernels. Large and small starch granules were enveloped in a protein matrix. In healthy wheat kernels, starch granules were densely packed in cells. Infected kernels were characterized by loosely arranged starch granules, a partial or even complete absence of the protein matrix, and a decrease in the number of small granules. Damage to large starch granules was also observed, in the form of fun-

nel-like holes located in the equatorial plane and in lateral surfaces. It is believed that the degree of endosperm damage reflects the rate of fungal infection. The type of damage noted in the analysed wheat species was the same as that reported for

common spring wheat and winter triticale, infected by *F. culmorum* (Jackowiak et al. 2005, Packa et al. 2008). The microscopic image of endosperm damage confirms that *F. culmorum* is able to produce extracellular enzymes which facilitate the colo-

Phot. 6. Common locations of *Fusarium* mycelium in ancient wheat kernels.
A, B – hyphae in the crease and under the seed coat (A – cv. ‘Kamut®’, B – Pol-4),
C, D – hyphae at the base of the crease (C – cv. ‘Terzino’, D – K-30), E, F – a thick layer of hyphae between the seed coat and the endosperm, damaged aleurone layer (E – Pol-4, F – cv. ‘Kamut®’);
h – hyphae, e – endosperm, sc – seed coat. SEM (photo by D. Packa)
nization of host plants. Our findings regarding α-amylase activity in the kernels of common wheat and ancient wheats infected by F. culmorum were presented at the 11th European *Fusarium* Seminar (Hošcik et al. 2010).

**Streszczenie**

BADANIA Z UŻYCIEM SKANINGOWEGO MIKROSKOPU ELEKTRONOWEGO ZIARNIKÓW DAWNYCH PSZENIC PORAŻONYCH PRZEZ *FUSARIUM CULMORUM*

Badano ziarniaki dawnych pszenic oplewionych: *Triticum monococcum*, *T. dicocccon*, *T. spelta* i wymłacalnych: *T. polonicum*, *T. turanicum* (‘Kamut®’). Ziarniaki porażone przez *Fusarium culmorum* kwalifikowane jako FDK (*Fusarium damaged kernels*)

były mniejsze, drobniejsze, pomarszczone, jaśniejszej barwy niż ziarniaki zdrowe, zwykle z uszkodzoną okrywą owocowo-nasienną i białą lub biało-różową grzybnią na powierzchni. Poszczególne ziarniaki w obrębie frakcji FDK wykazywały zróżnicowany stopień inwazji grzybowej. W komórkach bielma obserwowano charakterystyczne zmiany strukturalne, jak: luźny układ ziaren skrobiowych, częściowy lub całkowity brak białkowej matrycy spajającej ziarna skrobi, zanik małych ziaren skrobi oraz uszkodzenia dużych ziaren skrobi. Najmniejsze uszkodzenia komórek bielma obserwowano w ziarniakach pszenicy płaskurki i orkiszu.

**Literature**


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