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Efficiency of target protein production in a modified Nicotiana benthamiana-based expression system

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Plant-based expression systems, particularly those utilizing *Nicotiana benthamiana* are a promising platform for the production of recombinant proteins due to low costs and the absence of risks associated with mammalian pathogen contamination [1]. However, the widespread application of this technology is limited by the instability caused by degradation mediated by endogenous plant proteases such as phytaspase (NbSBT2) [2]. This is critical for therapeutic antibodies (e.g., anti-HIV antibody PG9), which are highly susceptible to proteolysis [3].

The aim of this study is to evaluate the potential of using plants with reduced activity of key apoplastic proteases, such as NbSBT2, for the production of therapeutically significant proteins.Two *N. benthamiana* lines are used: wild-type (wt) and a previously established stable NbSBT2 knockdown line (KD) carrying an RNAi construct. To evaluate system efficiency, agrobacterium-mediated transient transformation (strain GV3101) was performed using vectors carrying mRFP and the heavy chain of the PG9 antibody (PG9_hc). Proteolytic activity was assessed using the specific fluorescent substrate Ac-VEID-AFC. Protein purification and detection were performed using Protein A affinity chromatography, SDS-PAGE, and Western blot.The results showed that phytaspase activity in the KD line was significantly lower than in the wt. After transient transformation with mRFP construct, a higher density of fluorescent cells was observed in the KD line, indicating that phytaspase knockdown facilitates more efficient T-DNA delivery by agrobacteria, which aligns with the symmetrical results reported for agrobacterium mutagenesis [4]. Furthermore, upon expression of the PG9_hc antibody, both the yield and purity of the target protein were higher in the KD line compared to wild-type plants, as determined by affinity chromatography followed by Western blot analysis.

In conclusion, the modified system based on *N. benthamiana* with NbSBT2 knockdown increases delivery efficiency and reduces proteolytic degradation of recombinant proteins. These findings highlight the KD line as a promising platform for improving the stability and yield of therapeutic proteins, particularly the PG9 antibody.

Источники и литература

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