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CULTURE AND DIFFERENTIATION OF PLANTS OF ECONOMIC IMPORTANCE II. *AEGLE MARMELOS* L.[1]

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ABSTRACT

Callus tissues from *Aegle marmelos* L. were grown and maintained on Murashige and Skoog's medium. Callus and shoot formation were observed from cortical cells of the hypocotyl and stem explants. Shoots were developed on various concentrations (2.5 to 5.0 mg/l) of kinetin and NAA (0.0-0.5 mg/l) supplemented MS medium. The number of shoots per explant was enhanced by L-tyrosine (50-200 mg/l) when incorporated into the MS medium along with kinetin (5.0 mg/l) and NAA (0.5mg/l).

Embryoids and tracheids were fairly common in various treatments of kinetin.

INTRODUCTION

Tissue culture methods provide an important tool for rapid multiplication and propagation of plants. The organogenesis and differentiation of plants can be obtained through somatic embryogenesis in callus or cell suspension cultures, or through shoot bud formation (Narayanswami, 1977). The tissue derived from young and meristematic tissues remained more stable than those from differentiated explants and possessed higher potentialities for regeneration (Shimada, 1971 ; Ramawat and Arya, 1976). A large number of medicinal plants of arid and semi-arid areas have been grown on synthetic nutrient medium (Khanna, 1976 ; Zenk, 1978). In this communication we report the successful establishment of calli and subsequent regeneration of shoots and roots from the hypocotyl segments of 7 days old seedlings of *Aegle marmelos* L.

MATERIALS AND METHODS

Seeds of *A. marmelos* L. pretreated in 50% H_2SO_4 for 10 min., to remove hard seedcoat dormancy which prohibited it from germination, were surface sterilized in 70% ethanol for 1 min, then in commercial bleach (10% NaOCl) for 10 min and rinsed in 3 changes of sterile distilled water. Aseptically grown seed germinated within 3-7 days. The seedlings were cut aseptically into small segments and were placed on 40 ml medium of Murashige and Skoog (1962) (i.e. MS medium) supplemented with kinetin (0.1 mg/l) alpha-naphthalene acetic acid (NAA, 10.0 mg/l) in 0.8% bactoagar. The pH of the medium was adjusted to 6.0 before autoclaving at 20 psi for 20 min. Callus tissues were maintained in light or in darkness a $26 \pm 2°C$ and 60% relative humidity. A 2000 lux light was provided by fluorescent tubes and incandescent bulbs at culture level.

Kinetin (1.0, 2.5, 3.5, 5.0 mg/l) and NAA or Indole-butyric acid (I BA 0.0 to 0.5 mg/l) were incorporated in MS medium. L-tyrosine (25, 50, 100, 200 mg/l) was incorporated in Mo medium containing kinetin (5.0 mg/l) and IBA (0.5 mg/l). Hypocotyl and stem explants (0.5 to 1.0 cm) were placed asceptically. Twelve replicates were

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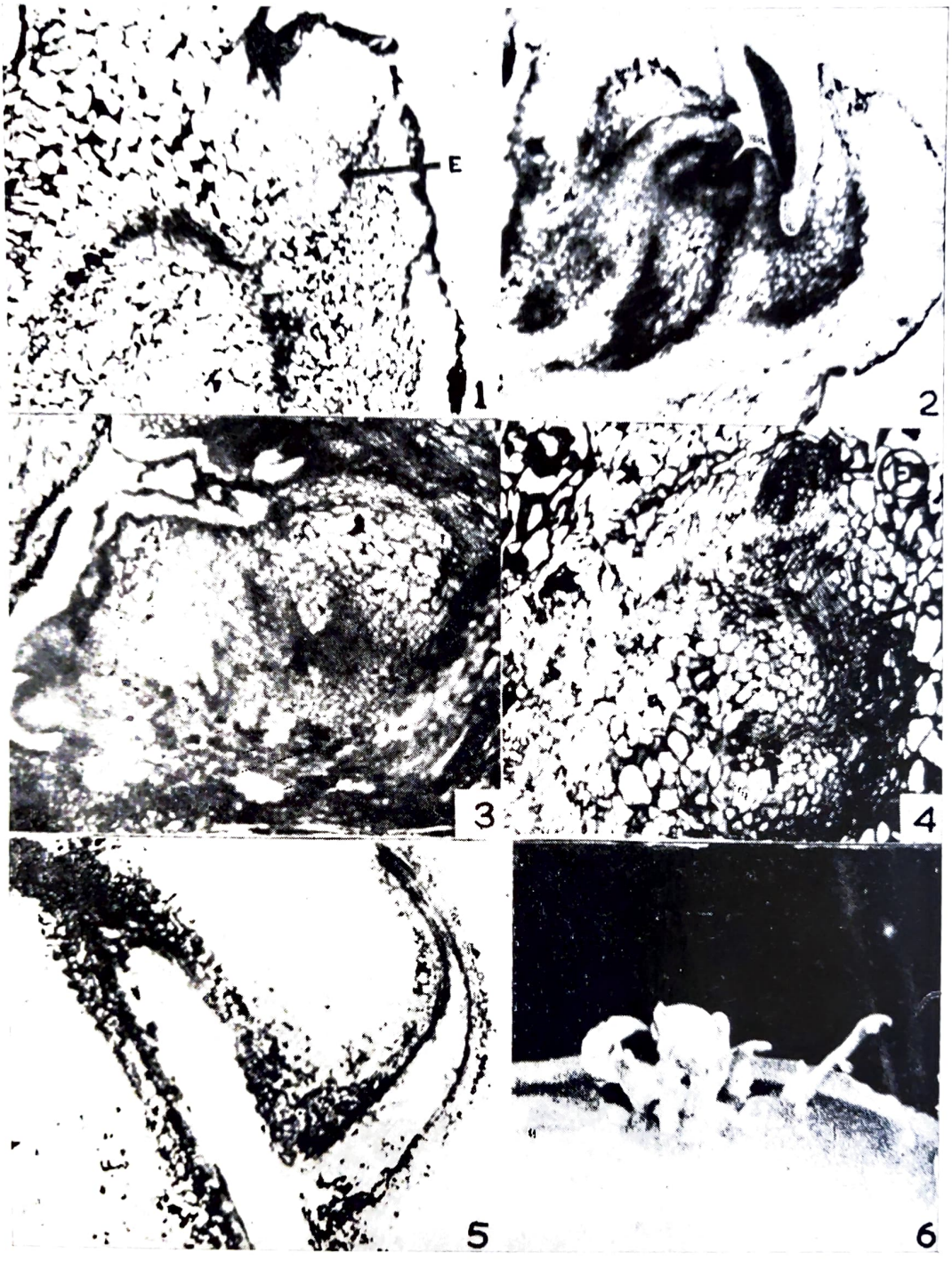

Figs. 1-8. Fig. 1. Callus development from cortex of hypocotyl. Note the embryoid (E) like structure in the cortex region X 100. Fig. 2. Shoot apex emerged out of callus tissues X 100. Fig. 3. Large number of shoot apices and developing vascular supply X 100. Fig. 4. Developing embryoid like structure (E) and vascular supply X 100. Fig. 5. Root developed from callus tissue X 32. Fig. 6. Large number of shoots developing from hypocotyl explant.

maintained in each treatment. The formation of shoot or plantlet was observed periodically and histogenesis of tissues was made at 10 μm using ethanol xylene series.

RESULTS AND CONCLUSION

Seeds of *A. marmelos* germinated within 3-5 days on MS medium. In the beginning, callus developed from the radicle and simultaneously large number of roots were produced. Callus development from hypocotyl was quicker (3.5 g/4 weeks) as compared to root and stem calli (2.0 g/4 weeks) on MS medium containing 0.5 mg/l kinetin and 10.0 mg/l NAA. The initial callus was creamish-yellow to light brown, globular, compact and hard.

Microscopic examination showed proliferation of cortex and callus development (Fig. 1). Several meristematic zones were observed interspersed in cortex parenchyma. The epidermal cells remained inactive. Proliferation in pith cells was also observed. Due to rapid proliferation of pith cells, vascular zone was disrupted. Explants transferred to kinetin-rich medium showed a lag period of 7-10 days and then grew very slowly. Callus growth ceased on MS medium supplemented with 5.0 mg/l kinetin devoid of auxin.

Organogenesis was observed after 3-4 weeks on MS supplemented with 2.5 to 5.0 mg/l kinetin. Six to ten shoot buds were produced in the medium containing 2.5, 3.0 and 5.0 mg/l, kinetin (Figs. 2-4). Shoots developed from proliferating cortex of hypocotyl and stem explants (Fig. 1) and emerged out later. The cells were small and compact. A large number of shoot buds developed from such cells (Figs. 2, 3). Tracheids forming embryoidal structures (Fig. 4) were observed. Such tracheids had scalariform secondary thickening. Roots were formed on low kinetin (0.04 to 0.5 mg/l) concentration (Fig. 5). Increased concentration (2.5 to 5.0 mg/l) of kinetin enhanced the number of shoots/explants, but with reduced size. Incorporation of 0.1-0.5 mg/l of auxin (IBA or NAA) in the medium reduced the number of schoots/explant from 6-7 to 2-3, but increased the vigour of shoots (in terms of its length). In some treatments of kinetin (5.0 mg/l) and IBA (0.5 mg/l) shoots produced roots. Such shoots were transferred to vermiculite.

L-tryosine (50-200 mg/l) increased the number of shoots/explants (from 2-3

Fig. 7. Plantlet formed on MS supplemented with 5.0 mg/l kinetin and 0.5 mg/l IBA, 8-weeks growth.

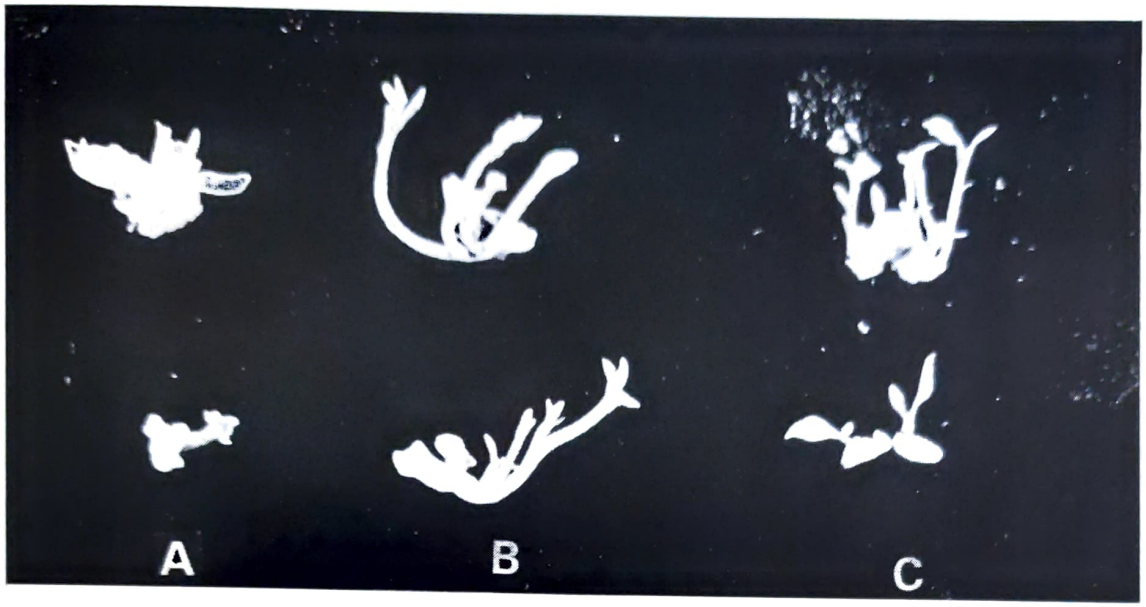

Fig. 8. Effect of concentrations of L-tyrosine incorporated in MS containing kinetin 5.0 mg/l) and (IBA (0.5 mg/l) A, 50 mg/l tyrosine, B, 100 mg/l, and C, 200 mg/l (at 4 weeks growth).

to 6-7) when incorporated in MS containing kinetin (5.0 mg/l) and IBA (0.5 mg/l) (Fig. 7).

Large number of herbaceous plants have been differentiated through tissue culture, but comparatively a few attempts have been made to differentiate and-multiply woody trees (Bonga, 1977). Proliferation of cortical and pith cells is a common feature and was also observed in *Salvadora* (Kant and Arya, 1967) and *Vigna* (Murthyreddy and Narayana, 1977). The marked effect of kinetin and auxin on shoot and root formation has also been observed in *Crotolaria* (Ramawat *et el.*, 1977) and *Albizzia* (unpublished). Further work on this aspect will make it possible to propagate the plant through tissue culture.

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