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Characteristics of selected strains of Stagonospora nodorum detected in Poland in terms of necrotrophic activity and production of proteinaceous toxins.

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Stagonospora nodorum is one of the major pathogens of wheat and triticale, and causes major losses. Following infection causes necrotic areas in which they are growing and from which spreads to the same plant, as well as to neighboring plants. The appearance 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. For genes encoding three of them the nucleotide sequence have been published and the population studies on the global sample of isolates *of S. nodorum* are being performed. Poster shows data on isolates from the Polish and complementary vision of Europe's population of *S. nodorum*

MATERIAL AND METHODS

147 isolates of *S. nodorum* were cultured 3 weeks on shaker in room temperature, in darkness on liquid medium at pH=5,7, containing sodium acetate, ammonium nitrate, magnesium sulfate, potassium dihydrogen phosphate, sucrose and yeast extract.
Culture media were filtrated on blotting paper and later on 0,45 µm filter. Mycelia were washed with deionized sterile water and were analyzed by PCR for presence of gene Tsn1 (SnToxA), Snn1 (SnTox1) and Snn3 (SnTox3) with use of specific primers. 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 or precipitated with acetone, dried and dissolved with 20 mM acetate buffer, pH=5.0. Partially purified extract was fractionated on HiTrap SP XL 1-mL column by elution with acetate buffers pH 5.0 with increasing ionic strength from 0 to 1 M of NaCl. Collected fractions were analyzed with HPLC method: Bioshell 400A Protein C4 column 15x4,6 mm, 3,4 µm UV detection λ=280 nm, gradient elution with acetonitrile:water +0,1% TFA.
for necrosis.

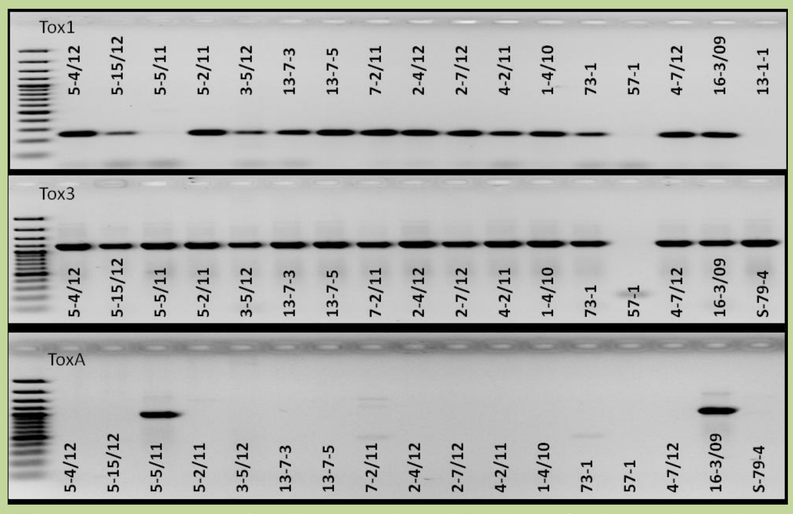

Figure 1. An example of separation of PCR analysis products for each toxin gene of *selected S.* nodorum isolates.

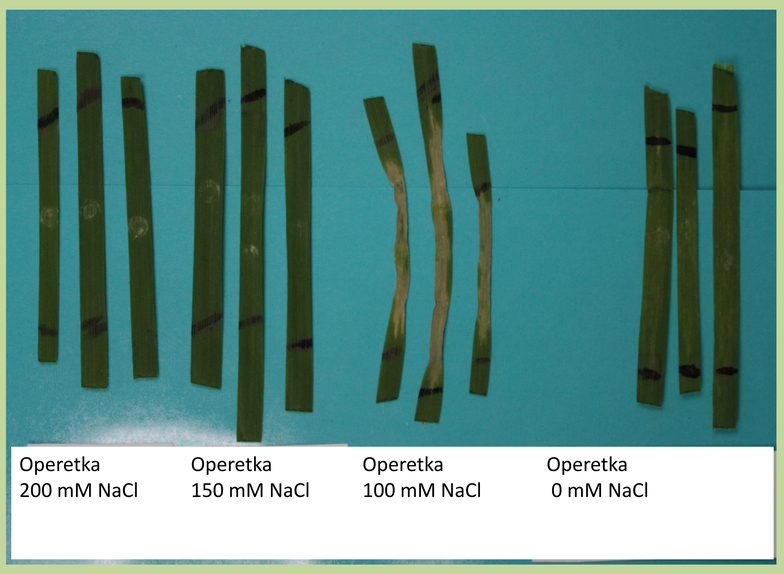

Figure 3. Results of infiltration of wheat (var. Operetka) leaves with fractions of S. nodorum isolate 5-5/11 culture filtrate purified on HiTrap SP XL.

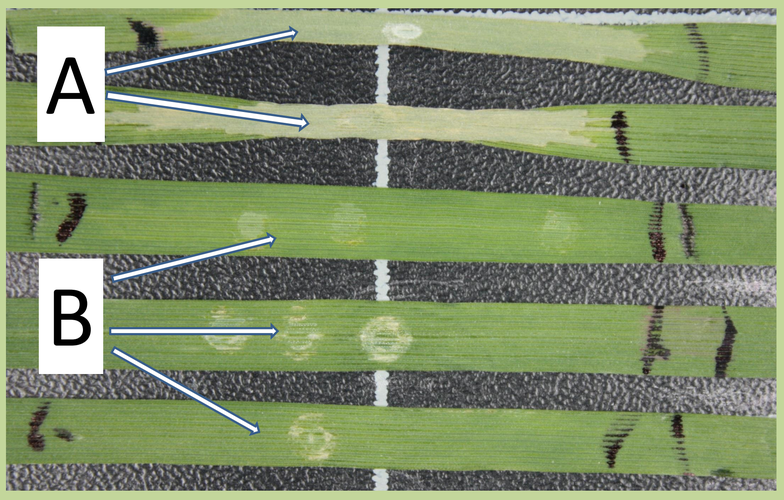

Figure 4. Results of infiltration of wheat (var. Operetka) leaves with fraction of *S. nodorum* isolate 5-5/11 culture filtrate purified on HiTrap SP XL 100mM NaCl: A - without Pronase; B- with Pronase.

Collected fractions and starting extract were infiltrated by syringe to the second leaves of wheat (var. Operetka) placed on agar with 50ppm benzimidazole. Extract treated with protease was used as a control. After six days of 16/8 photoperiod in room temperature leaves were checked

RESULTS

The DNA analysis of 147 isolates revealed comparable profile of the Polish and European population of *S. nodorum*. Among tested isolates the most numerous group consists of isolates producing Tox1 and Tox3 (54,4%) (Fig.2). All three toxins are produced by 10% and ToxA is produced by 11% of isolates. No one isolate producing ToxA and Tox1 or ToxA only was found. Tox1 and Tox3 is produced respectively by 81% and 74% of the isolates, and ToxA by 14,4%. The results confirm literature data on European populations of S.nodorum. In 18% of isolates genes producing these three toxins weren't detected.

Selected isolate (5-5/11) producing two toxins (ToxA and Tox 3) was grown in liquid medium and the resulting culture filtrate was tested for necrotic activity and fractionated on ion exchange columns to obtain partially purified fractions (Fig. 5). Toxicity of separated fractions tested on the leaves of selected wheat variety Operetka, sensitive to crude culture filtrate confirmed necrotic activity of fraction 100 mM) .

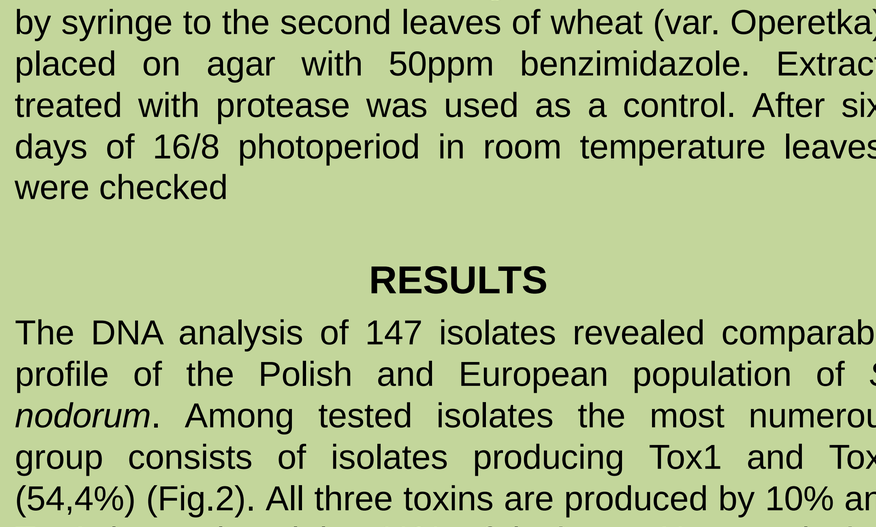

Figure 5. Fractionation of crude extract of 5-5/11 isolate on HiTrap SP XL 1 mL column

The HPLC chromatograms of selected culture filtrates and partially purified fraction were registered (Fig. 6) and proteinaceous nature of toxin(s) was confirmed (Fig. 4). Further work is conducted in order to separate single toxin(s) and evaluation of their properties.

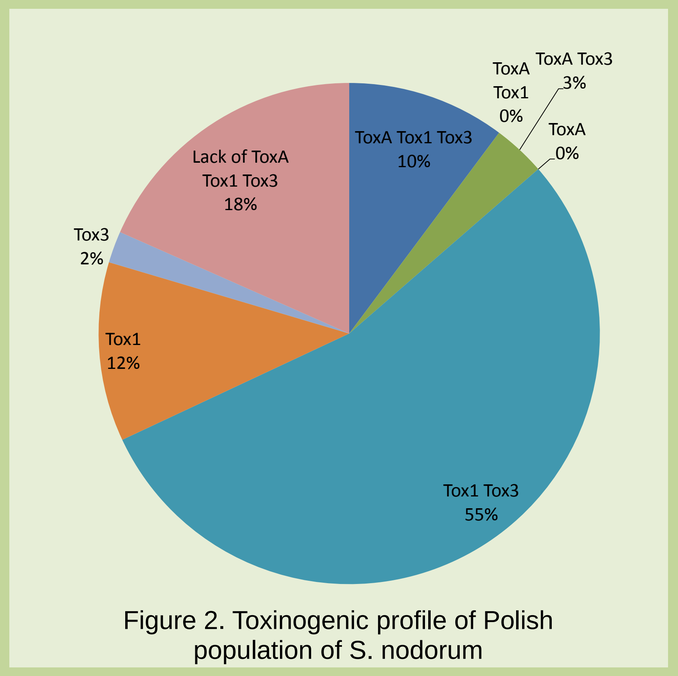

Figure 2. Toxinogenic profile of Polish population of S. nodorum

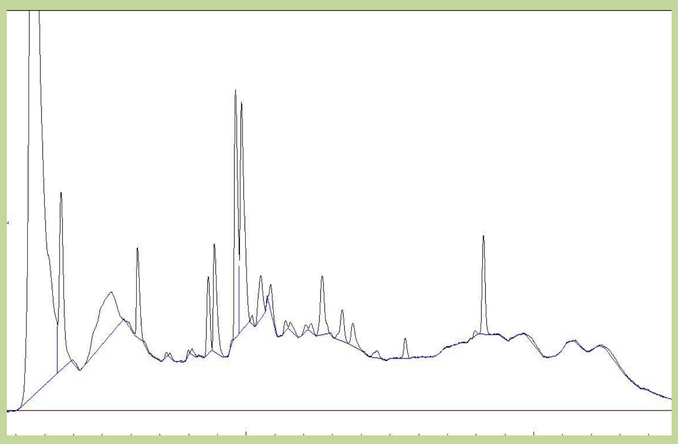

Figure 5. Chromatogram of crude extract of 5-5/11 isolate (Bioshell 400 Protein C4 column)

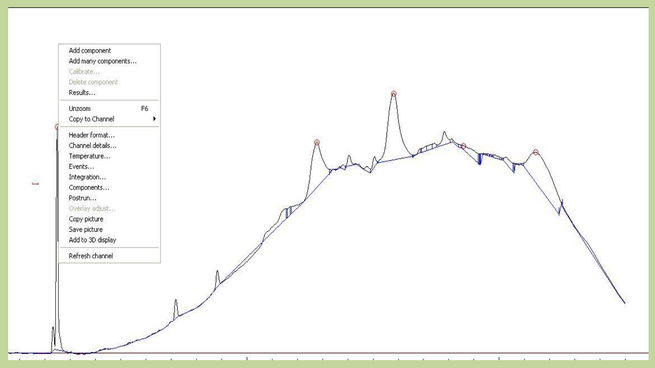

Figure 6. Chromatogram of fraction 100 mM NaCl of 5-5/11 isolate extract (Bioshell 400 Protein C4)

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