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Anther Culture in Glutinous Rice Varieties

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ABSTRACT

Haploid callus induction through anther culture was tried in two glutinous rice varieties of Assam. Maximum callus induction (19.0% in Mamonbora and 13.5% in Bejibora) was observed when cold pretreated anthers at 8oC for 3 days were cultured on Potato-2 medium supplemented with 2 mg/1 NAA under dark condition. Callus induction was higher in the uninucleate pollens than the binucleate pollens. When 30-35 days old calli were transferred to auxin free potato-2 medium supplemented with 1 mg/1 IAA plus 2 mg/1 kinetin, regeneration of green plants was achieved in Mamonbora and Bejibora to the tune of 4.8% and 2.1% respectively. Besides this, production of albino, green spots and roots only were also observed in some of the transferred calli.

Glutinous rice is grown mostly in south-east Asian countries including no th-eastern states of India. They form a special category and are used for preparation of delicious dishes, including daily breakfast in rural areas of Assam. So far this group of rice have got very little attention from geneticists and breeders. Although callus induction and plant regeneration from anthers have been studied in various categories of rice, so far it has not been reported in glutinous rice. Since genotype of the plant materials has the greatest influence on the frequency of pollen callus formation, study was made on the another culture capacity of two glutinous rice genotypes of Assam. The objective was to identify the appropriate media, the efficiency of cold pretreatment, cultural condition and the appropriate stage of pollen development for callus induction and regeneration of plants from callus so that the advantages of anther culture could be exploited for the improvement of this group of rice.

MATERIALS AND METHODS

Two glutinous rice varieties indigenous to Assam viz., Mamonbora and Bejibora were used for the investigation. Panicles at booting stage were collected for anther culture. Some Panicles were treated with cold temperature (8°C) for 3 days. Spikelets of both treated and untreated panicles were surface sterilized with 0.1% $HgC1^2$ for 1 min. Then under aseptic condition, anthers were taken out by separating the lemma and palea and inoculated on 5 different standard agar solidified basal media, viz., MS, SH, N6, Potato-2 and E10 and incubated under light (3000 lux) and dark cultural conditions. Since Potato-2 medium, pretreatment and dark cultural conditions were found to be more favourable for callus induction, in the following year, panicles were again collected, pretreated with cold temperature and anthers inoculated on Potato-2 medium modified with 2mg/1 IAA and 2mg/1 NAA and incubated in dark. In order to identify the stages of pollen capable of producing calli, two anthers of each spikelet were fixed in Carnoy's

solution and remaining anothers were cultured on Potato-2 medium. The stages of pollen development was determined by examining the fixed anthers of the spikelet under microscope through aceto-carmine squash technique. Percentage of callus produced based on the number of anothers inoculated were recorded. Calli produced were cut into pieces and subcultured on the same medium for callus multiplication. White, friable calli after 30-35 days of growth were transferred to MS and Potato-2 media without 2, 4-D but supplemented with 1mg/1 IAA and 2 mg/1 kinetin and incubated at 25°C under 12 hr light (3000 lux) and dark cycle. Differentiation of calli was noted. The experimental design was completely randomized and the effects of treatments were tested by analysis of Variance. Differences among means were tested by Duncan's Multiple Range Test DMRT (Duncan, 1955).

RESULTS AND DISCUSSION

Anther walls of the responsive anthers dried, turned black within 4-9 days and callus formed after 40-45 days from the microspores after bursting of the anther walls. Out of 5 media tested, callus formation was observed on Potato-2 and N6 media only. However, Potato-2 was found to be more effective for callus induction (9.3% in Mamonbora and 5.7% in Bejibora) than N6 medium (4.1 % in Mamonbora and 2.7% in Bejibora). The superiority of Potato-2 medium over other media may be due to the presence of low concentration of ammonium ions and the role of organic and inorganic constituents present in it.

Cold pretreated (at 8°C for 3 days) anthers yielded more pollen calli than the fresh untreated anthers in both the varieties. According to Sun (1978), the cold treatment reduces the metabolic activity of the microspores at the most suitable stage for androgenic development. Dark condition was found favourable for callus induction while none was observed in light. Vasil (1980) also noted that light was inhibitory for induction of androgenic callus.

In the following year when cold pretreated anthers were again cultured on modified Potato-2 medium, callus production was earlier (30-35 days) and frequency was also higher (Table 1). In both the varieties, percentage of callus formation was significantly higher on Potato-2 medium supplemented with 2 mg/1 NAA as against Potato-2 medium supplemented with 2 mg/1 IAA. Thus the combination of 2mg/1 NAA with 1.5 mg/1 2,4-D already present in the basal medium was efficient for callus formation. The result is in accordance with the findings of Liang (1978) in rice.

Regarding the stage of pollen, uninucleate pollens were more productive for callus induction (23.6% in Mamonbora and 18.1% in Bejibora) compared to the binucleate pollens (12.2% in Mamonbora and 6.2% in Bejibora) (Table 2). Similar results were also reported by several workers (Sun 1978; Mercy and Zapata, 1986).

On the regeneration media, some calli differentiated into roots only while some produced green spots only which did not differentiate further. On the Potato-2 regeneration medium 4.8% calli in Mamonbora and 2.1% calli in Bejibora differentiated to green plants after 25-35 days of transfer but on the MS regeneration medium, only Mamonbora produced (2.8%) green plants (Table 1). Thus differentiation to green plants was significantly higher in the callus of Mamonbora

Table 1. Response of anthers for callus formation and plant regeneration in glutinous rice varieties of Assam

Variety	Media	Callus induction: Anthers inoculated (No.)	Callus induction: Callus formation (No. %)	Plantlet regeneration (%)*: Regeneration	Roots only	Green spots	Albino plants	Green plants
Mamonbora	Potato 2+IAA (2mg/l)	152	19 (12.5)$_b$	MS	13.9	8.3	5.6	2.8$_b$
	Potato2+NAA (2mg/l)	168	32 (19.0)$_a$	Potato2	11.9	16.7	7.1	4.8$_a$
Bejibora	Potato 2+IAA (2mg/l)	160	12 (7.5)$_c$	MS	10.9	15.2	4.3	0$_c$
	Potato 2+NAA (2mg/1)	148	20 (13.5)$_b$	Potato2	14.6	12.5	6.2	2.1$_b$
CD (5%)			2.63					1.30

** Based on number of calli transferred for regeneration.*
No. of replications for each treatment=3
Means within columns separated by Duncan's multiple range test, P-5%
Means followed by the same letter are not significantly different.

Table 2. Callus induction from uninucleate and binucleate pollens of glutionous rice varieties cultured on Potato-2 medium supplemented with 2mg/1 NAA

Variety	Nuclear stages of pollen	Number of anthers inoculated	Callus formation Number (%)
Mamonbora	Uninucleate	72	17 (23.6)a
	Binucleate	90	11 (12.2)b
Bejibora	Uninucleate	83	15 (18.1)c
	Binucleate	65	4 (6.5)d
CD (5%)			0.067

Number of replications for each treatment = 3
Means within columns separated by Duncan's multiple range test, P-5%
Means followed by the same letter are not significantly different.

in Potato-2 medium. Increased shoot differentiation in Potato-2 medium may be due to higher concentration of sucrose (9%) in it. A high number of regenerated plants were albino which may be due to the presence of insufficient number of proplastids in the microspores (Wenzel and Foroughi-Wehr, 1984). The variety Mamonbora responded more than the variety Bejibora for both callusing and plant regeneration. Paulas and Sree Rangasamy (1995) also opined that genotype and hormonal level played an important role in the callus induction potential in rice.

In the present investigation, Potato-2 has been found to be a good medium for glutinous rice. Anther culture of wheat gave consistently higher number of green plants on Potato-2 medium as compared to N6 or Potato-1 media (Razdan, 1993). Though there may be great variability in the concentration of inorganic constituents of different samples of potato, reproducible response could be obtained in this medium because of the incorporation of 6 important salts at low concentration and low (10%) addition of potato extract (Razdan, 1993). However, frequency of callus formation and green plant regeneration was low in the present study. Therefore, techniques to induce more callus and more green plants from callus should be developed. Moreover, other varieties of this group may be tried since frequency depends on the particular rice genotype.

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