IMPACT OF DIFFERENT MEDIA AND GENOTYPES IN IMPROVING ANTHER CULTURE RESPONSE IN RICE (*Oryza sativa*) IN BANGLADESH

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Abstract

Rice is an important crop in Bangladesh.In vitro production of doubled haploid (DH) plants through anther-culture provides an efficient method for rapid production of homozygous lines. For this purpose, two different media MS and N6 supplemented with 1mg/L BAP +1mg/L 2,4-D+0.5mg/L NAA were evaluated for callus induction and green plant regeneration.Results showed that the highest percentages of calluses and green plants as a continuous process were obtained by N6 compared to MS. The best response to callus formation was obtained by BR48 and and minimum green plant obtained from OM576.

Keywords: Rice, anther culture, plant breeding

Introduction:

Rice is a major source of livelihood in terms of providing food, income and employment in Bangladesh. It covers near about seventy seven percent of the total cropped area in the country. Rice is the staple food for Bangladeshis, with a per capita consumption of approximately 143 kg. of milled rice per year, comprising around 75 % of the calorie supplied.

On the other hand, Bangladesh needs to increase rice yield further to meet the growing demand emanating from population growth. The United Nations (UNO, 1998) project that even by 2020 the Bangladesh population will grow at 1.2% per year and will reach 173 million. Nearly 46% of the population will live in urban areas in 2020 compared to 27% now. Farmers will have to generate larger marketable surplus to feed the growing urban population.

Thus no option remains but to bridge the gap between potential and actual yields., Although plant breeding methods have considerably contributed to increasing the productivity of modern rice, the advanced

technologies to complement conventional breeding techniques must be explored .Among these, biotechnological tools include the production of haploids plants via anther culture.

The advantage of the *in vitro* production of haploids over the conventional method is the shortening of time to achieve homozygosity. Since homozygosity is attained in one step, three to five years of selfing and reselection which is done in conventional breeding could be bypassed. Since no further segregation occurs, each plant derived from anther culture is a potential new variety and could be screened immediately for desirable characters.

Besides shortening the breeding cycle, there is also an increase in selection efficiency. In anther culture, the number of plants required to express the different recombinants is less than in conventional plant breeding. The probability of obtaining the desired phonotype from among the regenerated plants is higher since all possible gene combinations will be expressed.

While the anther culture technique is widely used for practical breeding, its application is still limited by many factors which influence culture efficiency, such as the genotype of the explants, the growing conditions of the donor plants, and the developmental stage of the microspores, pre-treatment of the panicles, the culture methods, the media and the culture conditions. One of the major constrains in the use of anther culture in rice improvement programmes is the identification of responsive anthers for callus formation.

Anther culture is one of the most extensively investigated areas of rice tissue culture. In China, for example, over a dozen important varieties and more than 100 improved lines have been developed using this technique. Anther culture-derived improved lines have also been developed in India, Japan, South Korea, Taiwan, Hungary and USA. However, most of the progress and achievements made pertain to japonica rice. Japonicas are easier to culture than indica cultivars. In addition, genotypic specificity to anther response and green-plant regeneration is a more serious problem in indica than japonica varieties. Thus, utilization of anther-culture breeding in areas predominantly planted with indica rice, such as the tropical/subtropical areas of Bangladesh, India, Pakistan, Philippines, Thailand etc., has not gained as much recognition and acceptance as an innovative breeding technology as in those areas where japonica rice is grown.In this study attempts were made to micropropagate F_1 crosses through callus induction of anther.

In the recent past anther culture in rice has been improved substantially.

However, detailed study on various factors governing culture response of anthersunder *in vitro* condition especially in *indica* rice is extremely limited.

By the use of these techniques, phenotypically and genotypically uniform plants were obtained, with a wideperspective to plant breeding for developing better varieties in a short time .

Materials and methods:

Explants Sterilization:

Boots of BR 48, BR 45,OM576 and CNI9024 were collected from BRAC rice field in the morning when the auricle distance of the flag leaf to that of the next leaf was of around 8-13 cm. The boots were kept under running tap water for about 30 minutes and then kept in a solution of liquid detergent (Teepol) for 2-3 minutes to remove surface dirt. They were then rinsed with sterile double distilled water at least thrice to remove the traces of detergent. The explants were then surface sterilized with 20% Chlorox (commercial bleach containing 5.2% (w/v) NaOCl solution) for 5-6 minutes. Surface sterilized explants were again treated with 0.1% mercuric chloride solution for 4-5 minutes under aseptic condition then washed five to six times with sterilized distilled water. Then panicles were wrapped in moistened paper and cold treated at 6ºC for 6 days.

Florets which have anther having length of less than half of the size of the floret were selected. Portion just above the anthers was cut and inoculated into nutrient media with different concentrations and combinations of growth regulators for callus induction. The experiment was conducted with three replications.

Culture media

Two basic media were used in this study with supplement of 1 mg/l BAP+ 1 mg/l 2,4-D +0.5 mg/l NAA

N6 medium of Chu (1978)

MS medium of Murashige and Skoog (1962)

Calli with the size ranging from 1.5-3 mm in diameter were regenerated in nutrient medium containing N6 medium supplemented with 1 mg/l BAP+ 1 mg/l 2,4-D +0.5 mg/l NAA .

All the cultures were grown in an air conditioned culture room at a temperature of 26±2ºc. The source of illumination was 40 w white florescent tubes light with intensity varying from 2000-3000 lux. The photoperiod was maintained at 16 h light and 8 h darkness and 55±5% humidity was maintained. Visual observation of culture was made every week and data were recorded after 4 weeks of inoculation.

The green regenerated plants were transferred to MS medium in the absence of phytohormones for root formation. Completely regenerated plants were kept for hardening and then cultivated in the greenhouse for further observation and evaluation.

Results and Discussion:

Effect of genotypes and nutrition media on callus induction

In this study, the medium was supplemented with various concentrations of different growth hormones for the induction of callus fromanthers.Though callus formation from anthers was observed in all treatments, the highest callus formation was found for BR48 (4.25 %). The lowest callus induction was found from OM576 (0.75%).Frequency of callus induction for all genotypes in N6 medium was higher than that in MS media and the average through four crosses was the highest also in N6 medium(table 1).

Table1: Effect of genotype and nutrient media on callus induction from anther culture

Genotypes	Percentage of anther formed callus (%) on different media		
	MS	N6	Average
BR5	2.0	1.8	1.9
BR48	4.0	4.5	4.25
OM576	0.5	1.0	0.75
CNI9024	3.5	4	3.75

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 is shown in table 2.

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

Genotypes	Callus forming roots /anthers (%)	Plant regeneration / anthers (%)	
		Albino plants	Green plants
BR5	0.8	0.4	0.5
BR48	1.5	1.6	1.1
OM576	0.25	0.43	0.2
CNI9024	1.8	1.6	0.4

Frequency of green plant regeneration was observed very low in all treatments. The highest green plants obtained from BR48 as 1.1%. Chen et al. (1991) reported that frequency of anthers producing callus, capacity of differentiation and chromosome number of regenerated plants were highly related to donor genotype. Anthers of some indica varieties forms no calli (Tsai and Lin 1977). This preliminary study reported here showed that

though green plant regeneration from anther was possible but more study needs for green plants regeneration from anther culture.

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