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Production of recombinant proteins in plant systems through transient gene expression technology

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Background/Aim. The transient gene expression (TGE) is a widely used method for plant biopharming that allows the production of high levels of recombinant proteins within days. The aim of the work was to investigate the influence of factors on TGE and to develop an effective protocol for the production of recombinant proteins in plant systems. **Methods.** Expression cassettes carrying the transgenes coding target recombinant proteins and *Agrobacterium*-mediated TGE technology were used to infect plant cells and to produce recombinant proteins. Infected leaves were harvested, proteins were extracted from tissues and protein analysis was performed. **Results.** The efficiency of recombinant protein production in plant systems depends on many factors. One of the most important factors is the natural stability of the target recombinant protein in plant cells, for example, the green fluorescent protein widely used for TGE experiments is a very stable protein, but many valuable biopharmaceuticals are not. The host plant is another important factor: the most effective species is *Nicotiana benthamiana*, but we have found that some other species of the *Nicotiana* genus and sweet basil can also produce high levels of recombinant proteins [1]. We used different expression cassettes for our experiments and found out that the inclusion of multiple genome elements of plant viruses, especially polymerases, in the expression cassettes can significantly increase the final yield of recombinant proteins: production of human interferon α -2b increased 80-fold when we constructed and used an improved viral-based expression vector compared to a simple expression vector. We elucidated that plant physiological state, such as growth stage and leaf

order, affects protein production: a higher yield of recombinant protein was observed in the upper leaves of mature plants [1]. Co-expression of viral suppressors of gene silencing can also greatly increase the production of recombinant proteins. Abiotic factors also affect the content of recombinant protein, for example, at high (above 28 °C) or low (below 18 °C) temperature the synthesis of recombinant protein decreases or even stops. We have also developed a new system for the recombinant protein production in plants through TGE under well-controlled *in vitro* conditions, which is preferable according to GMP regulations [2]. **Conclusions.** The production of recombinant proteins, including valuable ones, in plant systems can reach high levels after optimization of several parameters of biotic and abiotic nature. We selected the factors with the strongest effect on TGE and, after optimizing them, increased the levels of the target recombinant proteins.

Keywords: recombinant proteins, transient gene expression, plant expression systems, *Nicotiana benthamiana*, biopharming.

REFERENCES

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