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INFLUENCE OF METHYLOTROPHS ON GROWTH AND YIELD OF MUNGBEAN

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Abstract

The present investigation was conducted in pot house at Department of Microbiology, College of Basic Sciences and Humanities, CCSHAU Hisar, to study the influence of methylotrophs on growth and yield of mungbean. In our previous study, all the methylotrophs were tested for plant growth promoting traits including IAA production, phosphate solubilisation, siderophore production, HCN production, ACC utilization and ammonia excretion. The selected methylobacterium isolates MP12 (*Methylobacterium oryzae*), PP1 (*Ochrobacterium intermedium) and UP4 (Microbacterium ginsengisoli)* were assessed for plant growth promotion alone and as well as along with standard bioferlizers (*Rhizobium* MB703 and phospjorus solubilising bacteria P36) in the pot house experiment. The results of treatment MP12+ MB703 + P36 showed significant higher growth parameters *viz*., plant dry weight and higher number of nodules. Similar trend was observed in grain yield components other treatments and uninoculated control.

Key words: Bioferilizers, Methylotrophs, Mungbean and Plant growth promotion.

Introduction

Mung bean is one of the essential legume crops of short growing period and broadly cultivated in Asia during warm season. Mung bean has a great importance due to high protein content and ability to fix the atmospheric nitrogen by forming the root nodules. Root nodules harbours rhizobial and non rhizobial bacteria having direct or indirect influence on growth of plants like nodulation and grain yield of crop (Remans *et al.,* 2007). The nonrhizobial bacteria synergistically act with the rhizobial bacteria and improve plant growth possibly by producing growth hormone and by facilitation of nutrients (Mishra *et al.,* 2009; Rajendran *et al.*, 2008).

Methylotrophs are the nonrhizobial bacteria, can able to utilize utilize C1 (methanol) compounds as the sole source of carbon and energy. Methanol is principally generated from plant pectin and lignin during plant growth and decay. Methylotrophic have been reported to influence seed germination and seedling growth by producing different plant hormones like ACC deaminase (1-aminocyclopropane-1-carboxylate) which helps in lowering plant hormone ethylene levels (Deka Boruah *et al.,* 2010; Hong *et al.,* 2009; Madhaiyan *et al*., 2006 & 2007; Ryu *et al*., 2006) and indole acetic acid (IAA) production. These bacteria also help in nutrients acquisition by plants by solubilizing phosphate and also assist the rhizobia to fix more nitrogen. There have been extensivestudies on methylotrophs and their ability to promote plantgrowth and development by co-inoculation with a differentgroup of bacteria in pot and field conditions. Hence there is a possibility of increasing the effectiveness of the conventional bioinoculants by coinoculating with methylotrophs. Keeping all the above points in view an experiment was conducted to study the effect of three methylotrophic bacterial isolates *i.e*. *Methylobacterium oryzae, Ochrobacterium intermedium, Microbacterium ginsengisoli* (Ahlawat *et al*., 2018) on growth and yield of mungbean under pot culture conditions.

Material and Methods

Methylotrophic isolates MP12 (*Methylobacterium oryzae*), PP1 (*Ochrobacterium intermedium) and UP4 (Microbacterium ginsengisoli)* were assessed for growth of mungbean under pot culture conditions. The methylotrophic cultures were inoculated to mungbean seeds individually and in combination with standard biofertilizer cultures MB703 (*Rhizobium*) and P-36 (phosphate solubiliser). Soil was collected from dry land area of CCS Haryana Agricultural University Hisar. 5 kg of soil was taken in earthern pots. SSP and urea were added in pots at the rate of 100 kg/acre and 12 kg/acre respectively. Seeds of mungbean (MH 421) were surface sterilized by using 0.1% mercuric chloride and alcohol. Three replicates of each treatment were kept in each pot. All the seedswere inoculated with 1 ml inoculum of methylotrophic isolates. Four controls were also kept that included one absolute control without any treatment, one with addition of RDF, one with *Rhizobium* strain MB703 and one with phosphate solubilising strain P36. After germination, three plants in each pot were maintained. Pots were irrigated as and when required.

The treatments used were as under:

T1 Control

T2 RDF (Recommended dose of fertilizer)

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T3 *Rhizobium* (MB703) *(standard biofertilizer)*

T4 Phosphate solubilising bacterium (PSB) P36 *(standard biofertilizer)*

T5 Methylotroph(s) (MP12,PP1,UP4)
T6 *Rhizobium* (MB703) + PSB(P36)
T7 Methylotroph(s) + *Rhizobium*(MB703)
T8 Methylotroph(s) + PSB
T9 Methylotroph(s) + PSB+*Rhizobium*

Where MP12 is *Methylobacterium oryzae*, PP1 is *Ochrobacterium intermedium* and UP4 is *Microbacterium ginsengisoli*.

Following observations were taken:

a. Nodulenumber at 50% flowering

b. Plant dry weight at 50% flowering and at harvesting

c. Seed yield at harvesting

Statistical analysis

The experimental data was analysed by application of one factor CRD for pot house studies, by OPSTAT software available on CCS-HAU home page.

Results and Discussion

Effect of inoculation of methylotrophic isolates with standard biofertilizers on nodulation of mungbean

Nitrogen is the key nutrient for plant growth promotion (Hu and Schmidhalter, 2005). Nitrogen fixation is directly linked to nodulation, more is the number of nodules means the rate of nitrogen fixation will increased. Hence more will be the growth of plant. Therefore, in our study methylotrophic isolates (MP12, PP1 and UP4) and their coinoculation with standard cultures (MB703 and P36) to mungbean variety MH-421 was tested for nodule number. In our study it was observed that the nodule number of all the treatment were significantly higher than the absolute control treatment (15.60/plant) (Figure 1). When compared the individual inoculation among themselves the methylotroph MP12 (*Methylobacterium oryzae*) showed highest increase in nodule number (27.66/plant) followed by *Rhizobium* (MB703) (27.46/plant) and methylotroph UP4 (*Microbacterium ginsengisoli)* (26.70/plant) (Figure 1). These results are similar with the finding of Dorosinky and Kayrov, (1975) who workedon chickpea and observed that single inoculation of chickpea with *Rhizobium* has been reported to increase the nodulation. Co-inoculation, habitually improves the plant growth and yield, compared to single inoculation by improving absorption of nutrients like nitrogen, phosphorus and zinc (Araújo *et al*., 2009). In our study, among all bi-inoculation treatments, the treatment *Rhizobium* (MB703)+ MP12 (*Methylobacterium oryzae*) showed higher nodule number (43.20/plant) followed by treatment P36+ MP12 (*Methylobacterium oryzae*) (38.56/plant) whereas in the coinoculation study with the three inoculants, the treatment (MP-12, MB703 and P36) showed the highest nodule number (45.43/plant) (Figure 1). The enhancement in nodule number can be due to the presence of combined inoculation of rhizobia with methylotrophs which might have resulted in a synergistic way. Similarly, Stajkovic *et al*. (2011) reported in their findings that coinoculation of bean with *Rhizobium* and *Bacillus* strain SNji positively increased nodule (106.67 number) compared to inoculation with Rhizobium alone (54 nodule number/plant).

Effect of inoculation of methylotrophic isolates on plant biomass of mungbean at 50% flowering and harvest

Plant dry matter includes root and shoot dry weigh, is the important criteria for measuring the plant growth. The greater is the root length or density, higher is the nutrient and water uptake. As the absorption at root level is more intense, the higher the biomass and hence the plant growth. Therefore, in our study,all the three methylotrophic isolates (MP12, MP15, PP1 and UP4) was studied in mungbean with respect to plant dry weight at 50% flowering and at harvest. There was significant increase in plant dry weight at 50% flowering and at harvest on inoculation with different methylotrophic isolates when compared to uninoculated control 0.474 g plant^{-1} at 50% flowering and 1.776 g plant^{-1} at harvest (Table 2). Among the individual inoculation, *Rhizobium* (MB703) showed higher plant dry weight at 50% flowering as well as at harvest followed by inoculation with methylotroph MP12. Coinoculation of mungbean seeds with methylotroph MP12 with standard *Rhizobium* MB703 and standard phosphate solubiliser P36 showed highest plant dry weight 0.950 g/plant at 50% flowering and 3.300 g plant^{-1} at harvest followed by coinoculation of UP4 with standard MB703 and standard P36 was 0.931g plant^{-1} at 50% flowering and 3.117 g plant^{-1} at harvest (Table 1). Similar finding was observed by Radha *et al.* (2016) that coinoculation of methylobacterium (LE-1) with standard *Rhizobium* strain SB_{120} increase plant dry weight (root 3.76 cm and shoot 8.97cm).

Effect of inoculation of methylotrophic isolates on seed yield of mungbean at harvest under pot house conditions

Methylotrophs are known to play an important role in increasing crop yield and land fertility. In our study, effect of methylotrophic isolates (MP12, PP1 and UP4) alone and along with standard biofertilizers (MB703 and P36) was studied in mungbean with respect to seed yield. In the individual inoculation, *rhizobium* (MB703) and MP 12 (*Methylobacterium oryzae*) showed almost of similar seed yield 1.181 g/plant and 1.205 g/plant respectively (Figure 2). In the coinoculation study, (MP12) + *Rhizobium* (MB703) showed seed yield 1.504 and highest seed yield was observed with treatment Methylotroph (MP12)+*Rhizobium* (MB703)+PSB (P36) 1.635 g/plant (Table 2). The increase in the yield with coinoculation treatment may be due to compatible nature of Methylotroph and *Rhizobium* and phosphate solubilising bacteria. Similar finding was observed by Suresh Reddy *et al.* (2002) that combined inoculation of Methylotrophs and *Rhizobium* on groundnut significantly improves yield parameter of groundnut.

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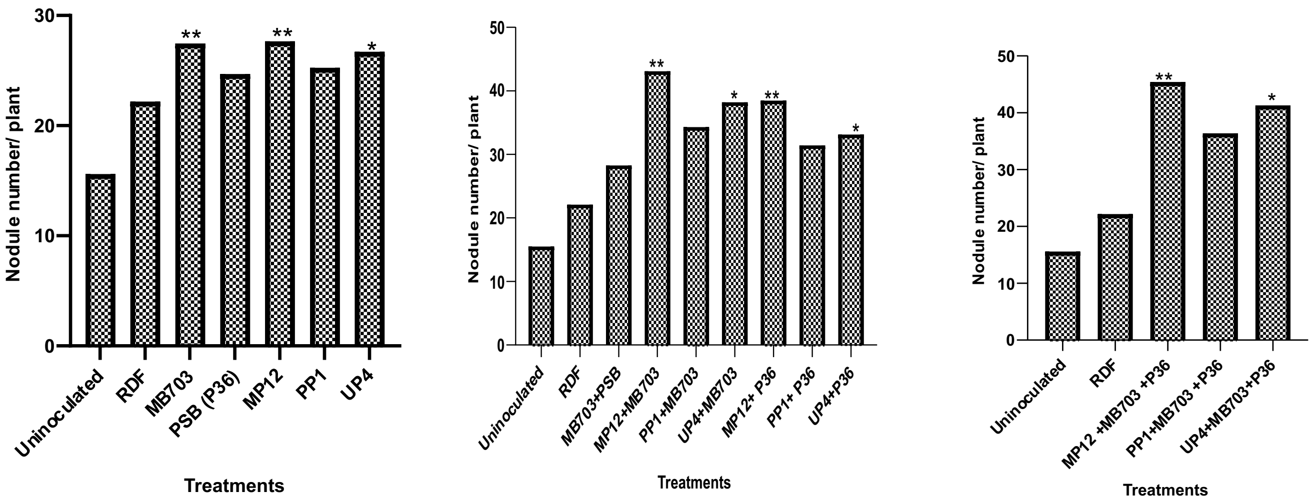
Table 1: Effect isolates on plant dry weight of mungbean under pot house conditions at 50% flowering and harvest

Treatment	Plant dry weight (g/plant) at 50% flowering	Plant dry weight (g/plant) at Harvest
Uninoculated	0.474	1.776
RDF	0.644	2.581
Rhizobium (MB703)	0.789	2.710
PSB (P36)	0.712	2.678
Methylotroph (MP12)	0.711	2.685
Methylotroph (PP1)	0.668	2.638
Methylotroph (UP4)	0.691	2.666
Rhizobium+PSB	0.864	2.828
Methylotroph (MP12)+*Rhizobium* (MB703)	0.847	3.085
Methylotroph (PP1)+*Rhizobium* (MB703)	0.773	2.966
Methylotroph (UP4)+*Rhizobium* (MB703)	0.802	3.012
Methylotroph (MP12)+ PSB (P36)	0.771	2.960
Methylotroph (PP1)+ PSB (P36)	0.693	2.857
Methylotroph (UP4)+ PSB (P36)	0.737	2.908
Methylotroph (MP12) +*Rhizobium*(MB703) +PSB (P36)	0.950	3.300
Methylotroph (PP1)+*Rhizobium* (MB703) +PSB (P36)	0.913	3.070
Methylotroph (UP4)+*Rhizobium* (MB703)+PSB (P36)	0.931	3.117
C.D. at 5%	0.104	0.108

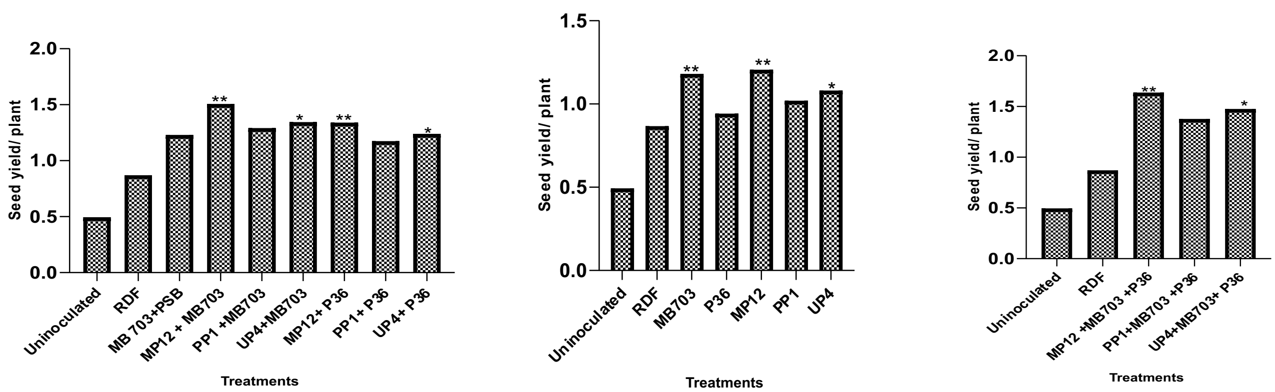
Table2: Effect of coinoculation of methylotrophic seed yield of mungbean under pot house conditions

Treatment	Seed yield/plant(g)
Uninoculated	0.493
RDF	0.867
Rhizobium (MB703)	1.181
PSB (P36)	0.942
Methylotroph (MP12)	1.205
Methylotroph (PP1)	1.019
Methylotroph (UP4)	1.080
Rhizobium+PSB	1.228
Methylotroph (MP12) + *Rhizobium* (MB703)	1.504
Methylotroph (PP1) + *Rhizobium* (MB703)	1.289
Methylotroph (UP4)+*Rhizobium* (MB703)	1.342
Methylotroph (MP12)+ PSB (P36)	1.337
Methylotroph (PP1)+ PSB (P36)	1.173
Methylotroph (UP4)+ PSB (P36)	1.238
Methylotroph (MP12) +*Rhizobium*(MB703) +PSB (P36)	1.635
Methylotroph (PP1)+*Rhizobium* (MB703) +PSB (P36)	1.374
Methylotroph(UP4)+*Rhizobium*(MB703)+ PSB (P36)	1.473
C.D. at 5%	0.101



(a) (b) (c)

Figure 1: Effect of coinoculation of methylotrophic isolates on nodulation of mungbean under pot house conditions (a) Single inoculation (b) bi-inoculation (c) Coinoculation with three different strain
****Highest significant difference from uninoculated control**
***Second highest maximum significant difference from uninoculated control**



(a) (b) (c)

Figure 2: Effect of coinoculation of methylotrophic seed yield of mungbean under pot house conditions (a) Single inoculation (b) bi-inoculation (c) Coinoculation with three different strain
****Highest significant difference from uninoculated control**
***Second highest maximum significant difference from uninoculated control**