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Effect of plant growth-promoting rhizobacteria at varying levels of N and P fertilizers on growth and yield of cauliflower in mid hills of Himachal Pradesh

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ABSTRACT

The use of plant growth promoting rhizobacteria (PGPR) is steadily increasing in agriculture and offers an attractive way to supplement chemical fertilizers and pesticides by a wide variety of mechanisms. The indigenous isolates of PGPR were isolated from cauliflower rhizosphere soil in different agro-climatic zones of Himachal Pradesh. Five efficient isolates designated as MK2, MK4, MK5, MK7 and MK9 were selected and characterized after successful experiments under in vitro and net house conditions at varying levels of N and P on the growth and yield of cauliflower. All the isolates induced the production of indole acetic acid (IAA) and were able to solubilize phosphorus. The conjoint use of PGPR and N and P fertilizers resulted in a significant increase in number of non-wrapper leaves, curd diameter, curd depth and curd weight of cauliflower. Furthermore, PGPR isolates remarkably increased the yield of cauliflower. Among the five isolates, three isolates were selected for field trials on the basis of the results obtained from net house studies in which MK5 with 100 per cent N and P proved better in all aspects of cauliflower wrt increase in growth, yield, IAA production and phosphate solubilization. The present study, therefore, suggested that the use of PGPR isolate MK5 with 100 per cent N and P as inoculant biofertilizer was beneficial for cauliflower cultivation as it enhanced growth of cauliflower, and induced IAA production and phosphorus solubilization.

Key words: IAA, PGPR, phosphorus solubilization, cauliflower.

INTRODUCTION

In the context of increasing international concern for food and environmental quality, the use of plant growth promoting rhizobacteria (PGPR) for reducing chemical inputs in agriculture is a potentially important issue. PGPR have been applied in various crops to enhance seed emergence, growth and crop yield, but only a few isolates have been commercialized. PGPR are also known to produce

antibacterial compounds that are effective against certain plant pathogens and pests (Dey et al 2004, Herman et al 2008, Minorsky 2008). PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus and production of siderophores that chelate iron and make it available to the plant root (Lalande et al 1989, Glick 1995, Bowen and Rovira 1999).

Cauliflower (*Brassica oleracea* var *botrytis* L) is a member of family cruciferae grown as commercial crop for vegetable purpose and as a seed crop in Himachal Pradesh. The high-yielding cauliflower varieties have resulted in an increase in cauliflower production but require large quantities of chemical fertilizers which lead to health hazards and environmental pollution. In order to make cauliflower cultivation sustainable and less dependent on chemical fertilizers, it is important to have effective PGPR isolates that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that can contribute to the higher production of cauliflower. Therefore, the present investigations were undertaken to screen the PGPR isolates with multifarious activities that are compatible with cauliflower for commercial cultivation in Himachal Pradesh.

MATERIAL AND METHODS

Collection of samples from cauliflower rhizosphere

Soil and root samples were taken from the rhizosphere of cauliflower plants collected from Bilaspur, Hamirpur and Kangra districts of Himachal Pradesh. The rhizosphere was dug out with intact root system. The samples were placed in plastic bags and stored at 4°C in the Soil Microbiology Laboratory, Dept of Soil Science and Water Management, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan for further analysis.

Isolation from the soil sample

Soil sample of 1 g was taken and added to 9 ml sterilized water blank for making stock solution. From this stock solution 0.1 ml of sample was taken and 9.9 ml sterilized water blank was added to it. It was marked as 10^{-2} dilution. It was serially diluted up to 10^{-10} dilution. 0.1 ml of each of the same 10^{-4}, 10^{-6}, 10^{-8} dilutions were taken and added to sterilized pre-poured petriplates containing NA and Pikovskaya's medium. The plates were incubated at 30± 2^0C for 48 hours.

Isolation from the root sample

The root sample (1 g) was taken and surface sterilized with 0.2 per cent

$HgCl_2$ for 3-5 minutes and then washed thoroughly (10 times) with sterilize distilled water to make it free from $HgCl_2$. The samples were immersed in the sterilized nutrient broth for 1-2 minutes in order to determine surface sterility. The 1 g of root samples were grinded with 9 ml sterilized water blank in sterilized pestle and mortar to make slurry under aseptic conditions. The grinded samples were then used for isolation and enumeration of endorhizobacteria on different media by using modified replica plating technique.

Characterization of isolates

Morphological characteristics (shape, elevation, surface, margin, etc) were examined on nutrient agar plates for the growth of selected bacterial isolates.

Growth under different temperature and pH conditions

Growth curves were drawn by growing the culture at various temperatures. Growth of the isolates at different pH was studied. The optimum pH and temperature suitable for the growth of bacterium was worked out.

Methods adopted for multifarious plant growth promoting activities of bacterial isolates

Phosphate solubilizing activity, nitrogen fixing ability, siderophore production, HCN production, IAA production and antagonistic activity were determined by the methods given by Pikovskaya (1948), Jensen (1987), Schwyn and Neilands (1987), Bric et a1 991, Bakker and Schippers (1987) and Gorden and Paleg (1957), respectively. The bacterial inoculants were prepared according to the method of Vincent (1970).

Field experimentation

Three bacterial isolates viz MK5, MK7 and MK9 were selected on the basis of their multifarious plant growth promoting activities under controlled conditions (growth chamber and net house). The experiment was conducted at Regional Horticultural Research Station, Bajaura (Kullu). The seeds were treated with bacterial isolates for 8 hours. Untreated seeds were designated as control. The seeds were sown in nursery and one month old seedlings were transplanted in the field. The bacterial culture was added at one month interval till harvesting. The treatments combinations viz:T1 (Control), T2 (MK5 + 50% NP), T3 (MK5 + 75% NP), T4 (MK5 + 100% NP), T5 (MK7 + 50% NP), T6 (MK7+75%NP), T7 (MK7 + 100% NP), T8 (MK9 + 50% NP), T9 (MK9 + 75% NP) and T10 (MK9 + 100% NP) were arranged in RBD design replicated thrice. The observations were recorded on different quantitative characters of cauliflower viz number of non-wrapper leaves, curd diameter, curd depth, curd

weight and curd yield. The data were analysed as per the method suggested by Gomez and Gomez (1984).

RESULTS

Morphological characteristics of PGPR isolates

The morphological characteristics of PGPR isolates (MK2, MK4, MK5, MK7 and MK9) varied widely. All the isolates produced round shaped and raised colonies having rough surface with undulated to erosive margins.

Microscopic observations of PGPR isolates

Microscopic observations were performed to investigate the characteristics of PGPR isolates such as shape, gram reaction and motility. All isolates were rod shaped, motile and gram positive in reaction. The growth of MK5 isolate was better in the temperature ranges of 25 to 40°C and pH 5-8 (Fig 1).

Production of multifarious activities

The multifarious activities revealed that all isolates induced the production of IAA. Isolates MK5, MK7 and MK9 were found to be the producers of IAA, siderophores and HCN. These isolates also had ability to solubilize the phosphorus and antagonistic activities against *Rhizoctonia solani*, *Pythium* sp and *Fusarium* sp (Table 1).

Plant Parameters

The PGPR isolates with different levels of N and P significantly affected

Table 1. Screening of bacterial isolates for multifarious plant growth promoting activities

Isolates	P-solubilization	N- free medium	Auxin production	Siderophore production	HCN production	Antagonism against		
						Fusarium sp	*Pythium* sp	*R solani*
MK2	+	+	+	-	-	-	-	-
MK4	+	++	+	-	-	-	-	-
MK5	+++	+++	++	++	++	++	+	++
MK7	++	++	+	+	+	+	-	+
MK9	++	++	+	-	-	-	+	-

+++ Very good activity, ++ good activity, + fair activity, - no activity

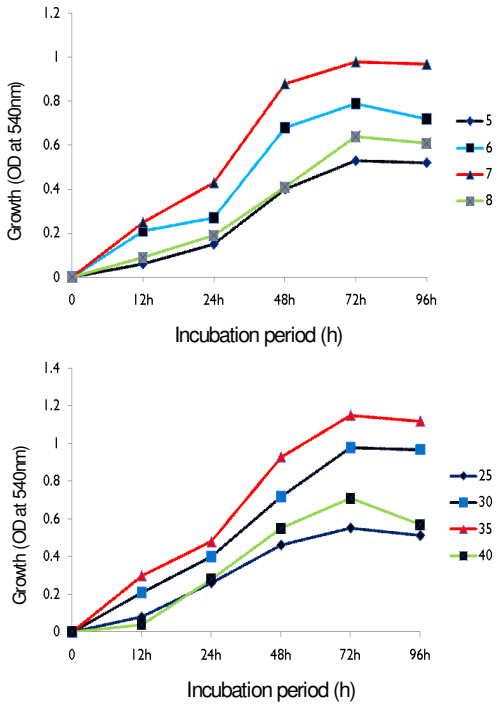

Fig 1. Effect of pH and temperature on growth of *Bacillus* sp (MK5)

number of non wrapper leaves, curd diameter, curd weight, curd depth and curd yield/plot as well as per hectare as compared to control (Table 2). Results revealed significant increase in plant parameters in treated plants over uninoculated control. The highest yield (355.56 q/ha) was recorded in MK5 isolate with 100 per cent N and P fertilizers which was statistically similar to isolates MK7 (315.80 q/ha) and MK9 (343.21 q/ha).

DISCUSSION

PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. The exact mechanism by which PGPR stimulate plant growth is not clearly established, although several hypothesis such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved (Lalande et al 1989, Liu et al 1992, Glick 1995, Bowen and Rovira 1999).

Of five isolates, one isolate MK5 was found to be good producer of IAA (Table 1). It has been reported that IAA production by PGPR can vary among different species

and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza et al 2001).

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants (Pradhan and Sukla 2006). PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to cauliflower that represents a possible mechanism of plant growth promotion under field conditions (Thakuria et al 2004, Verma et al 2001). In present study, all isolates were able to solubilize phosphate in the rhizosphere soil (Table 1). It is important to note that several phosphate-solubilizing bacilli occur in soil (Skrary and Cameron 1998) but their numbers are not usually high enough to compete with other bacteria commonly established in the rhizosphere (Lifshitz et al 1987).

A large body of evidence suggests that PGPR enhance the growth and crop yield, and contribute to the protection of plants against certain pathogens and pests (Dey et al 2004, Kloepper et al 2004, Kokalis-Burelle et al 2006, Herman et al 2008, Minorsky 2008). In this study, the effectiveness of PGPR isolates with different levels of N and P was investigated whether they could increase the yield or growth of cauliflower. Most of isolates significantly increased the different plant parameters. Of

Table 2. Effect of different PGPR and chemical fertilizers on plant parameters

Treatments	Number of non-wrapper leaves	Curd diameter (cm)	Curd weight (g)	Curd yield/ plot (kg)	Curd depth (cm)	Curd yield/ ha (q)
T1	9.33	11.01	401.00	8.02	6.90	148.52
T2	9.53	11.59	465.70	9.31	7.07	172.47
T3	9.80	13.46	700.00	14.00	8.31	259.26
T4	10.73	15.31	960.00	19.20	9.06	355.56
T5	9.73	13.21	506.00	10.12	7.64	187.41
T6	10.27	13.77	750.00	15.00	8.44	277.78
T7	10.53	13.89	852.70	17.05	9.00	315.80
T8	9.67	12.40	481.30	9.62	7.50	178.27
T9	10.07	13.53	750.00	15.00	7.94	277.78
T10	10.67	14.97	926.70	18.53	8.68	343.21
$CD_{0.05}$	1.13	2.51	267.59	5.33	1.50	98.78

three isolates, one isolate MK5 with 100 per cent N and P showed better performance in aspects of yield (Table 2). These results suggest that the increased growth of cauliflower by application of PGPR along with different concentrations of nitrogen and phosphorus is probably due to induction of IAA production and phosphorus solubilization.

Taken together, results suggest that PGPR are able to induce the production of IAA, solubilization of phosphorus, and resistance to pathogens and pests, thereby improving growth of plants. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable cauliflower cultivation in Himachal Pradesh.

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