Proteomic research on the resistance to *Fusarium* head blight in *Triticum aestivum*

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Numerous potential components involved in the resistance to *Fusarium* head blight (FHB) in cereals have been indicated, however, our knowledge regarding this process is still limited and further work is required. Two winter wheat (*Triticum aestivum* L.) lines differing in their levels of resistance to FHB were analyzed to identify the most crucial proteins associated with resistance in this species. The presented work involved analysis of protein abundance in the kernel bulks of more resistant and more susceptible wheat lines using two-dimensional gel electrophoresis and mass spectrometry identification of proteins, which were differentially accumulated between the analyzed lines, after inoculation with *F. culmorum* under field conditions (Fig. 1 and Tab. 1).

Tab. 1. The components of the resistance to FHB in the more resistant (RL) and more susceptible (SL) winter wheat (*Triticum aestivum*) lines and their yield levels in the control conditions.

Winter wheat line	Location	Conditions after inoculation					Control conditions	
		FHBi	% FDK (weight [g])	% FDK (number)	Total kernel number /head	Total kernel weight [g] /head	Total kernel number /head	Total kernel weight [g] /head
RL	Cerekwica	23.7 ± 0.58	45.5 ± 5.14	54.9 ± 5.70	27.9 ± 4.23	0.7 ± 0.25	39.9	2.0
SL		38.3 ± 0.58	83.5 ± 4.89	91.1 ± 4.90	16.6 ± 1.89	0.3 ± 0.06	38.4	1.9
RL	Radzikow	29.5 ± 2.65	19.5 ± 1.37	30.3 ± 10.01	29.1 ± 4.63	0.8 ± 0.13	42.3	1.6
SL		53.3 ± 10.07	34.1 ± 3.68	51.5 ± 6.13	21.9 ± 1.26	0.7 ± 0.07	44.5	1.8

FHBi - *Fusarium* head blight index, FDK - *Fusarium*-damaged kernels, RL - more resistant line, SL - more susceptible line; mean values and standard deviations of each parameter calculated after inoculation (three plots) and data from one plot calculated for the control conditions, are shown.

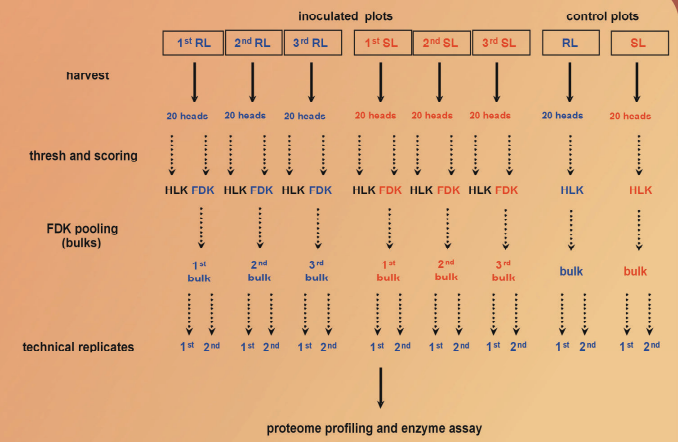

Fig. 1. The diagram demonstrating a workflow of sample preparation for proteome analysis. Abbreviations: FDK, *Fusarium*-damaged kernels; HLK, healthy-looking kernels; RL, line of winter wheat more resistant to FHB; SL, line of winter wheat more susceptible to FHB.

All the obtained two-dimensional patterns were demonstrated to be well-resolved protein maps of kernel proteomes (Fig. 2). Although, 11 proteins were shown to have significantly different abundance between these two groups of plants, only two are likely to be crucial and have a potential role in resistance to FHB (Fig. 3). Monomeric alpha-amylase and dimeric alpha-amylase inhibitors, both highly accumulated in the more resistant line, after inoculation and in the control conditions (Fig. 3). *Fusarium* pathogens can use hydrolytic enzymes, including amylases to colonize kernels and acquire nitrogen and carbon from the endosperm and we suggest that the inhibition of pathogen amylase activity could be one of the most crucial mechanisms to prevent infection progress in the analyzed wheat line with a higher resistance. Alpha-amylase activity assays confirmed this suggestion as it revealed the highest level of enzyme activity, after *F. culmorum* infection, in the line more susceptible to FHB (Fig. 4).

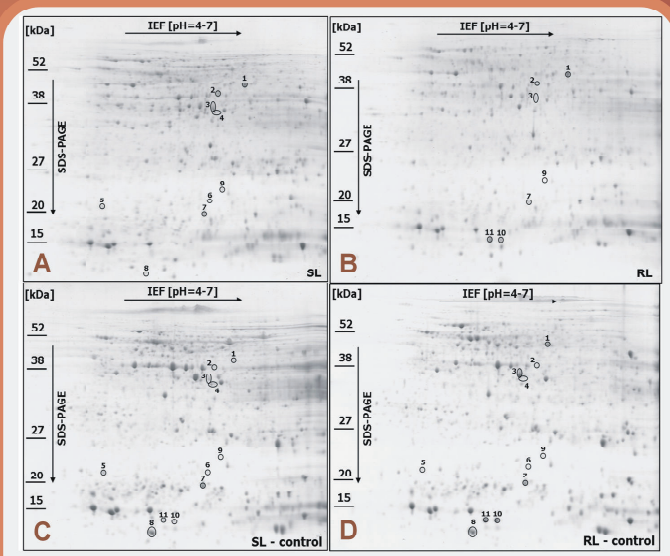

Fig. 2. Representative 2-DE protein maps of winter wheat (*Triticum aestivum*) kernel, A. after *Fusarium culmorum* infection (*Fusarium*-damaged kernels) for the line more susceptible (SL) to FHB, B. for the line more resistant (RL), C. without *Fusarium culmorum* infection for the line more susceptible (SL-control), D. for the line more resistant (RL-control). The spots (1-11) with differentially accumulated proteins ($p < 0.05$) are circled with the solid line.

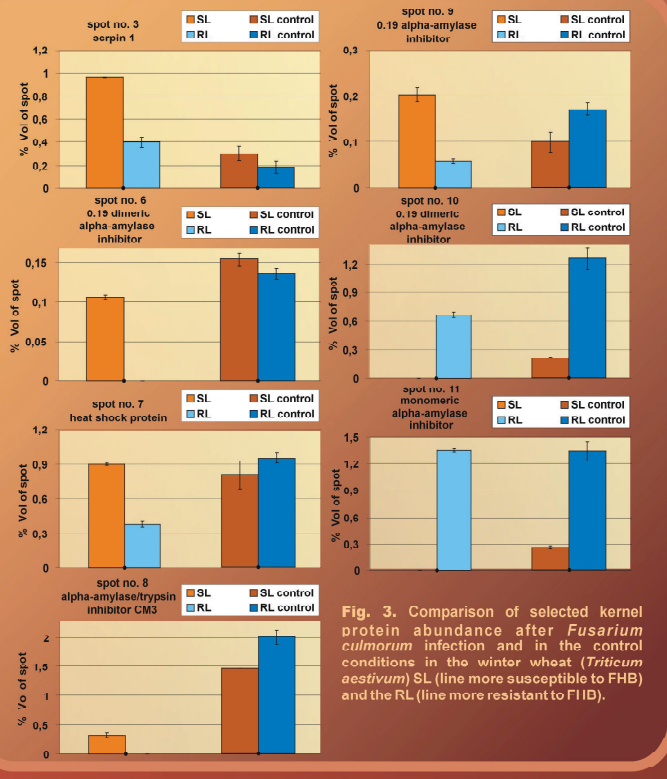

Fig. 3. Comparison of selected kernel protein abundance after *Fusarium culmorum* infection and in the control conditions in the winter wheat (*Triticum aestivum*) SL (line more susceptible to FHB) and the RL (line more resistant to FHB).

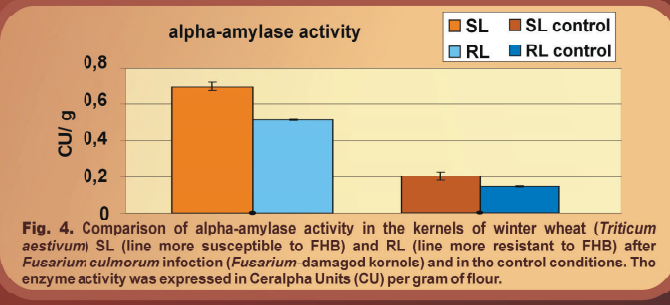

Fig. 4. Comparison of alpha-amylase activity in the kernels of winter wheat (*Triticum aestivum*) SL (line more susceptible to FHB) and RL (line more resistant to FHB) after *Fusarium culmorum* infection (*Fusarium*-damaged kernels) and in the control conditions. The enzyme activity was expressed in Ceralpha Units (CU) per gram of flour.

References

Perlikowski D., Wiśniewska H., Góral T., Kwiatek M., Majka M., Kosmala A. (2014). Identification of kernel proteins associated with the resistance to *Fusarium* head blight in winter wheat (*Triticum aestivum* L.). Plos One 9 (10): e110822. doi:10.1371/journal.pone.0110822.

Acknowledgements

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