Stable Brazzein Protein Production from Transgenic Carrot Cells by Air-lift Bioreactor Culture

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Recently, plant-based recombinant protein production has been considered as a promising system instead of microbial and animal cells. The aim of this study is to establish a stable production system of brazzein protein, a thermostable sweet protein, from carrot cell lines by efficient transformation. By Agrobacterium-mediated transformation, three transgenic lines (TL1, 11, 12) were obtained. The highest gene expression was showed in transgenic cell line TL11, however, considering the cell growth rate, TL12 was selected for the next experiment. In cell growth of the TL12 during culture period, the cell proliferation rapidly increased up to 15 days and showed the maximum dividing stage by entering the logarithmic phase at 5 days of culture. The cell FW was the highest at 1.3-fold in the stationary phase compared to the initial culture. However, the brazzein gene expression tended to decrease from the lag phase to the actively proliferated stage. The brazzein expression gene was also analyzed after treatments of various stress factors, and the effective treatment was ABA and H$_2$O$_2$. In particular, the higher gene expression was obtained 2.5-fold at H$_2$O$_2$ 220 µM, and 2.6-fold at ABA 50 µM compared to that of control. After optimizing the culture conditions, transgenic cells were transferred to the various types of air-lift bioreactor. Among them, column type bioreactor showed the highest biomass of 238.9 g·L$^{-1}$ compared to cone and balloon type bioreactors. The 0.26 mg·g$^{-1}$ of brazzein protein was obtained from 300 mg FW of TL12 by Bradford assay, and it show that around 61.63 g of protein could be produced by a column type bioreactor. Based on the results obtained in this study, we could suggest an efficient sweet protein production system for the food industrial use through stable brazzein production in air-lift bioreactors. (This work was supported by a grant from the Next-Generation BioGreen21 Program (Project No. PJ013689), Rural Development Administration, Republic of Korea)