Abstract

Developing genetically modified plants has been among widely used strategies to produce recombinant proteins. Prior to have a large-scale production of a recombinant protein in plant, there is a need to analyze protein expression in model hosts. Examination of the target protein in a bacterial host Escherichia coli and also transient expression in a model plant could lead us to have a proper insight of the subject.

In this research, interferon alpha 2b gene (INF α2b) as a protein with antiviral and antitumor activities was subjected to be cloned and expressed first in the bacteria then in the plant host. In order to do initial expression of multi-optimized INF α2b in the *Escherichia coli BL21 (DE3)*, target sequences (i.e. IFN or IFN:ELP) were cloned into pET28a+. Following bacterial protein induction (IFN & IFN:ELP), separation of proteins was carried out by SDS-PAGE in order to confirm the presence of the investigated proteins. Then, it was subjected to be analyzed via western blotting. The SDS-PAGE and western blotting analysis indicated that the target protein has been successfully expressed in *E. coli.*

Accordingly, two protein bands around 25 and 48 kDa as monomer and dimer forms of IFN α2b were observed via SDS-PAGE, and confirmed through western blotting. In order to investigate the protein of interest in plant host, the target sequences (IFN or IFN:ELP) were added in the C-

terminal side of the extension signal peptide (Ext) and the pBI121-based constructs were transiently transformed into the Tobacco and alfalfa plants via *Agrobacterium tumefaciens*. According to the real time PCR analysis, the highest level of transcripts obtained from alfalfa leaves containing pBI:IFN:ELP construct.

In proteomics level, analysis was focused on Tobacco and alfalfa leaves transformed with *Agrobacterium* harboring pBI:IFN:ELP construct. In consistent with real time PCR results, the level of expressed ELP-tagged IFN protein in alfalfa was found to be higher than Tobacco.

Purification of target proteins were done through non-chromatography method. The results of this research indicated that the optimized INF α2b gene was successfully expressed in both bacteria and plant host. On the other hand, considering the low level of recombinant protein production in plants, one remarkable achievement of this research would be application of ELP as a promising tag to purify a notable amount of INF α2b from plant.