RESEARCH PAPER

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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)