Abstract

The expression of recombinant proteins in seeds is especially advantageous due to the high concentration of storage proteins in a small volume. Recombinant proteins accumulate in seeds at high levels, remain stable for many years, and because of the low content of alkaloids and other substances in seed environment they can be easily extracted. There is also a minimal risk of contamination.

This bachelor thesis deals with the testing of transient expression of gene constructs directly in maturing soybean seeds. In this work, we have have prepared gene constructs for strong transient expression of the *uidA* gene in plant tissues. The impact of the acetosyringone and dithiothreitol additives and the impact of their combinations on the transient expression and activity of the *uidA* gene have been studied as well. Furthermore, the optimal length and environment of sonication have been studied. Finally, the effectiveness of transient expression by *in vitro* and *in vivo* has also been compared.

It has been confirmed that acetosyringone has a positive effect only on seeds that had not been previously sonicated. With prolonged sonication, the relative activity of the *uidA* gene decreases. Furthermore, the positive effect of dithiothreitol on seeds that had not been sonicated has been found. Higher values of the observed relative activity of the *uidA* gene have also been noted after adding both additives to the infiltration buffer. The optimum length of sonication was 30s for *in vitro* method and 60 s *in vivo* transformation of soybean seeds. It has been observed that sonication in the *Agrobacterium tumefaciens* cell suspension, especially with prolonged sonication lengths, has a negative effect on transient expression of the *uidA* gene. The sonication in water followed by addition of the cell suspension by syringe provides higher values of relative activity of the *uidA* gene. *In vivo* soybean transformation has been shown to provide 4 times more positive seeds and higher relative activity in comparison with the *in vitro* method.

(In Czech)

Keywords: transient expression; *Glycine max;* seed biology; *Agrobacterium tumefaciens*; *uidA* gen