

<https://doi.org/10.7124/bc.000B4C>

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COMPARISON OF DIFFERENT GENETIC CONSTRUCTS FOR THE PRODUCTION OF PLANT-MADE RECOMBINANT PROTEINS THROUGH TRANSIENT GENE EXPRESSION

Background/Aim. Transient gene expression (TGE) is considered to be a suitable technology for scalable production of recombinant proteins (RP) in plant systems. However, the yield of RP depends on many factors, including efficiency of genetic construct used. The aim of the work was to compare the suitability of genetic constructs with different backbones for high-yield production of various RPs through TGE. **Methods.** TGE, spectrophotometric, biochemical, immunological, and statistical methods were used in this work. **Results.** In our studies, three types of expression cassettes carrying different transgenes were used to produce RP in plants: a simple one where the transgene was controlled by 35S promoter of CaMV (35S), and two improved ones based on genome elements of plant viruses (PVX-based and TVCV-based). In general, the use of improved viral-based genetic constructs (PVX-based and TVCV-based) provides better RP yield than the use of simple ones (35S) [1]. However, yield also depends largely on the natural properties of the RP and the plant host, and the same genetic construct with different transgenes will give different results. For example, when the PVX-based expression cassettes were used, we obtained up to 47% of total soluble cell proteins (TSP) of jellyfish GFP [2], but only 9% of TSP of human interferon [1] and 3% of TSP of bacterial colicin. The 35S and PVX-based genetic constructs provided similar yield of recombinant colicin, but the production of recombinant interferon was 80-fold higher in the case of the PVX-based genetic construct. The TVCV-based genetic construct was most efficient for producing GFP in mutated *Nicotiana benthamiana* and basil plants, but in wild-type *N. benthamiana* plants GFP production was similar for both viral-based genetic constructs used. **Conclusions.** Overall, our results showed that the improved genetic constructs based on viral genome elements, especially TVCV-based constructs, are more suitable for TGE than simple 35S genetic constructs and yield higher levels of targeted RPs. However, the intrinsic properties of the protein molecule and the host plant also significantly influence the yield of RPs.

Keywords: recombinant proteins, transient gene expression, plant expression systems, viral-based genetic constructs.

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Received: 08.06.2026; Accepted: 08.09.2026; Published: 29.09.2026

Citation: Sindarovska Ya.R., Kuchuk M.V. (2026) Comparison of different genetic constructs for the production of plant-made recombinant proteins through transient gene expression. *Biopolymers & Cell*, Special issue(42), 20. <https://doi.org/10.7124/bc.000B4C>

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