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GROWTH, NODULATION AND YIELD OF MUNGBEAN (*VIGNA RADIATA* L.) AS INFLUENCED BY COINOCULATION WITH *RHIZOBIUM* AND PLANT GROWTH PROMOTING RHIZOBACTERIA

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A Pot experiment was conducted in wire house to study the effect of coinoculation with *Rhizobium* and two plant growth promoting rhizobacterial (PGPR) strains (Q7 & Q14) on mungbean (*Vigna radiata L.*). The *Rhizobium*, Q7 and Q14 showed better results over uninoculated control whether inoculated alone or in combination with each other. The coinoculation of Q7 and Q14 with *Rhizobium* showed better results over *Rhizobium* alone. PGPR Q7+ *Rhizobium* increased plant height, root length, number of nodules and number of grains pod^{-1} by 10.8, 5.5, 56.5 and 37.7%, respectively, over *Rhizobium* alone, while PGPR Q14 + *Rhizobium* decreased these parameters but increased number of pods plant^{-1}, 100 grain weight and number of grains plant^{-1} by 66.1, 43.1 & 68.6%, respectively, compared with *Rhizobium* alone. Q7 strain promoted vegetative growth but grain size was less as compared to other treatments while Q14 strain showed bold grain size and more yield.
Key words: Coinoculation, plant growth promoting rhizobacteria, *rhizobium*, mungbean

INTRODUCTION

Pakistan is an agricultural country producing a variety of crops. In legumes, pulses are important food crops with large protein contents and used widely in feeding masses but per acre yield is very low. Population is increasing at alarming rate, which demands urgent boost up in the crop yield from existing area. Mungbean (*Vigna radiata* L.) is an important pulse crop in many Asian countries including Pakistan, where the diet is mostly cereal based. The area under mungbean crop in Pakistan is 239.2 thousand hectares and producing about 115.4 thousand tons of grains annually (Anonymous, 2003). This low grain yield of the crop i.e. 477 kg ha^{-1} on an average as shown in the Agricultural Statistics of Pakistan (2002-2003) can be enhanced by proper utilization of biological and genetic potential of microbial and plant species. In recent years, free living soil bacteria are commonly used as inoculants in various parts of the world for improving the yield of agricultural crops. These bacteria, when applied to seeds or roots, colonize plant roots and stimulate plant growth and crop yield by multifarious mechanisms, ranging from direct influence (e.g. increasing solubilization and uptake of nutrient, production of plant growth regulators) to an indirect effect (e.g. pathogen suppression such as biocontrol, production of sidrophores and antibiotics). These have been termed as plant growth-promoting rhizobacteria (PGPR) (Bianca *et al.*, 2001). It is an established fact that rhizobia fix nitrogen and play role in improving plant growth. Several workers have reported that seed inoculation with *Rhizobium* has significantly increased the growth and yield of legume crops. (Goel *et al.*, 1999; Pathak *et al.*, 2001). It is highly likely that interactive effect of rhizobia and free living soil bacteria could be more beneficial due to a variety of growth enhancing mechanisms like creating additional infection sites by PGPRs that are later occupied by *Rhizobium*, producing some antibiosis in the rhizosphere and by producing sidrophores which chelate insoluble cations or colonize root surfaces (Plazinski and Rolfe, 1985). Few studies have shown that the inoculation of seed with mixed cultures have tremendous positive effects on plant growth compared to the single strain inoculation. (Xavier and Germida, 2002). Keeping in view the above discussion, a pot study was planned to evaluate the effect of co-inoculation on growth, nodulation and yield of mungbean.

MATERIALS AND METHODS

Preisolated plant growth promoting rhizobacterial (PGPR) strains (Q7 & Q14) and preisolated and preidentified *Rhizobium* strain "*Rhizobium japonicum*" were collected from Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Broth culture of *Rhizobium* and PGPR were prepared by using the composition of Yeast Extract Manitol media (YEM) (Vincent, 1970) and Salt Minimal media (Dworkin and Foster, 1998) except agar, respectively. Both broths were transferred to 200 mL flasks for each strain separately. After sterilization at 121°C for half an hour, YEM broth containing flask was inoculated with *Rhizobium Japonicum* and Salt Minimal broth containing flasks were inoculated with rhizobacteria Q7 and Q14, respectively. Inoculated flasks were incubated at 28 ± 1°C for four days with occasional shaking (4-5 times a day). Uninoculated media was

kept as control to check the contamination if any for both broths (YEM and Salt Minimal). After incubation, growth of bacteria in the broth was observed under microscope to ensure maximum population. An optical density of 0.5 recorded at λ 535 nm was achieved by dilution to maintain uniform cell density (10^8-10^9 CFU/mL).

Mungbean seeds were surface disinfected by immersing them in 95% ethanol for few seconds and then in 0.2% solution of $HgCl_2$ for four minutes, following several rinses with sterilized water to remove disinfectant (Russell *et al.*, 1982). A mixture of peat and clay (50% each) was sterilized at 121°C for half an hour in eight petri dishes (60 g in each). After sterilization, broth cultures (18 mL) and 10 mL sterilized sugar solution (10%) was added in each petri dish, in such a quantity that made the whole peat wet. Eighteen mL broths of *Rhizobium*, Q7 & Q14 strains alone, were added in each petri dish. Coinoculation was done by mixing equal volume of each strain to maintain 18 mL broth volume in each petri dish. These petri dishes were kept in the incubator for 48 hours and then sterilized mungbean seeds were coated with inoculated slurry. These inoculated/coated seeds were placed over night for drying.

Pot experiment was conducted during September - November in the wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Inoculated seeds of mungbean cv. NM-92 were sown in separate pots containing 14 kg soil each. Recommended N and P fertilizers @ 25 and 62 kg ha^{-1} were applied by broadcast method at the germination stage as urea and diammonium phosphate (DAP). Eight seeds were sown in each pot which were thinned to two plants pot^{-1} after fifteen days of germination. Irrigation requirements were fulfilled by canal water [EC = 0.03 dS m^{-1}, sodium adsorption ratio (SAR) = 0.26 $(mmol\ L^{-1})^{1/2}$ and residual sodium carbonates (RSC) = 0] meeting the irrigation quality criteria for crops (Ayers and Westcot, 1985). Treatments were replicated six times using completely randomized design (CRD). Three replications were used for recording data about root length, number of nodules and fresh and oven dry weight of nodules at the time of flowering. Rest of three replications were used for recording data regarding plant height, fresh and oven dry weight of plant biomass, number of pods, number of grains per pod and per plant and 100-grain weight after harvesting. A clay loam (typic haplocambids) soil was collected from a research field of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad and analyzed for physico-chemical characters. The analyses of a composite soil sample revealed a pH of 7.4, electrical conductivity of saturated soil extract (ECe), 1.6 dS m^{-1}; cation exchange capacity (CEC), 6.8 $cmol_c\ kg^{-1}$ and organic matter, 0.43%. Plant and grain samples were analyzed for N contents after harvesting. (Yoshida et al., 1976).

RESULTS

Results regarding plant height, root length, fresh and oven dry weight of plant biomass are summarized in Table 1. Data regarding plant height revealed that *Rhizobium* inoculation and coinoculation significantly increased plant height over control. *Rhizobium* inoculation alone increased plant height by 12% over control. Maximum increase in plant height (23.7% over control and 10.8% over *Rhizobium* inoculation alone) was recorded from coinoculation of *Rhizobium* + Q7 and it was statistically similar with Q7 + Q14 and *Rhizobium* + Q7 + Q14. The inoculation with Q7 and Q14 alone exhibited negative effect on plant height compared with *Rhizobium* inoculation alone.

Data regarding root length showed that *Rhizobium* inoculation increased root length by 29% over control and maximum increase in root length (36.4% over control and 5.5% over *Rhizobium* inoculation alone) was recorded with *Rhizobium* + Q7. While other coinoculation treatments (Q14 + Q7 & Q7 + Q14 + *Rhizobium)* were non-significant with each other and with *Rhizobium* inoculation alone but were significant in comparison with control. Inoculation of PGPR strain Q14 decreased the root length by 12% when compared with *Rhizobium* inoculation alone.

It was found that *Rhizobium* inoculation alone increased fresh plant biomass by 19% over control while coinoculation of *Rhizobium* + Q7 gave maximum total biomass which was 43.6% higher than control and 21.1% higher than *Rhizobium* inoculation alone. All other treatments were non-significant with each other but significant over control.

Results regarding oven dry weight of plant biomass revealed that *Rhizobium* inoculation alone increased oven dry weight by 11% over control while maximum oven dry weight of plant biomass was recorded from treatment Q7 + Q14 + *Rhizobium*, which was 30.7% higher than control and 23.7% higher than *Rhizobium* inoculation alone. Other coinoculation treatments (Q14 + *Rhizobium*, Q7 + *Rhizobium*, Q7 + Q14) were non-significant with each other but were significant with *Rhizobium* inoculation alone and uninoculated control.

Table 1. Effect of coinoculation on plant height, root length and plant biomass

Treatment	Plant Height (cm)	Root Length (cm)	Plant Biomass (g/plant)	Oven Dry Weight (g/plant)
Control	29.1 b	15.4 c	17.2 c	3.8 d
Q7	32.2 ab	19.6 ab	20.3 bc	4.0 c
Q14	31.3 ab	17.8 bc	19.8 bc	4.0 c
Rhizobium	32.5 ab	19.9 ab	20.4 bc	4.2 bc
Q14 + Q7	34.8 a	19.2 ab	22.6 ab	5.0 abc
Q7 + .Rhizobium	36.0 a	21.0 a	24.7 a	5.1 ab
Q14 + Rhizobium	32.3 ab	17.1 bc	21.4 ab	4.6 abc
Q7 + Q14 + Rhizobium	35.6 a	18.7 ab	22.9 ab	5.5 a

Nodule promoting effect of *Rhizobium* inoculation is evident from results (Table 2), it increased number of nodules, fresh and oven dry weight of nodules by 6 to 30 folds, respectively, over control and coinoculation of *Rhizobium* with PGPR strain Q7 increased these parameters by 56, 64 & 80%, respectively, in comparison with inoculation of *Rhizobium* alone. While inoculation of Q14 with *Rhizobium* decreased number of nodules by 15% but increased nodule weight and oven dry weight of nodule by 22 & 40% over *Rhizobium* inoculation alone. Coinoculation treatments (Q14 + Q7 & *Rhizobium* + Q14) decreased number of nodules over *Rhizobium* inoculation alone but coinoculation treatment, *Rhizobium* + Q7 + Q14, increased number of nodules by 17% over *Rhizobium* inoculation alone.

Table 2. Effect of coinoculation on number of nodules, weight of nodules and oven dry weight of nodules.

Treatment	No. of nodules	Nodule weight (g)	Oven dry weight of nodule (g)
Control	3 e	0.06 d	0.003 d
Q7	19 c	0.20 c	0.05 c
Q14	14 d	0.19 c	0.05 c
Rhizobium	23 bc	0.22 bc	0.05 c
Q14 + Q7	19 cd	0.22 bc	0.05 c
Q7 + Rhizobium	36 a	0.36 a	0.09 a
Q14 + Rhizobium	18 cd	0.27 ab	0.07 b
Q7 + Q14 + Rhizobium	27 b	0.26 ab	0.06 bc

Data in Table 3 indicated that *Rhizobium* and Q14 inoculation alone increased number of pods plant^{-1} by 13 & 63%, respectively, over uninoculated control. Maximum increase in number of pods plant^{-1} was recorded when *Rhizobium* and PGPR strain Q14 were coinoculated; it was 87 and 66% higher than control and *Rhizobium* inoculation alone, respectively.

Rhizobium inoculation alone increased number of grains pod^{-1} by 25% over uninoculated control. Coinoculation of Q7+*Rhizobium* showed 38% increase in number of grains pod^{-1} over *Rhizobium* inoculation alone followed by treatment Q14 alone where 13% increase over *Rhizobium* inoculation alone was recorded. Other coinoculation treatments (Q7+Q14, Q7+*Rhizobium*, & Q7+Q14+*Rhizobium*) showed non-significant effect over each other and over *Rhizobium* inoculation but were significant over control.

Maximum number of grains plant^{-1} were recorded with coinoculation of *Rhizobium*+Q14 which resulted in 15 & 62% increase over control and *Rhizobium* inoculation alone, respectively, followed by coinoculation of *Rhizobium*+Q7+Q14 which showed 120 & 42% increase over control and *Rhizobium* inoculation alone, respectively. While *Rhizobium* inoculation alone caused an increase of 54% over control

Rhizobium inoculation increased grain weight plant^{-1} by 64% over uninoculated control while coinoculation of *Rhizobium* with Q14 caused 157 & 56% increase over control & *Rhizobium* inoculation alone, respectively. Other coinoculation treatments were non-significant in comparison with Rhizobium inoculation alone but were significant over control.

Rhizobium + Q14 was the most effective treatment in increasing 100-grain weight which resulted in 55% increase over control and 43% increase over *Rhizobium* inoculation alone followed by coinoculation of *Rhizobium* + Q7 + Q14 and *Rhizbium* + Q7.

Data regarding nitrogen contents and its uptake in straw and grains (Table 4) revealed that *Rhizobium* inoculation alone increased N concentration in straw by 18% over control and with Q7, *Rhizobium* inoculation enhanced N contents by 42% over control while the effect of all other coinoculation treatments was non-significant over each other but were significant over control. Nitrogen contents in grain were increased by 8% with *Rhizobium* inoculation alone over uninoculated control, while coinoculation of *Rhizobium* with Q14

Table 3. Effect of coinoculation on reproductive aspects of mungbean

Treatment	No. of pods plant^{-1}	Grain weight plant^{-1} (g)	No. of grains plant^{-1}	No. of grains pod^{-1}	100 grain weight (g)
Control	5.5 c	1.4 c	30 d	5.5 b	4.7 c
Q7	6.3 c	2.6 bc	46.3 cd	7.3 ab	5.7 bc
Q14	9.0 ab	2.9 ab	56.3 bc	7.8 ab	5.8 bc
Rhizobium	6.2 c	2.3 bc	46.3 cd	6.9 b	5.1 bc
Q14 + Q7	7.8 bc	3.1 bc	60.0 bc	6.9 b	5.6 ab
Q7 + Rhizobium	7.5 bc	2.8 bc	58.3 bc	9.5 a	6.6 bc
Q14 + Rhizobium	10.3 a	3.6 a	75.3 a	7.7 ab	7.3 a
Q7 + Q14 + Rhizobium	9.0 ab	3.2 ab	66.0 ab	7.5 ab	6.6 ab

increased N contents in grain by 58% over control and 47% over *Rhizobium* inoculation alone.

Coinoculation of *Rhizobium* with Q7 increased N uptake in straw by 77% over control while this increase due to *Rhizobium* inoculation alone was 33% over control. Effect of all other coinoculation treatments was non-significant with each other but was significant over control.

Data regarding N uptake in grains showed that *Rhizobium* inoculation increased N uptake in grains by 75% over control while coinoculation of *Rhizobium* with Q14 increased N uptake in grain by 300% over control and 129% over *Rhizobium* inoculation alone. PGPR strain Q14 also showed 42% increase in N uptake in grains over *Rhizobium* inoculation alone.

Table 4. Effect of coinoculation on nitrogen uptake and percentage in grain and straw of mungbean

Treatment	Nitrogen in straw (%)	Nitrogen in grain (%)	Nitrogen uptake in straw (mg/plant)	Nitrogen uptake in grain (mg/plant)
Control	1.76 c	2.92 g	320 c	41 f
Q7	2.18 b	3.46 e	455 b	90 cd
Q14	2.09 b	4.39 b	435 bc	125 b
Rhizobium	2.07 b	3.14 f	428 bc	72 d
Q14 + Q7	2.17 b	4.15 c	445 b	128 b
Q7 + Rhizobium	2.49 a	3.60 e	568 a	101 bc
Q14 + Rhizobium	2.13 b	4.61 a	439 bc	165 a
Q7 + Q14 + Rhizobium	2.15 b	3.97 d	449 b	126 b

DISCUSSION

The plant rhizosphere is a major soil ecological environment for plant microbe interactions involving colonization of different microorganisms in and around the roots of the growing plant. This colonization may either result in associative, symbiotic, neutralist or parasitic interaction, depending upon the plant nutrient status in the soil environment. Inoculant microbes stimulate performance of crop by the production of plant growth regulators (Gaskin *et al.*, 1985), enhanced access to soil nutrients (Alagawadi and Gaur, 1992), suppressed deleterious rhizobacteria (Kloepper and Schroth, 1981), affected symbiotic nitrogen fixation by enhancing root nodule number or mass (Singh and Rao, 1979; Burns *et al.*, 1981; Polonenk *et al.*, 1987; Yahalom *et al.*, 1987) and by increasing nitrogenase activity (Iruthayathas *et al.*, 1983).

Results from present study showed more number of nodules and vegetative growth by PGPR strain (Q7) with *Rhizobium*, but no effect on yield which indicated that nodules formed in response to adjunct inoculant may be ineffective (Plazinski and Rolf, 1985). Another factor which may be an important determinant of growth promotion is the specific genotypic combination of plant and microbes (Rannie and Larson, 1979; Chenway *et al.*, 1988a). Q7 strain of PGPR with *Rhizobium* promoted plant height, root length, fresh and oven dry plant biomass while the second PGPR strain (Q14) increased the yield but not plant height, number of nodules and root length which may be due to some unspecified type of antagonism that prevented colonization of root surfaces by *Rhizobium* (Hely *et al.*,

1957 and Anderson, 1957). PGPR strain Q7 with *Rhizobium* might have increased nodule formation by stimulating the formation of epidermal cells that became infected root hair cells, creating additional infection sites that were later occupied by *Rhizobium*, producing some antibiosis in the rhizosphere, inducing phytoalexin production by plants and by production of sidrophores which chelated insoluble cations or colonized root surfaces (Plazinski and Rolfe, 1985; Burns *et al.*, 1981 and Yahalom *et al.*, 1987). The data regarding nitrogen contents in straw revealed that coinoculation of *Rhizobium* + Q7 showed more nitrogen contents in straw than coinoculation of *Rhizobium* + Q14 over *Rhizobium* inoculation alone while nitrogen contents in grain showed opposite behavior. Data regarding nitrogen uptake in straw and grain showed similar behavior. Similar results were reported by Chebotar et al., 2001.

CONCLUSION

The main objective of this study was the screening of effective PGPR strains and to test their effect alone or with *Rhizobium* on growth, nodulation and yield of mungbean. PGPR strain Q7 with *Rhizobium* only promoted vegetative growth while PGPR strain Q14 with *Rhizobium* promoted reproductive growth and yield significantly.

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