

## Expression of Tissue Plasminogen Activator in Transgenic Tobacco Plants by Signal Peptides Targeting for Delivery to Apoplast, Endoplasmic Reticulum and Cytosol Spaces

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**Abstract :** Tissue plasminogen activator (tPA) as a serine protease plays an important role in the fibrinolytic system and the dissolution of fibrin clots in human body. The production of this drug in plants such as tobacco could reduce its production costs. In this study, expression of tPA gene and protein targeting to different plant cell compartments, using various signal peptides has been investigated. For high level of expression, Kozak sequence was used after CaMV35S in the beginning of the gene. In order to design the final construction, Extensin, KDEL (amino acid sequence including Lys-Asp-Glu-Leu) and SP ( $\gamma$ -zein signal peptide coding sequence) were used as leader signals to conduct this protein into apoplast, endoplasmic reticulum and cytosol spaces, respectively. Cloned human tPA gene under the CaMV (Cauliflower mosaic virus) 35S promoter and NOS (Nopaline Synthase) terminator into pBI121 plasmid was transferred into tobacco explants by *Agrobacterium tumefaciens* strain LBA4404. The presence and copy number of genes in transgenic tobacco was proved by Southern blotting. Enzymatic activity of the rt-PA protein in transgenic plants compared to non-transgenic plants was confirmed by Zymography assay. The presence and amount of rt-PA recombinant protein in plants was estimated by ELISA analysis on crude protein extract of transgenic tobacco using a specific antibody. The yield of recombinant tPA in transgenic tobacco for SP, KDEL, Extensin signals were counted 0.50, 0.68, 0.69 microgram per milligram of total soluble proteins.

**Keywords :** tPA, recombinant, transgenic, tobacco

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