Papillar Resistance against Powdery Mildew Fungus in *Triticum* sp.

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Abstract: Race-nonspecific resistance to powdery mildew, often referred to as partial resistance, is characterised as a type of interaction between the host plant and pathogen in which plants show less disease severity compared with the susceptible control cultivar, despite compatibility or susceptible infection types. In our study, race-nonspecific resistance was evaluated in eight tetraploid *Triticum turgidum* ssp. *dicoccum* and two hexaploid *T. aestivum* wheats against powdery mildew (*Blumeria graminis* f. sp. *tritici*) in the laboratory and field conditions. Based on the comparison of the values of prehaustorial resistance in the supersensitive hexaploid control cultivar Ai-bian1 and in tetraploid wheat genotypes CGN 11486 and TRI 6158, we can characterize these two *dicoccum* samples as sensitive. Papillar resistance constitutes only a low portion at the whole resistance in these two genotypes. In the six tetraploid wheat genotypes the level of non-specific resistance was higher than in control resistant cultivar Amigo. Within three genotypes, resistance was determined to a relatively large extent by papillae formation. Resistance in the other three genotypes was ensured by other prehaustorial defense mechanisms. Disease severity estimated in the laboratory conditions was similar to the estimates obtained under field conditions.

Keywords: powdery mildew; race-nonspecific resistance; papillae; penetration resistance; *Triticum aestivum; Triticum turgidum*

Powdery mildew is one of the most consistently damaging diseases of the wheat in Europe. It is caused by the obligate biotrophic fungus *Blumeria graminis* DC. f. sp. *tritici* Speer. Consequently, much effort is devoted to the exploitation of genetic plant resistance for disease control. Most of the catalogued powdery mildew resistance genes (McIntosh *et al*. 2003) confer race-specific hypersensitive responses that remain effective for a short period of time, but may then become ineffective as races with virulence to the resistance genes develop in the pathogen population. In view of the frequent erosion of race-specific genes, race-nonspecific resistance can bring a more satisfactory answer to disease control problems. This type of resistance is characterized as partial, more or less effective against all races of pathogen and result in varying levels of resistance against them (Singh & Rajaram 2002).

In numerous studies of cereals attacked by powdery mildew, resistance has often been intimately associated with the occurrence of cell-wall appositions directly subtending the appressorium and penetration peg of the fungus (e.g. Belanger *et al*. 2002; Prats *et al*. 2005; Fric & Tamás 1993). Although the chemical composition of these appositions, termed papillae, shows considerable variability (Aist 1976), they commonly contain callose, lignin and phenolic substances (Nicholson & Hammerschmidt 1992). Other constituents found to accumulate in papillae are cellulose, pectin, suberin,

chitin, silicon, lipids and proteins usually found in cell walls, such as hydroxyprolin-rich proteins or peroxidases (reviewed by Schmelzer 2002). In addition, the essential role of reactive oxygen species (ROS) by several authors (e.g. Thordal-Christensen *et al*. 1997; Hückelhoven *et al*. 1999) has been described. They suggest that ROS may be involved in cross-linking reactions leading to papilla hardening. Generally, effectiveness of papillar resistance is related to the structure and chemical composition of papillae, multiplicity of their incidence, and timeliness of their formation.

Papilla formation around the penetration sites seems to be a general resistance in early defense reactions in both race-specific and race-nonspecific resistance. Papilla-based resistance in mlo barley cultivars that confers a durable race-nonspecific resistance against powdery mildew has been particularly well-documented (e.g. Jørgensen 1992; Büschges *et al*. 1997; Lyngkjær *et al*. 2000; Gálová *et al*. 2000).

The aim of present study was to evaluate race-nonspecific resistance in ten tetraploid *Triticum turgidum* ssp. *dicoccum* and in two hexaploid *T. aestivum* wheats against powdery mildew (*Blumeria graminis* f. sp. *tritici*) in the laboratory and field conditions. In addition, the importance of papillar resistance relative to the total resistance was evaluated.

MATERIAL AND METHODS

Plant and fungal material

Two hexaploid wheat (*Triticum aestivum* L.) cultivars – Amigo (good race-nonspecific resistance) and Ai-bian1 (supersensitive) were used as positive and negative controls to evaluate the degree of race-nonspecific resistance against powdery mildew in eight genotypes of tetraploid wheat (*Triticum turgidum* ssp. *dicoccum*). *Dicoccum* samples were kindly provided by IPK Gatersleben and country of their origin is in the Table 1.

For laboratory detection of their resistance, plants were grown in a chamber at 18°C and a photoperiod of 16 h light/8 h dark until full expansion of the primary leaf (10 days). The primary leaves were then inoculated with 20 conidia/mm^2 from *Blumeria graminis* DC. f. sp. *tritici*, race RK45.

For detection of resistance under field conditions, plants were grown in the field under environmental conditions to adult ontogenetic stage (spiking). The degree of powdery mildew attack was then evaluated.

Establishment of race-nonspecific resistance and papillae detection in laboratory conditions.

Race-nonspecific resistance is characterised by significant retardation of pathogen development on the host tissues. Consequently, each phase of the pathogen's development should be reached later in cultivars with good nonspecific resistance compared to control cultivars with low nonspecific resistance. In our study, stages of appressorium formation versus second germ tube elongation were used as a measure of the proportion of appressoria to all infection units (percentage of appressoria). In the case of resistance reaction, the attacked cell responds and various resistance mechanisms (including papilla formation) are initiated. In a compatible relationship, the infection peg often

Table 1. Summary of host-nonspecific resistance parameters detected in eight *Triticum turgidum* ssp. *dicoccum* wheat (their origin is in the notes) and control hexaploid cultivars Amigo and Ai-bian1: percentage of appressoria, papillae and race-nonspecific resistance evaluated in the field conditions

Genotype	Note	% appressoria	% papillae	Field resistance
Ai-bian1	control	55.1	13.2	2
Amigo	control	85.7	47.0	8
TRI 2883	DEU	98.9	55.7	9
TRI 5329	CH	96.9	38.9	9
TRI 11293	SVK	95.2	62.3	9
TRI 18201	FRA	94.5	18.5	8
TRI 17204	ITA	90.7	27.2	9
TRI A5100	YUG	89.3	52.5	9
CGN 11486	AUT	77.8	19.4	5
TRI 6158	IRN	68.7	19.0	3

penetrates, the haustorium is formed and epiphytically, second germ tube growth is observed 45–48 h after inoculation. Thus, a low proportion appressoria indicates lower degree of nonspecific resistance than a high proportion. The proportion of detected papillae to all infection sites was also determined.

For studies of rate of fungal development on various wheat genotypes, trypan blue staining followed by light microscopy observation of fungal structures and papillae were used as described previously Carver *et al*. (2001).

Establishment of race-nonspecific resistance in the field conditions.

In the field conditions values (1–9) of race-nonspecific resistance were evaluated with the consideration to percentage of disease extension in plant's tissues: 1 (100 – 90%), 2 (90 – 80%), 3 (80 – 70%), 4 (70 – 60%), 5 (60 – 50%), 6 (50 – 40%), 7 (40 – 30%), 8 (30 – 20%) and 9 (20% – no disease).

RESULTS AND DISCUSSION

Percentage of appresoria as a parameter of race-nonspecific resistance level, percentage of papillae as well as values of nonspecific resistance detected in the field conditions of eight *Triticum turgidum* ssp. *dicoccum* wheat and control cultivars Amigo and Ai-bian1 are presented in Table 1. Comparison of the values of prehaustorial resistance in the supersensitive hexaploid control cultivar Ai-bian1 and in tetraploid wheat genotypes CGN 11486 and TRI 6158 demonstrates that these two *dicoccum* samples are sensitive. As it is obvious from data in Table 1 (less than 80% of pathogen's units arrested in appressorial stage), more than 20% of pathogen's infection units were able to penetrate the host's cell and develop to secondary germ tube stage by 48 hours after inoculation. In addition, papillar resistance constitutes only a small portion of the total resistance in these two genotypes.

In the six tetraploid wheat genotypes the level of nonspecific resistance was higher than in control resistant cultivar Amigo. In three genotypes (TRI 2883, TRI 11293 and TRI A5100), the resistance was determined by papillae formation in relatively high measure. Proportion of papillar-based resistance at whole resistance of individual genotypes is presented in Figure 1. In the resistant genotypes the resistance was determined by papillae formation to varying extents. This means that there are other mechanisms ensuring protecting the host genotypes at the prehaustorial stage. Disease severity estimated in the laboratory conditions was similar to the degree of resistance in the field conditions (chart 2).

Together, our results demonstrate a host genotype-specific papillae-based resistance which is in accordance with their assumed quantitative nature. As has been described by several others authors (e.g. Thordal-Christensen 2003; Mysore & Ryu 2004), papillae are even formed in response to invading pathogens in cells of susceptible hosts, as well as in cells of non-host plants after inoculation by inappropriate fungus. In this view, penetration resistance seems to represent the first obstacle to the pathogen's development induced in the host induced after fungal attack. In the case of failure of this defense mechanism and successful penetration and haustorium formation, there are other resistance mechanisms (e.g. chemical elimination of the pathogen, hypersensitive reaction) inhibiting the pathogen's growth, well known in gene-for-gene interactions. It is interesting that even in host plants which possess race-nonspecific resistance there are several other defense mechanisms, as is also the case in the case of race-specific resistance.

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