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Studies on the Application of Tissue Culture for Plant Breeding

I. Effect of genotype and other combined factors on the callus induction and plant regeneration in anther culture of rice

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農作物 組織培養의 育種學的 利用에 관한 硏究

第 1 報 벼 葯培養效率에 대한 遺傳子型과 다른 要因間의 複合效果

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ABSTRACT

Rice (*Oryza sativa* L.) anthers collected from different genotypes were cultured to investigate the priority to increase callus production rate among several factors affecting on callus formation. Rice varieties, F_1 hybrids and F_2 plants differed greatly in their abilities to produce callus from anthers, and it was confirmed that the culturability of rice anther was a heritable characteristic. Cold shock treatment before plating anthers promoted callus formation rate, but combined effect with genotype having high culturability was more significant. The response to sucrose concentration in culture medium in callus induction rate was different between rice genotypes, but combined effect with genotype was not significant. Single supplement of NAA to R_2 medium increased callus production rate remarkably only in rice genotype having high culturability. Conclusively selection of genotypes is most important to increase callus initiation frequency from rice anthers.

INTRODUCTION

Niizeki and Oono (1968) first obtained haploid plants by culturing anthers *in vitro*. Since that time, investigators have attempted to produce large number of haploids for practical use because the time for creating new varieties from conventional cross breeding can be shortened by this method[4,9,10], and several new rice varieties have been developed through anther culture in China[10]. However, considerable research is needed to increase the efficiency of callus formation and plantlet regeneration and thus provide breeders with sufficient numbers of homozygous lines, generated from F_1 or F_2 plants from crosses, to make selections.

A number of variables have been found which affect the rate of callus and plant formation from

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anthers. In addition to genotype difference[1,3,4,6,9], constituents of culture medium[9,10], preplating treatment with cold temperature[5,7,10,11], sucrose concentration in culture medium[1,2,7]; choice of growth regulators[1,3,7,8,9], incubation temperature and light[10], and growth stage of anther or pollen [4,5,9] have been found to play important roles in callus and plant induction in rice anther culture. This study was conducted to investigate the priority among several factors in their importance for increasing callus formation rate and thus to apply rice anther culture to conventional breeding more effectively.

MATERIALS AND METHODS

GENOTYPE EFFECT. Thirteen cultivars and four F_1 were planted in greenhouse and seven F_2 populations were seeded in field condition. Rice panicles, which were tested for their pollen stage by staining with aceto-carmine, were collected at three to five days before heading from the boot, and they were pretreated at 8°C for 10 days. To prevent dessication during pre treatment, panicles were placed in plastic bags and wrapped in aluminum foil. Prior to plating anthers, panicles were surface-sterilized in a 7% solution of calcium hypochlorite for 15 minutes and then washed three times with sterile distilled water. Chaleff's R_2 medium (table 1) was used for callus and plantlet induction. R_2 medium contained 3% of sucrose, and supplented with 2mg/liter α-naphthaleneacetic acid (NAA). About 100 dissected anthers from the panicle identified as the uninucleate microspore stage were plated on the surface of 10 ml of solidified medium in a 8-cm diametered petrs-dish. To prevent dessication during incubation, inoculated petri-dishes were sealed with a wide strip of Parafilm. The sealed plates were incubated for three weeks at 25°C in the dark, and moved to a 25°C incubator with a 16-hour daily light period provided by cool-white fluorescent lights.

Table 1. Chaleff's R_2 medium used for rice anther culture

Constituent	Amount (mg/liter)	Constituent	Amount (mg/liter)
NH_4NO_3	400	$CoCl_2 \cdot 6H_2O$	0.025
KNO_3	2,530	Na_2-EDTA	37.2
$CaCl_2 \cdot 2H_2O$	440	$FeSO_4 \cdot 7H_2O$	27.8
$MgSO_4 \cdot 7H_2O$	370	Thiamine-HCl	1.0
KH_2PO_4	170	Inositol	100
H_3BO_3	6.5	Sucrose	30,000
$MnSO_4 \cdot H_2O$	16.8	NAA	2
$ZnSO_4 \cdot 7H_2O$	6.62	Kinetin	0.3
KI	0.83	Agar	8,000
$NaMoO_4 \cdot 2H_2O$	0.25	PH	5.8
$CuSO_4 \cdot 5H_2O$	0.025		

COMBINED EFFECT WITH OTHER FACTORS. Rice panicles collected from four genotypes were treated with different durations of cold shock at 8°C. Cold shock durations ranged 0 to 30 days with 5-day interval. All the culture procedure except cold shock duration were same as mentioned above.

Rice anthers of six genotypes were cultured on R_2 medium with 3, 4 and 5% sucrose to know the combination effect of genotype with sucrose concentration. Anther stage, cold shock condition, anther plating and incubating methods followed the culture method described in genotype difference.

R_2 medium was supplemented with NAA and/or 2,4-D in different ways, R_2 plus 2mg/liter NAA only, R_2 plus 2mg/liter 2,4-D only, and R_2 plus 2mg/liter NAA and 2mg/liter 2,4-D both. Anthers collected from five rice genotypes were plated to investigate the combined effect of genotypes with growth regulators in culture medium. Another factors were fixed as same as methods mentioned in

genotype difference.

About 2 weeks after callus initiation, they were transferred into fresh R_2 medium with 3% sucrose, 2mg/liter NAA, and 0.3mg/liter kinetin for plant regeneration in all trials conducted in this study. The cultures were maintained under the same conditions as for callus initiation.

RESULTS AND DISCUSSION

GENOTYPE EFFECT. Percentages of callus formation from rice anthers varied from 0 to 41 depending on the genotypes used as presented in table 2. Anthers collected from F_2 plants of seven crosses produced callus with 0 to 13 percentages depending on cross combination. Plant organ production from anther derived callus also showed great variation between genotypes as shown in table 3.

Taipei 309 and F_1 of T1040 (M401/Mars) showed high callus formation ability exceeding 30%, and Tx765433 and F_1 of T1040 (M401/Mars) produced more plant organs among rice genotypes used. After Niizeki and Oono(8), many investigators[1,3,4,6,9,10] have demonstrated the variability in callus and plant production ability among rice varieties and F_1 hybrid plants. They suggested that the ability to perform under anther culture condition would be heritable. In order to confirm whether the anther culturability is heritable, more than 100 panicles were collected from different F_2 plants, which showed almost same growth stage, in a cross combination, Tadukan/Starbonnet, and were tested for their callus production abilities. F_2 plants should greatly differed in callus formation rate each other if the anther culturability is heritable, because F_2 population is segregated genetically. Many of the plates were contaminated with fungus and others showed no callus initiation, and only 24 F_2 panicles could be compared in their callus production abilities (table 4). Percentages of callus formation were distributed from 0 to 48% among F_2 plants in the

Table 2. Genotypic difference of callus inducing rate in rice anther culture

Genotype	Anther no. (A)	Callus no. (B)	B/A (%)	Genotype	A	B	B/A (%)	Genotype	A	B	B/A (%)
Tx 765	341	58	17.0	Labelle	448	63	13.8	T/IR F_1	1234	277	22.4
PI 373	234	0	0.0	Nongbaek	1034	158	14.9	3d F_2	1160	0	0.0
Taducan	420	16	3.8	T 309	813	252	31.0	17d F_2	940	89	9.5
68 Cr3332	218	0	0.0	Minehik.	667	17	2.5	19d F_2	690	69	10.0
Nortai	231	28	12.1	IR 1317	700	21	3.0	20d F_2	6623	869	13.1
Cr 765	226	3	1.3	T1040 F_1	915	375	41.0	26d F_2	1040	54	5.2
IR 661	234	0	0.0	T1061 F_1	553	5	0.9	28d F_2	600	1	0.2
Starb.	228	55	24.1	N/T309F_1	1158	71	6.1	33d F_2	750	31	4.1

Table 3. Genotypic difference in plant organ differentiation from anther derived callus in rice

Genotype	Callus no.	Gr. pl.	Alb.	Others	Genotype	Callus no.	Gr. pl.	Alb.	Others
Tx765433	58	8	2	18	IR 1317	21	0	1	1
Taducan	16	0	0	7	T1040 F_1	375	8	12	152
Nortai	28	0	0	0	N/T 309 F_1	277	1	6	15
Cr765270	3	0	0	0	T/11317 F_1	277	1	6	15
Starbonnet	55	0	0	0	17d F_2	89	1	0	1
Labelle	63	1	0	0	19d F_2	69	0	8	2
Nongbaek	158	5	2	11	20d F_2	869	10	24	13
Taipei 309	252	5	2	52	26d F_2	54	0	1	1
Minehikari	17	0	0	0	33d F_2	31	0	0	0

Table 4. Variation of callus inducing rate among F_2 plants of a rice cross, "Taducan/Starbonnet"

Plant no.	Anther no.(A)	Callus no.(B)	B/A (%)	Plant no.	Anther no.(A)	Callus no.(B)	B/A (%)	Plant no.	Anther no.(A)	Callus no.(B)	B/A (%)
1.	318	154	48.4	9.	310	1	0.3	17.	180	3	1.7
2.	358	111	31.0	10.	290	31	10.7	18.	308	103	33.4
3.	175	22	12.6	11.	320	6	1.9	19.	170	7	4.1
4.	330	31	9.4	12.	160	32	20.0	20.	310	56	18.1
5.	295	4	1.4	13.	155	19	12.3	21.	165	1	0.6
6.	310	39	12.6	14.	340	69	20.3	22.	154	40	26.0
7.	310	0	0.0	15.	330	2	0.6	23.	346	41	11.8
8.	310	5	1.6	16.	163	23	14.1	24.	340	67	19.7

same cross. This means that the anther culturability of rice plant is a heritable characteristic, and that it may be possible to select for high culturability and transfer this characteristic into rice varieties and breeding lines as parents in crosses.

COMBINED EFFECT WITH OTHER FACTORS. Callus inducing rates at different durations of cold shock at 8°C before plating anthers on medium are shown in table 5. Five to ten-day treatment with cold temperature increased callus formation rate in four genotypes used. Genovesi and Magill[5], and othes[7,10,11] demonstrated that preplating treatments of anthers at 5 to 13°C for 5 to 14 days increased callus inducing rate remarkably. The result in this study agreed with them. The combination effect of genotype with cold shock treatment was significant like in Taipei 309 among 4 genotypes used. Taipei 309, showing 37% at 10-day treatment, maintained 20% callus induction frequency until 20-day treatment, and formed a few callus after 30-day storage in 8°C incubator. However, another genotypes showed less than 20% at 10-day treatment and near 0% of callus production even in case of 20-day treatment with could tmeperature.

The response to sucrose concentration in callus

Table 5. Callus inducing rate at different durations of cold shock before plating rice anthers on medium.

Genotype / Cold shock	N/T 309 F_1 Anther no.(A)	Callus no.(B)	B/A (%)	Nongbaek A	B	B/A (%)	Taipei 309 A	B	B/A (%)	Labelle A	B	B/A (%)
0 days	220	5	2.3	–	–	–	330	19	5.8	–	–	–
5	456	36	7.9	–	–	–	–	–	–	–	–	–
10	702	35	5.0	572	95	16.6	577	216	37.4	448	63	14.1
15	–	–	–	–	–	–	340	71	20.9	–	–	–
20	–	–	–	222	3	1.4	236	36	15.3	358	0	0.0
30	–	–	–	354	0	0.0	120	4	3.3	320	0	0.0

induction rate was different between rice genotypes (table6). For example, the highest callus formation rate was obtained in 4% sucrose and followed by 5% in a variety, Nongbaek, but no difference was found in Taipei 309 between 3 and 4%, and the highest performance was obtained in 3% in F_1 hybrid in T309/IR1317. In general, callus production in 3% sucrose medium was not different with that in 4%, but lower frequency of callus induction was found in 5% sucrose medium. Chen[2] reported that high sucrose concentration in modified MS medium promoted callus formation and subsequent organogenesis. General result in this study did not agree with him mainly due to the different genotypes and culture medium used, it is suggested. Combined effect of genotype with sucrose concentration was not significant in this study.

Table 6. Callus inducing rate at different levels of sucrose in rice anther culture medium

Sucrose / Geno-type	3% Anther no.(A)	3% Callus no.(B)	3% B/A (%)	4% A	4% B	4% B/A (%)	5% A	5% B	5% B/A (%)
Nongbaek	466	35	7.5	568	123	21.7	342	40	11.7
Taipei 309	577	216	37.4	226	80	35.4	–	–	–
IR 1317	356	15	4.2	344	13	3.8	358	11	3.1
T1040 F_1	457	174	38.1	458	201	43.9	354	65	18.4
N/T 309 F_1	576	34	5.9	582	37	6.3	572	31	5.4
T/I 1317 F_1	620	167	26.9	614	110	17.9	–	–	–

Single supplement of NAA to Chaleff's R_2 medium was best for callus formation from rice anthers as in table 7. Combined effect of genotype with NAA supplement was found, for example, percentage of callus formation of F_1 of T1040 (M401/Mars) was changed from 0.8 in 2,4-D adding to 38.1 in NAA. This result suggests that the response to growth regulators in culture medium may vary with rice genotypes used. Many investigators[1,3,7,8,9,10] found that growth regulators, like 2,4-D, IAA, NAA, MCPA, and 2,4,5,-T, increased callus induction frequency from rice anther, but the results were not consistnet because they used different genotypes and culture medica each other.

Table 7. Effect of NAA and 2,4-D on callus inducing rate in rice anther culture

Medium / Geno-type	Anther no.(A)	Callus no.(B)	B/A (%)	A	A	B/A (%)	A	B	B/A (%)
Minehikari	667	17	2.5	970	23	2.4	777	2	0.3
Nongbaek	466	35	7.5	320	3	0.9	232	0	0.0
IR 1317	356	11	3.1	232	0	0.0	–	–	–
T1040 F_1	457	174	38.1	125	1	0.8	–	–	–
N/T 309 F_1	346	20	5.8	346	8	2.3	346	6	1.7

Conclusively selection of genotypes is most important to increase callus initiation frequency from rice anthers. Cold shock treatment before plating anthers promoted callus formation rate, but combined effect with genotype having high culturability was more significant. NAA supplement to R_2 medium increased callus induction rate remarkably only in rice genotypes having high culturability. Genotype improvement for anther culturability may make easy to apply tissue culture techniques for plant breeding.

摘　　要

벼의 葯培養效率을 增進시키는데 관여하는 要因들의 重要性을 檢討하기 위하여 13個의 品種, 4個組合의 F_1, 7個組合의 F_2를 供試하여 이들의 葯을 R_2 培地에서 培養하였다. 遺傳子型間 葯培養能力의 差異와 함께 葯에 대한 低溫處理, 培地의 糖含量, 培地에 대한 生長調節劑 첨가 等의 單獨效果 및 遺傳子型과의 複合效果가 檢討될 수 있도록 實驗이 遂行되었다.

1. 同一한 環境에서 栽培된 벼品種 및 交配組合別 F_1들의 葯培養能力間에는 큰 差異가 認定되었으며 圃場에서 栽培된 F_2들도 交配組合間에 平均 葯培養能力이 크게 달랐다.

2. 同一한 交配組合의 F_2個體들 間에도 Callus分化率이 큰 變異를 보이고 있어 葯培養能力이 遺傳變異를 보이는 形質임이 確認되었다.

3. 葯을 培地에 置床하기 前 8℃에 5~10日間 溫度處理하는 것은 Callus分化率을 向上시키는데 效果的이었는데 그 效果는 葯培養能力이 높은 品種에서 더 컸다.

4. R_2培地의 糖含量은 3～4%인 것이 Callus分化에 좋았으며 3～5%사이에서는 遺傳子型과의 複合效果가 認定되지 않았다.

5. R_2培地에서는 NAA만을 첨가하는 것이 Callus分化率을 높이는데 效果的이었으며 葯培養能力이 높은 遺傳子型을 이용했을 때 그 效果가 훨씬 더 컸다.

6. 葯培養能力이 높은 遺傳子型을 選拔, 利用하는 것이 葯培養에 관여하는 要因들의 效率도 높일 수 있어 그 중요성이 가장 크다고 할 수 있다.

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