人類超氧歧化?基因與蕃茄植株上之表現及特性分析

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摘 要

胞外分泌型的超氧歧化?（extracellular superoxide dismutase, EC-SOD）和其他SOD最大的不同在於它可以將位於N端的訊息胜?鏈切割後再分泌至胞外的基質，其主要存在於細胞間隙與體液中藉以清除其中的超氧化物。本實驗乃利用基因轉殖的方式將來自於人類的EC-SOD轉殖於番茄植株中(Lycopersicon esculentum L. cv. Known-You 301)，觀察其在番茄中是否能正確表現蛋白及活性，及其對轉基因番茄於逆境下的影響。共設計有三種不同SOD轉基因的構築，第一種構築的SOD基因其在N端上仍保留有可將EC-SOD分泌至胞外的訊息胜?鏈；第二種構築是在SOD基因的C端上加入可將EC-SOD累積至endoplasmic reticulum (ER)上的訊息胜?鏈KDEL；第三種SOD基因的構築，其N端及C端上均不含有任何訊息胜?鏈。這些構築分別以農桿菌(Agrobacterium-mediated transformation)及基因槍(particle gun bombardment)的方式進行番茄轉殖。由Known-You 301番茄的再生試驗中知，MS培養基裡添加2 mgL-1 Benzylamino purine (BA)和0.02 mgL-1 naphthaleneacetic acid (NAA)可得到最多的再生芽體，且使用抗生素kanamycin來進行轉殖篩選的最佳濃度為40 mgL-1。總共使用2945個番茄子葉用來進行轉殖，共得到52個再生的擬轉殖株其轉殖率為1.5-1.8 %，以PCR偵測擬轉殖株中nptII及sod基因，共篩選出40個轉基因植株。轉基因植株進一步經RT-PCR及限制酵素SmaI 作用後，均可確認ec-sod在轉錄(transcript)層次上的確有表現。利用西方墨點法(western blotting)進行偵測，結果發現在轉基因植株中存在有兩條清楚的蛋白帶，其分子量大小分別為31 kDa和33 kDa。經由NBT活性染色分析發現，轉基因植株中出現一條非轉基因植物所沒有的透明帶，利用3 mM KCN處理後此透明帶即消失，因此我們推測其應為所轉殖進去的EC-SOD。在逆境的測試中將帶有胞外訊息胜?鏈之SOD轉基因植株8-2品系於37°C下觀察四天，之後分析其SOD蛋白表現，結果發現植物在第二天的逆境後出現了一條約31 kDa的蛋白，此蛋白在持續逆境處理四天後仍可偵測到，甚至將植株恢復到正常環境生長後三天依舊存在。

關鍵詞 : 人類超氧歧化? ; 番茄 ; 抗生素 ; 訊息胜 ; 條非轉 ; 轉殖率 ; 墨點法

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