Tox5 and its effect on SNB development in Polish wheat and triticale germplasm.

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Parastagonospora nodorum is an economically important pathogen of wheat and triticale. In host tissues, it is known to produce several protein effectors which lead to local necrosis and finally to yield loss through photosynthetic surface reduction. Effectors produced by the pathogen are interacting with dominant forms of specific host genes. In the case of effector recognition, necrosis is induced. Until now eight effectors and corresponding host genes are known. One of them is Tox5 which was described for the first time by Timothy Friesen in 2012. This effector is commonly produced by Polish isolates of *P. nodorum*, therefore its impact on disease development has been investigated. Tox5 was purified and used to phenotype wheat and triticale lines. Mix of *P. nodorum* isolates and diversified breading materials of wheat and triticale were utilized in disease evaluation trials. Tox5 insensitivity was positively correlated with phenotypic resistance. Elimination of Tox5 sensitive lines as well as lines sensitive to others effectors can be employed to increase *P. nodorum* resistance.

Figure 1

Tox5 purification steps: Ion exchange chromatography is followed by size exclusion chromatography. Fractions containing purified Tox5 are used for plant material screening.

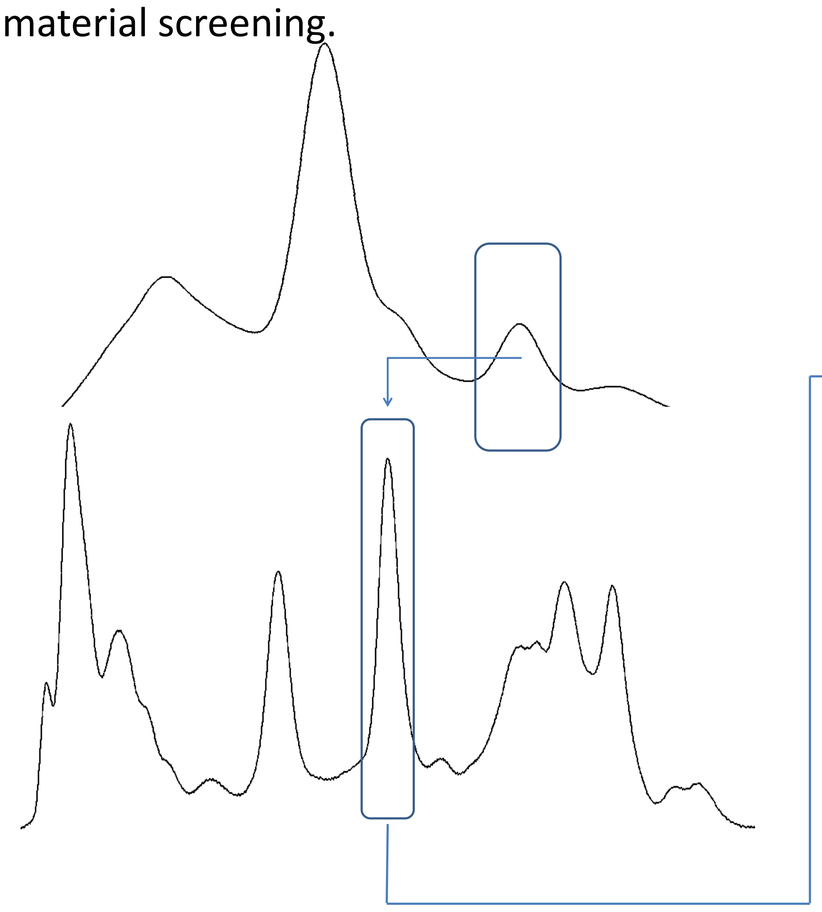


Figure 2

Example of sensitive and insensitive reaction, after infiltration with Tox5.

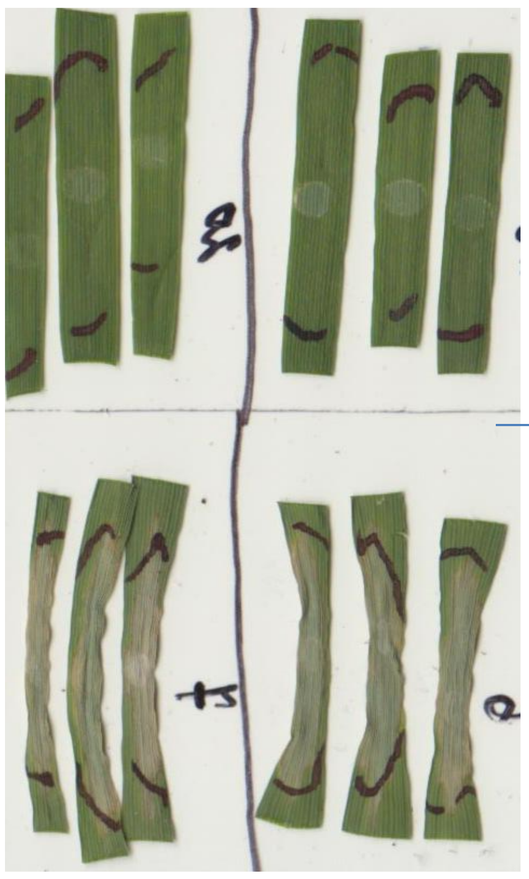
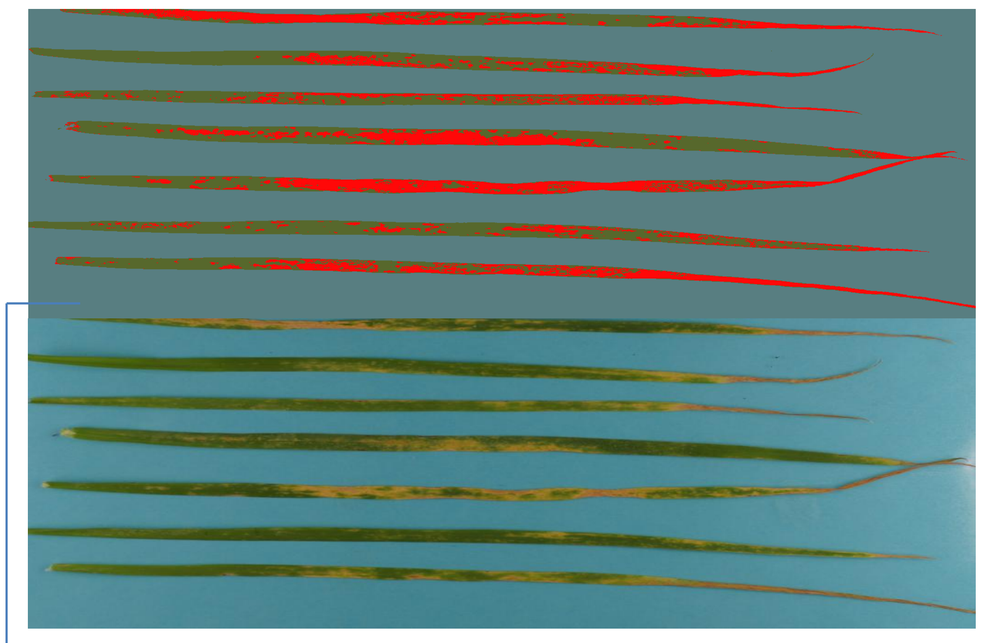


Figure 3

Symptoms of disease caused by *P. nodorum* in field trials.

Figure 4

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



Conclusion

Correlation coefficients between *P. nodorum* phenotypic resistance and effectors insensitiveness suggest that Tox3 insensitivity have biggest positive impact on resistance both in adult and seedling stage. Tox5 impact is far lesser and statistically correct mainly in seedling stage.

Figure 5

Correlation coefficients between *P. nodorum* phenotypic resistances: field conditions (adult), control environment (seedling) and effectors sensitivity. High value of coefficient means that effectors insensitivity has positive effect on plant resistance. Statistically correct correlations with levels of significances were marked with colors.

Wheat, field conditions 2017

Tox1	Tox3	Tox5	ToxA
0,071	0,304	0,141	0,576
p=,400	p=,000	p=,093	p=,019

2016

Tox1	Tox3	Tox5	ToxA
0,002	0,236	0,192	-0,097
p=,983	p=,004	p=,021	p=,376

2015

Tox1	Tox3	Tox5	ToxA
-0,036	0,227	0,058	-0,009
p=,661	p=,005	p=,604	p=,942

2014

Tox1	Tox3	Tox5	ToxA
0,020	0,130	0,089	--
p=,864	p=,250	p=,438	p= ---

Triticale, field conditions 2017

Tox1	Tox3	Tox5	ToxA
-0,060	0,212	0,210	-0,347
p=,495	p=,017	p=,019	p=,061

2016

Tox1	Tox3	Tox5	ToxA
-0,103	0,180	0,082	-0,036
p=,219	p=,031	p=,371	p=,813

2015

Tox1	Tox3	Tox5	ToxA
-0,000	0,216	0,088	-0,195
p=1,00	p=,016	p=,348	p=,179

2014

Tox1	Tox3	Tox5	ToxA
0	0,401	0,136	--
p=1,00	p=,001	p=,264	p= ---

Wheat, seedling 2017

Tox1	Tox3	Tox5	ToxA
0,093	0,785	0,253	0,231
p=,286	p=0,00	p=,003	p=,408

2016

Tox1	Tox3	Tox5	ToxA
-0,164	0,403	0,162	-0,002
p=,051	p=,000	p=,058	p=,989

2015

Tox1	Tox3	Tox5	ToxA
0,009	0,644	0,359	-0,079
p=0,912	p=0,000	p=0,000	p=0,508

2014

Tox1	Tox3	Tox5	ToxA
-0,085	0,234	0,030	--
p=,475	p=,046	p=,803	p= ---

Triticale, seedling 2017

Tox1	Tox3	Tox5	ToxA
-0,205	0,629	0,264	-0,266
p=,018	p=,000	p=,003	p=,148

2016

Tox1	Tox3	Tox5	ToxA
0,106	0,572	0,261	0,009
p=,203	p=,000	p=,001	p=,934

2015

Tox1	Tox3	Tox5	ToxA
0,000	0,437	0,287	-0,258
p=1,00	p=,000	p=,001	p=,068

2014

Tox1	Tox3	Tox5	ToxA
0,000	0,024	0,167	--
p=1,00	p=,844	p=,176	p= ---

P < 0,05

P < 0,1

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