IN VITRO PROPAGATION OF *ALBIZZIA LEBBECK* BENTH

T. M. VARGHESE and AMARJEET KAUR
Department of Botany, Haryana Agricultural University, Hisar 125 004, India.

In vitro technique has been successfully employed in propagation of a few tree species namely *Tectona grandis*[1], *Eucalyptus*[2], *Dalbergia sissoo*[3], *D. latifolia*[4], *D. lanceolaria*[5], *Leucaena leucophylla*[6] and *Prosopis cineraria*[7], etc. However, the technique has not been extended in most of the forest trees and plants used for afforestation programmes. The present investigation is based on *Albizzia lebbeck* Benth. commonly known as Black Siris, belonging to the family Leguminosae, one of the few fast growing plants introduced and grown in semi-arid regions of Punjab, Haryana and Rajasthan.

As there is no report on micro-propagation of *A. lebbeck* using plant tissue culture technique except from hypocotyl as explant[8], the present study was undertaken to find out the possibilities of raising plants from various vegetative clones of this species for finding out the variability and the morphotypes that can be regenerated through tissue culture technique and to evaluate the potentiality of various clones to regenerate the plants.

Seeds of *A. lebbeck* were surface-sterilized with 0.1% $HgCl_2$ solution for 5 min and rinsed twice in sterile distilled water. The seeds, thereafter, were dipped in absolute alcohol for 1 min and washed thoroughly with sterile distilled water. The seeds were germinated on agar medium in dark at 27 ± 2°C. Hypocotyl segments, segments of cotyledon, root, leaf and rachis (0.5–0.6 cm long) were excised from 7 to 10-day-old sterile seedlings and were transferred on Murashige and Skoog's (MS) medium[9]. Various concentrations of BAP and kinetin (1–4 mg/l) and auxins (NAA and IAA 1 to 2 mg/l) were incorporated in the MS medium as the basal medium. The pH was adjusted to 5.8 before autoclaving at 1.06 kg/cm^2 pressure for 20 min. Culturing was carried out in 150 ml corning flask, each containing 50 ml medium and the culture was maintained at 27 ± 2°C in temperature-controlled culture room with continuous light. The irradiance was approximately 2500 lux. The callus was transferred to a fresh medium after an interval of 15–20 days. The callus and shoot bud initiation took place as shown in table 1.

Shoot regeneration was observed in calli in all explants. As many as 20 shoots differentiated from the callus of hypocotyl and leaf explants (figure 1).

Table 1

Explants	Additives to MS medium	Days for initiation of callus	Days for induction of shoot buds
Hypocotyl	BAP (2 mg/l)+ NAA (1 mg/l)	7–9	40–45
Cotyledon	BAP (1 mg/l)+ NAA (0.5 mg/l)	8–10	50–52
Root	BAP (2.5 mg/l)+ NAA (1 mg/l)	8–10	60–65
Rachis	BAP (1 mg/l)+ NAA (0.5 mg/l)	7–10	48–50
Leaf	BAP (3 mg/l)+ NAA (1 mg/l)	12–15	70–80

When the shoots were 3–4 cm high, they were transferred to rooting medium i.e. MS supplemented with 0.5 mg/l BAP and 2 mg/l NAA. Root initiation took place after 20–25 days and the root system was well established after 40–45 days. The plants thus developed were transferred in 15 cm earthen pots containing a sterile mixture of sand, soil and farm yard manure (1:1:1). The plants were irrigated at regular intervals with nutrient solution (half MS solution) and were covered with glass beaker to maintain proper humidity.

The growtn of the plants has been successfully continued for the last three months (figure 2).

The plants regenerated, *in vitro* exhibited phenotypic variability and morphological variations; in the height of plants, type of leaves, branching behaviour, etc. Most of the plants had leaves comparable to those in nature. An interesting feature of some regenerants has been the production of a large number of prominent emergences on the stem (figure 3). Another phenotype had shorter leaves with secondary rachii at right angle to the primary one with the leaflets drooping down at 60 to 90° (figure 4 and 5). A few plants regenerated from rachis calli showed profuse branching from the basal nodes which were approximated due to the shortening of internodes (figure 6). Some plants were albinos with yellowish leaves. The plants did not develop beyond a height of 1 cm and leaves were all approximated due to suppression of internodes (figure 7). Some of the regenerants were dwarf with very short internodes. In these plants, the leaves were yellowish due to poor development of chlorophyll and pinnules were smaller but larger in number compared to normal plants. One of the

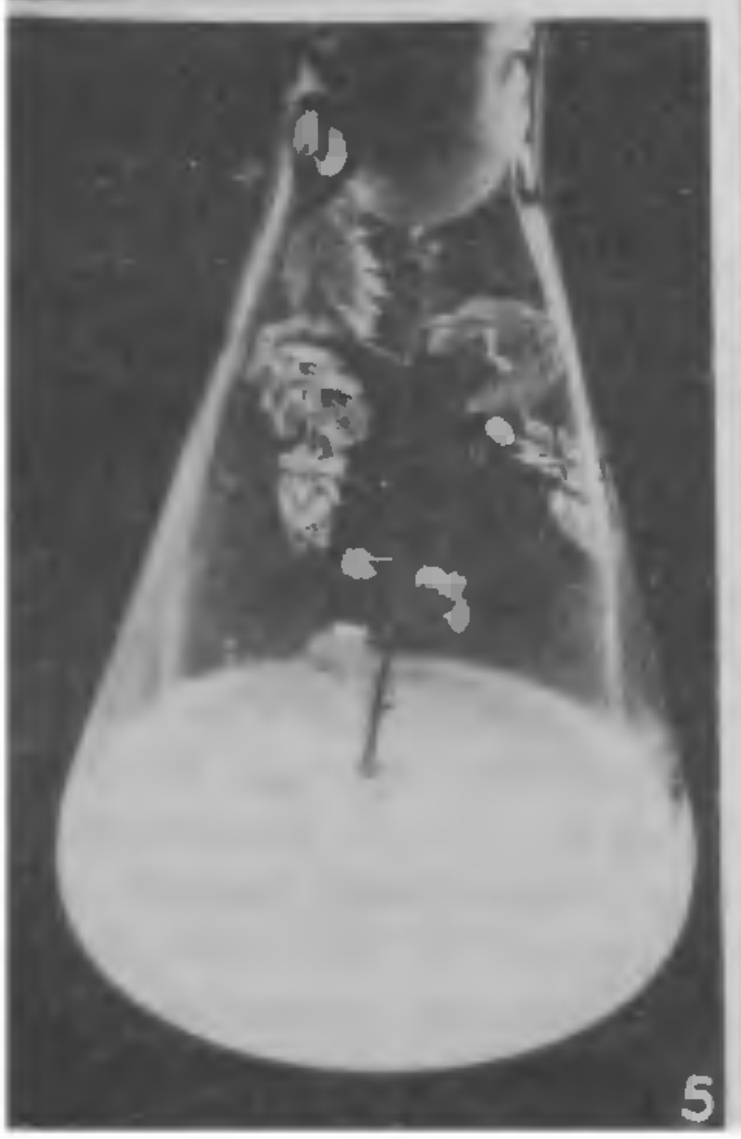

Figure 1–8. **1.** Multiple shoots from hypocotyl callus; **2.** Plants transplanted in the pot culture; **3.** Regenerants with prominent emergences on stems; **4–5.** Phenotype with drooping leaves; **6.** Plants with a number of branches at the base; **7.** Dwarf albino plants, and **8.** Plants with rudimentary leaves. **(1–6 and 8** $\times$ ½, **7** $\times$ ¾).

morphotypes developed from cotyledonary callus had rudimentary leaves on the nodes developed (figure 8).

The present study reveals that the *in vitro* technique is of immense use for induction of morphological variations in the species. Screening of such plants on the basis of their capacity to withstand drought, profuse growth and with better timber and fuel quality for selection of such phenotypes will be of practical significance. The study also provides hope for propagation of the selected genotypes of the species through *in vitro* culture technique as it is easy to regenerate multiple shoots from callus of any explant using tissue culture technique in the species.

Further studies are in progress to determine the cause of variability under *in vitro* conditions.

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A NEW HOST RECORD FOR *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) PENZ. AND SACC. FROM INDIA

M. NARAYANA BHAT, R. K. HEGDE and P. C. HIREMATH
Department of Plant Pathology, College of Agriculture, Dharwad 580 005, India.

PONGAMIA PINNATA is extensively grown not only as a shade plant but also for its green manure in agriculture. It is known to suffer from some diseases. In the recent past a new blight has been recorded in the forest nursery of this college.

The disease manifested as small isolated brown to dark brown, regular to irregular lesions on 10–12 months old seedlings. Such lesions rapidly enlarged, coalesced and formed irregular patches, more so, towards the tips and margins of infected foliage. Tissue isolation from the infected lesions consistently yielded *Colletotrichum* species. Pathogenicity of the isolate was tested on leaves of healthy plants. Typical symptoms appeared after 6 days of inoculation. Based on morphological characters, the pathogen was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and was confirmed with CMI (IMI 315759). Though *C. gloeosporioides* has been reported on several hosts in India[1], there is no record of the same on *P. pinnata* and hence this is a new record.

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AZIDE RESISTANCE AND ROLE OF VARIOUS METABOLITES ON AZOTOBACTER GROWTH

L. R. KASHYAP
Biotechnology Centre, Indian Agricultural Research Institute, New Delhi 110 012, India.

AZOTOBACTER species are free-living aerobic nitrogen-fixing bacteria which provide an attractive choice for beneficial use in agriculture. However, before this bacterium can be used, it is necessary to understand the factors that influence its growth characteristics. Biological nitrogen fixation, one of its useful properties, is mediated through nitrogenase which is repressed by ammonia[1]. Sodium azide acts as a substrate for this enzyme[2] and can be used for selecting nitrogenase derepressed strains of nitrogen-fixing organisms in combination with excess ammonia[3]. We have used this procedure for selecting derepressed strains in wild type and auxotrophic mutants of *Azotobacter chroococcum*. In this communication, we report the effect of some chemicals and metabolites on the growth of mutants