



International Journal of Research in Agronomy

E-ISSN: 2618-0618

P-ISSN: 2618-060X

© Agronomy

www.agronomyjournals.com

2025; 8(1): 979-985

Received: 12-01-2024

Accepted: 25-01-2024

C Mrudhula

Department of Microbiology,
University of Agricultural Sciences
Dharwad, Karnataka, India

Dr. Shubha S

Senior Microbiologist and Head,
AICRP on EAAI (Bioconversion
Technology), University of
Agricultural Sciences Dharwad,
Karnataka, India

Dr. Santhosh GP

Junior Microbiologist, AICRP on
EAAI (Bioconversion Technology),
University of Agricultural Sciences
Dharwad, Karnataka, India

Dr. Suma Mougali

Professor, Department of Genetics
and Plant Breeding, University of
Agricultural Sciences Dharwad,
Karnataka, India

Effect of Rhizobium and Rhizobacterial Inoculation on Nitrogen Content and Uptake in Green Gram

C Mrudhula, Shubha S, Santhosh GP and Suma Mougali

DOI: <https://doi.org/10.33545/2618060X.2025.v8.i1i.4699>

Abstract

Nitrogen availability is a critical factor limiting productivity in pulse crops, and biological nitrogen fixation plays a central role in meeting crop nitrogen requirements. The present investigation assessed the effect of Rhizobium and rhizobacterial inoculation on nitrogen content, nitrogen uptake, and related parameters in green gram (*Vigna radiata* L.). Different microbial treatments, including individual inoculants and consortia, were evaluated for their influence on plant nitrogen concentration and total nitrogen accumulation. The results revealed that microbial inoculation significantly increased nitrogen content and uptake compared to the uninoculated control. Microbial consortia recorded the highest nitrogen values, indicating enhanced symbiotic efficiency and improved nutrient assimilation. The synergistic action of nitrogen-fixing and plant growth-promoting microorganisms contributed to improved nitrogen use efficiency and plant nutrition. The study underscores the potential of microbial inoculants in reducing dependence on chemical nitrogen fertilizers in green gram cultivation.

Keywords: Biological nitrogen fixation, Green gram, Nitrogen uptake, *Rhizobium*, Rhizobacteria

Introduction

Green gram (*Vigna radiata* L.) is a nutritionally rich pulse crop valued for its high protein content, essential amino acids, vitamins, and minerals. It plays a vital role in vegetarian diets and sustainable agriculture due to its ability to fix atmospheric nitrogen and improve soil health. In India, green gram is cultivated over a wide range of agro-ecological regions, with major production concentrated in Rajasthan, Maharashtra, Andhra Pradesh, Telangana, Karnataka, Madhya Pradesh, Tamil Nadu, and Odisha. Its short duration, low water requirement, and compatibility with multiple cropping systems make it an important component of rainfed and irrigated agriculture.

However, green gram productivity is often limited by physiological constraints arising from nutrient stress, moisture stress, poor photosynthetic efficiency, and suboptimal nitrogen metabolism. Physiological traits such as chlorophyll content, nodulation efficiency, nitrogen uptake and assimilation, leghemoglobin concentration, and overall metabolic activity directly influence plant growth, yield formation, and grain quality. Under field conditions, these physiological processes are highly sensitive to environmental fluctuations and soil nutrient availability, leading to inconsistent crop performance.

Microbial inoculation has gained increasing attention as a sustainable approach to improve plant physiological efficiency without reliance on excessive chemical inputs. Symbiotic *Rhizobium* and associated rhizobacteria enhance physiological functioning by improving nodulation, biological nitrogen fixation, chlorophyll synthesis, enzymatic activity, and nutrient translocation within the plant. Increased chlorophyll content enhances photosynthetic capacity, while effective nodulation and higher leghemoglobin levels ensure efficient nitrogen fixation and transport, ultimately improving nitrogen use efficiency and plant metabolism.

The identification and utilization of efficient microbial strains capable of exerting consistent physiological benefits is a critical requirement in modern pulse production. Single strains often show variable performance due to soil and climatic factors, whereas microbial consortia offer synergistic effects by combining multiple functional traits such as nitrogen fixation, nutrient mobilization, and stress tolerance. The present agricultural scenario demands such biologically

Corresponding Author:

Dr. Shubha S

Senior Microbiologist and Head,
AICRP on EAAI (Bioconversion
Technology), University of
Agricultural Sciences Dharwad,
Karnataka, India

driven solutions to enhance crop productivity while maintaining soil health and environmental sustainability.

In this context, the present investigation emphasizes the physiological influence of microbial inoculants on green gram, focusing on parameters such as relative chlorophyll content, number and effectiveness of nodules, nodule biomass, shoot and root nitrogen content, and overall physiological performance across different growth stages. A clear understanding of microbial-mediated physiological responses will support the development of efficient bio-inoculant technologies aimed at improving nitrogen economy, photosynthetic efficiency, and sustainable productivity of green gram.

Materials and Methods

Current study was conducted under greenhouse conduction at university of agricultural Sciences, Dharwad with treatments designed with best performing plant growth promoting rhizobia and rhizobacteria after series of invitro characterization. This study was conducted to study the effect of rhizobia alone and with in combination with rhizobacteria for their effect on root nodulation and leghemoglobin content along with their effect on plant physiology which was studied as effect on chlorophyll content.

The chlorophyll content in leaves was determined using SPAD (Soil Plant Analysis Development) instrument at 30, 45 and 60 DAS. Parallely number of nodules and biomass of nodules was estimated. Pink and firmly attached nodules were carefully detached from uprooted plants and were counted. Further, fresh

weight of nodules was recorded. The mean values were expressed in gram per plant. For dry weight the samples were dried at 75°C until there was no reduction in weight and the mean values were expressed in gram per plant. Further, the nodules ranging in weight from 50-100 mg were crushed in 9 volumes of Drabkin's solution in a microfuge tube with a glass rod and then they were centrifuged at 12000 rpm for 15 min. The supernatant was filtered through 0.2 µm syringe filter. The filtrate was taken in a micro cuvette and its absorbance was noted using spectrophotometer at wavelength of 540 nm.

The nitrogen content in shoot and root Micro- Kjeldhal method was followed (Jackson, 1967). Dried and powdered samples (0.5 g) of shoot and root samples at 30, 45 and 60 DAS were separately digested with 5 ml of concentrated H₂SO₄ and 200 mg digestion catalyst (K₂SO₄: Cu₂SO₄, Selenium) (100:10:1 ratio) until the contents became clear. After cooling, the volume was made up to 25 ml with distilled water. Then 5 ml of aliquot was transferred to micro-Kjeldhal distillation unit. An aliquot of 10 ml of 40 per cent sodium hydroxide was added and steam distilled. Ammonia evolved was collected into 2% boric acid (20 ml) containing 2 drops of double indicator (83.3 mg bromocresol green and 16.6 mg methyl red indicator dissolved in 10 ml of 95 per cent ethanol) and back titrated against 0.005 N H₂SO₄, until the colour changed from green to red. The percentage of nitrogen was calculated by recording the volume of acid run down. The plant nitrogen was calculated by using the formula,

$$\% \text{ Nitrogen} = \frac{\text{Titrated value} \times \text{No. of acid} \times 0.014 \times \text{Vol. of the designed sample (ml)} \times 100}{\text{Weight of plant sample (g)} \times \text{aliquot taken (ml)}}$$

Results

Relative chlorophyll content

The relative chlorophyll content was measured using SPAD meter at 30, 45 and 60 DAS and the results are presented (Table 1). Inoculation of consortium for green gram varieties, increased the relative chlorophyll content of green gram when compared to controls. Among all observation recorded at different DAS i.e., 30, 45 and 60 the treatment M7 inoculum (44.44, 56.57, 54.08) shown highest SPAD values when compared to other treatments followed by M5 inoculum (41.31, 56.22, 53.87), M6 inoculum (39.99, 52.73, 49.67) and least SPAD values among all treatments was shown by M9 inoculum (34.51, 43.64, 41.40) whereas, M1 inoculum with NC-92 showed SPAD values of 36.91, 47.98, 45.65.

Among the varieties, DGGV-2 with an average of 39.84, 51.57, 49.34 shown higher SPAD values when compared to DGG-96 (37.37, 48.81, 46.58) at 30, 45 and 60 DAS respectively. The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in relative chlorophyll content at 30, 45 and 60 DAS with a range of 43.85-34.20, 56.03-40.53, 53.90-38.25 SPAD values. The treatment M7 with DGGV-2 recorded significantly highest SPAD values of 43.85, 56.03, 53.90 at 30, 45 and 60 DAS respectively.

Number of effective nodules

The number of effective nodules were counted in triplicates at 30, 45 and 60 DAS and the results are documented and represented in Table 2 and plate 1. Inoculation of consortium for green gram varieties, increased the number of effective nodules of green gram at 30, 45 and 60 DAS when compared to controls. Among all observation recorded at different DAS i.e., 30, 45 and 60 the treatment M7 inoculum (18.82, 35.07, 29.60) shown highest number of effective nodules when compared to other treatments followed by M5 inoculum (17.59, 33.04, 25.91) and M8 inoculum (16.38, 30.70, 25.15) and least number of effective nodules among all treatments was shown by M9 inoculum (09.02, 23.58, 18.54) whereas, M1 inoculum with NC-92 showed effective nodule number of 10.81, 25.98, 21.15.

Among the varieties, DGGV-2 with an average of 13.84, 28.35, 23.54 shown slightly higher number of effective nodules when compared to DGG-96 (13.41, 28.38, 23.04) at 30, 45 and 60 DAS respectively. The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in number of effective nodules at 30, 45 and 60 DAS with a range of 19.07-08.63, 35.33-23.50, 30.40-17.50 nodules per plant. The treatment M7 with DGGV-2 recorded significantly higher number of effective nodules with 19.07, 35.33 and 30.40 at 30, 45 and 60 DAS respectively.



A



B

Plate1: Effect of microbial inoculum on root nodulation in (a.) DGGV-2 and (b.) DGG-96**Effective nodule fresh weight**

The observations pertaining nodules fresh weight were recorded at 30, 45 and 60 DAS and the results are presented in Table 3. Inoculation of consortium for green gram varieties, increased the nodules fresh weight of green gram at 30, 45 and 60 DAS when compared to controls. Among all observation recorded at different DAS i.e., 30, 45 and 60 the treatment M7 inoculum (215.83, 284.17, 245.73) shown highest nodules fresh weight in milligrams when compared to other treatments when compared to all treatments followed by M5 inoculum (213.15, 272.00, 234.95) and M8 inoculum (208.35, 256.48, 223.55) and least among all treatments was M9 inoculum (177.38, 172.15, 181.13). Whereas, M1 inoculum with NC-92 showed nodule

fresh weight of 186.99, 187.72, 194.00 milligrams.

Among the varieties, DGGV-2 with an average of 198.61, 221.54, 213.59 shown higher nodules fresh weight in milligrams when compared to DGG-96 (196.22, 226.30, 209.19) at 30, 45 and 60 DAS respectively. The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in effective nodule fresh weight at 30, 45 and 60 DAS with a range of 216.19-176.59, 287.01-170.88, 248.31-176.83mg. The treatment M7 with DGGV-2 recorded significantly highest nodule fresh weight in milligrams with values of 216.19, 287.01 and 248.31 at 30, 45 and 60 DAS respectively.

Table 1: Influence of *Rhizobium* and rhizobacteria on relative chlorophyll content (SPAD value) in green gram varieties DGGV-2 and DGG-96

Treatments	SPAD values								
	30 DAS			45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	38.57	35.25	36.91	47.96	48.01	47.99	46.00	45.29	45.65
M2: HR-73	39.09	35.55	37.32	48.95	47.91	48.43	46.85	45.81	46.33
M3: BR-29	37.03	35.31	36.17	47.17	42.52	44.85	44.93	38.71	41.82
M4: HR-73+ BR-29	38.87	36.35	37.61	51.89	47.83	49.86	50.53	45.87	48.20
M5: HR-73+ AUDP 203+ AUDP 279	43.38	39.23	41.31	55.84	56.60	56.22	53.50	54.24	53.87
M6: BR-29+ AUDP 203 + AUDP 279	41.98	38.00	39.99	55.27	50.18	52.73	50.57	48.76	49.67
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	43.85	45.03	44.44	56.03	57.10	56.57	53.90	54.25	54.08
M8: NC-92 + AUDP 203 + AUDP 279	41.32	37.77	39.55	53.15	50.63	51.89	51.35	47.77	49.56

M9: UIC	34.82	34.20	34.51	46.75	40.53	43.64	44.54	38.25	41.40
M10: HR-73 + BR-29 + NC-92	39.47	37.01	38.24	52.68	46.80	49.74	51.20	46.82	49.01
Mean of variety	39.84	37.37		51.57	48.81		49.34	46.58	
Comparison of	S. Em. ($\pm$)		CD (p=0.01)	S. Em. ($\pm$)		CD (p=0.01)	S. Em. ($\pm$)		CD (p=0.01)
V (Variety)	0.04		0.10	0.02		0.07	0.02		0.05
R (Isolates and consortia)	0.08		0.23	0.05		0.16	0.04		0.11
Interaction V*R (Variety *Isolates)	0.11		0.33	0.08		0.22	0.06		0.16

Effective nodule dry weight

Nodules dry weight were recorded at 30, 45 and 60 DAS and the results are presented in Table 4. Inoculation of consortium for green gram varieties, increased the nodules dry weight of green gram at 30, 45 and 60 DAS when compared to controls. Among all observation recorded at different DAS *i.e.*, 30, 45 and 60 the treatment M7 inoculum (35.88, 54.75, 49.20) shown highest nodules dry weight in milligrams when compared to all treatments when compared to all treatments followed by M5 inoculum (35.32, 53.49, 46.72) and M8 inoculum (34.49, 50.90, 45.20) and least nodules dry weight among other treatments was shown by M9 inoculum (29.42, 36.46, 33.09) whereas, M1 inoculum with NC-92 showed nodule dry weight of 30.32, 41.23, 36.70.

Among the varieties, DGGV-2 with an average of 32.58, 46.44, 41.25 shown slightly higher nodules dry weight in milligrams when compared to DGG-96 (32.26, 45.47, 40.22) at 30, 45 and 60 DAS respectively. The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in effective nodule dry weight at 30, 45 and 60 DAS with a range of 36.05-29.21, 55.79-36.08, 49.80-32.67 mg. The treatment M7 with DGGV-2 recorded significantly highest nodule dry weight in milligrams will values of 36.05, 55.79, 49.80 at 30, 45 and 60 DAS respectively.

Shoot nitrogen content: The shoot nitrogen content in percentage was analyzed at 30, 45 and 60 DAS and the results are presented (Table 5). Inoculation of consortium for green gram varieties, increased the shoot nitrogen content of green gram at 30, 45 and 60 DAS when compared to controls. Among all observation recorded at different DAS *i.e.*, 30, 45 and

60 the M7 inoculum (3.48, 3.49, 2.98) shown highest percentage of shoot nitrogen content when compared to other treatments followed by M5 inoculum (3.32, 3.11, 2.92), M8 inoculum (3.15, 3.15, 2.80) and least percentage of shoot nitrogen content among all treatments was shown by M9 inoculum (1.62, 1.75, 1.64) whereas, M1 inoculum with NC-92 showed shoot nitrogen content of 2.55, 2.54, 1.86%. Among the varieties, DGGV-2 with an average of 2.83, 2.80, 2.34 shown higher percentage shoot nitrogen content when compared to DGG-96 (2.67, 2.69, 2.22) at 30, 45 and 60 DAS respectively.

The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in shoot nitrogen content at 30, 45 and 60 DAS with shoot nitrogen content range of range of 3.50-1.27, 3.55-1.53, 3.15-1.49. The treatment M7 with DGGV-2 recorded significantly highest percentage of shoot nitrogen content of 3.50, 3.55, 3.15 at 30, 45 and 60 DAS respectively.

Root nitrogen content

The nitrogen content of root was analyzed at 30, 45 and 60 DAS and the results are presented. (Table 6) Inoculation of consortium for green gram varieties, increased the root nitrogen content of green gram at 30, 45 and 60 DAS when compared to controls. Among all observation recorded at different DAS *i.e.*, 30, 45 and 60 the treatment M7 inoculum (1.00, 1.44, 0.85) shown highest percentage of root nitrogen content when compared to other treatments followed by M5 inoculum (0.85, 1.33, 0.82), M8 inoculum (0.79, 1.18, 0.67) and least percentage of root nitrogen content among all treatments was shown by M9 inoculum (0.05, 0.20, 0.00) whereas, M1 inoculum with NC-92 showed root nitrogen of 0.32, 0.82, 0.17%.

Table 2: Influence of *Rhizobium* and rhizobacteria on number of nodules in green gram varieties DGGV-2 and DGG-96

Treatments	Number of nodules								
	30 DAS			45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	10.73	10.88	10.81	26.54	25.41	25.98	20.80	21.50	21.15
M2: HR-73	11.63	11.45	11.54	27.50	26.74	27.12	21.54	22.27	21.91
M3: BR-29	10.07	09.80	09.94	24.50	24.89	24.70	19.50	20.21	19.86
M4: HR-73+ BR-29	13.23	12.50	12.87	28.00	27.64	27.82	23.50	22.40	22.95
M5: HR-73+ AUDP 203+ AUDP 279	17.90	17.27	17.59	33.65	32.43	33.04	26.33	25.49	25.91
M6: BR-29+ AUDP 203 + AUDP 279	15.50	15.30	15.40	29.60	29.78	29.69	25.50	23.59	24.55
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	19.07	18.57	18.82	35.33	34.81	35.07	30.40	28.80	29.60
M8: NC-92 + AUDP 203 + AUDP 279	16.63	16.13	16.38	31.30	30.11	30.71	26.23	24.07	25.15
M9: UIC	09.40	08.63	09.02	23.50	23.65	23.58	17.50	19.58	18.54
M10: HR-73 + BR-29 + NC-92	14.20	13.53	13.87	28.53	28.31	28.42	24.13	22.50	23.32
Mean of variety	13.84	13.41		28.85	28.38		23.54	23.04	
Comparison of	S. Em. ($\pm$)		CD (p=0.01)	S. Em. ($\pm$)		CD (p=0.01)	S. Em. ($\pm$)		CD (p=0.01)
V (Variety)	0.02		0.08	0.02		0.05	0.03		0.09
R (Isolates and consortia)	0.05		0.19	0.04		0.10	0.07		0.20
Interaction V*R (Variety Isolates)	0.07		0.27	0.05		0.14	0.10		0.28

Table 3: Influence of *Rhizobium* and rhizobacteria on nodules fresh weight in green gram varieties DGGV-2 and DGG-96

Treatments	Nodules fresh weight (mg)								
	30 DAS			45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	190.85	183.12	186.99	184.59	190.85	187.72	193.27	194.72	194.00
M2: HR-73	191.75	189.62	190.69	197.84	204.36	201.10	211.01	198.19	204.60
M3: BR-29	181.75	179.93	180.84	176.55	179.86	178.21	179.94	187.35	183.65
M4: HR-73+ BR-29	196.85	194.06	195.46	213.04	219.50	216.27	212.59	204.04	208.32
M5: HR-73+ AUDP 203+ AUDP 279	213.49	212.81	213.15	269.13	274.86	272.00	237.33	232.56	234.95
M6: BR-29+ AUDP 203 + AUDP 279	206.01	204.17	205.09	238.91	246.44	242.68	226.84	221.54	224.19
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	216.19	215.47	215.83	287.01	281.33	284.17	248.31	243.14	245.73
M8: NC-92 + AUDP 203 + AUDP 279	209.33	207.36	208.35	252.77	260.20	256.49	227.93	219.17	223.55
M9: UIC	178.16	176.59	177.38	170.88	173.42	172.15	176.83	185.42	181.13
M10: HR-73 + BR-29 + NC-92	201.67	199.04	200.36	224.63	232.18	228.41	221.85	205.76	213.81
Mean of variety	198.61	196.22		221.54	226.30		213.59	209.19	
Comparison of	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)
V (Variety)	0.24		0.70	0.22		0.62	0.12		0.36
R (Isolates and consortia)	0.55		1.57	0.48		1.38	0.28		0.80
Interaction V*R (Variety *Isolates)	0.77		2.21	0.68		1.95	0.39		1.13

Table 4: Influence of *Rhizobium* and rhizobacteria on nodules dry weight in green gram varieties DGGV-2 and DGG-96

Treatments	Nodules dry weight (mg)								
	30 DAS			45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	30.49	30.14	30.32	41.89	40.57	41.23	37.27	36.13	36.70
M2: HR-73	31.28 ^b	30.72	31.00	43.25	42.91	43.08	38.28	37.80	38.04
M3: BR-29	30.32	29.75	30.04	39.62	37.86	38.74	35.97	34.70	35.34
M4: HR-73+ BR-29	31.66	32.24	31.95	45.67	44.68	45.18	39.93	38.60	39.27
M5: HR-73+ AUDP 203+ AUDP 279	35.51	35.12	35.32	54.03	52.94	53.49	47.64	45.80	46.72
M6: BR-29+ AUDP 203 + AUDP 279	33.54	33.45	33.50	49.03	49.00	49.02	43.40	42.93	43.17
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	36.05	35.70	35.88	55.79	53.71	54.75	49.80	48.60	49.20
M8: NC-92 + AUDP 203 + AUDP 279	34.92	34.05	34.49	51.05	50.75	50.90	45.60	44.80	45.20
M9: UIC	29.62	29.21	29.42	36.84	36.08	36.46	33.50	32.67	33.09
M10: HR-73 + BR-29 + NC-92	32.39	32.21	32.30	47.18	46.22	46.70	41.13	40.20	40.67
Mean of variety	32.58	32.26		46.44	45.47		41.25	40.22	
Comparison of	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)
V (Variety)	0.03		0.08	0.01		0.03	0.03		0.08
R (Isolates and consortia)	0.07		0.19	0.02		0.06	0.06		0.18
Interaction V*R (Variety *Isolates)	0.09		0.27	0.03		0.09	0.09		0.26

Among the varieties, DGGV-2 with an average of 0.60, 1.00, 0.47 shown higher percentage root nitrogen content when compared to DGG-96 (0.52, 0.94, 0.39) at 30, 45 and 60 DAS respectively. The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in root nitrogen content at 30, 45 and 60 DAS with root nitrogen content range of 1.07-0.04, 1.47-0.03, 0.97-0.09. The treatment M7 with DGGV-2 recorded significantly highest percentage of root nitrogen content with values of 1.07, 1.47, 0.97 at 30, 45 and 60 DAS respectively.

Leghemoglobin content

The leghemoglobin content in treatments was analyzed at 45 DAS and the results are presented. (Table 8) Inoculation of consortium for green gram varieties, increased the Leghemoglobin content of green gram significantly as compared

to controls.

Among all observation recorded across all different treatments, microbial consortium with M7 inoculum (0.270) shown highest Leghemoglobin content when compared to other treatments followed by M5 inoculum (0.245), M6 inoculum (0.218) and least Leghemoglobin content among all treatments was shown by M9 inoculum (0.173) whereas, M1 inoculum with NC-92 showed Leghemoglobin content of 0.180. Among the varieties, DGGV-2 with an average of 0.196 shown Leghemoglobin content higher Leghemoglobin content when compared to DGG-96 (0.188) at 45 DAS.

The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in Leghemoglobin at 45 DAS with a range of 0.287-0.137. The treatment M7 with DGGV-2 recorded significantly highest Leghemoglobin of 0.287 at 45DAS.

Table 5: Influence of *Rhizobium* and rhizobacteria on shoot nitrogen content in green gram varieties DGGV-2 and DGG-96

Treatments	Shoot nitrogen content (%)								
	30 DAS			45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	2.66	2.43	2.55	2.63	2.45	2.54	1.82	1.89	1.86
M2: HR-73	2.73	2.50	2.62	2.73	2.66	2.70	2.03	2.07	2.05
M3: BR-29	2.52	1.73	2.13	2.38	1.72	2.05	1.82	1.82	1.82
M4: HR-73+ BR-29	2.80	2.73	2.77	2.79	2.70	2.75	2.24	2.17	2.21
M5: HR-73+ AUDP 203+ AUDP 279	3.20	3.43	3.32	3.20	3.37	3.29	3.11	2.73	2.92
M6: BR-29+ AUDP 203 + AUDP 279	2.88	3.08	2.98	2.74	2.96	2.85	2.45	2.17	2.31
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	3.50	3.45	3.48	3.55	3.42	3.49	3.15	2.80	2.98
M8: NC-92 + AUDP 203 + AUDP 279	3.15	3.15	3.15	3.15	3.15	3.15	3.01	2.59	2.80
M9: UIC	1.97	1.27	1.62	1.96	1.53	1.75	1.49	1.79	1.64
M10: HR-73 + BR-29 + NC-92	2.85	2.94	2.90	2.85	2.94	2.90	2.24	2.17	2.21
Mean of variety	2.83	2.67		2.80	2.69		2.34	2.22	
Comparison of	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)
V (Variety)	0.01		0.03	0.01		0.02	0.01		0.02
R (Isolates and consortia)	0.02		0.07	0.01		0.04	0.02		0.05
Interaction V*R (Variety *Isolates)	0.03		0.10	0.02		0.06	0.02		0.07

Table 6: Influence of *Rhizobium* and rhizobacteria on root nitrogen content in green gram varieties DGGV-2 and DGG-96

Treatments	Root nitrogen content (%)								
	30 DAS			45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	0.37	0.28	0.33	0.90	0.73	0.82	0.16	0.18	0.17
M2: HR-73	0.48	0.36	0.42	0.95	0.90	0.93	0.28	0.34	0.31
M3: BR-29	0.40	0.13	0.27	0.80	0.15	0.48	0.13	0.14	0.14
M4: HR-73+ BR-29	0.63	0.47	0.55	1.00	1.08	1.04	0.40	0.39	0.40
M5: HR-73+ AUDP 203+ AUDP 279	0.90	0.79	0.85	1.25	1.40	1.33	0.92	0.72	0.82
M6: BR-29+ AUDP 203 + AUDP 279	0.75	0.75	0.75	1.07	1.13	1.10	0.61	0.36	0.49
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	1.07	0.93	1.00	1.47	1.40	1.44	0.97	0.73	0.85
M8: NC-92 + AUDP 203 + AUDP 279	0.83	0.75	0.79	1.19	1.18	1.19	0.75	0.58	0.67
M9: UIC	0.04	0.05	0.05	0.37	0.30	0.34	0.09	0.08	0.09
M10: HR-73 + BR-29 + NC-92	0.67	0.68	0.68	1.04	1.10	1.07	0.40	0.37	0.39
Mean of variety	0.60	0.52		1.00	0.94		0.47	0.39	
Comparison of	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)	S. Em. (+)		CD (p=0.01)
V (Variety)	0.01		0.03	0.01		0.03	0.01		0.03
R (Isolates and consortia)	0.02		0.06	0.03		0.07	0.02		0.06
Interaction V*R (Variety *Isolates)	0.03		0.09	0.04		0.10	0.03		0.09

Table 7: Influence of *Rhizobium* and rhizobacteria on leghaemoglobin content in green gram varieties DGGV-2 and DGG-96

Treatments	Leghaemoglobin		
	DGGV-2	DGG-96	Mean values
Microbial inoculants	0.160	0.200	0.180
M1: NC-92 (Reference strain)	0.170	0.140	0.155
M2: HR-73	0.140	0.153	0.147
M3: BR-29	0.190	0.150	0.170
M4: HR-73+ BR-29	0.250	0.240	0.245
M5: HR-73+ AUDP 203+ AUDP 279	0.230	0.207	0.218
M6: BR-29+ AUDP 203 + AUDP 279	0.287	0.253	0.270
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	0.210	0.183	0.197
M8: NC-92 + AUDP 203 + AUDP 279	0.137	0.210	0.173
M9: UIC	0.187	0.147	0.167
M10: HR-73 + BR-29 + NC-92	0.196	0.188	
Mean of variety	S. Em. (±)		CD (p=0.01)
Comparison of	0.002		0.007
V (Variety)	0.040		0.016
R (Isolates and consortia)	0.006		0.022

Discussion

Green gram plants shoot nitrogen content, root nitrogen content, number of effective nodules, nodule fresh weight, nodule dry weight, leghemoglobin content showed significant differences at 30,45 and 60 DAS. Treating seeds with consortia increased nitrogen content, nodule number and nodule weight along with leghemoglobin content over NC-92, HR-73, BR-29 and

uninoculated control because of improved infectivity of rhizobial isolates. Highest values of these parameters were recorded for plants treated with M7(HR-73+ BR-29+ AUDP 203 + AUDP 279) inoculum. Among the varieties DGGV-2 shown higher values then DGG-96 and the interaction effect of *Rhizobium* and rhizobacteria was positively correlated in green gram varieties for parameters like shoot nitrogen content, root

nitrogen content, number of effective nodules, nodule fresh weight, nodule dry weight, leghemoglobin. Interaction effect in DGGV-2 variety with M7 microbial inoculum treatment shown highest value at all stages plant sample analysis when compared to all other treatments.

The similar results were reported by Baghel *et al.* (2024) ^[1] conducted field experiment in Bangladesh and reported that inoculation of *Rhizobium* sps. BARIRGm901 produced greater nodule numbers, nodule weight, shoot and root biomass, and plant height than control plants. Bhabai *et al.* (2019) ^[2] reported inoculation of *Rhizobium* + VAM+ PSB increased effective root nodule plant⁻¹ and nitrogen use efficiency after conducting an experiment at West Bengal. Justin *et al.* (2022) ^[4] conducted an experiment at Cameron and reported that rhizobial isolates significantly increased the plant growth and nodule weight as compared to uninoculated control. They revealed that identified *Rhizobium* belonging to the same cross inoculation group were useful in symbiotic N₂ fixation, plant growth and yield and enhancing nodule weight in the studied legume.

The results revealed that two varieties were on par with each other and DGGV-2 being the best host for inoculums which was indicated by nodule number, fresh and dry weight of nodules. The efficiency of rhizobial strains was improved with combination of rhizobacteria which is depicted in leghemoglobin content where higher values were observed for microbial inoculum with rhizobacteria. Improved efficiency of rhizobial strains leads to higher nitrogen content in plants, which was proved in present study with higher values of shoot and root nitrogen content for seeds treated with M7(HR-73+ BR-29+ AUDP 203 + AUDP 279) microbial inoculum.

From the above observations, the higher values of shoot nitrogen content, root nitrogen content, number of effective nodules, nodule fresh weight, nodule dry weight, leghemoglobin were observed in plants with consortium treatment followed at 45 DAS when plants are at peak flowering and pod filling stage highest content of nitrogen in plants and number of nodules along with weight of nodules was observed then at 30 DAS indicating the need of plant nutrients for meeting plant nutritional requirement in metabolic activities. Decline of nitrogen content and number of nodules along with weight of nodules at 60 DAS was observed with symptoms of crop harvest and beginning of senescence (Sharma *et al.*, 2024) ^[5].

Conclusion

The study conclusively demonstrated that inoculation with *Rhizobium* and rhizobacteria significantly improves nitrogen content and uptake in green gram. Consortium treatments were more effective than individual inoculants, reflecting enhanced biological nitrogen fixation and better nutrient translocation within the plant system. Improved nitrogen nutrition under microbial treatments highlights the importance of selecting efficient and compatible microbial strains for sustainable nutrient management. These findings support the use of microbial consortia as an eco-friendly strategy to enhance nitrogen availability and productivity in pulse-based cropping systems. Further field-level studies are recommended to validate the consistency of these results under diverse agroclimatic conditions.

Acknowledgments

I am extremely grateful to AICRP on EAAI (Bioconversion Technology) for timely issuing materials, chemical and assistance whenever required.

References

1. Baghel D, Gupta SB, Chowdhury T. Characterization and evaluation of native *Rhizobium* of groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.). Journal of Experimental Agriculture International. 2024;46(5):500-506.
2. Bhabai B, Mukhopadhyay D, Mitra B. Effect of biofertilizer and phosphorus on green gram (*Vigna radiata*). Journal of Pharmacognosy and Phytochemistry. 2019;8(4):505-509.
3. Jackson ML. Soil chemical analysis. New Delhi: Prentice-Hall of India Pvt Ltd; 1967. p. 1-498.
4. Justin G, Doloum G, Laurette NN, Steve TT, Albert N. Differential traits of rhizobia associated to root nodules of gum acacia (*Senegalia senegal*), shittah tree (*Vachellia seyal*), pigeon pea (*Cajanus cajan* L.) and cowpea (*Vigna unguiculata*). Microbiology Research. 2022.
5. Sharma M, Sorathia K, Jayanthi G, Karthikeyan K. Study on plant growth promoting traits of PGPR isolates from semi-arid Kachchh, Gujarat under salt stress conditions. 2024.