ABSTRACT

The subject of my thesis was to genetically modify a potato for increased resistance against its pathogens and pests. In developing a resistant plant, it is quite common to use the same type of molecules that plants use themselves in their defense reactions. In this work I used the gene *SPI-2* originating from a honeycomb moth (*Galleria mellonella*). The protein SPI-2 is a member of serine protease inhibitors. Since the previous attempts of the team to detect the protein in transformed plants haven´t been successful, the basic form of the gene was modified by adding a Kozak sequence near the start codon, which should have increased the translation initiation and hence increase the level of the protein. Two constructs were prepared for the transformation: *SPI-2-T* a *SPI-2-Y*. They differ by one amino acid, which slightly changes their inhibitory activity. First, the construct *SPI-2-T* was used for a transient transformation of tobacco *Nicotiana benthamiana* by agroinfiltration of its leaves. Then both constructs were used for a stable transformation of *Solanum tuberosum* cv. *Desireé*. The detection of the protein has not been successful, although the inserted gene was transcribed and his sequence was verified by sequencing. It is therefore most likely that the protein has a low stability in the cytoplasm. For that reason a third construct has been prepared (*SPI-2--T-apo*). Its´ product is targeted to the apoplast, where it should be more stable. This construct is now available for experimenting in plants. Adjacent, yet important practical output of the work was finding out that an increased concentration of claforan (from 300 mg/l to 500 mg/l) significantly accelerates regeneration of shoots from calluses after the transformation of potato.

Keywords: *Solanum tuberosum, Agrobacterium tumefaciens, Galleria mellonella*, genetic modification, protease inhibitor SPI-2, pathogen, pest, resistance, Kozak sequence