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Effect of Nitrogen, *Rhizobium* and Growing Environments on Nodulation, Nutrient Content and Uptake of *Albizia procera*. R. b.

Yogesh Kumar[1], V.M. Prajapati[1], M.B. Tandel[1], Govind[1], J.G. Pathak[1], M. R. Parmar[1] and Aditya Pratap Singh[2]*
[1]Department of Silviculture and Agroforestry, College of Forestry,
ACHF, NAU, Navsari, (Gujarat), India.
[2]Department of Genetics and Plant Breeding,
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, (West Bengal), India.
(Corresponding author: Yogesh Kumar)*
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ABSTRACT: The present study was carried out to investigate the effect of different levels of nitrogen (N) alone and nitrogen in combination with *Rhizobium* inoculation (R) on root nodulation, N content and N uptake by *Albizia procera* seedlings under variable environmental conditions. The experiment was conducted in polythene bags with eighteen treatment combinations, comprising of six levels of fertilizer *viz.*, 0 mg N, 25 mg N, 50 mg N, 0.50 ml R, 25 mg N + 0.5 ml R and 50 mg N + 0.5 ml R per seedling, respectively and three distinct growing environments (E) *viz.*, open, net house and poly house, respectively. The maximum number of nodule per plant (67.00), nodule diameter (3.72 mm) and fresh weight of nodule per plant (1.53 g) at 90 DAT were observed under the interaction of 0 mg N per kg of soil and seedlings kept in the Net house. However, maximum N content in both shoot (1.54%) and root (0.53%) were observed in 25 mg N kg^{-1} soil + 0.5 ml *Rhizobium* kg^{-1} soil in Net house condition. Whereas maximum N uptake by the plant (61.04 mg plant^{-1}) was observed in 50 mg N kg^{-1} soil + 0.5 ml *Rhizobium* kg^{-1} soil under Net house environment. Hence this treatment can be used for quality seedling production of *Albizia procera*.

Keywords: Nitrogen, *Rhizobium*, Growing environment, Nodulation, Nutrient uptake and content.

INTRODUCTION

Albizia procera commonly known as 'Safed siris' is a member of the sub-family Mimosaceae- family Fabaceae. It is a large, deciduous, straight-stemmed, fast-growing leguminous tree. *A. procera* is a multipurpose tree species and provides ~~an~~ excellent fuel, fodder and small timber for agricultural implements (Singh, 1982). It is a useful nitrogen-fixing tree species most suited for the semi-arid regions of central India (Kolhey, 2012). It is a popular agroforestry species for bund planting. This tree produces good quality fodder for cattle; however, the wood of this tree is also used for timber and fuel wood.

The Use of fertilizers in forestry, especially in the forest nurseries and early stage of raising plantations, has become a regular silvicultural practice in many countries (Baule and Fricker, 1970). The fertilizer trial conducted on forestry species in the past indicated that a balanced dose of fertilizers applied in proper time with suitable method had been found to have a positive response on survival and growth of plant both at nursery and in plantation stage (Prasad *et al.* 1984, Gangoo *et al.* 1997; Singh, 2001).

It has also been reported by many workers that the requirement of sunlight varies widely for the species and their growing conditions, particularly during the nursery stage. Understanding the response of seedlings of *A. procera* towards various growing environments in the nursery will help to produce a healthy nursery stock. This information will also enable foresters for proper site selection for establishing commercial nurseries of this species for large-scale seedling production.

The efficient and optimum use of fertilizers may be helpful in boosting up the growth of plant and meeting the additional requirement of crop and thereby sustain fertility as well as productive potential of soil ultimately resulting in enhancement of crop production without losing the inherent potential of native soil. In order to enhance the survival and growth of *A. procera*, proper fertilizer doses and combinations as well as growing environment have not been standardized particularly for South Gujarat region and, thus, the present trial has been conducted.

MATERIAL AND METHOD

A. Material

Study site:

The Present investigation was carried out at College of Forestry, ACHF, NAU, Navsari during the year 2020-2021. There were eighteen treatment combinations replicated thrice adopting a completely randomized design with the factorial concept.

Treatment details:

The treatments consisted of six levels of nitrogen and three types of growing environment as follows:

A) Nitrogen application:

N_1 – 0 mg N kg^{-1} soil

N_2 – 25 mg N kg^{-1} soil

N_3 – 50 mg N kg^{-1} soil

N_4 – 0.5 ml kg^{-1} soil *Rhizobium* inoculation

N_5 – 25 mg N kg^{-1} soil + 0.5 ml kg^{-1} soil *Rhizobium* inoculation

N_6 – 50 mg N kg^{-1} soil + 0.5 ml kg^{-1} soil *Rhizobium* inoculation

B) Growing environment:

E_1 – Open Environment

E_2 – Net house (75% Green shed net)

E_3 – Polyhouse (Open ventilated plastic sheets)

B. Methodology

Ripened pods of *A. procera* were collected directly from trees. Seeds were sown in trays, and seedlings were transplanted to polybags after germination and kept in various growing environments. Nitrogen was applied in two separate doses as urea. The first dose was administered one week after seedlings were transplanted into polybags. One month after the first dose, the remaining dose was administered. After preparing the aqueous solution by dissolving the desired amount of urea in water, a total of 54.3 mg and 108.7 mg urea per polybag (each bag containing approximately 1 kg of soil) was applied to supply 25 mg N kg^{-1}soil and 50 mg N kg^{-1} soil, respectively. Bio-fertilizer was used as per the treatment plan. After removing the plants from the polybags, nodulation parameters were measured. Plant samples of *A. procera* taken from polybags were oven-dried at 60$\pm$2^oC, ground and analyzed for nutrient uptake and content. For analysis, different standard procedure was used. The Kjeldahl method was used for the estimation of nitrogen content (Jackson *et al.* 1973). Nitrogen uptake was obtained by multiplying the nitrogen concentration with dry matter and dividing by 100.

RESULT

A. Nodulation Parameters

(i) Number of nodules per plant. The number of nodules per plant at 90 DAT was significantly affected by different treatments. It is evident that zero application of nitrogen (N_1) recorded the maximum number of nodules per plant (54.45) while, minimum (24.96) was recorded in treatment, 50 mg N kg^{-1} soil+ *Rhizobium* 0.5 ml kg^{-1} soil (N_6). The highest number of nodules per plant (46.18) was recorded in the Net house (75% green shed net) and the lowest number of nodules per plant was recorded with the open environment (24.55). The interaction effect of nitrogen and the growing environment was significant in altering the number of nodules per plant of *A. procera*. Controlled application of nitrogen (N_1) in the Net house (75% Green shed net) significantly increased the number of nodules per plant (67.00) (Table 1).

(ii) Nodule diameter (mm). Among different fertilizer treatments, control (N_1) recorded maximum nodule diameter (3.21 mm) while, minimum value (2.03 mm) was observed with 50 mg kg^{-1} soil + *Rhizobium* 0.5 ml kg^{-1} soil (N_6). The highest nodule diameter (2.91 mm) was recorded in the Net house (75% Green shed net) while, the lowest nodule diameter was recorded in open environment (2.00 mm). The interaction effect of nitrogen and the growing environment significantly increased the nodule diameter of *A. procera*. Non-application of N in conjunction with the Net house (75% Green shed-net) significantly increased nodule diameter (3.72 mm) (Table 1).

Table 1: Effect of nitrogen and growing environments on number of nodule per plant, nodule diameter (mm) and yield of nodule per plant (g) of *A. procera* R. b. seedling at 90 DAT (Days After Transplanting).

	Number of nodule per plant				Nodule diameter				Yield of nodule per plant ()			
Treatments	**E_1**	**E_2**	**E_3**	**Mean (N)**	**E_1**	**E_2**	**E_3**	**Mean (N)**	**E_1**	**E_2**	**E_3**	**Mean (N)**
N_1	45.13	67.00	51.20	**54.45**	2.86	3.72	3.04	**3.21**	1.057	1.537	1.177	**1.257**
N_2	21.73	46.60	41.40	**36.58**	1.87	2.88	2.73	**2.49**	0.633	1.110	1.010	**0.918**
N_3	19.53	37.13	31.60	**29.42**	1.81	2.54	2.28	**2.21**	0.590	0.907	0.813	**0.770**
N_4	24.73	54.53	48.47	**42.58**	2.01	3.29	2.91	**2.74**	0.680	1.290	1.130	**1.033**
N_5	23.27	39.00	26.87	**29.71**	1.96	2.59	2.10	**2.22**	0.657	0.960	0.730	**0.782**
N_6	12.87	32.80	29.20	**24.96**	1.52	2.42	2.17	**2.03**	0.460	0.877	0.773	**0.703**
Mean (E)	**24.55**	**46.18**	**38.12**		**2.00**	**2.91**	**2.54**		**0.679**	**1.113**	**0.939**	
	S. Em. ±	**C. D. at 5 %**	**C. V. %**		**S. Em. ±**	**C. D. at 5 %**	**C. V. %**		**S. Em. ±**	**C. D. at 5 %**	**C. V. %**	
N	0.56	1.61	4.65		0.017	0.048	2.00		0.014	0.040	4.540	
E	0.40	1.14			0.012	0.034			0.010	0.028		
N × E	0.97	2.80			0.029	0.082			0.024	0.068		

(iii) Yield of nodule per plant. The yield of nodule per plant recorded maximum (1.26 g) in nitrogen control (N_1) and minimum (0.70 g) was in 50 mg kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil (N_6). The highest yield of nodule per plant (1.11 g) was recorded in net house (75% Green shed-net) while lowest yield of nodule per plant was recorded in open environment (0.68 g). Interaction of fertilizer control (N_1) and net house environment (75% Green shed-net) significantly increased the yield of nodule per plant (1.537 g) (Table 1).

B. Nutrient Content and Uptake

(i) Nitrogen content (%) in shoot and root. Nitrogen content in shoot and root at 90 DAT was significantly influenced by different nitrogen/ nitrogen- *Rhizobium* treatments and growing environment. Among nutrient treatments, highest N content of shoot (1.34%) and root (0.48%) was recorded in 25 mg N kg^{-1} soil+ 0.5 ml *Rhizobium* kg^{-1} soil (N_5) while lowest was observed in controlled nutrient treatment (N_1). Growing environment significantly affected the N content in shoot and root. Highest N content of shoot (1.26 %) and root (0.40) was reported in the Net house (75% Green shed net) while lowest N content of shoot and root was recorded in the open environment.

In case of interaction effect, application of 25 mg Nkg^{-1} soil + 0.5 ml *Rhizobium* kg^{-1} soil in conjunction with the Net house (75% Green shed net) significantly increased the N content of shoot (1.54 %) and root (0.53%) (Table 2).

(ii) Nitrogen uptake by shoot, root and whole plant. N uptake by shoot, root and whole plant at 90 DAT was significantly influenced by different nutrient treatments and growing environment (Table 3). Maximum N uptake in the shoot (29.83 mg $plant^{-1}$), root (13.79 mg $plant^{-1}$) and whole plant (43.62 mg $plant^{-1}$) was recorded in 50 mg N kg^{-1} soil+ 0.5 ml kg^{-1} soil (N_6) while minimum uptake was observed in control (N_1). Maximum N uptake in shoot (26.85 mg $plant^{-1}$), root (11.74 mg $plant^{-1}$) and whole plant (38.59 mg $plant^{-1}$) were significantly observed in the Net house (75% Green shed-net). Application of 50 mg N kg^{-1} soil+ *Rhizobium* 0.5 ml kg^{-1} soil (N_6) in conjunction with the Net house (75% Green shed-net) significantly increased N uptake by shoot (42.70 mg $plant^{-1}$), root (18.34 mg $plant^{-1}$) and whole plant (61.04 mg $plant^{-1}$).

Table 2: Effect of nitrogen and growing environments on N content in shoot and root of *A. procera* R. b. seedling.

	Nitrogen content (%) in shoot				Nitrogen content (%) in root			
Treatments	**E_1**	**E_2**	**E_3**	**Mean (N)**	**E_1**	**E_2**	**E_3**	**Mean (N)**
N_1	0.55	0.83	0.78	**0.72**	0.20	0.26	0.25	**0.24**
N_2	0.83	1.08	0.89	**0.93**	0.27	0.32	0.28	**0.29**
N_3	0.82	1.31	1.18	**1.10**	0.27	0.38	0.36	**0.34**
N_4	1.01	1.37	1.25	**1.21**	0.35	0.42	0.40	**0.39**
N_5	1.03	1.54	1.45	**1.34**	0.40	0.53	0.50	**0.48**
N_6	1.09	1.45	1.19	**1.24**	0.38	0.47	0.40	**0.42**
Mean (E)	**0.89**	**1.26**	**1.12**		**0.31**	**0.40**	**0.37**	
	S. Em. ±	**C. D. at 5 %**	**C. V. %**		**S. Em. ±**	**C. D. at 5 %**	**C. V. %**	
N	0.008	0.022	2.100		0.004	0.010	2.99	
E	0.005	0.016			0.003	0.007		
N × E	0.013	0.038			0.006	0.018		

Table 3: Effect of nitrogen and growing environments on N uptake by shoot, root and whole plant in *A. procera* R. b. seedling.

	N uptake by shoot (mg $plant^{-1}$)				N uptake by root (mg $plant^{-1}$)				N uptake by whole plant (mg $plant^{-1}$)			
Treatments	**E_1**	**E_2**	**E_3**	**Mean (N)**	**E_1**	**E_2**	**E_3**	**Mean (N)**	**E_1**	**E_2**	**E_3**	**Mean (N)**
N_1	3.45	12.76	10.63	**8.95**	2.44	5.85	5.06	**4.45**	5.89	18.61	15.69	**13.40**
N_2	8.93	18.74	11.16	**12.94**	4.74	8.00	5.35	**6.03**	13.68	26.74	16.51	**18.97**
N_3	9.02	32.53	24.99	**22.18**	4.82	12.89	10.67	**9.46**	13.84	45.43	35.66	**31.64**
N_4	9.19	22.84	17.53	**16.52**	5.38	10.18	8.73	**8.10**	14.57	33.02	26.27	**24.62**
N_5	10.30	31.52	26.10	**22.64**	6.65	15.15	13.55	**11.78**	16.95	46.67	39.65	**34.42**
N_6	21.07	42.70	25.71	**29.83**	10.47	18.34	12.56	**13.79**	31.55	61.04	38.26	**43.62**
Mean (E)	**10.33**	**26.85**	**19.35**		**5.75**	**11.74**	**9.32**		**16.08**	**38.59**	**28.67**	
	S. Em. ±	**C. D. at 5 %**	**C. V. %**		**S. Em. ±**	**C. D. at 5 %**	**C. V. %**		**S. Em. ±**	**C. D. at 5 %**	**C. V. %**	
N	0.34	0.97	5.38		0.16	0.46	5.35		0.37	1.05	3.95	
E	0.24	0.69			0.11	0.32			0.26	0.74		
N × E	0.59	1.68			0.28	0.79			0.63	1.82		

DISCUSSION

A. Nodulation Parameters

The inhibitory effect of combined nitrogen on *Rhizobial* infection, nodule development and nitrogen fixation by *Rhizobium* legume symbiosis has been well documented by Bisseling *et al.* (1978); Gibson and Jordan (1983). In the present study the same trend was found, From results, it can be inferred that the seedlings under tap water treatment (N_1- control) recorded the maximum number of nodules per plant (54.45), maximum nodule diameter per plant (3.21 mm) and also the maximum yield of nodules per plant i.e. the maximum fresh weight of nodules per plant (g) (1.26 g) at 90 DAT. The spontaneous appearance of nodules in non-fertilized and non-inoculated treatments may be due to the higher susceptibility of plants under nutrient stress to be colonized even by parasitic bacteria (Dumroese e*t al.*, 2009). In addition, growth conditions in this work were in the open air; consequently, the presence of *rhizobia* in non-inoculated treatments may be also due to deposition of soil particles by wind, transference in the nursery via workers, precipitation or irrigation events. Ntambo *et al.* (2017), the result showed that the application of fertilizers with the doses of 50, 100 and 200 kg N ha^{-1} on soybeans caused a decline in the number and weight of dry nodules. Chaukiyal *et al.* (2000) also reported minimum values in nodule number per plant *viz.* 36 in N-0, 28 in N-40, 31 in N-80 and 24 in N-100 in *Pongamia pinnata* all in May, whereas, this trend was opposite during the rainy season i.e. treatments with higher N doses recorded more number of nodules per plant during the rainy season. Nitrogen availability is an important environmental regulator of nodulation and the inhibitory effects of high nitrate contents on nodulation are complex and still not fully understood. A few hypothesis have been proposed to explain this process, such as deprivation of carbon skeletons in nodules (Streeter, 1988), feedback inhibition by-products of nitrate metabolism, (e.g., glutamine and/or asparagines), and reduced O_2 availability in nodules, restricting respiration of bacteroids (Saito *et al.,* 2014).

B. Nutrient content and uptake

Among different seedling components shoot showed higher N content (%) than root. In the shoot, the N content ranged from 0.55- 1.54 percent, whereas in root it was 0.20- 0.53 percent under different treatments at 90 DAT. Significantly higher N content in shoot and root i.e., 1.54 and 0.53 percent, respectively were observed under nitrogen application at 25 mg urea kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil (N_5) under Green shed-net (E_2). *Rhizobium* inoculation singly showed N content of 1.21 and 0.39 percent in shoot and root. Among two levels of N, lower levels of N influence significantly over higher N level when applied with *Rhizobium*. Inoculated seedlings showed significantly higher N content over only N fertilization and control. N uptake by the shoot of *A. procera* was 29.83 mg $seedling^{-1}$ at 50 mg urea kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil (N_6) compared to control i.e. with no application of nitrogen (8.96 mg $seedling^{-1}$) at 90 DAT.

A similar trend in nutrient content was reported by Vasishth *et al.* (2003) in *Acacia catechu*; Kaushik *et al*. (2003); Darpan (2011) in *Pongamia pinnata* and Kolhey, (2012) in *Albizia procera*. Vasishth *et al*. (2003) summarized that combined application of *Rhizobium* and nitrogen fertilizer was found to be more effective in accumulating the amount (163.2 mg $plant^{-1}$) of total N, whereas un-inoculated plant in absence of N could accumulate only about 59.2 mg N $plant^{-1}$ in *Acacia catechu* seedlings. Kaushik *et al.* (2003) reported that an application of *Rhizobium* also increased the uptake of nutrients, which enhanced the growth and development of seedlings. The *Rhizobium* alone and along with N, showed higher N content than un-inoculated ones. N uptake by seedlings is also more in inoculated ones over un-inoculated ones. The adequate supply of moisture, mineral, nutrients, ensure the better growth and development of seedlings**.**

The better uptake of nutrients might be facilitated through microbial inoculants. The *Rhizobium* inoculation only and *Rhizobium* + N treatments not only increased the nutrient status but also resulted in higher biomass production. The interaction between nitrogen x growing environment was also found to be significant in influencing the N content and uptake by plants.

CONCLUSION

The maximum number of nodules per plant, maximum nodule diameter per plant and also the maximum yield of nodules per plant i.e. maximum fresh weight of nodules per plant (g) were observed under the interaction of N_1 (Control- Tap water) and E_2- Net house (75% Green shed net). Higher nutrient (N) content in both shoot and root was recorded in treatment N_5 (N-25 mg kg^{-1} soil+ Bio-fertilizer 0.5 ml kg^{-1} soil) in conjunction with E_2- Net house (75% Green shed net). Whereas; maximum N uptake by the plant was observed in treatment N_6 (N-50 mg kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil) kept under E_2- Net house (75% Green shed net). It is also concluded that nitrogen affects nodulation at the initial stage of *A. procera* seedling. *Rhizobium* + N treatments increased the nutrient status. Therefore, this combination can be used for quality seedling production of *A. procera.*

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Conflict of Interest. None.

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