Impact of ubiquitin moiety at N-terminus on the expression of hG-CSF in transgenic tobacco. Expression cassettes of Constructs H and UH, both without a signal peptide, were presented in panel A and immunoblot analysis of total soluble protein (20 µg) extracted from transgenic H and UH leaves was shown in panel B. For Construct H, i.e. hG-CSF without the addition of signal peptide and ubiquitin, no visible hG-CSF signal was detected in the total soluble protein by immunoblot (panel B, lanes H). For construct UH, it was hypothesized to show similar expression pattern as Construct H because both of them would direct the synthesis of hG-CSF in cytosol where ubiquitin should be cleaved from the final protein product. Interestingly, different from the H transgenic plants, although in relatively low amounts, hG-CSF was detectable in some UH transgenic plants with the same molecular weight (MW, 18.6 kD) as commercially available hG-CSF produced in E.coli (panel B, lanes UH and +), suggesting that ubiquitin moiety at N-terminus may protect the protein from proteolytic attack in transgenic plants. Construct H, hG-CSF chimeric gene without phaseolin signal peptide and ubiquitin sequence; UH, as H but fusion with ubiquitin; RB, right border; LB, left border; CaMV35S pro, cauliflower mosaic virus 35S gene promoter; and NOS ter, nopaline synthase gene terminator. Lanes 1-3, individual plants and +, positive control (10 ng commercial hG-CSF).