9th International Triticale Symposium, May 23-27, 2016. Szeged, Hungary

Effect of proteinaceous toxins on *Parastagonospora nodorum* blotch development in triticale.

Jakub Walczewski, Edward Arseniuk, Piotr Ochodzki

Department of Plant Pathology, Plant Breeding and Acclimatization Institute – NRI, Radzików, 05-870 Błonie, Poland

Introduction

Parastagonospora nodorum is a necrotrophic pathogen of all assimilative green plant parts of wheat and triticale as well as of other cereals and grasses. With development of the disease, called Stagonospora nodorum leaf and glume blotch, on necrotic lesions appears pycnidial sporulation. Destruction of green plant parts affects adversely photosynthesis, what results in grain yield loss, quantitative and qualitative in nature. Proteinaceous host selective toxins produced by *P. nodorum* in infected plant tissue play crucial role in induction of tissue necrosis. Toxins interact with specific host genes. Positive recognition with dominant allele in affected plant leads to necrosis induction, while absence of dominant allele causes toxin insensitivity. So far, eight pairs of *P. nodorum* toxin/host genes were reported and described. Tests conducted under controlled environment as well as in field conditions confirmed that protein toxins are important factors in *P. nodorum* leaf and glum blotch of wheat.

Figure 1.
Example of scoring method used in phenotypic resistance trials. Green - healthy tissue, red – *P. nodorum* affected tissue. Two series of seven second leaves were inoculated and average % of damaged tissue was calculated with WinCAM software.

Table 1.
P. nodorum phenotype resistance [% of damaged tissue] at seedling stage compared with Tox3 resistance. [„R" – resistant, „S" – susceptible]

Triticale cultivar	Tox3 reaction	Necrosis surface
Moderato	R	1,1
TODAN	R	1,2
MAH 33097-4	R	1,2
Mikado	R	1,5
Borwo	R	1,5
MAH 33098-21	S	2,1
LEONTINO	S	2,2
DANKO 25/13	S	2,3
LM 18-12	S	2,5
Cyrkon	R	3,3
Alekto	S	3,3
DANKO 1/13	R	3,4
Elpaso	R	3,4
Fredro	R	4,2
Maestozo	R	4,7
Gniewko	S	4,9
LM 1-12	S	5,3
SUBITO	R	5,4
Atletico	S	5,6
DANKO 20/13	R	6,5
Baltiko	S	6,6
MAH 28944-5	S	6,7
Magnat	S	6,7
DANKO 15/13	S	6,9
MAH 32726-1-1	S	7,0
TOMKO	S	7,9
LM 22-11	S	8,0
Amorozo	S	8,4
DANKO 23/13	R	8,6
DANKO 13/13	S	8,6
DANKO 19/13	S	8,7
PALERMO	S	8,8
LM 15-12	S	8,9
Bereniko	S	8,9
KWS TRISOL	S	9,1
DANKO 14/13	R	9,5
DANKO 18/13	R	10,5
DANKO 22/13	S	10,6
MAH 33116-6-3	R	11,0
DANKO 16/13	S	11,7
ALGOSO	S	11,7
DAST 1-12	S	12,6
LM 8-12	S	12,8
Aliko	S	13,0
DANKO 2/13	R	13,1
DANKO 24/13	S	13,4
DANKO 21/13	R	14,7
Grenado	S	15,2
Cerber	S	16,5
DANKO 6/13	R	16,5
Borowik	S	16,9
DANKO 17/13	S	18,1
Pizarro	S	18,2
PIGMEJ	S	24,8
SORENTO	S	26,9
Presto	S	27,5
PAWO	S	28,7

Correlation coefficient	0,348
Critical value α-0,1	0,218
n	58

Objectives

Examination of *P. nodorum* isolates collected in Poland for ToxA, Tox1 and Tox3 genes. Evaluation of Polish triticale varieties and breeding lines reactions to semipurified Tox3.

Materials & methods

PCR: 170 isolates were grown on liquid medium, DNA was isolated from hyphae. ToxA, Tox1, and Tox3 primers were similar to ones used by McDonald et al (2013).

Selected Tox3 producing isolate (5-5/11) was grown 4 weeks on Fries liquid medium. Resulting culture medium were dialysed in 3,5 kDa tubing against 20mM NaOAc, pH 5.

Purification: dialysate was concentrated on cation exchanger (HiTrap SPXL) and eluted with NaCl gradient (0-200 mM). Fractions were collected and infiltrated in BG220 leaves for screening of necrotic activity. Active fractions were combined and further purified on gel filtration column (Superdex75) (Fig.2.).

Infiltration: Three second leaves for every cultivar or breeding line were infiltrated with 20µl of semi-purified Tox3 preparation. Plants were cultivated in 12/12 day/night in 20ºC for five days after infiltration (Fig.3.).

Figure 2.
Chromatograms of Tox3 purification with ion-exchange chromatography (IEC) and gel filtration (GF)

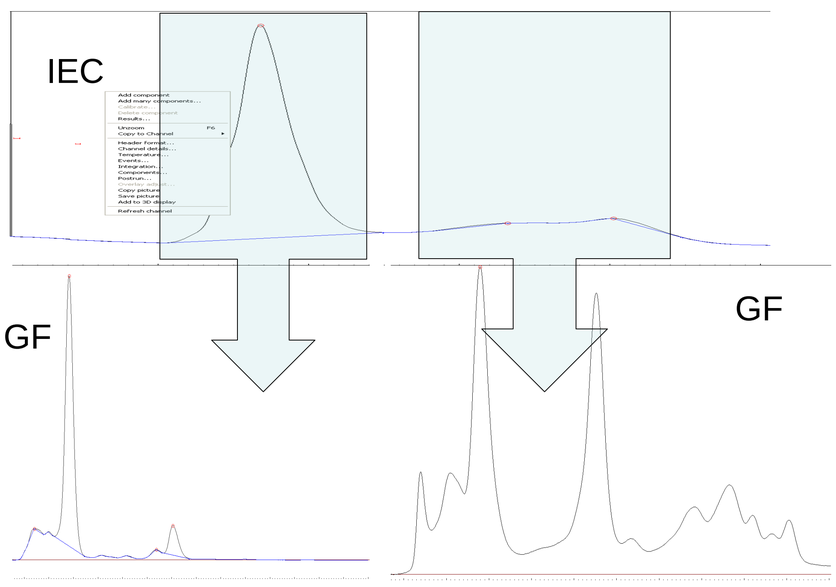


Figure 3.
Example of triticale cultivar leaves infiltrated with semi purified Tox3

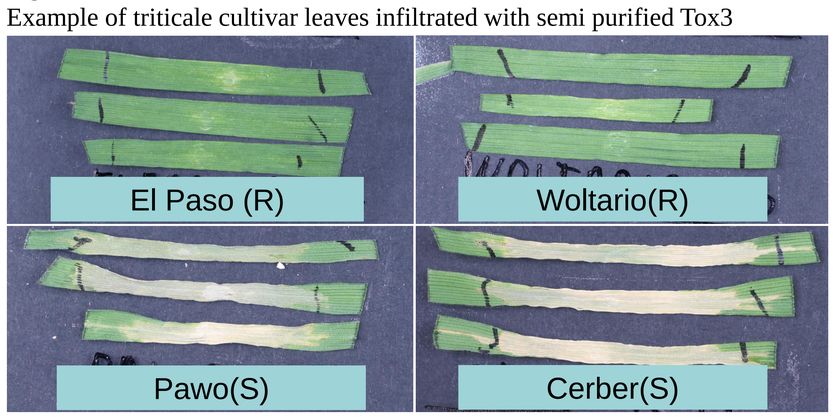
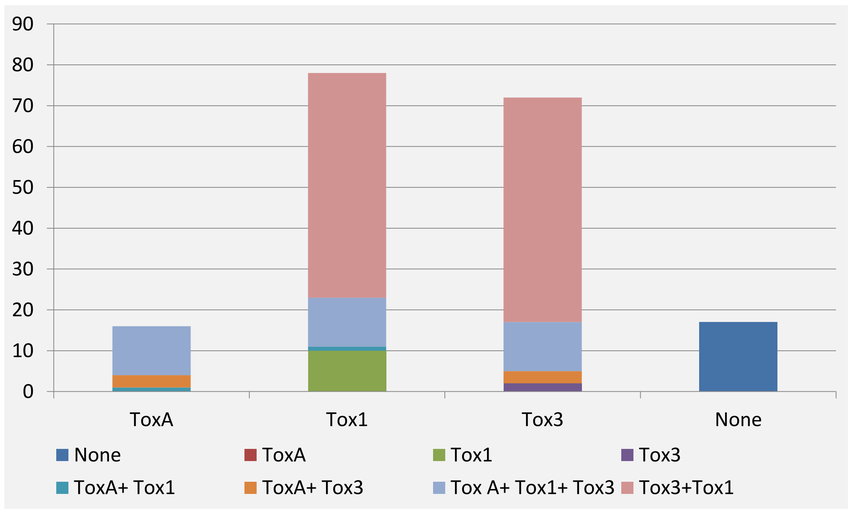


Figure 4.
Presence of ToxA, Tox1, and Tox3 genes in Polish *P. nodorum* isolates n = 170



Phenotypic resistance trial: seedlings were cultivated in plates in controlled environment chamber for approximately two weeks. The second leave of seedling were inoculated with water suspension of pycnidiospores of eight isolates at concentration of $6*10^6 ml^{-1}$ was applied. Inoculated plants were cultivated at high air humidity for 6 days and then inoculated leaves were photographed. Scoring was done by WinCAM software and area of damaged tissue (%) (Fig. 1.) was calculated from two series of seven leaves for each tested line.

Results

In Polish *P. nodorum* isolates genes coding Tox1 and Tox3 toxins are significantly more frequent (78 % and 73%) than ToxA gene (16%). Majority of tested isolates contain both Tox1 and Tox3 (55%) (Fig.4.).

58 of 85 inoculated seedlings (66%) were susceptible.

Seedling leaves of 58 Polish triticale varieties were infiltrated with chromatographically semi-purified preparation of Tox3.

Toxin resistance was compared with P. nodorum phenotypic resistance and significant correlation between these two types of reactions was found.

Conclusions

Correlation between Tox3 resistance and *P. nodorum* phenotype resistance is in agreement with literature data. This fact suggests the need to incorporate *P. nodorum* effectors resistance tests to triticale breeding programs.

Main purpose of the project is to screen Polish triticale germplasm lines against susceptibility to known proteinaceous toxins produced by *P. nodorum* and to validate their effect on SNB development in triticale and wheat. Data on this poster represent the first step undertaken to achieve our goal. In upcoming years we are planning to purify more effectors and to continue genotyping of the cereal lines.

Project was financially supported by Ministry of Agriculture and Rural Development.