P. nodorum effectors resistance among Polish wheat and triticale germplasms

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Overview: A group of wheat and triticale breading lines were screened for reaction to purified effectors, and for phenotypic resistance to *P. nodorum* in seedling (controlled environment) and in adult plant growth stages (field conditions). Correlations between effectors and phenotypic resistances suggest that the largest impact on disease development have Tox3 and Tox5 and ToxA.

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. Necrotic tissue is essential for fungal growth and reproduction. Fungus is causing necrosis by production of several host-selective necrotrophic effectors proteinaceous in nature. Positive recognition with dominant allele of specific gene in host plant, leads to necrosis induction, while absence of dominant allele causes effectors insensitivity. There are clear evidences of positive influence of selection against dominant alleles on resistance. Today eight effectors are known; therefore it is desirable to know their contribution to disease development.

Methods: Extracts of effectors were prepared and tested mostly according to Timothy Friesen works. Three second leaves for each line were infiltrated on length of around 2cm and marked with a nonphytotoxic pen. Second seedling leaves fully developed were inoculated under controlled environment conditions and rated for lesion length after 7 days with image analysis method. In field trials plants were inoculated at boot, heading, and flowering stages. Phenotypic resistance was tested with water suspension of pycnidiospores derived from 11 *P. nodorum* isolates capable to produce multiple effectors**.** Population studies of *P. nodorum* were conducted with PCR primers designed for Tox1, Tox3 and Tox5 on 170 isolates derived from crop plantations grown in geographically different places in Poland.

Figure 1. Symptoms of disease caused by *P. nodorum* on glumes and leaf.

Figure 3.
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.

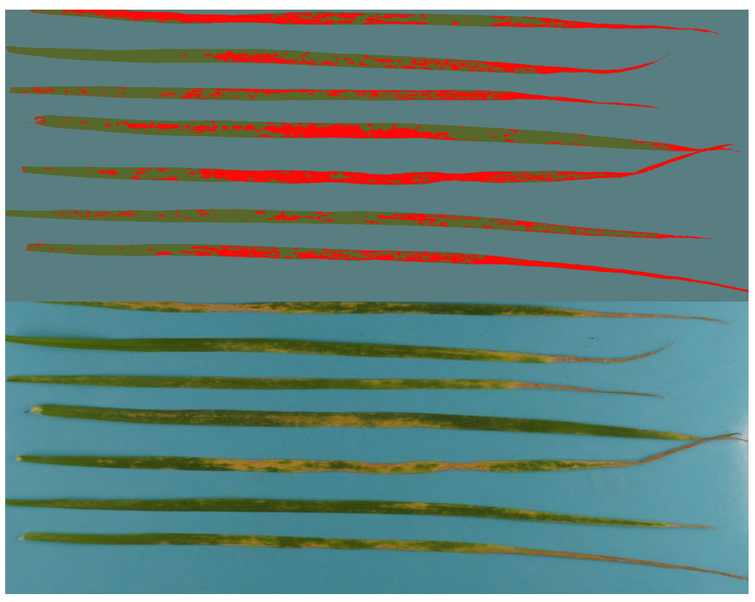

Figure 6. Presence of ToxA, Tox1, and Tox3 genes in Polish *P. nodorum* isolates n = 170

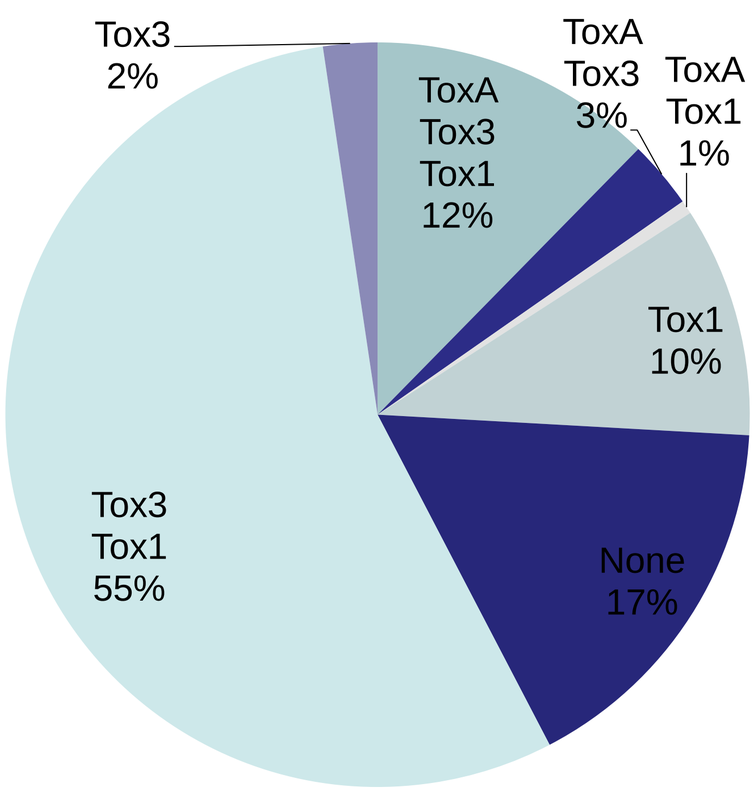

Figure 2. Effectors resistance among Polish breading lines of wheat and triticale. Leaves of breading lines of wheat and triticale (n=88) were infiltrated with separated effectors and scored after 5 days. Reaction type were shown **(R)**esistant – no change, **(I)**ntermediate susceptible – chlorosis or light necrosis and **(S)**usceptible – complete necrosis of tissue,.

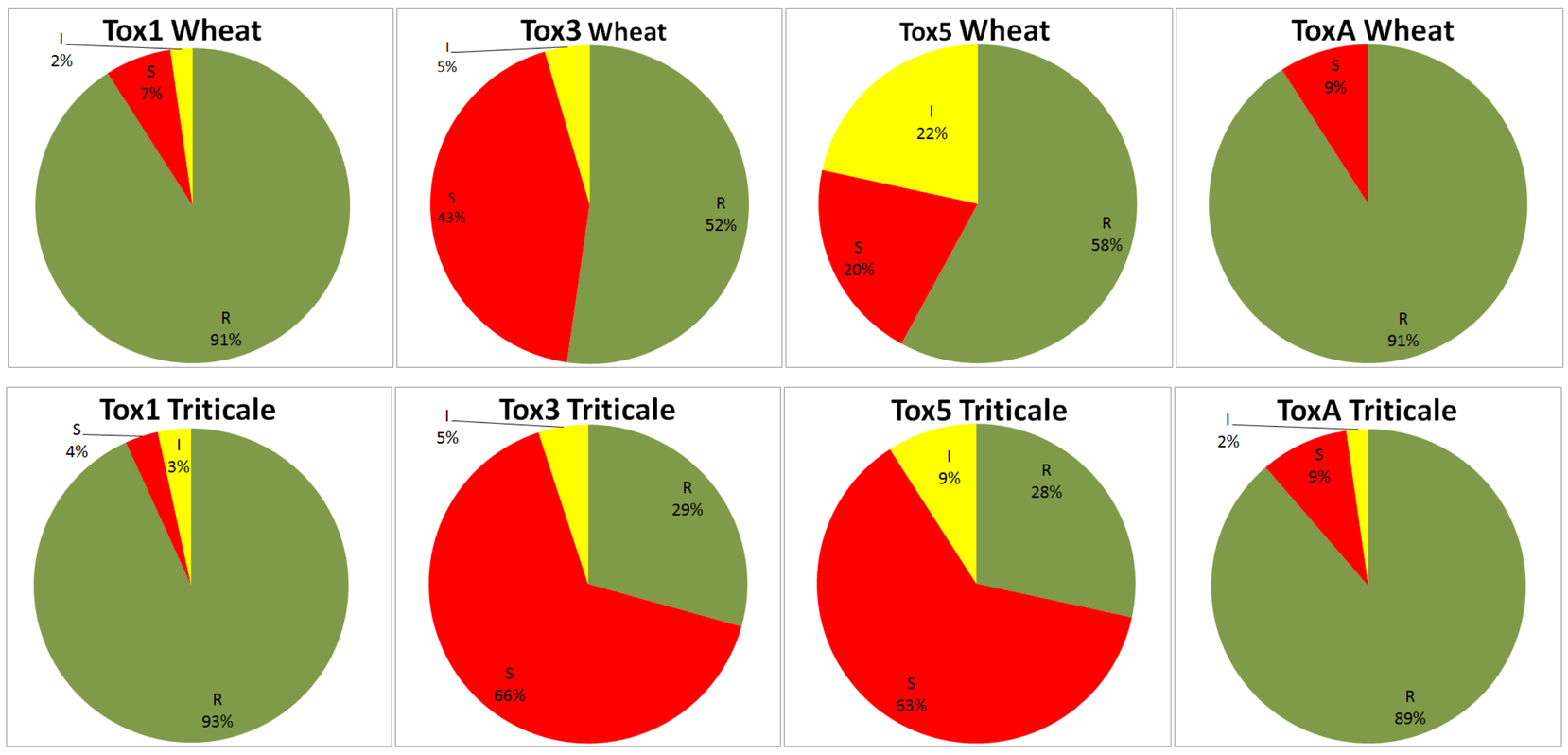

Figure 4. Correlation between effectors insensitivity and phenotypic resistance. Field scoring was done separately for spikes and leaves. Statistically correct correlations with levels of significances were marked with colors.

	Tox1		Tox3		Tox5		ToxA	
Wheat n=88		Correlation		Correlation		Correlation		Correlation
	Seedling	0,074	Seedling	0,425	Seedling	0,318	Seedling	-0,07
	Field leaves	-0,153	Field leaves	0,198	Field leaves	0,255	Field leaves	0,185
	Field spikes	-0,071	Field spikes	0,075	Field spikes	0,139	Field spikes	0,268
Triticale n=68 Field		Correlation		Correlation		Correlation		Correlation
	Seedling	-0,229	Seedling	0,439	Seedling	0,286	Seedling	0,025
	Field leaves	-0,139	Field leaves	0,213	Field leaves	0,222	Field leaves	0,168
	Field spikes	-0,27	Field spikes	0,235	Field spikes	0,2	Field spikes	0,221

Statistically correct with significance 0,05
Statistically correct with significance 0,1

Figure 6. Examples of wheat leaves infiltrated with Tox3

Conclusions: Among tested breeding lines only some were susceptible to ToxA and Tox1, susceptibility to Tox3 and Tox5 was far more frequent. The largest positive correlation between insensitivities and phenotypic resistance of adult and seedling stages was observed between Tox3 and Tox5. ToxA resistance appears to have no impact on phenotype resistance at the seedling stage. In contrast to the other screened effectors resistance to Tox1 seems to lower the phenotypic resistance in triticale, however, in tested breading lines there was only small number of such ones susceptible to Tox1 and ToxA. Thus, final conclusions will be made after larger number of lines will be tested. Tox3 gene is widely widespread in population of pathogen in Poland. Therefore it seems notably important to focus on excluding Snn3 and Snn5 dominant alleles from wheat and triticale germplasms in Poland.

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