

growth inhibition under Zn deficiency was also reported<sup>1,2</sup>.

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## WIDESPREAD INCIDENCE OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* ON COTTON IN PUNJAB (INDIA)

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ROOT-KNOT nematodes are widely distributed pests of agricultural and horticultural crops and cause enormous loss in the yield throughout the world. First incidence of root-knot nematode on cotton (*Gossypium arboreum* var *neglectum* f. *bengalensis*) in

India, was observed in 1939 from Punjab state<sup>1</sup>. Abu Bucker and Seshadri<sup>2</sup> detected infestation of *M. incognita* on cotton in a field of South Arcot district. *M. javanica* was also encountered from 7 to 8% samples of cotton from some parts of Haryana and Punjab states<sup>3</sup>.

During the *Kharif* season of 1985, while analyzing the cause of the large scale occurrence of wilt of cotton in Punjab, root-knot nematode infestation was found to be widespread. The nematode parasitized American (*G. hirsutum* L) as well as Desi Cotton (*G. arboreum* L), but the extent of galling was more on the latter. Subsequently a preliminary survey of Punjab was conducted and root-knot nematode infestation was observed in all the five districts, forming the cotton belt of the state.

Infestation of root-knot nematode, ranging from light to heavy on cotton was recorded from Dhilwan, Tapa Mandi and a number of other places.

Laboratory investigations revealed the occurrence of mature females with egg sacs and different developing stages of root-knot nematodes in the galled roots. Critical examination of the perineal region of females dissected out from acid fuchsin stained, galled cotton roots revealed the pattern to be similar to that of *M. incognita*. The head region of the males also resembled those of *M. incognita* as described by Eisenback *et al*<sup>4</sup>. In USA and some other countries, nematodes belonging to this species are recognized as a serious pest to cotton. Out of the four races, occurring in *M. incognita*, only race 3 and 4 attack cotton<sup>4</sup>. So far race 4 has not been found to occur in India<sup>5-7</sup>. From the northern part of the country only race 1 and 2 have been recorded so far<sup>5,6</sup>. Further studies are in progress to identify the race, widespread on cotton in Punjab.

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**PLANTLET REGENERATION FROM CALLUS CULTURES OF *EUCALYPTUS GRANDIS* HILL EX MAIDEN**

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THERE has been an increasing awareness throughout the world in using tissue culture methods towards crop improvement programmes. Some of these techniques are currently being used in the propagation of forest tree species. Application of callus and cell cultures in tree-breeding programmes has far-reaching effect and has advantages over conventional methods.

*Eucalyptus* species are widely used in paper industry as raw material and there is need for better clones to meet the future demands. Tissue culture researches have been carried out on many *Eucalyptus* species. Callus cultures and regeneration of plantlets were attempted by many<sup>1-5</sup> but successful plantlet regeneration was achieved only in a few<sup>6-8</sup>. Plantlets developed via embryoids in callus in selected trees of *Eucalyptus*<sup>9</sup>. Callus produced from cotyledons of *E. citriodora* developed shoots which were rooted; embryoids were formed in suspension cultures of shoot callus of *E. citriodora* and also in callus of *E. grandis*<sup>10</sup>. Induction of shoot buds and plantlet regeneration from the callus derived from the terminal vegetative buds is reported here.

Terminal vegetative buds from 3-year-old *E. grandis* trees raised at Kottappara were collected and routine procedures of sterilization were employed. Explants were transferred onto Murashige and Skoog's<sup>11</sup> (MS)

medium under aseptic conditions. Liquid medium with a filter paper support as well as solid medium gelled with 0.8% Difco agar were used inside 25 x 150 mm (Borosil) culture tubes. The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were incubated at 26 ± 1°C under continuous illumination (1000 lux) for 8 hr followed by 16 hr dark period.

Combinations of the medium used were:

- (1) MS basal + 3% sucrose + 2,4D (0.5 mg/l) + pCPA (2.0 mg/l) + BAP (0.1 mg/l)
- (2) MS basal + 3% sucrose + BAP (1 mg/l) and
- (3) MS basal (½ strength) + 2% sucrose + NAA (0.2 mg/l) + BAP (0.2 mg/l)

*Callus and organogenesis:* After the explants (shoot buds) were transferred onto medium 1 callus initiation started at the cut edge within a week. White and healthy callus mass grew in considerable quantity within 4 weeks. Subculturing was done every four

**Table 1** Effect of auxins on rooting of *E. grandis* shoots in culture

| Medium composition              | Auxin (mg IBA) | Rooting response   |  |
|---------------------------------|----------------|--|--|
| MS (full strength) + 2% sucrose | —              | —  |  |
| MS (full strength) + 2% sucrose | 0.8            | —  |  |
| MS (half strength) + 2% sucrose | —              | —  |  |
| MS (half strength) + 2% sucrose | 0.2            | Roots differentiated on the callus which proliferated at the cut edge of the shoots.               |  |
| MS (half strength) + 2% sucrose | 0.4            |  |  |
| MS (half strength) + 2% sucrose | 0.6            |  |  |
| MS (half strength) + 2% sucrose | 0.8            |  |  |
| MS (half strength) + 2% sucrose | 1.0            |  |  |
| MS (half strength) + 2% sucrose | 2.0            |  |  |
| MS (¼ strength) + 2% sucrose    | —              |  | —  |
| MS (¼ strength) + 2% sucrose    | 0.8            |  | Plantlets though healthy, roots differentiated on the callus proliferated at the base. |
| MS (¼ strength) + 2% sucrose    | 0.9 mg NAA     | No callus proliferated at the cut edge. Roots directly differentiated. All plantlets were healthy. |  |

Abbreviations used: BAP, N<sub>6</sub> benzylaminopurine; 2,4D, 2,4 dichlorophenoxyacetic acid; p-CPA, parachlorophenoxy acetic acid; IBA, indole-3-butyric acid; NAA, naphthalene acetic acid.