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Effect of facultataive methylotrphs on tissue culturing of rice

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The effect of facultative methylotrophs (FMs) on tissue culturing of rice cultivar Co43 was studied under *in vitro* conditions. Facultative methylotrophic isolates obtained from calli tissues of various tropical plants were screened based on growth hormone production and their effect on seedling vigour of rice cultivar Co43. Efficient isolates *viz*., PPFMs-LE1, PPFMs-OS, PPFMs-Co47, PPFMs-Vu, PPFMs-OS and NPFMs-OS along with standard strain *Methylobacterium extorquens* AM1 were inoculated by following various methods. Upon inoculation, the calli tissues were found to be well colonized by facultative methylotrophs and this led to proliferation of calli tissues and increased the regenerating ability of calli tissues. Chlorophyll and soluble protein content were also found to be increased significantly over the uninoculated control.

Key words : Facultative methylotrophs, Chlorophyll, Auxin, Cytokinins, Tissue culture

INTRODUCTION

Facultative methylotrophs belonging to the genera *Methylobacterium* are ubiquitous in the phyllosphere and rhizosphere of the plants. These bacteria have the ability to synthesize phytohormones such as IAA, cytokinin and gibberellic acid and hence, actively promoted the callus induction and regenerating ability of rice cultivar Co43.

Plant development is modulated by hormonal interactions (Carimi *et al.*, 2003) and so harmonizing the endogenous hormone level in tissue culture is vital for its success. In plant tissue culture auxin together with cytokinins promote cell differentiation and induce the formation of roots (Trotsenko *et al.*, 2001). Aerobic methylobacteria were found to be able to synthesize cytokinins necessary for plants (Ivanova *et al.*, 2000). Ivonova et al, (2001) reported that methylobacteria are able to produce IAA. Kalyaeva *et al.* (2001) demonstrated that *Methylovorus mays*, which obligately utilizes methanol as a source of carbon and energy and synthesizes phytohormones, actively promoted growth and morphogenesis in several dicot plant species propagated *in vitro*.

Kalyaeva *et al.* (2003) studied the effects of four aerobic methylotrophic bacteria on the morphogenesis of soft wheat (*Triticum aestivum*) in vitro using immature embryo as exlants. The colonization of the explants with the strains of *Methylobacterium* sp. D10 and *Methylophilus glucoseoxidans* stimulated the formation of morphogenic calli and shoots and also promoted development of the regenerated plants. The present study was undertaken with the aim to assess the effect of facultative methylotrophs on tissue culturing of rice.

MATERIALS AND METHODS

Bacterial strains:

The reference strain *M. extorquens* AM1 along with six strains of facultative methylobacteria *viz*., PPFMs-Os1(C1), PPFMs-Vu(C2), PPFMS-LE1(C3), PPFMs-Co47(C4), PPFMs-Os2(C5) and NPFMs-Os(C6) were employed. Ammonium mineral salt (AMS) medium (Whittenbury *et al.*, 1970) supplemented with 0.5 % (v/v) methanol was used for culturing the isolates. Cultivars were grown at 30°C in a shaker at 150 rpm. Logarithmic phase cultures (10^8 cells/ml) of facultative methylobacterial isolates were used for effective colonization of explants and callus tissues (Kalyaeva *et al.*, 2003).

Source of explants:

Field grown mature seeds of rice cultivar Co43 were used as source of explants. Dehusked mature seeds were used as explants for callus induction. Standard MS medium with optimal concentration of 2, 4-D and kinetin was used as the callus induction medium. Callus inducing and regenerating ability of the explants were assessed after one and two months of culturing, respectively. The chlorophyll and soluble protein content of the regenerated plantlets were assessed by following the method of Lowry *et al.* (1951) and Wellbern Lichtenthaler (1984), respectively.

Method of inoculation:

Various methods of inoculation of facultative methylobacterial isolates were followed and they were as follows: (i) Seed imbibition with FM isolates (SI) (ii)

Callus treatment with FM isolates (CT) (iii) Seed imbibition with FM isolates culture filtrate (SICF) and (iv) Callus treatment with FM isolates culture filtrate (CTCF). In the method of seed imbibition, the seeds were imbibed in bacterial suspension for 5 minutes and in the callus treatment method, the calli tissues were immersed for 3 minutes for effective colonization. After treating with methylobacterial isolates, the explants were dried off on sterile tissue paper (Kalyaeva *et al.*, 2003). Treated explants were cultured on appropriate MS medium for callus induction and regeneration. Along with all sets of treatments an untreated control was also maintained.

Histological analysis:

Histological analysis of the inoculated and uninoculated calli tissues were done according to the methods of Johansen (1940) to find out the colonization of calli tissues by methylotrophic isolates.

RESULTS AND DISCUSSION

The results of the callus inducing and regenerating ability of rice cultivar Co43, upon inoculation with FM isolates are presented in Table 1 and Plate 1. The facultative methylotrophic isolate PPFMs-LE1 significantly increased the callus induction and regeneration ability of the rice cultivar Co43 over the other methylotrophic isolates. In case of rice cultivar Co43, among the various methods of inoculation, the method seed imbibition with culture proved to be the best and this may be due to the fact that FMs colonized well in the seeds rather than calli tissues. Treatment with culture filtrate had a positive influence in the earlier stages, whereas by time calli tissues turned brown which may be due to instability of culture filtrate hormones and production of phenolics by the calli tissues.

The chlorophyll and protein contents were also found to be increased significantly (Table 2). Endogenous hormones play a major role in callusing and regenerating ability. Since methylotrophic isolates are capable of synthesizing plant growth promoting substances like gibberellic acid and IAA, they can be cocultured in plant tissue culture to enhance the callusing and regenerating ability.

Our findings were in accordance with Kalyaeva *et al.* (2001) who demonstrated that methylotrophic bacteria can provide *in vitro*-cultured plants with both growth regulators and vitamins. Furthermore, according to the authors, "the ability of the colonized plants and explants to grow normally on the sucrose-free medium and the

Table 1 : The effect of facultative methylotrophs on callus induction and regeneration of rice

Treatment	Callusing percentage			
	Method of inoculation			
	S1	CT	SICF	CTCF
Effect of facultative methylotrophs on callus induction of rice				
X+C1	70.3	69.0	69.2	67.7
X+C2	69.0	68.6	67.3	67.0
X+C3	75.2	73.4	70.1	71.2
X+C4	71.8	69.3	68.7	68.0
X+C5	72.0	69.7	69.0	69.0
X+C6	68.8	68.3	67.4	66.9
X+*M. extorquens* AM1	73.0	70.8	69.2	69.8
X+control	67.0	66.3	67.3	66.7
Effect of facultative methylotrophs on regeneration of rice				
Y+C1	88.9*	87.6	77.0	83.7
Y+C2	79.0*	78.3	68.9	76.0
Y+*M. extorquens* AM1	84.3*	82.0	70.2	79.3
Y+control	-	-	-	-
Y1+C1	82.3	90.2	74.3	89.6
Y1+C2	71.2	79.2	68.0	76.3
Y1+*M. extorquens* AM1	79.0	85.6	70.1	70.1
Y1+control	47.3	72.0	51.1	73.2
Y2+C1	89.8	83.7	83.0	78.3
Y2+C2	80.1	74.2	76.3	68.8
Y2+*M. extorquens* AM1	83.1	76.3	79.1	72.1
Y2+control	52.3	61.7	47.6	62.1

X= MS basal+ 2,4-D 2.0 mg l^{-1} + kinetin 0.50 mg l^{-1}
Y= MS basal
Y1= MS basal+BAP 3.0 mg l^{-1}
Y2= MS basal+ BAP 3.0 mg l^{-1}+NAA 0.5 mg l^{-1}
- : Drying of calli
*: Multiple shoot induction

Table 2 : Chlorophyll and soluble protein content of regenerated plantlets of rice

Crop	Treatment	Chlorophyll content (mg g^{-1} fresh weight)			Soluble protein content (mg g^{-1} fresh weight)
		Chl a	Chl b	Total Chl	
Rice Co43	T_1	1.12	0.75	1.87	8.15
	T_2	0.98	0.58	1.56	7.15
	T_3	0.92	0.52	1.44	7.45
	T_4	0.82	0.50	1.32	6.85

T_1 - LE1 T_2 - OS2 T_3 – *M. extorquens* AM1 T_4 - Control

bright green coloration of these plants infer that, by producing cytokinins, the methylobacteria promote chloroplast development and activity".

Histological analysis (Plate 2) shows the effective colonization of calli tissues by the facultative methylotrophic isolates. Histological analysis reveals the positive interaction of facultative methylotrophs and plants. The regenerated plantlets of rice cultivar Co43 obtained from inoculated calli tissues had significantly increased the amount of chlorophyll and protein content when compared to the uninoculated control.

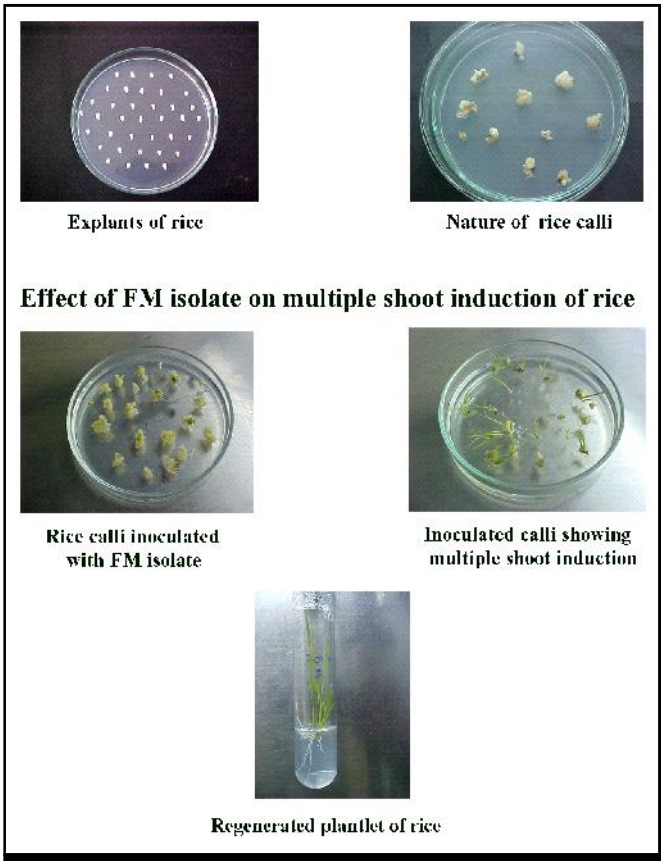

Plate1 : Influence oc PPFMs on callus induction of rise cultivar Co-43

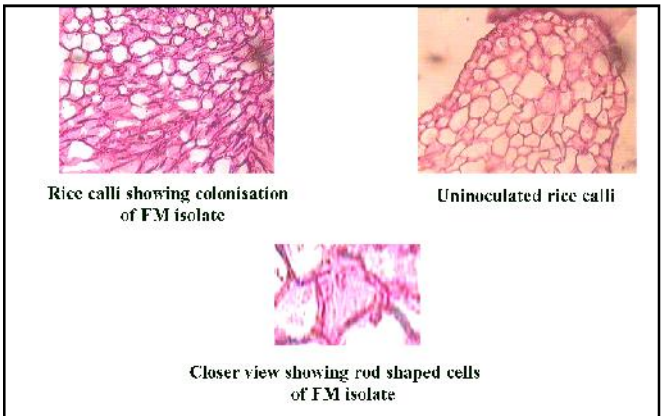

Plate2 : Pattern of colonization of FM isolate on ricecalli tissues

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