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# 水稻品種間原生質體融合培養及植株再生

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**摘要：** 本試驗的目的，在探討水稻原生質體融合、培養及植株之再生，並嘗試此法轉移細胞質雄不稔性之可行性。細胞質雄不稔性狀採用Reimei A之KR型，栽培種採用與KR型細胞質可親和的高雄141號，分別由成熟胚及未成熟胚誘導懸浮培養細胞，經酵素法分離原生質體，將兩者之原生質體用PEG法融合，並利用營養細胞餵養法促進其生長，再誘導植株再生，經克服原生質體培養與再生的困難後，獲得39株再生植株，陸續移入土中栽培，經調查後發現，再生植株之株高、穗長、穗重及一粒穗數，均比親本植株減少。但是分蘗數、穗數有增加的趨勢，花粉異常率約達50-60%左右，所有植株均無法結實，這些特性較偏向於雄不稔性植株。本試驗以原生質體融合技術，可以不受傳統雜交育種的種間限制，探討細胞質與細胞核相互作用之基礎研究，並且將可實際應用於水稻的育種研究。

**關鍵語：** 水稻、細胞質雄不稔、原生質體融合。

## Protoplast fusion, culture and plant regeneration between rice cultivars

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**Abstract. Rice production can be increased by utilizing hybrid vigor. Because rice is self-pollinated crop, the breeding of hybrid F1 needs tedious work of emascultation, which fortunately can be devoid by using male sterility of mother plant. In traditiona l breeding, the cytoplasmic male sterility (CMS) character was transferred by back cross which is time- and labor-consuming. This study is thus designed to examine**

**the possibility of transferring CMS by protoplast fusion method. KR type CMS of Reimei A was used. Cultivar, Kaoshung141, which nuclei are compatible to the KR type of CMS cytoplasm, was used as nuclear donor. Protoplasts were isolated from suspension culture cells derived from mature embryos of Reimei A and immature embryos of Kaoshung 141. Two sources of protoplasts were induced for fusion by PEG treatment, and then to culture and plant regeneration. At present, 39 plants were regenerated. The general agronomic characters and male sterility of regenerated plants were examined. It showed that all regenerated plants have lower plant height, panicle length, panicle weight and spikelet number per panicle than the parent rice plants. However, they are similar to CMS type plants as their tiller number and panicle number are increased, and all of them can not get seeds. From this study, it is expected to establish rice CMS transfer system through protoplast fusion technique, and to understand the relationship between nucleus and cytoplasm. Both of them are very useful for rice plant improvement.**

**Key words: Rice, Cytoplasmic male sterility, Protoplast fusion**

## 前　言

作物性狀的改良，過去均利用傳統雜交育種方法，而雜交親本的選擇，常受到種間的限制；目前所發展的原生質體融合技術，可以不受種間的限制，更擴展性狀的改良範圍，同時亦可用於細胞質性狀的轉移，不僅是作物育種的重要研究途徑，亦可作爲體細胞雜種植株的遺傳分析，及細胞質和細胞核相互作用的重要基礎研究。

自1980年代以來，有關原生質體融合的報告相當多，發展非常快速。在*Nicotiana* (Liao et al.1990a,b)、*Solanum* (Perl et al.1990)、*Lycopersicon* (Wijbrandi et al.1990)、*Brassica* (Landgren and Glimelius 1990, Sakai and Imamura 1990)等屬植物中，均已得到種間雜種，甚至屬間雜種(Bonnett and Glimelius 1990, Kushnir et al.1991, Landgren and Glimelius 1990)，並且在*Nicotiana*等屬中，亦有轉移CMS性狀的報告(Asashi et al.1988, Sakai and Imamura 1990)。而有關水稻原生質體融合的報告，仍然非常有限，最主要的原因是水稻原生質體的培養較爲困難，融合後植株再生率極低且不穩定，成爲主要的瓶頸，亟待改善。

利用雜種優勢提高作物產量，是作物生產上極有效的途徑。水稻雖然是自交作物，卻仍具有極強的雜種優勢(Stansel and Craigmiles 1966)，雜種水稻的產量可提高15-20% (Tseng and Huang 1987, Yuan and Mao 1991)，然而在育成雜種F1的過程中，必須利用雄不稔性，才可免除去雄 的繁重工作，傳統上細胞質雄不稔性之轉移均利用回交方式，往往需回交7至8代才能穩定，極爲費時費工。因此本試驗擬測試是否可以原生質體融合的方法，轉移細胞質雄不稔性(CMS) 。自1986年以後才陸續有報告指出，水稻原生質體培養成再生植株

(Kyozuka et al.1987, Lee et al.1989, Liu et al.1989, Wen et al.1991, Yamada et al.1986)。而融合方面，Terada等(1987)得到水稻及稗子雜種細胞，並再生爲小苗；Hayashi等(1988)得到水稻與野生稻的體細胞雜種植株；Yang等(1988,1991)、Akagi等(1989)與Kyozuka等(1989)並經由原生質體融合的途徑轉移水稻的CMS性狀。

本試驗中發展不同水稻品種間原生質體的融合，基本上是以PEG (polyethylene glycol)法誘融，將PEG-蓋玻片法改良，採用PEG-培養皿法，並利用營養細胞培養法，可明顯促進原生質體生長(Liu et al.1989)，形成細胞團，經移入分化培養基後，已得到再生植株，經移入土中栽培，可成長至開花成熟，進一步對這些再生植株進行性狀之分析及探討。

## 材料與方法

本試驗材料選用栽培品種高雄141(KH141)，與具有KR型細胞質雄不稔性狀之Reimei A (RM)品種。分別將KH141的幼胚，及Reimei A的成熟胚，接種於$MSD_{10}$培養基(MS培養基添加10 $\mu$M 2,4-D )，四週後誘導癒合組織形成，再將癒合組織移入$AAD_{10}$培養基(AA培養基添加10 $\mu$M 2,4-D)， 經每週固定繼代培養，建立懸浮培養細胞。此懸浮細胞經酵素處理，可分離出原生質體(Liu et al.1989)。酵素溶液採用1% macerozyme R10，2% cellulase RS和0.1% pectolyaze Y23配製於cpw 13溶液(Frearson et al.1973)。

分離後之原生質體，以$1x10^6$原生質體/ml密度，平埋培養於直徑9cm的培養皿，每皿培養9ml，故每皿之總量爲$9x10^6$原生質體，經培養5-6週後，計算每皿形成之小細胞團數目，並選取小細胞團移至MSKN分化培養基(MS培養基添加20 $\mu$M Kinetin及10 $\mu$M NAA)，誘導植株再生。

原生質體之融合採用PEG-培養皿法，即將兩品種分離之原生質體，調整密度爲$2x10^6$/ml，以1:1混合後，取200 $\mu$l（約含有$4x10^5$個原生質體）滴入直徑5cm 的塑膠培養皿中，每次重覆10個培養皿，靜置10分鐘，滴加600 $\mu$l 25% PEG-6000溶液，靜置10分鐘，再滴加700 $\mu$l以1:1新鮮混合之高鹼及高鈣溶液(高鹼液爲10ml溶液中含0.54g glucose及75mg glycine, pH 10.2；高鈣液爲10ml溶液中含0.54g glucose及0.147g $CaCl_2 \cdot 2H_2O$)，靜置10分鐘後，吸除所有塑膠培養皿中的溶液後，加入1ml的cpw13溶液，靜置20分鐘後洗除，重覆清洗三次，完成誘融處理。原生質體再以含有1.2% agarose之KPR培養基進行平埋培養，並加入0.25ml餵養細胞、1.25ml KPR 培養基和1.25ml AA培養基，以餵養細胞法促進原生質體生長(Jan 1991, Liu et al. 1993)。經2-3週後，待有小細胞團形成，即洗除營養細胞並計算每碟所形成之細胞團數目，以估算小細胞團的形成率。而後再加入1.25ml AA培養基，培養2-3週後，挑取細胞團，移至固態$MSD_{10}$培養基使細胞團增殖，2-3週後，再移至固態MSKN分化培養基，誘導植株分化，待植株長至10cm左右，移入土中栽培，置於30℃溫室中生長至抽穗、開

花。並進行各項調查。

再生植株的調查，包括基本農藝性狀：株高、分蘗數、穗數、穗重、穗長、每穗粒數、結實率等，此外，並採取花藥，依Alexander(1969)之方法進行差異染色法，檢定花粉活力，每處理約計算100粒花粉，紫紅色爲正常花粉，綠色爲異常花粉，計算其異常花粉率。

## 結　果

### 一、原生質體融合、培養及植株再生：

本試驗進行多次原生質體融合與培養處理，已得到再生植株，結果列於表一。其中親本KH141及Reimei A的原生質體是以平埋法培養於直徑9cm 的培養皿，每次試驗有十碟重覆。KH141進行二次培養，平均所得小細胞團數分別爲每碟98及43個，經再生後移植盆栽的植株共有七株。Reimei A共進行三次培養，所形成小細胞團數分別爲每碟523，36及62個，沒有得到再生植株(表一)。

在原生質體融合培養方面，原生質體經PEG誘融處理後，會黏著在培養皿底部，因此將含有agarose 的培養基滴在原生質體上，待agarose凝固後，原生質體被包埋住，此時若直接培養，原生質體很難生長，若在agarose周圍，加入餵養細胞(圖1a)，則可明顯促進原生質體的生長。顯微鏡觀察得知agarose完全將原生質體與餵養細胞區隔，沒有混淆的情形。經2-3週後，可將餵養細胞洗除，僅保留平埋於agarose內由原生質體長出的小細胞團(圖1b)。融合部分共有三次試驗，每次有十碟的重覆，最初每碟培養量爲$4x10^5$原生質體，結果每碟所形成的小細胞團，三次試驗中分別爲平均每碟104，127及117個小細胞團，經移到分化培養基

**Table 1.** Plant regeneration from rice protoplast culture

| Cultivar | Exp. | Initial protoplasts / dish | Ave. callus No. / dish | Plants in soil |
|---|---|---|---|---|
| KH141 (fertile) | 1* | $9x10^6$ | 98 | 6 |
| | 2 | $9x10^6$ | 43 | 1 |
| Reimei A (CMS) | 1 | $9x10^6$ | 523 | 0 |
| | 2 | $9x10^6$ | 36 | 0 |
| | 3 | $9x10^6$ | 62 | 0 |
| KH141 + Reimei A | 1 | $4x10^5$ | 104 | 13 |
| | 2 | $4x10^5$ | 127 | 22 |
| | 3 | $4x10^5$ | 117 | 4 |

* There are 10 dishes in each experiment(Exp.).

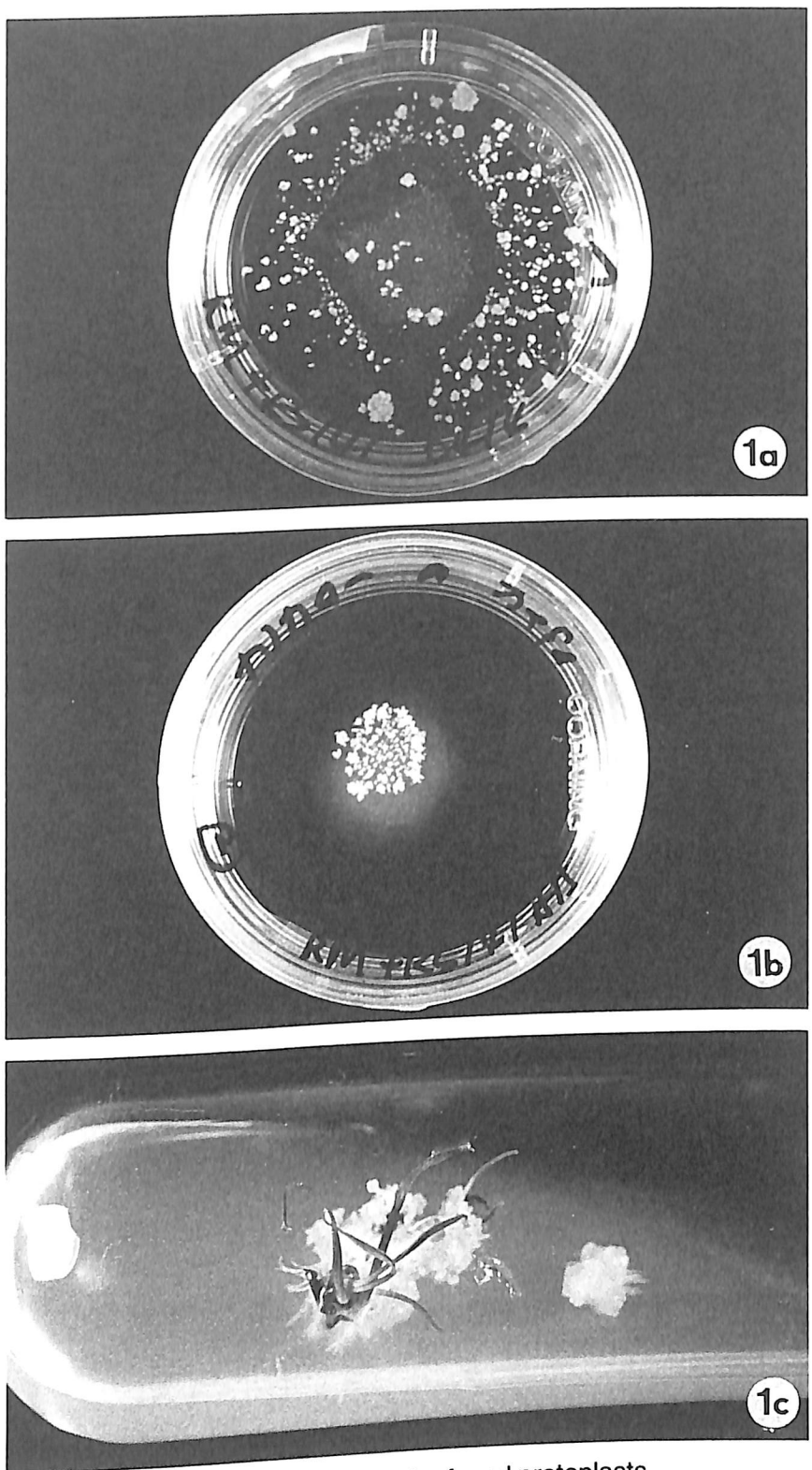



Fig. 1. Nurse cell culture and plant regeneration for fused protoplasts.
1a. Fused protoplasts were embedded in agarose and nurse cells were suspended around agarose.
1b. Callus formation from fused protoplasts after nurse cells were washed out.
1c. Plant regeneration from callus derived from fused protoplasts.

後，觀察到少數細胞團有再生植株(圖1c)，整體而言，植株再生率不高，三次試驗總計得到39株再生植株(表一)。

**二、再生植株農藝性狀之觀察：**

經融合後之再生植株由試管中移出，以1/2000a之栽培盆種植在溫室中，植株生長良好，株型直立且分蘗極多，並可生長至抽穗開花，但所有融合植株均無法結實，並且至生育後期，葉色仍保持綠色，且不斷分蘗(圖2)。 進一步對這些植株進行農藝性狀觀察及花粉活力測試，其中六株的 調查結果列於表二。以親本KH141及Reimei A的一般特性作爲比較，結果發現，融合後的體細胞再生植株，在株高、穗長、穗重及一穗粒數，都明顯的減少，而分蘗數、穗數有增加的趨勢；此外，因這些植株的種子都無法充實，故穗重只是內外穎及小枝梗的重量。另外，以差異染色法檢定花粉活力，正常花粉粒之細胞質可染成紫紅色，而異常花粉粒只有細胞壁染上綠色(圖3)， 結果發現，這些植株的花粉異常率約達50-60％左右。其他未調查的植株表現，均與此六株相似。

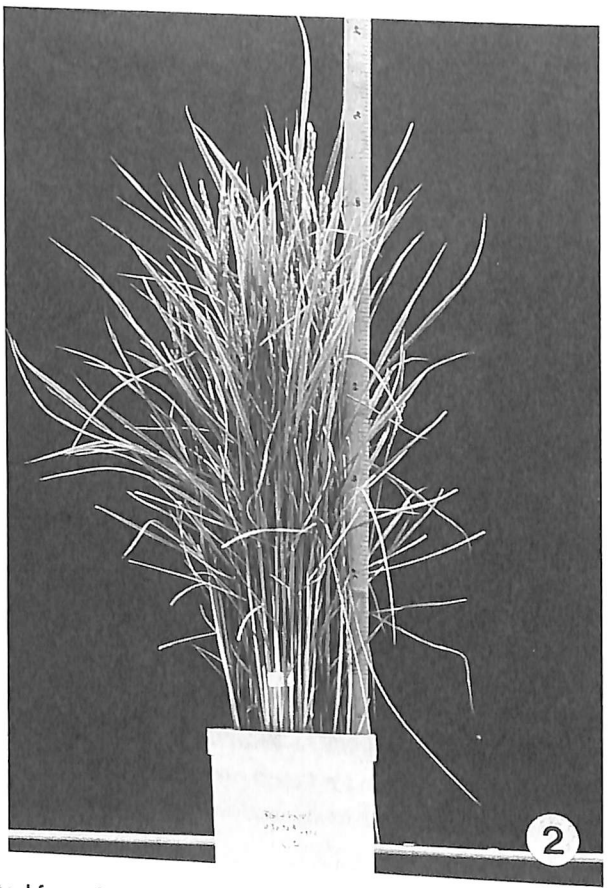



Fig. 2. Plant regenerated from fused protoplasts showed vigious growth with increased tiller number.

**Table 2.** Agronomic characters of the rice plants regenerated from the protoplast fusion products between KH141 and Reimei A

| Cultivar | Plant height (cm) | No. of tiller /plant | No. of panicle /plant | Panicle length (cm) | Panicle weight (g) | Spiklets/ panicle | Seed -set (%) | Aborted pollen (%) |
|---|---|---|---|---|---|---|---|---|
| KH141 | 93 | - | 21 | 20 | 1.9 | 80 | 95 | - |
| Reimei A | 81 | - | 12 | 18.5 | 0 | 75 | 0 | - |
| KH141+Reimei A | | | | | | | | |
| 1 | 71 | 30 | 18 | 9.8 | 0.27 | 35 | 0 | 61.2 |
| 2 | 79 | 42 | 36 | 11.2 | 0.26 | 47 | 0 | 61.8 |
| 3 | 68 | 38 | 25 | 13.3 | 0.37 | 44 | 0 | 54.4 |
| 4 | 69 | 47 | 20 | 13.3 | 0.31 | 43 | 0 | 59.2 |
| 5 | 78 | 59 | 38 | 12.9 | 0.23 | 56 | 0 | 65.0 |
| 6 | 71 | 31 | 26 | 13.1 | 0.23 | 42 | 0 | 52.3 |

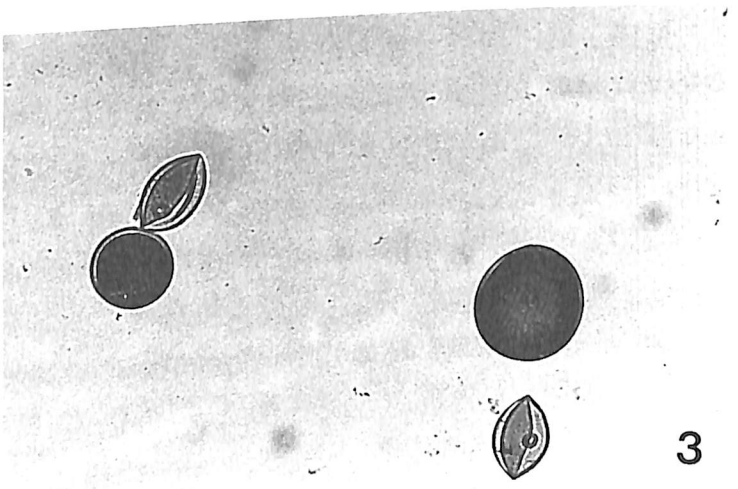



Fig. 3. Pollen viability test by differential staining method. Red color indicates viable pollen and blue color indicates aborted pollen.

## 討　論

本研究室經過深入探討，對融合及培養條件多方面改善，得以突破水稻原生質體培養與再生的困難，得到品種間原生質體融合後的再生植株。

本試驗曾將PEG-蓋玻片的誘融法(Kao et al.1974)，改爲PEG-培養皿法，即將原生質體直接滴加於培養皿底部，而不用蓋玻片，發現操作更爲簡便，並可提高培養之效率。此外，利用餵養細胞培養法(Jan 1991, Liu et al.1993)亦是培養是否成功的重要關鍵，若不添加餵養細胞，則融合處理後的原生質體，完全不會生長。而經PEG融合處理後，可觀察到原生質

體的生長較快，根據Liao等(1990)在菸草種間原生質體融合及Jan(1991)在水稻和稗子間原生質體融合的試驗結果，均顯示有融合後的優勢存在，此現象值得進一步探討。目前水稻原生質體的分離及培養，均以所建立之懸浮培養細胞，作爲原生質體分離的材料；一般栽培品種可利用未成熟胚爲材料誘導癒合組織，但對於細胞質雄不稔系統，則因未成熟胚不易取得，故採用成熟胚爲材料。本試驗中Reimei A的懸浮培養細胞生長情形與一般栽培種相似，若培養於基本的$AAD_{10}$培養基，並不能誘導再生植株，若經滲透壓處理則可得到再生植株，並保存其植株特性(資料整理發表中)。而由懸浮細胞分離原生質體後，雖可經培養得到細胞團，但沒有得到再生植株。另一個親本高雄141，由原生質體培養僅得到少數再生植株，且生長不太正常，株型瘦 弱、分蘗少、穗小。經PEG誘融處理後再生的植株，其外表型態及農藝性狀，與親本有很大不同，株型雖較偏向親本高雄141，但株高降低、穗變小、完全不稔實，其分蘗多、且持續不斷生出，這些特性則較偏向於雄不稔性植株，顯示有極高的可能性是經由融合所產生的結果，將進一步檢查 。

由以上之經驗，本研究室將更積極著手進行水稻細胞質雜種(Cybrid)的試驗，細胞質雜種適用於細胞質性狀之轉移，略不同於一般原生質體融合，宜採用Donor-Recipient方法(Sidorov et al.1981, Zelcer et al.1978)，即(1)提供細胞質者，必須先將細胞核去掉，早期使用 r- 射線照射(Sidorov et al.1981, Zelcer et al.1978)，近來則可用梯度離心的方法，將核甩掉(Wallin et al.1989)，或用UV照射，破壞部分染色體(Hall et al.1992a,b)。(2)提供細胞核者，則必須先將細胞質破壞，一般可利用Iodoacetate 處理(Sidorov et al.1981, Zelcer et al.1978)。然後再將二者原生質體進行融合，如此可有效獲得眞正的細胞質雜種。水稻方面雖有少數關於CMS性狀轉移的報告(Akagi et al.1989, Kyozuka et al.1989, Yang et al.1 988)，但關於Donor-Recipient方法中，原生質體處理的各種情形，則沒有詳細的報告，甚值得進一步的研究。未來將以栽培種爲提供細胞核者，先用Iodoacetate處理，抑制細胞質的活性，另一具CMS性狀者，將以UV燈照射，破壞部分染色體。再將此兩種經過處理的原生質體進行融合培養，希望能夠得到眞正的細胞質雜種植株。

預期本試驗不僅可作爲水稻細胞質與細胞核關係之基礎研究，並可提供一個快速有效的細胞質轉移方法，實際應用於水稻之育種。

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