Genetic Variability in Progenies of *Acacia nilotica* (L.) ex DEL. ssp. *indica* (BENTH.) BRENAN for Nitrogen Fixing Ability

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(Received 15th September 1994)

Summary

Nitrogen fixing abilities of 7 provenances and 21 progenies of *Acacia nilotica* (L.) WILLD ex DEL. ssp. *indica* (BENTH.) BRENAN were assessed in nitrogen free sterilized sand in "chillum" jars at Hisar (29°10'N Lat., 75°46'E long., 100 m alti.) in arid India. There were significant differences ($P < 0.05$) in plant growth and their nitrogen contents between provenances. Maximum growth and nodulation occurred in Behrampur provenance and the least in that of Gurgaon and Patna. Significant variations were also observed among the progenies of each provenance. Progenies of Gurgaon provenance exhibited maximum variation. Interactions between progenies and provenances were significant ($P < 0.05$). Overall best interaction was observed between Behrampur provenance and P_1. Wide genetic diversity in nitrogen fixation ability of *Acacia nilotica* ssp. *indica* can be of great potential in tree improvement programes particularly selection of genotypes for nitrogen deficient soils.

Key words: Acacia nilotica ssp. *indica,* progenies, nitrogen fixing ability.

FDC: 232.11; 165.5; 161.11; 176.1 *Acacia nilotica;* (540).

Introduction

The dominant woody legumes of arid and semi-arid regions of India comprise of species of *Prosopis, Acacia, Albizia* and *Dalbergia.* In most of tree legumes a few root nodules are formed on surface soil and the importance of nitrogen fixation is therefore, questioned (FARNSWORTH et al., 1978). Different provenances of *Acacia albida* exhibit considerable variation in the growth which may be attributed to their nodulation pattern (SNIEZKO, 1987). NGULUBE (1990) reported that nodulation was highly variable within provenances of *Acacia auriculiformis.*

Acacia nilotica (L.) WILLD ex DEL. ssp. *indica* (BENTH.) BRENAN locally called "babul" is a co-dominant associate of *Prosopis* in semi-arid regions. It is a small sized tree with strong vertical and horizontal root system (TOKY and BISHT, 1992), and a long growing period of more than 300 days with 4 peaks of leaf flush and monolayered canopy (BISHT and TOKY, 1993). This species has high potential for nitrogen fixation (TOKY et al., 1994), and has been considered as one of the fast growing species on the wastelands.

Our earlier study (BENIWAL et al., 1994) reported significant differences in the abilities for nitrogen fixation between provenances of *Acacia nilotica* ssp. *indica.* The present study reports genetical variations in nitrogen fixing ability of progenies of different provenances. Selection of superior progenies for nitrogen fixation ability would greatly increase the species potential.

Material and Methods

In an earlier study on *Acacia nilotica* ssp. *indica* (KRISHAN, 1992), the seeds of 23 provenances were collected in 1986 from 11°N to 31°N latitude in India. Each seed lot comprised of 2 kg to 5 kg seed. A provenance trial was laid out at Hisar, located in arid region of north-western India. Each provenance consists of 81 trees, split up into 3 blocks. After 6 years of age in 1993, the trees started bearing seeds. For the present study, 7 best performing provenances i.e. Varanasi, Gurgaon, Khajuraho, Behrampur, Jaipur, Patna and Hisar were selected, and during June, 1993, seeds of 21 trees (3 trees of each provenance selected randomly) were collected.

To study nitrogen fixing ability "chillum" jar technique was used. The "chillum" jars are earthern pots specially designed to study nodulation behaviour in legume species under net house condition (DAHIYA and KHURANA, 1981). The "chillum" jars assemblies containing sand were autoclaved for 2 hours. The seeds were soaked in boiling water, sterilized with 0.1% mercuric chloride solution and sown in "chillum" jars after inoculation with *Rhizobium* strain AC-I. *Rhizobium* AC-I was isolated from healthy and pink nodules of *Acacia nilotica* (BALA et al., 1990). The plants were provided sterilized water daily and Sloger's nitrogen free nutrient solution once a week.

Every progeny was represented by 3 replicates of "chillum" jars and each "chillum" jar contained 3 plants. "Chillum" jars were arranged in a complete randomised design in a net house. The plants were uprooted after 45 days of seed sowing, and the dry weights of shoots and nodules were estimated by keeping them in oven at 60 °C for 24 hours. Shoots were ground and nitrogen contents were determined by NESSLER's reagent method. Nitrogenase activity (acetylene reduction assay) was estimated in intact nodules using a gas chromatograph (Nucon Amil 5500), as described by HARDY et al. (1968). The nitrogenase activity was expressed as a u moles C_2H_2 reduced per hour per plant.

The data were analysed using complete randomised design and ANOVA was calculated to find out significant differences between provenances and progenies.

Results

Nodulation behaviour

Significant differences ($P < 0.05$) occurred between provenances for nodulation behaviour (*Table 1*). Maximum number of nodules and their weights were observed in Behrampur provenance followed by that of Varanasi provenance. These 2 provenances differed significantly ($P < 0.05$) from the remaining provenances.

Significant differences ($P < 0.05$) for nodule number and their biomass were also observed between progenies of most of the provenances (*Table 1*). Variation for nodule number (CV = 22%) and nodule weight (CV = 24%) were the greatest in progenies of Gurgaon provenance, and the least were in those of Varanasi provenance (CV = 12%, CV = 13%, respectively).

Interactions between provenances and progenies (*Table 1*) were also significant ($P < 0.05$). The most efficient interactions were observed for Behrampur provenance and P_1.

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Table 1. – Variation between provenances and progenies of *Acacia nilotica* ssp. *indica* for nodule number and nodule dry weight (mg/seedling). P_1, P_2 and P_3 represent progenies of a provenance.

Provenance	Nodule number (n=3)			Mean (n=9)	CV (%)	Nodule dry weight (n=3)			Mean (n=9)	CV (%)
	P 1	P 2	P 3			P 1	P 2	P 3		
Varanasi	28	27	31	29	12	26	28	31	28	13
Gurgaon	26	32	20	26	22	30	33	20	28	24
Khajurah	24	24	28	26	14	23	29	27	26	16
Behrampur	35	31	26	31	17	37	29	28	31	16
Jaipur	26	21	29	25	16	23	21	28	24	17
Patna	18	23	22	21	17	19	24	21	22	14
Hisar	24	28	30	27	14	22	30	30	27	17

LSD at 5% level

Provenance:	3.5	3.4
Progeny :	2.3	2.2
Provenance X Progeny:	6.0	5.9

Table 2. – Variation between provenances and progenies of *Acacia nilotica* ssp. *indica* for shoot dry weight (mg/seedling) and nitrogen content (mg/seedling). P_1, P_2 and P_3 represent progenies of a provenance.

Provenance	Shoot dry weight (n=3)			Mean (n=9)	CV (%)	Nitrogen content (n=3)			Mean (n=9)	CV (%)
	P 1	P 2	P 3			P 1	P 2	P 3		
Varanasi	293	338	367	333	9.7	9.1	9.7	9.0	9.3	5.9
Gurgaon	303	296	336	311	5.7	6.3	7.0	9.2	7.5	16.8
Khajuraho	298	368	346	337	9.0	10.9	14.9	12.6	12.6	13.8
Behrampur	451	360	450	420	13.0	16.8	11.8	13.8	14.1	17.2
Jaipur	330	294	387	327	15.8	10.0	7.4	12.2	9.9	20.9
Patna	337	314	428	360	15.2	9.0	7.3	9.8	8.7	14.3
Hisar	292	341	327	320	8.1	9.9	10.6	9.6	10.0	7.0

LSD at 5%

Provenance	21.4	0.8
Progeny	14.0	0.5
Provenance X Progeny	37.1	1.4

Table 3. – Variation between provenances and progenies of *Acacia nilotica* ssp. *indica* for nitrogen concentration (%) and nitrogenase activity (u moles/plant/h). P_1, P_2 and P_3 represent progenies of a provenance.

Provenance	Nitrogen concentration (n = 3)			Mean (n=9)	CV (%)	Nitrogenase activity (n = 3)			Mean (n = 9)	CV (%)
	P 1	P 2	P 3			P 1	P 2	P 3		
Varanasi	3.1	2.9	2.5	2.8	9.7	3.8	2.2	0.9	2.3	64.9
Gurgaon	2.1	2.7	2.7	2.5	11.6	3.5	6.0	3.4	4.3	28.4
Khajuraho	3.7	4.0	3.5	3.7	6.6	6.0	4.7	6.3	5.7	12.5
Behrampur	3.7	3.3	3.1	3.4	8.5	3.6	3.5	6.0	4.3	26.6
Jaipur	3.0	2.8	3.2	3.0	5.8	1.4	1.4	2.1	1.6	23.2
Patna	2.7	2.3	2.3	2.4	7.8	1.6	1.6	1.4	1.5	19.7
Hisar	3.4	3.1	3.0	3.1	5.6	3.5	1.5	5.6	3.5	48.1

LSD at 5%

Provenance:	0.1	0.5
Progeny:	0.1	0.3
Provenance X Progeny:	0.2	0.8

Table 4. – Analysis of variance for nodulation and nitrogenase activity in *Acacia nilotica* ssp. *indica* between and within provenances.

		F Values					
Source	Degree of Freedom	Nodule number/ seedling	Nodule weight/ (mg/seedling)	Shoot weight (mg/seedling)	Nitrogen content (mg/seedling)	Nitrogen conc. (%)	Nitrogenase activity (u moles/plant/h)
Provenance	6	6.40*	6.75*	24.22*	68.95*	92.15*	85.03*
Progeny	2	0.25	1.31	34.40*	7.88*	12.30*	9.61*
Provenance X Progeny	12	3.82*	4.59*	7.98*	13.84*	11.82*	19.65*

* $P > 0.05$

Shoot weight and nitrogen content

Shoot weight ranged from 311 mg/plant in Gurgaon provenance to 420 mg/plant in Behrampur provenance. Patna provenance was the second best. Two top ranking provenances varied statistically ($P<0.05$) among themselves and from the remaining provenances. The low ranking provenances did not differ significantly among themselves ($P>0.05$) (*Table 2*).

Progenies of a provenance also differed significantly among themselves except that of Behrampur and Gurgaon provenances (*Table 2*). Maximum variation among progenies was in Jaipur provenance (CV=15.8%), and the least in Gurgaon provenance (CV=5.7%). The most efficient interaction between provenances and progenies was observed in Behrampur provenance and P_1 and Behrampur provenance and P_3.

Nitrogen concentration in the shoot ranged from 2.4% in Patna provenance to 3.7% in Khajuraho provenance, and nitrogen content from 7.5 mg/plant in Gurgaon provenance to 14.1 mg/plant in Behrampur provenance. Significant differences ($P<0.05$) were also observed between progenies (*Table 2, 3*).

Nitrogenase activity

Maximum nitrogenase activity was recorded in Khajuraho provenance, and least that in Patna provenance (*Table 3*). The least nodule number and nodule weight were also observed in Patna provenance (*Table 1*).

Maximum variation among progenies was observed in Varanasi provenance (0.9 u to 3.8 u moles/plant/h; CV=65%). Interactions between provenances and progenies were also significant ($P<0.05$). Maximum interaction was in Khajuraho provenance and P_3, and the least in Varanasi provenance and P_3 (*Table 3*).

There were significant and positive correlations between nitrogen content and shoot weight ($r=0.73$, $P<0.01$), nitrogen content and nodule weight ($r=0.47$, $P<0.05$), and nitrogen content and nitrogenase activity ($r=0.69$, $P<0.05$).

Analysis of variance for nodulation and nitrogenase activity between and within provenances has been given in *table 4*.

Discussion

The present study was initiated to evolve high nitrogen fixing combination of *Acacia nilotica* and *Rhizobium* sp. In an earlier study (BENIWAL et al., 1994) we observed a significant variation for nitrogen fixation among different provenances of *A. nilotica* in association with *Rhizobium* spp. *Acacia* strain AC-I. This strain was salinity tolerant and highly efficient for nitrogen fixation in *Acacia nilotica*. In the present study we selected 7 best provenances out of 27 provenances which were grown in the field (KRISHAN, 1992; KRISHAN and TOKY, 1995). We also observed that growth and biomass accumulation in 6-year old trees were different in progenies of the same provenance. Genetic variability for nitrogen fixation potential could be the one of the reasons for variation between progenies. To test this possibility we tested 3 mother trees (progenies) each of the 7 provenances for nitrogen fixation in sterilized nitrogen free sand medium.

Variability was observed among different provenances. Provenance from Behrampur was genetically superior than rest of the provenances in terms of nodule number, nodule dry weight, shoot dry weight and nitrogen content. On the other hand, provenance from Patna was genetically inferior to other provenances and showed poor nodulation and nitrogen fixation. Extent of nodulation therefore, is a factor for higher biomass production. These results confirmed our field observations for genetical variability among 23 provenances. Such genetic variability for nodulation and nitrogen fixation were also reported in *Leucaena leucocephala, Acacia albida* and *Gliricidia sepium* (ATTA-KRAH, 1987; SANGINGA et al., 1990).

The progenies of Behrampur provenance were overall superior to others in terms of biomass and total N content. P_1 of Behrampur provenance was the best progeny among all the tested progenies. These differences might be due to outcrossing nature of *Acacia nilotica* as TYBIRK (1988) reported 28% outcrossing in *A. nilotica* ssp. *subulata* in Kenya.

The ability of nitrogen fixation is limited by supply of photosynthate (HARDY and HAVELKA, 1976; GUNAWARDENA et al., 1993; SANGINGA et al., 1994). A positive correlation between net rate of photosynthesis and nodule weight and nitrogen fixation has been reported in *Alnus glutinosa* (GORDON and WHEELER, 1978). More growth in Behrampur provenance of the present study gave more nitrogenase activity as compared to other provenances. The poor growth in some provenances may be due to the limited supply of photosynthate.

Low available P is a common feature in most tropical soils and addition of P improves nodulation and nitrogen fixation in trees. Recent studies on *Gliricidia sepium* have shown large variability in growth, P uptake and use efficiency and N_2 fixation (SANGINGA et al., 1994). Genetical variability that exists among provenances of *G. sepium* for P uptake in turn strongly affects its nodulation and nitrogen fixation ability. We have not taken this parameter into consideration, however, this could be one of the reasons for genetic variability for nitrogen fixation.

This kind of study can help to identify the better genotypes of *Acacia nilotica* as a matter of fact other tree legumes which can form effective symbiosis with indigenous or inoculated *Rhizobium*. The compatible combinations of host genotypes and *Rhizobium* stains will improve the establishment and growth of NFT's on N poor soils for agroforestry systems and energy plantations on the wastelands.

Acknowledgements

Dr. BAL KRISHAN provided the seeds of *Acacia nilotica,* and to Mr. LAWAN JAIN typed the manuscript.

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Genetic Analysis on the Hybrid Origin of *Populus Tomentosa* CARR.

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(Received 13th October 1994)

Abstract

Results supported the hypothesis that *P. tomentosa* CARR. was the natural hybrid of *P. alba* and *P. davidiana*. Evidence supporting hybrid origin came from studies in which comparisons of floral bracts of parents and progenies were made as follows: 1) from control-cross progenies of *P. alba* x *P. davidiana*, 2) from segregation studies of backcrossed progenies of both *P. alba* x *P. tomentosa* and *P. davidiana* x *P. tomentosa*, 3) from open-pollinated populations of female and male *P. tomentosa* plants possessing different bract types. Additional support for hybrid origin came from quantitative analysis of flower organ measurements.

Key words: P. tomentosa, P. alba, P. davidiana, control-pollination, backcrossing, open-pollination, origin, inheritance.

FDC: 165.1; 165.3; 165.71; 176.1 *Populus tomentosa.*

Introduction

P. tomentosa, belonging to the section of Leuce Duby, is a fast growing timber species native to China. It has been cultivated more than any other poplar species because of its fast growth, excellent wood quality and resistance to plant diseases and insect pests, especially the roundheaded borer (YANG, 1991). In China it is cultivated as timber species in Hebei, Shandong, Henan, Shanxi, Shaanxi, southeast Gansu, the north of Jangsu and Anhui (ZHANG, 1958). It is quite important to the economy of northern China. The species is researched in the provinces mentioned above and in Ningxia and Beijing. Most research concentrates on selection of plus trees and clonal testing.

The existence of *P. tomentosa* was first acknowledged as a species in Revue Horticole in 1867 (CARRIERE, 1867). Since then, many plant taxonomists have discussed its taxonomic position. CARRIERE (1867), LEE (1935), SCHENCK (1939), LIU (1955), and CHEN (1959) all consider it to be an independent species and call it *Populus tomentosa* CARR. HENRY (1903) also acknowledges it as an independent species but calls it *Populus peking* L. HENRY. MAXIMOWICZ (1879) and WESMAEL (1887) regard it as a variety of the white poplar. After morphologically comparing the variation in bracts of *P. alba, P. tremula, P. davidiana, P. x canescens* and *P. tomentosa,* BARTKOWIAK (1961) first suggests *P. tomentosa* originates from a natural hybrid. When comparing 25 characters of *P. tomentosa* with those of *P. alba, P. alba* var. *bachofenii, P. alba* var. *bolleana, P. tremula, P. davidiana, P. x canescens,* BIALOBOK (1964) suggests that *P. tomentosa* is a natural hybrid of *P. alba* and *P. davidiana.* This paper is an important and valuable taxonomic report about the taxonomic position of *P. tomentosa.* WAN and FANG et al. (1984) still believe that *P. tomentosa* is an independent species, but the hybrid origin of *P. tomentosa* is acknowledged by most scholars. BIALOBOK (1964) also points out *P. tomentosa* is similar to *P. adenopoda.* There are now 3 viewpoints about this origin. First, *P. tomentosa* originates from *P. alba* and *P. davidiana.* This view is supported by NIU and QU (1980), YU and ZHANG (1992). Second, *P. tomentosa* originates from *P. alba* x *P. adenopoda.* This view is supported by BAI and MA (1990). Third, *P. tomentosa* may result from the combination of several natural hybrids, mainly *P. adenopoda* x *P. davidiana* and its parents, also include *P. alba* and *P. hopeiensis.* ZHAO (1987) and XUE (1981) believe the last viewpoint. No genetic studies on *P. tomentosa* have been reported.

The studies mentioned above represent a valuable information source on the possible origin of *P. tomentosa.* Their conclusions, however, are inferred from morphological comparisons of *P. tomentosa* with assumed parents, or are judged on the basis of the overlapping distributions of assumed parents. AYALA and KIGER, JR. (1984) point out that "Nothing in biology is understandable except in the light of genetics." In the following paper, we investigated the origin of *P. tomentosa* by genetic studies.

Materials and Methods

The genetic analysis of the origin of *P. tomentosa* was made from 4 studies: (1) comparing floral bracts of control-pollinated F1 progenies from *P. alba* x *P. davidiana* with those of *P. tomentosa* and both parent species; (2) analyzing segregation of floral bracts of backcrossed offspring and comparing them with tentative parent species, *P. alba* and *P. davidiana,* and

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