



PHOSPHORUS NUTRITION OF MUNGBEAN (*Vigna Radiata* L.) IN RELATION TO MYCORRHIZOBACTERIAL INOCULATION

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ABSTRACT

Microbial phosphate solubilization substantially supplements chemical phosphatic fertilization. Pakistani soils are generally P-deficient and require adequate P fertilization. However, due to very low P-use-efficiency, it becomes indispensable to devise workable strategies to address these issues. We conducted a field experiment to compare the response of mungbean (*Vigna radiata* L.) to ACC-deaminase, phosphate-solubilizing rhizobacteria (PSRB), *Pseudomonas fluorescens* and arbuscular mycorrhizal (AM) fungi under varying levels of inorganic P (P₁ = No P fertilizer, i.e. control, P₂ = 30.0 kg P ha⁻¹, and P₃ = 60.0 kg P ha⁻¹). We noted that mungbean showed significant enhancements in various plant attributes when supplied with P nutrition ranging from 8.2 to 45.9% at 50% recommended P fertilizer dose, i.e. 30 kg P ha⁻¹ and 17.8 to 76.1% at 100% recommended P fertilizer dose, i.e. 60 kg P ha⁻¹. In comparison to 30 kg P ha⁻¹, 60 kg P ha⁻¹ significantly increased (1.6 to 3.9 times) plant traits of mungbean. Microbial inoculants showed positive effects, with *P. fluorescens* increasing plant attributes from 5.2 to 31.2%, and AM fungi *Glomus mossaea* from 13.5 to 40.0%. The mycorrhizal inoculation was significantly better than rhizobacterial inoculation, with enhancements of 1.2 to 2.7 times in various growth attributes of mungbean. We conclude that microbial inoculation of arbuscular mycorrhizal fungi was found to be more efficient than rhizobacterial inoculation.

Keywords: *Vigna radiata* L., Phosphorus, ACC-deaminase, *Pseudomonas fluorescens*, mycorrhizal fungi, Phosphate-solubilization

INTRODUCTION

Phosphorus (P) is an essential nutrient for the proper growth and development of crop plants. It is a building block of adenosine triphosphate (ATP), the energy currency of plants and of deoxyribonucleic acid (DNA), the hereditary materials for all living things (Dissanayak *et al.*, 2021). Adequate amount of P plays vital roles in different metabolic and developmental processes of plants, viz. membrane synthesis, carbon metabolism, and energy production. In addition, P is involved in various other physiological processes necessary for plant growth i.e. respiration, activation and inactivation of enzymes, and biological nitrogen (N) fixation of legumes, as reported by Khan *et al.* (2023). Its deficiency affects primary productivity in cropping systems (Kaya *et al.*, 2020).

Pakistani soils are generally P-deficient and contains <10 mg kg⁻¹ Olsen P with total P (organic and inorganic) in the plow layer of soil ranges from around 0.02 to 0.5% (Vishandas *et al.*, 2006; Memon, 1996). Moreover, >90% of total P is fixed with calcium which causes low P in soil solution and the

applied soluble P fertilizers become fixed or precipitated, especially in alkaline-calcareous soils (Ibrahim *et al.*, 2022), leading to severe yield losses. Hence, efficient P fertilization becomes highly indispensable for crop production in Pakistan. However, it has been reported that world P reservoirs may end by 2050 (Balemi and Negisho, 2012). Moreover, at one hand P fertilization is a very costly business while at the other hand farming community faces severe difficulties in relation to its timely availability (Vishandas *et al.*, 2006). The efficiency of P fertilizers has also <25% (Ahmad and Rashid, 2003). Thus, phosphate availability to plants in appropriate amount is a global issue, which if deficient, can affect about 30-40% of the crop yield. For that, the continuous supply of P is important to fulfill crop requirement.

Mung bean (*Vigna radiata* L.) belongs to the leguminosae family, and is a main source of proteins, minerals, vitamins and dietary fiber (Hou *et al.*, 2019). Mung bean is a phosphorus responsive crop, it needs P for biochemical process, growth and yield improvement (Ahmad *et al.*, 2021).

Microorganisms are microscopic creatures which play huge roles in enhancing nutrients bioavailability and their supplies to plants (Beattie, 2015). A diverse group of bacteria commonly called 'plant growth promoting rhizobacteria (PGPR), solubilize the form of phosphorus which is unavailable to plants (Pii et al., 2015). Microorganisms containing phosphate solubilizing activity enhance P solubility and increase crop growth by releasing growth hormones/regulators. The utilization of PGPR for altered growth, development and ultimately the yield is now well established (Saleem et al., 2007; Arshad et al., 2008; Belimov et al., 2009) and increase biological nitrogen fixation, the number of nodules in legume crops, dry biomass, yield, yield attributes, availability of nutrients in soil and plant nutrient uptake (Beattie, 2015). Many microbial strains solubilize insoluble rock phosphate in P pool, P fixed in soil colloid, and organic P and thus they are known as P solubilizing microorganisms (Backer et al., 2018).

Microorganisms with the activities of solubilizing phosphate along with ACC deaminase increased yields of many crops (Beattie, 2015) including mungbean (Shaharooni et al., 2006; Ali et al., 2010; Rahman et al., 2012). Some PGPR contain the ACC (enzyme 1-amino-cyclo-propane-1-carboxylate) deaminase, which hydrolyses into α -ketobutyric acid and ammonia, and controls the biosynthesis of ethylene within plant roots under biotic and abiotic stress environment (Glick, 2005). Inoculation with beneficial rhizobacteria improves progress in the development of inoculated plants by reducing the higher levels of ethylene and enabling plants to develop strong and dense root system (Stearns et al., 2005).

The determination of potential mutual symbiotic association of rhizobacteria for N_2 fixation and vesicular arbuscular mycorrhizal (VAM) fungi for nutrient particularly P accumulation (Yasmeen et al., 2012). Some bacterial strains closely associate with VAM fungal spores can play a key role in enhancing colonization (mycorrhizal) with host plants by the exudation of metabolites which helps in production of the hyphae and colonization in roots of host plants (Khan et al., 2010). We conducted this study to understand growth, yield and P-use-efficiency of mungbean in relation to myco-rhizobacterial inoculation with the hypothesis that this strategy may offer promising results for sustainable low-P-input mungbean production.

MATERIAL AND METHODS

Experiment details: The field experiment was conducted at the research fields of Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan. Soil samples from the experimental area were collected before conducting the experiment to analyze selected soil properties following standard methods (Ryan et al., 2001). The experimental soil was poorly fertile

(organic matter content 0.68%, Olsen P 7.7 mg kg⁻¹, electrical conductivity 1.40 dS m⁻¹, alkaline in reaction (pH 7.9), with clay loam textural class. The experiment followed a two-factor randomized complete block split plot design with three repeats. Factor (A) comprised of three P treatments, i.e. P₁ = No P fertilizer, i.e. control, P₂ = 30.0 kg P ha⁻¹, and P₃ = 60.0 kg P ha⁻¹, while factor (B) comprised of three soil microbes, viz. SM₁ = control (no soil microbe), SM₂ = *Pseudomonas fluorescens* biotype G containing both ACC-deaminase and P-solubilizing activities, SM₃ = Mycorrhizal fungi *Glomus mosseae*. The size of each experimental unit was 3.0 m × 5.0 m = 15 m². The pre-isolated strains of ACC-Deaminase and P solubilizing rhizobacteria *Pseudomonas fluorescens* biotype G were used in this study. This ACC-Deaminase and phosphate solubilizing rhizobacterial strain was selected on the basis of varying plant-growth-promoting attributes, as described by Shaharooni et al. (2008). The maize fine roots containing VAM spores of *Glomus mosseae* as inoculum was chosen. It was properly combined with mungbean seeds prior to sowing. The rhizobacterial inocula were prepared and seed inoculation was carried as prescribed by Shaharooni et al. (2008). Phosphorus supplementation was done through soil application using DAP (46% P₂O₅). The recommended rate of N @ 20 kg ha⁻¹ was also supplied to mungbean through urea. Half dose of N was applied with full basal dose of P and remaining of half N was applied with first irrigation.

Collection of data: At maturity, 10 plants were harvested from each experimental plot at random, for recording plant height, dry biomass, branches plant⁻¹, pods plant⁻¹, grains pod⁻¹, pod size, chlorophyll content (SPAD), seed index and grain yield plant⁻¹. Phosphorus accumulation from the leaves of selected mature plants harvested from each plot, was determined following the standard method (Westerman, 1990).

Statistical Analysis: The data were analyzed using Statistix software (version 8.1) to perform analysis of variance, mean separation, and correlation. Differences among treatments means were determined using Tukey's Honestly significance difference (HSD) test at alpha < 0.05.

RESULTS

The mungbean crop responded to different P doses and soil microbes as in Table 1. The data apparently highlights that P dose affected shoot length, chlorophyll content and pod size significantly (p < 0.05) while all the leftover traits highly significantly (p < 0.01 to p < 0.001) i.e., number of branches, number of pods, number of grains, seed index/1000-grain weight, grain yield and phosphorus concentration. Likewise, soil microbial inoculants highly significantly (p < 0.001) affected all the above-mentioned plant traits except number of grains that was significant to a lesser extent (p < 0.05). It was,

however, noteworthy that both P doses and microbial inoculants did not have a significant effect on most of these parameters in an interactive manner ($p > 0.05$). Nonetheless, their interaction was found significant ($p < 0.05$) for some plant traits i.e., shoot length, chlorophyll content and P concentration.

The data further depicted that the P application at 50% and 100% of the recommended doses (30 and 60 kg ha⁻¹) increased shoot length by 8.2% and 19.2%, respectively, over control (Table 2). The latter P treatment (60 kg ha⁻¹) increased shoot length up to 2.3-fold over its counterpart (30 kg ha⁻¹). Soil microbial inoculant ACC-deaminase, phosphate-solubilizing rhizobacteria (PSRB) *Pseudomonas fluorescense* biotype G. increased 10.8% and 23.9% in case of AM fungus *Glomus mosseae*, capable of forming symbiotic relationship with plant roots for enhanced P solubilization. Hence, AM fungus *Glomus mosseae* increased shoot length of by 2.2-fold as compared to ACC-deaminase, PSRB *P. fluorescense*. Both main sources of variation i.e., phosphorus doses and soil microbial inoculants affected shoot length of in an interactive manner. The highest increase in shoot length (57.4%) was recorded between half of the RDF (30 kg P ha⁻¹) and AM fungus *Glomus mosseae*, followed by the increase in shoot length recorded where full RDF (60 kg P ha⁻¹) was applied either without any soil microbial inoculant (49.8%) or with AM fungus *Glomus mosseae*. Hence, AM fungus *Glomus mosseae* performed more significantly even at 50% of the recommended P level as against its counterpart with 100% recommended P level (60 kg ha⁻¹) for enhancing the shoot length. The application of P doses (30 and 60 kg ha⁻¹) increased number of branches (plant⁻¹) by 15.9% and 25.4%, respectively, over control (Table 2). The latter P treatment (60 kg P ha⁻¹) alone increased up to 1.6 times when compared to its counterpart (30 kg P ha⁻¹). This increase was up to 12.6% in case of ACC-deaminase, PSRB *P. fluorescense* and up to 22.7% in case of AM fungus *Glomus mosseae*, capable of forming symbiotic relationship with plant roots for enhanced P solubilization. The AM fungus *Glomus mosseae* enhanced the number of branches by 1.8 time over ACC-deaminase, PSRB *P. fluorescense*.

The chlorophyll content enhanced by 9.3% and 17.8% as P doses increased to 30 and 60 kg ha⁻¹, respectively, over control (Table 2). The latter P treatment (60 kg ha⁻¹) increased to 1.9-fold compared to 30 kg ha⁻¹. ACC-deaminase, PSRB *P. fluorescense* and AM fungus *Glomus mosseae* augmented chlorophyll content up 6.2% and 14.8%, respectively. AM fungus increased chlorophyll content by 2.4-fold as compared to ACC-deaminase, PSRB *P. fluorescense*. The highest increase in chlorophyll content (40.2%) was recorded in the case of the interactive effect between full recommended dose of P (60 kg ha⁻¹) and AM fungus.

The P use at 30 and 60 kg ha⁻¹ amplified the number of pods (plant⁻¹) by 21.8% and 32.7%,

respectively, over control (Table 2). The application of phosphorus at 60 kg ha⁻¹ increased number of pods upto 1.5-fold as compared to 30 kg ha⁻¹. ACC-deaminase and AM fungus increased 14.7% and 31.2% number of pods (plant⁻¹). Arbuscular mycorrhizal fungus *Glomus mosseae* increased number of pods (plant⁻¹) by 2.1-fold as compared to ACC-deaminase, PSRB *P. fluorescense*.

Pod size (cm) significantly affected by different levels (30 and 60 kg ha⁻¹) of P and enhanced pod size by 13.7% and 24.1%, respectively, over control (Table 2). The latter P treatment (60 kg ha⁻¹) increased up to 1.8-fold as compared to its counterpart (30 kg ha⁻¹). Soil microbial inoculants PSRB *P. fluorescense* and AM fungus *Glomus mosseae* increased pod size 10.3 and 18.3% over control. Hence, AM fungus *Glomus mosseae* increased stem diameter by 1.8-fold as compared to PSRB *P. fluorescense*.

Grains per pod significantly influenced by application of P levels (30 and 60 kg ha⁻¹) and augmented by 27.2% and 43.1%, respectively, over control (Table 3). The latter P treatment (60 kg ha⁻¹) increased grains up to 1.6-fold as contrast to 30 kg ha⁻¹. The increase in grains pod⁻¹ up to 11.4% and 18.9% were recorded with PSRB *P. fluorescense* inoculum and AM fungus *Glomus mosseae* over control. Arbuscular mycorrhizal fungus *Glomus mosseae* improved number of grains by 1.7-fold as compared to PSRB *P. fluorescense*. However, the two main sources of variance i.e., phosphorus doses and soil microbial inoculants did not affect grains pod⁻¹ in an interactive manner.

The weight of a thousand grains is an important parameter enhancing the grain yield of certain crops. The application of different P levels (30 and 60 kg ha⁻¹) increased 1000 grain weight by 15.1% and 36.6%, respectively, over control (Table 3). The P 60 kg ha⁻¹ promote 1000 grains weight up to 2.4-fold as compared to 30 kg ha⁻¹. Soil microbial inoculants PSRB *P. fluorescense* and AM fungus *Glomus mosseae*, also improved grain weight 5.2% and 13.5% over control. Hence, AM fungus *Glomus mosseae* increased by 2.6-fold as compared to PSRB *P. fluorescense*.

The grain yield was significantly affected by P application at (30 and 60 kg ha⁻¹) as it increased the grain yield by 32.5% and 51.0%, respectively, when compared to no P application (Table 3). Phosphorus application at 100% RDF (60 kg P ha⁻¹) boosted grain yield up to 1.6 times over 50% RDF (30 kg P ha⁻¹). Soil microbial inoculants contributed to increasing stem diameter over no P application. The relative increase was 8.6% in case of PSRB *P. fluorescense* and 20.4% in case of AM fungus *Glomus mosseae*, which is capable of forming symbiotic relationship with plant roots for enhanced P solubilization. Hence, AM fungus *Glomus mosseae* increased grain yield of *Vigna radiata* L. by 2.4-fold as compared to PSRB *P. fluorescense*.

Phosphorus concentration of mungbean was also significantly affected by varying doses of P (30 and 60 kg ha⁻¹) and increased phosphorus concentration by 45.9% and 76.1%, respectively, over control (Table 3). The higher dose of P (60 kg ha⁻¹) elevated P concentrations up to 1.7 times over its lower dose (30 kg ha⁻¹). Soil microbial inoculants also enhanced plant P concentration over no P application. This increase noticed was 7.1% with PSRB *P. fluorescence* and 19.1% with AM fungus *Glomus mosseae*. Arbuscular mycorrhizal fungus *Glomus mosseae* helped enhance phosphorus concentration, by 2.7-fold as compared to

ACC-deaminase, PSRB *P. fluorescence*. The two main sources of variance i.e., phosphorus doses and soil microbial inoculants affected phosphorus concentration in an interactive manner. The maximum phosphorus concentration (153% or 2.54-fold) was recorded where the full RDF (60 kg ha⁻¹) was applied with AM fungus *Glomus mosseae*. It was followed by the increase in phosphorus concentration recorded either with full recommended dose of phosphorus (60 kg ha⁻¹) alone or its integration with ACC-deaminase, PSRB *P. fluorescence* (146% or 2.46-fold in each case).

Table 1. P-values from analysis of variance of various parameters of mungbean as affected by different phosphorus levels (P), ACC-deaminase- phosphate-solubilizing *Pseudomonas* and mycorrhizal fungi (M) and their interaction (P × M).

Parameter	P	M	P × M
Shoot length (cm plant ⁻¹)	0.0104	0.0024	0.0302
Number of branches (plant ⁻¹)	0.0001	0.0001	0.2700
Chlorophyll content (SPAD)	0.0293	0.0017	0.0490
Number of pods (plant ⁻¹)	0.0065	0.0003	0.4268
Pod size (cm)	0.0265	0.0009	0.1418
Number of grains (pod ⁻¹)	0.0086	0.0118	0.2303
Seed index/1000-grain weight (g)	0.0011	0.0006	0.1631
Grain yield (g plant ⁻¹)	0.0089	0.0002	0.0521
Phosphorus concentration (%)	0.0025	0.0045	0.0147

Table 2. Response of mungbean to ACC-deaminase rhizobacteria, without† or with‡ AM phosphate solubilizing activity, at different phosphorus levels.

Shoot length (cm plant ⁻¹)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	22.3c	25.5bc	33.4a	27.1
<i>P. fluorescence</i> †	28.2abc	29.2abc	32.5ab	30.0
<i>Glomus mosseae</i> ‡	32.5ab	35.1a	33.0a	33.5
Phosphorus dose mean	27.7	29.9	33.0	
Number of branches (plant ⁻¹)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	5.5	6.7	7.6	6.6C
<i>P. fluorescence</i> †	6.6	7.6	8.1	7.4B
<i>Glomus mosseae</i> ‡	7.4	8.3	8.7	8.1A
Phosphorus dose mean	6.5C	7.5B	8.1A	
Chlorophyll content (SPAD)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	34.8b	42.3ab	46.3a	41.1
<i>P. fluorescence</i> †	40.0ab	43.5ab	47.5a	43.7
<i>Glomus mosseae</i> ‡	46.3a	46.5a	48.8a	47.2
Phosphorus dose mean	40.3	44.1A	47.5	
Number of pods (plant ⁻¹)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	14.50	17.80	21.50	17.93C
<i>P. fluorescence</i> †	17.50	21.55	22.65	20.57B
<i>Glomus mosseae</i> ‡	20.50	24.60	25.50	23.53A
Phosphorus dose mean	17.50B	21.32A	23.22A	
Pod size (cm)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	5.1	6.3	7.2	6.2B
<i>P. fluorescence</i> †	6.1	6.9	7.4	6.8B
<i>Glomus mosseae</i> ‡	6.9	7.3	7.8	7.3A
Phosphorus dose mean	6.0B	6.8AB	7.5A	

Table 3. Response of mungbean to ACC-deaminase rhizobacteria, without† or with‡ AM phosphate solubilizing activity, at different phosphorus levels.

Number of grains (pod ⁻¹)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	5.2	7.1	8.7	7.0B
P. fluorescence†	6.2	8.1	9.1	7.8AB
Glomus mosseae‡	7.3	8.6	9.0	8.3A
Phosphorus dose mean	6.2B	7.9A	8.9A	
Seed index/1000-grain weight (g)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	30.2	36.8	45.2	37.4B
P. fluorescence†	34.6	38.6	44.9	39.3B
Glomus mosseae‡	36.9	41.7	48.8	42.4A
Phosphorus dose mean	33.9C	39.0B	46.3A	
Grain yield (g plant ⁻¹)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	20.2	30.0	36.5	28.9C
P. fluorescence†	25.1	32.8	36.3	31.4B
Glomus mosseae‡	29.1	35.8	39.5	34.8A
Phosphorus dose mean	24.8B	32.8A	37.4A	
Phosphorus concentration (%)				
Microbial inoculant	Phosphorus dose (kg ha ⁻¹)			Microbial inoculant mean
	00	30	60	
Control	0.13d	0.26bc	0.32ab	0.24
P. fluorescence†	0.18d	0.27abc	0.32ab	0.25
Glomus mosseae‡	0.24c	0.27abc	0.33a	0.28
Phosphorus dose mean	0.18	0.27	0.32	

DISCUSSION

Microbial plant nutrition has been considered as the best possible option both under nutrient stress and normal conditions for the enhanced plant growth, and increased yield and product quality of several crops (Beattie, 2015), including mungbean (Shaharoon et al., 2006; Ali et al., 2010; Rahman et al., 2012). It was concluded that P solubilizing bacteria significantly enhanced phosphorus nutrition by solubilizing P from active and passive pools through the process of mineralization (Bashan et al., 2013).

The findings of this field experiment clearly demonstrated the benefit of using rhizobacterial and mycorrhizal fungal inoculants, in integration with chemical phosphatic fertilizers, for the enhanced growth, yield and phosphorus accumulation of mungbean (Table 1 to 3). Both the adequate phosphorus nutrition and microbial inoculants significantly ($p < 0.05$ to < 0.0001) improved various growth traits and yield of mungbeans (Table 2 to 3). These two main sources also significantly affected plant phosphorus accumulation (Table 3). Furthermore, the interaction of two main sources of variation was also found to be significant in case of shoot length, chlorophyll content and phosphorus concentration of plants (Table 1) which reflected that the two sources of variance governed these plant traits of mungbean in an interactive manner.

These results advocate the findings of many early studies highlighting significant impacts on the growth, yield and nutritional quality of mungbean crop to phosphate solubilizing bacterial inoculums and AM fungi (Yasmeen et al., 2012; Ahmad et al., 2021). The potential of establishing mutualistic association with rhizobacteria for atmospheric N₂ fixation and AM fungi for P accumulation (Yasmeen et al., 2012) has established effects on leguminous plants in natural and agri-ecosystems. According to Kumar et al. (2015), the P solubilizing rhizobacteria significantly helps to increased P uptake. However, in present study high utilization efficiency of P solubilization was observed by application of dual inoculation of bacteria and fungi. Related finding was noted in maize crop, by application of co-inoculation of VAM fungi and PSB (Suri et al., 2011).

The application of PGPR inoculum with ACC deaminase activities substantially altered the negative influences on peas crop under water stress (Arshad et al., 2008). Additionally, Rahman et al. (2012) confirmed the positive effects of rhizobacterial inoculants in a field experiment on the yield attributes of two mungbean cultivars. Likewise, significant enhancement in agronomic traits was observed under rhizobacterial inoculation (Jha et al., 2012). The integrated application of P and microbial inoculation illustrated higher P and its uptake by crop plants. Similar results were obtained by using four PSRB

strains isolated from the rhizosphere of pearl millet, mungbean and sesame (Shahzad *et al.*, 2010).

The above discussion clearly emphasizes that both the ACC-deaminase rhizobacteria and arbuscular mycorrhizal fungi, capable of phosphate-solubilization, possess the ability to alter the growth traits and yields of mungbean by way of their enhanced phosphate solubilization under P deficiency stress. In addition, this study further demonstrated the usefulness of these soil microbes even under P adequate conditions.

We conclude that microbial inoculation of arbuscular mycorrhizal fungi was found to be efficient over rhizobacterial inoculation. Microbial inoculants, when used with optimal phosphorus levels, augmented the growth, yield and phosphorus accumulation under both P-deficient and P-adequate environments.

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