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ANTHER CULTURE FROM CROSSES BETWEEN IR64 AND NEW PLANT TYPE CULTIVARS

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ABSTRACT

Anthers of F_1 plants from crosses of IR64 and new plant type cultivars: IR69146-15, IR69146-25, IR68530 and IR70441 were used for culture to study the role of media, plant regulators in callus induction and plant regeneration The highest frequency of callusing response obtained in IR64/IR69146-25 cultured in MS medium with 0.5 mg/l 2,4-D + 2 mg/l NAA (8.85%). MS medium and N6 medium promoted higher callusing frequency (6.648% and 6.25%) than LS medium did (4.31%). IR64/IR68530 exhibited the highest frequency of green plant regeneration (5.73%) when cultured in the N6 medium. Plant regeneration efficiency of N6 medium (4.64%) was 1.5 times higher than that of MS medium (2.82%). The best medium for plant regeneration was found to be N6 medium supplemented with 2mg/l BAP + 0.5mg/l NAA (5.29%).

Key word: Anther culture, calli, media, new plant type, plant grow regulators.

INTRODUCTION

"New plant type" was conceptualized to increase yield potential by 20% with many positive attributes: low tillering capacity, no unproductive tillers, large panicles (200-250 grains), very sturdy stems, dark green thick and erect leaves, vigorous root system, 100-130 day growth duration, multiple disease and insect resistance, and acceptable grain quality (Khush 1995). Major germplasm to identify donors for these traits entries came primarily from Indonesia, called *bulus* referred to as tropical japonicas. Many *bulu* varieties were crossed with semidwarf japonica from China to obtain numerous new plant type breeding lines with ideal type, but many of them found to have poor grain filling and yield potential was not realized. Moreover, these lines have short grains and lack of resistance to brown plant hopper, green leafhopper, and tungro (IRRI 2001).

Anther culture is one of the best breeding methods with numerous advantages: shortening breeding cycle by immediate fixation of homozygosity, increasing in selection efficiency, widening of genetic variability through the production of gametoclonal variants, and allowing early expression of recessive genes (Zapata 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 genotypes of the explants (Niizeki and Oono 1968; Shen et al. 1982; Li 1991), growth condition of the donor plants (Chen 1988), developmental stage of microspores (Chen 1977; Genovesi and Magill 1979), pre-treatment (Qu and Chen 1983), culture methods (Yang and Zhou,1979; Chen 1988), media (Chen 1988, Sun et al. 1990), and culture conditions (Wang et al. 1977; Qu and Chen 1983). Among these influencing factors, genotype of the donor plants has been reported to be the most important factor in anther culture (Chen 1988; Henry et al. 1994). The results indicated that the order of culturability is: japonica / waxy > japonica / japonica > japonica > indica / japonica > indica / indica > indica (Shen et al. 1982). Besides the genotypic influence, role of media and plant growth regulators in callusing and plant regeneration from anthers of indica rice has been identified (Lenka and Reddy 1994).

In this study, anthers of F_1 plants from crosses between IR64 and new plant type varieties were used to evaluate the culture efficiency of

genotypes in different media and plant growth regulators.

MATERIALS AND METHODS

F_1 progenies of crosses between IR64 and new plant type cultivars as IR64 / IR69146-15, IR64 / IR69146-25, IR64 / IR68530 and IR64 / IR70441 were grown under greenhouse condition. Panicles from primary tillers 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 5-9 cm. Washed the panicles in tap water and wrapped in moistened paper before keeping in the incubator at 8^0C for 8 days. Selected florets in the middle of panicle having anther length of less than half of the floret size and dipped into 70% ethanol for a minute before immersing in 0.1% $HgCl_2$ for 10 minutes. The explants were rinsed 3-5 times by sterile distilled water. Cut the portion just above anthers and then held each floret with a sterile forceps to tap at the edge of a vessel containing the callus induction medium (as designed in experiment 1) to release the anthers, incubated cultures in the dark at 25^0C. Calli with the size of 2-3 mm were subcultured to regeneration media as designed in experiment 2. *In vitro* propagated plant regeneration in MS medium with supplement of 2 mg/l BAP was conducted. Then we separated cluster of shoot and transferred into MS medium without phytohormones for root induction. Completely regenerated plants were acclimatized in Yoshida solution (Yoshida et al. 1972) for 15 days prior to individualize the plants. We allowed them to grow for a few days before cultivating in the greenhouse for further observation and evaluation.

Culture media

Experiment 1: Effect of different media and genotypes on efficiency of callus induction from anther of four crosses. Three basic media were used in this study with supplement of 0.5 mg/l 2,4-D (2,4 – dichlorophenoxy acetic acid) + 2mg/l NAA (Acid α - naphthalen acetic)

(1): N6 medium by Chu (1975)
(2): MS medium by Murashige and Skoog (1962)
(3): LS medium by Linsmaier and Skoog (1965)

Experiment was conducted in split-plot design with three replications (Gomez and Gomez 1984). Each plot was carried out 10 vessels with 180 inoculated anthers.

Experiment 2: Study on role of media, and plant regulators on plant regeneration: Calli with size of 2-3 mm in diameter were regenerated in two nutrition media combining with two formularies of plant regulator.

Media:
(1): N6 medium by Chu (1975)
(2): MS medium by Murashige and Skoog (1962)

Regulators:
(1) 1mg/l NAA + 1mg/l BAP + 1mg/l Kinetin
(2) 0,5 mg/l NAA + 2 mg/l BAP (6 – benzynlamino purine)

The experiment was conducted in RCD (Randomized Completely Design) with three replications (Gomez and Gomez 1984). Each plot was carried out 8 vessels with 4 calli in a vessel. The cultures were kept in the condition of 25^oC and 16/24 h shine bright per day.

RERULTS AND DISCUSSION

Effect of genotypes and nutrition media on callus induction

It has been demonstrated that the earliest calli formed at 10-15 days after inoculating anthers (Wang et al. 1974; Li 1992). Because visible size of the callus is not standardization, in the experiment the earliest calli occured after 18 days for IR64 / IR69146-25 cultured in MS medium with the mean of 22.2 days (table 1). But in other genotypes, callus formation time was the shortest when cultured in N6 medium (26-33 days). Except IR64 / IR69146-25, the callus initiation was expedited at 5-10 days earlier in N6 medium as compared to MS or LS ones. The interaction effect of genotype by media was defined at significance of $P<0.01$.
General trend of variation in anther culture among rice genotypes was reported: japonica > indica/japonica > indica/indica >indica (Shen et al. 1982). Four crosses used as materials in this study were genotypes of indica/japonica so culture efficiency was not high. Among the three media evaluated, both

MS and N6 were equally efficient in callus induction, while LS was the least effective. Number of calli in each vessel varied from 4.8-15.9% equally to 2.7-8.9%. High callusing frequency of 8.9% and 7.3% was observed for IR64 / IR69146-25 and IR64 / IR69146-15, respectively, in MS medium. Callusing frequency of 7.8% and 7.3% was noticed for IR64 / IR69146-25 and IR64 / IR68530 in N6 medium. The extent of response by different genotypes in each medium varied in table 2. In almost crosses, the callusing response was considerably high with one or two media, while the response was low in others. For example, IR64 / IR69146-25 exhibited high callusing frequency of 8.9% and 7.8% in N6 and MS media, respectively as compared to LS (2.7%). Likewise, IR64 / IR68530 showed high callusing response in N6 medium (7.3%), while the response was low in MS medium (3.6%). The mean of callusing frequency in LS medium (4.3%) was lower than in MS (6.6%) or N6 (6.3%). These results were much lower as compared to results by Chaleff and Stolarz (1982), which obtained 25.2-42.9% callusing frequency in LS medium using *japonica* rice. But frequency of callus in this study was the same results by Chung (1982) using Usen (*indica*) / Palkweng (*japonica*) (4.5%).

Table 1: Effect of genotype and nutrition media on callus formation time

No	Crosses (V)	Callus formation time (days) in different media (E)			Mean (V)
		MS	LS	N6	
1	IR64/IR68530	36.3 b	31.7 b	26.0 b	31.33
2	IR64/IR69146-25	22.2 c	35.0 a	28.0 b	28.40
3	IR64/IR69146-15	41.0 a	30.0 b	33.3 a	34.77
4	IR64/IR70441	40.0 a	35.0 a	32.7 a	35.90
	TB (E)	34.88	32.93	30.0	32.60
	CV (%)				5.2
	F V				565.24 **
	E				25.15 **
	VxE				30.32 **

*Note: E: nutrition media factor V: genotype factor **: significant at P<0.01*
Means within a column sharing the same letter are not significant different at $P_{0.05}$

Table 2: Effect of genotype and nutrition media on ratio of callus formation to anthers inoculated

Media (E)	Crosses (V)	No of calli in a vessel of 180 anthers	Percentage of anthers formed calli
MS	IR64/IR68530	6.57	3.647 b
	IR64/IR69146-25	15.93	8.853 a
	IR64/IR69146-15	13.13	7.297 a
	IR64/IR70441	12.23	6.797 a
LS	IR64/IR68530	8.43	4.683 ab
	IR64/IR69146-25	4.80	2.667 b
	IR64/IR69146-15	10.30	5.723 a
	IR64/IR70441	7.50	4.167 ab
N6	IR64/IR68530	13.13	7.297a
	IR64/IR69146-25	14.07	7.813 a
	IR64/IR69146-15	8.43	4.683 b
	IR64/IR70441	9.37	5.207 b
	CV% V		21.7
	F E		12.10**
	V x E		7.58**
	$LSD_{2\text{-E means}}$ (5%)		2.154

*Note: E: nutrition media factor V: genotype factor **: significant at P<0.01*

Means within a block of column sharing the same letter are not significant different at $P_{0.05}$

The role of media, and plant regulators on plant regeneration

Different genotypes, nutrition media were studied to determine their role on plant regeneration from anther calli. The interaction of genotype by nutrition media showed that the highest frequency of plant regeneration was observed in IR64/IR68530 in N6 medium (5.73%). IR64/IR69146-25 exhibited high plant regeneration response in both MS and N6 media (5.21%). In general, N6 medium was found to be superior for plant regeneration in almost crosses as compared to MS medium (table3). The plant regeneration frequencies of IR64/IR68530, IR64/IR69146-15 and IR64/IR70441 were 3.12%, 1.91%, and 2.26%, respectively.

Table 3: Interaction between genotype by nutrition media on green plant regeneration

Crosses (V)	% Green plant regeneration in different media (E)		Mean (V)	Difference
	MS	N6		
IR64/IR68530	2.605 b	5.728 a	*4.167*	- 3.123**
IR64/IR69146-25	5.210 a	5.210 ab	*5.210*	0.000ns
IR64/IR69146-15	2.258 bc	4.168 bc	*3.213*	- 1.910**
IR64/IR70441	1.213 c	3.472 c	*2.343*	- 2.258**
Mean (E)	*2.822*	*4.645*	*3.733*	*- 1.823***
F (VxE)				4.36*
LSD (5%) of VxE				1.286

*Note: E: nutrition media factor V: genotype factor *: significant at P<0.05*
***: significant at P<0.01 ns: none significant difference at P<0.05*
Means within a column sharing the same letter are not significant different at $P_{0.05}$

Studying the role of media and plant growth regulators on plant regeneration from anther calli, in different two media and two levels of plant regulator combinations were performed in the experiment. The efficiency of plant regulator was similar in MS medium but it was higher in N6 with 0.5 mg/l NAA + 2 mg/l BAP (5.296%), as compared to 1 mg/l NAA + 1 mg/l BAP + 1 mg/l Kinetin (3.99). N6 medium was better than MS with both two levels of plant growth regulator combinations (table 4).

Table 4. Interaction efficiency of nutrition medium and plant regulator on regeneration

Plant regulator (L)	% Green plant regeneration in different media (E)		Mean (L)	Difference
	MS	N6		
1NAA+1BA+1Kin	2.952	3.993	3.473	-1.041*
0.5 NAA + 2 BAP	2.691	5.296	3.993	-2.605**
Mean (E)	2.822	4.645	3.733	-1.823**
Difference	0.262ns	- 1.303**	- 0.520	
F (ExL)				6.14*
LSD (5%) of 2- ExL				0.909

Note: E: nutrition media factor L: Plant regulator factor
**: significant at P<0.05 **: significant at P<0.01*

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SUMMARY IN VIETNAMESE

Nuôi cấy túi phấn cây lúa F_1 của các tổ hợp lai giữa giống IR64 với các giống lúa dạng hình mới

Túi phấn của cây lúa F_1 từ bốn tổ hợp lai giữa giống IR64 với các giống lúa dạng hình mới: IR69146-15, IR69146-25, IR68530 và IR70441 được sử dụng trong nuôi cấy để nghiên cứu hiệu quả của các môi trường và công thức chất điều hòa sinh trưởng khác nhau trong hai giai đoạn tạo mô sẹo và tái sinh cây xanh. Kết quả cho thấy tỉ lệ tạo mô sẹo cao nhất được tìm thấy ở tổ hợp IR64/IR69146-25 cấy trên môi trường MS có bổ sung 0,5 mg/l 2,4-D + 2 mg/l NAA (8,85%). Môi trường MS và N6 cho tỉ lệ tạo mô sẹo (6,648% và 6,25%) cao hơn môi trường LS (4,31%). Tổ hợp IR64/IR68530 cho kết quả tái sinh cây xanh cao nhất (5,728%) trên môi trường N6. Hiệu quả tái sinh cây xanh của môi trường N6 (4,645%) cao hơn 1.5 lần so với môi trường MS (2,822%). Kết quả khảo sát hiệu quả tái sinh cây xanh của môi trường và các công thức chất điều hòa sinh trưởng cho thấy môi trường N6 có bổ sung 2mg/l BAP + 0.5mg/l NAA cho tỉ lệ tái sinh cây xanh cao nhất (5,296%).