Economic Affairs
Citation: EA: 61(3): 495-499, September 2016

Efficacy of *Rhizobium* inoculation on graded N levels and net return from cluster bean seed production under hot arid regions

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ABSTRACT

Effectiveness of seed inoculation with *Rhizobium* on cluster bean grain yield was assessed in field experiments under graded doses of nitrogen application *viz*., 15, 30, 45 and 60 kg N/ha. The data revealed that inoculation significantly increased the cluster bean seed grain yield at all levels of nitrogen application including control. Application of 60 kg N/ha along with *Rhizobium* inoculation maximized cluster bean seed yield (13.28 q/ha) which was 152.47% higher and significantly better than the yield obtained at same level of N application without *Rhizobium* inoculation. However, it was statistically at par with 60 kg N application and 45 kg N/ha + *Rhizobium* (12.26 q/ha). Higher value yield, nitrogen use efficiency and apparent nutrient recovery were optimized under seed inoculation with *Rhizobium* as compared to non inoculated seeds. The results depicts the beneficial effects of *Rhizobium* inoculation on cluster bean grain yield resulting in saving of 13.37 to 21.73 kg/ha nitrogen and an enhanced seed yield ranging from 2.34 to 8.05 q/ha along with nitrogen application compared to control.

Keywords: Cluster bean, hot arid region, nitrogen, *Rhizobium*, seed yield

Fertilizers contribute a lot in fulfilling the nutrient requirement of crops but their regular, excessive and unbalanced use may lead to health and ecological hazards, depletion of physiochemical properties of the soil and ultimately poor crop yields. Nitrogen is the first limiting plant nutrient in arid region, has a great influence on crop growth, yield and its quality. The desert soils are generally deficient in organic matter thus unable to release N at a rate required to maintain adequate N supply to the growing plant. Further, the problem of nutrient drain from the soil is becoming so acute that it is beyond the capacity of any single fertilizer to accept the challenge of appropriate nutrient supply. Hence, alternate sources of nutrients which may enhance crop yield without having adverse effects on soil properties and the use of bio-fertilizers seems to be a better proposition in this aspect.

Access this article online	
Publisher	Website: http://www.ndpublisher.in
	DOI: 10.5958/0976-4666.2016.00062.0

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Cluster bean has acquired a status of vegetable crop due to its high yield and suitability for arid region. Water stress causes both reductions in the rate of protein synthesis as well as changes in the type of proteins produced. It is believed that these stress induced proteins allow plants to make biochemical and structural adjustments that enable plants to cope with the stress (Pratap and Sharma, 2010). Though the area under cluster bean is maximum in Rajasthan yet its average productivity is very low as compared to Gujarat, Haryana and Punjab (Henry, 2003 and Rathore, *et al.*, 2007). Limited moisture and improper nutrient management are considered to be the major limiting factors for achieving higher seed yield of cluster bean in arid region. Rainfall pattern for major portion of the year supports short duration crops like legumes and they achieve higher grain yield than the long duration crops (Rao and Singh, 2004).

The nutrient management thus assumes importance for higher seed yield. Given the poor socio-economic conditions of farmers coupled with low and erratic rainfall distribution, intensive use of chemical fertilizers is a risky preposition in these regions. Low-cost nutrient supplementation through biofertilizers as integrated nutrient supply system may be a better option to fulfill nutrient requirement of the seed crops. Bio-fertilizers play a vital role in maintaining long term soil fertility and sustainability, increasing yield of crops by 10-30 percent (Khandelwal *et al.*, 2012 and Sammauria *et al.*, 2009). Hence, the present study was undertaken to assess the the effectiveness of *Rhizobium* cultures for improving growth and seed yield of cluster bean under fertilized conditions.

Materials and Methods

The field experiment was conducted at ICAR-Central Institute for Arid Horticulture, Bikaner during rainy season of 2014-2015. The soil was sandy loam in texture, alkaline in reaction with pH 8.4, organic carbon 0.11%, available N 110 kg/ha, available P 12 kg/ha and available K 320 kg/ha . The experiment was laid out in randomized block design with 3 replications, nutrient management treatments comprised of five N levels (0, 15, 30, 45 & 60 kg/ha) with and without *Rhizobium* inoculation, thus making a total of 10 treatments in all. Cluster bean variety "Thar Bhadavi" was sown with hand plough at row-to-row spacing of 45cm and plant to plant distance of 20 cm. Recommended dose of fertilizers (60 kg of P_2O_5 +60 K_2O /ha) was applied as basal dose through single superphosphate and MOP, respectively. Nitrogen was applied as per treatments. Seed was inoculated with *Rhizobium* culture mixed in 5% sucrose solution for 10 minutes and dried in shade before planting. Likewise, the rest of Seed was treated in similar way but without bio-fertilizers. Other cultural practices were followed as per recommendation for the crop in the region. Three life saving irrigations was applied as and when needed to obtain a satisfactory crop stand. Data was statistically analyzed as per Gomez and Gomez (1984).

Crop was harvested at maturity and cluster bean-grain yield was recorded. Cluster bean-grain yield at varying levels of nitrogen with or without *Rhizobium* were fitted in quadratic response model (Y = ax^2 + bx + c) and amount of nitrogen required (kg/ha) for target yield (T) was calculated using the relationship in absence and presence of *Rhizobium*:

Nitrogen (kg/ha) required in presence or absence of *Rhizobium* to obtain different-target yields (T)

$$= \frac{-b \pm \sqrt{b \;-\; a(c-T)}}{}$$

Where a, b and c are regression co-efficients of the quadratic equation

Percent yield response and nitrogen use efficiency (NUE) were computed (Fageria *et al.*, 2008) using the cluster bean grain yield data and rates of fertilizer nutrients applied for cluster bean as given below:

1. Yield Response:

$$\frac{\text{Yield in fertilized plot} - \text{Yield in unfertilized plot}}{\text{Yield in unfertilized plot}}$$

2. Nitrogen use efficiency (NUE):

$$\frac{\begin{pmatrix}\text{Cluster bean-grain yield} & - & \text{Cluster bean-grain yield} \\ \text{in fertilized plot} & & \text{in unfertilized plot}\end{pmatrix}}{\text{Quantity of total nutrient applied}}$$

Net return for all the treatments was calculated taking nitrogen price ₹ 13.04/- for urea/kg N, ₹ 43.75/- for SSP/kg phosphorus and ₹ 32/- for MOP/kg K and seed price as ₹ 200/kg.

Results and Discussion

Yield and yield response: Seed inoculation of cluster bean with *Rhizobium* biofertilizer + 60 kg N/ha gave highest seed yield followed by *Rhizobium* biofertilizer + 45 kg N/ha as compared to other treatment combinations. Saving of N requirement for different target yields by seed inoculation of *Rhizobium* ranged in between 13- 21 kg/ha N in the present set up.

Effect of graded dose of N application increased the yield of the cluster bean with or without inoculation of *Rhizobium*. Application of 60 kg N/ha along with *Rhizobium* inoculated seeds gave highest grain yield (13.28 q/ha) which was 8.58 percent q/ha more as compared to 60 kg N/ha application (Table 1). This treatment gave 8.02 and 5.21% higher yield as compared to absolute control and only *Rhizobium* application, respectively. The grain yield obtained with 60 kg N/ha (12.23 q/ha) was statistically at par with that of 45 kg N/ha + *Rhizobium* (12.26 q/ha). Similarly, yield under 45 kg N/ha was statistically at par with that of 30 kg N/ha + *Rhizobium*. 18% higher mean yield (10.88 q/ha) was observed under seed inoculation with *Rhizobium* as compared to without *Rhizobium* (9.20 q/ha). Maximum increment in cluster bean grain yield was observed in inoculated cluster bean seeds by *Rhizobium* over absolute control (no N was applied).

Table 1: Effect of N and *Rhizobium* inoculation on seed yield of cluster bean crop

Nitrogen level (kg/ha)	Yield (q/ha)	
	Without *Rhizobium*	With *Rhizobium*
0	5.26	7.57
15	8.58	9.89
30	9.87	11.42
45	10.06	12.26
60	12.23	13.28
Mean	9.20	10.88
CD at 5%	0.85	

Percent rise in yield by the application of 15, 30, 45 and 60 kg N/ha was 63.12, 87.64, 91.31 and 132.51%, respectively whereas, that of yield with added seed inoculation by *Rhizobium* was 88.02, 117.08, 133.08 and 152.47%, respectively. Application of 15, 30, 45 and 60 kg N/ha, the percent increase in yield by the seed inoculation with *Rhizobium* was 24.90, 29.44, 41.77 and 19.96%, respectively and maximum increase in yield was at 15 kg N/ha application with *Rhizobium*. The legumes are able to meet much of their nitrogen requirements from atmospheric nitrogen through symbiotic relationship with bacteria (Herridge *et al.,* 1993).

Table 2: Effect of N levels and *Rhizobium* on N use efficiency (NUE, kg seed/kg N) and percent yield response (ANR, %)

Nitrogen level (kg/ha)	NUE (kg seed/kg N)		Yield response (%)	
	Without *Rhizobium*	With *Rhizobium*	Without *Rhizobium*	With *Rhizobium*
15	22.13	30.87	63.12	88.02
30	15.37	20.53	87.64	117.08
45	10.67	15.56	91.31	133.08
60	11.62	13.37	132.51	152.47
Mean	14.95	20.08	93.65	122.66

The increased availability of nutrients through application of chemical fertilizers and biological nitrogen fixation by *Rhizobium*, might have favored the plant growth characters under aforesaid treatments. Deshmukh *et al.* (2014) also reported improvement in growth attributes of cluster bean with application of nutrients. Better growth associated with increased availability of plant nutrients might have resulted in better development of yield attributes under aforesaid treatments. Similar observations by Ganie *et al.* (2010) in garden pea and Sajitha (2007) in cluster bean do confirm the trend in the present experimental setup. From the above results, it is evident that added biofertilizers might have resulted in improvement of soil physical, chemical and biological properties leading to better nutrient absorption by the plants and higher assimilation of photosynthates which resulted in better yield.

Nitrogen use efficiency: Nitrogen use efficiency (NUE) is the increase in economic yield obtained per unit increase in nutrient applied. NUE of cluster bean crop

Table 3: Contribution of *Rhizobium* to N nutrition of cluster bean crop as derived from quadratic ($y= ax^2+bx+c$) model

Treatments	Quadratic equation	Fertilizer N requirement for different target yield (q/ha)					
		8 q/ha	9 q/ha	10 q/ha	11 q/ha	12 q/ha	13 q/ha
Without *Rhizobium*	$y = -0.001x^2 + 0.167x + 5.629$	15.67	23.49	32.50	43.48	58.98	—
With *Rhizobium*	$y = -0.001x^2 + 0.154x + 7.651$	2.30	9.32	17.17	26.21	37.25	52.92
Saving of N requirement for different target yield by the seed inoculation of *Rhizobium* (kg/ha)		13.37	14.17	15.33	17.28	21.73	—

Table 4: Net return and B: C ratio under treatments

Nitrogen level (kg/ha)	Cost of cultivation		Gross return		Net return		B:C ratio	
	Without *Rhizobium*	With *Rhizobium*	Without *Rhizobium*	With *Rhizobium*	Without *Rhizobium*	With *Rhizobium*	Without *Rhizobium*	With *Rhizobium*
0	46545	46545	105200	151400	58655	104855	1.26	2.25
15	46741	46741	171600	197800	124859	151059	2.67	3.23
30	46936	46936	197400	228400	150464	181464	3.21	3.87
45	47132	47132	201200	245200	154068	198068	3.27	4.20
60	47328	47328	244600	265600	197272	218272	4.17	4.61
Mean	46936	46936	184000	217680	137064	170744	2.9	3.6

was significantly influenced by the graded doses of nitrogen application with or without *Rhizobium* (Table 2). NUE (kg grains/kg N) showed considerable variation. Higher mean NUE (20.08 kg grains/kg N) was observed in inoculated seeds with *Rhizobium* as compared to non-inoculated (14.95 kg grains/kg N). NUE was maximum (30 kg grains/kg N) under lower dose of N with *Rhizobium* (15 kg N/ha + *Rhizobium*) as compared to only 15 kg N/ha application without *Rhizobium* (22.13 kg grains/kg N). NUE was inversely related to N application. In general, increasing dose of N decreased the N use efficiency for all the treatments wherein per cent decrease in NUE was more when only graded dose of N was applied as compared to its usage on *Rhizobium* inoculated seeds. *Rhizobium* forms an endosymbiotic nitrogen fixing association with roots of cluster bean. The bacteria colonize plant cells within root nodules where they convert atmospheric nitrogen into ammonia and then provide organic nitrogenous compounds such as glutamine or ureides to the plant. The plant in turn provides the bacteria with organic compounds made by photosynthesis. This clearly indicates that the treatments involving nitrogen application to *Rhizobium* inoculated seeds of cluster bean channelized more of the absorbed nutrients particularly N to economic yield in a better way. Similar results have been reported by Bakulin *et al.* (2007) and Kumar *et al.* (2012).

Saving of N through Seed inoculation

The grain yield obtained in presence or absence of *Rhizobium* with respect to varying N levels fitted well in quadratic model. For different target yields, N required in presence and absence of *Rhizobium* was worked out (Table 3 & Fig. 1&2).

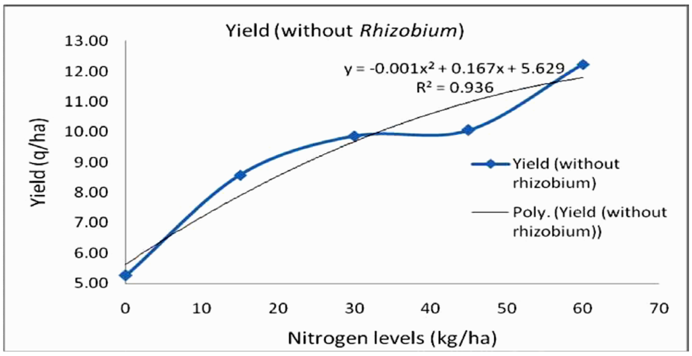

Fig. 1: Yield response of cluster bean without *Rhizobium* inoculation

For producing 8 to 12 q/ha target yield of cluster bean grains, 2.30 to 37.25 kg N/ha is needed with *Rhizobium* inoculation (Table 3). Whereas, for same target yield

15.67 to 58.98 kg N/ha is required without *Rhizobium* inoculation. Maximum yield (13 q/ha) was not achievable with recommended dose of N, whereas, this yield target can be achieved by 52.92 kg N/ha with *Rhizobium*. The results of this study indicated the beneficial effects of *Rhizobium* inoculation on grain yield and 13.37 to 21.73 kg N/ha can be saved to get yield from 8 to 12 q/ha with seed inoculation by *Rhizobium*. Significant response to inoculation of biofertilizer was reported by Meena *et al*., (2002).

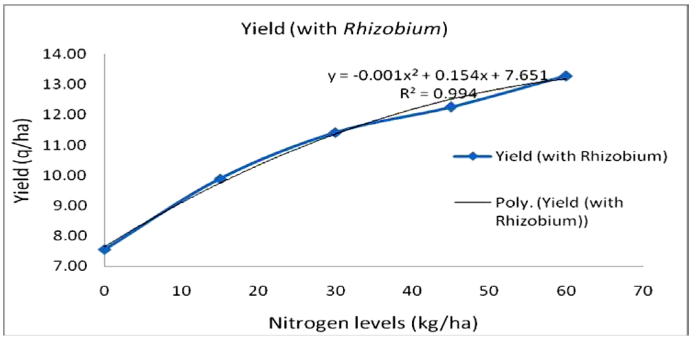

Fig. 2: Yield response of cluster bean with *Rhizobium* inoculation

Net return and benefit cost ratio of the *kachri*

Net return from seed inoculation with or without *Rhizobium* followed similar trend (Table 4) as that of seed yield with highest values of ₹ 218272/ha was observed in 60 kg/ha N with *Rhizobium* treatment. This was closely followed by 60 kg/ha N with *Rhizobium* treatment (₹ 198068 /ha) and 60 kg/ha N application (₹ 197272 / ha). *Rhizobium* treatment gave net return of ₹ 104855/ ha as compared control (₹ 58655/ha). The benefit: cost ratio was highest in the treatment receiving 60 kg/ha N with *Rhizobium* treatment (4.61) closely followed by 60 kg/ha N with *Rhizobium* treatment (4.20). Whereas, control gave the lowest B: C ratio (1.26). Application of 0, 15, 30, 45 and 60 kg/ha with *Rhizobium* inoculation have 0.99, 0.56, 0.66, 0.93 and 0.44 higher B: C ratio as compared to without *Rhizobium* inoculation. Mean net return Rs 33680/ ha was more with seed inoculation with *Rhizobium as compared* without *Rhizobium*.

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