Int.J.Curr.Microbiol.App.Sci (2019) *8*(11): 134-139

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 11 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.811.016

Effect of *Rhizobium* Inoculation on Yield and Nodule Formation of Cowpea

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Keywords

Rhizobium inoculation, Genotypes of cowpea, Nodulation

Article Info

Accepted:
04 October 2019
Available Online:
10 November 2019

A B S T R A C T

A pot experiment was carried out at the Department of Genetics and Plant Breeding, Uttar Banga Krishi Viswavidalaya, Pundibari, Cooch Behar (West Bengal) under natural condition during the rabi season of 2011-2012 and 2012-2013 to find out the response of inoculation by three strains of *Rhizobium* with different plant genotypes of cowpea. Five varieties of cowpea viz. Sundari Bangla, Reenu, Lafa Barbati, Baijanti and Kashi Kanchan and three strains of rhizobium viz. RH41, Cowpea 3 and Cowpea 23 were used in the experiment. Each variety was tested with and without inoculation. Inoculated plants gave significantly higher nodule number, nodule weight, root weight, shoot weight, seed yield and nitrogen in soil after harvest compared to non-inoculated plants. Among the five varieties, Sundari Bangla produced the highest yield in both the years.

Introduction

The growing demand for food associated with rising population has necessitated the increased need for fertilization. But too much reliance on chemical fertilization has led to various environmental hazards as well as the cost of chemical fertilizers is becoming un-affordable for the farmers. Thus in order to make farming system more sustainable, some means are to be evolved to reduce the need for chemical fertilizers, in particular the nitrogen fertilizers. Legume has long been known for their unique ability to enrich the soil through biological nitrogen fixing system in symbiotic association with rhizobial strains.

There is a great possibility to increase production of legume plants by exploiting better colonization of their root and rhizosphere through rhizobial inoculation, which can fix atmospheric nitrogen and protect nature from pollution. Of the several legumes, cowpea is also known for their high

quality nutritional values and they are strongly dependent on the nitrogen fixation for their nitrogen nutrition.

Rhizobium species invade the root hairs of cowpea and result in the formation of nodules, where free air nitrogen is fixed. These bacteria, which are present in most of the soils, vary in number, effectiveness in nodulation and N-fixation. Although this crop is capable of fixing atmospheric nitrogen through *Rhizobium* species living in root nodules, however, under the agro-ecological conditions of North Bengal, the nodulation of cowpea is poor and is a major cause of its lower yield. The present study was conducted to assess the relative ability of the three *Rhizobium* strains viz. RH41, Cowpea 3 and Cowpea 23 for nodulation and subsequent effect on soil nitrogen content and yield in five different genotypes of cowpea.

Materials and Methods

To evaluate the response of five selected genotypes of cowpea to *Rhizobium* inoculation, pot experiments were conducted under natural at Uttar Banga Krishi Vishwavidalaya, Pundibari, Cooch Behar during the period 2011 to 2013. The experiment was laid out in Completely Randomized Design (CRD) with two factors viz., genotype and *Rhizobium* culture.

Five cowpea genotypes viz. Sundari Bangla, Reenu, Lafa Barbati, Baijanti and Kashi Kanchan were sown in the earthen pots 30 cm height with 30 cm top and 15 cm bottom diameter. The pots were filled with 5 kg dry sandy loam soil with minimum water holding capacity of the experimental site and sterilized by autoclaving at 121^0C temperature and 15 psi pressure for 30 minutes.

Three peat based *Rhizobium* strains viz. RH41, Cowpea 3 and Cowpea 23 used in the experiment were collected from Nodule Research Laboratory, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. Seeds of *Vigna unguiculata* with uniform shape, size and weight were surface sterilized with 3% sodium hypo chloride solution for 10 minutes followed by 4 washings with sterilized water. For inoculation, the seeds were moistened with a small amount of water and pelted with peat based *Rhizobium* containing 10^7 per gram with concentrated sugar solution as an adhesive. Ten (10) seeds were sown in each sterilized pots following drying. A basal dose of 20 mg kg^{-1} N as urea, 30 mg kg^{-1} P_2O_5 as triple super phosphate and 30 mg kg^{-1} K_2O as potassium sulphate was applied before sowing. Un-inoculated plants with N-fertilizer.and without N-fertilizer were included for comparison.

During the course of the experiment, growth and developments of plants in the pot were carefully observed. Intercultural operations and other management practices were done as per requirements. Nodulation was recorded during 45 days after sowing (DAS) at 50% flowering stage by selecting five plants from each pot at random. The plants were up-rooted taking care not to disturb the roots and root hairs. They were washed under running tap water. The number of nodules per plant was counted after which the nodules were carefully removed from the roots and their fresh weight recorded. The data regarding nitrogen content in soil and grain yield/plant were recorded after the harvest of the crop at physiological maturity. Observations were recorded on five plants in each replication. Total nitrogen content of the soil after the harvest of the crop was determined by modified Kjeldhal method. The collected data were subjected to statistical analysis using Fisher's analysis of variance techniques. Duncan's Multiple Range (DMR) Test (Steel & Torrie, 1980) was used to compare the differences among treatment means..

Results and Discussion

Table 1 represents data on nodulation, nodule weights and root weights of the five cultivars as influenced by the different *Rhizobium* strains. Nodulation data was recorded at flowering stage because at maturity most of the nodules were disintegrated and decomposed. Seed inoculation significantly affected number of nodules and the treatments significantly differed from each other as compared to the control. The highest nodule number per plant (166 and161.34 plant^{-1}) and nodule weight (1142.11 and 1131.23mg plant^{-1}) were recorded by the genotype Kashi Kanchan in the year 2011-2012 and 2012-2013 respectively in response to *Rhizobium* culture RH41. The same culture also produced the maximum value for nodulation in the remaining varieties except in Lafa Barbati where the highest value of nodulation was observed with the inoculation of the *Rhizobium* strain cowpea 3. Nodulation in terms of nodule number and nodule fresh biomass was observed lower in control with nitrogen fertilizer. Similar kinds of observations were reported by Subasinghe *et al.*,(2001) and Oteino *et al.*,(2009) who revealed the inhibitory effect of nitrogen fertilizer on nodule formation in grain legumes and cowpea respectively. For the character root weight, the maximum value (3.57 and 3.35 g plant^{-1}) was recorded by the inoculated Kashi Kanchan variety with the culture cowpea 23 in the year 2011-2012 and 2012-2013 respectively.

Data regarding shoot weight, seed yield and N-content in soil after the harvest of the crop was presented in Table 2. The maximum shoot weight was observed by the inoculation of culture RH 41 in all the cowpea genotypes where the highest value was recorded by the variety Reenu (11.32 and 10.92 g plant^{-1}) in the year 2011-2012 and 2012-2013 respectively. When the seed yield per plant was considered the inoculant RH41 was observed to produced maximum yield in four of the five cowpea genotypes under investigation where the highest seed yield ((17.99 and 17.67 g plant^{-1}) was recorded in the cultivar Sundari Bangla in the year 2011-2012 and 2012-2013 respectively. The lowest seed yield (12.12 g plant^{-1}) was observed in un-inoculated Reenu variety without N-fertilizer. These results are in line with the findings of Pawar and co-workers(1998) and Giri *et al.*,(2010)who reported that seed inoculated plants exhibited significantly greater number of nodules per plant, root weight, shoot weight and seed yield as compared to un-inoculated control plants in chickpea. The results pertaining to soil N-content after harvest showed significant interaction between the treatments and varieties. The investigation showed that almost all the *Rhizobium* strains significantly improved the soil N contents after harvesting of the crop except the culture RH 41. Maximum soil N-content (0.21%) were recorded in the variety Sundari Bangla and Kashi Kanchan with the seed inoculation of *Rhizobium* culture cowpea 3 while minimum value were observed in case of control without N-fertilizer. Similar results were reported Rshid *et al.,* (1999) and Zammurad *et al.,* (2008) who found that soil N-content was significantly affected by *Rhizobium* inoculation.

On the basis of this study, it is concluded that inoculation of seed with *Rhizobium* significantly affected the nodule formation of cowpea as well as N-content of soil which consequently led to improved soil fertility and can reduce the production cost of next crop through reduced input in the form of N-fertilizers, which in turn also minimize the health hazard effects. *Rhizobium* inoculation was found to be more effective and produced better yield when compared with no inoculation.

Table.1 Effects of inoculation on nodulation and root weight of selected cowpea genotypes

Treatment	Nodule no. per plant		Nodule weight (mg plant^{-1})		Root weight (g plant^{-1})	
	2011-12	2012-13	2011-12	2012-13	2011-12	2012-13
Sundari Bangla x UWN	40.13d	39.08d	162.14d	155.20e	2.79b	2.65b
Sundari Bangla x UN	30.01e	27.32d	80.34NS	79.32NS	1.32c	1.26c
Sundari Bangla x RH41	100.00b	95.34b	905.00a	897.00b	2.20b	1.13c
Sundari Bangla x cowpea 3	95.12b	90.22b	759.11b	747.55b	3.10a	3.00a
Sundari Bangla x cowpea 23	78.32c	72.34c	749.10b	638.24c	3.08a	2.98a
Reenu x UWN	37.00e	33.66e	100.23e	92.43f	1.57c	1.43c
Reenu x UN	33.23e	27.43e	56.10NS	44.56NS	1.99b	1.79c
Reenu x RH41	77.50c	72.50c	611.13b	595.53c	2.47b	2.25b
Reenu x cowpea 3	59.00d	53.00d	374.00c	362.00d	1.30c	1.14c
Reenu x cowpea 23	50.00d	45.34d	435.00c	423.00d	1.10c	0.90d
Lafa Barbati x UWN	36.24e	32.42e	120.00e	110.13e	1.05c	0.93d
Lafa Barbati x UN	40.00d	38.00e	239.22d	223.44e	1.40c	1.26c
Lafa Barbati x RH41	71.14c	67.52b	600.34b	593.00c	1.34c	1.14c
Lafa Barbati x cowpea 3	75.13c	71.53c	783.00b	769.00b	2.20b	2.04b
Lafa Barbati x cowpea 23	65.00c	61.00c	549.11c	534.23c	3.18a	2.98a
Baijanti x UWN	47.00d	43.00d	340.00d	322.00d	1.58c	1.36c
Baijanti x UN	33.00e	29.00d	193.00e	183.00e	1.47c	1.29c
Baijanti x RH41	164.00a	156.00a	1132.00a	1034.66a	3.33a	3.13a
Baijanti x cowpea 3	71.00c	63.00c	570.24c	554.42c	1.56	1.34c
Baijanti x cowpea 23	61.00c	55.00d	529.11c	513.55c	2.65b	2.35b
Kashi Kanchan x UWN	68.00c	62.00c	336.34d	324.32d	1.36c	1.20c
Kashi Kanchan x UN	34.33e	28.00e	100.00e	91.34f	1.64c	1.38c
Kashi Kanchan x RH41	166.00a	161.34a	1142.11a	1131.23a	1.76c	1.52c
Kashi Kanchan x cowpea 3	98.00b	92.00b	741.00b	732.34b	2.00b	1.84c
Kashi Kanchan x cowpea2 3	94.00b	88.00b	680.00b	660.00c	3.57a	3.35a
S.E.(m)	6.59	5.99	76.18	75.00	0.20	0.18

Means followed by common letter are not significantly different at 5% DMRT
NS= non-significant, UWN= un-inoculated without nitrogen, UN = Un-inoculated with nitrogen

Table.2 Effects of inoculation on shoot weight, seed yield and N-content in the selected cowpea genotypes

Treatment	Shoot weight (g plant^{-1})		Seed yield(g plant^{-1})		N content in soil (%)	
	2011-12	2012-13	2011-12	2012-13	2011-12	2012-13
Sundari Bangla x UWN	5.00c	4.80d	15.10b	14.78b	0.11c	0.10d
Sundari Bangla x UN	6.54c	6.10c	16.00a	15.18b	0.13c	0.11c
Sundari Bangla x RH41	9.30a	9.08a	17.99a	17.67a	0.12c	0.10c
Sundari Bangla x cowpea 3	7.86b	7.42b	17.80a	17.54a	0.21a	0.19a
Sundari Bangla x cowpea 23	6.81c	6.33c	16.32a	16.10a	0.18a	0.16b
Reenu x UWN	6.79c	6.35c	13.30c	12.12c	0.11d	0.10d
Reenu x UN	8.00b	7.76b	15.78b	15.35b	0.15b	0.13c
Reenu x RH41	11.32a	10.92a	16.40a	16.20a	0.12c	0.10d
Reenu x cowpea 3	6.70c	6.00c	15.60b	15.40b	0.17a	0.15b
Reenu x cowpea 23	5.91c	5.31c	16.55a	16.25a	0.18a	0.16b
Lafa Barbati x UWN	6.76c	6.10c	13.75c	13.53c	0.11d	0.10d
Lafa Barbati x UN	6.67c	6.23c	13.55c	13.25c	0.14c	0.12c
Lafa Barbati x RH41	9.45a	8.93b	17.45a	17.29a	0.12c	0.10b
Lafa Barbati x cowpea 3	5.50c	5.06c	17.08a	16.90a	0.18a	0.16b
Lafa Barbati x cowpea 23	7.49b	7.03b	14.22b	14.12b	0.20a	0.18a
Baijanti x UWN	5.24c	8.14b	13.31c	13.11c	0.11d	0.10d
Baijanti x UN	8.52b	4.90d	13.84c	13.66c	0.15b	0.13c
Baijanti x RH41	10.09a	9.77a	15.90b	15.76b	0.12c	0.10d
Baijanti x cowpea 3	7.41b	7.17b	14.40b	14.22b	0.18a	0.16b
Baijanti x cowpea 23	7.67b	7.13b	13.28c	13.16c	0.16b	0.14c
Kashi Kanchan x UWN	5.60c	5.12c	13.29c	13.15c	0.11d	0.10d
Kashi Kanchan x UN	8.00b	7.74b	14.95b	14.85b	0.13c	0.11c
Kashi Kanchan x RH41	9.11a	8.83b	17.80a	17.64a	0.13c	0.11c
Kashi Kanchan x cowpea 3	5.52c	5.18c	16.00a	15.88b	0.21a	0.19a
Kashi Kanchan x cowpea2 3	8.00b	7.62b	16.45a	16.35a	0.18b	0.16b
S.E.(m)	0.69	0.61	0.60	0.56	0.011	0.009

Means followed by common letter are not significantly different at 5% DMRT
NS= non-significant, UWN= un-inoculated without nitrogen, UN = Un-inoculated with nitrogen

Among the *Rhizobium* strain, the culture RH41 was found to be the most promising one for inoculation in the cowpea genotype as it proved its efficiency in nodulation and yield quality in almost all the varieties under investigation. More field trial is imperative in the agro-ecological regions of Pundibari District (West Bengal) to confirm these findings.

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How to cite this article:

Thiyam Rebika and Nabakishor Nongmaithem. 2019. Effect of *Rhizobium* Inoculation on Yield and Nodule Formation of Cowpea. *Int.J.Curr.Microbiol.App.Sci.* 8(11): 134-139.
doi: https://doi.org/10.20546/ijcmas.2019.811.016