Development of Functional Transgenic Tomato with Hypotyglyceridemic Action

Lin Huang-chien, You Chi-wen
E-mail: 9511398@mail.dyu.edu.tw

ABSTRACT
Globin digest (GD), hydrolyzed by acidic protease in diet is evidenced to reduce the serum triglyceride levels in blood. Molecular dissection of the underlying mechanism revealed that the peptide "Val-Val-Tyr-Pro", designated as VVYP, was the prominent constituent having hypotyglyceridemic action in GD. Hence, the present study was conducted with a view to developing transgenic tomato plants with hypotyglyceridemic action. Using overlapping PCR, modified ammonium transporter 1 (Amt1) gene of tomato containing 1, 2, or 3 copies of the VVYP peptide was obtained without altering the original protein configuration. After sub-cloning the modified Amt1 gene into the plant expression vector pBI121, Agrobacterium-mediated transformation system was employed to transform the tomato to overexpress Amt1 gene. In addition to tomato, to preview the feasibility of this work, transgenic tobacco plants with hypotyglyceridemic action were also developed. Transgenic plants were selected on medium containing kanamycin as selectable marker and the transformants were analysed for the integration and expression of transgene by using molecular tools.

Keywords: overlapping PCR, serum triglyceride, tomato, transformation, VVYP peptide
基因組DNA抽取法（傳統法）

2.2.17.3 聚合酶鏈鎖反應

2.2.17.4 植物Total RNA抽取法

2.2.17.5 甲醛變性瓊脂凝膠電泳

2.2.17.6 逆轉錄酵素-聚合酵素鏈鎖反應

2.2.18 轉基因株系表現外源性蛋白質之分析

3.1 利用Overlapping PCR技術改造Amt1 gene

3.2 構築pGEM-T/Amt1質體

3.3 AMT1蛋白質結構之分析

3.4 構築植物表現載體pBI121/Amt1

3.5 載體pBI121/Amt1轉型至農桿菌

3.6 基因轉殖菸草之育成

3.6.1 菸草轉殖Amt1基因

3.6.2 菸草轉基因株系馴化及健化

3.6.3 菸草轉基因株系之分子解析

3.7 基因轉殖番茄之育成

3.7.1 番茄轉殖Amt1基因

3.7.2 番茄轉基因株系馴化及健化

3.7.3 番茄轉基因株系之分子解析

3.8 轉基因株系表現外源性蛋白質之分析

4.0 結論

參考文獻

附錄一、Amt1蛋白3D結構預測

附錄二、Amt1蛋白3D結構預測

表目錄

表一 菌種特性及來源

表二 overlapping PCR原理


