The Bioscan 10(3): 1401-1407, 2015 (Supplement on Agronomy)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

FIELD EFFICACY OF *BRADYRHIZOBIUM JAPONICUM* ISOLATES AND THEIR IMPACT ON CROP GROWTH, NUTRIENT CONTENT AND PRODUCTION OF SOYBEAN IN VERTISOL

F. C. AMULE*, A. K. RAWAT AND R. K. SAHU
Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur, INDIA
e-mail: amule.jnkvv@gmail.com

KEYWORDS
Soybean
Bradyrhizobium
Isolates
Efficacy
Growth
Nutrient Content
Vertisol)

Received on :
17.06.2015

Accepted on :
24.09.2015

***Corresponding author**

ABSTRACT
A field experiment was conducted at research station, JNKVV, Jabalpur (M.P.) during 2009-10 to test the efficacy of *B. japonicum* under field conditions and evaluates their impact on crop growth of soybean in vertisol. The said experiment was the field testing part of research which was conducted during 2002-2007. The efficacy of 36 isolates were tested and also compared with the USDA isolates, FUI (Fertilized uninoculated control) and UFUI (Unfertilized un inoculated control) in field condition. Results revealed that the maximum seed germination were observed with R_{11} isolate (48 and 65%) followed by R_{35} (46 and 63%) at 4^{th} and 5^{th} DAS. Similarly nodule number (66 $plant^{-1}$), nodule ODW (0.36 g $plant^{-1}$), nodule N (3.72%), grain yield (3350 kg ha^{-1}) and total N uptake (429 kg ha^{-1}) were recorded significantly superior over FUI. Only eight isolates could increase N content of plants at 45 days over FUI while phosphorus and potassium content were significantly higher only with R_{11} (3.78, 0.085, 0.59 % NPK respectively) fallowed by R_{35} (3.70, 0.082, 0.58% NPK respectively) over FUI. Rest of the isolates except BR_{11}, R_{12}, R_{16}, R_{17}, R_{21} and R_{35} were statistically at par to FUI toward their promise for seed yield.

INTRODUCTION

Leguminous crops are of great importance throughout the developing world, providing a valuable source of protein in the human diet as well as animal fodder and pollen for honey production. However, they are also key assets for sustainable agriculture, thanks to their root nodules. These nodules contain bacteria called rhizobia, which fix atmospheric nitrogen, making it available to the plant. One consequence of this is a reduction in the plant need for nitrogen fertilizer. Madhya Pradesh is known as soybean state of India and soybean is principal high protein grain legume of Madhya Pradesh is presently grown in about 1.68 million ha area in 2008-09 (Agricultural Statistics at a glance, 2010). Soybean was initially introduced in Madhya Pradesh in mid sixties, and due to absence of native effective rhizobial strains in most of the soils, it was inoculated with bradyrhizobial inoculants imported from USA. Later on, the indigenous biofertilizer production units came into existence in the state, which resulted in increased native Indian strains. Use of such inoculants leads to better establishment of native rhizobial population in the soil (Catroux *et al.*, 2001).

The various soil types and agroclimatic zones in state are expected to provide differential ecological niches for proliferation of rhizobial strains. The high soil temperature in surface soil during summer, varying moisture status during monsoon, different cropping patterns and diverse soil types may be a region of particularly high rhizobial diversity which lead to selection of particular strains from the existing soybean rhizobial biodiversity and this basic information remains poorly explored and under exploited. Initially only *Bradyrhizobiumjaponicum* was reported as slow growing soybean rhizobia but more different species of soybean rhizobia have been identified all over the world which falls under the category of *Rhizobium* and *Bradyrhizobium*. *B. japonium*, *B. liaoningense*, *B. elkani*, *Rhizobium S. fredii*, *S. xinjiangensis*, *Rhizobium tianshanense* (moderately fast growing). Most of the strains introduced in Madhya Pradesh belong to the slow growing species *Bradyrhizobium japonicum*. Soybean rhizobia and their biodiversities were broadly studied on a systematic investigation of microbial flora at different locations (Chen and Huang, 2002). Due to considerable agricultural and environmental significance of rhizobia, these legume symbionts have been extensively studied during the years back, the assessment of diversity within rhizobial natural populations in various regions of the world has received increased attention (Madrzak *et al.*, 1995; Zhang, *et al.*, 1999; Ando and Yokoyama, 1999; Chen *et al.*, 2000). Soybean rhizobial populations are common in the soil of soybean cropping areas. The fast growing and extra slow growing (ESG) soybean rhizobia were isolated from the nodules of soybean plants in several provinces and have been proposal as *Sinorhizobium fredii*, *S. xinjiangense* (Chen *et al.*, 1988) and *Bradyrhizobium liaoningense* sp. (Xu *et al.*, 1995) respectively. Soybean was an exotic plant introduced in Paraguay (South America). Commercial cropping of soybean expanded after 1970s with inoculation practice in just 15-20% of the cropping areas but root nodulation occurs in most sites where soybean grow (Chen *et al.,* 2000).The occupation of sero group in the nodules on the tap root of the inoculated plants was in the range of 77-100% suggestion that the *Bradyrhizobium japonicum* strain USDA 110 infected tap roots immediately after inoculation (Payakapong *et al.,* 2004). The occupation ratios on the lateral roots were 53-67%. The results showed that with the expansion of the root area of host plants, the occupation ratio varies by different group of rhizobia. (Yamakawa *et al.*, 2003). The diversity and symbiotic effectiveness of soybean rhizobial isolates from different locations. T hey characterized in relation to several parameters in vitro (colony morphology) and in vivo (nodulation). T hey found that out of 78 isolates 58 had slow growth rates and an acid reaction (Chen *et al.*, 2002). The best known and most exploited symbiotic N_2 fixing bacteria are those belonging to the family Rhizobiaceae and include the following genera: *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobim*, *Mesorhizobium* and *Allorhizobium* (Graham and Vance, 2000). The high temperature during summer, varying moisture status during monsoon, different cropping pattern and diverse soil types may be a reason for rhizobial biodiversity. Looking at the need to isolate effective soybean rhizobial strains from various geographical areas of Madhya Pradesh and to study their genetic diversity, surveys during 2002-2007. On the basis of laboratory and green house screenings only 40 isolates were short-listed to further test their efficiency under field conditions and to get more efficient soybean isolates. In this study, screening approaches were employed to select the most potential effective rhizobial isolates.

MATERIALS AND METHODS

A field experiment was carried out at Research station, Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur during kharif 2009 -10. Previously survey work were conducted in different phases during soybean cropping season from 2002-2007 jointly by JNKVV, Jabalpur centre and Indian Institute of Soil Science, Bhopal (M.P.) under All India Network Project on Soil Biodiversity and Biofertilizers. Soybean nodules were sampled form 159 sites of 119 blocks belonging to 40 districts. Initially these isolates were screened out under laboratory and glass house conditions for their nitrogen fixing capacity, nodulation and plant biomass to shortlist their number for field screening. Out of 990 isolates, best 36 were evaluated under field conditions and their performance was compared with proven exotic strains (USDA, Parbhani, Pantnagar, Palampur, and Ludhiana strains) and indigenous JNKVV strains used as a check. Overall the isolates performance was compared with fertilized uninoculated control. On the basis of laboratory and green house screenings at Jabalpur and Bhopal 40 isolates were short-listed to further test their efficiency under field conditions. Therefore, in the present investigation it is proposed to screen the symbiotic performance of 36 soybean rhizobial isolates under field conditions against USDA and JNKVV check in order to further shortlist their number and to get more efficient soybean isolates. The experiment was laid out in augmented RBD without replication.

The soil of experimental site having 0.22 dSm^{-1}EC, 6.9 g kg^{-1} soil organic carbon (OC), available nutrients 140 mg N, 12 mg P and 153 mg K kg^{-1} soil. All the isolates were provided with recommended dose of NPK (20:80:20 kg ha^{-1}) along with fertilized uninoculated control where the conditions were same except seed inoculation. The sources of NPK were applied by urea, single super phosphate and muriate of potash. The Soybean variety JS - 9752 was sown in last week of June 2009 and harvested at 13 October 2009. The short listed isolates were purified by streaking on YEMA plates and transferring the single colony on the YEMA slants. Liquid formulations of all the isolates were prepared at Indian Institute of Soil Science, Bhopal using the YEMA liquid medium. Seed treated of particular isolates, total number of 40 isolates (34 isolates from M.P.and6 USDA strains) and one JNKVV check was taken for seed treatment. They were sown separately. All the packages of practices were followed as per recommendations to grow the crop.

From the next day of sowing critical observations were made on early seed germination and plot wise visual rating (%) was done at 4th and 5th day of sowing. The five plants per plot were randomly tagged for plant height at 6th, 13th and 20th day of sowing while nodulation were done at 45 days after sowing by uprooting 5 plants $plot^{-1}$. The rhizosphere soil was washed in the running water. After proper washing per plant nodules were counted. After counting, the nodules were detached from the roots and were kept in small paper bags. Then the nodules were oven dried in hot air oven at 65 ^{0}C for 3-4 days (till constant weight) to record their oven dried weight. Dried plant samples were grinded by electric grinder for further analytical work. Leaf chlorophyll (a, b and total) content was estimated by acetone extraction method (Arnon, 1949) in fresh plant leaves at 15th and 21st DAS. The soil EC was analyzed using 1:2.5 (soil: water) suspension, organic carbon by Walkley and Black (1934).Plant samples were analyzed for N by micro-Kjeldhal method (Jackson, 1973). Phosphorus content was determined by vanadomolybdo phosphoric acid yellow colour method (Jackson, 1973) and K content by using flame photometer as described by Jackson, 1973.

RESULTS AND DISCUSSION

Plants absorb nutrients and moisture from soil through their root system. When plants grow in soil they also release a wide range of organic compounds. These compounds and sloughed-off root tissues attract millions of microbes around the root zone of plants and create a region of vigorous microbial activity. These microbes interact with plant's roots and the nutrients in soil. Among these rhizobacteria, *Rhizobium* is one of them. Although rhizobia that infect and fix atmospheric nitrogen in legumes are the most well studied and longest commercially exploited as the plant growth promoting rhizobacteria (Fred *et al.,* 1932). The nitrogen fixing compatibility of rhizobia varies greatly; therefore, selection of the best strains must take rhizobia-host compatibility into account. Therefore, a large number of isolations were made from various geographical areas of Madhya Pradesh (Rawat *et al.*, 2007) and have been

Table 1: Field efficiency evaluation of *B.japonicum* isolates on soybean germination (4thand 5thDAS),nodulation, ODW of nodules, nodule N content at 45 DAS, grain yield and total N uptake by crop

Isolate No.	Germination (%) 4 th DAS	5 th DAS	Nodule no. plant^{-1}	O.D.W (g plant^{-1})	Nodule N(%)	Grain yield (kg ha^{-1})	Total N uptake (kg ha^{-1})
R1	11	20	29	0.16	3.05	2400	269
R2	18	36	39	0.25	3.44	2700	314
R3	14	32	34	0.20	3.36	2550	286
R4	21	40	49	0.28	3.64	2700	352
R5	8	16	22	0.12	2.58	2250	240
R6	9	18	25	0.12	2.66	2250	242
R7	13	28	33	0.20	3.33	2550	286
R8	18	38	41	0.26	3.50	2700	315
R9	10	18	27	0.13	2.77	2250	260
R10	13	28	32	0.19	3.28	2475	283
R11	48	65	66	0.36	3.72	3350	429
R12	36	52	56	0.33	3.70	2850	385
R13	8	15	18	0.10	2.09	2100	203
R14	18	37	40	0.26	3.44	2700	315
R15	20	38	47	0.27	3.53	2700	352
R16	26	52	53	0.32	3.67	2850	369
R17	25	48	53	0.32	3.67	2850	367
R18	12	27	31	0.18	3.25	2400	278
R19	13	28	32	0.19	3.30	2475	285
R20	12	25	31	0.18	3.22	2400	273
R21	24	48	51	0.31	3.66	2850	364
R22	22	42	49	0.28	3.64	2700	353
R23	14	30	34	0.20	3.30	2550	284
R24	8	15	19	0.11	2.72	2175	252
R25	19	38	44	0.26	3.53	2700	342
R26	15	33	35	0.22	3.36	2550	290
R27	17	36	38	0.25	3.44	2625	303
R28	19	38	42	0.26	3.50	2700	327
R29	9	17	22	0.12	2.75	2250	257
R30	16	34	36	0.22	3.42	2625	302
R31	11	20	28	0.16	3.05	2325	268
R32	10	19	27	0.13	2.88	2250	268
R33	12	27	31	0.18	3.14	2400	273
R34	23	47	50	0.31	3.64	2775	353
R35	46	63	62	0.36	3.70	3150	417
R36	12	27	31	0.19	3.14	2475	270
R37	10	19	28	0.15	2.86	2250	266
R38	16	34	36	0.23	3.39	2625	293
R39	12	25	31	0.18	3.08	2400	269
R40	14	32	34	0.21	3.36	2550	287
JNKVV Check	12	20	30	0.15	2.30	2500	331
FUI control	8	15	18	0.10	2.09	2100	202
SEd	3.16	8.54	4.55	0.03	0.58	322.68	70.16
LSD (p=0.05)	6.89	18.60	9.91	0.07	1.26	703.13	152.88
CD (%)	16.22	23.80	10.46	13.34	14.14	10.07	17.99

screened under field conditions to answer the following hypothesis: first area specific inoculants are needed looking to adoptability of nodule forming bacteria to a particular agroclimatic zone? Second is there any relationship between nodules number, nodular biomass and seed yield and nitrogen uptake?

The data related to the early vigor of crop growth (4thand 5th DAS) nodulation and ODW of nodules nodule N content at 45 DAS and grain yield and total N uptake by soybean crop (Table 1 and Fig. 1). The maximum germination was recorded at 4th and 5thDAS which may be due to better production of plant growth regulators (Bashan *et al.*, 1990) by isolates R_{11} (48 and 65%) followed by R_{35} (46 and 63%) respectively. Similar findings were reported by Kolhapure *et at.*, (2003) and Kamdi *et al.*, (2014). Early germination and seedling vigor can complete successfully influencing stand establishment and ultimately grain yield (Biswas *et al.*, 2000).

Nodulation was better with all the isolates over FUI or native ones. Most of the nodules gave significantly better nodule occupancy per plant over FUI. Highest number of nodules was found where seed inoculated by isolate R_{11} (66 nodules plant^{-1}) followed by R_{35}, R_{12}, R_{16}, R_{17} and R_{21} (62, 56, 53 and 53 nodules plant^{-1}) respectively. These isolates reflected the same trend towards oven dried nodular biomass similar trend was found ThiThi *et al.* (2013) enhanced soybean biomass by co-inoculation of *Bradyrhizobium japonicum* and plant growth

Table 2: Field efficiency evaluation of *B.japonicum* isolates on NPK content (%) in soybean plant at 45 DAS

Isolate No.	N content	P content	K content
R1	2.88	0.056	0.38
R2	3.16	0.064	0.48
R3	3.02	0.059	0.46
R4	3.39	0.065	0.50
R5	2.69	0.044	0.35
R6	2.74	0.045	0.35
R7	3.00	0.059	0.46
R8	3.28	0.065	0.49
R9	2.77	0.051	0.36
R10	2.94	0.058	0.45
R11	3.78	0.085	0.59
R12	3.70	0.075	0.57
R13	2.10	0.044	0.30
R14	3.28	0.065	0.48
R15	3.33	0.065	0.50
R16	3.70	0.072	0.57
R17	3.64	0.071	0.55
R18	2.93	0.058	0.45
R19	3.00	0.059	0.46
R20	2.91	0.058	0.45
R21	3.58	0.069	0.54
R22	3.50	0.066	0.54
R23	2.94	0.059	0.46
R24	2.77	0.047	0.35
R25	3.33	0.065	0.49
R26	3.08	0.061	0.46
R27	3.16	0.064	0.48
R28	3.30	0.065	0.49
R29	2.77	0.047	0.35
R30	3.14	0.063	0.48
R31	2.83	0.056	0.38
R32	2.80	0.055	0.36
R33	2.88	0.058	0.44
R34	3.53	0.068	0.54
R35	3.70	0.082	0.58
R36	2.88	0.058	0.39
R37	2.80	0.052	0.36
R38	3.11	0.062	0.47
R39	2.88	0.057	0.39
R40	3.02	0.060	0.46
JNKVV Check	2.73	0.058	0.49
FUI control	2.10	0.040	0.30
SEd(C×S)	0.57	0.017	0.10
LSD (p=0.05)	1.25	0.037	0.21
CV (%)	15.35	22.49	16.67

promoting rhizobacteria and its effects on microbial community structures.

N content in nodules is considered one of the important parameters for determining the nitrogen fixing ability of a plant. Data indicated that out of 40 strains 21 were able to give significantly better nodule nitrogen under FUI (Table 1) supported by Ramaswami and Oblisami (1986). The increment in nitrogen content as well as number of nodules per plant due to the application of inoculation in combination with nitrogen fertilizer were also reported by Rashid *et al.*, (1999). At 45 days after sowing, nitrogen, phosphorus and potassium contents in plant samples were determined (Table 2). Only eight isolates (R_4, R_{11}, R_{12}, R_{16}, R_{17}, R_{21}, R_{34} and R_{35}) increased the nitrogen content in plants significantly over control (FUI) while rest of the isolates were statistically at par to FUI while strains R_{11} and R_{35} only could increase the phosphorus content significantly over FUI.

The maximum soybean seed yield (Table 1) was recorded with R_{11} (3350 kg ha^{-1}) followed by R_{35} (3150 kg ha^{-1}) isolate while minimum grain yield was recorded with FUI (2100 kg ha^{-1}). It was also seen that only six isolates (R_{11}, R_{12}, R_{16}, R_{17}, R_{21} and R_{35}) yielded significantly better seed yield over FUI while rest of the isolates were statistically at par to it. The increase in soybean seed yield due to inoculation was also reported by (Prashad *et al.*, 2014). It is very apparent that the USDA strains or Parbhani, Pantnager, Palampur and Ludhiana strains were not found to be compatible with the native isolates of Madhya Pradesh soils. Nodulation, shoot biomass, and grain yield can be improved by seed inoculation with Bradyrhizobium (Fatima *et al.*, 2007; Hayat *et al.*, 2008). Positive effect of *Bradyrhizobium japonicum* on growth, yield, and nodules of soybean after inoculation have also been reported (Egamberdiyeva *et al.*, 2004). Improving nitrogen fixation and growth of crop can be achieved by co-inoculation of plant growth promoting rhizobacteria such as *Pseudomonas putida* with symbiotic rhizobial bacteria (Tilak *et al.*, 2006).

With regard to total nitrogen uptake (Table 1) by soybean,

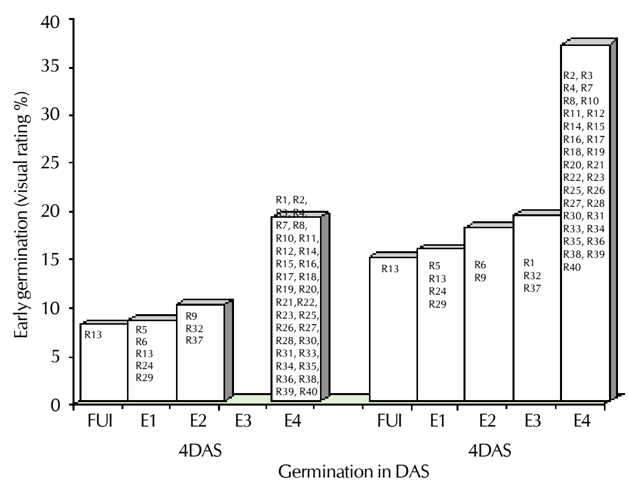

Figure 1: Early germination (visual rating %) at 4th and 5th DAS

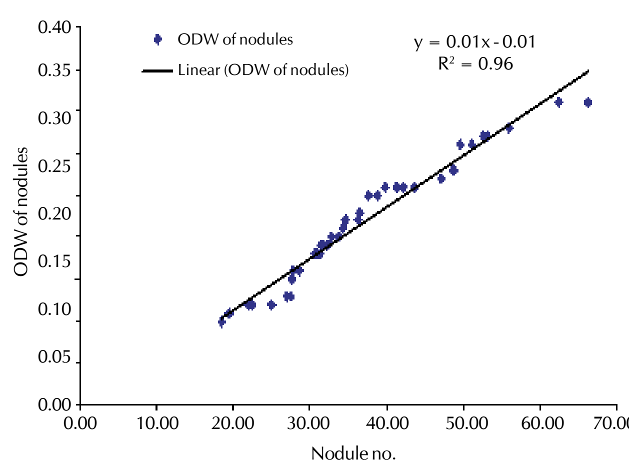

Figure 2: Correlation between no. of nodules and ODW of nodules.

Table 3: Effect of seed inoculation with *B.japonicum* isolates on chlorophyll content (mg/g of fresh leaves) at 15 and 21 DAS.

DAS	15 DAS			21 DAS		
Isolate No.	Chlorophyll 'a'	Chlorophyll 'b'	Total 'a+'b'	Chlorophyll 'a'	Chlorophyll 'b'	Total 'a' +'b'
R1	1.04	0.60	1.65	2.16	1.41	3.57
R2	1.09	0.75	1.84	2.22	1.59	3.81
R3	1.07	0.70	1.78	2.21	1.54	3.76
R4	1.10	0.83	1.93	2.24	1.62	3.85
R5	1.01	0.44	1.44	1.98	1.31	3.29
R6	1.01	0.48	1.48	1.99	1.31	3.31
R7	1.07	0.69	1.76	2.21	1.52	3.73
R8	1.09	0.76	1.85	2.23	1.59	3.82
R9	1.03	0.53	1.55	2.07	1.34	3.41
R10	1.07	0.68	1.75	2.21	1.47	3.68
R11	1.14	0.97	2.11	2.38	1.79	4.17
R12	1.13	0.94	2.07	2.26	1.70	3.97
R13	0.99	0.41	1.40	1.96	1.29	3.25
R14	1.09	0.76	1.85	2.23	1.59	3.82
R15	1.10	0.80	1.90	2.24	1.61	3.85
R16	1.12	0.89	2.01	2.26	1.68	3.94
R17	1.12	0.88	2.01	2.25	1.67	3.92
R18	1.07	0.67	1.74	2.19	1.46	3.65
R19	1.07	0.68	1.76	2.21	1.49	3.71
R20	1.05	0.65	1.71	2.19	1.45	3.63
R21	1.12	0.87	1.99	2.24	1.65	3.89
R22	1.10	0.85	1.95	2.24	1.64	3.88
R23	1.07	0.68	1.75	2.21	1.47	3.68
R24	1.01	0.48	1.48	2.02	1.33	3.35
R25	1.10	0.79	1.88	2.23	1.61	3.84
R26	1.08	0.73	1.81	2.22	1.55	3.77
R27	1.09	0.75	1.83	2.22	1.58	3.81
R28	1.10	0.79	1.88	2.23	1.61	3.84
R29	1.01	0.51	1.52	2.06	1.34	3.40
R30	1.09	0.75	1.83	2.22	1.56	3.79
R31	1.04	0.60	1.64	2.09	1.39	3.48
R32	1.03	0.58	1.61	2.09	1.36	3.44
R33	1.05	0.64	1.69	2.18	1.43	3.62
R34	1.12	0.86	1.98	2.24	1.64	3.88
R35	1.13	0.94	2.07	2.27	1.72	3.98
R36	1.05	0.63	1.68	2.18	1.43	3.61
R37	1.03	0.56	1.59	2.08	1.35	3.43
R38	1.09	0.74	1.82	2.22	1.55	3.77
R39	1.05	0.63	1.67	2.18	1.42	3.60
R40	1.08	0.72	1.80	2.22	1.54	3.76
JNKVV Check	1.06	0.68	1.74	2.17	1.46	3.63
FUI control	0.99	0.41	1.40	1.96	1.29	3.25
SEd(C×S)	0.12	0.15	1.28	0.28	0.21	0.19
LSD (p=0.05)	0.26	0.32	2.79	0.60	0.46	0.41
CV (%)	8.87	16.38	56.93	10.18	11.22	4.14

similar isolates were responsible to reflect significantly better uptake over FUI which increased the seed yield. Maximum total nitrogen uptake by soybean was recorded with R_{11} followed by R_{35} and R_{12} (which belong to Nimar Valley agro-climatic zone of Madhya Pradesh). Several other worker have repeated the significant contribution of *Rhizobium* (Bisht and Chandel, 1996, Rao *et al.*, 1997,Kumrawat *et al.*,1997, Jain *et al.*, 1999, Raut and Fohire, 1991, LI *et al.*, 1993 and Dobert and Blevins, 1993). The increase in nitrogen uptake by plant due to inoculation and nitrogen fertilizer application was also reported by Pasricha and Kar (1983), Moawad*et al.*, (1988), Basu and Bandyopadhyay (1990) and Rashid *et al.*, (1999).However, chlorophyll 'a' in soybean leaves at 15 and 21 DAS (Table 3) was not affected significantly but same isolates significantly favored chlorophyll 'b' content at 15 DAS. Shaobing*et al.*, 2002 found a significant increase in single-leaf net photosynthetic rate by rhizobial inoculation. The increase in grain yield was due to an increase in total biomass production. They also concluded that certain strains of rhizobia can promote different growth and yield through mechanisms that improve single-leaf photosynthetic rate. Gharib*et al.*, 2007also foundthat the chlorophyll content significantly increased in the leaves of *Phaseolus vulgaris* due to *Rhizobium* inoculation.

The correlation between number of nodules and ODW of nodules (Fig. 2) revealed that the value of $R^2$is found to be 0.96 which suggests that 96% variability in ODW of nodules by nodulation can be explained due to number of nodules while remaining 4% variability may be due to other factors.

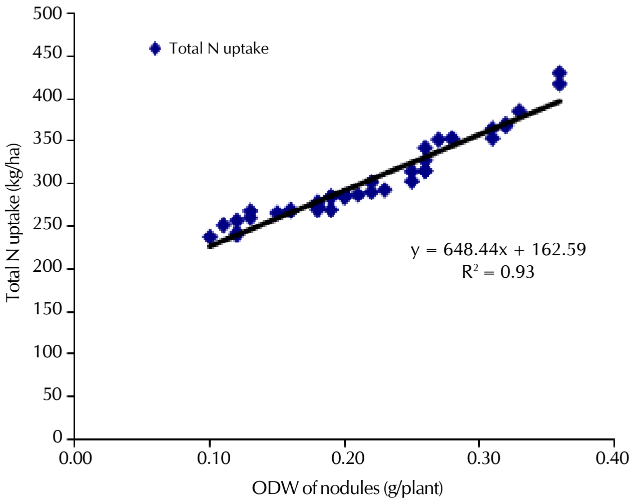

Figure 3: Correlation between ODW of nodules and total N uptake by crop

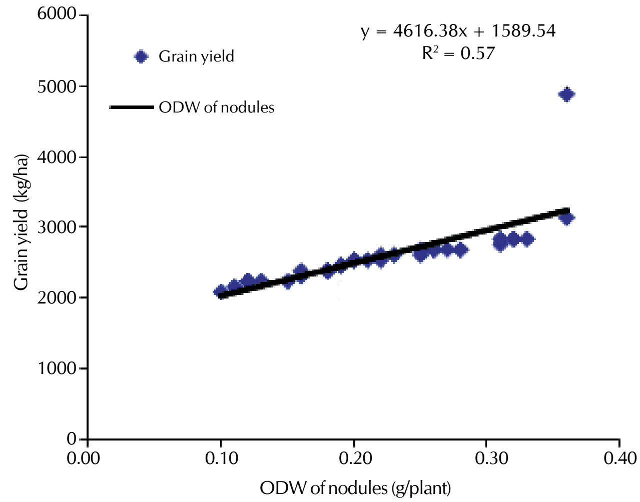

Figure 4: Correlation between ODW of nodules and grain yield of soybean

Nodule dry weight could have a positive correlation with nodule number. This means that if inoculation had increase nodule number, it would have resulted in increased in nodule dry weight. Similar observation was reported by Sarkodie-Addo *et al.* (2006). Similarly correlation was recorded between oven dried weight of nodules and total nitrogen uptake by crop (Fig. 3). The value of $R^2$is found to be 0.93 which suggests that 93% variability in total nitrogen uptake by crop can be explained due to oven dried weight of nodules while remaining 7% variability may be due to other factors. While the correlation value of R^2-is found to be 0.57 between oven dried weight of nodules and grain yield by crop (Fig. 4). This suggests that 57% variability in grain yield by crop can be explained due to oven dried weight of nodules while remaining 43% variability may be due to other factors. Rosario *et al.* (1997) reported that the proportion of the nitrogen in the plant contributed by fixation was highly correlated with all nodulation and BNF traits. It is also of interest to note that the amount of nitrogen fixed was found to be correlated with the entire selected parameters. This means that any increase in nodulation, nitrogen uptake as well as any improvement in one or more of the BNF-associated agronomic variables should be accompanied by a corresponding increment in the amount of N_2 fixed. This is also an indication that any of the above characters could appropriately be used as an index for biological nitrogen fixation potential. Tejera *et al.* (2005) also confirmed a significant positive correlation between nodule number and shoot dry weight and nodule number and N % confirming the importance of symbiosis in N accumulation in legumes.

REFERENCES

Ando, S. and Yokoyama, T. 1999. Phylogenetic analyses of Bradyrhizobium strains nodulating soybean (Glycine max) in Thailand with reference to the USDA strains of Bradyrhizobium. *Can. J. Microbiol.* **45:** 639-645.

Arnon, D. I. 1949. Copper enzyme polyphenoloxides in isolated chloroplast in Beta vulgaris. *Plant Physiology*. **24:** 1-15.

Agriculture Statistics at a Glance 2010. Annual report Directorate of Economics and Statistics, Department of Agriculture and Cooperation and Farmer's Welfare,Ministry of Agriculture and farmers Welfare, India. *eands. dacnet.nic.in/Advance_Estimate-2010.htm.*

Bashan, Y., Harrison, S. K. and Whitmoyer, R. E. 1990. Enhanced growth of wheat and soybean plant inoculated with Azopirillumbrasilense is not necessary due to general enhancement of mineral uptake. *Applied environmental microbiology*. **56:** 769-775.

Basu, T. K. and S. Bandyopadhyay 1990. Effect of *rhizobium* inoculation and nitrogen application on some yield attributes of mung. *Environ. Ecol.* **8:** 650-784.

Bisht, J. K. and Chandel, A. S. 1996. Effect of integrated nutrient management on yield and quality of soybean. *Annual Agricultural Research*. **17(4):** 360-365.

Biswas, J. C., Ladha, J. K. and Dazzo, F. B. 2000. Rhizobial inoculation improves nutrient uptake and growth of low land rice. *Soil Sci. Soc. Am. J.* p. 64.

Black, C. A. (ed.) 1965. Method of Soil Analysis, Part 2,Chemical and Microbiological Properties, American Society of Agronomy, Inc, Publisher, Madison, Wisconsin USA. p.1578.

Chen, L., Figueredo, S. A., Villani, H., Michajluk, J., Hungria, M. and Chen, L. S. 2002. Diversity and symbiotic effectiveness of rhizobia isolated from field grown soybean nodules in Paraguay. *Biol., Fert. Soils*. **35:** 448-457.

Chen, L. S., Figueredo, A., Pedrosa, F. O. and Hungria, M. 2000. Genetic characterization of soybean rhizobia in pagaguay. *Appl. Environ. Microbiol.* **66:** 5099-5103.

Chen, W. X., Yan, G. H. and Li, J. L. 1988. Numerical taxonomic study of fast growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium*gen. *Nov. Int. J. Syst. Bacteriol.* **38:** 392-397.

Catroux, G., Hartmann, A. and Revellin, C. 2001. Trends in rhizobial inoculants production and use. *Plant and soil.* **230:** 21-30.

Dobert, R. C. and Blevins, D. G. 1993. Effect of seed size and plant growth on nodulation and nodule development in lima beans (*Phaseoluslunatus L.*). *Plant and Soil.* **148(1):** 11-19.

Egamberdiyeva, D., Qarshieva, D. and Davranov, K. 2004. Growth and yield of soybean varieties inoculated with Bradyrhizobium spp. in N-deficient calcareous soils. *Biol Fert Soils.* **40:**144-146.

Fatima, Z., Zia, M. and Chaudhary, M. F. 2007. Interactive effect of Rhizobium strains and P on soybean yield, nitrogen fixation and soil fertility. *Pak J. Bot.* **39(1):** 255-264.

Fred, E. B., Baldwin I. L., and McCoy E., 1932. Root Nodule Bacteria and Leguminous Plants. University of Wisconsin Studies in Science, number 5. University of Wisconsin Press, Madison.

Gharib, A. A., Shahen, M. M. and Raqab, A. A. 2007. Influence of *Rhizobium* inoculation combined with *Azotobacterchrococcum* and *Bacillus megaterium* VAR *Phosphaticum* on growth, nodulation, yield and quality of two snap been (*Phasealus vulgaris L.*) cultivars. *Soil, Water and Environment research institute, ARS, Giza, Egypt. (4th conference on Recent technologies in Agriculture, 2009.)* pp. 650-661.

Graham, P. H. and Vance, C. P. 2000. Nitrogen fixation in perspective: An over view of research and extension needs. *Field crops research.* **65:** 93-106.

Hayat, R., Ali, S., Siddique, M. T. and Chatha, T. H. 2008. Biological nitrogen fixation of summer legumes and their residual effects on subsequent rainfed wheat yield. *Pak. J. Bot.* **40(2):** 711-722

Jackson, M. L. 1973. Soil Chemical Analysis. *Prentice hall of India Pvt. Ltd.*, New Delhi.

Jain, P. C., Kushwah, Dhakad, P. S., Khan, H. and Trivedi, S. K. 1999. Responce of chickpea (*Cicer arietinum*) to phosphorus and biofertilizers. *Legume Res.* **22(4):** 241-244.

Kamdi, T. S., Sonkamble, Priti. And Joshi, S. 2014. Effect of organic manure and biofertilizers on seed quality of groundnut (*Arachishupogaea L.*). *The Bioscan.* **9(3):** 1011-1013.

Kolhapure, D. J., Memane, S.A. Rasal, P.H. and Pawar K.B. 2003. Verietal response of soybean to different strains of *Bradyrhizobium japonicum*. *J. Maharashtra Agril. University*. **28(2):** 161-163.

Kumarawat, B. S., Dighe, T. M., Sharma, R. A. and Katiki, G. V. 1997. Response of soybean to biofertilizer in black clay. *Soils.Crop Res. Hissar.* **14(2):** 209-214.

Li, X. M., Dou, X. T. and Li, X. M. 1993. The evaluation of nodulation and nitrogen fixation abilities in soybean cultivars grown in the field. *Soybean Science*. **12(4):** 308-312.

Moaward, H., El-Din, S. M. S. B. and Khalafullah, M. A. 1988. Field performance of rhizobial inoculants for some important legumes in Egypt, In: *Nitrogen fixation by legumes in Mediterraneen Agriculture, D.P. Beck and L.A. Moteron (eds)* Aleppo, Syria: ICARDA. pp. 235-44.

Madrzak, C. J., Golinska, B., Kroliczak, J., Pudelko, K., La zewska, D., Lampka, B. and Sadowky, M. 1995. Diversity among field populations of *Bradyrhizobium japonicum* in Poland. *Appl. Environ. Microbiol*. **61:** 1194-1200.

Pasricha, P. C. and Kar 1983. Improving symbiotic nitrogen fixation and productivity in black gram (*Vigna mungo* L.). *Current Sci.* **52:** 1143-1145.

Payakapong, W., Tittabutr, P., Teaumroong N.and Boonkerd, N., 2004. Soybean cultivars affect nodulation competition of Bradyrhizobium japonicum strains. *World J. Microbiol. Biotechnol.* **20:** 311-315.

Prashad, S. K., Singh, M. K. and Singh, J. 2014. Response of *Rhizobium* inoculation and phosphorus levels on mungbean (*Vignaradiata*) under guava-based agri-horti system. *The Bioscan.* **9(2):** 557-560.

Ramaswami, P. P. O. and Obliswami, G. 1986. Influence of nitrogen, Phosphorus and inoculation on nitrogen fixation and yield in black gram (*Vignamungo*L.) *National Seminar on Microbial Ecology*. p. 23-24.

Rao, L. J., Rao, J. P. Reddy, D. P. and Reddy, B. B. 1997. Performance at soybean varieties of different spacing and rhizobium inoculation under rainfed condition. *J. Res. APAU.* **19(2):** 75-76.

Rashid, A., Musa, M. Aadal, N. K. Yaqub, M. and Chaudhry, G. A. 1999. Response of groundnut to *Bradyrhizobium* and a Diazotroph bacterial inoculation under different levels of nitrogen. *Pakistan J. Soil*. **16:** 89-98.

Raut, R. S. and Fohire, O. D. 1991. Phosphorus response in chickpea with *Rhizobium* inoculation. *Legume Res*. **14(2):** 78-82.

Rosario, E. E. D., Hautea, A., Hautea and Lantican, R. M. 1997. Genetic effects of quantitative traits on nodulation and nitrogen fixation in Mungbean (Vigna radiate (L.) Wilczek). Phillipp. *J. Crop Sci*. **22(2):** 74-82.

Sarkodie-Addo, J., Adu-Dapaah, H. K., Ewusi-Mensah, N. and Asare, E. 2006. Evaluation Of medium-maturing soybean (*Glycine max* (L) Merrill) lines for their nitrogen fixation potentials. *J. Science and technology.* **20(2)**: 34-39.

Shaobing, P., Jatish, C., Biswas, J., Ladha, K., Prasad,G. and Yizhuchen. 2002. Influence of Rhizobial inoculation on photosynthesis and grain yield of Rice. *Agronomy J.* **94:** 925-929.

Tejera, N.A., Campos, R., Sanjuan, J. and Lluch, C. 2005. Effect of sodium chloride on growth, nutrient accumulation, and nitrogen fixation of common bean plants in symbiosis with isogenic strains. *J.Plant Nutrition.* **28:**107-1921.

ThiThi, Aung., Bancha, Buranabanyat., Pongdet, Piromyou., Aphakorn, Longtonglang., Panlada, Tittabutr., Nantakorn, Boonkerd and Neung and Teaumroong 2013. Enhanced soybean biomass by co-inoculation of *Bradyrhizobium japonicum*and plant growth promoting rhizobacteria and its effects on microbial community structures, *Afric. J. Microbiol. Res.* **7(29):** 3858-3873.

Tilak KVBR, Rauganayaki, N. and Manoharachari, C. 2006. Synergistic effects of plant-growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeon pea (Cajanuscajan). *Eur. J. Soil Sci.* **57**: 67-71

Vance, C. P. 2000. Legume symbiotic nitrogen fixation: agronomic aspects, in H.P. Spaink, A. Kondorosi, and P.J.J. Hooykaas (eds.), The *Rhizobiaceae*Dordrecht, the Netherlands: Kluwer Academic Publishers. pp. 509-530.

Verrnon, L. P. 1960. Spectrometric determination of chlorophylls and pheophytins in plant extracts. *Anal. Chem*. **32:** 1144-1150.

Walkley, A. and Black, C. A. 1934. An examination of different method for determination of soil organic matter and proposel modification of chromic acid titration method. *Soil Sci.* **37:** 29-38.

Xu, L. M., Ge, C. and Cui, Z. 1995. *Bradyrhizobium liaoningense sp.* Nov. isolated from the root nodules of soybean. *Intl. J. Syst. Bacteriol.* **45:** 706-711.

Yamakawa, T., Hussin, A. and Ishizaka, J. 2003. Soybean preference for *Bradyrhizobiumjaponicum* for nodulation: occupation of serogroup USDA 110 in nodules of soybean plants harboring various Rj-genes grown in a field. *Soil, Sci. and Pl. Nutri*. **49:** 835-841.

Zhang, X., G. Nick, S. Kaijalainen, Z. Terefework, L. Paulin, S.W. Tighe, P. H. Grahma and Lindstrom, K. 1999. Phylogeny and diversity of Bradyrhizobium strains isolated from the root nodules of peanut (Arachis hypogaea) in Sichuan, China *Syst. Appl. Microbiol*. **22:** 378-386.