

Effect of Concentration of Sucrose on the Frequency of Callus Induction and Plant Regeneration in Anther Culture of Rice (*Oryza sativa* L.)

Sharmin Shahnewaz¹ and M. A. Bari

Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

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Abstract

Effect of various concentrations of sucrose in culture media on the frequency of callus induction and regeneration of green plantlets from anther culture of rice were investigated. Cold pretreated anthers of BRRRI Dhan-29 at 6°C for seven days were cultured on N6 medium containing sucrose at concentrations of 0, 1, 2, 3, 4, 5, 6%; 2.0 mg/l 2,4-D; 0.5 mg/l Kn and 2.5 mg/l NAA. Results revealed that 4% sucrose was suitable for inducing high frequency (7.5%) callus induction and high green plant regeneration (65%). Highest concentration of sucrose (6% and above) in the culture medium not only resulted in an increase in the percentage of callus formation but also prompted the regeneration of albino plants.

Introduction

Anther culture is an important technique for immediate fixation of homozygosity and shortening of breeding cycle. Haploids obtained from anther culture are potential breeding material in crop improvement (Collins et al. 1981). Several scientists (Niizeki and Oono 1968, Hu Han 1978, Wang et al. 1979, Hakim and Mansur 1991) have successfully produced callus and plants from anther cultures and isolated pollen. Doubled haploid plants have been produced from single gametes by using anther culture technique. For its effective utilization in breeding programs, the haploid production technique should allow genotype independent production of large numbers of haploid. In rice production of haploid through anther culture has major obstacle with low regeneration rate and albino production (Lentini et al. 1995). Many factors that affect the frequency of callus formation and green plant regeneration from

¹Author for correspondence.

in vitro anther culture of rice are composition of culture media, pretreatment of panicles, anther condition, genotype, growing conditions of the donor plants and development stage of microspores (Yamada et al. 1967, Pande and Bhojwani 1999, Chaleff and Stolarz 1981 and Sharmin and Bari 2001).

Anther culture has been well integrated into rice breeding programs, specially in China and a number of high yielding, disease resistant and quality rice varieties have been developed from the anther derived plants (Shih-Wei and Zhi-Hong 1991), still remain problems. Albino plants production from rice anther culture is a serious problem in *indica* rice varieties (Chen et al. 1991). Many investigators reported the effect of sucrose concentrations upon the frequency of callus formation and regeneration of albino plants from cultured rice anthers (Matsubayashi and Kuranuki 1975, Sopory 1979, Chen 1978, Wang et al. 1974). Wang et al. (1977) reported that sucrose concentration of above 6% in the induction medium increased proportion of albino plants. Elevated level of sucrose in culture medium not only increase the frequency of callus formation but also promote the regeneration of albino plants.

Sucrose has been used as a major carbohydrate source in the induction medium. In rice, higher level of sucrose besides promoting the induction and growth of callus, is also useful in organogenesis (Chi-Chang-Chen 1978) but the callus developed in a medium containing 9% sucrose differentiated into more of albino plants than those from low concentration of sucrose. Wang et al. (1974) reported the increase of frequency of callus formation by regulating organic supplements and osmotic pressure in the medium without changing the basic composition.

Reinert et al. (1977) suggested that sucrose level of 2 - 5% is good for rice anther culture. Sandhu et al. (1993) reported a high frequency regeneration of green plant in rice when lower concentration of sucrose was used (3% w/v) for callus induction of anther.

Maltose has been reported to be a superior source of carbohydrate than sucrose for androgenesis in several species, including cereals (Last and Brettel 1990, Pande and Bhojwani 1999).

Several cultivars of rice have been studied for callus induction and regeneration of plantlets. The effect of genotypes, influence of phytohormones and effect of cold treatment were studied by Sharmin and Bari (2001). They found BRR1 Dhan-29 was more responsive to callus induction and plant regeneration among the tested varieties. So, the cultivar BRR1 Dhan-29 has been chosen to investigate the effect of various concentrations of sucrose in

culture media on the induction of callus and green plant regeneration frequency through anther culture of rice.

Materials and Methods

Rice cultivar BRR1 Dhan-29 was selected for conducting the experiment. The panicles were collected from the healthy plants when the distance between the auricle of flag leaf and penultimate leaf was 5 - 7 cm. In rice the peak response is reported to occur at the late uninucleate stage (Chaleff 1979). The panicles were warped in polythene bags and kept in the dark for seven days at 6°C. Panicles were rinsed with ethanol (70%) for 5 minutes. Spikelets were then removed from the sheath leaf, surface sterilized with 95% alcohol for 3 minutes and rinsed thoroughly with sterilized distilled water.

Anthers were picked up from the spikelets by sterilized forceps and placed horizontally on the N6 medium for callus induction. Anthers were cultured on N6 (Chu 1978) medium containing 0, 1, 2, 3, 4, 5 and 6% sucrose; 2.5 mg/l 2,4-D, 2.5 mg/l NAA and 0.5 mg/l Kn for callus induction.

There were three dishes per replication depending upon the availability of anthers. The culture was maintained in the dark at 26 ± 2 °C. The cultures were examined every week for up to six weeks.

Data were recorded on the percentage of anthers forming calli in six weeks (callus induction frequency) to evaluate the effect of concentration of sucrose on the frequency of induction of callus.

For regeneration of plantlets anther derived calli obtained from the above mentioned culture media were transferred to a modified MS (Murashige and Skoog 1962) medium supplemented with 2.0 mg/l BAP, 1.0 mg/l NAA and 1.0 mg/l Kn and incubated in light for four - six weeks.

Data on percentage of calli regenerating green and albino plantlets were recorded after six weeks of total incubation.

Results and Discussion

It was observed from results in Table 1 that sucrose was observed to be an essential component for induction of callus in anther culture of rice. A significant effect of sucrose concentration in culture media on the frequency of callus formation was noticed.

Maximum callus induction frequency (7.5%) was obtained in medium having 4% sucrose (Fig. 1, Table 1), and the frequency declined considerably at low concentration of sucrose (Fig. 2). Two to 6% sucrose was found suitable for

callus induction and regeneration of green plantlets (Table 2). These findings are consistent with the previous reports (Reinert and Bajaj 1977, Chen 1978, Chen et al. 1991, Sandhu et al. 1993, Pande and Bhojwani 1999). The effect of sucrose concentration on the callus induction frequency might be due to its contribution to the osmotic potential of the medium rather than its utilization as a carbon source.

Table 1. Effect of concentrations of sucrose on the frequency of induction of callus in BRRI Dhan-29.

Culture medium	Conc. of sucrose (%) in N6 medium	No. of anthers plated	Per cent of anther forming calli	Colour of callus	Texture of callus
N6 + 2.0 mg/l 2,4-D	0	300	0	-	-
+ 0.5 mg/l Kn	1	295	1.69	White	Compact
+ 2.5 mg/l NAA	2	150	4.6	"	"
+ sucrose	3	200	6.5	"	"
	4	210	7.5	"	"
	5	150	7.3	"	"
	6	150	7.3	"	"

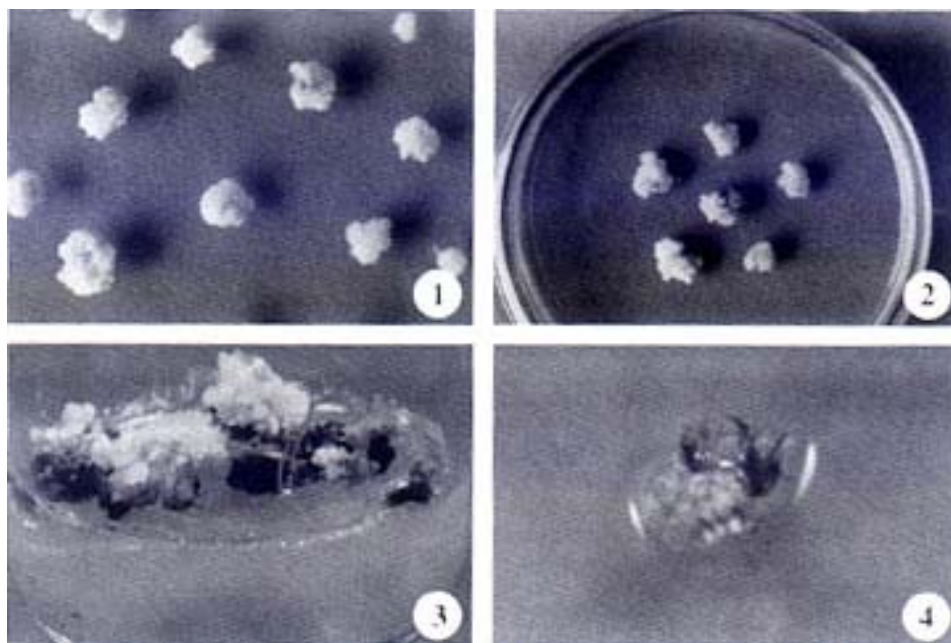
Table 2. Frequency of regeneration of green and albino plants from anther derived calli obtained from various concentrations of sugar in culture media.

Culture medium	Callus obtained from media with various conc. of sucrose		% of plants regenerated		Total (%) G = A
	Sucrose (%)	No. of callus plated	Green plant (G)	Albino plant (A)	
MS + 2.0 mg/l BAP	0	0	0	0	
+ 1.0 mg/l Kn	1	5	4.0	6.0	10.0
+ 1.0 mg/l NAA	2	7	8.0	4.0	12.0
+ sucrose	3	12	30.0	12.0	42.0
	4	14	65.0	12.0	77.0
	5	10	22.0	38.0	60.0
	6	15	18.0	42.0	60.0

From the statistical analysis, we get t-value of 3.30 with 10 degrees freedom. It is evident from the t-value that the calculated value is highly significant at 1% level ($p > 0.01$). So, there is a significant effect of concentration of sucrose on the frequency of callus induction in rice.

Slightly higher callus formation frequency (7.5%) was found in medium containing 4% sucrose than on medium with 3% sucrose (frequency 6.5%) because of an osmotic effect of additional sucrose present in culture media.

Sucrose in culture medium functions both as a carbon source and osmotic regulator. Both the functions are critical for embryoid and callus formation (Last and Brettell 1990). Sucrose is rapidly hydrolyzed to glucose and fructose, nearly doubling the osmolality of the medium. Navarro-Alvareg et al. (1994) reported that sucrose could be detrimental to embryoid production in wheat, although glucose seems to help initiate or promote the early stage of development in anther culture.



Figs. 1 - 4: Anther culture of rice. 1. Development of callus from anther of BRR1 Dhan-29 cultured with 4% sucrose. 2. Development of callus from anthers of BRR1 Dhan-29 culture with 2% sucrose. 3. Regeneration of green plantlets from anther derived calli obtained from 4% sucrose. 4. Regeneration of green plantlets from anther derived calli obtained from 2% sucrose.

Results of regeneration from anther derived callus are shown in Table 2. The regeneration frequency of green plantlets was found to be higher (30 - 65%) from calli obtained from medium containing (3 - 4%) sucrose compared to (18 - 22%) calli obtained from medium having (5 - 6%) sucrose (Table 2, Figs. 3, 4). With the increasing level of sucrose in the induction medium the regeneration frequency of albino plant increased significantly (Table 2). Similar findings were also reported by (Wang et al. 1977, Chen et al. 1991 and Sandhu et al. 1993).

From the statistical calculation the observed t-value is 5.58 with 10 degrees freedom which is positive and highly significant at 1% level. This indicates that there is a strong relationship between the sucrose concentration and the percentage of regeneration of green plants. But the t-value is 5.78 with 10 degrees freedom in case of albino plants. When the concentration of sucrose is increased above 4% then the rate of production of albino plants also increased. The results suggested that medium with low concentration of sucrose (3 - 4%) is suitable for callus and regeneration of green plantlets in anther culture of rice.

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