Investigation of the role of ORA59 transcription factor during *Pyrenophora teres f. teres* infection in barley

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Barley (*Hordeum vulgare* L.) is in the four most important cereals in the world. It has a significant role in the world's food supply and livestock feed and it is a model plant for research. Deployment of resistant cultivars is the most economic and eco-friendly method to control plant diseases. *Pyrenophora teres f. teres* (PTT), the causal agent of net form of net blotch disease of barley, is one of the most important fungal pathogens of barley.

The plant hormones has a key role in defence mechanism against plant pathogenes. The defence reaction induced by salicylic acid (SA) and jasmonic acid (JA) are the two most important pathways, which enable the plant to respond to the pathogenic attack properly. The activation of the JA-signalling pathway is required for resistance against necrotrophic pathogens. SA can antagonize JA signalling and vice versa. Their interaction provides an opportunity to the fine-tuning of the response. The APETALA2/Ethylene-Responsive Factor (AP2/ERF) superfamily of transcription factors (TFs) are implicated in the responses to both biotic and abiotic stress. ORA59 is one of the member of this family, which has been shown to increase its expression as a result of infection in model plants (Arabidopsis). The production of ORA59 is stimulated by JA and repressed by SA. In our previous experiment significant increase in JA was observed as a result of PTT infection in barley.

Therefore we investigate the role of *ORA59* gene in defence mechanism against *Pyrenophora teres f. teres* infection in barley where it has not been studied yet.

Two barley genotypes cv. Golden promise and cv. Mv Initium was involved in the experiment. H-947 *Pyernophor teres f. teres* isolate was used for artificial infection. The barley ORA59 gene (HORVU4Hr1G000700.2 in Plant Ensembl) was identified based on Blast searches, it showed 69% AA identity to the Arabidopsis protein.

In our experiment, significant increase in the barley *ORA59* gene expression was observed after seven and fifteen days of *Pyrenophora teres f. teres* infection (Figure 1.).

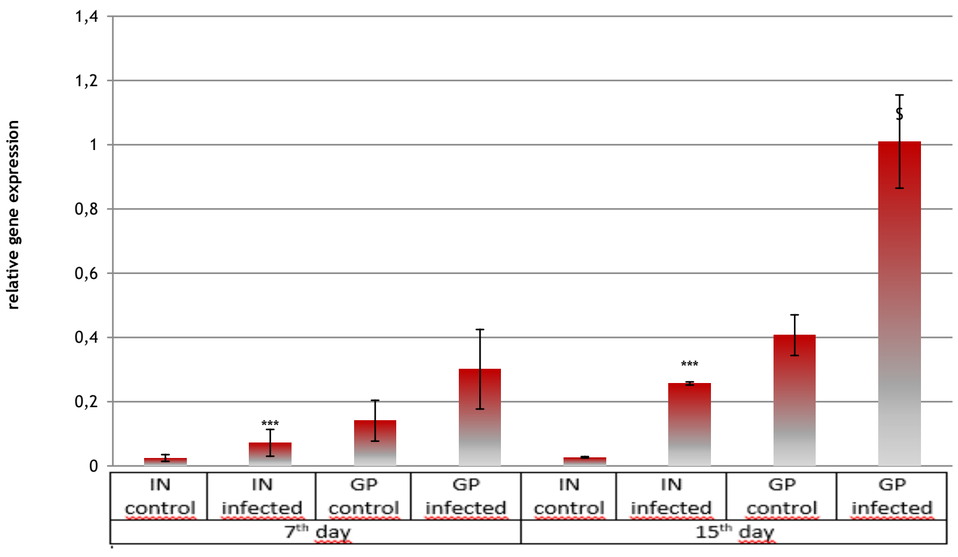

Figure 1. ORA59 gene expression on PTT infected Mv Initium (IN) and Golden promise (GP) barley cultivars. (p = 0.01 ***). Normalized to EF1 and α-tubulin genes.

The connection between *ORA59* expression and PTT resistance of barley genotypes was studied by gene knock-out using CRISPR/Cas9 system. *ORA59* edited barley plants were produced by Agrobacterium-mediated transformation of immature embryos of Golden Promise. One of the transformed plant proved to be *ORA59* edited.

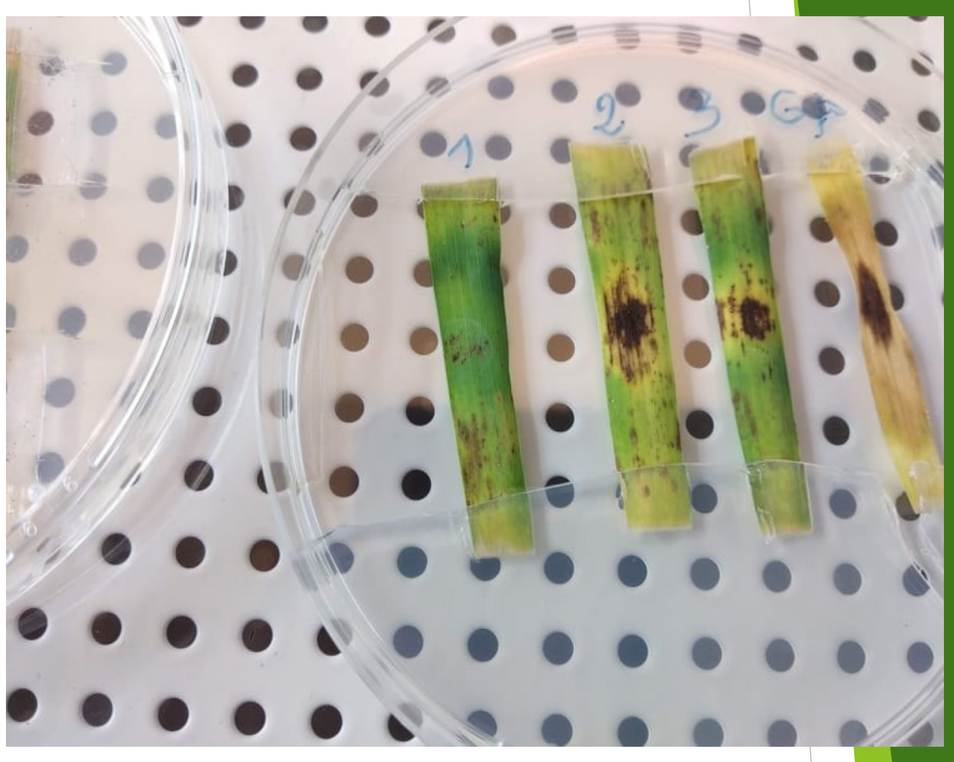

Figure 2.: Deteached leaves assay: 1,2,3 ORA59 CP edited leaves of GP, wild type GP.

The resistance of gene edited barley plants against PTT was characterized by detached leaves assay. Two leaf pieces were separated from three shoots of the *ORA59* edited plant and placed on agar and then infected with H-947 PTT isolate. As a result of infection, necrosis appeared in the detached leaf test on the leaves, which were recorded according to the Tekauz scale on days 1, 2, 4, 6, and 9 after infection (Figure 2.). Based on the lesion type, the AUDPC curve of ORA59 shoots was calculated (Figura 3.). The three samples, unable to produce the transcription factor ORA59, showed significantly higher infection than the wild type.

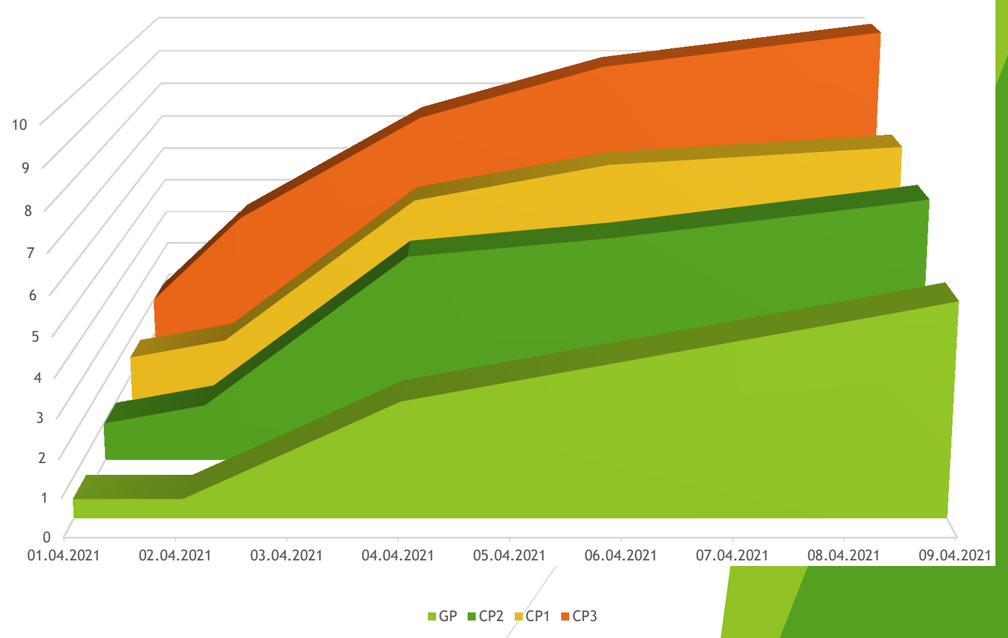

Figure 3.: AUDPC curve of CP1, CP2, CP3 ORA59 edited leaves, wild type GP.

Acknowledgement

The research was supported by NKFI.119276 and GINOP-2.3.2-15-2016-00029.