利用蕪菁嵌紋病毒表現人類胞外型超氧歧化?※褲] = Expression of a human extracellular superoxide dismutase gene by turnip..

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摘 要

以氧維生的生物體受到自然環境或人為因素的影響，在體內會產生超氧自由基 (superoxide; O2.)，超氧歧化?SOD (superoxide dismutase; SOD) 為清除體內O2.的第一道防線，其中胞外型SOD (extracellular SOD; EC-SOD) 大多存在於血液及胞外基質中。由於SOD是具有高經濟價值的蛋白質，因此本實驗，以蕪菁嵌紋病毒 (Turnip mosaic virus; TuMV) 載體攜帶人類胞外型超氧歧化?基因並利用植物系統生產EC-SOD蛋白。實驗首先將TuMV病毒載體中所帶有番茄斑點凋萎病毒 (Tomato spotted wiltvirus; TSWV) NSs基因片段與EC-SOD基因進行置換，置換所用的四種含SOD基因構築均由聚合?鏈鎖反應 (polymerase chain reaction; PCR) 擴增而來，包括其N端是否含分泌至胞外的訊息胜?鏈及C端是否含可累積於內質網的KDEL四元胜?鏈，所得到的四種TuMV重組病毒構築為: TuMV-ECSOD-S (N端帶有訊息胜?鏈)、TuMV-ECSOD-SK (N端帶有訊息胜?鏈及C端帶KDEL胜?鏈)、TuMV-ECSOD-N (N端及C端均不帶有胜?鏈) 及TuMV-ECSOD-K (C端帶有KDEL胜?鏈)。構築完成的四種pTuMV-ECSOD質體，再以機械接種方式接種到單斑寄主奎藜 (Chenopodium quinoa willd.)，四種構築中的三種重組病毒質體所接種的植物約5至7天出現單斑病徵，但接種TuMV-ECSOD-SK的奎藜，其單斑病徵出現的時間約慢一天，奎藜上病斑大小均與TuMV-NSs質體接種的結果相同。之後收集單斑再將其汁液接種至系統性寄主小白菜及青江菜上，約在10至21天可出現系統性病徵，植物葉片呈現嵌紋皺縮及深色壞疽小點，其質地變硬變脆且全株矮化。利用西方墨點法 (western blotting) 分別以EC-SOD及TuMV抗血清偵測罹病葉片，可偵測到有 EC-SOD及病毒鞘蛋白的條帶，顯示所構築含有SOD的TuMV重組病毒具有感染力且可正確表現出EC-SOD蛋白。利用NBT及Riboflavin進行染色並分別以KCN及H2O2進行SOD活性測試時，發現在TuMV-ECSOD接種的植物中其CuZn-SOD比未接種的健康植物或接種不含EC-SOD病毒 (如TuMV-NSs) 的植物相比，均具有較強的SOD活性表現。此外，在CuZn-SOD條帶的下方也出現一條淡淡的SOD條帶，推測可能是EC-SOD蛋白。另外也以巴拉刈溶液噴灑於EC-SOD重組病毒接種的青江菜葉片上，於噴灑後26、52及78小時可發現野生型TuMV接種的青江菜及未接種的健康植株明顯比接種TuMV-ECSOD-N及TuMV-ECSOD-K植株萎凋許多，證明了EC-SOD可降低植株被巴拉刈的傷害。

關鍵詞 : 人類胞外型超氧歧化? ; 蕪菁嵌紋病毒載體 ; 奎藜 ; 青江菜 ; 巴拉刈

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