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Effect of nitrogen, *Rhizobium* and growing environments on the growth and biomass production of *Albizia procera* R. b.

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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 seedling growth and vigour of *Albizia procera* 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. Seedlings treated with N @ 50 mg kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil (N_6) and kept under Net house (75% Green shed-net) (E_2) recorded higher survival (97.07%), shoot height (23.20 cm), collar diameter (3.67 mm), number of rachis (16.00), total leaflet area (250.47 cm^2), length of main root (44.27 cm), total fresh weight of plant (12.17 g), dry weight of plant (6.84 g) and seedling quality index (1.92). On the other hand maximum root: shoot ratio (1.93) was recorded by the interaction of N_1- (Control- Tap water) and E_1- (Open Environment); whereas, maximum sturdiness quotient (6.65) was recorded under treatments N_1- (Control-Tap water) and E_2- Net house (75% Green shed net). Hence this treatment can be used for quality seedling production of *Albizia procera*.

Keywords: Nitrogen, *Rhizobium*, growing environment, nodulation, nutrient uptake and content

1. Introduction

Albizia procera commonly called white siris, is one of an important species belonging to the family Fabaceae. It is a fast-growing medium to large sized deciduous tree distributed throughout moist and dry deciduous forests of India. This species provides wood for a variety of end users, palatable and nutritious fodder for livestock. It is extensively planted in farm lands, agricultural boundaries as wind breaks and for wasteland reclamation and roadside avenues. Thus, it is an important reforestation and agro forestry species. Due to its multipurpose use and good nitrogen fixing ability it is considered as one of the priority species in plantation programs. Use of fertilizers in forestry, especially in the forest nurseries and early stage plantations, has become a regular silvicultural practice in many countries (Baule and Fricker, 1970) [1]. The fertilizer trial conducted on forestry species in the past years indicated that a balanced dose of fertilizers applied in proper time with suitable methods had been found to have a positive response on survival and growth of plants both at nursery and in plantation stage (Prasad *et al.* 1984, Gangoo *et al.* 1997 and Singh, 2001) [15, 7, 20].

It has also been reported by many workers that the requirement of sunlight varies widely from species to species and their growing conditions, particularly during the nursery stage. Hence, 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. In order to enhance the survival and growth of *A. procera* and to provide superior and healthy planting stock, proper fertilizer doses and combinations as well as growing environment have not been standardized for South Gujarat region and, thus, the present trial has been conducted.

2. Material and Method

2.1 Material

2.1.1 Study site

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 completely randomized design with factorial concept.

2.1.2 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)

2.2 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 by preparing an aqueous solution by dissolving the desired amount of urea in water. A total of 54.3 mg and 108.7 mg urea per poly bag (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 in accordance with the treatment plan. Seedling growth attributes such as shoot height, collar diameter, number of rachis per plant were measured or counted on monthly interval up to 90 days. At the end of the experiment, total five seedlings per treatment per replication were selected randomly and destructive sampling was made to measure the fresh biomass, dry biomass and root to shoot (R:S) ratio (Biomass basis). Sturdiness quotient and seedling quality index were estimated as per Thompson (1985) [23] and Dickson *et al.* (1960) [6], respectively.

2.2.1 Data analysis

Data obtained were subjected to statistical analysis following Completely Randomized Design (CRD) and analysis of variance (ANOVA) was worked out with the help of online statistical package, OPSTAT, as per Sheoran *et al.* (1998) [19].

3. Result

Varying levels of nitrogen treatments combined with different growing environments showed significant influence on various growth parameters of *A. procera* seedlings at 90 days after transplanting (DAT). Seedlings fertilized with N 50 mg kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil (N_6) recorded higher growth and biomass in terms of shoot height (20.33 mm), collar diameter (3.27 mm), number of leaves (14.96), total leaf area (230.08 mm^2), length of main root (40.71 mm), total fresh weight of plant (10.64 g) and dry weight of plant (5.62 g) along with maximum survival percentage (91.18%).

It is evident that every subsequent increase in nitrogen dose increases the foliage growth of the plants and hence the chlorophyll content is also increased. This further explains the increased shoot height, collar diameter and biomass growth of the plant due to enhanced photosynthetic activity as was reported by Husen and Pal (2004) [9] in *Tectona grandis* and Singh *et al.* (2003) in *Manilkara zapota*. The higher growth in N_6 + *Rhizobium* treated seedlings might be due to synergistic effect of lower N levels as starter dose with *Rhizobium* inoculation which mediated the efficient uptake of N in seedlings. Such synergistic effect of increased uptake of one nutrient facilitated by other nutrients in leguminous species was also reported by Prasad, (1998). Current study showed that an application of 50 mg N kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil exhibited better seedling growth. The outcome of the present study is supported by several authors such as Prakash and Oraon (2020) [14] in *Albizia lebbeck*; Das *et al.* (2018) [5] in *Tamarindus indicus*; Kumar *et al.* (2013) [13] in *Dalbergia sissoo*; Chauhan and Pokhriyal (2002) [4] in *Albizia lebbeck*.

Albizia lebbeck attained maximum shoot height (40.73 mm) and root length (29.10) under the influence of *Rhizobium* and Urea combination (Prakash and Oraon, 2020) [14]. Further maximum plant height (31.42 mm), number of leaves (21.68 $seedling^{-1}$), stem girth (1.30 mm), root length (29.52 mm), shoot dry biomass accumulation (2.07 g $seedling^{-1}$) were recorded at a combination of *Rhizobium* and N in *Tamarindus indicus* (Das *et al*, 2018) [5]. Likewise, a combined effect of inoculation along with lower level of N showed maximum seedling height (64.8 mm), shoot biomass (20.4 g/seedling) and collar diameter (8.27 mm) in *Dalbergia sissoo* (Kumar *et al.*, 2013) [13]. Furthermore, Chauhan and Pokhriyal (2002) [4] reported that when the seedlings of *Albizzia lebbeck* were treated with both nitrogen and *Rhizobium*, they performed better than those which received either one of them or none (control). Among seedling vigour parameters, maximum root: shoot ratio of 1.63 was recorded in control (N_1), lowest SQ value of 5.95 was noticed in treatment N_2 (N 25 mg kg^{-1} soil). Moreover, important seedling vigour parameter i.e. SQI recoded highest value of 1.62 in N 50 mg kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil (N_6) treatment at 90 DAT.

The root: shoot ratio is an important measure for seedling survival. It relates the water absorbing area of roots to the transpiring area of shoot. A good ratio indicates a healthy plant (Jaenicke, 1999) [10]. The balanced root to shoot ratio of seedlings in nursery should be of 1<Root Shoot Ratio>2 (Takoutsing *et al.*, 2013) [22] and seedlings with a low root to shoot ratio (1< Root Shoot Ratio>2) have a greater survival rate than large seedlings with higher root to shoot ratios (Gregorio *et al.*, 2007) [8]. The higher root shoot ratio in the present investigation was recorded in control (N_1: 1.63) at 90 DAT which was quite high in comparison to the findings of Kumar *et al.* (2013) [13] which was recorded as (0.84) in T_5 (inoculated + N_2). Contrary in results may be due to change in species as well as the environmental conditions in which the transplanted recruits were growing.

The sturdiness quotient (SQ) value signifies the sturdiness i.e., ability of a seedling to withstand in stress condition in terms of field establishment. The SQ value of less than 6 is the most desired value of forest tree species seedlings grown in nursery and sturdiness quotient higher than 6 is undesirable (Jaenicke, 1999) [10]. Seedlings with sturdiness quotient greater than 6 were basically tall, thin and etiolated, while a small quotient indicates sturdy plants with a greater chance of survival, especially on windy or dry sites (Takoutsing *et al.*, 2013) [22]. In current study minimum SQ (5.95) was observed in N 50 mg N kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil (N_6). Present finding is supported by Jaishankar *et al.* (2013) [11], they found minimum SQ to be 9.60 under the influence of

Bio-fertilizers + N: P: K fertilizers in *Dalbergia sissoo*.

Seedling Quality Index (SQI) is considered a promising integrated measure of morphological traits and used as good indicator for assessment of seedling quality as it computes the robustness and biomass distribution of seedlings as compared to individual growth parameters like shoot length, collar diameter etc. (Binotto *et al.*, 2010) [3]. Normally higher the value of SQI, better is the ability of the seedling to survival and establish in the field condition. In the study, maximum SQI was observed in seedlings fertilized with N 50 mg kg^{-1} soil+ Bio-fertilizer 0.5 ml kg^{-1} soil (N_6:1.62), and its value depends on dry biomass of the seedlings and SQ. The present outcome of the study was supported by Kumar *et al.* (2013) [13] in *Dalbergia sissoo* who reported the SQI to be maximum (0.475) in inoculated + N_1 treatment. Further, *Dalbergia sissoo* seedlings showed maximum SQI of 3.76 under the influence of Bio-fertilizers + N: P: K fertilizers (Jaishankar *et al.*, 2013) [11]. Light gradients are ubiquitous in nature, so all plants are exposed to some degree of shade during their lifetime. The minimum light required for survival and shade tolerance is a crucial life-history trait that plays a major role in plant community dynamics (Valladares and Niinemets, 2008) [24]. The present study also investigates the response of *A. procera* seedlings to the various shade levels under different growing environments. Among different growing environments seedlings subjected to Net house recorded higher growth and biomass in terms of shoot height (18.91 mm), collar diameter (3.02 mm), number of leaves (14.36), total leaf area (217.29 mm^2), length of main root (38.60 mm), total fresh weight of plant (9.79 g) and dry weight of plant (4.96 g) along with maximum survival percentage (88.54%). However, seedlings subjected to Control (Open environment) i.e. E_1 exhibited maximum root: shoot ratio (1.65) at 90 DAT.

In the present experiment, relatively vigorous growth and development was observed in *A. procera* seedlings kept under Net house conditions which may be attributed to the microclimatic condition (e.g. low light intensity, moderate temperature, high humidity, better water holding capacity and soil moisture etc. compared to the other transplantation sites) favourable for the growth. Plants grown in full sunlight suffer from limitation of low soil moisture, minimum water holding capacity and high temperature, high light intensity which affects the growth and survival of the seedlings. Bhuyan (2002) [2] also reported lowest relative growth of *Elaeocarpus ganitrus* seedlings in full sunlight conditions compared to the intermediate light. Seedlings needs lots of shade during their early growth period for better survival and growth that can be understood from several studies related to green house and transplantation experiments. The results of this study are also comparable to the studies carried out by Prasad (2002) in *Terminalia tomentosa* and *Terminalia bellirica*; Khan *et al.* (2000) [12] in four species of conifers; Rezende *et al.* (1998) [16] in *Cryptocaria aschersoniarla*; Saxena *et al.* (1995) [17] in *Dalbergia sissoo* and *Acacia catechu* and Sharma *et al.* (1994) [18] in *Enicostemma littorale*. In all the above mentioned studies increment in height, stem diameter, number of leaves and biomass was greater when grown under shaded environments as compared to full sunlight. Hence, in the recent study, better performance of *A. procera* under Net house suggests that species require medium exposure to sunlight for its growth and biomass production.

The interaction between nitrogen and growing environments was also found to be significant in influencing the growth and biomass of *A. procera* seedlings. Varying levels of nitrogen treatments combined with different growing environments showed significant influence on various growth parameters of *A. procera* seedlings at 90 days after transplanting (DAT). Seedlings fertilized with N 50 mg kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil in conjugation with Net house recorded higher growth and biomass in terms of shoot height (23.20 mm), collar diameter (3.67 mm), total fresh weight of plant (11.44 g) and dry weight of plant (6.84 g). However, for number of leaves, total leaf areas, length of main root, survival percentage and vigour indices the interaction effect of nitrogen and growing environment was found to be non-significant. These results are in conjugation with the studies conducted by Jackson *et al.* (2012) where they studied the response of container *Pinus palustris* seedlings to nitrogen supply and their performance inside a greenhouse and in an outdoor compound and observed that seedling N rate in addition to nursery type often interacted to affect results. They reported that RCD of greenhouse seedlings was 7 per cent greater than those grown outdoors. Although total biomass of seedlings was similar regardless of growing area (2.95 g indoors vs. 2.85 g outdoors), shoot biomass was 28 per cent greater for seedlings grown outdoors, whereas root biomass was 29 per cent greater for seedlings grown inside the greenhouse. In contrast to these results Walters and Reich (1996) [25] while working on effects of low light and nitrogen on hardwood seedlings of *Betula papyrifera*, *Betula alleghaniensis*, *Ostrya virginiana*, *Acer saccharum*, and *Quercus rubra* examined effects of light and nitrogen on the interrelationships among survival, growth, and shade tolerance. The results of their study revealed that nitrogen amendments did not have any significant effect on any plant trait at either light level.

Table 1: Effect of nitrogen and growing environments on survival percentage (%), seedling height (cm) and collar diameter (mm) of *A. procera* R. b. seedlings at 90 DAT (Days after Transplanting)

	Survival Percentage				Seedling height (cm)				Collar diameter (mm)			
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	74.73	83.33	81.40	79.82	11.73	16.33	15.00	14.35	2.07	2.60	2.47	2.38
N_2	78.93	85.60	80.80	81.78	13.87	17.26	14.87	15.33	2.33	2.86	2.53	2.58
N_3	79.40	92.20	88.80	86.80	14.33	20.60	19.47	18.13	2.33	3.27	2.93	2.84
N_4	77.53	84.60	82.40	81.51	13.13	17.00	15.74	15.29	2.20	2.67	2.67	2.51
N_5	78.40	88.47	87.00	84.62	13.60	19.07	18.07	16.91	2.33	3.06	2.87	2.75
N_6	87.27	97.07	89.20	91.18	18.27	23.20	19.53	20.33	3.07	3.67	3.07	3.27
Mean (E)	79.38	88.54	84.93		14.16	18.91	17.11		2.39	3.02	2.76	
	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	0.99	2.84	3.52	0.99	0.27	0.79	4.92		0.025	0.071	2.73	
E	0.70	2.01		0.70	0.19	0.56			0.018	0.050		
N X E	1.71	NS		1.71	0.48	1.36			0.043	0.123		

Table 2: Effect of nitrogen and growing environments on no. of rachis, total leaf area (mm^2) and length of the main root in *A. procera* R. b. seedlings at 90 DAT (Days After Transplanting)

	No. of rachis per plant				Total leaf area (mm^2)				Length of the main root (cm)			
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	11.66	13.27	12.87	12.60	0.20	0.26	0.25	0.24	29.67	35.27	34.27	33.07
N_2	12.53	13.80	12.87	13.07	0.27	0.32	0.28	0.29	32.47	36.80	33.66	34.31
N_3	12.60	15.07	14.53	14.07	0.27	0.38	0.36	0.34	32.73	40.93	39.07	37.58
N_4	12.26	13.67	13.07	13.00	0.35	0.42	0.40	0.39	31.47	36.00	34.87	34.11
N_5	12.34	14.33	13.93	13.53	0.40	0.53	0.50	0.48	32.07	38.33	37.67	36.02
N_6	14.20	16.00	14.67	14.96	0.38	0.47	0.40	0.42	38.20	44.27	39.67	40.71
Mean (E)	0.679	1.113	0.939		0.31	0.40	0.37		32.77	38.60	36.53	
	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	0.18	0.51	3.92		0.004	0.010	2.99		0.64	1.82	5.30	
E	0.13	0.36			0.003	0.007			0.45	1.29		
N X E	0.31	NS			0.006	0.018			1.10	NS		

Table 3: Effect of nitrogen and growing environments on total fresh weight (g) and total dry weight (g) of *A. procera* R. b. seedlings at 90 DAT (Days after Transplanting)

	Total fresh weight (g) of plant				Total dry weight (g) of plant			
Treatments	E_1	E_2	E_3	Mean (N)	E_1	E_2	E_3	Mean (N)
N_1	6.08	8.39	7.93	7.46	1.83	3.79	3.38	3.00
N_2	7.23	8.92	7.66	7.94	2.82	4.24	3.16	3.41
N_3	7.34	10.78	9.95	9.36	2.86	5.88	5.09	4.61
N_4	6.84	8.74	8.12	7.90	2.45	4.09	3.57	3.37
N_5	7.06	9.75	9.20	8.67	2.65	4.90	4.49	4.01
N_6	9.50	12.18	10.25	10.64	4.72	6.85	5.27	5.61
Mean (E)	7.34	9.79	8.85		2.89	4.96	4.16	
	S.Em.±	C.D. at 5%	C.V. %		S.Em.±	C.D. at 5%	C.V. %	
N	0.10	0.28	3.42		0.04	0.10	2.71	
E	0.07	0.20			0.03	0.07		
N X E	0.17	0.49			0.06	0.18		

Table 4: Effect of nitrogen and growing environments on vigour indices of *A. procera* R. b. seedling

	Root Shoot Ratio				Seedling Quotient (SQ)				Seedling Quality Index (SQI)			
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	1.93	1.47	1.49	1.63	6.32	6.65	6.15	6.37	1.33	1.44	1.43	1.40
N_2	1.61	1.44	1.53	1.52	6.22	6.31	5.85	6.13	1.40	1.65	1.50	1.51
N_3	1.60	1.37	1.40	1.46	6.31	6.09	5.68	6.03	1.39	1.61	1.37	1.46
N_4	1.69	1.46	1.54	1.56	6.33	6.37	5.97	6.22	1.46	1.70	1.54	1.57
N_5	1.65	1.40	1.49	1.52	6.38	5.91	5.97	6.09	1.53	1.60	1.45	1.53
N_6	1.43	1.33	1.44	1.40	6.03	5.87	5.95	5.95	1.46	1.92	1.49	1.62
Mean (E)	1.65	1.41	1.48		6.26	6.20	5.93		1.43	1.65	1.46	
	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	0.03	0.09	6.40		0.11	NS	5.54		0.03	0.07	5.07	
E	0.02	0.07			0.08	0.23			0.02	0.05		
N X E	0.06	NS			0.20	NS			0.04	0.13		

4. Conclusion

The present study emphasizes on the production of healthy and hardened seedlings of *Albizia procera* showing better seedling growth and vigour using different nitrogen treatments and growing environments. N- 50 mg kg^{-1} soil + Bio-fertilizer 0.5 ml kg^{-1} soil in combination with Net house is one among the best treatments that has provided good survival and seedling growth under nursery condition and the same may be used for large scale seedling production.

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