利用莖頂培養與農桿菌基因轉殖方式發展轉花色基因之菊花

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摘 要

由於植物莖頂培養具有高度的分化全能性，遺傳性狀穩定且可使培殖體快速增殖，因此本實驗乃 利用莖頂培養技術生產無變異之菊花種苗，來提供基因轉殖材料。另外亦將CHI及DFR花色基因轉殖至 菊花中，以觀察其對菊花花色表現的影響。由田間採得之菊花植株，經次氯酸鈉消毒後，切取其莖頂 進行紙橋培養，約15天至2個月左右可得菊花芽體，再移殖至固態MS培養基中進行大量繁殖。由於所切 莖頂大小會影響存活率，本實驗共得158無菌菊花苗，存活率為39 %。此外，由田間採集來及莖頂培養 所得之菊花植株，利用反轉錄聚合?連鎖反應檢測菊花矮化類病毒（Chrysanthemum stunt viroid, CSVd），均發現有CSVd的感染。菊花轉殖前的再生培養基，以MS加入1 mg/l BA及0.5 mg/l NAA的條件 對黃秀芳及白東陽兩種品種的菊花葉片的再生能力最佳，28個黃秀芳培殖體均可全部再生，平均每個 培殖體可再生出6.3株不定芽；48個白東陽培殖體中有36個培殖體有再生能力，平均每個培殖體可再生 出2.7株不定芽。而在菊花對hygromycine最低致死濃度的測試中發現，10 mg/l 的濃度即可使葉片停 止再生，並於第28天全部死亡，因此目前所進行之轉殖實驗即以基礎MS培養基含1 mg/l BA及 0.5 mg/l NAA及10 mg/l之hygromycine進行篩選。而轉殖過程中菊花培殖體與農桿菌的共培養時間， 則以四天為最佳。農桿菌的轉殖試驗中，共取109個菊花培殖體進行CHI基因轉殖，經篩選後得25個再 生芽體，以CHI專一引子進行PCR分析，有9個芽體可偵測到CHI基因，其大小為541 bp，此PCR產物再以 南方墨點法分析，更進一步證實其為轉基因植物。另外取83個培殖體進行DFR基因轉殖，經篩選後共得 13個再生芽體，經PCR分析，有9個再生芽體可偵測到相對的DFR基因，其大小為586 bp，以南方墨點法 分析，也均可偵測到正反應。為了進一步了解轉基因併入染色體的數目，因此再進行植物基因體南方 點漬法，發現所轉殖的轉DFR基因菊花，其基因體均可偵測到DFR基因的存在，而且均只有單一個插入 位置，目前轉基因菊花已形成花苞，於溫室中觀察其開花情形。

關鍵詞 : 莖頂培養，菊花矮化類病毒，反轉錄聚合?連鎖反應，農桿菌轉殖，共培養，南方點漬法。

目錄

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