Omonrice 10: 107-109 (2002)

*SHORT COMMUNICATION*

# Effect of different media and genotypes on anther culture efficiency of $F_1$ plants derived from crosses between IR64 and new plant type rice cultivars

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## ABSTRACT

Anthers of $F_1$ plants derived from four crosses of new plant type and improved rice cultivars were cultured in N6, LS and MS media supplemented with 2,4-D (0.5 mg/L) + NAA (2.0 mg/L) for callus induction. The calli were subcultured in N6 medium supplemented with NAA (0.5 mg/L) + BAP (2 mg/L) for plant regeneration. Frequency of callus formation was the best in N6 medium (3.53%) as compared to MS (2.63%) or LS medium (2.37%). Anther-derived calli from the cross of IR64/IR68530 showed the highest response in plant regeneration (1.12% in total of inoculated anthers), and none green plant obtained in cross of IR64/IR70441.


## INTRODUCTION

Since the first haploid rice plants were produced through anther culture by Niizeki and Oono (1968), plant breeders began directing their attention to anther culture and its possible application in breeding. The advantages of incorporating anther culture in rice breeding are shortening the breeding cycle by immediate fixation of homozygosity, increase selection efficiency, widening genetic variability through the production of gametoclonal variants, and allowing the early expression of recessive genes. In China, a large number of rice varieties have been developed through anther culture and released for cultivation over thousands of hectare (Chen 1986). In Korea, haploid breeding has been elevated to a national rice breeding program in 1977. Four rice varieties derive from anther culture have been released for seven years (Chung 1992). However, many difficulties of anther culture applying in rice breeding have been counted: genotype dependent, low frequency of callus induction, low frequency of plant regeneration, low ratio of green plants to albino, and high frequency of haploid plants. Several influencing factors have been studied such as genotype of explants (Shen et al. 1982, Li 1991), growth condition of donor plants (Chen 1998), culture methods (Qu and Shen 1983). Application of these protocols, anthers of $F_1$ plants of IR64 and new plant type varieties were used in this experiment to evaluate the culture efficiency of different media on each combination.

## MATERIALS & METHODS

$F_1$ progenies of crosses between IR64 and new plant type cultivars as IR64/IR68530, IR64/IR69146-25, IR64/IR69146-15, and IR64/IR70441 were grown under greenhouse condition. Panicles of these $F_1$ plants were collected in the morning when the auricle distance of the flag leaf to that of the next leaf of around 5-10 cm. Wrapped panicles in moistened paper and cold treated at 8$^{o}$C for 8 days. Florets having anther length of less than half of the size of the floret were selected and dipped into 70% ethanol for 2 minutes before immersing in 0.1% $HgCl_2$ for 10 minutes. The explants were rinsed three times by sterile distilled water. Portion just above the anthers was cut and inoculated onto the vessel containing nutrition medium and kept in the darkness at 25$^{o}$C for callus induction. The experiment was conducted in RCB design with three replications.

### *Culture media*

Three basic media were used in this study with supplement of 0.5 mg/l 2,4-D + 2mg/l NAA

N6 medium of Chu (1978)
MS medium of Murashige and Skoog (1962)
LS medium of Linsmaier and Skoog (1965)

Calli with the size of 2-3 mm in diameter were regenerated in nutrition medium containing N6 medium supplemented with 0.5 mg/l NAA + 2 mg/l BAP and kept in the condition of 25$^{o}$C and 16/24h shine bright per day.

The green regenerated plants were transferred to MS medium without phytohormones for root induction. Completely regenerated plants were acclimatized in Yoshida solution for 15 days prior to cultivate in the greenhouse for further observation and evaluation.

Percentage of callus induction and differentiation were observed and calculated.

## RESULTS & DICUSSION

### * Effect of genotypes and nutrition media on callus induction

Though anthers in all treatments, formed calli were observed, the data up to now have showed that the earliest and highest number of anther formed callus was from the cross of IR64/IR68530 (4.48 %). The lowest callus induction was from IR64/IR69146-15 (1.00 %). An average frequency of callus induction from all crosses in N6 medium was higher than that in MS and LS media and the average through four crosses was the highest also in N6 medium (table 1).

Table 1: Effect of genotype and nutrition media on callus induction from anther culture

| Designation | Percentage of anther formed callus (%) on different media | | | |
|---|---|---|---|---|
| | MS | LS | N6 | Average |
| IR64/IR68530 | 3.67 | 3.79 | 5.98 | 4.48 |
| IR64/IR69146-25 | 2.00 | 0.80 | 2.40 | 1.73 |
| IR64/IR69146-15 | 0.85 | 0.90 | 1.25 | 1.00 |
| IR64/IR70441 | 4.00 | 4.00 | 4.50 | 4.17 |
| Average | 2.63 | 2.37 | 3.53 | 2.84 |

### * Response of different genotypes on plant regeneration

Regeneration of green plants is greatly influenced by age and size of callus. Wang et al. (1977) and Chen et al. (1986) reported that rice callus induced in early stage (around 30-50 days after inoculation), offers high differentiation for green plants. Frequency of plant regeneration from different genotypes was given in table 2.

Table 2: Plant regeneration from anther-derived calli induced on N6 medium

| Designation | Callus forming roots /anthers (%) | Plant regeneration / anthers (%) | |
|---|---|---|---|
| | | Albino plants | Green plants |
| IR64/IR68530 | 1.12 | 1.68 | 1.12 |
| IR64/IR69146-25 | 0.87 | 0.43 | 0.22 |
| IR64/IR69146-15 | 0.25 | 0.50 | 0.46 |
| IR64/IR70441 | 1.39 | 1.39 | 0.00 |

Frequency of green plant regeneration was very low in all treatments, but that of albino and root was much higher. The highest green plants obtained from IR64 / IR68530 as 1.12% and none green plant regenerated from IR64/IR70441. Chen et al. (1991) reported that frequency of anthers producing callus, capacity of differentiation and chromosome

number of regenerated plants were tightly related to donor genotype. Anthers of some indica varieties form no calli (Tsai and Lin 1977). This initiation study showed that though green plant regeneration from anther of crosses of IR64 and new plant type cultivars was possibly obtained but many problems created need to be solved for higher efficiency of green plants regenerated.

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***SUMMARY IN VIETNAMESE***

## Nuôi cấy túi phấn cây F1 của các tổ hợp lai giữa các giống lúa new plant type với lúa cao sản


Túi phấn của cây lúa F1 từ bốn tổ hợp lai giữa lúa new plant type với lúa cao sản (loại hình indica) được nuôi cấy trong môi trường N6, MS vá LS, có bổ sung thêm 2,4-D (0.5 mg/L)+ NAA (2mg/L) để kích thích tạo mô sẹo. Các mô sẹo sau đó được cấy truyền sang môi trường N6 có bổ sung NAA (0.5 mg/L) + BAP (2 mg/L). Tỷ lệ hình thành mô sẹo trong môi trường N6 (3.53%) cao nhất so với môi trường MS (2.63%) và môi trường LS (2.37%). Những mô sẹo được hình thành từ túi phấn của tổ hợp lai IR64/IR68530 cho tỷ lệ tái sinh cây xanh cao nhất (1.12% số túi phấn cấy) trong khi tổ hợp lai IR64/IR70441 không thu được cây xanh.