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OBSERVATION ON IN VITRO PROPAGATION OF M9 APPLE ROOTSTOCK (*MALUS SYLVESTRIS* MILL)

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A comparative study was conducted to vindicate micropropagtion and callus culture in Malling 9 apple rootstock. Supplemented Murashige and Skoog basal salts were used as culture medium. Callus from nodal segments was readily initiated in 1.0 mg l^{-1} IBA and 0.5 mg l^{-1} BA, on subculturing in 2.0 mg l^{-1} NAA embryoids and then shoots were developed. Direct shoot formation from nodal segments was obseved in 1.0 mg l^{-1} BA and the most acceptable shoot proliferation was in 1.0 mg l^{-1} BA plus 1.0 mg l^{-1} NAA. Rhizogenesis in the shoots obtained from both methods was noted superior in 2.0 mg l^{-1} IBA when exposed for short duration. Survival was 100 percent in sand or silt. Both the methods were found very effective and equally poised.

INTRODUCTION

Apple pomology in our country, is still relying on conventional footings. During recent years there has been a trend towards intensification of apple production system. That system was made possible by the introduction of dwarf rootstock for apple in Malling series (Malling 1 through 27). The inherent advantages include easy pruning, spraying and harvesting, greater production per unit area and better fruit quality (Halfacre and Barden, 1979).

Malling 9 (M9) is a dwarfing apple rootstock that suits our farming systems better than tall rootstocks. It is propagated by stooling (Hartmann and Kester, 1983). This method is cumbersome and time consuming. In vitro techniques provide an answer to such problems being rapid and economical in their manipulation.

In a comparison of solid and liquid MS media Snir and Erez (1980) worked on apple rootstock Malling-Merton 104, 106 and 109 and concluded that 1.0 mg l^{-1} BA and 1.0 mg l^{-1} IBA proved better for shoot proliferation and liquid medium was superior than solid one. Warner and Boe (1980) used shoot tips of apple rootstock M7 and observed best shoot regneration on half strength MS medium with 0.5 mg l^{-1} BA. Similar results were reported by Loreti et al. (1981) in M27 apple rootstock with 1.0 mg l^{-1} BA and 0.1 mg l^{-1} GA_3. Rapid shoot proliferation in crab apple was obtained in MS medium containing 1.0 mg l^{-1} BA (Singha, 1982). Le (1985) deduced the same effect with 7.5 uM BA in apple cv. Gravenstein.

Indirect embryogenesis has also been established in various apple cultivars: in leaf and fruit tissue of five apple cultivars with MS medium having 100 mg l^{-1} asparagine, 2.0 mg l^{-1} NAA and 1.0 mg l^{-1} kinetin (Gladysheva and Koshelev, 1983) in different explants of Golden Delicious in supplemented White's medium (Mehra and Sachdeva, 1984) and Akero cultivar with MS medium containing 1.0 mg l^{-1} IBA and 0.1 mg l^{-1} BA (Evacdsson, 1985).

Rhizogenesis of apple shoots has been achieved by using different media with auxins like IBA (Snir and Erez, 1980; Loreti et al 1981; Jacobani and Standardi, 1982; Pua et al., 1983 and Le, 1985) and NAA (Ancora *et al.*, 1981; Singha, 1982 and Mehra and Sachdeva, 1984). Jacobani and Standardi (1982) kept shoots of apple cv. Wellspur in dark for eight days before transferring it to MS medium with 2.0 m g l^{-1} IBA. Travers et al. (1985)put more emphasis on sucrose (1.5 percent) for rhizogenesis in peatvermiculite growing medium in apple rootstock An 313.

Keeping in view the commercial application of in vitro propagation of apple rootstock the present study was designed to standardize a suitable medium and compute a comparison of the two methods.

MATERIALS AND METHODS

Nodal segments of M9 rootstock were removed from green house grown plant and surface sterilized with 0.01 percent mercuric chloride solution, containing one drop of Tween-20 for five minutes followed by 3-4 water rinses. Modified Murashige and Skoog (1962) basal medium was used with various addenda. Medium was sterilized before use at 121 °C and 1.40 kg cm^{-2} pressure for 15 minutes. From here to onward the experimental procedure was bifurcated:

I. Callogenesis-using nodal segments for callus initiation on MS medium with 0-5.0 mg l^{-1} BA, 0-2.5 mg l^{-1} BA and 0-3.0 mg l^{-1} NA-A and 0-1.0 mg l^{-1} kinetin. The callus thus produced was put in media for shoot initiation in basal medium having 0-3.0 mg l^{-1} BA and 0-1. 0 mg l^{-1} NA-A or 0-1.0 mg l^{-1} IBA. II . Micropropagation-nodal segments were inoculated in basal medium with similar supplements as in callogenesis for shoot initiation. After initiation the shoots were transferred to a full strength medium containing 0-1.0 mg l^{-1} BA and 0-2.0 mg l^{-1} BA and to a half strength basal medium with same phytohormones.

For root initiation the adventitious shoots were cultured in basal medium with 0-4.0 mg l^{-1} IBA or 0-3.0 mg l^{-1} NAA alone and in combinations. The rooted plants were transferred to different rooting media like sand, silt and peat moss.

All the cultures were incubated at 25 ± 2 °C in a 16 hours day at an irradiance of 12 Wm^{-2}.

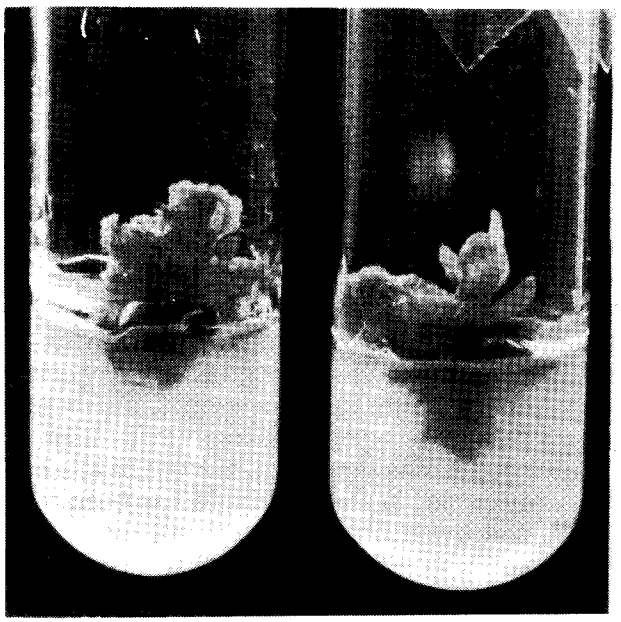
RESULTS AND DISCUSSIONS

Of all the combinations used for callogenesis in nodal segments, 1.0 mg l^{-1} IBA and 0.5 mg l^{-1} B A resulted in callus initiation in 85% cultures followed by 70 percent in cultures having 1.0 mg l^{-1} NA-A and 0.5 mg l^{-1} kinetin.

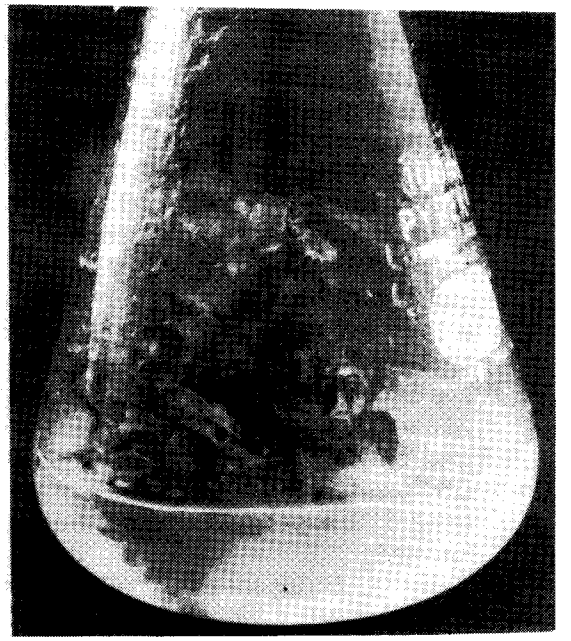
Callus initiation started readily in the former treatment with IBA which seconds the findings of Evacdsson (1985) who declared IBA ineluctable for callus initiation in apple nodal segments. Reversal transfer initiated embryoid formation within first week in 2.0 mg l^{-1} BA and 2.0 mg l^{-1} NAA. Other treatments showed late and less frequent embryogenesis (Photograph I). Embryogenesis involves initiation of embryogenic cells and subsequent development of these cells into embryoids and both of these stages need auxins (Ammirato, 1983). The role of cytokinins in embryogenesis has been reported with conflicting response and is, some times, shown to activate RNA synthesis and to stimulate protein and enzyme activity in certain tissues (Torres, 1989).

1.0 mg l BA was the best cytokinin level to obtain direct shoots in all the culture tubes within four weeks. These shoots conical flasks. Maximum number of shootlets with acceptable size were resultant of 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NA-A (Photograph II), similar reports have been alluded by Jacobani and Standardi (1982) and Pua *et al.* (1983). Exogenous cytokinin and cytokinin: auxin ratio is very important for shoot initiation and proliferaton, Street (1977) found it inevitable for shoot induction basing on multiple physiological reasons.

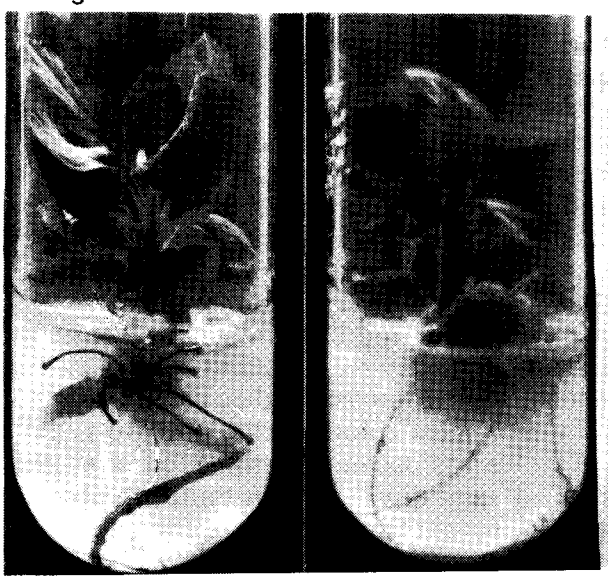
The shoots obtained in both the methods were inoculated in fresh medium for rhizogenesis for short period of time. 100 percent cultures rooted in 2.0 mg l^{-1} IBA & a less degree success was observed in the same quantity of NAA, the results of combination of these auxins were not satisfactory. Pua *et al.* (1983) and Travers et al. (1985) obtained the same result with a little higher concentration of IBA. Auxins are helpful in the induction of roots when used in less amount. Hu and Wang (1983) described the



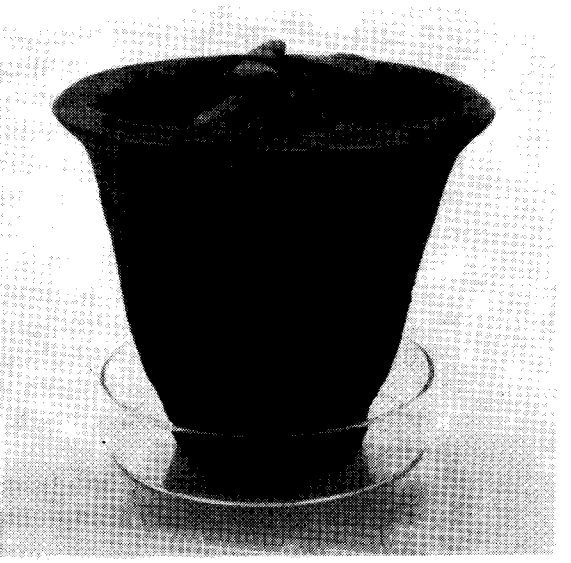
Photograph I. Embryogenesis from callus on MS medium with 2.0 mg l^{-1} BA and 2.0 mg l^{-1} NAA.



Photograph II . Shoot proliferation on MS medium added with 1.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA.



Photograph III. Roo t formation with 2.0 mg l^{-1} IBA. Right ;roots emerging from callus. Left; roots emerging from single shootlet.



Photograph IV. Free living plant in sand.

role of auxins in rhizogenesis when these were used in less concentrations for a short exposure. Long exposure and high concentration results in callus production and inhibited root elongation. There was a difference in the rooting pattern in shoots from both the methods; on an average six roots were produced in shoots emerging from callus and in other procedure only a single root with root hairs was seen in most of the cultures (Photograph III).

Survival percentage of the rooted plants was 100% in sand or silt and 80% in sand plus peat moss(Photograph IV). These results are similar to Ancora et al. (1981).

In conclusion, both the methods have proved very effective and equally poised. The difference lies in their use as several million plants can be produced from a single isolated shoot tip by micropropagation (Wilkins and Dodds, 1983) and callus culture could be a prodigious source of epigenetic changes (Meins, 1983).

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