PRODUCTION OF RECOMBINANT EXTRACLLULAR MATRIX MIMICKING PEPTIDES IN TOBACCO PLANT

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Application of the plant cultivation in the closed system (*in vitro*) technology for production of recombinant proteins provides a cost and product quality effective alternative as compared to traditional sources and it could be easily adapted for the large-scale industrial production [1]. Tobacco is one of the most popular plant-based expression systems, and it is widely used in producing antibodies, pharmaceutical and industrial proteins [2]. Composite mixtures of polymers and animal tissue specific extracellular matrix proteins – such as collagen, fibronectin, laminin – are being used for biomaterial production. However, specific functions of the extracellular matrix proteins could be readily reproduced using relatively short segments of their structure – peptides mimicking extracellular matrix. Therefore, aim of our research was to develop a closed type plant tissue cultivation system using tabaco plants (*Nicotiana tabacum*) dedicated for production for production of the recombinant peptide mimicking fibronectin typeIII domain 9-10 segments (FN9-10).

DNA construct with constant p35S promotor and t35S terminator was prepared and pDGB3_alpha1 based plasmid vector was developed using the GoldenBraid 2.0 cloning system. The construct included a green fluorescent protein (avGFP) marker for the recombinant protein expression analysis, polyhistidine (6xHis) tag for protein affinity purification and a cleavage site for the WELQut protease for the separation of target peptide (Fig. 1). Tobacco leave tissues were transformed using *Agrobacterium tumefaciens*. Selection of tabaco transformants was carried out using different concentrations of antibiotic. It was established that concentration of kanamycin and geneticin suitable for transformed callus tissue selection was 150mg/L and 40 mg/L, respectively. To eliminate agrobacteria, thimetin was used at 300 mg/L with no adverse effect on tabaco callus viability. Further, selection of transformed tabaco callus tissues was carried out based on fluorescence of the avGFP marker.

Expression of the recombinant peptide construct in different transformed callus and cell suspension lines was estimated quantitatively using a combined avGFP fluorescence and fluorescein diacetate based cell metabolic activity assay. Expression of recombinant FN9-10 fragments was further confirmed by immunoblot analysis using antibodies specific to the FN9-10 domain of fibronectin. Purification experiments were carried out and specific binding of the recombinant protein to the Ni-ion affinity matrix was demonstrated using fluorescence and immunoblot analysis.

Fig. 1. DNA construct used for tobacco plant transformation.

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