COMPARATIVE GROWTH, NODULATION AND TOTAL NITROGEN CONTENT OF SIX TREE LEGUME SPECIES GROWN IN IRON MINE WASTE SOIL

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THATOI, H., MISRA, A.K. & PADHI, G.S. 1995. Comparative growth, nodulation and total nitrogen content of six tree legume species grown in iron mine waste soil. Six tree legume species, namely *Sesbania grandiflora, Leucaena leucocephala, Acacia nilotica, Acacia auriculiformis, Prosopis juliflora* and *Albizia lebbeck*, were grown in iron mine waste soil to study their comparative growth, nodulation and total nitrogen contents with a view to select suitable plant species to be used for reclamation of mine waste soils. Growth responses of plants as determined from shoot length, root length, fresh and dry weights were found to be highest in *S. grandiflora*, followed by *L. leucocephala* and *A. nilotica*. Although nodule number per plant was highest in *S. grandiflora, A. nilotica* showed better rooting and nodulation capacity with larger nodule size and dry weight per nodule over others. Total nitrogen content of plant was highest in *L. leucocephala*. From the overall performance from all parameters *L. leucocephala, S. grandiflora* and *A. nilotica* were seen to be the best responding plants in iron mine waste soils. However, *S. grandiflora* was unsuitable for reclamation purpose because of its high water demand, soft wood and lower hardiness. Hence, the other two tree legumes, *L. leucocephala* and *A. nilotica*, were found to be most suitable for growing in iron mine areas.

Key words: Reclamation - legumes - iron mine waste soil

THATOI, H., MISRA, A.K. & PADHI, G.S. 1995. Perbandingan pertumbuhan, nodulasi dan jumlah kandungan nitrogen enam spesies pokok kekacang yang tumbuh di tanah bekas lombong besi. Enam spesies pokok kekacang iaitu *Sesbania grandiflora, Leucaena leucocephala, Acacia nilotica, Acacia auriculiformis, Prosopis juliflora* dan *Albizia lebbeck* ditanam di tanah bekas lombong besi untuk mengkaji secara perbandingan bagi pertumbuhan, nodulasi dan jumlah kandungan nitrogen tanaman untuk memilih spesies tanaman yang sesuai bagi memulihguna tanah bekas lombong. Tindakbalas pertumbuhan tanaman yang ditentukan daripada ketinggian pucuk, ketinggian akar, pengaruh segar dan kering didapati paling tinggi di dalam *S. grandiflora*, diikuti oleh *L. leucocephala* dan *A. nilotica*. Walaupun jumlah nodul bagi setiap tanaman adalah paling tinggi di dalam *S. grandiflora, A. nilotica* menunjukkan keupayaan pengakaran dan nodulasi yang lebih baik dengan saiz nodul yang lebih besar dan pengaruh kering bagi satu nodul berbanding dengan yang lain. Jumlah kandungan nitrogen di dalam tanaman adalah paling tinggi di dalam *L. leucocephala*. Dari keseluruhan performans daripada kesemua parameter, *L. leucocephala, S. grandiflora* dan *A. nilotica* menunjukkan tindakbalas paling baik bagi tanaman di tanah bekas lombong. Bagaimanapun, *S. grandiflora* tidak sesuai untuk tujuan memulihguna kerana memerlukan air yang banyak, kayu lembut dan ketahanan yang rendah. Oleh itu, dua lagi pokok kekacang iaitu *L. leucocephala* dan *A. nilotica* didapati paling sesuai ditanam di kawasan lombong besi.

Introduction

The Eastern Ghats region of Orissa, India, is rich in various types of minerals, iron being chief among them. Most of the iron mines are located along the hills within dense forest covers. In recent years, the area has been facing a serious environmental problem due to large scale deforestation and depletion of soil quality, along with the problem of toxic heavy metal ion accumulation leading to the formation of vast stretches of waste lands that need immediate attention for reclamation. But revegetation is difficult because of harsh climate and poor fertility along with low microbial activity of mine waste soil (Jasper *et al.* 1988).

Nitrogen, one of the most important nutrients for the growth and establishment of plants, is generally deficient in mine waste soil (Bradshaw *et al.* 1975). Since legumes have the ability to fix atmospheric nitrogen in symbiotic association with the *Rhizobium*-forming nodule (Vincent 1974), they can be introduced as pioneer species into mine revegetation programme. The establishment of nitrogen-fixing plants has two advantages : (1) they utilise atmospheric as well as soil nitrogen and have low available N requirements, (2) they improve the soil physical environment by decreasing the bulk density and increasing moisture retention capacity. These factors make legumes an ideal plant community that can be adoptive to most hostile soil conditions.

However, in any revegetation programme selection of suitable plant species for a particular soil is very important. This is because different species of plants have different growth responses and have their own selection for soil. Besides, in legumes, soil plays a major role in the nodulation behaviour and nitrogen fixing ability of the plants. Therefore, prior to their introduction to any soil, legumes are to be screened for their suitability and potential for nitrogen enrichment (Pokhriyal *et al.* 1993) In the present work a nursery experiment was conducted on six tree legume species, namely *Sesbania grandiflora, Leucaena leucocephala, Acacia nilotica, Acacia auriculiformis, Prosopis juliflora* and *Albizia lebbeck,* to study their comparative growth, nodulation and total nitrogen contents with a view to selecting the most suitable plant species capable of growing in iron mine waste soil.

Materials and methods

The experiment was carried out in the nursery of the Post-Graduate Department of Botany, Utkal University, Bhubaneswar, Orissa. Iron mine waste soil was brought from a iron mine area of Daitari (21° 06' to 23° 24'N and 85° 52 E) in the district of Keonjhar, Orissa during January. Polypots of size 15 × 27 cm were filled with 4 kg pure mine soil that had been sieved through 3 × 3 mm wire mess to separate large pieces of laterite. In total, 120 polypots were filled with mine soil arranged in the nursery in six sets with 20 replicates in an randomised block design method.

Seed germination and seedling raising

Ripe seeds of the six tree legume species were collected from healthy matured plants in the locality, sterilized with 0.1% $HgCl_2$ for 3 min, followed by rectified spirit (95% alcohol) for 1 min and germinated in large Petri dishes over a water-soaked cotton bed. The hard coated seeds of *A. nilotica* and *P. juliflora* were immerssed in hot water (taken a few minutes after boiling) and then soaked overnight to improve germination before putting in Petri dishes. The germinating seeds were shown in polypots and the seedlings were raised in the nursery. Adequate watering was done regularly and seedlings were maintained for a period of two months.

Sampling and analysis

During sampling three seedlings from each species were randomly picked and brought to the laboratory to study their growth, nodulation and total nitrogen content. While growth of the plant was measured in terms of shoot length, root length and fresh and dry weight of plants, nodulation was studied as nodule number, nodule size (maximum) and dry weight per nodule for each plant. The polypots were gently torn and the soil adhering to the roots of the plants was separated; the root portion was washed carefully to remove the soil particles and was dried with a blotting paper. Immediately the fresh weights of the plants were taken and then shoot and root lengths were measured. The nodules were separated carefully with foreceps and their number and size and dry weights were recorded. Plant and nodule dry weights were taken after drying the samples at 60 °C for 48 h in an oven. The dried plant samples were ground with a Wiley mill (0.2 mm mesh) and total nitrogen content was determined using the micro-Kjeldhal method (Bremner 1965).

The data were subjected to analysis of variance to test for significant differences in different parameters between the plant species. The means were compared using the least significant difference test.

Results and discussion

Data for comparative growth, nodulation and total nitrogen content of the six tree legume species, viz., *S. grandiflora, L. leucocephala, A. nilotica, A. auriculiformis, P. juliflora* and *A. lebbeck,* are presented in Table 1 and Figures 1 and 2. Growth of plant with respect to shoot length, fresh weight as well as dry weights was found to be highest in *S. grandiflora,* followed by *L. leucocephala* and *A. nilotica.* However, root length showed a reverse trend among these three plants, being highest in *A. nilotica.* The other three species showed significantly poor growth responses, with *A. auriculiformis* exhibiting the lowest shoot length, *A. lebbeck* the lowest root length and *P. juliflora* the least value for fresh as well as dry weights. The plant species vary greatly in their growth habits due to their genotypic characteristics. While some exhibit fast growth others are slow growing. Besides,

Table 1. Comparative growths, nodulations and total nitrogen contents of six tree legumes grown in iron mine waste soil (pure) at 60 days after sowing (mean value ± SE)

Plant species	Shoot length plant $^{-1}$ (cm)	Root length plant $^{-1}$ (cm)	Fresh weight plant $^{-1}$ (g)	Dry weight plant $^{-1}$ (g)	Nodule no. plant $^{-1}$	Dry wt nodule $^{-1}$ (mg)	Maximum advantage nodule size (mm)	Total N content plant $^{-1}$ (%)
Sesbania grandiflora	31.1 ±1.83	29.33 ±1.22	5.57 ±0.34	2.98 ±0.3	24.67 ±2.4	3.23 ±0.05	4.5 ±0.05	1.75 ±0.02
Leucaena leucocephala	24.3 ±1.58	30.46 ±1.68	4.7 ±0.38	2.41 ±0.02	18.33 ±2.19	3.37 ±0.04	0.5 ±0.11	2.1 ±0.1
Acacia nilotica	22.36 ±1.52	32.73 ±2.35	3.37 ±0.21	1.01 ±0.15	11.3 ±1.76	4.66 ±0.04	6.0 ±0.34	1.75 ±0.01
Acacia auriculiformis	10.93 ±1.05	22.67 ±0.86	2.57 ±0.07	0.98 ±0.01	15.67 ±1.45	3.0 ±0.07	4.5 ±0.05	1.4 ±0.01
Prosopis juliflora	13.36 ±0.58	28.9 ±1.85	1.87 ±0.12	0.62 ±0.03	2.67 ±0.33	2.05 ±0.57	3.0 ±0.20	0.21 ±0.01
Albizia lebbeck	12.36 ±0.69	19.9 ±1.08	2.9 ±0.16	1.04 ±0.03	12.0 ±2.1	3.1 0.18	4.0 ±0.26	2.03 ±0.1
F Values	**	*	**	**	**	**	**	**
LSD (5%)	4.035	4.90	0.734	0.1125	6.2	0.281	0.616	0.137

*, ** are significant at $p < 0.01$, $p < 0.001$ respectively.

growth of plant in any soil is dependant on soil nutrient status, microbial activity, moisture retention capacity as well as presence and absence of stress factors, if any.

Iron mine waste soil is generally of poor quality with low nutrient content, low microbial activity along with a high concentration of iron oxides (Misra *et al.* 1990). Plants cannot produce optimum growth in such soil condition. Further, presence of a high concentration of iron in iron mine waste soil may also interfere with the growth of the plants. Iron is an essential plant nutrient mostly needed for synthesis of chlorophyll and iron-containing enzymes such as cytochrome, oxidages, ferridoxins, etc. Plants tend to store iron for future use as ferritin which is a ferric phosphate coated with protein (Smith 1984). While iron is essential for life in small amounts, higher concentrations of iron are toxic. In nature iron is usually present in ferric form but it is taken up in ferrous form (Smith 1984). Neutral to basic soil conditions with good aeration facilitates iron uptake (Mozafar & Oertli 1986). On the other hand, iron toxicity is associated with water-logged, low pH soil or soil with a higher concentration of ferrous iron (Smith 1984).

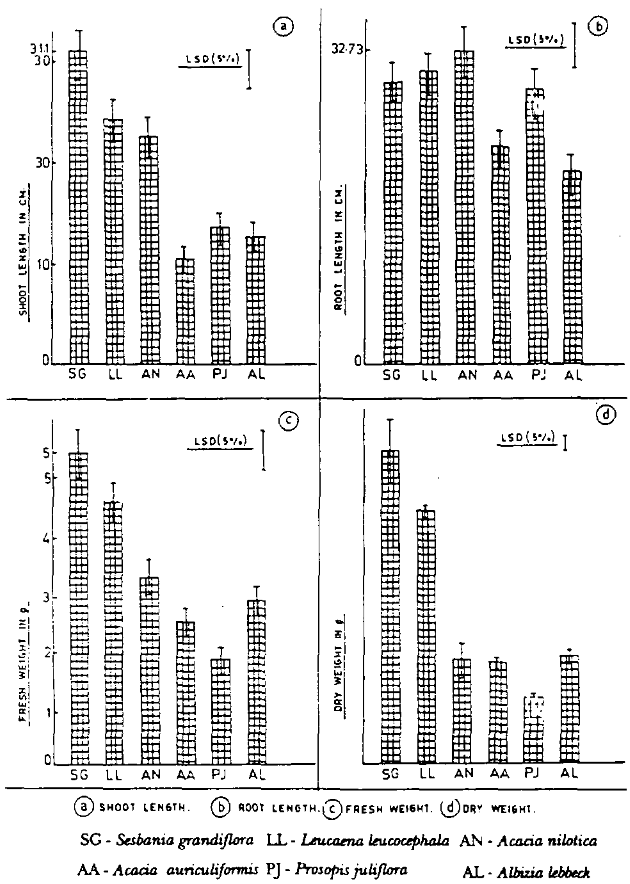

Figure 1. Comparative growth responses of six tree legume species grown in iron mine waste soil at 60 days

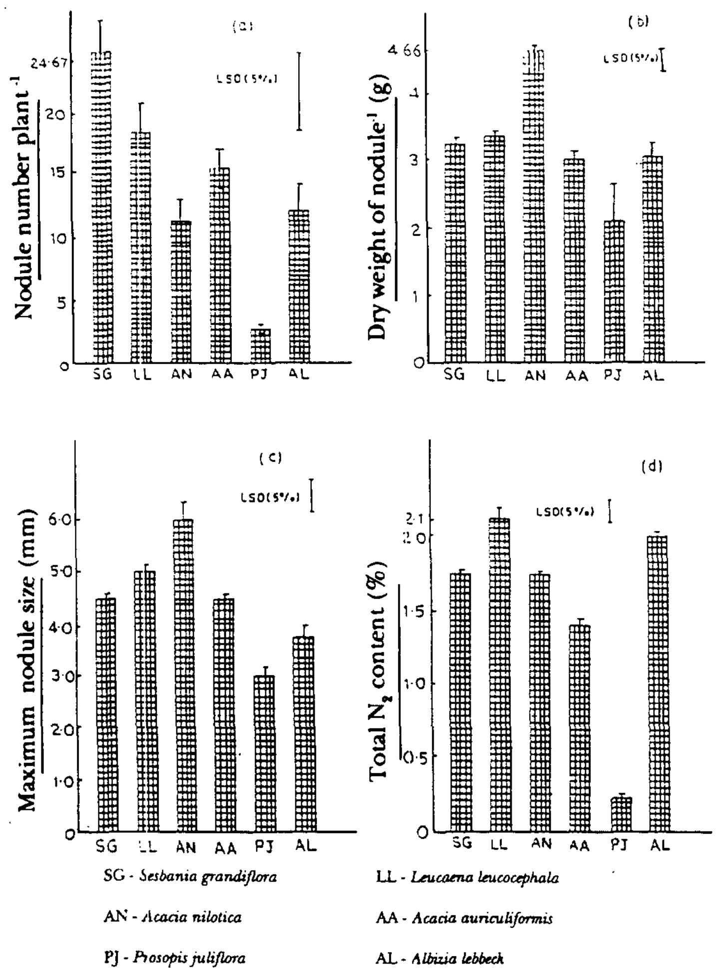

Figure 2. Comparison of nodulation and total nitrogen content of six tree legume species grown in iron mine waste soil at 60 days

It was observed from the nursery trial that *S. grandiflora, L. leucocephala* and *A. nilotica* showed significantly better growth responses in comparison to the other species. In the present study the differential growth responses of the plants were mainly due to the growth habit of the plants and the varying impact of soil on the different plant species. Besides, the tolerance capacity of the tree species to the heavy metal content of the soil, particularly iron, also played a very important role.

Nodulation (nodule number, dry weight per nodule and nodule size) and total nitrogen contents of the plants are represented in Table 1 and Figure 2. The highest nodule number was found in *S. grandiflora,* followed by *L. leucocephala* and *A. auriculiformis.* Dry weight per nodule and maximum size of nodule were highest in *A. nilotica* followed by *L. leucocephala* and *S. grandiflora.* The total nitrogen content was found to be highest in *L. leucocephala,* followed by *A. lebbeck.* This indicates that *L. leucocephala* and *A. lebbeck* are high nitrogen-fixing plants. *S. grandiflora* and *A. nilotica* both had the same total nitrogen content value, followed by *A. lebbeck.* Among the six species, *P. juliflora* showed the least value for all the parameters for nodulation. However, *S. grandiflora, L. leucocephala, A. nilotica* and *A. auriculiformis* showed better nodulating capacity in the iron mine soil than the other two legumes studied.

All the six tree legumes also showed variation in their nodulating ability as well as total plant nitrogen content. Nodulation and nitrogen fixation in legumes are the result of effective symbiotic association formed between legumes and the bacterium *Rhizobium* (Vincent 1974).

Large variations in nodulating ability exist between different species of various legumes. Both host and *Rhizobium* genomes affect the time taken to form nodules and their number and effectiveness in fixing nitrogen (Dart 1975). *Rhizobium* spp., which form nodules in symbiotic association with legumes, are extremely sensitive to environment stress situations (Sprent 1976). In mine soils environmental stress due to metal ions is a major problem. Therefore, in iron mine waste soil stress due to iron metal ions may have great influence on nodulation behaviour of the legumes.

Total nitrogen content of plant was highest in *L. leucocephala* (Table 1) indicating the great nitrogen-fixing potential of that plant. Out of the remaining five species, *S. grandiflora, A. lebbeck* and *A. nilotica* showed comparatively better nitrogen-fixing potential than the others. Development of successful symbiotic association between legume *Rhizobium* results in fixation of atmospheric nitrogen to NH_3 (Dart 1975). More nitrogen is fixed in legumes through symbiosis than is taken up from the soil by roots (Nutman 1957). Total nitrogen in legumes is the sum total of the nitrogen fixed and taken up by the plant, hence it also reflects the nitrogen fixing potential of the plants.

From the data it was difficult to know the responses of plants in chronological order if all the parameters were considered at a time. Therefore, a numerical ranking system was followed taking into consideration the total number of plants and parameters studied. All the six plants were graded one to six considering their responses from higher to lower order for the individual parameters and finally the scores obtained by each plant for all the parameters were added and percentage value was calculated. From this percentage scoring, performance ranking was prepared as given in Table 2. From this table it is found that *S. grandiflora* and *L. leucocephala* jointly scored the highest, followed by *A. nilotica.* The other three species scored much less showing significantly poorer performance of the plants in iron mine soil. From this ranking, *S. grandiflora, L. leucocephala* and *A. nilotica* came out as the best plant species capable of growing in iron mine waste soil. However, although *S. grandiflora* scored highest it is not very suitable for growing because of the soft wood of the plant without any timber value at all. From our experiment we observed that *S. grandiflora* is a highly water demanding species. Since the experiment was conducted under nursery conditions with adequate watering the plant could show good performance. It is difficult to get such response in iron mine waste soil where water stress is a common feature because

Table 2. Comparative performance gradation of six tree legumes grown in iron waste soil

Plant species	SL	RL	PFW	PDW	TN	NN	NDW	NSZ	TOT	PER	Rank*
Sesbania grandiflora	6	4	6	6	5	5	4	4	41	85.42	1
Leucaeana leucoephala	5	5	5	5	6	5	5	5	41	85.42	1
Acacia nilotica	4	6	4	4	5	2	6	6	37	77.08	2
Acacia auriculiformis	1	2	2	2	2	4	1	3	17	35.42	4
Prosopis juliflora	3	3	1	1	1	1	3	1	14	29.17	5
Albizia lebbeck	2	1	3	3	3	3	2	2	19	39.58	3

SL - shoot length, RL - root length, PFW - plant fresh weight, PDW - plant dry weight, TN - total N content of plant, NN - nodule number, NDW - nodule dry weight, NSZ - nodule size, TOT - total points, PER - percentage.
* Rank 1 to 5 in the order of highest to lowest.

of the low water holding capacity of the soil (Misra *et al.* 1990). Further, in field condition regular watering is not practically possible. Due to these reasons, *S. grandiflora* may not be that suitable for reclamation of iron mine waste soil as the other two plants. *L. leucocephala* and *A. nilotica* are thus the best plant species for growing in iron mine area. *Leucaena* is a fast-growing tree with varied utility such as fodder, firewood and timber values (National Academy of Science 1977). It has also a great nitrogen-fixing potential (Guevarra 1976), whereas *A. nilotica* is a native tree legume species which has relatively fast-growing habit and is also a drought resistant, multipurpose tree (Michelsen & Rosendahl 1990). Use of leguminous plants like *L. leucocephala* and *A. nilotica* will improve soil fertility in a mine area and will help promote succession of a plant community gradually through their soil amelioration. This will help a great deal in reclamation of mine areas.

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