INDUCEPROT – Induced Accumulation of Recombinant Proteins in Barley Endosperm

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- **Industry partner:** ORF Genetics Ltd (Iceland), Nomad Bioscience GmbH (Germany)

INDUCEPROT wird eine "Treiberlinie" aus Gerste als Produktionsplattform für hochwertige Proteine generieren. Eine Reduzierung des körpereigenen Samenspeicherproteins HORDEIN wird die Produktion und Akkumulation von rekombinanten Targetproteinen begünstigen. Der dreistufige Prozess umfasst a) Korn-spezifische (Endosperm) Expression des Zielproteins, b) induzierte Inaktivierung der HORDEIN-Akkumulation und c) Vergleich mit bestehenden Protein-produktionssystemen. Die Kombination von innovativen in vivo-Protein Modulation und gezielte Mutagenese ermöglichen eine Verlagerung von der Akkumulation von endogenem HORDEIN zur Akkumulation von Zielproteinen durch Veränderung der Wachstumstemperatur der Pflanze. Dies ermöglicht eine Hochskalierung der pflanzlichen Produktion von Proteinen/Arzneimitteln und leistet somit einen Beitrag zur Bioökonomie.

I. Topic, open questions and purpose

Together with two industry partners and three interdisciplinary members of the Science Campus Halle, we will generate a barley ‘driver line’ as a production platform for high-value proteins. Conditional depletion of the endogenous grain storage protein HORDEIN will favour production and accumulation of recombinant target proteins directly in the grain. The three-step process comprises a) grain-specific (endosperm) expression of the target protein, b) induced inactivation of HORDEIN accumulation, and c) comparison to existing protein production systems. The combination of innovative in vivo protein modulation and site-directed mutagenesis tools will allow shifting from an accumulation of endogenous HORDEIN towards the accumulation of target proteins by altering plant growth temperature. This will allow an upscaling of plant-based production of protein pharmaceuticals such as antibodies and enzymes from a system capable of proper posttranslational modification and free of

human pathogens. This pipeline ensures the production of functional and safe products at low cost and simple downstream purification.

II. Theory and methods

The barley grain is an ideal production host for recombinant proteins, bypassing entirely the use of bacteria or animal or human cells. This bioreactor system provides a biochemically inert and risk-free environment as well as a way of long-term protein storage before processing of the target proteins. The applicants (PI1) and other groups showed that recombinant protein accumulation of rather complex proteins like antibodies can be achieved in a competitive amount (Hensel et al 2015). The European company ORF Genetics Ltd. has very successfully commercialized several products using the endosperm-specific expression of various recombinant proteins in barley used in the cosmetic industry and life science research. PI1 use genome engineering (CRISPR RNA/Cas9 endonuclease technology) to alter seed storage protein gene *Hordein B1*. HORDEINs are major storage proteins that accumulate up to 30-50% of the total protein content of the grain which we will exploit by using seed storage promoters and protein bodies as the natural accumulation compartment. PI2 developed a method that allows to conditionally stabilize/destabilize proteins and to turn on/off levels of protein abundance in living plants. This “switchable” protein technique (the low-temperature (lt)-degron) is now patented (WO2016135185A1) and can be used as protein accumulation tool in vivo in various species (Arabidopsis, tobacco, cell culture, yeast, insects; Faden et al. 2016).

III. Results and perspectives

Using the IPK genomic resources and knowledge accumulated over previous targeted mutagenesis projects performed by PI1, target sequences specific for *HorB1* genes were selected and integrated into the modular vector system (Koeppel et al, unpublished). In total four different constructs were generated. Immature embryos of barley cv. Golden Promise was transformed by agrobacteria carrying the respective constructs. Regenerants were tested by PCR for the presence of the target sequence and Cas9 to ensure integration of the necessary elements in the genome. T1 grains of the primary mutants were comprehensively analysed concerning grain size, thousand-grain weight, total protein content and amount of HORDEINs in the grain. As a result, high variation in all parameters tested was observed (exemplarily shown in Fig. 1 for the parameter grain area).

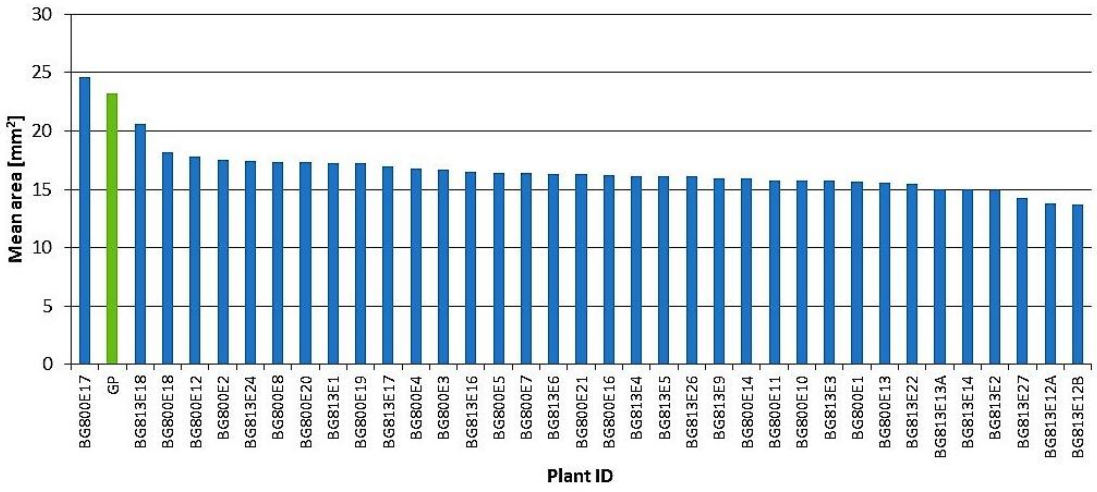

Figure 1: *Grain size area of horb1 mutant families analysed by MARVIN device. All grains of primary mutant plants were evaluated for length and width of the grains. Between 4 and 467 grains were evaluated per family.*

Based on the assumption that reduced storage proteins leads to smaller grains, selected T1 populations were sown and transgene-free plants were identified and further analysed. While in T1 grains as expected just minor reductions in HORDEIN proteins were detected, within the T1 families nice segregation was observed (Fig. 2A).

To test, if the *horb1* mutants were hampered in germination, respective tests were performed. Differences in shoot and root length were detected (Fig. 2B).

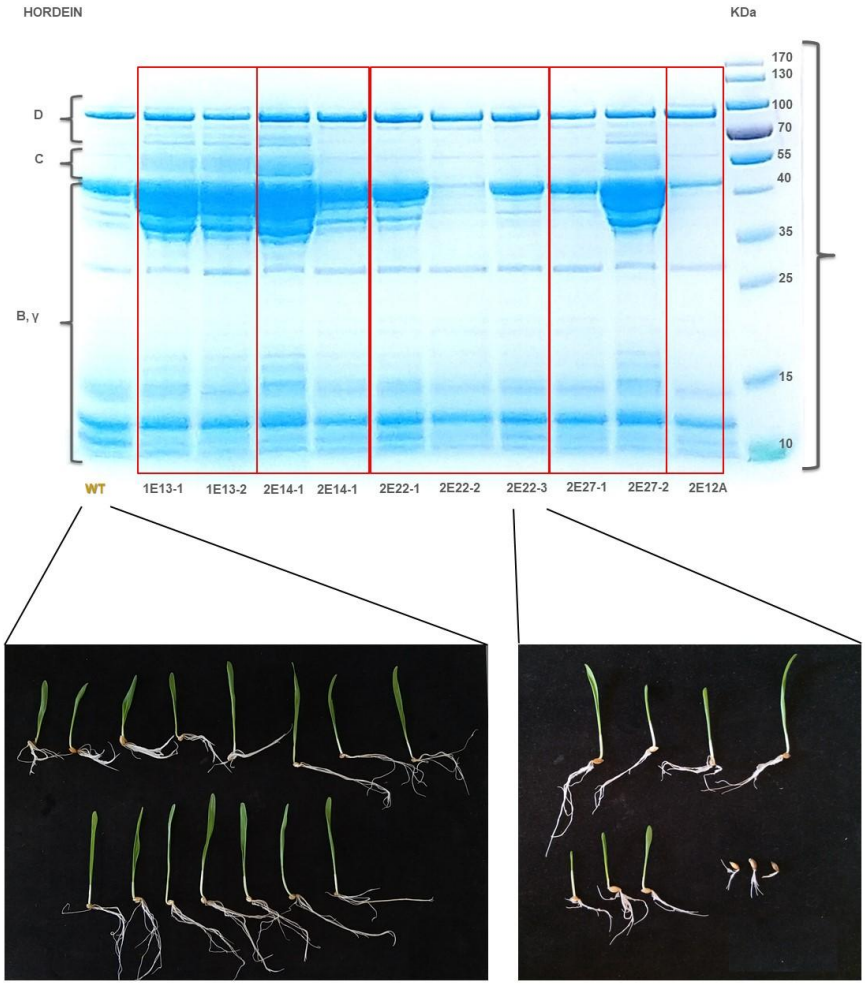

Figure 2: *Analysis of selected horb1 T1 mutant grains. A) Coomassie stain of SDS page of alcohol-soluble protein fraction of segregating T1 families. Proteins were extracted and separated on a 12% PAGE gel before stained in Coomassie staining solution. B) Germination test of selected families. Fifteen grains were surface sterilized and germinated on wet filter paper. Growth were examined 8 days after sowing. (Pouneh Pouramini)*

Comprehensive sequence analysis and phenotyping are necessary to become a consistent picture if the altered growth parameters can be linked to the corresponding genotypes.
For complementation and enabling the temperature-dependent degradation of HORDEIN proteins, respective fusion proteins were synthesized. Cloning to drive the expression of this fusion protein under the control of the endosperm-specific AsGlo1 promoter are ongoing. Preselected *horb1* mutants will be used as donor material for complementation experiments using immature embryos and *Agrobacterium*-mediated transformation. Besides, collaboration partner ORF Genetics Ltd. provided constructs for the endosperm-specific accumulation of the human epidermal growth factor (EGF) and fibroblast growth factor b (FGFb) protein, which will be used as controls to existing protein production platforms established at Iceland.

IV. Index

Publications

Marzec M, Brąszewska-Zalewska A, Hensel G (2020) Prime editing – a new way for genome editing. Trends in Cell Biology, accepted.

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Dissmeyer N (2019) Conditional Protein Function via N-Degron Pathway-Mediated Proteostasis in Stress Physiology. Annu Rev Plant Biol 70: 83-117.

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Mot AC, Prell E, Klecker M, Naumann C, Faden F, Westermann B, and Dissmeyer N (2018) Real-time detection of N-end rule-mediated ubiquitination via fluorescently labeled substrate probes. New Phytol 217: 613-624.

Faden F., Mielke S., and Dissmeyer N. (2018) Switching toxic protein function in life cells. bioRxiv, https://doi.org/10.1101/430439.

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Dissmeyer N (2019) Conditional protein function via N-degron pathway mediated proteostasis in stress physiology. 11th General Meeting of the International Proteolysis Society ´Interfaces in Proteolysis´ - September 2019, Mariánské Lázně/Czech Republic

Dissmeyer N (2019) Conditional protein function via N-degron pathway mediated proteostasis in stress physiology. 9th International Symposium on Plant Senescence - April 1, 2019, Berlin/Germany

Dissmeyer N (2019) Molecular Farming in Plants. Faculty of Natural Sciences I - Biosciences, Martin Luther University of Halle-Wittenberg - November 11, 2018, Halle/Germany

Dissmeyer N (2018) Plant Toxins. Faculty of Natural Sciences I - Biosciences, Martin Luther University of Halle-Wittenberg - November 11, 2018, Halle/Germany

Poster

Pouramini P, G Hensel (2019) Targeted knockout of barley endosperm-specific storage proteins as prerequisite for molecular farming purposes. Workshop Molecular Breeding, September 5–6, Geisenheim/Germany

Pouramini P, G Hensel (2019) Targeted knockout of barley endosperm-specific storage proteins as prerequisite for molecular farming purposes. Plant Biotechnology: Green for Good V, June 10-13, Olomouc/Czech Republic