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Influence of different *Bradyrhizobium japonicum* isolates on growth parameters and yield of soybean.

KEYWORDS

Bradyrhzobium japonicum, growth parameters, soybean.

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ABSTRACT *The field experiment was conducted during 2008 to 2010 to see the effect of different Bradyrhizobium japonicum isolates on root, shoot, nodule number, N content, N uptake and yield of soybean at departmental field of Department of Plant Pathology, Dr.PDKV, Akola (M.S.). The results revealed that maximum shoot length was recorded in isolates BJ-28 after 30 days where as B29, BJ5, BJ4 showed maximum shoot length after 60 days. Maximum root length was recorded in BJ25 followed by BJ2 and BJ26 similarly, maximum number of nodules per plant were recorded in BJ9 and least in BJ13 at 30 DAS. Isolates BJ21 (107.83) and BJ23 showed maximum nodules (100.83) per plant at 60 DAS. Highest yield i.e. 10.31 q/ha, 10.13 q/ha and 10.05 q/ha was obtained due to inoculation of BJ-28,BJ2 and BJ1 respectively. Maximum N content was recorded in BJ1 and BJ2 i.e.0.82% during first year and BJ21, BJ4 and BJ3 during 2009-10. Where as in all BJ4 and BJ1 showed maximum nitrogen content as compared to other isolates. Maximum nitrogen uptake was observed in BJ4, BJ1,BJ3 and BJ21. BJ32 showed minimum uptake as compared to other isolates.*

Introduction

Soybean the "Golden bean" (*Glycine max* (L.)Merrill) is an important oil seed as well as pulse crop and played a significant contribution to yellow revolution in India and as a food plant it forms an important part of routine diet of the people in India. According to survey conducted by Soybean Processors Association of India (SOPA) during 2007, Maharashtra ranks 2nd both in area and production *i.e.* 26.28 million ha and 1200 mt respectively.

Among the five leguminous crops grown in Maharashtra *viz.* pigeonpea, chickpea, soybean, black gram and ground nut. Soybean is most the important crop of the Vidarbha region. (Meshram et.al., 2004). In spite of wide area coverage the productivity of this legume is very low compared to national average. Poor productivity in this region is mainly attributed to poor availability of native rhizobia in soil due to diverse climatic conditions. It is essential that soybean seeds should be inoculated with suitable efficient strains of *Brayrhizobium japonicum*. Soybean plant can fix up to 200 kg N/ha per year, reducing the need for expensive and environmentally harmful nitrogen fertilizers (Javaid and Mahmood,2010). The leguminous plants were known nitrogen accumulators.The increasing cost of fertilizers and their impact on the environment have forced people to look for other possible source of plant nutrient.

The nitrogen demand of soybean can be supplied via biological nitrogen fixation through the inoculation with selected Bradyrhizobium japonicum. Biological nitrogen fixation can reduced the need for N fertilizers .Symbiotic nitrogen fixation (SNF) resulting from mutual beneficial interaction between soybean and soil nodule bacteria provides a significant role of N fertilization.

Symbiotic nitrogen fixation in agriculture can be attributed mainly to legumes, the plants in the Leguminosae contains more than 200 genera and 20000 species, which range from small plants such as clover to the large plants, *Acasia* species. Approximately 90% of them fix nitrogen from the atmosphere with *Rhizobiaceae,* either with *Azorhizobium, Bradyrhizobium, and Rhizobium* or with *Sinorhizbium* in root nodules. *Rhizobium* is relatively more effective and widely used in association with legumes which fixes atmospheric nitrogen. Selection and choice of appropriate strains of rhizobia for biofertilizer production is essential

Microbes are an integral part of natural fertility cycles and play crucial role in the mineralization of nutrients. Thereby making nutrient in organic matter available to plants for growth. Soil is the best medium for microorganisms to grow and flourish containing effective and ineffective strains. Biological nitrogen fixation is the process that changes inert N_2 to biologically useful NH_3. This process is mediated in nature only by bacteria.

Chemical fertilizers were used intensively around the world to increase crop yield. However, they started displaying their harmful effects to the environment. Therefore, the biofertilizers are good option to reduce the use of commercial N fertilizers and to keep the environment unpolluted which lead to maintain the good soil health.

Therefore, the present investigation was undertaken with an objective to select an efficient biological nitrogen fixer for soybean crop to reduce the use of nitrogenous fertilizer.

Material and Methods

Collection of plant samples

The soybean plant samples were collected in the month of July, 2008 from different districts of Vidarbha region. Status of nodulation in different areas was recorded by uprooting five soybean plant at 40-45 days. The number of nodules per plant were recorded..

Isolation of *Bradyrhizobium japonicum*

Isolation of *Bradyrhizobium japonicum* was done by direct inoculation method. Roots of collected samples were washed carefully with water.

The nodules from the top, middle part of the tap root and from the secondary roots were selected randomly for isolation.

These nodules then surface sterilized with sodium hypochlorite (NaOCl) solution for 1 minute. Sterilized nodules were washed with three changes of distilled sterilized water to remove the traces of sodium hypochlorite .

The nodules were kept on the sterilized blotter paper to drain

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out the excess water from the nodules. These nodules were crushed in watch glass with sterilized glass rod in few drops of distilled sterilized water. Thus obtained bacterial suspension was streaked on the solidified yeast extract mannitiol agar with congo red medium. For each nodule samples three replications were made and incubated at room temperature in inverted position for 2-4 days. After 2-4 days white translucent colonies were separated and sub-cultured for further studies.

Field experiment

The field experiment on "Influence of different *Bradyrhizobium japonicum* isolates on growth parameters and yield of soybean" was undertaken during (*kharif*) 2008-09 and 2009-10 to assess the performance of different isolates on nodulation and yield of the soybean at Departmental field.

Details of field experiment

Crop	: Soybean
Variety	: JS-335
Design	: RBD
Replications	: 3
Treatments	: 32
Plot Size	: 3.50 x 2.5m^2
Date of sowing	: 07/07/2008 and 30/06/2009
Sowing method	: Dibbling
Date of harvesting	: 16/10/2008 and 20/09/2009

Inoculation of seed with *Bradyrhizobium japonicum* isolates

Seed of JS 335 were treated with different *Bradyrhizobium japonicum* isolates before sowing. Each isolate was mass multiplied in 100 ml YEMA broth 7 days before sowing. The observations on nodulation, root and shoot length were recorded at 30,60 and 90 days after sowing and finally the yield kg/ha. All recommended agronomical and plant protection practices were adopted during the crop growth period for both the *kharif* season.

Nitrogen uptake and content

Plants at harvest were used for estimation of nitrogen content. The plants were ground and nitrogen in straw was estimated. The total nutrient uptake in kg/ha was calculated by using formula

Uptake of nutrient (kg/ha) = Yield (kg/ha) x Nutrient content/ 100

Total Nitrogen

Total Nitrogen in plant samples was determined by Kjeldahl method in which complex nitrogenous compound in plant samples were converted to ammonia and then ammonium sulphate. The ammonia in the ammonium sulphate was released with NaOH during distillation and absorbed in a known volume of standard sulphuric acid. The unutilized excess of standard H_2SO_4 was determined by a back titration with standard sodium hydroxide. The total nitrogen was then calculated from amount of the standard H_2SO_4 neutralized by absorbed ammoia during distillation. Statistical analysis was made wherever necessary as per standard methods.

Results and Discussion

The isolation of *Bradyrhizobium japonicum* was carried out from root nodules of soybean plant samples collected from different locations of Vidarbha region. Isolates were designated as BJ1 to BJ32. (Table-1). The range of nodule number per plant was 26 to 121. The highest nodules per plant were recorded in BJ 8 followed by BJ 28, BJ 4, BJ 15 and BJ 14.The large variation exhibited on the basis of diverse geographical situation.

Effect of different isolates on shoot length

Field experiment was designed to assess the effect of different isolates of *Bradyrhizobium japonicum* on root, shoot, nodule number, N content, N uptake and yield of soybean during 2008-09 and 2009-10.

During kharif 2008 maximum shoot length were recorded in BJ28 (29.80cm) followed by BJ2 (29.73cm), where as minimum shoot length observed in BJ19 and BJ32 i.e. 23.93 and 23.87cm respectively. At 60 days after sowing maximum shoot length were recorded in BJ29 and BJ21 *i.e.* 36.93 cm and 35.47cm respectively. The least shoot length was recorded in BJ26 (29.93 cm) as compared to other isolates. At 90 days similar trend of observations were observed and each treatment were at par with each other as no significant differences were observed.

During 2009-10 the maximum shoot length 32.17cm was recorded in BJ22 while BJ6 (25.93 cm) showed minimum shoot length as compare to other isolates at 30 days after sowing. At 60 and 90 days after sowing all the treatments were at par with each other with minimum differences among each other.

Table 1. Nodulation status of *Bradyrhizbobium japonicum* collected from different locations of Vidarbha.

Sr.no	Isolates		Nodule number
1	Karanja	BJ -1	65
2	Risod	BJ – 2	70
3	Mangrulpir	BJ -3	88
4	Ambashi	BJ -4	90
5	Dongaon	BJ -5	72
6	Malegaon	BJ -6	63
7	Vadap	BJ -7	70
8	NARP,Yavatmal	BJ -8	121
9	Wani	BJ -9	32
10	Darwha	BJ -10	56
11	NGP	BJ -11	72
12	Umred	BJ -12	66
13	Malkapur	BJ -13	53
14	Sindkhed	BJ -14	52
15	Chikhali	BJ -15	97
16	Nandura	BJ -16	94
17	Khamgaon	BJ -17	43
18	Mehkar	BJ -18	40
19	Wardha	BJ -19	52
20	Pulgaon	BJ -20	47
21	Hinganghat	BJ -21	53
22	BARS Diwthana	BJ -22	81
23	Barshi takli	BJ -23	62
24	Telhara	BJ -24	41
25	Balapur	BJ -25	59
26	Patur	BJ -26	57
27	RRC Amravati	BJ -27	89
28	Chandur Rly	BJ -28	93
29	Anjangaon	BJ -29	30
30	Tiwasa	BJ -30	26
31	Daryapur	BJ -31	32
32	Warora	BJ -32	34

From the pooled results (Table 2) it is evident that the BJ10 (30.07 cm) recorded maximum shoot length at 30 days. While BJ29 (36.00 cm), BJ5 (35.33) and BJ4 (35.10cm) showed maximum shoot length after 60 day. After 90 day BJ29 (37.38 cm) and BJ5 (35.98 cm) exhibited higher shoot length.

Table 2: Effect of *B.japonicum* isolates on Shoot length (cm) of soybean (JS 335)

Treatment	2008 – 09			2009 - 2010			Pooled		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
BJ1	27.73	32.67	33.77	31.00	33.13	33.67	29.37	32.90	33.72
BJ2	29.73	33.80	34.90	28.40	33.67	35.00	29.07	33.73	34.95
BJ3	27.70	34.47	35.57	26.00	34.33	35.73	26.85	34.40	35.65
BJ4	28.33	34.80	35.90	29.60	35.40	35.97	28.97	35.10	35.27
BJ5	27.53	35.07	36.17	30.20	35.60	35.80	28.87	35.33	35.98
BJ6	24.47	34.07	35.17	25.93	33.90	35.23	25.20	33.98	34.37

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BJ7	26.87	34.67	35.77	28.57	35.00	35.77	27.72	34.83	35.77
BJ8	24.47	33.27	34.37	30.80	33.17	34.80	27.63	33.22	34.58
BJ9	28.93	32.80	33.90	30.20	34.73	34.53	29.57	33.77	34.22
BJ10	28.47	32.20	33.30	31.67	32.93	32.97	30.07	32.57	33.13
BJ11	26.27	34.00	35.10	29.23	35.27	34.93	27.75	34.63	35.02
BJ12	26.53	34.40	35.50	28.73	34.27	35.97	27.63	34.33	35.73
BJ13	25.67	33.73	34.83	30.40	33.27	34.90	28.03	33.50	34.87
BJ14	24.67	33.93	35.03	28.70	33.80	35.17	26.68	33.87	35.10
BJ15	26.20	34.20	35.30	28.30	34.53	35.20	27.25	34.37	35.25
BJ16	24.20	34.67	35.77	28.50	34.67	35.43	26.35	34.67	35.60
BJ17	27.23	32.80	33.90	27.40	33.00	33.37	27.32	32.90	33.63
BJ18	27.33	34.13	35.23	30.63	34.80	34.67	28.98	34.47	34.95
BJ19	23.93	34.13	35.23	31.63	34.70	34.90	27.78	34.42	35.07
BJ20	24.93	33.87	34.97	32.13	34.20	34.53	28.53	34.03	34.75
BJ21	27.53	35.47	36.57	31.13	33.67	35.27	29.33	34.57	35.92
BJ22	24.20	33.40	34.50	32.17	34.40	33.50	28.18	33.90	34.00
BJ23	27.27	33.60	34.70	30.13	33.10	35.00	28.70	33.35	34.85
BJ24	25.20	31.07	32.17	30.67	31.40	33.73	27.93	31.23	32.95
BJ25	29.53	34.80	35.90	31.80	35.07	35.63	30.67	34.93	35.77
BJ26	24.93	31.60	32.70	29.63	31.67	33.40	27.28	31.63	33.05
BJ27	25.43	29.93	31.03	27.63	30.77	30.17	26.53	30.35	30.60
BJ28	29.80	33.53	34.63	29.20	33.53	34.93	29.50	33.53	34.78
BJ29	26.87	36.93	38.03	27.47	35.07	36.73	27.17	36.00	37.38
BJ30	24.47	33.00	34.10	30.20	32.97	35.20	27.33	32.98	34.65
BJ31	26.87	34.73	35.83	28.30	35.03	33.53	27.58	34.88	34.68
BJ32	23.87	33.20	34.30	27.00	33.53	33.27	25.43	33.37	33.78
Interaction Y x T	NS	NS	NS	Sig	NS	NS	Sig	NS	NS
SE (m)				0.69			1.71		
CD @ 5%				1.95			4.90		

Maximum root length 19.53 cm was recorded due to BJ25 followed by BJ2 (18.47 cm) and BJ8 (18.47 cm) while the minimum root length was found in BJ15 (15.47 cm) at 30 days after sowing during 2008-09. Significant increase in root length was observed at 30 and 90 days after sowing.

Pooled results indicated significant differences in the root length after 30 days. Where as all the isolates were at par with each other after 60 and 90 days. After 90 days of sowing maximum root length was recorded due to BJ21 (35.12 cm).(Table - 3)

At 30 days after sowing maximum nodules per plant were recorded in BJ9 (23.00) followed by BJ23 (19.33), where least number were exhibited in BJ13 (8.73) during 2008-09. At 60 days after sowing significant increase in nodule numbers per plant were observed. Highest number of nodules per plant were recorded in BJ21 (107.83) followed by BJ23 (100.83). The least number of nodules were recorded in BJ27 (27.67) and BJ13 (33.83). Similar observations were recorded after 90 days.

During year 2009-10, significant increase in nodule number per plants were recorded i.e.108, 100.33 and 95 in BJ21, BJ23 and BJ19 respectively after 60 days. Where least number were observed in BJ24 i.e.40.67. After 90 days BJ23 recorded 120 nodules followed with 104 in Bj23.

The pooled results (Table 4) showed that maximum nodules were shown by BJ9 *i.e.* 29.80 after 30 days. Isolates BJ 21 (114.50), BJ23 (101.00) showed highest nodules per plant after 60 days. Slight increase in nodule number due to all isolates was recorded after 90 days. While 107.9 nodules were observed in BJ21. In general BJ21 and BJ23 proved as efficient isolates in respect to nodulation and shoot/ root length. Similar observations were reported by Dwivedi (2003) who evaluated ten strains of *Bradyrhizobium japonicum* and reported that strain MB2P3 was superior in respect to nodulation. Further,Tamiru et.al. (2012) also concluded that the nodulation rating, nodule number per plant, nodule volume per plant, and nodule dry weight were significantly influenced by *Bradyrhizobium japonicum* strains. Inoculation of *B.japonicum* significantly improved the dry weight of shoots and roots, nodulation, yield and yield components of soybean (Khalid ,2011) . The present results are corroborate with the results of Manochehr (2013) and Workneh Bekere (2013).

Table 3: Effect of *B.japonicum* isolates on root length (cm) in soybean (JS 335)

Treatment	2008 – 09			2009 - 2010			Pooled		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
BJ1	16.63	30.20	32.13	17.53	30.10	32.20	17.08	29.95	32.17
BJ2	18.47	30.53	32.93	16.10	28.43	32.10	17.28	28.75	32.52
BJ3	16.93	31.80	33.73	18.10	30.40	33.57	17.52	31.15	33.65
BJ4	17.67	31.67	33.80	19.40	31.30	33.20	18.53	33.90	33.50
BJ5	17.33	31.53	34.27	18.07	31.57	33.37	17.70	30.80	33.82
BJ6	16.93	31.47	33.60	16.60	31.43	33.30	16.77	33.95	33.45
BJ7	17.07	32.33	34.53	16.23	32.77	34.23	16.65	32.85	34.38
BJ8	18.47	31.07	33.20	17.27	32.07	33.67	17.87	30.55	33.43
BJ9	18.40	30.13	31.73	20.73	30.53	31.83	19.57	29.85	31.78
BJ10	17.93	30.00	32.60	19.97	30.73	31.60	18.95	30.15	32.10
BJ11	16.20	31.27	33.53	17.83	33.47	33.57	17.02	32.05	33.55
BJ12	16.80	31.93	33.67	16.00	31.40	32.80	16.40	30.95	33.23
BJ13	15.93	30.60	32.93	15.50	31.70	31.77	15.72	33.40	32.35
BJ14	16.20	30.40	32.80	19.47	27.40	31.23	17.83	29.45	32.02
BJ15	15.47	31.40	33.93	17.53	30.00	33.40	16.50	30.40	33.67
BJ16	16.73	31.40	33.93	19.87	30.67	32.40	18.30	30.90	33.17
BJ17	17.40	30.07	32.47	18.87	29.67	31.50	18.13	31.00	31.98
BJ18	16.67	31.07	32.73	17.90	31.00	33.03	17.28	31.00	32.88
BJ19	17.53	31.67	34.20	16.73	30.67	33.47	17.13	30.80	33.83
BJ20	15.40	31.27	33.20	16.60	30.73	31.97	16.00	33.80	32.58
BJ21	17.93	32.47	35.87	19.20	31.80	34.37	18.57	31.10	35.12
BJ22	16.40	30.60	33.00	17.60	31.10	32.43	17.00	29.15	32.72
BJ23	17.67	29.20	31.27	19.53	29.57	30.10	18.60	28.60	30.68
BJ24	17.87	28.27	30.33	20.20	29.27	31.90	19.03	25.40	31.12
BJ25	19.53	27.40	31.07	18.13	28.83	31.77	18.83	27.60	31.42
BJ26	18.33	28.13	30.00	19.63	29.77	31.90	18.98	27.85	30.95
BJ27	15.20	28.20	30.27	17.57	30.43	31.27	16.38	27.90	30.77
BJ28	18.13	30.80	33.27	18.33	31.27	33.57	18.23	30.25	33.42
BJ29	17.60	31.93	35.13	20.23	32.30	32.67	18.92	30.40	33.90
BJ30	17.60	30.47	32.80	16.33	31.03	32.10	16.97	29.85	32.45
BJ31	17.27	31.13	34.13	17.67	31.60	33.17	17.47	31.60	33.65
BJ32	15.53	31.67	34.20	18.23	31.77	34.23	16.88	35.20	34.22
Interaction Y x T	NS	NS	NS	Sig	N S	Sig	Sig	NS	NS
SE (m)				0.57		0.71	0.50		
CD @ 5%				1.61		2.00	1.49		

Table 4: Effect of *B. japonicum* isolates on nodule number of soybean (JS 335)

Treatment	2008 – 09			2009 - 2010			Pooled		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
BJ1	15.53	54.70	49.53	32.47	54.00	61.87	48.00	108.70	111.40
BJ2	21.43	57.40	58.73	32.13	62.00	72.53	53.57	119.40	131.27
BJ3	13.47	70.83	53.13	32.10	71.33	76.00	45.57	142.17	131.13
BJ4	17.60	90.23	79.00	33.23	88.00	93.33	50.83	178.23	169.20

BJ5	17.73	64.67	76.80	31.40	85.33	89.00	49.13	150.00	161.27
BJ6	12.67	90.10	75.67	34.57	86.80	92.67	47.23	176.90	167.67
BJ7	15.80	59.33	62.33	32.60	71.00	80.00	48.40	130.33	142.33
BJ8	13.93	35.80	55.40	36.60	64.33	78.00	50.53	100.13	127.53
BJ9	23.00	41.00	42.60	36.60	67.00	70.00	59.60	108.00	112.60
BJ10	14.73	36.27	33.07	32.93	65.00	80.00	47.67	101.27	117.73
BJ11	11.93	60.53	57.00	36.80	70.33	86.33	48.73	130.87	147.33
BJ12	11.93	51.20	53.87	28.37	71.00	80.67	40.30	122.20	140.20
BJ13	8.73	33.83	32.00	31.27	74.67	87.00	40.00	108.50	119.67
BJ14	14.13	72.97	52.33	31.63	85.33	89.00	45.77	158.30	143.07
BJ15	16.63	58.37	54.33	32.97	76.33	86.67	49.60	134.70	141.00
BJ16	14.43	66.37	70.33	32.13	75.00	76.33	46.57	141.37	143.60
BJ17	18.13	42.63	41.07	30.07	75.67	83.33	48.20	118.30	123.07
BJ18	16.40	44.63	70.93	32.60	66.67	73.67	49.00	111.30	129.33
BJ19	13.93	75.80	79.07	31.30	95.00	102.67	45.23	170.80	181.73
BJ20	12.53	77.17	71.33	34.40	75.00	80.67	46.93	152.17	151.67
BJ21	13.60	107.83	98.00	33.60	108.00	120.00	47.20	215.83	215.67
BJ22	12.07	67.40	68.53	32.43	68.87	72.33	44.50	136.27	142.47
BJ23	19.33	100.83	96.67	33.83	100.33	104.67	53.17	201.17	185.00
BJ24	10.00	39.50	34.27	34.33	40.67	51.67	44.33	80.17	85.60
BJ25	14.20	75.17	39.00	32.20	72.33	74.67	46.40	147.50	105.13
BJ26	16.43	43.70	23.53	33.57	55.33	62.33	50.00	99.03	92.80
BJ27	18.77	27.67	29.13	34.00	58.67	67.33	52.77	86.33	96.47
BJ28	14.80	48.40	48.87	31.43	62.20	79.00	46.23	110.60	131.07
BJ29	12.80	47.03	37.87	33.60	44.93	60.33	46.40	91.97	93.87
BJ30	16.47	55.03	54.53	35.17	63.27	69.33	51.63	118.30	123.87
BJ31	15.27	72.90	53.67	33.07	58.87	71.20	48.33	131.77	104.00
BJ32	14.60	45.60	45.40	31.37	59.33	56.33	45.97	104.93	104.43
Int. Y x T	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE (m)	2.29	1.91	2.58	1.21	3.2	2.09	0.49	1.30	0.84
CD @ 5%	6.46	5.38	7.27	3.4	9.0	5.91	1.23	3.79	2.4

Grain Yield

Highest yield i.e 9.62 q/ha was induced by BJ23 followed by 9.06q/ha and 8.84q/ha due to, BJ21 and BJ2 respectively. The lowest yield was recorded in BJ32 (5.81q/ha) and BJ8 (5.98q/ha) during 2008-09.

Significant increase in yield was achieved where BJ1 showed maximum yield i.e. 12.64q/ha, followed by BJ2 (11.42 q/ha) and BJ6 (11.08 q/ha) over other isolates. The lowest 8.81q/ha was recorded in BJ10 as compared to other isolates during 2009-10.

From the pooled results (Table 5) significant differences in yield q/ha were observed. Amongst the isolates BJ28, BJ2 and BJ1 gave significantly highest yield i.e. 10.31,10.13 and 10.05 q/ha respectively. Most of the isolates were found efficient but at par with each other. Lowest yield was achieved due to BJ32 (7.26 q/ha) and BJ 22 (7.42 q/ha).

Maximum nitrogen content was observed in BJ1 an BJ2 i.e. 0.82 per cent, while reduced nitrogen content was recorded in BJ32 (0.42%),BJ9 (045%), BJ25 (0.45%) and BJ22 (0.49%). Increase in nitrogen content was observed in second year. Two year pooled data (Table 5) showed that maximum N content induced by BJ4 (2.09%) followed by BJ1 (1.75%).

Regarding nitrogen uptake, maximum uptake was obtained in BJ1 (7.88 kg/ha), BJ2 (7.28 kg/ha) and BJ15 (7.02 kg/ha) where as least uptake was recorded in BJ32 (2.44 kg/ha),BJ 25 (2.83) and BJ22 (2.96 kg/ha) during 2008-09. during 2009-10 maximum nitrogen uptake i.e. 13.94 kg/ha was recorded in BJ4 and 13.28kg/ha in BJ21. Regarding pooled data (Table 5) BJ4 showed highest nitrogen uptake by BJ1 i.e. 10.27kg/. Significant effect of *B.japonicum* on dry matter yield, N and P uptake in soybean was also reported by Lukiwati and Simanungkalit (2002) in field experiment.

Table 5 : Effect of *B.japonicum* isolates on Yield (q/ha), n content (%) and N uptake kg/ha on soybean (JS 335)

Treatment	2008 – 09			2009 - 2010			Pooled		
	Yield	N content	N uptake	Yield	N content	N uptake	Yield	N content	N uptake
BJ1	7.39	0.82	7.88	12.64	0.93	11.80	11.13	0.88	9.84
BJ2	8.84	0.82	7.28	11.42	0.89	10.17	10.13	0.86	8.72
BJ3	7.68	0.65	5.00	8.58	1.06	9.09	8.13	0.86	7.05
BJ4	8.75	0.75	6.60	10.37	1.34	13.94	9.56	1.05	10.27
BJ5	8.16	0.69	5.60	10.59	0.72	7.65	9.37	0.71	6.63
BJ6	8.81	0.79	6.95	11.08	0.67	7.39	9.94	0.73	7.17
BJ7	7.62	0.67	5.10	9.84	0.74	7.30	8.73	0.71	6.20
BJ8	5.98	0.80	4.79	7.40	0.68	5.06	6.69	0.74	4.92
BJ9	6.86	0.45	3.09	9.38	0.56	5.29	8.12	0.51	4.19
BJ10	6.24	0.56	3.50	8.81	0.59	5.19	7.53	0.58	4.35
BJ11	8.32	0.61	5.08	10.76	0.66	7.11	9.54	0.64	6.10
BJ12	7.87	0.54	4.24	10.50	0.77	8.08	9.18	0.66	6.16
BJ13	7.90	0.52	4.11	9.29	0.81	7.52	8.60	0.67	5.82
BJ14	7.77	0.52	4.04	10.36	0.58	6.04	9.07	0.55	5.04
BJ15	6.80	0.78	7.06	10.48	0.54	5.65	9.77	0.66	6.35
BJ16	8.23	0.74	6.08	9.87	0.65	6.39	9.05	0.70	6.23
BJ17	7.89	0.51	4.02	10.05	0.72	7.26	8.97	0.62	5.64
BJ18	7.93	0.55	4.35	10.22	0.54	5.56	9.07	0.55	4.95

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BJ19	8.62	0.77	6.63	11.18	0.59	6.49	9.90	0.68	6.56
BJ20	7.90	0.69	5.45	10.20	0.95	9.70	9.05	0.82	7.58
BJ21	9.06	0.59	4.00	9.61	1.38	13.28	8.20	0.99	8.64
BJ22	6.10	0.49	2.96	8.75	0.61	5.34	7.42	0.55	4.15
BJ23	9.62	0.56	4.14	10.08	0.47	4.78	8.73	0.52	4.46
BJ24	6.93	0.51	3.53	9.32	0.83	7.73	8.12	0.67	5.63
BJ25	6.29	0.45	2.83	9.68	0.69	6.67	7.98	0.57	4.75
BJ26	8.65	0.75	6.49	9.54	0.63	6.05	9.10	0.69	6.27
BJ27	7.39	0.52	3.84	9.88	0.63	6.17	8.63	0.57	5.01
BJ28	8.76	0.78	6.82	11.86	0.64	7.59	10.31	0.71	7.20
BJ29	7.62	0.56	4.25	9.26	0.57	5.30	8.44	0.57	4.77
BJ30	7.35	0.74	5.45	9.69	0.67	6.49	8.52	0.71	5.97
BJ31	7.71	0.78	6.00	10.01	0.67	6.63	8.86	0.73	6.32
BJ32	5.81	0.42	2.44	8.72	0.57	4.93	7.26	0.50	3.69
Int. Y x T	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE (m)	0.39	0.01	0.30	0.47	0.03	0.37	0.43	2.16	1.92
CD @ 5%	1.19	0.05	0.87	1.32	0.08	1.0	0.59	0.00	0.00

Highest nodulation, nitrogen content (2.86%), yield and N fixation with combination of various exotic *B.japonicum* strains were recorded by Zarrin Fatima, *et. al.* (2007). Similarly, Morshed, *et. al.* (2008) concluded that inoculation of *B.japonicum* could give maximum seed yield, and N uptake by soybean. Further, Asadi Rahmani, *et. al.* (2009) demonstrated that thermotolerant *Bradyrhizobium* isolate were superior in nitrogen fixation, nodule number and dry weight, shoot dry matter and nitrogen uptake of soybean. Rahman and Sampa (2012) also stated that application of *B.japonicum* strain BAU 101 performed best in terms of N content and uptake, and yield of soybean. Dwivedi (2003) also reported similar results.

All these results in respect to development of nodules and other yield contributing characters proved the significance of same efficient isolates studied during the course of investigation. Particularly isolate BJ21,BJ23,BJ4,BJ29 were proved superior among all isolates.

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