

Figure 2. a, Cross-section of carbonized fossil wood: *Laurinoxylon Felix*, showing vessels, paratracheal parenchyma and oil-cells ( $\times 50$ ). b, Tangential-longitudinal section of the same specimen showing 2-3 seriate rays with swollen oil-cells ( $\times 100$ ). c, Intervessel pits ( $\times 400$ ). d, Tangential-longitudinal section of extant *Litsea reticulata* wood to show similar rays with swollen upright (oil) cells.

with vegetal matter in dark, cold and high-pressure conditions prevailing in the deep-sea basin. In a situation analogous to that prevailing for modern deep-sea fan deposits, the vegetal matter together with coarse clastics was flushed down the submarine canyons and transported by episodic high-density turbidity currents. Depending on the quantum of vegetal matter available at a site, carbonized laminae of less than a millimetre to a couple of centimetre thick coal seams, laterally pinching could be formed. The entire process of carbonization starting from raw vegetal matter took place after being flushed in the deep-sea basin, as suggested by the interstratified and compressed nature of thin carbonized wood and coal pockets frequently observed within the flysch sediments, demanding more intensive studies.

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## Evaluation of VA mycorrhizal inoculation in micropropagated *Populus deltoides* Marsh clones

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Using the method of axenic establishment, the effect of mycorrhizal inoculation at three different stages of



micropropagated plants of *Populus deltoides* Marsh (clones G-3 and D-121) was evaluated, i.e. shoots given rooting stimulus, rooted but nonhardened plantlets and rooted and hardened plantlets. Marked beneficial effects for all three stages were noted in short-term experiments (20 weeks). For consistent positive effects in terms of early shoot-bud break, establishment and survival, it was found that the rooted and hardened plantlets were most suited in long-term studies (60 weeks).

*IN VITRO* micropropagation techniques are increasingly being applied to large scale micropropagation of fruit and timber trees. However, one of the greatest challenges which such techniques are required to meet, is to identify and overcome factors affecting the survival and growth of micropropagated plants following their transfer from culture vessels to the greenhouse or field conditions. It has been established that tissue culture plantlets have very divergent leaf anatomy and physiology and therefore, require an acclimatization period during the transition from culture to greenhouse or field conditions, where desiccation and wilting are the main causes of low survival.

To overcome this problem, we have an alternative strategy of hardening the plants with a visually identifiable character of 'hardened' plants through the induction of temporary bud dormancy. This strategy has been employed quite successfully for many *in vitro* propagated temperate tree species in natural environments where maximum day temperatures range from  $28 \pm 2^\circ\text{C}$  (ref. 1). However, these procedures with poplars under high-day temperature conditions ( $35\text{--}45^\circ\text{C}$ ) resulted in survival problems.

Perusal of the literature shows that vesicular-arbuscular mycorrhizae (VAM) are associated with several woody species including poplars in natural ecosystems<sup>2,3</sup>. The development of certain plants, especially tree species like *Liquidambar*<sup>4</sup>, *Citrus*<sup>5</sup> or *Malus*<sup>6,7</sup>, depends directly on the presence of VAM fungi which play an important role in stress/disease resistance, nutrient uptake and establishment of plant cutting/seedlings. Our preliminary studies indicated that rooted plantlets showed marked improvement in growth and survival when inoculated with VAM fungus<sup>8</sup>. The aim of the present study was: (i) to determine if VAM inoculation helps in the hardening, survival, establishment and growth of micropropagated plants following transplantation, and (ii) to determine the stage at which micropropagated plants should be VAM inoculated.

The VAM spores were isolated from natural soils of established poplar plantations (Lalkuan Dist, Nainital) having neutral pH and low phosphorus (4.05 kg/ha) and rich organic matter (0.8%). These isolated spores were a mixture of *Glomus* and *Acaulospora* species. The relative proportions of such species in the soil was 30

spore/g soil and 10 spore/g soil simultaneously. This isolate mixture as such, multiplied in sterile coarse sand in the presence of *Zea mays* var. D 765 in pot cultures under controlled conditions with a temperature ranging from  $28^\circ\text{C}$  to  $38^\circ\text{C}$  providing Hoagland solution containing half of the recommended phosphorus. VAM spores were isolated following wet sieving and decanting method<sup>9</sup>. The spores were always surface-sterilized prior to *in vitro* inoculation using 0.4% streptomycin sulphate and 200 mg/l chloramine T solution further washed repeatedly with sterile distilled water. The spore suspension was made in sterile distilled water containing a spore density of 200 spores/ml; 2 ml suspension was used for inoculation to each axenically propagated shoot/plantlet in sterilized sand (M). The controls (C) with axenically propagated shoot/plantlet in sterile sand were provided with 2 ml sterile distilled water. Viability of these spores was tested using 0.5 mg 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)/g.

The explants for micropropagation were taken from field-growing selected trees of *Populus deltoides* Marsh (clones G-3 and D-121). The leader shoots were used for this purpose. They were cut into small pieces of three to four nodes each and the first five nodes, including shoot tops, were discarded. The trimmed petioles and cut ends of explants were sealed in molten wax. The explants were surface-sterilized in 0.2%  $\text{HgCl}_2$  with a few drops of Teepol detergent to aid thorough wetting for 15 to 20 min and washed several times with sterile distilled water. The explant consisting of a single node segment with a short internode at each end was planted in a modified<sup>10</sup> medium (containing half the amount of the major salts) and supplemented with 2% sucrose (half Murashige and Skoog, HMS). The healthy noninfected explants were given rooting stimulus on a similar medium supplemented with 5 mg/l indolebutyric acid (IBA) for 15 days. The cultures were incubated in a temperature-controlled room at  $25 \pm 2^\circ\text{C}$  in 16 h light and 8 h darkness. The intensity of light at the level of culture was 4400 lux.

The explants given rooting stimulus were treated as follows:

1. Placed in sterile sand with disinfected VAM fungal spores for two weeks (day 16);
2. Placed in HMS medium without sucrose until rooting occurred followed by treatment as in (1) for two weeks (30 days);
3. Placed in the medium as in (2) until induction of characteristic dormant bud-like structure (DBLS), followed by treatment as in (1) for two weeks (60 days).

The colonization of roots after two weeks and prior to transplanting was checked<sup>11</sup> by clearing and staining of roots.

These were finally transplanted in autoclaved field soil (field soil of poplar plantation) mixed with autoclaved

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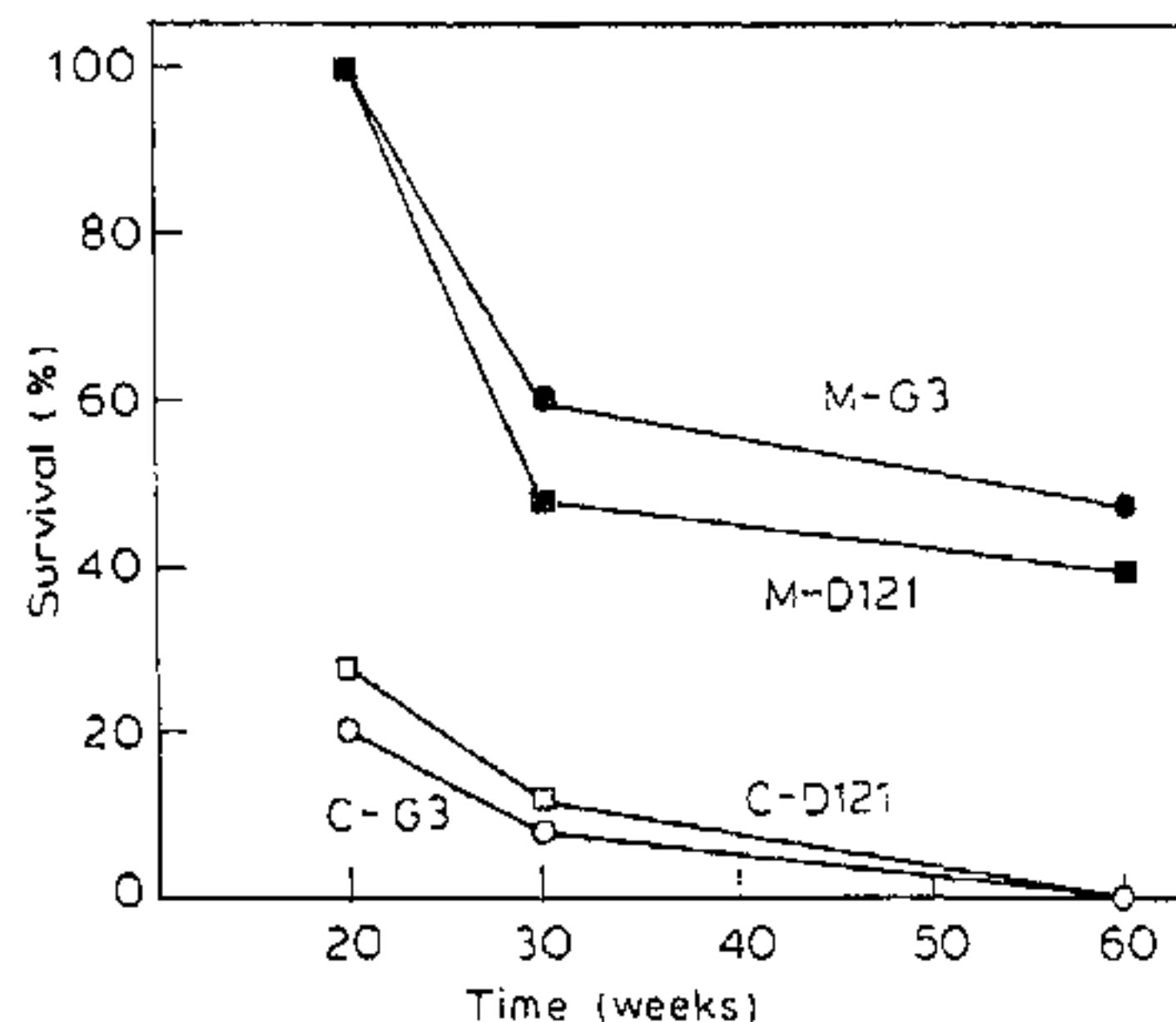
coarse sand in equal proportion (1:1). These were provided with nutrient solution consisting of modified Hoagland solution.

The data pertaining to different sets of experiments show the positive response of micropropagated plants to VAM inoculation in all the three stages. While the responses looked quite marked in the stage I and II plants, these did not survive, during the extended period of trials. The stage III plants seem to be best suited for VAM inoculation. The beneficial effect evident here showed a marked reduction in survival by the end of 60 weeks. The colonization of roots by VAM fungi in inoculated micropropagated plants was observed to be 30-40% after two weeks of *in vitro* inoculation prior to transplantation (Table 1, Figure 1). The

**Table 1.** Effect of VAM inoculation on per cent survival of *Populus deltoides* clone G-3 and D-121.

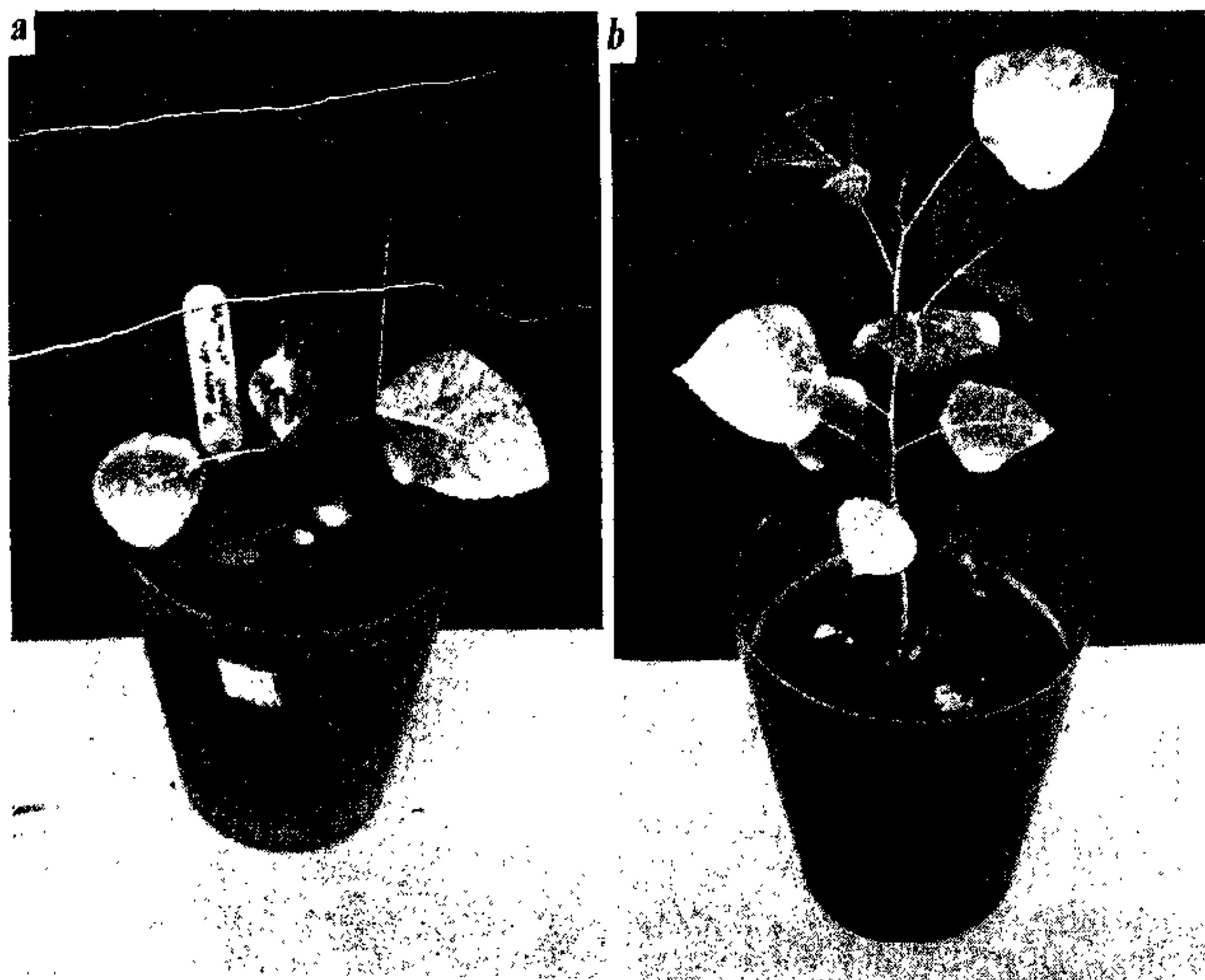
Culture type	Time (weeks) following transplantation											
	20		30		60		20		30		60	
	SI	SII	SIII	SIII	SIII	SIII	SI	SII	SIII	SIII	SIII	SIII
Clone												
G-3	0	5	0	12	20	100	8	60	0	48		
D-121	0	8	0	16	28	100	12	48	0	40		

Per cent survival calculated on the basis of 25 replicates. Culture type: C, Control; M, VAM inoculated. SI, Shoot given rooting stimulus; SII, Rooted but nonhardened plants; SIII, Rooted and hardened characteristic plants.



**Figure 1.** Effect of mycorrhizal inoculation on survival of micropropagated *Populus deltoides* clones.

colonization and positive response of VAM association were manifest in terms of active root growth. This seems to have supported shoot bud break and its growth into leafy shoots after 1-2 weeks following transplantation. The controls, however, showed delayed bud break ranging from 8 to 22 weeks (Figure 2). This is in sharp contrast to no-difference in growth *in vitro* observed in cherry<sup>12</sup>. Inoculation also markedly affected survival, establishment and growth of transplants in field soil mix in polypots under natural conditions. Comparisons over an extended period of time showed that control plants could not withstand



**Figure 2.** Effect of VAM inoculation on growth in *Populus deltoides* D-121 clone. **a**, Control. **b**, VAM inoculated.



outdoor ambient summer (day) temperature ranging between 40 and 45°C while the VAM-inoculated plants successfully survived such harsh conditions (Table 1). VAM colonization of plant roots *in vitro*, raises the possibility of producing effective propagules (vesicles/spores/mycelium) which could be recycled and used for inoculating micropropagated plants while maintaining aseptic conditions.

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## Ethephon-induced gum production in *Acacia senegal* and its potential value in the semi-arid regions of India

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Gum arabic has multifarious applications and is imported to India annually in large amounts. For some unknown reasons the source tree, *Acacia senegal*, which occurs abundantly in our country, does not yield gum. A preliminary study revealed that 0.8 to 0.9 kg of good

quality gum can be obtained per tree, by introducing 4 ml of ethephon solution containing 960 mg of the active substance through a hole in the sapwood in April/May. A detailed study could extend the value of this finding to utilize a source presently untapped, generate revenue from unproductive semi-arid lands and substitute imports.

*ACACIA SENEGAL* Willd. is a tree with multiple uses that occurs naturally in the barren, arid and semi-arid tracts of India. When the tree's main stem or its gnarled spiny branches are wounded, the tissues beneath the bark produce a viscous exudate that dries up to walnut-sized spherical mass. This constitutes gum arabic, an important trade item since biblical times<sup>1</sup>. The Egyptians used it in ceramic pottery and medicines over 4000 years ago<sup>2</sup>.

Gum arabic is highly soluble in water and is a good emulsifier, with low viscosity. The gum is odourless, flavourless, safe and finds widespread use in important industries such as paper, textile, adhesives, minerals, fertilizers, explosives, pharmaceutical, cosmetic, soap, ceramics, food, beverage and confectionary<sup>1-4</sup>. Throughout the Old World, gum arabic has been used in foodstuffs and beverages since antiquity. In 1961, gum arabic was classified by the US Food and Drug Administration as being 'generally recognized as safe', as a food stabilizer and affirmed its direct use in foodstuffs<sup>2</sup>. When present in the diet, at levels less than 10%, gum arabic is completely digested and absorbed<sup>5</sup>. Comprehensive dietary and toxicological tests carried out on gum arabic in humans have led to its acceptance as a component of foodstuffs and medicines<sup>6</sup>.

Extensive stands of *A. senegal* trees, both wild and planted, occur in the arid and semi-arid regions of Kutch (Gujarat) and Rajasthan. Strangely, the trees exude little or no gum. Natural wounds (e.g. breaking of branches by wind, injuries by birds, etc.) also cause little or no exudation. India is consequently importing annually around 5000 tonnes of gum arabic worth 7.3 million rupees from Sudan which provides 75-85% of the world's supply<sup>7</sup>. With the rising consumption of gum arabic in India, there is a definite need for tapping indigenous plants. Economical methods of tapping must be evolved and standardized to achieve optimum annual production of gum arabic on a sustained basis and also to ensure the survival of the trees in the wild.

Ethephon (2-chloroethylphosphonic acid, a synthetic chemical compound which releases ethylene in plant tissues) has been used for enhancing latex flow in para rubber (*Hevea brasiliensis*)<sup>8,9</sup> and resinosis, gummosis and gum-resinosis in several plants<sup>10-13</sup>.

In the present study, we have extended its use for inducing gummosis in *A. senegal* trees. We have worked out the optimum concentration of ethephon for maximum yield of the exudate without visible adverse effects on the plants. Ghosh and Purkayastha<sup>14</sup> have