Chinese Agron. J. 2: 69-81 (1992)
Chinese Society of Agronomy © 1992

# 洋菜及糖類誘導之水份逆境對水稻花藥培養的影響

温世政　莊志弘　葉常青　蔡新聲

台灣省農業試驗所

**摘要**：水稻花藥培養之培養基，洋菜濃度提高至1.8%，有利於提高癒合組織之形成及綠苗之分化率。培養基中6%蔗糖配合適量的甘露醇(0.53-1.06%)，亦有助於花藥癒合組織的形成及分化。液體培養基雖可大幅提高癒合組織形成率，但因所形成之癒合組織分化能力過低，因此不適於實際之水稻育種。

**關鍵語**：水稻、花藥培養、水份逆境

**Effect of agar- and sugar-induced water stress on rice anther culture**

S. C. Wen, C. H. Chuang, C. C. Yeh and H. S. Tsay

Department of Agronomy, Taiwan Agricultural Research Institute, Taichung, Taiwan 41301, Republic of China

**Abstract. Rice anthers at uninucleate stage were cultured in medium containing high concentrations of agar and sugars for water stress treatment. The results indicated that the callus and green plant formation ability increased significantly on the treatment of agar-induced water stress. Best result was obtained from anthers cultured in a medium containing 1.8% agar. Medium containing 6% sucrose and 0.53–1.06% mannitol was beneficial to callus induction as well as the subsequent differentiation of green plants. Although high percentage of callus induction was recorded, liquid suspension culture was not recommended for rice anther culture due to the low differentiation rate of the produced callus.**

**Key words: Rice, Anther culture, Water stress.**

## 前　言

水稻(*Oryza sativa* L.)屬一年生禾本科雌雄同花之天然自交作物，爲我國最主要的糧

食作物。本省的水稻有粳型和籼型兩種，籼粳稻品種間，可以傳統雜交方式獲得後代，但其雜種後代性狀的連續分離和部分不稔性，限制了遺傳性狀的互換與後代品種的迅速固定(Woo and Tung 1972)。自1968年Niizeki and Oono首先在水稻花藥培養成功後，以花藥培養產生單倍體的水稻育種技術，已廣爲先進國家採用。利用單倍體進行染色體倍加，能迅速獲得同質雙倍體植株，理論上可減少5～6代固定雜種$F_1$成純系的時間，避免雜種子代發生特性分離的現象，且可提高選拔效率，是目前世界上頗爲流行的作物品種改良新方法(Tsay 1986)。

水稻花藥培養癒合組織的誘導及綠苗分化受許多因素的影響，如花藥培養前的低溫、高溫及離心處理、小胞子發育時期、培養基成分、滲透壓、培養環境等(Chen et al. 1986)。而提高花藥癒合組織之誘導率，癒合組織之綠苗分化率及增加試管苗移植成活率，是使水稻花藥培養技術實用化的必備條件。近年來許多學者以逆境處理(stress treatment)提高癒合組織再生植株的能力，例如高濃度的蔗糖能增加玉米(Lu et al. 1983)、黑麥(Lu et al. 1984)及甘蔗(Ahloowalis and Maretzki 1983)等癒合組織再生植株之能力。利用不同濃度的洋菜(agar)和甘露醇(mannitol)調節培養基的水分逆境，可使水稻未成熟胚癒合組織再生芽和根的能力提高(Lai and Liu 1988, Liu and Lai 1991)。許多報告亦認爲稍高濃度的蔗糖(5～9%)，對禾本科作物花藥癒合組織的形成有促進的效果(Chen 1978, Ouyang et al. 1973, Tsay et al. 1986)，因此尋求一提高水稻花藥癒合組織形成率與綠苗分化率的水分逆境處理，即爲本試驗之研究動機。

## 材料與方法

### 花藥的消毒與接種

花藥培養所用材料爲粳稻台農67號品種。於劍葉葉耳距下位葉之葉耳間長約3-5cm時連同劍葉取下，以溼潤之衛生紙包裹於稻桿基部，裝入塑膠袋再置於10±1°C恆溫箱中進行一週低溫前處理。接種前先以70%酒精擦拭葉鞘外部，取出幼穗浸漬於70%酒精中30秒，再以0.5%次氯酸鈉消毒3分鐘，經無菌水沖洗3-4次後，將屬於單核期之花藥，以Chen and Chen(1979)所述之方法接種於癒合組織誘導培養基上。

### 培養基種類與培養環境

誘導水稻花藥形成癒合組織之固體培養基，其成份爲MS(Murashige and Skoog 1962)培養基之主要有機成份，$N_6$ (Chu et al. 1975)培養基之無機鹽類，加上4 mg/l NAA, 2 mg/l kinetin, 6%蔗糖及0.9%洋菜(Difco agar)（簡稱NK培養基）。誘導水稻花藥形成癒合組織之液體培養基，其成份與上述NK培養基相同，唯不含洋菜或以10%Ficoll取代洋菜。誘導癒合組織分化之培養基，其成份爲MS培養基，加上2 mg/l

kinetin, 0.5 mg/l NAA, 40 mg/l adenine sulfate, 3%蔗糖及0.9%洋菜（簡稱R-2培養基）。以上三種培養基之pH皆爲5.7±0.1 (Chen et al. 1982)。

在上述NK固體培養基中，加入表二所示不同濃度之洋菜及表六所示不同濃度組合之蔗糖及甘露醇；在NK液體培養基中加入Ficoll及表十所示不同濃度組合之蔗糖及甘露醇；以上述培養基誘導水稻花藥癒合組織，再將所形成的癒合組織接種於R-2分化培養基上，進行植株分化之誘導。由所得結果詳細探討水分逆境處理對水稻花藥培養之影響。

將接種後的花藥置於恆溫25±1°C，黑暗下培養，約三十天後開始形成癒合組織，60天後調查癒合組織形成率。而誘導癒合組織分化植株之方式，則取自花藥長出10日齡，大小約2～4mm的癒合組織，接種於R-2分化培養基上，置於25±1°C之恆溫、光照1,500 lux (100 $\mu$E/ms)，每日照光16小時的環境下培養，4週後調查綠苗及白苗之植株分化率。

各項資料以二項分佈統計法，計算95%顯著水準之信賴介值(95% confidence limits)，供不同處理間之比較。

## 結 果

### 不同洋菜濃度對水稻花藥培養的影響

1990年一期作之試驗結果得知，在0.6, 0.9, 1.2及1.5%四種洋菜濃度處理中，水稻花藥形成癒合組織的能力並無明顯差異，癒合組織的誘導率大約在45～51%之間（表1）；若將不同洋菜濃度誘導所得之水稻花藥癒合組織，繼代培養於R-2分化培養基上進行植株之誘導，則發現較高濃度的洋菜所誘導的水稻花藥癒合組織，綠苗或白苗的形成能力都較高（表2），表2顯示0.6%洋菜所誘導的水稻花藥癒合組織綠苗分化率僅4.2%，白苗分化率僅2.7%，總分化率爲6.8%。隨著洋菜濃度的增高，癒合組織分化成植株的百分比大增，洋菜濃度至1.5%時，有28.6%的綠苗形成率，13.9%的白苗形成率，總分化率爲

**Table 1.** Effect of agar concentration on callus formation of rice anther culture (1st crop, 1990)*

| Agar conc. (%) | No. of anthers cultured | Anthers producing callus | | |
|---|---|---|---|---|
| | | Number | % | 95%CL** |
| 0.6 | 581 | 296 | 50.9 | 45-57 |
| 0.9 | 565 | 278 | 49.2 | 44-54 |
| 1.2 | 540 | 242 | 44.8 | 40-50 |
| 1.5 | 581 | 265 | 45.6 | 41-51 |

*Callus induction medium: $N_6$ inorganic salts and MS organic substances supplemented with 2 mg/l kinetin, 4 mg/l NAA and 6% sucrose.
**95% confidence limits.

**Table 2.** Effect of agar concentration of the callus induction medium on the regeneration ability of rice anther-derived callus (1st crop, 1990)*

| Agar conc. (%) | No. of callus cultured | Callus producing green plants | | | Callus producing albino plants | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | 95%CL** | No. | % | 95%CL | No. | % | 95%CL |
| 0.6 | 262 | 11 | 4.2 | 2- 7 | 7 | 2.7 | 1- 6 | 18 | 6.8 | 4-11 |
| 0.9 | 246 | 31 | 12.6 | 9-18 | 12 | 4.9 | 3- 9 | 43 | 17.7 | 13-23 |
| 1.2 | 236 | 28 | 11.9 | 8-17 | 24 | 10.2 | 7-14 | 52 | 22.0 | 17-28 |
| 1.5 | 266 | 76 | 28.6 | 23-35 | 37 | 13.9 | 10-19 | 113 | 38.7 | 33-45 |

*10-day old anther-derived callus induced from the media shown in Table 1 were transferred to R-2 medium for plant regeneration.
**95% confidence limits.

38.7%，幾乎爲0.6%洋菜濃度處理的五倍以上。

由於表2的資料顯示，1.5%洋菜濃度處理所誘導的水稻花藥癒合組織分化率最高，且分化率有隨洋菜濃度升高而增加的趨勢，因此有必要將洋菜濃度的上限加大，所以於1990年二期作再以0.9, 1.2, 1.5, 1.8及2.1%五種不同洋菜濃度，測試其對水稻花藥培養之影響，結果如表3所示，在0.9～1.8%的洋菜濃度範圍內，隨著洋菜濃度的增加，水稻花藥癒合組織的誘導率漸增，而2.1%的洋菜濃度，則顯現濃度過高之害。其中以1.8%洋菜濃度，水稻花藥癒合組織形成率最高，可達62.4%。將表3不同洋菜濃度誘導所得之水稻花藥癒合組織，繼代培養於R-2分化培養基上進行植株之誘導，結果如表4所示，綠苗的形成率有隨洋菜濃度升高而增多的趨勢，其中以2.1%洋菜所誘導之癒合組織綠苗形成率最高，達33.3%。連同白苗之總分化率爲61.1%。綜合表1至表4的結果可知，爲兼顧癒合組織之誘導率及綠苗分化率，誘導水稻花藥癒合組織的培養基以含有稍高濃度的洋菜（約1.8%）較爲適當。

**不同糖類及濃度對水稻花藥培養的影響**

表5的資料顯示，在不含甘露醇的情況下，6%的蔗糖對水稻花藥癒合組織的誘導優於3%，若能在含6%蔗糖的培養基中，加上0.53%的甘露醇則效果更佳，癒合組織誘導率可達51.4%；甘露醇濃度超過1.6%以上則呈現濃度過高之害。將不同糖類及濃度所誘得的水稻花藥癒合組織，繼代培養於R-2分化培養基上進行植株之誘導。結果如表6所示，水稻花藥癒合組織的綠苗形成率也以6%蔗糖優於3%。若能配合0.53%的甘露醇則更能使綠苗的形成率由13.2%升高至21.9%。綜合表5與6的結果可知，誘導水稻花藥癒合組織的培養基以6%的蔗糖配合0.53%甘露醇較佳，由此培養基形成之癒合組織所具有的綠苗分化力亦最強。

因1990年一期作測試之甘露醇濃度級距過大，所以於1990年二期作，設計如表7所示

**Table 3.** Effect of agar concentration on callus formation of rice anther culture (2nd crop, 1990)*

| Agar conc. (%) | No. of anthers cultured | Anthers producing callus | | |
|---|---|---|---|---|
| | | Number | % | 95%CL** |
| 0.9 | 458 | 203 | 44.3 | 39-49 |
| 1.2 | 472 | 271 | 57.4 | 52-62 |
| 1.5 | 504 | 266 | 52.8 | 48-58 |
| 1.8 | 466 | 291 | 62.4 | 57-67 |
| 2.1 | 471 | 228 | 48.4 | 43-53 |

*Callus induction medium: same as Table 1.
**95% confidence limits.

**Table 4.** Effect of agar concentration of the callus-induction medium on the regeneration ability of rice anther-derived callus (2nd crop, 1990)*

| Agar conc. (%) | No. of callus cultured | Callus producing green plants | | | Callus producing albino plants | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | 95%CL** | No. | % | 95%CL | No. | % | 95%CL |
| 0.9 | 156 | 34 | 21.8 | 15-30 | 31 | 19.9 | 14-28 | 65 | 41.7 | 33-51 |
| 1.2 | 186 | 39 | 21.0 | 15-28 | 20 | 10.8 | 6-18 | 59 | 31.7 | 25-40 |
| 1.5 | 150 | 39 | 26.0 | 19-35 | 34 | 21.5 | 15-30 | 73 | 48.7 | 40-58 |
| 1.8 | 178 | 50 | 28.1 | 21-36 | 30 | 16.9 | 11-24 | 80 | 44.9 | 37-53 |
| 2.1 | 162 | 54 | 33.3 | 25-41 | 45 | 27.8 | 20-36 | 99 | 61.1 | 52-70 |

*10-day old anther-derived callus induced from the media shown in Table 3 were transferred to R-2 medium for plant regeneration.
**95% confidence limits.

**Table 5.** Effect of sugar concentration on callus formation of rice anther culture (1st crop, 1990)*

| Sugar conc. (%) | | No. of anthers cultured | Anthers producing callus | | |
|---|---|---|---|---|---|
| Sucrose | Mannitol | | No. | % | 95%CL** |
| 3 | 0 | 507 | 186 | 36.7 | 32-42 |
| 3 | 0.53 | 591 | 260 | 44.0 | 39-49 |
| 3 | 1.60 | 546 | 166 | 30.4 | 25-35 |
| 3 | 4.80 | 447 | 39 | 8.7 | 6-12 |
| 6 | 0 | 576 | 246 | 42.7 | 38-48 |
| 6 | 0.53 | 473 | 243 | 51.4 | 46-56 |
| 6 | 1.60 | 435 | 186 | 42.8 | 38-48 |
| 6 | 4.80 | 517 | 63 | 12.2 | 9-16 |

*Callus induction medium: $N_6$ inorganic salts and MS organic substances supplemented with 2 mg/l kinetin, 4 mg/l NAA and 0.9% agar.
**95% confidence limits.

**Table 6.** Effect of sugar concentration of the callus-induction media on the regeneration ability of rice anther-derived callus (1st crop, 1990)*

| Sugar conc. (%) | | No. of callus cultured | Callus producing green plants | | | Callus producing albino plants | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sucrose | Mannitol | | No. | % | 95%CL** | No. | % | 95%CL | No. | % | 95%CL |
| 3 | 0 | 196 | 23 | 11.7 | 7-18 | 11 | 5.6 | 3-10 | 34 | 17.3 | 12-23 |
| 3 | 0.53 | 198 | 19 | 9.6 | 6-16 | 13 | 6.6 | 4-11 | 32 | 16.1 | 11-12 |
| 3 | 1.60 | 138 | 15 | 10.9 | 6-18 | 7 | 5.1 | 2-11 | 22 | 15.9 | 10-24 |
| 3 | 4.80 | 66 | 6 | 9.1 | 3-19 | 4 | 6.1 | 1-16 | 10 | 15.1 | 7-27 |
| 6 | 0 | 234 | 31 | 13.2 | 9-18 | 14 | 6.0 | 3-10 | 45 | 19.2 | 14-24 |
| 6 | 0.53 | 192 | 42 | 21.9 | 15-26 | 21 | 10.9 | 7-17 | 53 | 27.6 | 20-36 |
| 6 | 1.60 | 166 | 30 | 18.1 | 12-25 | 14 | 8.4 | 4-14 | 44 | 26.5 | 20-36 |
| 6 | 4.80 | 86 | 8 | 9.3 | 4-17 | 16 | 18.6 | 11-29 | 24 | 27.9 | 18-40 |

*10-day old anther-derived callus induced from different media shown in Table 5 were transferred to R-2 medium for plant regeneration.
**95% confidence limits.

**Table 7.** Effect of mannitol concentration on callus formation of rice anther culture (2nd crop, 1990)*

| Mannitol conc. (%) | No. of anthers cultured | Anthers producing callus | | |
|---|---|---|---|---|
| | | No. | % | 95%CL** |
| 0 | 353 | 193 | 54.7 | 49-61 |
| 0.53 | 487 | 253 | 52.0 | 47-57 |
| 1.06 | 454 | 268 | 59.0 | 54-64 |
| 1.60 | 461 | 171 | 37.1 | 32-42 |
| 2.13 | 514 | 167 | 32.5 | 27-38 |

*Callus induction medium: $N_6$ inorganic salts and MS organic substances supplemented with 2 mg/l kinetin, 4 mg/l NAA, 6% sucrose and 0.9% agar.
**95% confidence limits.

之培養基，測試五種不同甘露醇濃度對水稻花藥培養之影響。結果如表7,8顯示，誘導水稻花藥癒合組織以培養基中加入1.06％的甘露醇較爲適當，濃度在1.60％以上則有不利的影響；而綠苗之分化率則以0.53～1.60％之甘露醇濃度較佳，但含有1.06％甘露醇的培養基中所誘導的癒合組織，其綠苗分化率卻低於其他處理，其原因有待查明，初步推斷可能和取材之差異及不同人員接種及操作有關。綜合表5至表8的結果可發現，當培養基中不含甘露醇時，加入6％蔗糖對水稻花藥癒合組織之誘導及綠苗之分化均較3％的蔗糖濃度爲佳。以6％蔗糖配合0.53～1.06％甘露醇，則對綠苗之形成有利，當甘露醇濃度高於2.13％則顯現不利的結果。爲兼顧水稻花藥癒合組織形成率及綠苗分化率，培養基中宜以6％蔗糖配合0.53～1.06％之甘露醇較爲適當。

**Table 8.** Effect of mannitol concentration of the callus-induction media on the regeneration ability of rice anther-derived callus (2nd crop, 1990)*

| Mannitol conc. (%) | No. of callus cultured | Callus producing green plants | | | Callus producing albino plants | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | 95%CL** | No. | % | 95%CL | No. | % | 95%CL |
| 0 | 212 | 48 | 22.6 | 17-30 | 31 | 14.6 | 10-20 | 79 | 37.3 | 30-44 |
| 0.53 | 166 | 44 | 26.5 | 19-36 | 27 | 16.3 | 10-23 | 72 | 43.3 | 34-52 |
| 1.06 | 184 | 33 | 17.9 | 12-25 | 22 | 12.0 | 7-18 | 55 | 29.9 | 22-38 |
| 1.60 | 66 | 21 | 31.8 | 21-45 | 17 | 25.8 | 16-40 | 38 | 57.6 | 45-68 |
| 2.13 | 110 | 22 | 20.0 | 13-29 | 28 | 25.5 | 17-36 | 50 | 45.5 | 35-55 |

*10-day old anther-derived callus induced from different media shown in Table 7 were transferred to R-2 medium for plant regeneration.
**95% confidence limits.

## 不同糖類及濃度組合之液體培養基對水稻花藥培養的影響

表 9 顯示水稻花藥癒合組織之誘導，液體培養基優於固體培養基（表9之1號處理和表1比較），含有6% 蔗糖之液體培養基，花藥癒合組織誘導率爲62.9%，若能配合10%Ficoll更可使誘導率提高至81.4%（表9之6號處理），若和甘露醇搭配則宜適度將蔗糖濃度減爲3%。例如0.53～1.60% 甘露醇配合10%Ficoll及3% 蔗糖之培養基，癒合組織誘導率高達90% 以上（表9之3,4號處理），而6% 蔗糖和相同濃度之甘露醇及Ficoll搭配的培養基，癒合組織誘導率則降爲59.9～76.8%，超過4.8% 以上之甘露醇則顯現濃度過高之害，不論蔗糖及Ficoll如何組合，癒合組織之誘導率，均低於20%（表9之5及9號處理）。將

**Table 9.** Effect of different sugar concentration in liquid medium on callus formation of rice anther culture (1st crop, 1990)*

| Treatment code | Ficoll (%) | Sugar conc. (%) | | No. of anthers cultured | Anthers producing callus | | |
|---|---|---|---|---|---|---|---|
| | | Sucrose | Mannitol | | No. | % | 95%CL** |
| 1 | 0 | 6 | 0 | 518 | 326 | 62.9 | 58-68 |
| 2 | 10 | 3 | 0 | 595 | 485 | 81.5 | 76-86 |
| 3 | 10 | 3 | 0.53 | 442 | 415 | 93.9 | 91-97 |
| 4 | 10 | 3 | 1.60 | 526 | 477 | 90.7 | 88-94 |
| 5 | 10 | 3 | 4.80 | 507 | 96 | 18.9 | 15-23 |
| 6 | 10 | 6 | 0 | 473 | 385 | 81.4 | 77-85 |
| 7 | 10 | 6 | 0.53 | 542 | 416 | 76.8 | 72-81 |
| 8 | 10 | 6 | 1.60 | 446 | 267 | 59.9 | 55-65 |
| 9 | 10 | 6 | 4.80 | 452 | 79 | 17.5 | 13-22 |

*Callus induction medium: $N_6$ inorganic salts and MS organic substances supplemented with 2 mg/l kinetin, 4 mg/l NAA and 6% sucrose.
**95% confidence limits.

**Table 10.** Effect of different sugar concentration of callus-induction liquid medium on the regeneration ability of rice anther-derived callus (1st crop, 1990)*

| Composition of callus induced medium (%) | | | No. of callus cultured | Callus producing green plants | | | Callus producing albino plants | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ficoll | Sucrose | Mannitol | | No. | % | 95%CL** | No. | % | 95%CL | No. | % | 95%CL |
| 0 | 6 | 0 | 208 | 6 | 2.9 | 1- 6 | 8 | 3.8 | 2- 7 | 14 | 6.7 | 4-12 |
| 10 | 3 | 0 | 254 | 3 | 1.2 | 0- 5 | 4 | 1.6 | 1- 5 | 7 | 2.7 | 1- 6 |
| 10 | 3 | 0.53 | 272 | 9 | 3.3 | 1- 6 | 5 | 1.8 | 1- 5 | 14 | 5.1 | 3- 9 |
| 10 | 3 | 1.60 | 266 | 11 | 4.1 | 2- 7 | 6 | 2.3 | 1- 5 | 17 | 6.4 | 3-10 |
| 10 | 3 | 4.80 | 44 | 2 | 4.5 | 0-15 | 1 | 2.3 | 3-11 | 3 | 6.8 | 1-19 |
| 10 | 6 | 0 | 262 | 9 | 3.4 | 1- 5 | 9 | 3.4 | 1- 6 | 18 | 6.9 | 4-11 |
| 10 | 6 | 0.53 | 292 | 10 | 3.4 | 1- 6 | 15 | 5.1 | 3- 9 | 25 | 8.6 | 6-12 |
| 10 | 6 | 1.60 | 256 | 23 | 9.0 | 6-13 | 30 | 11.2 | 7-16 | 53 | 20.7 | 15-26 |
| 10 | 6 | 4.80 | 54 | 0 | 0.0 | 0- 7 | 8 | 1.5 | 0-11 | 8 | 14.8 | 7-28 |

*10-day old anther-derived callus induced from different media shown in Table 9 were transferred to R-2 medium for plant regeneration.
**95% confidence limits.

表9所獲得之水稻花藥癒合組織，繼代培養於R-2分化培養基上進行植株之誘導，結果如表10所示，所有處理之綠苗形成率均低於10%，此種結果顯示液體培養基雖對水稻花藥癒合組織之誘導有利，但卻不利於植株之分化，在九種誘導癒合組織之液體培養基中，只有在6%蔗糖、10%Ficoll及1.6%甘露醇的培養基，植株分化率爲9%，其餘八種處理之植株分化率均低於5%。

## 討　論

### 不同洋菜濃度對水稻花藥培養的影響

植物組織培養是一種藉人工培養基誘導培植體形成癒合組織或分化的生物技術，因此能否篩選到合適的培養基將影響組織培養的成敗。一般植物組織培養常使用洋菜作爲膠體化物質，洋菜在培養基中除了可提供支持力量外，並可維持滲透壓，故對組織培養之影響不可忽視。Arnold and Erikson (1984)及Pasqualetto et al. (1986, 1988)報導指出，提高洋菜濃度可增加癒合組織分化植株之能力，及減少形成的植物體產生玻璃質化(vitrification or glassification)之現象。Lai and Liu (1988)利用不同濃度的甘露醇(mannitol)和洋菜(agar)調節培養基的水分逆境，可使水稻未成熟胚癒合組織再生芽和根的能力提高。本研究的結果顯示，提高洋菜濃度至1.8%對於水稻花藥癒合組織的誘導有幫助，而且可以增加由此培養基所誘得癒合組織之植株分化率，對實際將花藥培養技術應用於育種工作

上極有幫助。

**不同糖類及濃度對水稻花藥培養的影響**

培養基中糖類的主要功能是提供能量來源及調節滲透壓潛勢(osmotic potential)。禾穀類作物組織培養癒合組織的誘導一般都使用蔗糖，用量約爲2～6%(Vasil and Vasil 1985)。Mercy and Zapata (1986)指出高濃度糖類能增加水稻花藥誘導擬胚狀癒合組織的形成。Lu et al. (1983)進行七個玉米雜交品種之未成熟胚培養，發現6～12%高濃度的蔗糖對擬胚狀癒合組織的形成有幫助。許多報告亦認爲稍高濃度的蔗糖(5～9%)，對禾本科作物花藥癒合組織的形成有促進的效果，Wang et al. (1974)及Chaleff and Stolarz (1981)則以爲此種促進效果可能與培養基的滲透壓有關。本研究結果顯示蔗糖濃度由3%提高至6%有助水稻花藥癒合組織的誘導，而6%的蔗糖配合0.53%甘露醇，則可使水稻花藥癒合組織的誘導率提高至51.4%（表5）。多次試驗結果顯示，提高培養基中糖類濃度對於誘導癒合組織有很大幫助，與上述學者之論點極爲接近。

在癒合組織分化植株的能力方面，許多學者均認爲適當的提高滲透壓，有助於癒合組織的分化能力。Brown et al. (1979)及Brown and Thorpe (1980)均認爲菸草莖髓(stem pith)培養，輕微的提高滲透壓逆境，可增加癒合組織形成芽的能力。Lai and Liu (1988)利用不同濃度洋菜和甘露醇調節水分逆境，發現培養基中含有1.2%洋菜和0.4M（約8%）甘露醇，可提高水稻未成熟胚來源癒合組織分化根和芽的比率。Liu and Lai (1991)更進一步探討，發現適當的調節培養基滲透壓，可降低水稻未成熟胚癒合組織細胞的含水量，及因細胞proline、polyamine及ABA之累積而改變其代謝反應，因此提高其分化能力。Kavi Kishor (1987)認爲滲透壓爲300m Osmol時，最適合水稻癒合組織生長，但200m Osmol時，即提高滲透壓（如降低糖的濃度）有利於水稻植株的分化。本試驗亦顯示相同的結果（表5及6），利用6%蔗糖所誘導出之癒合組織，接種於含3%蔗糖分化培養基上，有促進植株分化之效果。Close and Ludeman (1987)的報告也指出，14個玉米雜交種未成熟胚癒合組織形成體胚的能力，有其特殊的滲透壓需要，而培養基滲透壓可經由添加山梨糖醇(sorbitol)或甘露醇調節。表8的結果顯示適當的添加甘露醇以降低培養基之滲透壓，有增加癒合組織綠苗分化率的趨勢；若以濃度大於1.6%以上的甘露醇處理水稻花藥癒合組織，其分化率反而降低。由此可知，雖然水份逆境處理對於癒合組織的分化有促進的效果，但處理程度卻不可太過嚴重，否則反而抑制綠苗分化。以上結果均顯示，培養基中適當的滲透壓對促進癒合組織的分化極爲重要。所以利用糖類適當的調節滲透壓，以提高癒合組織之分化能力，其功效值得肯定。

**液體培養對水稻花藥培養的影響**

水稻花藥培養的成敗，花藥壁的影響很大。在花藥培養初期，花藥壁可產生促進小胞子發育的物質(Nitsch and Norreel 1973)。Sharp et al. (1972)在培養於培養基之蕃茄花藥上，隔以一層濾紙，再將蕃茄小胞子培養於濾紙上之保護培養法(nurse culture)，成功

的獲得小胞子的癒合組織及植株。Nitsch (1972)報導培養基中加入曼陀羅經培養一週後之花藥抽出液，可成功的獲得曼陀羅小胞子培養植物體。以上報告均顯示花藥壁所產生的未知成份對小胞子的發育極爲重要。但花藥壁對於花藥培養亦有不良的影響，Loomis and Battaile (1966)認爲切離組織所含酚類物質(phenolic compound)會馬上氧化，使苯環上之氫氧基，被氧化形成quinone之有毒物質，造成組織的褐變及傷害，因而抑制癒合組織之形成。Vasil (1980)認爲固體培養易受老化花藥壁釋出的抑制物所影響，因此建議在培養基中添加活性碳，或利用液體培養可得較好的結果；Nitsch (1981)也認爲液體培養有利於胚之發育。有些報告指出在液體培養時，由於培養之花藥較易藉擴散作用吸收養分，且將花藥壁褐化時所產生之有毒物質洗去，所以癒合組織的形成效果較好(Heberle and Reinert 1979, Sunderland 1978, Wernicke and Kohlenbach 1976)。本研究顯示水稻花藥行液體培養對癒合組織之誘導極有助益（表9之1號處理和表1比較），當培養基中加入10%Ficoll、3%蔗糖配合0.53%甘露醇，癒合組織誘導率可高達93.9%，比表9之1號處理（對照組）高出甚多。推測其原因可能是花藥處於液體之培養環境中，其內小胞子可藉擴散作用充分獲得所需之養分及花藥壁所產生促進小胞子發育的物質；而且花藥壁所釋出之有毒物質會擴散到培養基中，不易因累積過量而造成毒害；且因加入Ficoll,使得花藥上浮，故小胞子生長時所需的氧氣亦可充分供應，這些條件提供的良好生長環境，可能是癒合組織形成率提高的原因。

水稻花藥培養可自同一花藥中不同的小胞子產生許多癒合組織，這些不同小胞子來源的癒合組織，含有不同基因型，若能將此不同基因型之癒合組織均予以誘導分化成植物體，則小量的花藥培養即可產生各種基因型之後代，在遺傳育種上極具意義；然因固體培養花藥所佔的空間有限，而使癒合組織形成後擠在一起，造成競爭而失去許多優良基因型；液體培養雖可克服上述缺點，但所形成的癒合組織分化能力卻大爲降低。Tasy and Chen (1984)指出液體培養所產生之水稻花藥癒合組織，其綠苗分化力幾近於零。故若能針對此問題詳加研究，予以克服，將可使應用花藥液體培養進行水稻育種的可行性大爲提高。

其他水稻花藥培養成敗之因子，如花藥來源母株之營養及生理狀況；Tsai and Lin (1977)報導以開花初期之水稻花藥進行培養，效果較佳；Hu et al. (1978)認爲水稻孕穗期之溫度及日照爲影響花藥培養的重要因子；本研究中二期作花藥癒合組織之分化能力明顯高於一期作，與我們早期之研究結果相同(Yeh and Tasy 1987)，可能係花藥來源母株之營養及生理狀況不同所致，實值得進行更進一步之研究。

# 引用文獻


Ahloowalis, B. S. and Maretzki, A. (1983) Plant regeneration via somatic embryogenesis in sugarcane. *Plant Cell Rep.* 2:21-25.

Arnold, S. V. and Erikson, T. (1984) Effect of agar concentration on growth and anatomy of adventitious shoots of *Picea abies* (L.) Karst. *Plant Cell Tissue and Organ Culture* 3:257-264.

Brown, D. C. W., Leung, D. W. N. and Thorpe, T. A. (1979) Osmotic requirement for shoot formation in tobacco callus. *Physiol. Plant.* 46:36-41.

Brown, D. C. W. and Thorpe, T. A. (1980) Changes in water potential and its components during shoot formation in tobacco callus. *Physiol. Plant.* 49:83-87.

Chaleff, R. S. and Stolarz, A. (1981) Factors influencing the frequency of callus formation among cultured rice (*Oryza sativa*) anthers. *Physiol. Plant.* 51:201-206.

Chen, C. C. (1978) Effects of sucrose concentration on plant production in anther culture of rice. *Crop Sci.* 18:905-906.

Chen, C. C. and Chen, C. M. (1979) A method for anther culture of rice. *TCA Manual* 5:1051-1053.

Chen, C. C., Tsay, H. S. and Huang, C. R. (1991) Factors affecting androgenesis in rice (*Oryza sativa* L.). In: Biotechnology in Agriculture and Forestry, Vol. 14, Rice (Ed. by Y. P. S. Bajaj). pp. 107-122. Springer Verlag, Heidelberg.

Chen, L. J., Lai, P. C., Liao, C. H. and Tsay, H. S. (1982) Medium evaluation for rice anther culture. *J. Agric. Res. China* 31:283-290.

Chu, C. C., Wang, C. C., Sun, C. S., Chen, H., Yin, K. C., Chu, C. Y. and Bi, F. Y. (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Sic. Sinica* 17:657-668.

Clapham, D. (1973) Haploid *Hordeum* plants from anthers in vitro. *Z. Pflanzenzucht.* 69:142-155.

Close, K. R. and Ludeman, L. A. (1987) The effect of auxin plant growth regulators and osmotic regulation on induction of somatic embryogenesis from elite maize inbreds. *Plant Sci.* 52:81-89.

Heberle, E. and Reinert, J. (1979) Androgenesis in isolated pollen cultures of *Nicotiana tabacum*: dependence upon pollen development. *Protoplasma* 99:237-245.

Hu, C., Huang, S. C., Ho, C. P. and Liang, H. C. (1978) On the inductive conditions of rice pollen plantlets in anther culture. In: Proc. Symp. Plant Tissue Culture. pp. 87-95. Science Press.

Kavi Kishor, P. B. (1987) Energy and osmotic requirement for high freuency regeneration of rice plants from long-term cultures. *Plant Sci.* 48:189-194.

Lai, K. L. and Liu, L. F. (1988) Increased plant regeneration frequency in water-stressed rice tissue cultures. *Jap. J. Crop. Sci.* 57:553-557.

Liu, L. F. and Lai, K. L. (1991) Enhancement of regeneration in rice tissue culture. In: Biotechnology in agriculture and forestry, vol. 14, Rice (Ed. by Y. P. S. Bajaj), pp. 47-57. Springe-Verlag Berlin Heidelberg.

Loomis, W. D. and Battaile, J. (1966) Plant phenolic compounds and the isolation of plant enzymes. *Phytochemistry* 5:423-438.

Lu, C. Y., Chandler, S. F. and Vasil, I. K. (1984) Somatic embryogenesis and plant regeneration from cultured immature embryos of rye (*Secale cereale* L.). *J. Plant Physiol.*

115:237-244.
Lu, C., Vasil, V. and Vasil, I. K. (1983) Improved efficiency of somatic embryogenesis and plant regeneration in tissue cultures of maize ( *Zea mays* L.). *Theor. Appl. Genet.* 66:285-289.
Mercy, S. T. and Zapata, F. J. (1986) Effect of sucrose on callus induction and plant regeneration in Taipei 309. *Int'l Rice Res. Newsl.* 11:25.
Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15:473-479.
Niizeki, H. and Oono, K. (1968) Induction of haploid rice plant from anther culture. *Proc. Jap. Acad.* 44:554-557.
Nitsch, J. P. (1972) Haploid plants from pollen. *Z. Pflanzenzchtg.* 67:3-18.
Nitsch, C. and Norreel, B. (1973) Effect d'un choc thermique sur le pouvoir embryogene du pollen de *Datura innoxia* cultuve dans l'anthere ou isole de l'anthere. *C. R. Acad. Sci.* D 276:303-306.
Nitsch, C. (1981) Production of isogenic lines: Basic technical aspects of androgenesis. In: Plant tissue culture, methods and application in agriculture. (ed. by T. A. Thorpe) pp. 241-252. Academic Press.
Ouyang, T. W., Hu, H., Chuang, C. C. and Tseng, C. C. (1973) Induction of pollen plants from anthers of *Triticum aestivum* L. cultured in vitro. *Sci. Sinica* 16:79-95.
Pasqualetto, P. L., Zimmerman, R. H. and Fordham, I. (1986) Gelling agent and growth regulator effects on shoot vitrification of "Gala" apple in vitro. *J. Amer. Soc. Hort. Sci.* 111:976-986.
Pasqualetto, P. L., Zimmerman, R. H. and Fordham, I. (1988) The influence of cation and gelling agent concentrations on vitrification of apple cultivars in vitro. *Plant Cell Tissue and Organ Culture* 14:31-40.
Sunderland, N. (1978) Strategies in the improvement of yields in anther culture. In: Proc. Symp. Plant Tissue Culture. pp. 65-86. Science Press, Peking.
Sharp, W. R., Raskin, R. S. and Sommer, H. E. (1972) The use of nurse culture in the development of haploid clones in tomato. *Planta* 104:357-361.
Tsai, S. C. and Lin, M. H. (1977) Production of rice plantlets by anther culture. *J. Agric. Res. China* 26:100-112.
Tsay, H. S., Miao, S. H. and Widholm, J. M. (1986) Factors affecting haploid plant regeneration from maize anther culture. *J. Plant Physiol.* 126:33-40.
Tsay, H. S. (1986) Improvement of cereal crops through anther culture. *J. Agric. Associ. China* 134:1-23.
Tsay, H. S. and Chen, L. J. (1984) Effect of cold treatment on the rice anther culture. *J. Agric. Res. China* 127:8-17.
Vasil, I. K. (1980) Androgenetic haploid. In: Perspectives in plant cell and tissue culture. (Ed. by I. K. Vasil), pp. 195-223. Academic Press, New York.
Vasil, V. and Vasil, I. K. (1985) Induction and maintenance of embryogenic callus cultures of gramineas. In: Cell culture and somatic cell genetics of plants. (Ed. by I. K. Vasil), pp. 36-42. Academic Press, Orlando.
Wang, C. C., Sun, C. S. and Chu, C. C. (1974) On the conditions for the induction of rice pollen plantlets and certain factors affecting the frequency of induction. *Acta Bot. Sinica* 16:43-53.
Wernicke, W. and Kohlenbach, H. W. (1976) Investigations on liquid culture medium as a means of anther culture in *Nicotiana*. *Z. Pflanzenphysiol.* 79:189-198.

Woo, S. C. and Tung, I. J. (1972) Induction of rice plants from hybrid anthers of indica and japonica cross. *Bot. Bull. Acad. Sinica* 13:67-69.

Yeh, C. C. and Tsay, H. S. (1987) Effect of inoculation time during the year on the culture efficiency of rice anthers. *J. Agric. Res. China* 36:247-257.

（收稿日期：80年11月7日，接受刊登日期：80年12月26日）