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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5 (1): 25-28, 2002

Effect of Rhizobial Management upon Rhizobial Population, Nodulation and Growth of Yard Long Beans (*Vigna sesquipedalis* L.): A New Approach to Maximize Benefits from *Rhizobium*

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Abstract: The work aimed to investigate some benefits derived from *Rhizobium* technology. The results showed that management methods in retaining native *Rhizobium* being tested had a tremendous effect on population management, i.e. native *Rhizobium* could be maintained. However, *Rhizobium* has preferential level of selectivity to host. KKU 25 yard long bean plants had the highest level of rhizobia population followed by white seeded cultivar at day 30 and thereafter the amount of rhizobia population declined with time in all subplots whilst no population was detected from the control subplots. Native *Rhizobium* had significant potentiality for growth of yard long bean crop plants by supplying some certain amount of nitrogen being fixed from the atmosphere and the growth of yard long bean crop plants was similar to that of the control subplots that received nitrogen from chemical nitrogen fertilizer.

Key words: Native *Rhizobium* strain, nitrogen fixation, population management, *Rhizobium,* yard long bean

Introduction

It has been advocated by a number of workers that *Rhizobium* has its significant role on growth and crop yield of many leguminous crops by contributing some amounts of nitrogen to the crop plants from their biological fixation. This includes native populations or indigenous rhizobia of both effective and ineffective strains. Amongst the population available in soils, they obviously have possessed the various qualities with respect to survival capability and competition among the various strains (Wynne *et al.,* 1980: Reenie and Dubetz, 1984; and Srisouvakontorn, 1989). Growers of crop plants obviously apply some large amounts of chemical nitrogen fertilizer to their soils for high crop production in past decades, when rhizobial technology was inadequately available. Nowadays, a number of growers had realized how *Rhizobium* technology could contribute to their crop yields particularly growing leguminous crops such as peanut (*Arachis hypogeae*), mungbean (*Vigna radiata*) cowpea (*Vigna unguiculata*) and others. Most of these leguminous crops have been classified as promiscuous crops in their rhizobial requirements. Nevertheless, it has been reported by Toomsan *et al.* (1989a) that seed inoculation of peanut crop obviously gave no responses on growth of the crop plants. Similarly, Toomsan (1990) again with peanut crop reported that total dry weights and shoot dry weights/plant of three peanut cultivars being grown at two different villages in Northeast Thailand did not statistically differ from one another due to seed inoculation and un-inoculation of *Rhizobium*. On the contrary, Wadisirisak and Boonkerd (1990) reported that seed inoculation of peanut cv. Thainan 9 grown at Kalasin Province, Northeast Thailand gave significantly greater nodule numbers and seed yields over the control treatment and they recommended that seed inoculation must be practiced whenever legume seed crop has to be sown and the population of *Rhizobium* could be lasted in soil for a few years after each inoculation (Tantichareon, 1996). Population of soil rhizobia at a given site could be influenced by many factors such as soil temperature, moisture content, pH, soil type and the presence of antagonistic microorganisms. This finding confirms the work reported by Caldwell and Vest (1970), and Subba Roa (1995). Whilst Luangchaisri (1995) showed that the populations of soil rhizobia of soil where soybean crop (*Glycine max* L.) had been grown continuously were significantly higher than the sites where non-legume crops had been cultivated yet Anderson and Ingram (1993) showed that host of soil rhizobia has its significant effect on the amount of population due to the substances being released from the plant roots (exodus of plant substances by roots) and it was reported by Nurhayati *et al.* (1988) that indigenous or native strains of rhizobia possessed high potential in nitrogen fixation. Therefore, it is of a tangible value to investigate how population of indigenous rhizobia could be managed under *in vitro* condition when yard long bean plants act as the host plants for their population multiplication and it was anticipated that the results attained could possibly provide some useful data for future field experiments in order to maximize potential benefit derived from nitrogen fixation.

Materials and Methods

This *in vitro* investigation was carried out under controlled laboratory condition at the Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand during July 1999 to March 2000. The experimental design being used was a split plot design arranged in CRD with four replications. The main plot consisted of two cultivars of yard long bean, i.e. white seeded and KKU 25. A number of glass test tubes of 32 units were used for four subplot managements, each contained the same amount of sterilized coarse sand particles. The four subplot managements being used were:

1 Control, (the experimental test tubes were added with 70 ppm of nitrogen solution).
2 Non-managed treatment (the tubes were added with 1 ml of native yard long bean *Rhizobium* derived from soil where yard long bean plants had been grown continuously for two years at the dilution rate of 10^{-1}).
3 Test tubes filled with materials as that of the management number 2 plus one seedling of white seeded yard long bean for each test tube.
4 Test tubes were filled with materials similar to that of the management number 2 plus one seedling of KKU 25 yard long bean cultivar for each test tube.

The seedlings being used were germinated in a separated plate at room temperature. All test tubes being used were sterilized before use and the plant seedlings of yard long bean were supplied with N free modified solution of Long Ashton nutrient solution throughout the experimental period (Summerfield *et* *al.,* 1977). All experimental test tubes were fitted with test tube stands and they were placed in an environmental growth chamber where humidity, light and temperature had been controlled. The plants in all test tubes were allowed to grow for 60 days whilst other test tubes with or without yard long bean plants were completely enclosed. Within this period, management methods were applied, i.e. rhizobial population of all test tubes were determined at days 1, 30 and 60 after planting using MPN plant infection technique (Fisher and Yates, 1963). At 60 days after emergence, all yard long bean plants were removed from the test tubes then sterilized seedlings of both yard long bean cultivars were planted into all test tubes and allowed to grow again in growth chamber (14 and 10 hours day and night within a day, respectively) with the application of nutrient solution as did with the first period of management method of both yard long bean cultivars. The plants were allowed to grow for 60 days after planting. The cotyledons of seedlings of both sets of plants were removed before transplanting as that stated by Toomsan *et al.* (1984). At the last day of growing period (60 days), all yard long bean plants were removed from the test tubes and they were used for the determination of fresh weights, numbers of nodules and *Rhizobium* population/plant. The obtained data were statistically analyzed using Duncan's Multiple Range Test (DMRT) with the application of MSTAT-C computer programme (Nissen, 1988).

Results and Discussion

Rhizobial population: With rhizobial population at the initial day, the results showed that rhizobial population of the managed subplots were significantly greater than the control subplots (added with N at 70 ppm only). However, the amounts of rhizobial population among the subplots being added with some certain amounts of native rhizobia taken from soil where yard long bean crop plants had been grown continuously possessed some similar amounts of rhizobial population whether with or without the growth of yard long bean plants. The results revealed that the average values of rhizobial population were within the range of Log MPN 2.53 to 2.57 for the three managed subplots (Table 1). The results indicated that native rhizobial population could always be available in soils where yard long bean plants had been previously grown hence, the results could presumably be applied to some other host crops such as cowpeas, soybeans and others. Therefore, legume hosts by nature could manifest their significant effects on rhizobial population in soils. Nambiar *et al.* (1984) stated that most of agricultural soils being used to grow peanut crop contained rhizobial population of approximately 10^3 cells/g dry weight of soil and they further added that *Rhizobium* of peanut could be grouped as those found with cowpea crop. Similarly, Subba Rao (1995) claimed that rhizobial species of yard long bean crop plants could be grouped as those of cowpeas.

At day 30 after planting of both yard long bean cultivars, the results showed that amounts of rhizobial populations of those managed subplots were significantly greater than the control subplots. The differences were large and statistically significant (Table 2). Rhizobial population of the non-managed subplots was similar to that of the managed subplots (with the growth of white seeded yard long bean plants) with the mean values of Log MPN of 2.94 and 2.75, respectively. Whilst that of the managed subplots (with the growth of KKU 25 yard long bean cultivar), the results revealed that rhizobial population was greater than the rest. The results suggested that KKU 25 yard long bean plants had the highest potential in promoting the growth of rhizobial

Table 1: Rhizobial population being determined at day 1 after added with *Rhizobium* taken from soil being used to grow yard long bean plants for two years as influenced by population management methods.

Managed methods	Log MPN (day 1)		Mean
	White seeded	KKU25	
Control (Nitrogen 70 ppm.)	0	0	0 b
Non-managed (with no plant)	2.57	2.57	2.57a
Managed using white seeded cultivar	2.50	2.57	2.53a
Managed using KKU 25 cultivar	2.57	2.57	2.57a
Mean	1.95	1.96	

F-test:
Main plots (A: cultivars) NS
Subplots (B: managed methods) **
AxB NS
C.V. % 3.39
Remarks: NS = Non significant
Letters indicate significant differences of DMRT at **P = 0.01

Table 2: Rhizobial population being determined at day 30 after added with *Rhizobium* taken from soil being used to grow yard long bean plants for two years as influenced by population management methods.

Managed methods	Log MPN (day 30)		Mean
	White seeded	KKU 25	
Control (Nitrogen 70 ppm.)	0	0	0 c
Non-managed (with no plant)	2.66	3.16	2.94 b
Managed using white seeded cultivar	2.91	2.59	2.75 b
Managed using KKU 25 cultivar	5.45	5.54	5.49 a
Mean	2.79	2.86	

F-test:
Main plots (A: cultivars) NS
Subplots (B: managed methods) **
AxB NS
C.V. % 28
Remarks: NS = Non significant
Letters indicate significant differences of DMRT at **P = 0.01

Table 3: Rhizobial population being determined at day 60 after added with *Rhizobium* taken from soil being used to grow yard long bean plants for two years as influenced by population management methods.

Managed methods	Log MPN (day 60)		Mean
	White seeded	KKU25	
Control (Nitrogen 70 ppm.)	0	0	0 b
Non-managed (no planting)	2.25	2.17	2.21 a
Managed using white seeded cultivar	2.33	2.27	2.30 a
Managed using KKU25 cultivar	2.99	2.91	2.95a
Mean	1.93	1.87	

F-test:
Main plots (A: cultivars) NS
Subplots (B: managed methods) **
AxB NS
C.V. % 28
Remarks: NS = Non significant
Letters indicate significant differences of DMRT at **P = 0.01

population compared with that of the white seeded yard long bean cultivar and also the non-managed subplots. The results also indicated that KKU 25 yard long bean plants could have performed some exudation processes of some certain amount of substances together with the adequate amount of O_2 that could favour most the growth of *Rhizobium* than that of the white seeded yard long bean plants. The finding confirms the work reported by Anderson and Ingram (1993). It may be

Table 4: Nodule numbers of yard long bean cultivars being determined at day 60 after transplanting yard long bean seedlings as influenced by rhizobial population management methods.

Management methods	Nodule numbers (nodule/plant)		Mean
	White-seeded	KKU25	
Control (Nitrogen 70 ppm.)	0	0	0c
Non- managed (no plant)	4	2	3b
Managed using white-seeded cultivar.	7	4	5ab
Managed using KKU25 cultivar.	6	7	7a
Mean	5	3	
F-test:			
Main plots (A: cultivars)	NS		
Subplots (B: managed methods)	*		
AxB	NS		
C.V. %	39		

Remarks: NS = Non significant

Letters indicate significant differences of DMRT at *P = 0.05

Table 5: Total fresh weights of white seeded and KKU 25 yard long bean cultivars as influenced by rhizobial population management methods.

Management methods	Total fresh weights (mg./plant)		Mean
	White-seeded	KKU25	
Control (Nitrogen 70 ppm.)	778	822	800
Non- managed (no planting)	731	663	697
Managed using white-seeded cultivar.	795	889	842
Managed using KKU25 cultivar.	785	783	784
Mean	772	789	
F-test:			
Main plots (A: cultivars)	NS		
Subplots (B: managed methods)	NS		
AxB	NS		
C.V. %	18		

Remarks: NS = Non significant

possible that the two cultivars of yard long bean had produced different properties of substances from the exudation process as stated by Subba Rao (1995). The period of growth of 30 days after planting of yard long bean plants could presumably be the peak period for growth of rhizobial population and this period could have coincidentally happened during the rapid growth period of the yard long bean plants. However, it might be of better justification if rhizobial population had been determined at least one week interval after the sowing of yard long bean seeds to the test tubes, hence more details of rhizobial population could be intensively evaluated. Residual *Rhizobium* being retained from the soil where yard long bean plants have been used to grow from time to time had some significant value for the survival and availability of rhizobial population in soil environments. Luangchaisri (1995) confirmed this *Rhizobium* technology with his experiments on soybean crop plants.

At 60 days after planting of yard long bean seedlings, the results showed that the amount of rhizobial population slightly decreased with time for the non-managed subplots and the managed subplots of white seeded yard long bean cultivar but largely decreased with the managed subplots of KKU 25 yard long bean cultivar and became similar for all treated subplots (those added with native rhizobia) but significantly greater than that of the control subplots (Table 3). The results suggested that growth stages of the yard long bean crop plants could possibly have some effects on rhizobial population. For this work, it was found that the amount of rhizobial population reached peak period at day 30 after the planting of yard long bean seedlings. Nevertheless, it was observed that the non-managed subplots (no plants) retained a similar amount of rhizobial population as found with those being grown with yard long bean plants of both cultivars. The results indicated that at this stage of crop growth, the yard long bean plants those acting as the hosts of rhizobia may have declined in releasing their exudation substances, hence tremendously affected the growth of rhizobial population. Therefore, it might be inferred that rhizobia population could possibly reach its peak stage coincidentally within the period of the most rapid growth of the yard long bean plants when the crop plants were able to release most of their exudation substances.

Nodulation and growth of yard long bean plants: The results showed that the control management subplots (added with nitrogen) gave no nodule at all whilst the rest produced some certain amounts of nodulation numbers, e.g. the non-managed subplots (no yard long bean plants) gave the mean value of nodule numbers of 3 whilst those planted with yard long bean cultivars had produced mean nodule numbers of 5 and 7 for white seeded and KKU 25 cultivars, respectively. The differences were large and of statistical significance (Table 4). The results suggested that indigenous *Rhizobium* manifested some preferential symbiotic responses for their hosts, which KKU 25 yard long bean plants had performed the highest quality and greater than that of the white seeded cultivar. Therefore, selective quality of *Rhizobium* with respect to host could possibly be the element for biologists to consider when *Rhizobium* technology must be applied within the range of field grown legume crops.

Fresh weights of yard long bean cultivars: With fresh weights of the yard long bean plants, the results showed that fresh weights/plant of the crop plants of both white seeded and KKU 25 cultivars were similar in all methods of management subplots with the mean values of 800, 697, 842, and 784 mg/plant for control (added with 70 ppm N), non-managed, and managed with the growth of white seeded yard long bean plants, and managed with the growth of KKU 25 yard long bean plants, respectively (Table 5). There were no statistically significant differences among the management subplots. The results indicated that the growth of yard long bean plants of the control subplots had received adequate amount of nitrogen from the added amount of 70 ppm to the growth medium. However, the advantage in using native rhizobial population being added to the test tubes instead of the application of nitrogen could be of important value in growing this legume crop since no expenses on nitrogen chemical fertilizer had been required for the growth of the crop plants. The finding confirms the work reported by Rungrattanakasin *et al.* (2000). Therefore, this approach in evaluating rhizobia population, nodulation and growth of yard long bean plants could possibly be of valuable choice for growers of field grown leguminous crops in at large.

To sum up, an added amount of *Rhizobium* taken from soil where yard long bean had been continuously grown for two years to each of the managed test tubes at the initial day produced some large amounts of rhizobial population, whilst the subplot added with nitrogen (control subplots) produced none of rhizobial population and the differences were large and of statistical significance. All managed subplots added with native rhizobial population gave some similar amounts of rhizobial populations. At day 30 after planting of both cultivars of yard long bean seedlings, the treated management subplots (native *Rhizobium*) gave significantly greater amounts of rhizobial population over the control management subplots. The highest amount of rhizobial population was found with the management subplot being used for the growth of KKU 25 yard long bean plants while both white seeded managed subplots and the non-managed subplots ranked the second and none was observed with the control subplots. In spite of the amounts of rhizobial population at day 60 after planting decreased with time yet all native *Rhizobium* added managed subplots had significantly greater amounts of rhizobial population than that of the control subplots. Nodule numbers were highest with KKU 25 cultivar followed by white seeded cultivar and the lowest was found with the non-managed subplots but none of them was found with the control subplots. There were no statistical differences found among the four managed subplots since the control subplot received some amount of nitrogen from chemical fertilizer (70 ppm) whilst the managed subplots had some amounts of nitrogen from rhizobial fixation hence the amounts of yard long bean plant fresh weights were similar in all of the four management subplots.

Acknowledgments

The authors wish to thank Drs. Thepparit Tulaphitak and Banyong Toomsan for their invaluable advices and assistance and most of all, Dr. Amnuaysilpa Suksri for his kind assistance in reviewing the manuscript.

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