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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (3): 984-987, 1999 Research Article

Optimization of Different Media for Plant Regeneration from Callus Culture of Indica Rice (*Oryza sativa*) Genotype DM. 25

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Abstract

Different Media were optimized for plant regeneration from callus initiated from mature embryos of Indica rice on medi with various levels of 2,4-D alone and in combination with various concentrations of Benzyle adenine. Plant regeneratio was achieved in both MS and N6 media with and without various levels of growth regulators. In addition to green plants production of albino plants was also observed in all the media tested for regeneration. The composition of each Callu induction and regeneration media was found to be responsible for the recovery of higher number of plants.

Introduction

Plant regeneration from cultured tissues is an asexual process. It could be expected to give rise to clonal uniformity. Maintenance of useful and important genotypes, gene transfer between species and production of novel verities are a few important aspects in plant breeding, to which a tissue culture has recently approached(Kavi Kishor & Reddy, 1993). In rice plant regeneration has been reported from callus initiated from mature embryos (Suresh Kumar *et al.*, 1993, Zafar *et al.*, 1992; Rueb *et al.*, 1994). Plant regeneration in grasses seems to occur largely and perhaps exclusively from a white, compact, smooth surfaced, knobby callus composed of isodiametric cells, usually termed as embryogenic. Almost no regeneration occurs from non-embryogenic Callus, which is yellow, or brown, loose rough surfaced and crystalline in appearance. Among the two methods of regeneration of plants from tissue culture, the somatic embryogenesis is recognized as a superior method and has initially been limited to a number of dicot species(Chen *et al.*, 1985). This method enables the rapid production of large number of plants within a relatively short period of time and developing plantlets are more easily manipulated then those derived through organogesis (Rao *et al.*, 1985). Regeneration of plants from rice protoplasts has been reported from a number of japonica varieties (Kyzuka *et al.*, 1987). In Pakistan, exclusively indica varieties are grown which occupy 80 per cent of the cultivated rice in the world but little work been reported on these varieties. (Kyzuka *et al.*, 1988). Recent interest in indica type of rice resulted in certain reports of successful regeneration from elite varieties (Datta *et al.*, 1990; Zafar *et al.*, 1992; Rueb *et al.*, 1994).

A great deal of work is still required to be done on indica varieties, commonly cultivated in Pakistan. They are well known for their fine grains, aroma, high kernel elongation and cooking qualities, which constitute "Basmati Characteristics". Before cell culture technique can be fully applied to crop improvement, efficient regeneration methods have to be developed in Callus culture for evaluating the totipotency of callus. It will potentially offer a versatile technology for the production and selection of new desirable phenotypes of some crop plants.

Materials and Methods

MS basal medium containing sucrose, agar and differen growth regulators was used for callus induction in rice (Murashige and Skoog, 1962). Effects of differen concentrations of auxin (2, 4-D) and Cytokinin(BA) in callu induction medium were studied in terms of regeneratio potential. Different combinations of auxin and cytokini were used in MS medium for callus induction and both M and N6 basal media (Chu *et al.*, 1975) were used with an without plant growth regulators for regeneration.

Effect of Auxin Concentration on Callus Initiation: M medium was supplemented with different concentrations 2-4-Dichlorophenoxyacetic acid(2,4-D) viz., 0.0, 0.5, 1.0 2.0 and 4.0 mg/l respectively. Experimental treatmen were as under:

T_1A	MS + 0.0 mg/l	2,4-D
T_2A	MS + 0.5 mg/l	2,4-D
T_3A	MS + 1.0 mg/l	2,4-D
T_4A	MS + 2.0 mg/l	2,4-D
T_5A	MS + 4.0 mg/l	2,4-D

Effect of Auxin-Cytokinin combinations on Callus initiatio MS medium was supplemented with 2mg/l 2,4-D pl different levels of Benzyl adenine (BA) viz., 0.0, 0.5, 1. 2.0, and 4.0 mg/l respectively. Treatments were as und

T_1B	MS + 2 mg/l	2,4-D + 0 mg/l	BA
T_2B	MS + 2 mg/l	2,4-D + 0.5 mg/l	BA
T_3B	MS + 2 mg/l	2,4-D + 1.0 mg/l	BA
T_4B	MS + 2 mg/l	2,4-D + 2 mg/l	BA
T_5B	MS + 2 mg/l	2,4-D + 4.0 mg/l	BA

Plant regeneration from Callus: Five different media w MS0, MSR, N6-0, N6-R and MS containing 4mg/l IAA 2mg/l K were used to obtain maximum regeneration fr Callus. Compositions of the media tried were as under

i. MSO = MS + no plant growth regulator.
ii. MSR = MS + 0.5 mg/l BAP +).005 mg/l NAA
iii. N6-0 = N6 + No plant growth regulator
iv. N6-R = N6 + 0.05 mg/l BAP + 0.05 mg/l NAA
v. MSIK = MS + 4mg/l IAA + 2mg/l K

IAA = Indole acetic acid NAA = Naphthalene acetic acid. K = Kinetin BAP = Benzyl amino purine.

Explant Preparation and Culturing: Well known rice genotype DM-25 was selected for this morphogenetic study. The seeds were taken and dehusked manually and were washed in tap water for half an hour. Dis-infection of these seeds were done with 0.01 per cent Mercuric chloride plus one drop of tween-20 as emulsifier agent for 10 minutes in flasks by shaking with hands. Similarly two or three shakings of 10 minutes each in autoclaved distilled water were followed to remove the traces of sterilant. Then mature embryos/explants were placed in jars containing medium. With the help of disinfected forceps. These jars were kept in growth chamber having temperature 25 to 27 °C and 16/8 hours day/night (2,000 lux) cycle for Calli initiation. Data were recorded on various characters during the course of experiment.

For Callus Initiation: Effect of various levels of 2,4-D separately and 2,4-D in combination with BA in different combinations was studied on Callus initiation. Response of embryo derived callus on different media was observed in each case. The growth and morphology of initiating Calli were noted. Both white, compact smooth surfaced knobby and yellow or brown loose rough surfaced, crystalline calli called embryogenic and non-embryogenic respectively were observed. The best plant growth regulator combination for callus initiation was used for callus proliferation and maintenance for further use. The sub-culturing of callus was done after every three weeks.

Plant Regeneration from Callus: For regeneration studies, the secondary calli induced on different media were not pooled together but tested separately in terms of regeneration potential on different media. On these media, embryogenic calli of different origin were cultured in each case. The number of plants produced from calli were counted to compute regeneration percentage by the formula,

$$\frac{\text{Number of Calli regenerated}}{\text{Number of Calli cultured}} \times 100$$

The growth and morphology of regenerating plants were observed and percentage of green and albino plants initiated in different media was also determined by the formula

$$\frac{\text{No. of green/albino plants produced}}{\text{Total No. of plants produced}} \times 100$$

Data were recorded after four weeks of calli cultured on different regeneration media in each case.

Results and Discussion

The basic method of plants regeneration by tissue culture has been employed by the production of callus as a first step followed by embryoids development and plantlets formation from the callus , by using the mature embryos as explants of (*Oryza sativa L.*) rice genotype BM-25. Different responses of various regeneration media from embryo-derived calli raised at various callus induction media achieved in each case. Data collected in relation to regeneration potential (Table 1-5) as effected by different media indicate that all the regenerating media were significantly different from one another in terms of regeneration potential a well as in phenotypic characteristics off regenerated plantlets. The best response

Table 1: Effect of MSO, MSR, N6-O, N6-R, and MSIK medium on plant regeneration from callus culture of indica rice DM.25.

Original callus induction media	No. of calli cultured	No. of cali regenerated	Regeneration frequency%	Total No. of plants	Green plants%	Albino plants %
MSO						
$MS_{0.5}$	14	4	28.57	8	7	1
MS_1	15	2	13.33	4	3	1
MS_2	14					
MS_4	16					
$MS_2B_{0.5}$	15	2	13.33	6	4	2
MS_2B_1	17	2	11.76	4	3	1
MS_2B_1	16					
MS_2B_4	17					

MSR						
$MS_{0.5}$	18	5	27.78	15	12	3
MS_1	17	4	23.53	6	4	2
MS_2	19	3	15.78	8	5	3
MS_4	16	2	12.50	7	4	3
$MS_2B_{0.5}$	17	7	41.17	18	13	5
MS_2B_1	16	5	31.25	14	8	5
MS_2B_2	15	3	20.00	8	4	4
MS_2B_4	18	2	11.00	5	3	2
N6-O						
$MS_{0.5}$	15	2	13.33	5	30	2
MS_1	14					
MS_2	16					
MS_4	16					
$MS_2B_{0.5}$	18	4	22.22	7	4	3
MS_2B_1	17	2	11.76	3	2	1
BS_2B_2	16					
MS_2B_4	15					
N6-R						
$MS_{0.5}$	18	7	38.89	20	9	11
MS_1	17	5	29.41	17	8	9
MS_2	16	2	12.5	7	2	5
MS_4	14					
$MS_2B_{0.5}$	16	9	56.25	23	10	13
MS_2B_1	17	7	41.18	19	8	11
MS_2B_2	14	4	28.57	10	3	7
MS_2B_4	15	2	13.33	6	2	4
MSIK						
$MS_{0.5}$	17	7	41.18	23	9	14
MS_1	19	5	26.32	13	5	8
MS_2	18	3	16.67	8	3	5
MS_4	12					
$MS_2B_{0.5}$	17	12	70.59	33	15	18
MS_2B_1	18	9	50.00	25	11	14
MS_2B_2	16	5	31.25	14	5	9
MS_2B_4	19	2	10.53	6	3	3

was obtained in MSIK medium from the calli initiated on MS medium supplemented with 2mg/l 2,4-D plus 0.5 mg/l BA followed by the N6-R, MS-0, MS-R and N6-0 media respectively. In addition to green plants, some albino plants were also obtained. On MS-0 regeneration medium, ratio of green to albino plants was greater, compared to other regeneration media used where the ratio of green to albino plants was reduced. Callus induction with higher concentration of Auxin- Cytokinin responded relatively poor for regeneration, although in these media Callusing frequency and proliferation of Calli was extensive. A higher frequency of albino plants has already been reported in B-370 rice genotype (Zimny and Lorz, 1986; Zafar *et al.*, 1992). These phenotypic discrepancies may also be due to tissue culture procedures, for example, media used or the tissue cultured. A final possibility is that the discrepancies may reflect genotypic differences, involving differences i poloidy of starting material, structural chromosom changes, particularly interchanges during growth of plan cell under the influence of plant growth regulators (Zafar *al.*, 1992; Karp and Maddaock, 1984).

From the results, it can be concluded that rapidly growin and highly proliferating calli do not necessarily yield mo number of plants but it the frequency of embryogen callus (which is white, compact, smooth surfaced knob and composed of isodiametric cells) which is responsible f higher plant regeneration frequency. Less proliferating an slow growing calli may have a higher frequency embryogenic callus, thus higher regeneration potenti Therefore composition of both the callus induction an regeneration media is important for the recovery of plan from callus. It has also been found that lowest percenta

of albino plants was observed on regeneration media without growth regulators(MS-0). From the Calli which were raised at lowest 2,4-D level i.e. .5 mg/l. It can also be concluded that higher 2,4-D level as well as complex regeneration media may interfere with some vital functions, thus resulting in higher frequency of albino plants.

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