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Radzików, 2015-12-01

PhD thesis:
"Analysis of selected genetic and biochemical-physiological factors in wheat (*Triticum aestivum* L.) resistance to leaf rust (*Puccinia triticina*)".

to obtain the degree of PhD in agricultural sciences, field of knowledge: Agricultural Science, discipline: agronomy
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Summary

Wheat (*Triticum aestivum* L.) is a cereal crop cultivated worldwide. Global production ranks wheat on the third place after maize and rice. Wheat breeding is focused on high yield, pathogen resistance and abiotic stress tolerance. One of the most important diseases of wheat is leaf rust or brown rust which is caused by the fungus *Puccinia triticina*. The leaf rust epidemics may cause yield losses up to 50%. The most important method of prevention of this disease is to use resistant cultivars. Understanding the mechanisms involved in resistance is important for breeding strategies and crop improvement.

The aim of the study was to analyse the early stages of wheat – *Puccinia triticina* interaction. This included: pathogen development, micronecrotic reaction of host cells, hydrogen peroxide accumulation and the determination of its source, callose deposition and lignification, and analysis of expression of selected genes and SSH clones.

Observations of disease symptoms allowed for the separation of the tested lines into three groups: susceptible (Thatcher and Tc*Lr34*), intermediate resistant (Tc*Lr24*, Tc*Lr25* and Tc*Lr29*) and highly resistant (Tc*Lr9*, Tc*Lr19* and Tc*Lr26*). The following components of host – pathogen interaction were assessed by microscopic observations of stained leaf samples: pathogen structures were visualized by staining with calcofluor white, host cell necrosis was specifically stained with Evans blue, accumulated hydrogen peroxide was stained with DAB, callose depositions by aniline blue and cell wall lignification by phloroglucinol. The results showed that low number of pathogen structures i.e. haustorium mother cells, rapid hydrogen peroxide accumulation in mesophyll and rapid micronecrotic reaction characterized lines highly resistant to brown rust. Thus these features were considered as indicators of the efficient resistance. The lignification found in infection sites was observed in both intermediate resistant and highly resistant plants. This process, considered as a component of basic resistance is correlated with the accumulation of hydrogen peroxide in mesophyll and

with micronecrotic reaction. Callose deposition was observed in resistant as well as susceptible plants, so presumably it was not crucial for this type of resistance. The expression of genes encoding peroxidases and NADPH oxidases combined with the results obtained with the use of enzyme-specific inhibitors revealed which enzymes were responsible for the production of hydrogen peroxide. The use of sodium azide, which is an inhibitor of peroxidases, indicated that this enzyme was involved in the hydrogen peroxide accumulation in seven lines (Tc*Lr9*, Tc*Lr19*, Tc*Lr24*, Tc*Lr25*, Tc*Lr29*, Tc*Lr34* and Thatcher). The use of diphenylene iodonium, an inhibitor of NADPH oxidase, indicated the role of this enzyme Tc*Lr9*. The use of 2-bromoethylamine, an inhibitor of diamine oxidase, proved the role of this enzyme in intermediate resistant (Tc*Lr25* and Tc*Lr29*) and highly resistant (Tc*Lr9*, Tc*Lr19* and Tc*Lr26*) lines. The use of 1,12-diaminododecane, an inhibitor of polyamine oxidases, showed that the enzymes were active in Tc*Lr9*. The analysis of expression of peroxidases and NADPH oxidases indicated that these genes were involved in production of hydrogen peroxide and their expression patterns are correlated with microscopic observation. The expression patterns of the six from the eight tested SSH clones indicated their participation in the resistance of wheat to brown rust. The bioinformatics analysis of the full coding sequence of JG968933 clone has shown that the gene encodes wall associated kinase (*TaWAK*). Expression pattern of the *TaWAK* gene as well as the strong induction during rust infection strongly indicates its role in wheat resistance.

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