

WHEAT PATHOGEN RESISTANCE AND CHITINASE PROFILE

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ABSTRACT

The powdery mildew and leaf rust caused by *Blumeria graminis* and *Puccinia recondita* (respectively) are common diseases of wheat throughout the world. These fungal diseases greatly affect crop productivity. Incorporation of effective and durable disease resistance is an important breeding objective for wheat improvement. We have evaluated resistance of four bread wheat (*Triticum aestivum*) and four spelt wheat (*Triticum spelta*) cultivars. Chitinases occurrence as well as their activity was determined in leaf tissues. There was no correlation between resistance rating and activity of chitinase. The pattern of chitinases reveals four isoforms with different size in eight wheat cultivars. A detailed understanding of the molecular events that take place during a plant–pathogen interaction is an essential goal for disease control in the future.

Keywords: Disease resistance, Chitinases, Leaf rust, Powdery mildew, Wheat

INTRODUCTION

Wheat (*Triticum aestivum* and *Triticum spelta* – AABBDD, 2n = 42) is the most widely grown crop plant, occupying 17 % of all the cultivated land (Gill et al., 2004). Biotic and abiotic stresses are important limiting factors for yield quality in wheat production. Powdery mildew caused by obligate biotrophic fungus *Blumeria graminis* f.sp. *tritici* (Bgt), is one of the most devastating diseases of wheat, that causes significant yield losses. Well known are also rust diseases of wheat, the most common leaf or brown rust is caused by the fungus *Puccinia recondita* f.sp. *tritici*. Leaf rust produces orange-brown uredinia (fruiting bodies) in pustules primarily on the upper surface of leaves. Heavy rusting causes early loss of infected leaves (Griffey et al., 1993).

To reduce the damage caused by biotic factors, plants have evolved many sophisticated adaptive response mechanisms. A plant-pathogen interaction may therefore be considered as an open warfare, of which major weapons are proteins and low-molecular-mass compounds synthesized/accumulated by both organisms (Ferreira et al., 2007). Inducible defence mechanisms mainly involve synthesis of pathogenesis-related (PR) proteins including chitinases (PR-3 group).

Chitinases (EC 3.2.1.14) constitute the second largest group of antifungal proteins (Kasprzewska, 2003) and are glucanhydrolases that catalyze the hydrolysis of chitin. Since this polymer of unbranched β -1,4-linked 2-acetamido-2-deoxyD-glucose is absent in plants, but present in many fungal pathogens, the role of these enzymes in plants has primarily been established in defense responses against (mainly fungal) pathogen (Meins et al. 1989, Van Loon et al., 2006). Massive induction of chitinases has been described in many plant species against various microorganisms, but important role under abiotic stress (e.g. metals, drought, heat etc.) has also been described (Mészáros et al., 2013). Moreover, specific chitinase isoforms appear to play role in normal plant growth and development (Regalado et al., 2000). Chitinases, as with many other PR proteins, may be synthesized in both a constitutive and an inducible manner.

In this work, the profile and overall activity of chitinase enzymes was studied in selected set of bread wheat (*Triticum aestivum*) and spelt wheat (*Triticum spelta*). The data were compared between the individual hexaploid wheat types. Further, since conventional assays of chitinase activities revealed correlation with sensitivity to biotic as well as abiotic stresses, we traced the correlation of chitinase enzyme activity in wheat accessions with available data on resistance to selected diseases. Chitinases once proven as reliable molecular markers might be

very helpful for traditional plant breeding approaches in efforts to identify tolerant varieties.

MATERIAL AND METHODS

Plant material and growth conditions

The set of 8 wheat genotypes from species *T. aestivum* and *T. spelta* of different origin was used (Table 1). The collection was provided by Gene Bank in Plant Production Research Center, Piešťany (Slovakia). Wheat seeds were germinated in Petri dishes lined with two layers of water-moistened filter paper (Whatman No. 1) in cultivation room at 25 °C and daily length of 16 hours for 6 – 7 days. For analyses young wheat leaves were used.

Chitinase quantitative assay

A fluorimetric assay was used to detect endochitinase activity in crude protein extracts using the synthetic substrate 4-methylumbelliferyl- β -D-N,N',N''-triacetylchitotrioside [4-MU-(GlcNAc)₃] (Sigma, USA) as described previously (Libantova et al., 2009). The reaction mixture contained 20 μ l of protein extracts mixed with 30 μ l of 300 μ M substrate in 0.1 M sodium citrate buffer (pH 3.0). The assay was carried out in 96-well black-sides assay plates. After incubation at 37°C for 1 h, the reaction was stopped by adding 150 μ l of 0.2 M Na₂CO₃ and fluorescence was measured by Fluoroskan II microtiterplate reader (TITERTEK, Finland) using excitation and emission filters 355 nm/450 nm (Cohen et al., 2006). Based on the standard curve, the chitinase activity was calculated as picomoles of methylumbelliferone (4-MU) generated per hour per microgram of soluble protein.

Detection of chitinases activity in gel

Total proteins were extracted from young leaves according to Hurkman and Tanaka (1986), and their concentration was determined by Bradford (1976). Separation of proteins (aliquots of 20 μ g) on 1.0 mm thick minigels was done using Mini-PROTEAN Tetra Cell apparatus (Bio-Rad laboratories, USA) according to Laemmli (1970). The 0.01% (w/v) glycol chitin obtained by acetylation of glycol chitosan (Sigma, USA) (Trudel and Asselin, 1989) was used as a substrate for chitinases and was added directly into 12.5% polyacrylamide

gels. Molecular weights of proteins were estimated using protein ladder (Mark 12 Unstained Standard, Invitrogen). Electrophoretic conditions during separation of proteins were 18 mA in stacking gel and 24 mA in separation gel under constant current for cca. 3-4 h. After electrophoresis, proteins were re-natured by shaking the gels in 50 mM sodium acetate buffer (pH 5.0) containing 1% (v/v) Triton X-100 overnight. The chitinase profile was detected according to Pan et al. (1991) upon staining with 0.01% (w/v) Fluorescent Brightener 28 (Sigma, USA) in 250 mM Tris-HCl (pH 8.9) for 15 min, and subsequently visualized under UV light using UVP Bio Doc-It System. The active chitinases appeared as dark bands on a bright background. The gels were photographed and contrast was adjusted using Scion Image software (<http://www.scioncorp.com>). After detection of chitinases, the gels were stained for detection of total proteins with 5% (w/v) Coomassie Brilliant Blue R-250 in 7% (v/v) acetic acid and 20% (v/v) methanol, and photographed again.

Evaluation of descriptors

For evaluation of wheat resistance to fungal pathogens, descriptor list for genus *Triticum* L. available at Gene Bank in Plant Production Research Centre, Piešťany (Slovakia), was used for plants grown in years 2010, 2011 and 2012. According to this descriptor, degree of disease/pests resistance is based on the scale from low (0) to high (10) degree of resistance. Leaf and spike powdery mildew (*Blumeria graminis* f.sp. *tritici*) and brown rust (*Puccinia recondita* f.sp. *tritici*) was chosen as most common wheat fungal pathogens.

RESULT AND DISCUSSION

Generally, selected cultivars of *Triticum aestivum* were less resistant to leaf and spike powdery mildew (*Blumeria graminis* f.sp. *tritici*) and brown rust (*Puccinia recondita* f.sp. *tritici*) comparing to cultivars of *Triticum spelta* (Tab. 1).

Table 1 Data on disease resistance in studied wheat accessions

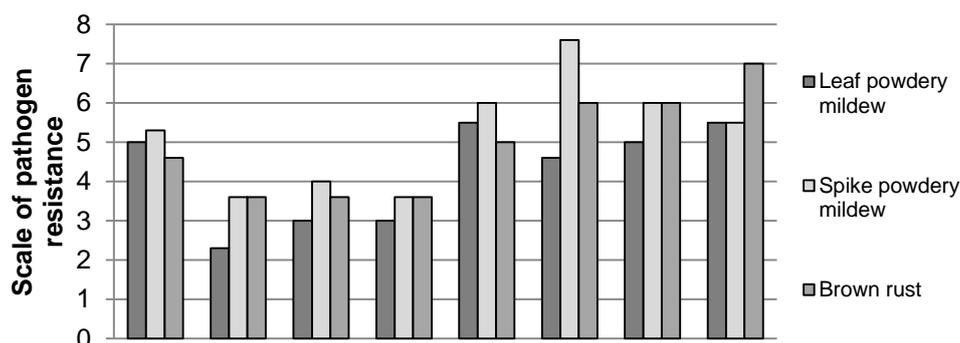
Wheat variety	Powdery mildew ^a		Leaf rust ^a
	leaf	spike	
<i>Triticum aestivum</i>	1 - Astella	medium	medium
	2 - Radošínska Norma	low	low
	3 - Šamorínska	low	low
	4 - Vigľašská Červenoklasá	low	low
<i>Triticum spelta</i>	5 - Epeautre Nain	medium	low
	6 - Kipperhaus Wiesser Spelz 3/2	low	high
	7 - Spelt, Lad Grasort 1/9	medium	medium
	8 - White Spelt	medium	medium

Disease resistance based on 3-year field experiments

In the experimental plants the total chitinase activities were measured fluorimetrically (Fig. 1). No differences between chitinase activities of *Triticum aestivum* and *Triticum spelta* representatives were noticed. Further, the obtained

data show that degree of disease resistance does not correlate with total chitinase activity in wheat tissue (Fig. 1).

A



B

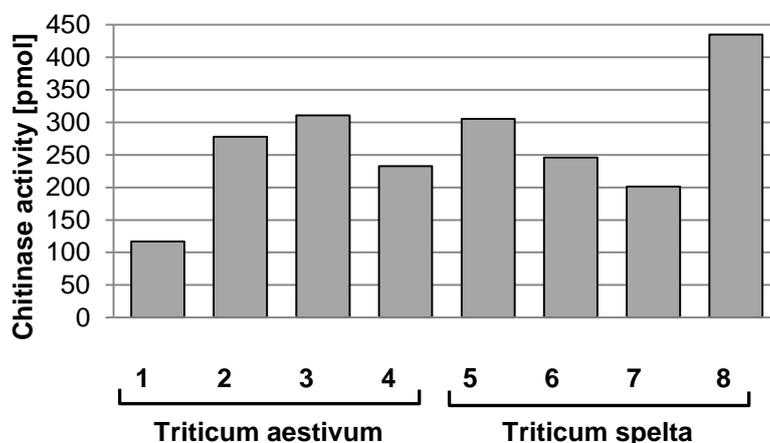


Figure 1 Representatives of bread wheat (*Triticum aestivum*) (1-4) and spelt wheat (*Triticum spelta*) (5-8) are differently sensitive to various fungal diseases (A/upper graph).

The cultivars were analyzed for activity of chitinases in leaf tissue (B/lower graph). The data show no sound interaction between resistance against pathogens and chitinase activity in different wheat cultivars. The individual wheat cultivars 1-8 are listed in Table 1.

The overall enzyme activity of chitinase in plant tissue, however, not necessarily reflects the behaviour of individual isoforms, especially under changing environmental conditions (Piršelová et al., 2011). For this reason, studying the behaviour of chitinase fractions after separation in gels might be more informative. Previously, existence of multiple intercellular chitinase isoenzymes were detected in wheat plants under non-denaturing conditions. In wheat, 50% of

the chitinase isoenzymes were found in the basic fractions and the other 50% was located in acidic fractions (Botha et al., 1998). Moreover, chitinases can be synthesized both constitutively as well as in inducible manner (Ferreira et al., 2007).

In the eight tested wheat cultivars we studied and compared the pattern of chitinases as typical plant defense molecules. Our study had identified four chitinase isoforms with different sizes of 40 kDa, 35 kDa, 25 kDa and ~20 kDa in all wheat plants (Fig. 2).

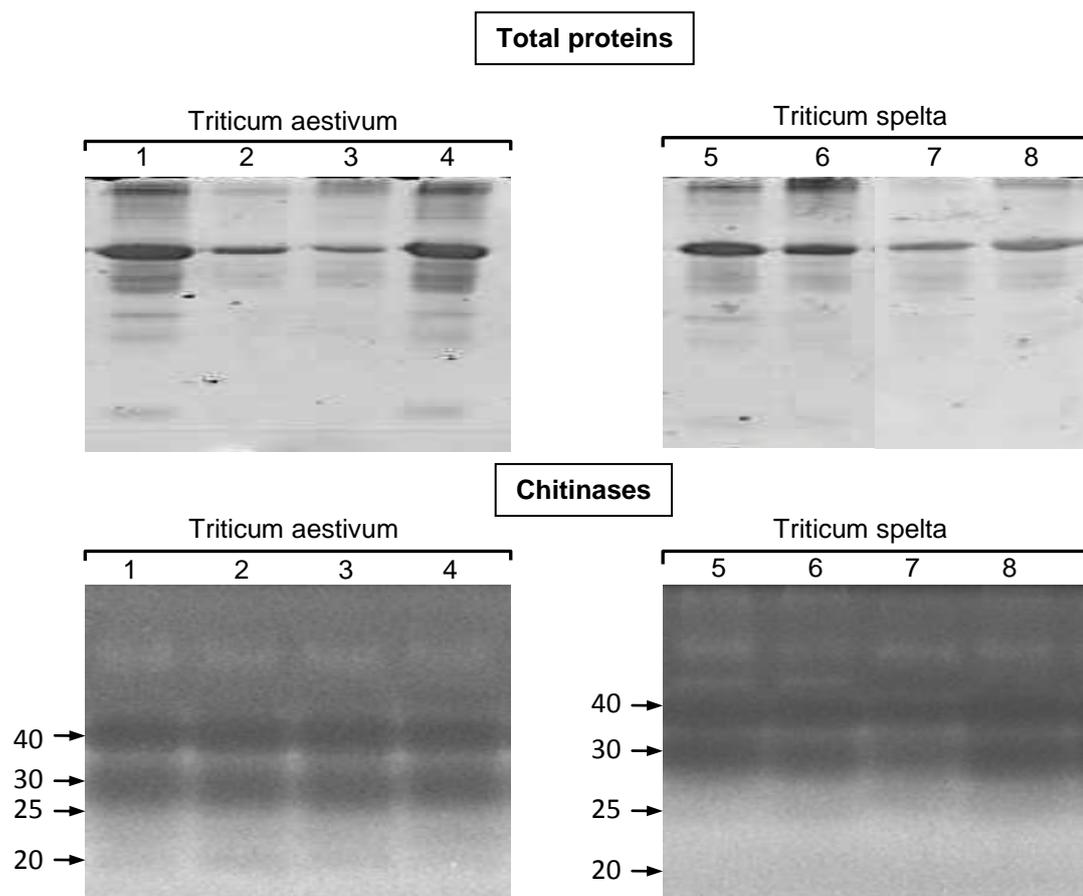


Figure 2 Chitinase pattern in representatives of bread wheat (*Triticum aestivum*) and spelt wheat (*Triticum spelta*). Proteins were separated on glycol chitin containing gels using SDS-PAGE. Subsequently gels were stained for chitinase activities with Fluorescent Brighener 28 and visualised under UV-light. Arrows indicate the molecular mass of corresponding protein bands in kDa.

Other authors have observed similar profiles in their studies. However, while in susceptible plants five different chitinase activity peaks were detected, in the resistant counterparts there were six activity peaks identified (Sahai and Manocha, 1993). The resistant and susceptible plants differ in the presence of two acidic isoenzymes (Botha et al., 1998). Furthermore, after different plant treatments (e.g. pathogen attack, ethylene treatment) the number of chitinase isoforms increased, while still higher number was present in the resistant variety. Additive isoforms might be indicators for better defense equipment against fungi. Previously, a chitinase (30.8 kDa) with antifungal activity has been isolated from mung bean (*Phaseolus mungo*) seeds (Wang et al., 2005), whereas two 28-kDa chitinases designated chitinase A and chitinase B also exhibiting antifungal activity were characterized in maize (*Zea mays*) seeds (Huynh et al., 1992). For comparison, 8 chitinase isoforms were identified in tobacco (Pan et al., 1992), 3 chitinase isoforms in cucumber (Zhang and Punja 1994). In celery, two chitinases were strongly induced by fungi (Krebs and Grumet 1991). Temporally affected expression profiles (with maxima after 36-72 hpi) were observed for two endochitinases in wheat upon infection of wheat-Fusarium, yellow dwarf virus (YDV) and Hessian fly (Wu et al., 2013). Previously, similar amplitudes of expression for two different chitinase genes were detected in a susceptible- and sensitive wheat cultivars (Li et al., 2001). Kinetics not only amplitude of chitinase induction might be crucial for efficient defense against stress (Mészáros et al., 2013), since fast response can affect the outcome of plant-pathogen interaction in favour of plant. Nevertheless our results showed that the basic pattern of chitinases is not a reliable indicator of pathogen resistance (susceptibility). Similar study on plants exposed to pathogen infection might reveal such markers of cultivars with good/bad defense equipment.

CONCLUSION

In conclusion, we have found that tested bread wheat cultivars are less resistant to common wheat fungi as well as powdery mildew and brown rust comparing with the analysed representatives of spelt wheat. The cultivars also differ in the overall chitinase activity in leaf tissue. However, there is no sound correlation between

chitinase activity and level of resistance. In each wheat cultivar up to four chitinase isoforms with different size were detected. Studying these profiles upon pathogen infection might reveal isoforms that are inducible during abiotic stress and/or can be evaluated as markers of overall resistance/susceptibility.

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