**Construction of scFv-antibodies to the active center of the Sunn bug (*Eurygaster integriceps* Put)
gluten-hydrolyzing protease GHP3**

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Key message. This work describes the preparation of immune library and selection of recombinant single-chain (scFv) antibodies to the active center of the gluten-destroying proteinase GHP3 of the *Eurygaster integriceps* Put. bug.

Keywords: Sunn bug, proteinases, wheat, scFv-antibodies, phage display

Proteinases of the Sunn bug *E. integriceps* Put. excreted into the wheat grain during nutrition violates the structure of the gluten proteins responsible for the most important qualities of bread flour, which cause enormous damage to the crop in Russia and other countries.

The aim of the work was to create a genetic construct encoding scFv antibodies to the active center of the *E. integriceps* Put. GHP3 proteinase [1], potentially capable of inhibiting the activity of this protein. These constructs allows potential creation of transgenic plants that are resistant to grain damage caused by *E. integriceps*.

Extraction of total RNA from the spleen of mice immunized by chimeric protein consisting of fragments of GHP3 active center [2] and cDNA synthesis were performed with Trizol reagent and oligo-dT primers. PCR amplification of the entire variety of variable fragments of heavy (VH) and light (VL) chains of murine IgG was performed using 22 pairs of specially selected primers (Progen). A gene library of mice scFv antibodies (VH+VL) was created in pSEX81 phagemid vector. Selection of anti- GHP3 scFv antibodies was performed using phage display technology and Hyperphage M13 K07ΔpIII (Progen). To express the selected scFv fragments in bacteria *E. coli*, their genes were re-cloned from pSEX81 into pOPE101 expression vector.

Previously, the data of structure analysis of the GHP3 proteinase of *E. integriceps* [1] were used to design and synthesize the chimeric gene encoding several copies of polypeptide chain involved in the formation of the active center of the protein [2]. The recombinant product overexpressed in *E. coli* was used to immunize mice, and B-cells (mouse spleen) were isolated after four cycles of immunization, which allow us to obtain mRNA from these cells. This resulted sample, contained all transcripts encoding murine antibodies including GHP3-specific molecules, was used for cDNA synthesis and PCR amplification of the variety of VH and VL fragments of murine IgG. The amplified fragments were inserted into pSEX81 phagemid vector and transformation of *E. coli* by the pool of constructed plasmids resulted in creation of immune library containing about 1 million of bacterial clones carrying scFv genes with different VH/VL combinations.

To select from this library sequences encoding scFv-antibodies to GHP3 proteinase by phage display technology, we used the recombinant chimeric protein and native GHP3 from *E. integriceps* salivary glands immobilized on nitrocellulose membrane. Two rounds of panning were followed by re-cloning of selected scFv genes into pOPE101 expression vector. It allowed us to start the analysis of antigen-binding and GHP3-inhibiting activities of selected antibodies.

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1. Konarev AV et al. J. Agric. Food Chem. 59, 2462-70 (2011)
2. Dolgikh V et al. Food Science & Nutrition. 8, :703-8 (2019)