

From the Professorship of Agrobiotechnology and Risk Assessment of Bio- and Gene Technology of the Faculty of Agriculture and Environmental Sciences

Hypotheses of the Dissertation

## Establishing the greenhouse-production of FGF21-Transferrin in tobacco seeds and leaves for oral treatment of non-alcoholic steatosis hepatis NASH

to obtain the academic degree of Doctor of Agricultural Sciences (doctor agriculturae, Dr. agr.)

at the Faculty of Agriculture and Environmental Sciences of University of Rostock

> submitted by M. Sc. Hsuan-Wu Hou from Leuven

Defence on 14 June 2024

FGF21 is a potential PD drug candidate to treat NAFLD and NASH, but it needs to be exclusively targeted to the liver, which can be achieved by oral administration, since the portal vein goes from the intestine directly to the liver before entering the blood system.

The translocation from the intestine to the blood might be achieved by the fusion to transferrin (FGF21-Tf). However, Tf prolongs the half-life of the fusion protein and promotes circulation in the blood, but after translocation, subsequent separation of FGF21 and Tf can be achieved by introducing a furin cleavage site (FGF21-F-Tf).

Moreover, for oral administration, FGF21 needs to be protected from the acidic conditions in the stomach and therefore plant matrices like leave or seed tissue, providing protection from acids and enzymes in the stomach by *in planta* bioencapsulation, might be utilized via generating transgenic plants, stably expressing FGF21-F-Tf.

However, therapeutic proteins (i.e. biopharmaceuticals) like FGF21 and thus transgenic plants, expressing the therapeutic protein, need to be produced in a contained environment such as greenhouses, but while leave production is already established, only few information are available about seed production in greenhouses.

Consequently, the aim of the PhD thesis was to establish a seed-based oral delivery system for a FGF21-F-Tf fusion protein with the following hypotheses:

- 1. Tobacco is a suitable crop for seed production in greenhouses.
- 2. FGF21-F-Tf fusion protein can be produced in seeds of stably transformed, transgenic tobacco for oral delivery.
- 3. Transient expression can be exploited to optimize the FGF21-f-Tf fusion protein in terms of the accumulation and stability.
- 4. FGF21-F-Tf can be transiently produced for bioavailability and bioactivity studies.