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Rhizobial Nitrogen Fixation Efficacy by Chickpea Genotypes in Pot Culture and Field Experiment

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Abstract

Two potential *Rhizobium* strains, namely RhCh-7 and RhCh-8, isolated from chickpea grown in Jammu region, were tested for their nitrogen fixing abilities with ten different chickpea cultivars. The efficiency study was carried out by pot culture and field experiment. The nitrogen fixation capacities were assayed in the respect to nodulation capacity, host plant protein content, plant dry mass and number of pod formation. Variation in nitrogen fixation efficiencies was found with the chickpea varieties in both the experiments. In the field experiment, controls also showed significant values but *Rhizobium* strains treated samples always revealed higher values compared to their corresponding control values and hence showed their effects. Among the RhCh-7 strain treated chickpea cultivars, nitrogen fixing capacity was significantly high with the cultivars SCS-13, SUS-5, SCS-2 and C-235 in which SCS-13 had maximum efficiency ; while the cultivars SCS-2 (with highest value), C-235, SUS-5 and SCS-13 were observed to be statistically at par in the efficiency against RhCh-8 *Rhizobium* treatment. But in both the treatments, SCS-12 showed lowest and the cultivars PBG-1, B-801, IBL-933, SCS-3, Gaurav with moderate nitrogen fixation capacities. These trends were maintained irrespective of the nitrogen fixation measuring parameters viz. nodule formation capacity, plant dry mass, plant leave protein and pod number and also the experiments conducted viz. pot culture and field experiment. But field experiment always depicted higher values compared to that of pot culture experiment.

Key words : *Rhizobium*, Nitrogen fixation, Chickpea cultivars, Pot culture, Field experiment.

Chickpea, most widely consumable pulses among the Indian, is best known for its quality protein and good nutritive value. Nitrogen is a major limiting factor for the plant growth. In India, chickpea are generally grown in the dry areas and dependent normally on the nitrogen fixing symbiotic *Rhizobium* for their nitrogen requirement. The symbiotic nitrogen fixation is a complex process involving bacterial recognition, infection to the host plant, bacteriods formation within the plant cell as root nodules, and then reduction of aerial nitrogen into ammonia by bacterial nitrogenase enzyme (1, 2), followed by ammonia assimilation to plant amino acids and other nitrogenous biomolecules (3). So, nitrogen fixation efficiency depends upon the combined effect of how compatible the legume-*Rhizobium* association (4, 5), and the performance of nitrogenase activity and ammonia assimilation there after (6, 7). These factors are established by the plant genetics and genetic compatibility of both the host plant and *Rhizobium* bacteria (8, 9). Therefore, the selection of suitable *Rhizobium* strain(s) and its competent host chickpea cultivar(s) for optimal nitrogen fixation is necessary to get maximum productivity. In this paper the fixation efficacy was reported in ten chickpea cultivars by potential *Rhizobium* strains tested under pot culture and field experiment in Jammu sub-tropics.

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Methods

Rhizobium Strain Selection

Two most potential and effective chickpea *Rhizobium* strains namely RhCh-7 and RhCh-8 were screened out from the strains isolated from root nodule of chickpea grown in the Jammu region. It was

Table 1. Nitrogen fixation efficiencies of *Rhizobium* strain RhCh-7 and RhCh-8 with different chickpea cultivars in pot culture experiment.

	RhCh-7 strain treatment				RhCh-8 strain treatment			
Cultivars	Nodule number	Nodule dry mass (mg)	Plant dry mass (mg)	Plant protein (%)	Nodule number	Nodule dry mass (mg)	Plant dry mass (mg)	Plant protein (%)
SCS-12	8.33	11.93	453.33	3.74	5.67	11.0	463	3.85
SCS-3	13.33	18.67	496.33	3.86	15.33	24.33	495.33	3.9
PBG-1	15.33	21.33	538.33	3.88	17.33	25.83	548.33	4.02
SCS-13	30.33	42.67	613.67	4.26	19.33	28.0	575.33	4.07
C-235	20.33	31.67	562.67	4.13	22.00	34.67	611.0	4.22
IBL-933	13.33	16.0	531.0	3.96	13.33	23.67	517.0	3.9
B-801	15.33	21.33	543.00	3.95	18.33	27.67	540.0	4.0
SCS-2	22.67	28.33	570.67	4.13	24.33	34.33	605.33	4.27
SUS-5	24.67	35.67	587.0	4.35	20.33	30.0	603.0	4.07
Gaurav	13.33	19.67	507.0	3.85	13.33	20.33	514.0	3.9
CD (P = 0.05)	9.93	7.95	78.63	0.13	8.12	10.20	80.02	0.11

done on the basis of their tolerance to high temperature (55 C), high salt concentration (1% sodium chloride) and low pH, and better nitrogen fixing ability (tested by Leonard Jar assembly experiment).

Nitrogen Fixing Capacity Measurement

Healthy seeds of ten chickpea cultivars viz. SCS-3, SCS-12, PBG-1, C-235, SCS-13, B-801, IBL-933, SCS-2, SUS-5 and Gaurav were surface sterilized by 2% sodium hypochlorite and then inoculated with RhCh-7 and RhCh-8 *Rhizobium* strains separately. These inoculated seeds were sown in the pot containing potassium sulfate treated sand. The system was kept in open sun light according to pot culture method (10). The same experiment was conducted in the field under RB design with three replications and allowing them to grow under Jammu field soil and environment conditions. Controls were taken for all the cultivars against each *Rhizobium* treatment. To assess the strain-cultivar interactions in context to their nitrogen fixing abilities under pot culture and field conditions, number of nodules, host plant dry mass, plant leaf protein content were assayed after one month growth. Pod numbers were also taken in count in addition under field experiment. Host protein content was estimated by Lowry method (11). The data were statistically analyzed using analysis

Table 2. Nitrogen fixation efficiencies of *Rhizobium* strain RhCh-7 with different chickpea cultivars under field conditions.

	Nodule number		Plant dry mass (mg)		Plant leaf protein (%)		Pod number	
Cultivars	Treated	Over control	Treated	Over control	Treated	Over control	Treated	Over control
SCS-12	8.33	5.33	848.7	436.7	7.103	1.347	65.0	31.0
SCS-3	11.33	7.33	842.3	443.0	7.843	1.483	79.67	40.0
PBG-1	13.0	8.60	883.3	504.0	8.20	1.80	102.33	38.67
SCS-13	26.0	21.0	1426.7	1073.3	8.697	2.70	151.0	100.67
C-235	19.0	14.0	1174.0	686.7	8.4	2.02	124.0	72.67
IBL-933	16.0	11.33	953.3	438.3	7.53	1.97	100.0	49.67
B-801	12.33	8.33	1057.7	561.3	6.78	1.6	103.0	39.67
SCS-2	17.0	13.66	1491.3	960.0	7.77	1.99	133.33	59.67
SUS-5	20.66	18.0	1243.3	799.0	8.4	2.24	119.33	77.33
Gaurav	7.66	5.0	1082	516.7	8.39	1.833	105.6	52.0
CD (P = 0.05)	4.43	3.84	341.55	172.83	0.30	0.31	38.26	31.27

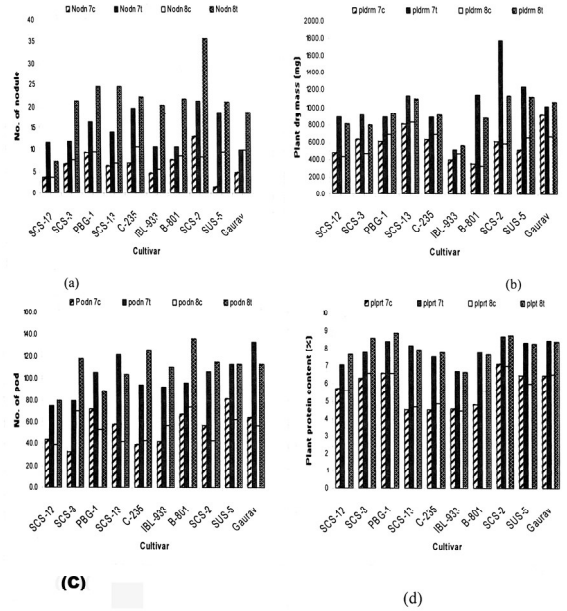

Figure 1. Comparison of RhCh-7 and RhCh-8 strains treated nitrogen fixation efficiency with regard to (a) nodule formation (b) plant dry mass (c) pod number and (d) plant protein content with the variation of chickpea cultivar under field conditions.

of variance techniques (ANOVA).

Results and Discussion

In the pot culture experiment, the number of nodules formed per plant and their dry mass against each chickpea variety were found to be closely related and were statistically significant in both *Rhizobium* treatments. Plant dry mass and plant protein content showed different sequential trend of efficiency from that of nodule formation capacity, but were also statistically significant with chickpea cultivars in both treatments. The data of the experiments were analyzed by ANOVA (Table 1).

In RhCh-7 treatment, the values for nodule number, nodule dry mass, plant dry mass and plant protein in the cultivars ranged from 8.33 to 30.33, 11.93 to 42.67 mg, 453.33 to 613.67 mg and 3.74 to 4.35% respectively. Among the cultivars, SCS-13 was statistically superior with values 30.33, 42.67 mg, 613.67 mg (all maximum) and 4.26% (had second highest value but statistically at par with SUS-5 which carried the highest value 4.35%) respectively and hence possessed highest nitrogen fixing efficiency. The cultivar SUS-5 (24.67, 35.67 mg, 587.0 mg and 4.35%), SCS-2 (22.67, 28.33 mg, 570.67 mg and 4.13%), C-235 (20.33, 31.67 mg, 562.67 and 4.13%), PBG-1 (15.33, 21.33 mg, 538.33 mg and 3.88%) and B-801 (15.33, 21.33 mg, 543.0 mg and 3.95%) (values arranged in above mentio-ned parameters sequence) had shown small variation in order in parameter to parameter but were found statistically at par. SCS-12 registered minimum values in all parameters viz. 8.33, 11.93 mg, 453.33 mg, and 3.74% respectively and hence showed lowest efficiency.

In RhCh-8 treatment, the nitrogen fixation parameters viz. nodule number, nodule dry mass, plant dry mass and plant leaf protein percentage in the cultivars were statistically significant and values ranged from 8.33 to 24.33, 11.0 to 35.67 mg, 463.0 to 611.0 mg and 3.85 to 4.27% respectively. Maximum value was found in SCS-2 cultivar (24.33, 34.67 mg, 605.33 mg, and 4.27%) followed by C-235, SUS-5, SCS-13, B-801, PBG-1 and SCS-3 which were statistically at par but showed small variation in nitrogen fixation efficiency when expressed plant dry mass and plant pro-

Table 3. Nitrogen fixation efficiencies of *Rhizobium* strain RhCh-8 with different chickpea cultivars under field conditions.

Cultivars	Nodule number		Plant dry mass (mg)		Plant leaf protein (%)		Pod number	
	Treated	Over control	Treated	Over control	Treated	Over control	Treated	Over control
SCS-12	7.66	4.33	573.3	290.0	7.65	1.63	80.0	36.0
SCS-3	17.33	13.66	708.7	300.0	7.82	1.85	89.0	42.67
PBG-1	18.66	15.0	830.0	326.67	7.67	2.08	118.0	62.33
SCS-13	29.33	24.0	1096.7	496.7	8.86	2.62	132.67	89.0
C-235	27.66	23.0	913.3	564.33	8.40	2.27	126.33	79.0
IBL-933	19.0	15.33	800.0	369.67	7.93	1.74	110.0	53.33
B-801	21.66	16.66	713.3	350.0	6.66	1.82	122.0	47.67
SCS-2	38.33	34.0	1220.0	733.33	8.26	2.25	138.67	106.0
SUS-5	24.0	17.67	1136.7	676.67	8.77	2.28	136.67	73.67
Gaurav	11.66	6.66	946.7	310.0	8.29	1.93	106.67	51.67
CD (P = 0.05)	6.55	6.76	163.45	206.17	0.34	0.46	35.90	27.09

tein. Here also cultivar SCS-12 showed minimum efficiency as possessing lowest values (5.67, 11.0 mg, 463.0 mg, and 3.85%) in all aspects. The cultivars SCS-2, C-235, SUS-5, SCS-13, B-801, PBG-1 and SCS-3 with the values ranging 24.33 to 15.33, 34.33 to 24.33 mg, 611.0 to 548.33 mg and 4.27 to 4.0% for nodule number, nodule dry mass, plant dry mass and plant protein respectively, showed medium range of nitrogen fixation efficiencies.

In comparison study, nitrogen fixation efficiency was found to be maximum with chickpea variety SCS-13 followed by SUS-5, SCS-2, and C-235 (statistically at par to each other) with RhCh-7 strain treatment ; whereas SCS-2 possessed highest efficiency and C-235, SUS-5, SCS-13, B-801, PBG-1 and SCS-3 were in decreasing order in RhCh-8 strain treatment. But in both treatments, the cultivars PBG-1, B-801, IBL-933, SCS-3, Gaurav and SCS-12 had lower nitrogen fixation capacity ; in which SCS-12 was the poorest. A variation in efficiency order observed for plant protein percent and plant dry mass viz. SUS-5 cultivar showed maximum efficiency in plant protein percent against RhCh-7 strain, whereas C-235 cultivar was maximum in plant dry mass (611 mg) and second highest in protein content against RhCh-8 strain. Again SCS-2 which was highest in nodule number/dry mass and plant protein values carried second highest value in plant dry mass. Biosynthesis of protein is a genetic controlled process and varies with genotype (12). Besides, nitrogen fixation and ammonia assimilation for conversion of amino acid play roles for protein content. Genetic compatibility between cultivars and *Rhizobium* strain which leads to nodulation and its efficiency (13, 14, 7) may also be responsible for variation of nitrogen fixation in different chickpea variety.

In the field experiment, pod number was also included as nitrogen fixing parameter in addition to nodule formation, plant dry mass and leaf protein content. The statistically analyzed values of treatments and values over controls are given in Tables 2 and 3 for RhCh-7 and RhCh-8 respectively and in Figure 1. Values over their respective controls (as an effect of experimental *Rhizobium* strains) were taken for interpretations. In field experiment also, both RhCh-7 and RhCh-8 strain treatments revealed similar trends in nitrogen fixation capacities with corresponding chickpea cultivars which were statistically significant and having same efficiency order to that of pot culture experiment i.e. the values for nodule formation, plant dry mass and protein content and pod number in cultivar SCS-13 (21.0, 1073.3 mg, 2.70% and 100.0) and SCS-12 (5.33, 436.7 mg, 1.347% and 31.0) were maximum and minimum respectively and SUS-5 (18.0, 79.0 mg, 2.24%, 77.33), C-235 (14.0, 686.7 mg, 2.02%, 72.67) and SCS-2 (13.66, 960.0 mg, 1.99%, 59.67) which were statistically at par and having moderate nitrogen fixing in RhCh-7 treatment. In RhCh-8 *Rhizobium* strain treatments, cultivar SCS-2 with the values 34, 733.33 mg, 2.62%, and 106 respectively possessed maximum nitrogen fixing efficiency. The cultivars SCS-13 (24, 496.7 mg, 2.25%,

and 89), C-235 (23, 564.33 mg, 2.27%, and 79) and SUS-5 (17.67, 676.67 mg, 2.28% and 73.67) had medium efficiencies. Here also, cultivar SCS-12 with minimum values (4.33, 290.0 mg, 1.63% and 36.0 respectively) showed lowest nitrogen fixation capacity. But the parameters against chickpea cultivars under field experiment were carrying higher values compared to values of respective treatments of pot experiment. The figure 1 had showed the variation in values from cultivar to cultivar and also the compared the responses between RhCh-7 and RhCh-8 strains.

In this experiment, nitrogen fixation efficiency in ten chickpea varieties by two potential *Rhizobium* strains isolated from Jammu region, were tested in pot culture and under field conditions in Jammu to find out most potential combination of *Rhizobium*-chickpea for nitrogen fixation. Result trends are similar in both the experiments and even the nitrogen efficiency in cultivars with *Rhizobium* strain are of same order. But the values of field experiment are higher in general compared to that of pot culture experiment where nutrients were negligible (as pot sand was washed and K_2SO_4 treated). The controls under field conditions also showed some effects which were absent pot culture. Protein percentages and plant dry mass were also higher in field experiment. It indicates that nitrogen fixation process was more vibrant under field soil conditions. It may be due to presence of inherent rhizobacteria and also some important nutrients/elements in soil which may involve in boosting up nitrogen fixation process by enhancing nodulation, nitrogenase activity and ammonia assimilation directly or indirectly. Molybdenum, iron, sulfur and magnesium are the essential elements for nitrogenase constituent and activity, whereas metal like zinc, boron, phosphorus, and potassium are reported to have vital effects on nodulation and overall nitrogen fixing abilities (15—19). Besides, enriched soil causes effective photosynthesis (by activating different enzymes) which enhances production of carbohydrates, lipids and other biomolecules. These biochemicals mainly carbohydrates and lipids provide energy, reducing power and ketoacids via respiration for nitrogen fixation and ammonium assimilation processes ; which lead to enhance the plant protein contents and plant biomass levels (15, 20, 21). So, the elements enriched soil and their effective rations, in addition to soil rhizobacteria and organic nutrients, may be the causes for better nitrogen fixation efficiencies especially in context of plant protein and plant dry mass under field conditions (for both *Rhizobium* strains) than pot culture. Apart from those nitrogen fixation efficiencies trends of the cultivars were maintained in both experiments with same *Rhizobium* strain ; in which SCS-13 was most efficient in RhCh-7 strain whereas the cultivar SCS-2 showed highest nitrogen fixation capacity in RhCh-8 *Rhizobium* strain. The nitrogen fixation efficiency order with other cultivars also varied accordingly. This variation in efficiencies was due to genetic compatibility in the interaction of cultivars and respective *Rhizobium* strains ; which reflected to nodulation capacity and efficient nitrogen fixation. But deviation in nitrogen efficiency order in context of plant protein, pod number and plant dry mass values may be due to variation of ammonia assimilation potency and other related metabolisms (6) among the cultivars. These physiological characters are fully genetic specific and also may be influenced by the genetic compatibility of host plant and *Rhizobium* strain (8, 22).

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