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

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## 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. Oval or lens-shaped, first chlorotic and later in the season redbrown spots develop along the leaf blade and sheath and affect the entire leaf and/or glume and awns. 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. In past several years appeared quite a number of reports on proteinaceous host selective toxins produced by *P. nodorum* in infected plant tissue. These 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.**
Sample of wheat cultivars infiltrated with semi purified Tox3.
22% „R" resistant
68% „S" susceptible

| Wheat cultivar | Tox3 Reaction |
|---|---|
| Akteur | R |
| Alcazar | R |
| Arkadia | R |
| Arktis | R |
| Askalon | R |
| Astoria | S |
| Bagou | R |
| Baletka | R |
| Bamberka | S |
| Banderola | R |
| Batuta | R |
| Begra | S |
| Belenus | R |
| Bockris | S |
| Bogatka | R |
| Boomer | R |
| Bystra | S |
| Cubus | R |
| BL1 | R |
| BL2 | R |
| BL3 | R |
| BL4 | R |
| BL5 | R |
| BL6 | R |
| Dorota | R |
| Elipsa | R |
| Estivus | S |
| Fakir | S |
| Fidelius | R |
| Figura | R |
| Finezja | R |
| Forkida | R |
| Forum | R |
| Fregata | R |
| Garantus | R |
| Henrik | R |
| Jantarka | S |
| Jenga | S |
| Kampana | R |
| Kepler | R |
| Kobiera | S |
| Kohelia | R |
| Kranich | R |
| Kredo | S |
| KWS Dacanto | S |
| KWS Magic | R |
| KWS Ozon | R |
| Legenda | R |
| Linus | R |
| Liwilla | R |
| Look | R |
| Ludwig | R |
| Markiza | R |
| Meister | R |
| Meteor | R |
| Mewa | S |
| Mikula | R |
| Mulan | S |
| Muszelka | R |
| Muza | R |
| Naridana | R |
| Natula | S |
| Nutka | R |
| Operetka | S |
| Ostka Strzelecka | R |
| Ostroga | S |
| Oxal | S |
| Patras | S |
| Platin | S |
| Praktik | R |
| Rywalka | S |
| Sailor | R |
| Satyna | R |
| Skagen | R |
| Smaragd | S |
| Smuga | S |
| Speedway | R |
| Sukces | R |
| Tonacja | R |
| Torrild | R |
| Tulecka | R |
| Türkis | R |
| Turnia | R |
| Wydma | S |
| Zawisza | R |
| Zyta | R |
| BL7 | S |

**Figure 2.**
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.

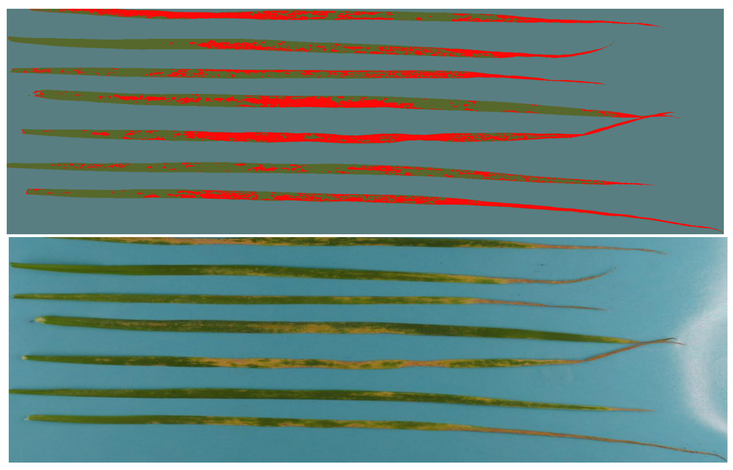


**Figure 3.**
*P. nodorum* phenotype resistance [% of damaged tissue] at seedling stage compared with Tox3 resistance. [„R" – resistant, „S" – susceptible]

| Wheat cultivar | Tox3 reaction | % of damaged tissue |
|---|---|---|
| Fidelius | R | 0,8 |
| Askalon | R | 1,0 |
| Liwilla | R | 1,2 |
| Boomer | R | 1,3 |
| Forum | R | 1,3 |
| Legenda | R | 1,8 |
| Finezja | R | 2,0 |
| Figura | R | 2,1 |
| Mikula | S | 2,1 |
| Nutka | R | 2,4 |
| KWS Ozon | R | 2,4 |
| Arktis | R | 2,5 |
| Kepler | R | 2,5 |
| Bogatka | R | 2,7 |
| Bystra | R | 2,7 |
| Look | R | 2,8 |
| Cubus | R | 3,0 |
| Banderola | R | 3,2 |
| Fregata | R | 3,2 |
| Elipsa | R | 3,3 |
| Kampana | R | 3,3 |
| Jenga | R | 3,5 |
| Kredo | S | 3,5 |
| Muszelka | R | 3,5 |
| KWS Dacanto | S | 4,2 |
| Henrik | R | 4,3 |
| Markiza | R | 4,6 |
| Kohelia | R | 4,9 |
| Patras | R | 5,3 |
| Alcazar | R | 5,4 |
| Akteur | R | 5,5 |
| Dorota | R | 5,7 |
| Begra | S | 5,9 |
| Ludwig | R | 5,9 |
| KWS Magic | R | 6,0 |
| Ostka Strzelecka | R | 6,4 |
| Arkadia | R | 6,4 |
| Garantus | R | 7,2 |
| Bagou | R | 7,3 |
| Kranich | R | 8,3 |
| Forkida | R | 8,6 |
| Platin | S | 8,9 |
| Belenus | R | 8,9 |
| Batuta | R | 9,0 |
| Operetka | S | 9,9 |
| Kobiera | S | 10,3 |
| Bamberka | S | 10,3 |
| Natula | S | 11,1 |
| Bockris | S | 11,2 |
| Fakir | S | 11,5 |
| Oxal | S | 12,7 |
| Rywalka | S | 12,8 |
| Jantarka | S | 14,8 |
| Estivus | S | 15,3 |
| Baletka | R | 17,4 |
| Astoria | S | 17,8 |
| Ostroga | S | 19,2 |
| Muza | R | 21,9 |
| Mewa | R | 23,6 |

| | |
|---|---|
| Correlation coefficient | 0,445 |
| Critical value α-0,1 | 0,216 |
| n | 59 |

## Objectives

Examination of ToxA, Tox1 and Tox3 genes of *P. nodorum* isolates collected in Poland. Evaluation of Polish wheat varieties and breading lines reactions to semi purified Tox3.

## Materials & methods

Isolate 5-5/11 were grown for 4-5 weeks on Fries liquid medium [g/L] : 5g ammonium tartrate; 1g $NH_4NO_3$; 0,5g $MgSO_4$ *$7H_2O$; 1,3g $KH_2PO_4$; 2,6g $K_2HPO_4$ 30g sucrose; 1g yeast extract.

**Chromatography:** After dialysis against 20mM NaOAc pH 5 in 3,5kDa tubing, portion of dialysate was separated on column HiTrap SPXL. Fractions were infiltrated in BG220 leaves to screen for active fractions. Active fractions were concentrated and separated on gel filtration column with Superdex75.

**Infiltration:** Three second leaves for every wheat cultivar or breading line were infiltrated with approximate 20µl of semi purified Tox3 preparation. Plants were cultivated in 12/12 day/night in 20°C for five days after infiltration.

**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).

**Phenotype resistance trial:** Seedlings were sown in 8x13cell plates and cultivated in 12/12h day/night in 20°C in controlled environment chamber for approximate two weeks. After the second leave complete development seedlings were inoculated with water suspension of pycnidiospores at concentration of 6mln/ml, mixture contained the same amount of eight isolates. After inoculation plants were cultivated at high air humidity for 6 days and then second leaves were photographed. Scoring was done by WinCAM software, average % of damaged tissue was calculated from two series of seven leaves for each tested line.

**Figure 4.**
Example of wheat cultivars leaves infiltrated with semi purified Tox3

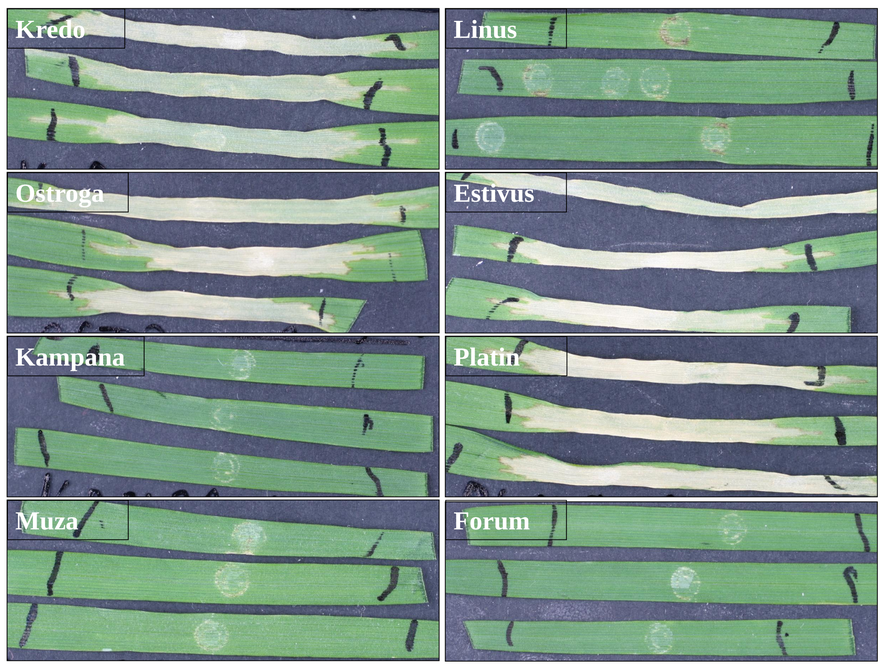
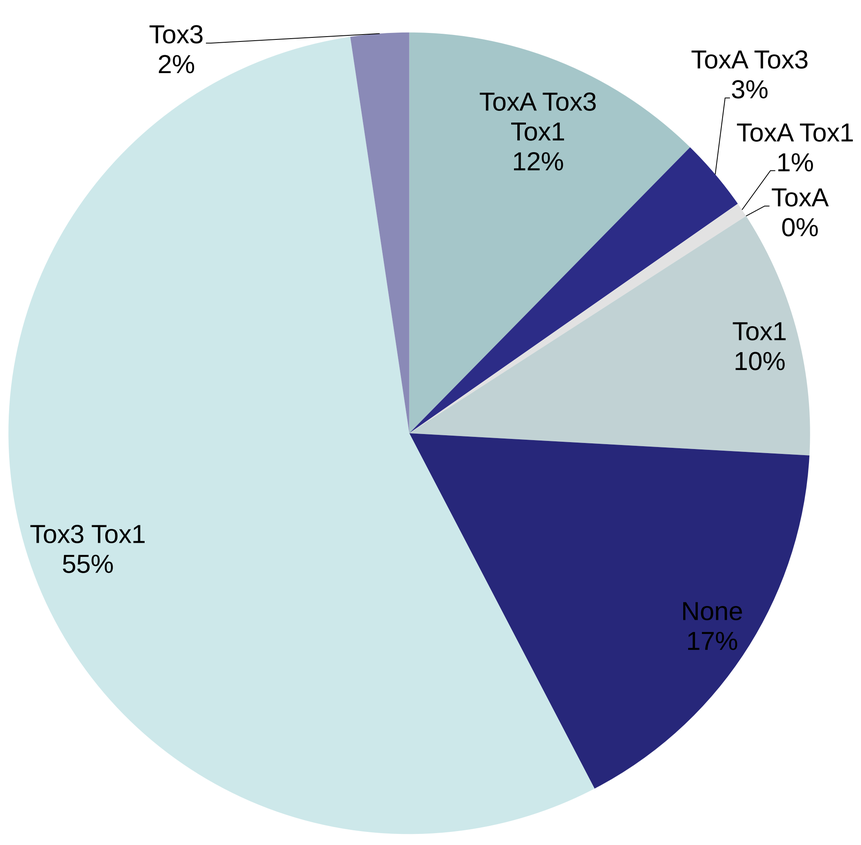



**Figure 5.**
Presence of ToxA, Tox1, and Tox3 genes in Polish *P. nodorum* isolates n = 170




## Results

Genes coding Tox1 and Tox3 toxins are significantly more frequent (78 % and 73%) in Polish isolates than ToxA gene (16%). Majority of tested isolates contain both Tox1 and Tox3 (55%).

Seedling leaves of a number of Polish wheat varieties were infiltrated with chromatographically purified preparation of Tox3, 22% of wheat seedlings were susceptible and 68% were resistant.

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

## Conclusion

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 wheat breeding programs.

Main purpose of the project is to screen Polish wheat germplasm lines against susceptibility to known proteinaceous toxins produced by *P. nodorum* and to validate their effect on SNB development in wheat and triticale. 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.

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