Production, isolation and necrotrophic activity of Parastagonospora nodorum proteinaceous toxin Tox5.

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One of the major pathogens of triticale and wheat is *Parastagonospora nodorum (Stagonospora nodorum).* Infection causes necrotic spots on leafes in which they are growing, and can spread to the same plant, as well as to neighboring plants. The presence of this pathogen during high humidity and temperature can lead to a serious reduction in the yield by reducing the photosynthetically active surface of the leaf and damage to the chaff. In the last few years there have been reports of production by *S. nodorum* protein toxins, which play a key role in the induction of necrotic changes in infected tissues of the host. These toxins affect the specific host genes. The positive interaction of the toxin with the products of dominant form of the gene leads to the induction of necrosis, and in the presence of only a recessive form is the insensitivity to a particular toxin. Experiments conducted under both laboratory and field conditions confirmed that the toxins are a major factor in the development of leaf and chaff septoria on wheat. There were no negative effects of the elimination of the dominant allele of susceptibility to the toxin, while the increase in resistance was found, which positively corresponds with the research Oliver and others (2014). Until now seven different proteinaceous toxins have been described. One of them is Tox5 toxin. The aim of the studies was production and purification of necrotrotrophic efector Tox5 in amount which allowed to conduct studies on susceptibility of wheat and triticale genotypes.

MATERIAL AND METHODS

Tox5-producing isolate Sn 16-8-05 was grown on liquid medium at pH=5.7, containing sodium acetate, ammonium nitrate, magnesium sulfate, potassium dihydrogen phosphate, sucrose and yeast extract on shaker in room temperature, in darkness during 3 weeks (Fig.1). Culture media were filtrated on blotting paper and later on 0,45 µm filter. Filtrates were dialyzed against 20 mM acetate buffer, pH=5.0 to remove LMW compounds (cut-off MW<3 kDa). Particles larger than 3 kDa were concentrated on centrifugal filter and dissolved with 20 mM acetate buffer, pH=5.0. Partially purified extract was fractionated on HiTrap SP XL 5-mL column by elution with acetate buffers pH 5.0 with increasing ionic strength from 0 to 300 mM of NaCl. The obtained fractions were tested for necrotoxicity on the leaves of selected wheat genotype. Fractions with necrotrophic activity were combined and further separated on 80 cm Superdex 75 chromatography column with 20 mM acetate buffer. Collected fractions were tested for necrotrophic activity and active fractions were pooled.

Figure 1. Growing of *Parastagonospora nodorum* cultures on liquid media

Collected fractions and starting extract were infiltrated by syringe to the second leaves of wheat and triticale genotypes placed on agar with 50ppm benzimidazole. After six days of 16/8 photoperiod in room temperature leaves were checked Active fractions were combined and leaves of wheat and triticale genotypes were infiltrated.

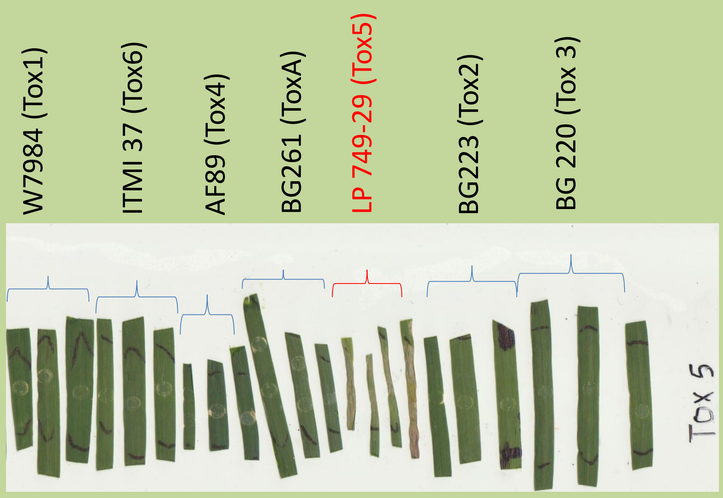

Figure 2. Results of infiltration of set of segregating wheat lines with fractions of S. nodorum isolate 16-8-05 culture filtrate

Figure 4. Results of infiltration of wheat **LP 749-29** leaves with fractions of *S. nodorum* isolate 16-8-05 culture filtrate purified on HiTrap SP XL. * - necroprytic active fractions (see Fig.3)

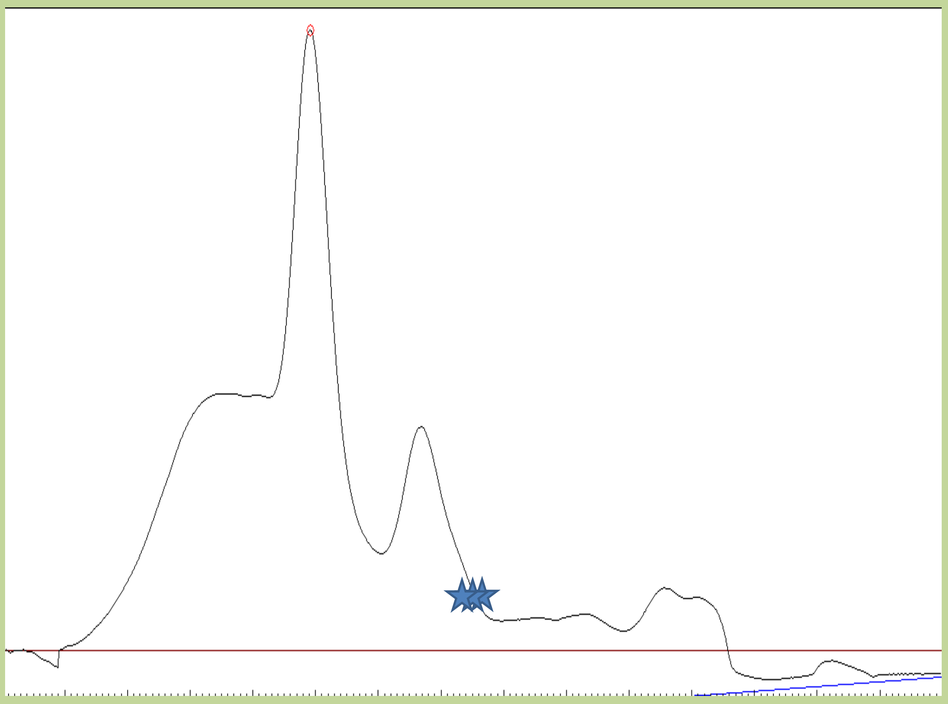

Figure 3. Fractionation of crude extract of 16-8-05 isolate on HiTrap SP XL 5 mL column

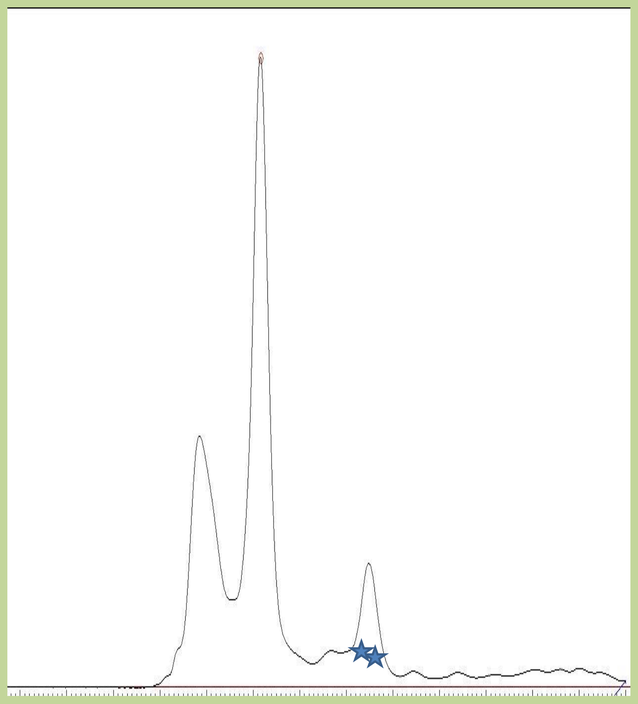

Figure 5. Chromatogram of gel permeation (Superdex G 75 column) of semipurified extract

RESULTS

Selected isolate Sn16-8-05 produced only Tox5 toxin. Resulting culture filtrate was tested for necrotic activity and fractionated on ion exchange and gel permeation column to obtain partially purified fractions (Fig. 3 and 5). Toxicity of separated fractions tested on the leaves of selected wheat genotype, sensitive to crude culture filtrate confirmed necrotic activity of fractions (Fig 2 and 4). Susceptibility to Tox5 toxin was observed in 42% of wheat and in 71% of triticale genotypes.

Table 1. Susceptibility of wheat and triticale genotypes to Tox 5 toxins.

	Plant reaction	Number of plants	%
Wheat n=88	Resistant	51	58,0
	Susceptible	37	42
Triticale n=88	Resistant	25	28,4
	Susceptible	63	72

Figure 6. Results of infiltration of set of wheat genotypes with purified Tox 5 toxin.

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