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高溫處理及培養基成分對水稻花藥培養之效果[1]

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摘要：水稻花藥培養初期以 35±1°C 的高溫處理 1～2 天，再移至 25±1°C 恆溫培養，可提高癒合組織的誘導率，且不影響其分化率。培養後期的高溫處理則無效。

1mg/l NAA 及 4mg/l kinetin 之植物生長素能提高花藥癒合組織之綠苗形成率，但不利於形成後綠苗之生長。

N_6 無機鹽較 MS 無機鹽更能抑制花藥癒合組織的褐化，且癒合組織有較旺盛之生長勢。

1968年 Niizeki and Oono [16] 首先利用水稻花藥培養誘得單倍體植株後，十幾年來，經由許多研究人員不斷努力的結果，已顯着提高了水稻花藥培養的育種效率 [2,7,9]。

培養環境為決定花藥培養成功的重要因子之一，主要包括溫度及光線。不同作物所需的溫度及光線亦各異 [11,13,14,18,20]。水稻花藥培養，黑暗培養較照光者有利 [8]。培養溫度影響水稻癒合組織形成及分化能力，30°C 之高溫比 23～26°C 之低溫，對癒合組織誘導之效果較好，唯綠苗分化率降低，且有形成較多白苗之不良影響 [19,22]。

培養基成分對花藥培養更具有舉足輕重的地位 [1,3,12,17,21]。水稻花藥培養癒合組織誘導培養基所用之植物生長素，若以 NAA 替代 2.4—D 有助於植物體的分化 [1,5,6]。分化培養基中添加 1～2 mg/l NAA 能提高癒合組織分化能力 [5]。培養基無機鹽類的氮源，以低濃度的 NH_4^+ 配合高濃度的 NO_3^- 有助於水稻花藥癒合組織的形成 [6,7]。

本研究之目的在探討高溫處理及培養基成分之效果，以期能有助於水稻花藥培養育種效率。

材料與方法

一、花藥的接種

花藥培養所用材料為粳稻臺農67號品種。於劍葉葉耳距下位葉之葉耳間長約 3～5 公分時連同劍葉取下，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，以無菌水冲洗 3～4 次，將屬於單核期之花藥以 Chen and Chen [4] 所述之方法接種於培養基中。

二、培養基種類與培養環境

(一) 誘導花藥形成癒合組織之培養基，簡稱 NK，為 MS (Murashige and Skoog, 1962) 培養基之主要有機鹽類 [15]，及 N_6 (Chu *et al.*, 1975) 培養基之無機鹽類 [7]，加上 4mg/l NAA, 2mg/l kinetin, 6% sucrose 及 0.8% Phytagar [6]。

(二) 誘導癒合組織分化的培養基，簡稱 R—2，為 MS 培養基，加上 4mg/l kinetin, 1mg/l NAA, 40mg/l adenine sulfate, 3% sucrose 及 0.8% Phytagar。

1.臺灣省農業試驗所　研究報告第1415號。本文承蒙臺灣省農業試驗所農藝系研究員劉大江博士斧正，特此申謝。

2.臺灣省農業試驗所農藝系助理及研究員兼農藝系主任。臺灣省　臺中縣　霧峰鄉。

花藥培養於 25±1°C 恆溫，黑暗下，進行癒合組織之誘導。誘導癒合組織分化植物體之方式則取10天日齡之癒合組織，在 25±1°C 恆溫，光照 1,500 lux，光期16小時的環境下培養。

三、綠苗的移植

由癒合組織分化出來的綠苗生長經20天，具有較佳之根系後，移植至消毒過之混合土壤（壤土：泥炭土＝3：1），20天後調查移植成活率。

四、統計分析

花藥癒合組織形成率，以每支試管為重複（每根試管約接種60個花藥），當變方分析中處理效應顯著時，以鄧肯氏多變域測驗法（Duncan's new multiple range test）進行處理平均值間之差異顯著性測驗。由於癒合組織形成率為百分比，屬二項分布數據，故均先以反正弦函數變型（Arcsin transfermation）後，才進行資料之統計分析。

至於癒合組織分化率及植株移植成活率，因都以每個癒合組織及每棵植株為單位，故均視之為二項分布資料，以卡方測驗法（Chi-square test）比較處理間之差異。

結　　果

一、高溫培養花藥對癒合組織誘導及其分化之影響：

（一）培養初期高溫處理之影響：

表1結果顯示，各處理花藥褐化數並無差異。經過1天及2天 35±1°C 的高溫處理能有效提高花藥癒合組織形成率，而不影響癒合組織的分化能力。處理超過兩天則反而降低癒合組織形成率，且隨着處理時間延長，植株分化率有綠苗減少白苗增加之趨勢。

Table 1. Influence of high temperature-shock on anther culture of rice.

Treatment*	Num. of anthers cultured	Num. and % of anthers browning	Num. and % of anthers forming callus	Num. of callus cultured	Num. and % of callus forming plants		
					Green	Albino	Roots & abnormal shoots
CK	718	355(49.4)[a1]	156(21.7)[b1]	100	27(27.0)[a2]	(9.0)[a2]	18(18.0)[ab2]
H—1	840	396(47.1)[a]	265(31.5)[a]	116	24(20.7)[a]	14(12.1)[a]	25(21.6)[a]
H—2	675	291(43.1)[a]	230(34.1)[a]	118	24(20.3)[a]	13(11.0)[a]	27(22.9)[a]
H—4	596	304(51.0)[a]	99(16.6)[bc]	92	6(6.5)[b]	15(16.3)[a]	9(9.8)[b]
H—8	602	317(52.7)[a]	70(11.6)[c]	70	3(4.3)[b]	13(18.6)[a]	12(17.1)[ab]

*: CK: Cultured anthers were incubated at 25±1°C constantly.
H: Cultured anthers were first incubated at 35±1°C for 1 day(H-1), 2 days(H-2), 4 days(H-4), and 8 day(H-8)and then transferred to 25±1°C.

[1]: Means with the same letter are not significantly different at 5% level by Duncan's new multiple range test.

[2]: Means with the same letter are not significantly different at 5% level by Chi-square test.

（二）培養後期高溫處理之影響：

將培養於正常溫度（25±1°C）20天的花藥移至 35±1°C 高溫下培養，結果如表2。顯示後期的高溫處理，對癒合組織的形成並無助益，僅使花藥褐化率大幅度增加及降低癒合組織分化率。

Table 2. Influence of high temperature to anthers after 20 days in culture on the ability of callus formation and plant regeneration.

Treatment*	Num. of anthers cultured	Num. and % of anthers browning	Num. and % of anthers forming callus	Num. of callus cultured	Num. and % of callus forming plants		
					Green	Albino	Roots & abnormal shoots
CK	2087	692(33.2)[b1]	639(30.6)[a1]	120	23(19.2)[a2]	18(15.0)[a2]	23(19.2)[a2]
HT	1155	591(51.2)[a]	380(32.9)[a]	100	10(10.0)[a]	15(15.0)[a]	9(9.0)[a]

*: CK: Cultured anthers incubated at 25±1°C.

HT: Anthers were cultured for 20 days at 25±1°C followed by 35±1°C for 25 days.

1, 2: Same as Table 1.

二、培養基成分

(一) 植物生長素對癒合組織分化能力之研究：

表3顯示，含15%馬鈴薯抽出液之 PMS 培養基及植物生長素之 R—2 培養基均能提高綠苗的形成率，而以 R—2 培養基綠苗形成率最高，唯綠苗可能受植物生長素影響，在試管中之生長勢及移植成活率稍差。

Table 3. Effect of culture medium on the regeneration ability of anther-derived callus and the survival rate of the derived green plantlets after transplanting.

Culture* medium	Num. of callus cultured	Num. and % of callus forming plants			Num. of seedling transplanted	Num. and % of seedling survived
		Green	Albino	Roots & abnormal shoots		
MS	300	62(20.7)[b1]	46(15.3)[ab]	56(18.7)[a]	62	53(85.5)[a]
R—2	600	195(32.5)[a]	103(17.2)[a]	64(10.7)[b]	70	54(77.1)[a]
PMS	250	71(28.4)[a]	26(10.4)[b]	40(16.0)[a]	71	60(84.5)[a]

*: MS: Murashige and Skoog (MS) basic salts.

R-2: MS basic salts with 4ppm kinetin, 1ppm NAA and 40mg/l adenine sulfate.

PMS: MS basic salts with 15% potato extract.

1: Means with the same letter are not significantly different at 5% level by Chi-square test.

(二) 無機鹽類對癒合組織分化之研究：

表4顯示，含 N_6 無機鹽之 RN 培養基不論綠苗，白苗，及不定芽體分化率均稍高於 R—2 培養基，唯差異不大。且 RN 培養基能明顯抑制癒合組織的褐化，癒合組織有較旺盛之生長勢。

Table 4. Effect of inorganic salts on the differentiation ability and growth vigor of anther-derived callus in rice. (F_1 hybrid of TNG 67/IR 4227-18-3-2//TNG 67///TNG 67).

Culture* medium	Num. of** callus cultured	Num. and % of callus turned into					Mean of*** fresh weight (mg)	Mean of dry weight (mg)	% of dry matter of callus
		Browning	Yellowing	Green plants	Albino plants	Roots & abnormal shoots			
R—2	202	71(35.1)[a1]	32(15.8)[a]	31(15.3)[a]	37(18.3)[a]	21(10.4)[a]	221.41[b2]	33.27[b2]	15.03
RN	202	22(10.9)[b]	48(23.8)[a]	38(18.8)[a]	45(22.3)[a]	34(16.8)[a]	309.62[a]	42.53[a]	13.74

*: R-2 medium: Same as Table 3.

RN medium: The R-2 medium with it's inorganic salts substituted by those of N_6 medium.

**: 10-day old anther-callus induced on NK medium were cut into two halves, one was transferred to R-2 medium and the other to RN medium.

***: Yellow callus were weighted for both fresh and dry weight determinations.

[1]: Same as Table 3.

[2]: Means with the same letter are not significantly at 5% level by Duncan's new multiple range test.

（三）植物生長素對植株移植成活率之研究：

將由 R—2 培養基剛誘導出之小綠苗，分別移入新鮮之 R—2 及 MS 培養基中，經過20天的生長後移植。由表 5 中可見含植物生長素之 R—2 培養基，綠苗植株移植成活率僅及 MS 培養基的一半，顯見植物生長素不利於綠苗植株的生長。

Table 5. Influence of the final culture medium on the survival rate of rice anther callus-derived plants after transplanting.

Culture medium	Num. of plant* transplanted	Plants survived	
		Num.	— % —
R—2	63	29	46.03[b1]
MS	63	54	87.71[a]

*: Plant were first induced in R-2 medium and then transferred onto R-2 or MS medium for 20 days before transplanting.

[1]: Same as Table 3.

討　　論

培養溫度能影響花藥培養單倍體的形成，如 Keller and Armstrong [13,14] 以培養初期 35°C 高溫處理十字花科的 *Brassica campestris* 1～3 天，*Brassica oleracea* 2 天，而後再移至 25°C 低溫的處理方式，可提高單倍體之形成率。本試驗亦發現水稻花藥培養初期以 35°C 之高溫處理 1～2

天可提高癒合組織的形成率。但培養後期的高溫處理僅增加花藥褐化百分比，對癒合組織的形成並無助益。這說明了培養溫度影響癒合組織形成與否應該是在小孢子發育早期便已決定。培養溫度不僅影響水稻花藥癒合組織形成率的高低同時亦影響癒合組織的分化能力。Song *et al.*[19] 報導 30°C 高溫下形成之水稻花藥癒合組織其綠苗分化率低下，且增加白苗數。 Wang *et al.*[22] 也指出於水稻花藥小孢子第一次有絲分裂期的高溫處理，會減少綠苗，提高白苗之分化率，本試驗初期培養的高溫處理，隨着時間加長亦有減少綠苗，增加白苗分化率之現象。而後期的高溫處理，僅降低綠苗及不定芽體分化率，對白苗分化率則無影響。綜合之結果與 Song *et al*[19], Wang *et al.*[22] 等學者之研究吻合。亦即，水稻花藥培養初期施以適當之高溫處理確實可提高花藥培養之育種效率，唯提高之比例不大。如何尋找更適當的高溫處理時期，使在不影響分化能力下，提高癒合組織的形成率，以提高育種效率，則有待更進一步的努力。

許多報告指出水稻花藥培養基中所含之植物生長素爲影響花藥培養成敗的重要因子。 如 2,4—D 造成癒合組織不易分化；而若以 NAA 替代則可使植株在花藥癒合組織誘導培養基中直接產生[1,5,6,10]。Chen and Lin[5] 指出在分化培養基中添加 1～2mg/l NAA 能提高癒合組織分化能力。本研究之結果在含 4mg/l kinetin 及 1mg/l NAA 之 MS 基本鹽類培養基，與含15%馬鈴薯抽出液之 MS 培養基，其癒合組織綠苗的分化率均高於不含植物生長素之 MS 培養基。而另一個試驗中却發現含植物生長素之 R—2 培養基不利於綠苗的生長，移植成活率僅及 MS 培養基的一半。因此花藥所形成之癒合組織應移至含植物生長素的分化培養基中進行綠苗植株誘導，以提高綠苗分化率，所誘導之綠苗，則應儘速移至不含植物生長素的培養基中繼續生長，以提高綠苗的存活率。

培養基中另一個重要成分爲無機鹽中的氮源。高濃度的 NO_3^- 配合低濃度的 NH_4^+ 能有助於水稻花藥形成癒合組織[6,7]。本試驗結果亦顯示含較高濃度 NO_3^-，較低濃度 NH_4^+ 之 N_6 無機鹽分化培養基，較含 MS 無機鹽的分化培養基，更能明顯減少癒合組織的褐化，且使癒合組織有較佳的生長勢。

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The Effects of Temperature Treatment and Medium Composition on Rice Anther Culture

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Summary

Rice anthers at uninucleate stage were cultured in medium and incubated at 25°C under darkness for heat trea tment and medium composition studies. It was found that heat treatment of 35°C to the cultured anthers for 1-2 days at the initial culture stage incr eased the percentage of callus inducti on. Nevertheless, no effect was found to the subsequent differentiation of anther-derived callus. There was no beneficial effect either on callus induction or plant regeneration when heat trea tment was given to anthers which have been cultured for 20 days.

The plant growth regulators of 1mg/l NAA and 4mg/l kinetin increased the formation of green plants out of anther callus, but adversely affected the subsequent growth of the green plants.

Inorganic salts of N_6 medium were more effective in delaying the browning, and concomitantly accelerating the growth of anther callus as compared to those of MS medium.

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