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RICE BREEDING FOR HIGH GRAIN QUALITY THROUGH ANTHER CULTURE

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ABSTRACT

Anther culture technique offers great opportunities for accelerating breeding progress and improves grain quality characters. From four crosses between OM4900 and OM3536, OM5930, OMCS2000, OM5992; the frequency of callus induced exhibited the lowest in case of OM3536/OM4900 (5.13%) and the highest in OM5992/OM4900 (9.27%). Green plant regeneration rates varied from 6.17% (OM3536/OM4900) to 14.00% (OMCS2000/OM4900). Further evaluations of these lines under greenhouse and field conditions addressed the successful selection of 22 outstanding DHLs. Aside from giving stable performance, these lines were selected for their yield, grain quality and uniformity.

Key words. amylose content, anther culture, callus, green plant regeneration.

INTRODUCTION

The creation of high yielding rice varieties, good grain quality begins with knowledge of biotechnology combined with traditional experiences will produce results faster and more accurate. Many varieties of rice produced by tissue culture techniques and *in vitro* selection has improved some of the characteristics of shape, nutritional quality, pest resistance and the harsh conditions of the environment (Sun et al. 1992).

One of advantages of tissue culture techniques that the protocols may permit the recovery of variants not easily obtained by conventional breeding practice. The probability for the in vitro recovery of benefit homozygous genotype reasonably high when anther culture, which provides the benefits of haploid event, is used to provide cells for biochemical selections. However, variation may be benefit or deleterious.

Production of double haploids through anther culture is a rapid approach to homozygosity that shortens the time required for the development of new rice cultivars as compared to conventional methods, which require at least 6-7 generations.

Most of these applications have been limited to crosses involving at least one japonica parent due to the recalcitrant nature of indica rice varieties. Japonica cultivars are generally easier to culture than indica ones. Early anther necrosis, poor callus proliferation and albino-plant regeneration are currently recognized as the major problems in indica rice varieties (Chen et al. 1991).

The high grain quality of rice meets the demand of both domestic and international markets for tasting and grain appearance. Country can be benefited by earning foreign exchange by production and export of rice. However, there are some limitations for farmers to cultivate good quality rice. It needs to develop high yielding varieties, with disease or pest resistance, salt tolerance and proper cultural management. The conventional breeding techniques are time consuming and self in-compatibility act as barrier for distant hybridization and fertilization. The quality variety can be improved (with disease and pest resistance, and salt tolerance) through tissue culture techniques *viz.* anther culture or genetic manipulation like protoplast fusion (hybrid and cybrid) and through gene transfer

In this paper, we describes the frequency androgenesis and green plant regeneration from the anthers and quality characters in the double haploids lines of heterotic F_1 plants of some novel indica rice crosses.

MATERIALS AND METHODS

Plant Materials

F_1 plants from crosses between OM4900 and OM3536, OM5992, OM5930 genotypes, were used in this study. Anther donor rice plants were grown in green house. Planting was done at 1-

week interval for five sowing dates, which served as replication.

Preparation of Planting Materials

Panicles at booting stage were collected when the anthers occupy 1/3 to 1/2 of the spikelet length or when the auricle distance between the flag leaf and subtending leaf of the primary and secondary tillers reach 5-10 cm depending on genotypes.

The boots were placed inside the refrigerator with an average temperature of 7-10^{0}C for 5-10 days. After cold treatment, panicle were surface sterilized in ethanol 70% for 1 minutes, and 3% sodium hypochlorite for 15 minutes under aseptic condition; individual spikelets were cut to expose the anthers.

Callus Induction and Plant Regeneration

Anthers containing pollen at the mid-uninucleate to early binuclear stage of development were plated onto the N6 medium (Chu et al. 1975), supplemented with 2mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 2mg/L napthalenic acetic acid (NAA) and 6% sucrose. Cultures were kept under dark condition with the temperature range of 25-28^{0}C. Induced calli were subculture onto the plant regeneration medium consisting of MS medium with combination 1mg/L BA + 2mg/L Kinetin + 3% sucrose. Cultures were kept under 16 hrs daily illumination with 80-watt fluorescent bulb.

Grain quality analysis

Grains harvested from experiment were milled and grinded. Powder sample were analysis in laboratory of Genetics and Plant Breeding Department, CLRRI by using biochemistry method for amylose content (AC), gelatinization temperature (GT), gel consistency (GC) analysis.

The simplified amylose assay was used for amylose content determination. 100 mg of milled rice flour was gelatinized with 1 N NaOH in a boiling water bath for 10 min. The absorbance of the amylose-iodine mixture was determined colorimetricly at 590 nm, and the amylose content of the sample was calculated based on the calibration curve of standard samples of which the amylose contents were determined previously by the method of Sadavisam and Manikam (1992).

The protocol was used for gelatinization temperature determination. Six milled whole grain samples were placed in petri dish containing 10 ml 1.7% KOH. The petri dish were covered and incubated at 30°C for 23 hours. The appearance and disintegration of the kernels were rated visually after incubation based on the 7 scales. A rating of 1 to 3 was classified as high final gelatinization temperature (>75°C); 4 to 5, intermediate (70-74°C); and 6 to 7, low final gelatinization temperature (< 70°C).

The gel consistency test was based on the method of Tang et al. (1991). 100 mg of 100-mesh screen passed rice flour containing 12% of moisture was placed in a 13 x 100 mm test tube and gelatinized with 0.2 N KOH in a boiling water bath for 8 min. After being cooled in an ice-water bath, the tubes were laid fiat on the laboratory table for one hour. The length of the gel from the bottom of the tube to the gel front was measured in millimeters and regarded as the gel consistency of the samples.

Data Analysis

The data were analyzed based on the transformed date using the Cropstat program. Mean comparisons were conducted using the Duncan Multiple Range test (DMRT) at 5% probability level.

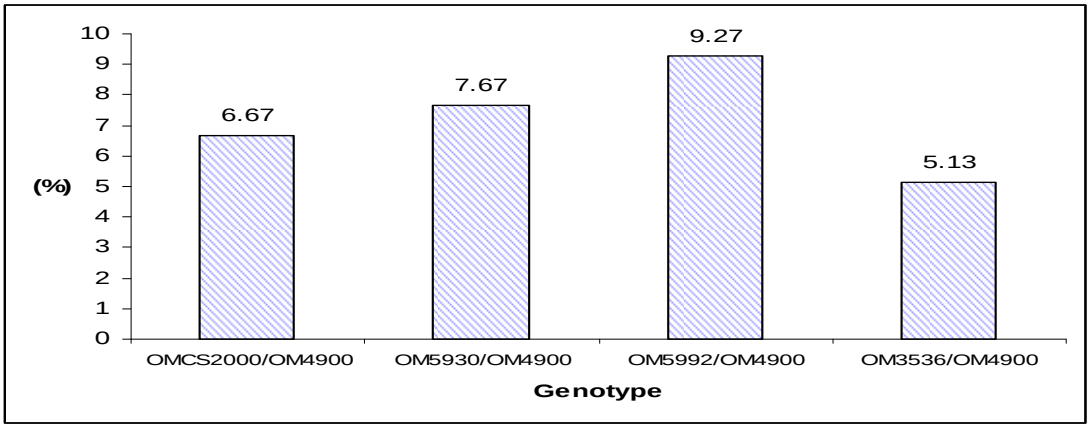
RESULTS AND DISCUSSION

Anther Culture Response

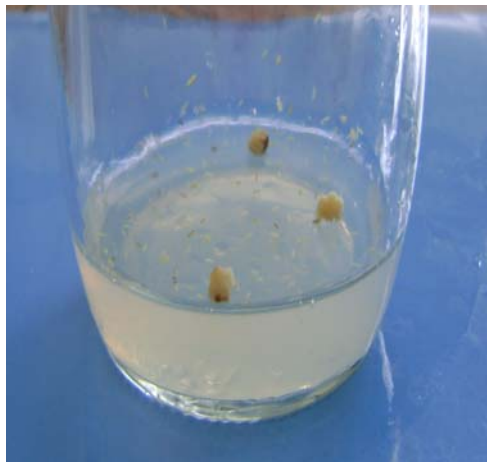
Callus induction: Callus induction started when anther color changed from yellow to brown three to four weeks after culture. Callus formation continued up to many weeks to three month. Anthers yielded embryogenic calli were compact, globular in shape, rapidly growing and with clearly formed somatic embryos. Anther form callus after 21-30 days.

Table 1. Percentages callus formation and green plant regeneration

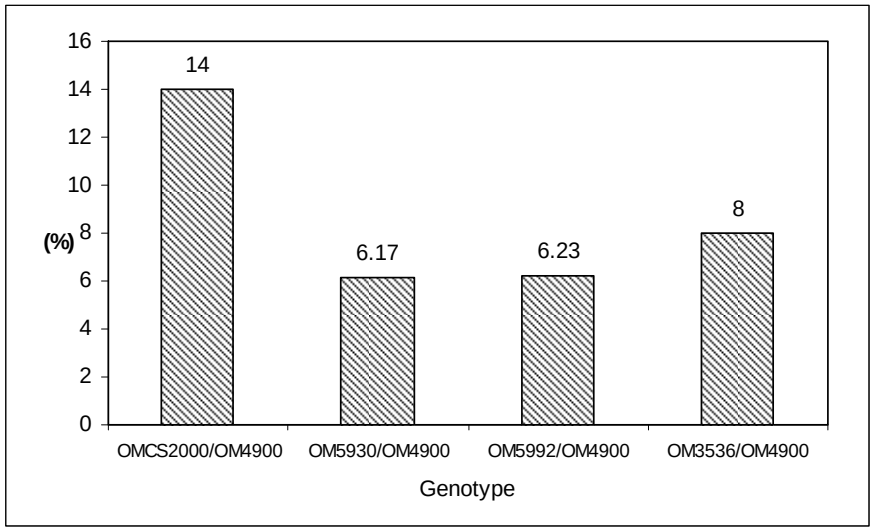
Genotype	Callus induction (%)	Green plant regeneration (%)
OMCS2000/OM4900	6.67 b	8.00b
OM5930/OM4900	7.67 b	6.17b
OM5992/OM4900	9.27 a	6.23b
OM3536/OM4900	5.13 c	14.00a
CV%	10.38	10.02



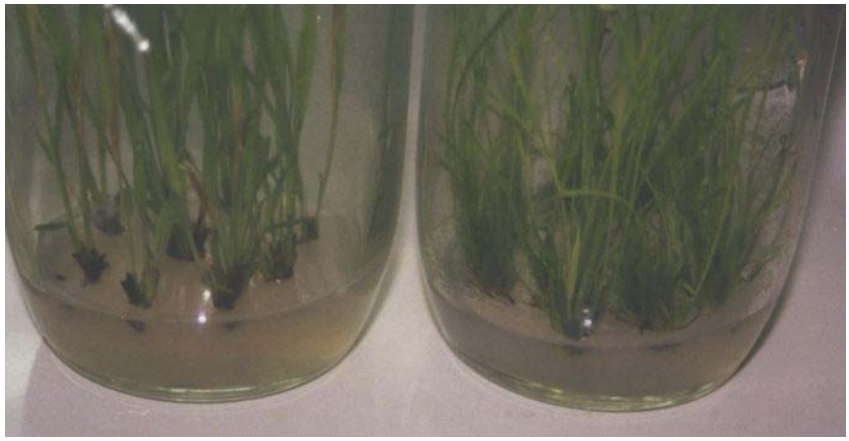
The frequency of the anthers forming calli varied between 5.13 to 9.27% depending upon the genotype (Table 1). The data on callus formation revealed that anthers from OM5992 / OM4900 produced the highest number of calli (9.27%) and the lowest number of calli inducted OM3536 / OM4900 (5.13%). Crosses of OMCS2000 / OM4900, OM5930 / OM4900 ranked second and the difference was significantly different from OM5992/OM4900 and OM3536/OM4900. There were strong genotypic effects on callus induction frequency among four crosses.



Green plant regeneration: In spite of the levels of callus formation, plant regeneration was very low. High callus formation did not result high levels of plant regeneration efficiency. The result showed that callus with soft shape, light yellow color were given high green plant regeneration, the callus with hard shape, yellow color were died after transfer to green plant regeneration medium.



The assessment results can green plant regeneration showed differences between four crosses. Green plant regeneration rates varied from 6.17% to 14.00% (Table 1). OMCS2000 / OM4900 rates of green plant regeneration reached the highest of 14.00%, combined with high rates of OM5992 / OM4900 green plant regenerated the lowest, at 6.17%. The result showed that the difference in the rate of green plant regeneration of the various crosses were significantly different. Compared with the results of a study by Gioi (2003), anther culture of F_1 plants from crosses between IR64 and new plant types cultivars reached the highest green plant regeneration in the experiment of 5.72%. The ability to form green plant regeneration of all four crosses is superior.



Grain quality analysis: Among rice genotypes of the same apparent amylose type, alkali spreading value and gel consistency may be used as quality indices. Among high-amylose rices, intermediate GT and soft gel consistency are preferred by consumers over low GT and hard gel consistency (Juliano 1985b). Among intermediate-amylose rices with an intermediate GT value are preferred to those with a low GT value, as the cooked rice is softer. Gel consistency values are similar among these intermediate-amylose rices. Among low-amylose and waxy rices, a low-GT type is preferred to a type with a high GT value. In terms of rice improvement breeding, hard gel consistency is dominant over medium and soft gel, and medium gel consistency is dominant over soft (Tang, Khush and Juliano 1989).

Table 2. Summary of the analysis for grain quality

Genotype	Total (lines)	AC (%)			GC (mm)			GT (score)		
		Low (lines)	Medium (lines)	High (lines)	Low (lines)	Medium (lines)	High (lines)	Low (lines)	Medium (lines)	High (lines)
OM3536 / OM4900	40	5	31	4	2	26	12	7	24	9
OMCS2000/OM4900	30	0	26	4	0	17	13	12	15	5
OM5992/OM4900	30	8	20	2	1	7	22	2	16	12
OM5930/OM4900	33	9	23	1	0	7	23	13	13	4

AC: low <20%, medium=20-25%, high >25%; GC: low <40mm, medium = 41-60mm, high > 60mm; GT: low= 1-3 (score), medium= 4-5 (score), high= 6-7 (score).

The gel consistency values increased consistently for all crosses studied as compared to their parents. The percentage of GT in four crosses also increased. Of 22 selected lines from 133 genotypes exhibited low amylose content, high gel consistency and gel temperature. Especially, nine selected lines from OM5930/OM4900 gained low amylose content, which exhibited the better improvement as compared to the parent OM5930. The grain quality analysis addressed that the DHLs were better than the parents.

REFERENCES

Chen CC, HS Tsay, CR Huang. 1991. Factors affecting androgenesis in rice (*Oryza sativa* L.). *In*: YPS Bajaj (Ed.), Rice. Biotechnology in Agriculture and Forestry, Vol. 14, pp: 192–215, Springer, Berlin, Heidelberg, New York.

Chu CC, CC Wang, CS Sun, C Hsu, CY Chu, and FY Bi. 1975. Establishment of an efficient medium for anther culture of rice, through comparative experiments on the nitrogen sources. Science Sinica 18, pp: 659-668.

Gioi TD and VD Tuan. 2004. Anther culture from crosses between IR64 and new plant type cultivars. OMonRice 12: 27-32.

Juliano BO (ed). 1985b. Rice: Chemistry and Technology, 2nd Ed. St Paul, MN, USA, Am. Assoc. Cereal Chem. 774 pp.

Sadavisam and Manikam. 1992. Biochemical methods for agricultural sciences. Wiley Eastern Ltd. India.

Sun ZX, HM Si, XY Zhan and SH Chen. 1992. The effect of thermo- photoperiod for donor plant growth on anther culture of Indica rice, *In* CB You (Ed), Biotechnology in Agriculture, Proceedings of the first Asian Pacific Conference on Agriculture Biotechnology, Beijing, China, 20-24 August 1992, Current plant Science and Biotechnology in Agriculture 15, pp.361-364.

Tang SX, GS Khush, BO Juliano. 1989. Diallel analysis of gel consistency in rice (*Oryz. sativa* L.). SABRAO J., 21: 135-142.

Tang SX, GS Khush, BO Juliano. 1991. Genetic of gel consistency in rice. India J. Genet. (101): 823-829.

Chọn giống phẩm chất bằng phương pháp nuôi cấy túi phấn

Nghiên cứu kỹ thuật nuôi cấy túi phấn rất có ý nghĩa cho việc chọn giống và cải tiến phẩm chất gạo. Thông qua phương pháp này, chúng tôi sử dụng 4 tổ hợp lai F_1 của OM3536 / OM4900, OMCS2000 / OM4900, OM5930 / OM4900, OM5992 / OM4900. Tỉ lệ hình thành mô sẹo biến thiên từ 5,13% (OM3536/OM4900), đến 9,27% (OM5992/OM4900). Tỉ lệ cây xanh tái sinh cao nhất ở tổ hợp lai F_1 OMCS2000 / OM4900. Cây xanh được thích nghi, trồng trong nhà lưới và ngoài đồng. Qua quá trình theo dõi, chúng tôi chọn được 22 dòng nuôi cấy túi phấn từ 4 tổ hợp lai có năng suất cao, phẩm chất tốt.