Nodulation and Nitrogen Fixation in Tree Legume (*Albizia procera*) as Affected by *Rhizobium* Inoculation and N-fertilizer

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A nursery experiment was conducted to assess the performance of *Albizia procera* to *Rhizobium* inoculation alone and along with 3 levels of N fertilization (N_1, N_2 and N_3 as 50mg, 150mg and 400mg N/seedling). Also to study the influence of Nitrogen on biological fixed nitrogen by *A. procera*. The *A. procera -Rhizobium* isolate tested as acidic (5.0 pH) tolerant and salt (30000ppm) tolerant. Due to inoculation in *Albizia procera,* 2.6 times increase in biomass accumulation was seen, N uptake was increased from 9.293 to 41.326 mg/seedling at 120DAT and nodulation increased from 0.117 to 0.0.412 g/seedling at 90DAT. However significantly maximum biomass accumulation (10.96g/seedling), extra N gain (55.44 mg/seedling over control at 120DAT), nodule no. (68/seedling) and nodule dry weight (0.483g/seedling) at 90DAT were obtained at *Rhizobium*+N_2 treatment. Inoculation of *Albizia procera* was significantly effective and nitrogen application (150mg/seedling) along with inoculation further significantly enhanced symbiotic traits in *Albizia procera*.

Key words: *Albizia procera , BNF, N uptake, Nodulation and Rhizobium.*

Planting leguminous trees may be an important option to enrich the soil nitrogen status (Khan *et al*., 2004, Hossain,2003) in degraded land and *Rhizobium* inoculation of NFTs at seedling stage helps in producing healthy nursery stocks capable of growing successfully when planted in field (Totey *et al*. 2000). *Albizia procera*, commonly known as 'Safed siris' is one of the important Nitrogen Fixing Tree (NFT) species belongs to family Fabaceae, sub-family Mimosoideae. It provides an excellent fuel, small timber and fodder especially during lean periods of summer, when there is scarcity of green fodder in the region (Singh, 1982). Besides due to its nitrogen fixing ability and fast rate of litter decomposition; it helps in ameliorating the soil. The unavailability of the quality planting stock of the species is big snag in the success of tree planting. Hence production of high quality tree seedlings at nursery is a dare need for successful establishment in plantation programmes. Use of inorganic fertilizers is costly and cumbersome to use over vast plantation areas besides causing soil pollution if not judiciously used (Prasad, 1998). This necessitates the integrated nutrient management involving biological sources of nutrient for sustainable crop production. However, in order to ensure optimal exploitation of their economic and agricultural potential, it is necessary to go for inoculation using effective symbionts. The inoculation effects of some forest legumes has been studied indicating tremendous potentiality of biofertilizers in improving health of soil as well as plant stock (Kumar *et al*., 2013 and Karupasamy *et al* 2012). One of the major factors that can optimize inoculation is availability of mineral N in soil. Diouf et.al 2003 reported that addition of N as starter is required for enhancing BNF. At the same time the evaluation of optimum level of nitrogenous fertilizer for improving BNF is also necessary (Kumar *et al* 2013**).** Hence the present investigation was carried out with objectives to characterize *A. procera* -rhizobia, to assess the response of legume tree seedling (*A. procera*) to *Rhizobium* inoculation and the

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influence of N fertilization on biologically fixed amount of nitrogen by *A. procera* .

MATERIALS AND METHODS

The experiment was conducted in the Glass house of Dept. of Agricultural Microbiology, Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during rabi season of 2011-2012 with *Albizia procera* (Safed siris). The experiment was laid out in complete randomized design (CRD) with 8 treatments, replicated thrice. Treatments were T_1 -Control (Un-inoculated) , T_2 - Inoculated, T_3 - Inoculated + N_1, T_4 -Inoculated + N_2, T_5 -Inoculated + N_3, T_6 - Un-inoculated + N_1, T_7 -Un-inoculated + N_2, T_8-Un-inoculated + N_3. (50mg, 150mg, 400mg/ seedling as N_1, N_2 and N_3 respectively). Seeds of *A. procera* collected from naturally grown area of *A. procera* nearby Agriculture College, Raipur were allowed to germinate in trays containing field soil and sand (2:1) and then uniform seedlings were selected for experiment purpose. Raipur is situated in plains of Chhattisgarh at $21^0$16' N latitude and $81^0$36' E longitude with an altitude of 289.60 meter above mean sea level (MSL).

Rhizobium was isolated from fresh nodule of a locally grown nodulated Albizia procera plant & culture broth was prepared by inoculating in sterilized YEM media and shaken for 7 days. (Vincent ,1970). A well mixed 5 Kg mixture of soil, sand and compost in 3:1:1 ratio was filled in each polythene bag (12"x 10" size). Soil was vertisol , slightly alkaline in reaction (pH 7.6), having medium organic carbon status (6.3g/kg soil), low mineralizable Nitrogen status (240.4 kg/ ha.), low in available (Olsen's) Phosphorus content (11.6 kg/ha.) but high with respect to available Potassium content (416 kg/ha.). The analysis was done as per Page et.al (1982). Initial Rhizobium population Cfu Per g. of soil was 4.8×10^2. After inoculation with matured broth of A. procera – Rhizobium, seedlings were transplanted so that a single healthy seedling was maintained in each polybag. In uninoculated pots, seedlings were dipped in same amount of nutrient broth but not inoculated with Rhizobium. Nitrogen through urea was given in water soluble form after seven days of transplantation of seedlings as per treatment description. Phosphorus and Potassium were applied commonly to all polybags @ 2.0g phosphorous and 1.5g potassium per seedling through SSP and MOP respectively. The seedlings were allowed to grow up to 120 Days.

A. procera -Rhizobium isolate was characterized by Gram staining, sensitivity to antibiotics , acidity and temperature tolerance. Sensitivity against antibiotics was tested by placing the antibiotic disc (Gentamicin, Streptomycin, Kanamycin, Tetracycline and Chloramphenicol) on the inoculated agar plates using sensi- disc dispenser and based on the inhibition zone after 2days incubation, the test organism is determined to be resistant, intermediate or sensitive to the antibiotic. For acidity tolerance, the *p*H level of YEMbroth was adjusted to 5, 5.5, 6, 6.5, 7, 7.5, 8 and inoculated with species *Rhizobium* isolate. After incubation survival of *Rhizobium* were recorded. Likewise for temperature tolerance, the survival of isolate was tested after subjected to thermal shock at different temperatures (Aneja,2003). Seedling biomass accumulation and nodulation at monthly interval and N uptake by plants after harvest on 3 randomly selected plants were recorded in each treatment. All observations were statistically analyzed using ANOVA for completely randomised design (CRD). The significant difference were tested through F-test at 5% level of significance (Panse and Shukhatme (1978)).

RESULTS AND DISCUSSIONS

Characterization of *Albizia procera - Rhizobium*

Albizia procera- Rhizobium isolate was Gram negative, on YEMA media produced translucent colonies of circular shape and raised, smooth surface with milky white in colour. The authentication of the isolates was performed using sub culturing method. Results for antibiotic indicated that Tetracyclin (zone of inhibition 28mm) was most sensitive for growth of *Albizia procera-Rhizobium*. The isolate was found somehow resistance to Chloramphenicol and Gentamicin. It shows intermediate sensitivity towards Kanamycin and Streptomycin (Table 1). It can tolerate salt(NaCl) concentration upto 30,000 ppm however maximum growth was seen at 10,000ppm. The *Albizia procera- Rhizobium* isolate can tolerate acidity upto pH 5.0, so it may be useful for tropical acidic rainfed areas of Chhattishgarh plain to support the growth of *Albizia procera.* pH 6 to 7 was found most suitable for its growth but shows survivelity upto pH 8. The isolate can growth well at temperature 30 ^{0}C and 35 ^{0}C while with increase in temperature growth reduces, but it can also tolerate upto 50 ^{0}C temperature (Table 1). Gupta *et al.* (2002) also characterized rhizobial isolates with respect to their sensitivity towards antibiotics and salt tolerance, found that *Rhizobium* strains tolerated salt concentration up to 40,000ppm. While Mpepereki *et al*. (1997) observed growth of lathyrus-*Rhizobium* upto even upto pH 4 and were at salt tolerance (NaCl) upto 55,000ppm.

Biomass accumulation in *Albizia procera*:

Biomass of *Albizia procera* ranged from 0.75-2.29 (g/seedling), 0.99-4.36 (g/seedling), 1.81-6.84 (g/seedling), 3.47-10.96 (g/seedling) under different treatments at 30, 60, 90, and 120 DAT respectively Fig. 1. *Rhizobium* inoculation along with lower doses of N fertilizer (N_1 and N_2) showed maximum accumulation of total dry biomass *i.e.* 10.41 and 10.96 g/seedling which were 3.00 and 3.16 times greater than control at 120 DAT while only *Rhizobium* inoculation produced 9.13 g/seedling which was at par with T_5 9.24 g/seedling Table2. This is in line with the findings of Morques *et al.* (2002), where a higher biomass was reported in inoculated seedlings of *Centrolobium tomentosum*. Biofertilizer helps boost microbial population present in soil which in turn makes the insoluble nutrients available for growth of plant. Less N application along with *Rhizobium* influence in increasing *Rhizobium* symbiosis and similar observations were also found by Chauhan and Pokhriyal (2002) in *Albizzia* and Kumar *et al* (2013) in *D. sissoo*. Further with application of only inorganic N, significant increase in biomass being maximum of 7.58g/seedling at T8 followed by T7, 6.87g/seedling and T6, 5.67 g/seedling where at control it was 3.47 g/ seedling at 120 DAT.

Nodulation study

With *Rhizobium* inoculation nodules number increased from 13 to 45/seedling and further increased to 68 and 56 due to starter dose of N_2 and N_1 respectively whereas with higher Nitrogen dose N_3 with *Rhizobium* inoculation nodulation did not increase considerably (39/ seedling) Table 3. While among uninoculated

Table 1. Characteristics of *Albizia procera- Rhizobium* isolate

A. Antibiotics sensitivity							
Gentamicin (10 µg)	Streptomycin (10 µg)	Tetracycline (30 µg)	Kanamycin (30 µg)	Chloramphenicol (30 µg)	Control		
12mm	18mm	28mm	15mm	11mm	-		
B. Salt tolerance							
10,000	20,000	30,000	40,000	50,000	60,000	70,000	Control
+++	++	+	-	-	-	-	+++
C. pH tolerance							
5	5.5	6	6.5	7	7.5	8	Control
+ +	+ +	+ + +	+ + +	+ + +	+	+	+++
D. Temperature tolerance							
30 ^{0}C	35 ^{0}C	40 ^{0}C	45 ^{0}C	50 ^{0}C	Control		
+ + +	+ + +	+ +	+ +	+	+++		

(+++ - Very good growth, ++ - Medium growth, + - Poor growth) treatments, maximum nodule no. was found (25/seedling) at T_8, followed by (21/seedling) at T_7, and (18/seedling) at T_6 whereas minimum was seen in control 13/seedling (at 90DAT). It was seen that nodulation decreased considerably in the control, but could not be completely eliminated as is evident from sparse nodulation in the control plants (2-13/seedling from 30-90DAT). At 90 DAT significantly maximum nodule dry weight (g/seedling), 0.483 was found at T_4 followed by 0.456 at T_3, 0.412 at T_2 and 0.387 at T_5 while at control it was minimum (0.117). Nodule dry wt. ranged from 0.215 to 0.341g/seedling with application of inorganic N only. Lower levels of N dose N_1 and N_2 when applied with *Rhizobium* inoculation influenced the nodulation and ensure to increase BNF, At 90 DAT, significantly maximum nodulation was observed in *Albizia procera* plants in all treatments which becomes slightly senescent at latter stage (at 120 DAT) of *Albizia procera*. *Albizia procera* nodules were irregular in shape, milky white colored and somewhat medium size nodules. Similar observation was also found by Chauhan and Pokhriyal (2002) who studied nodule behaviour in *Albizia* tree species.

Nitrogen status in *Albizia procera*

N uptake varied from 9.293 to 64.729 mg/seedling being significantly maximum in T_4 which gains 55.436 extra N through BNF at 120 DAT (Table 3). Inoculated seedlings showed significantly higher N content over only N fertilization and control.. In $R + N_2$ *Albizia procera* plants showed significantly maximum Nitrogen content 64.729mg/seedling followed by 56.986 mg/seedling at *Rhizobium* + N_1 while 44.217mg/seedling at T_5 which were significantly higher than at *Rhizobium*

Table 2. Effect of *Rhizobium* inoculation with different levels of N on nodulation behaviour of *Albizia procera* plants at different stages

Treatment	No. of nodules/seedling Days after transplant				Dry weight of nodules (g/seedling) Days after transplant			
	30	60	90	120	30	60	90	120
T_1(Control)	2	10	13	-	0.018	0.090	0.117	-
T_2(Inoculated	6	25	45	12	0.045	0.301	0.412	0.105
T_3(Inoculated + N_1)	6	25	56	11	0.044	0.304	0.456	0.108
T_4(Inoculated + N_2)	8	29	68	12	0.063	0.34	0.483	0.108
T_5(Inoculated + N_3)	5	20	39	8	0.041	0.276	0.387	0.081
T_6(Un-inoculated + N_1)	4	10	18	5	0.034	0.185	0.215	0.072
T_7(Un-inoculated + N_2)	4	12	21	6	0.034	0.198	0.234	0.071
T_8(Un-inoculated + N_3)	4	18	25	6	0.036	0.245	0.314	0.063
SEm(±)	0.540	0.577	1.154	0.735	0.001	0.004	0.001	0.001
CD (5%)	1.619	1.730	3.461	2.206	0.004	0.014	0.003	0.004

Table 3. Effect of *Rhizobium* inoculation with different levels of N fertilization on biomass and N uptake (mg/seedling) in *Albizia procera* plants at 120 DAT.

Treatment	Biomass accumulation (g/seedling)	N Uptake (mg/seedling)	Extra N gain through BNF (mg/seedling)
T_1(Control)	3.469	9.293	-
T_2(Inoculated (*Rhizobium*)	9.130	41.326	32.033
T_3(Inoculated + N_1)	10.409	56.986	47.693
T_4(Inoculated + N_2)	10.965	64.729	55.436
T_5(Inoculated + N_3)	9.238	44.217	34.924
T_6(Un-inoculated + N_1)	5.666	16.933	9.372
T_7(Un-inoculated + N_2)	6.867	22.852	10.239
T_8(Un-inoculated + N_3)	7.579	29.238	12.279
SEm (±)	0.174	0.652	-
CD (5%)	0.523	1.956	-

alone 41.326mg/seedling. Again among only N fertilization, the effects were in order of T8 29.238 > T7 22.852> T6 16.933mg/seedling. However, all treatments showed significant effect over control.. Extra N gain through BNF was maximum at T_4 (55.436mg/seedling) followed by T_3 and $T_{5,}$ 47.693 and 34.924mg/seedling respectively at 120 DAT (Table 3). The higher growth in *Rhizobium* + N_2 treated seedling 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. Similar observations were recrded by Chaukiyal, S.P. and Pokhriyal, T.C. (2005) for N fixation in *Pongamia pinnata*.. Such synergistic effect of increased uptake of one nutrient facilitated by other nutrients in leguminous species was also reported by Prasad, (1998).

***Rhizobium* population in rhizosphere soil of *Albizia procera* plants**

Initially *Rhizobium* population per g of soil in rhizosphere soil of *Albizia procera* was 4.8 x 10^2 before planting, after at 90 DAT, *Rhizobium* population(/g soil) in rhizosphere soil of *Albizia procera* was 1.65 x10^3 at control due to influence of legume tree, further due to inoculation it increased significantly to 2.52 x 10^3 and further increased to 2.96 x 10^3 and 3.24 x 10^3 when *Rhizobium* inoculation was given along with N_1 and N_2 doses respectively Table 4. At 120 DAT, *Rhizobium* population decreased considerably as compared to 90DAT. N at less level N_1 and N_2 has a positive correlation in increasing *Rhizobium* population at rhizosphere zone of *Albizia procera* but in case of higher N dose *i.e.*, N_3 was not so effective when applied along with *Rhizobium* inoculation. The more microbial population in rhizosphere soil was established by planting of legume tree, which also reported by Singh *et al.*, 2000, Revati *et.al.*, 2013.

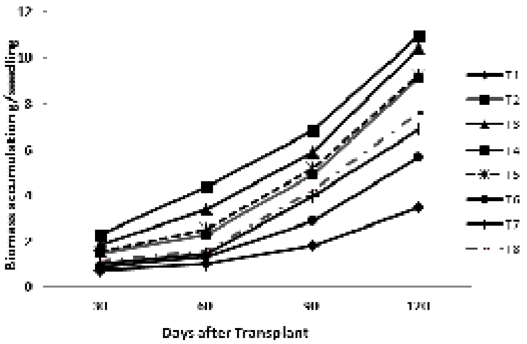

Fig. 1. Effect of *Rhizobium* inoculation with different levels of N on biomass accumulation in *A. procera*

Application of N as starter dose along with *Rhizobium* inoculation showed significant effect over only *Rhizobium*. Bora *et al.*, 2006 observed both root and shoot biomass was considerably high in *A. procera* inoculation seedling growth and in LLU (low level urea). The value was significantly reduced in case HLU (High level of urea) amended soil. This may be, due to the fact that the higher rates of N fertilizer may have produced nutrient imbalance thus rendering unavailable the other elements in soil. (Jha *et al.*, 2000). This type of study was also carried out by Chauhan and Pokhriyal (2002) in *Albizzia lebbeck*.

Based on results it can be inferred that Inoculation of *Albizia procera* plants with *species-Rhizobium* was found significantly effective and application of N fertilizer at the rate of 150mg/

Table 4. *Rhizobial* population in rhizosphere soil of *Albizia procera* plant as affected by different treatments at 60, 90, 120 DAT

Treatment	Rhizobium population cfu per g of soil 60 DAT	90 DAT	120 DAT
T_1(Control)	1.52 x 10^3	1.65 x 10^3	1.40 x 10^3
T_2(Inoculated (*Rhizobium*)	2.46 x 10^3	2.52 x 10^3	2.34 x 10^3
T_3(Inoculated + N_1)	2.73 x 10^3	2.96 x 10^3	2.64 x 10^3
T_4(Inoculated + N_2)	3.01 x 10^3	3.24 x 10^3	2.86 x 10^3
T_5(Inoculated + N_3)	2.24 x 10^3	2.36 x 10^3	2.28 x 10^3
T_6(Un-inoculated + N_1)	1.74 x 10^3	1.84 x 10^3	1.71 x 10^3
T_7(Un-inoculated + N_2)	1.82 x 10^3	1.93 x 10^3	1.74 x 10^3
T_8(Un-inoculated + N_3)	1.87 x 10^3	1.96 x 10^3	1.80 x 10^3
SEm (±)	0.018 x10^3	0.027 x 10^3	0.013 x 10^3
CD at 5%	0.056 x10^3	0.082 x 10^3	0.040 x 10^3

seedling as starter dose along with *Rhizobium* inoculation has tremendous potential in improving initial growth response of *Albizia procera* plants in nursery. This finding may be helpful in producing quality planting stock of *Albizia procera* for afforestation programmes in waste and degraded land.

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