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In vitro Plantlet Regeneration in American Cultivar of Apple (*Malus pumila* Mill.) Through Seed Culture for Root stock Purpose

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ABSTRACT

Seeds from fresh mature American cultivars of apple were inoculated on half strength MS medium supplemented with growth regulators. Adventitious shoot proliferation was seen from the base of embryonic shoot after seed germination when medium was enriched with BAP (5µM) and PG (10µM).Rich microshoot proliferation was observed when adventitious shoots were subcultured in small lumps bearing 5 to 7 microshoots on the fresh medium of same composition. But when BAP (5µM) and IBA (1µM) supplemented medium was used proliferation recorded was nearly 2 fold lesser. Rootless shoots were subcultured on MS (x1/2) + IBA (2.5µM) which resulted in development of elongated profuse adventitious roots. Plantlets regenerated were normal and healthy looking with strong root system fit to be used for rootstock purposes.

Keywords: Seed culture, adventitious shoots, plantlets, rootstocks.

Abbrevations: MS - Murashige and Skoog; BAP - 6 benzylamino-purine; IAA - indole acetic acid; IBA - indole butyric acid; PG - pholoroglucinol

INTRODUCTION

Plant tissue culture, an important tool of biotechnology, has opened numerous possibilities for plant propagation. Attempts have been made to use tissue culture technique to achieve propagation of trees for obtaining genetically pure elite population (Mehra & Sachdeva, 1979). Nowadays, this technique is increasingly being applied for mass clonal propagation of woody plants inclusive of fruit and nut crops which are otherwise difficult to propagate through conventional methods of propagation (Nemeth, 1981).

The valley of Kashmir occupies prominent position in cultivation and production of temperate fruits. During the past decade work on commercially important woody species has invoked worldwide interest - Apple (*Malus pumila* Mill.) being one amongst them. The apple industry of today needs an intensive research and management so that this industry becomes the real backbone of rural economy of the mountain locked state. There are over 8000 apple varieties in the world but fewer than 100 are involved in extensive production. Although micropropagation of apples is rapidly becoming an established commercial technique, certain important problems remain to be solved. One of these is the extension of the technique to a number of important cultivars. The production of

young apple trees from seeds more quickly and cheaply by this technique would be of considerable commercial value for root stock purpose.

MATERIAL AND METHODS

Healthy seeds collected from fresh mature fruits of American Cultivar of Apple were surface sterilized with 5% Labolene and tween–20 (2-4 drops) with regular brushing of seeds for 30 minutes. The washed seeds were refrigerated for 2 weeks to 2 months. Before inoculation both the seed coats were removed from the seeds. The excised seeds were separately kept in flasks containing lab detergent, tween 20 (2-4 drops) to which was added 2% v/v of sodium hypoclorite solution in 100 ml of water for 6 to 8 minutes followed by rinsing with tap water 20 to 30 minutes and then by double distilled water. Final sterilization treatment was carried out with $HgCl_2$ (0.1%) for 2 minutes under the laminar air flow cabinet and finally rinsed with autoclaved double distilled water for 3 to 4 times. The seeds were first allowed to dry and then inoculated onto the agar medium.

RESULTS

The refrigerated seeds (without seed coat) inoculated on half strength MS medium supplemented with Kn, BAP+PG, BAP+IBA expressed various responses which were recorded and are listed in Table 1.

The cotyledons of cultured seeds on BAP (5μM) +PG (10μM) opened up followed by development of hypocotyl within 4 to 6 days. Apart from the formation of embryonic shoot, two additional adventitious shoots developed from the base of embryonic shoot on the same medium after 23 days of its culture (Fig.1a). Adventitious shoot proliferation continued and the number reached to 27 within 59 days of its culture. The shoots were sub-cultured in 2 groups – one group on the same supplements on which these were growing previously i.e. BAP (5μM) + PG (10μM) and increase in the number of shoots was 4 folds within a month (Fig.1b). The other group of adventitious shoots was sub-cultured on BAP (5μM) +IBA (1μM) supplemented medium where only 2.3 folds was noted. But apart from this, the initiation and development of adventitious roots was also recorded on this medium. The roots were thick, white and elongated after a month of its subculture (Fig.1c).

The adventitious shoots in both the groups were further separated in small lumps and sub-cultured on their respective supplements which showed increase in their multiplication within next 20 days. Apart from adventitious shoots, axillary shoots also developed in both the cases and a total of 305 shoots were formed after 109 days. The remarkable observation found was that when adventitious shoots were subcultured in small lumps of 5-7 shoot each on to fresh medium of same composition, it resulted in further proliferation of adventitious shoots whereas when individual adventitious shoots were subcultured it proliferated in the form of axillary shoots only. Such shoots were maintained for further proliferation for more than six months. The shoot multiplication rate in

this study has been found to be very quick. Total number of adventitious and axillary shoots formed within 109 days is depicted graphically (Fig.2).

Rootless shoots either individually or in lumps were inoculated on root inducing medium. The shoots which were cultured under the influence of NAA+IBA+IAA (2.5μM each) resulted in the formation of white friable callus at its base. The differentiation of roots took place through the callus whereas the shoots growing in presence of IBA (5 μM) produced small and thick direct adventitious roots (Fig.1d). If IBA concentration was reduced to half (i.e. 2.5μM) thin, elongated direct adventitious roots were produced (Fig.1e). Response of subcultured shoots of different rooting media composition is graphically depicted in Fig.3 and Table 2.

The complete plantlets formed *invitro* were allowed to remain on the same medium without any transfer for 1-2 months so that the adventitious roots developed become hard enough to resist the change of deflasking. Care was taken during deflasking to remove the nutrient medium from the roots (Fig.1f). Deflasked plantlets were transplanted in small pots filled with compost rich soil. The whole scheme of initiation, multiplication of adventitious shoots and their rooting from the embryonic shoot of the seed of American cultivar of apple is depicted in the form of flowchart (Fig.4).

Table1: Morphogenetic response of seeds of American cultivars to cytokinin and auxins

S.No	Growth Medium* (μM)	Nature of response*	Average no of shoots	Percentage response
1.	MS + BAP(5) + PG(10)	Profuse multiple shooting	27	50
2.	MS + BAP(5) +IBA(1)	-	-	-
3.	MS +Kn (15)	-	-	-

* 10 replicates per treatement; data scored after 59 days

* MS half strength

Table 2: Response of microshoots to rooting medium after 59 days.

S.No.	Growth Medium* (μM)	Nature of response	Percentage of rooting
1.	MS + NAA + IBA + IAA (5 each)	White friable callus at the base	10
2.	MS + NAA + IBA + IAA (2.5 each)	White friable callus at base. Rooting through callus	25
3.	MS + IBA (5)	Small and thick adventitious roots (direct)	45
4.	MS + IBA (2.5)	Long adventitious roots (direct)	73

* MS half strength

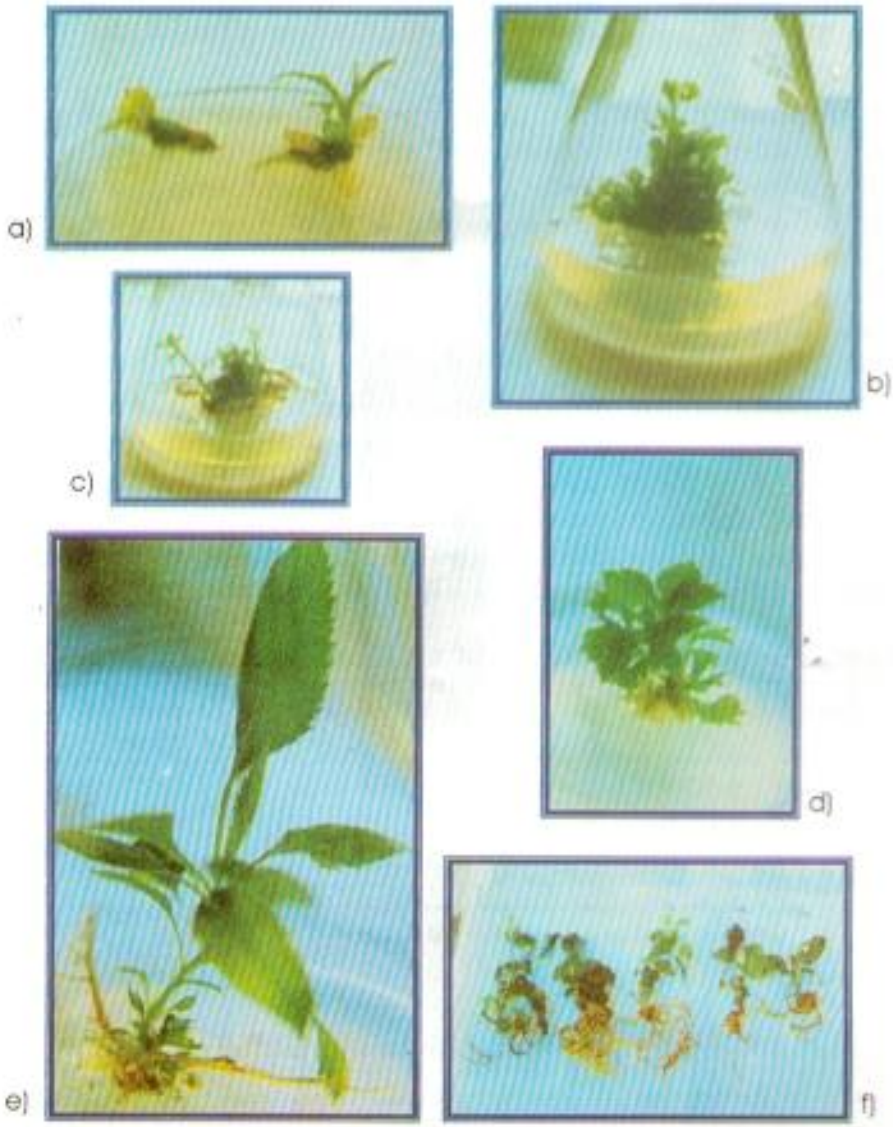

Fig. 1: (a-f). Invitro Plantlet Regeneration in American Cultivar of Apple (*Malus pumila* Mill) Through Seed Culture for Root Stock Purpose

a) Initiation of shoot multiplication on MS(x1/2)+BAP(5μM)+PG(10μM) {after 23 days}
b) Adventitious shoot proliferation after sub culturing on MS(x1/2)+BAP(5μM)+PG(10μM) {after 59 days}.
c) Proliferation of adventitious shoots and root formation in sub cultured adventitious shoots on MS(x1/2)+BAP(5μM)+IBA(1μM) {after 30 days}
d) Adventitious shoots showing small and thick adventitious roots on MS(x1/2)+IBA(5μM) {after 20 days}
e) Adventitious shoot showing thin elongated adventitious roots on MS(x1/2)+IBA(2.5μM) {after 15 days}
f) Thin plantlets taken fron vials ready for transplantation.

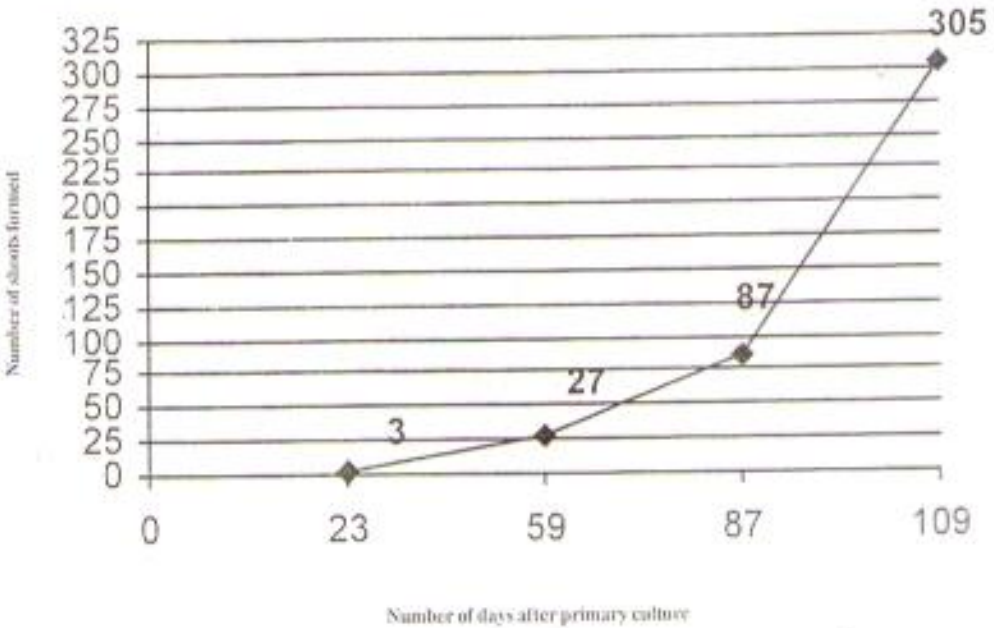

Fig. 2: Multiplication of adventitious and axillary shoots from embryonic axis of American apple seed.

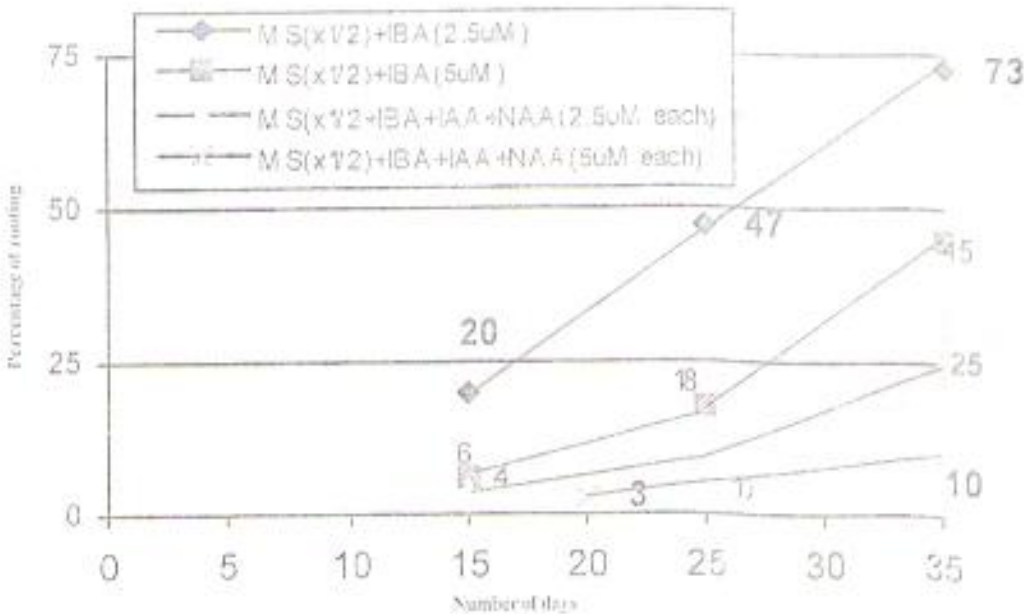

Fig. 3: Response of sub-culture shoots on rooting medium.

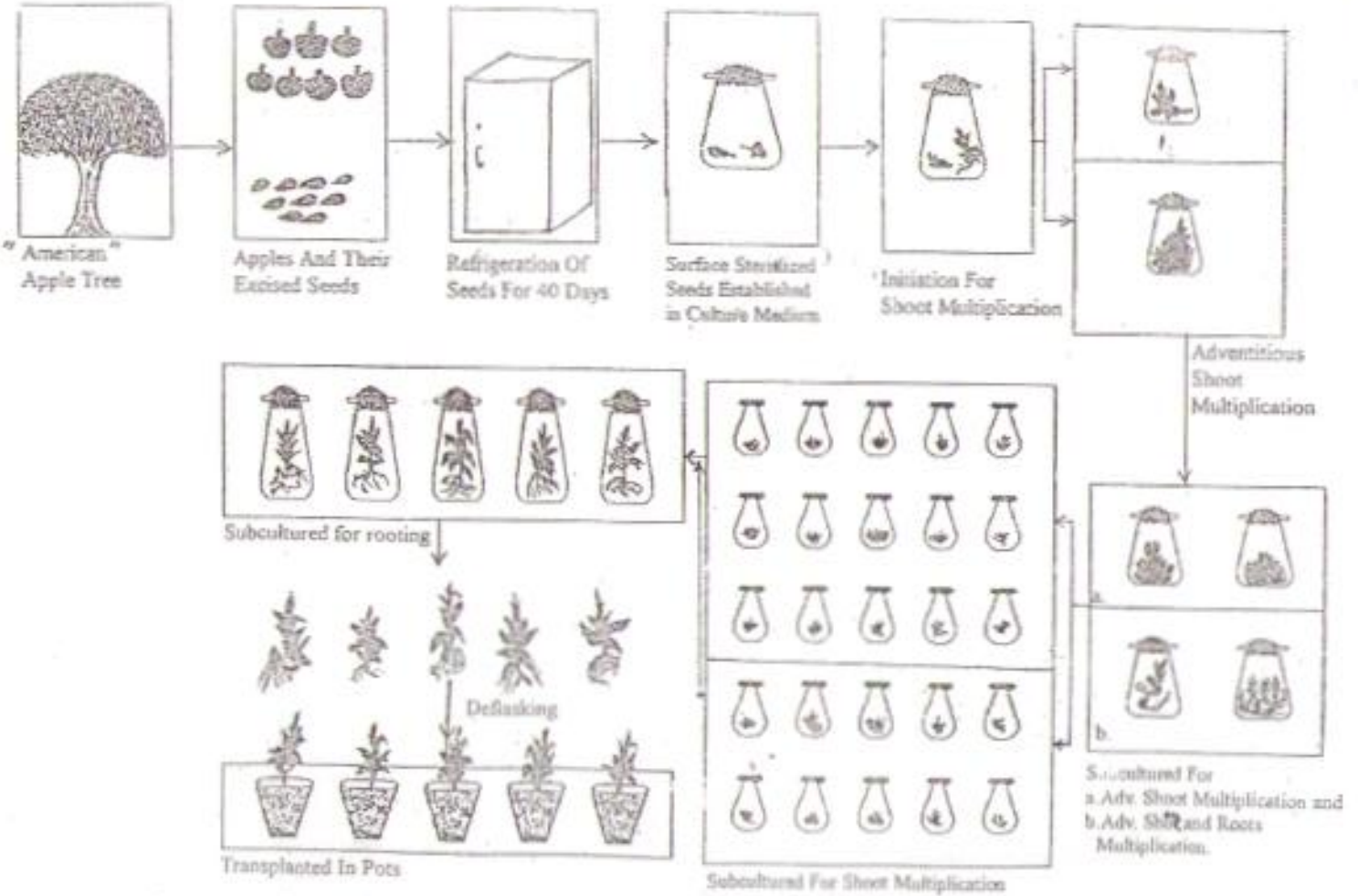

Fig. 4: Flow Diagram Scheme For Initiation And Multiplication Of Adventitous Shoots and their rooting from Seeds of Apple (Cv. American)

DISCUSSION

The present findings on American apple cultivars suggest that by applying *invitro* propagation methodology, with the help of growth adjuvants there is definitely a great potential for such fruit cultivars to have these in large numbers. By standardizing this technique, a big nursery of such fruit trees could be exploited on commercial level for root stock purposes.

Embryonic tissues of American cultivar from chilled seeds were found morphogenetically very responsive for micropropagation. It was observed that thousands of shoots can be propagated from single embryonic shoot within a year. The remarkable observation found was that when 5-7 adventitious shoots were subcultured together, it resulted in further proliferation of adventitious shoots whereas when individual adventitious shoots were subcultured it proliferated in the form of axillary shoots only. Rootless shoots growing in presence of IBA (5μM) produced small and thick direct adventitious roots. It was observed that when IBA concentration was reduced to half (i.e. 2.5μm) thin, elongated direct adventitious roots were produced.

The success in the micropropagation of apple lies in the type of explant used, control over oxidative browning and composition of nutrient medium used (Jones, 1967 and Zimmerman, 1984). Mehra and Saroj (1979) have reported White's and MS medium equally effective for differentiation of apple cultivars. Use of MS was found quite responsive in present findings also.

Organogenetic potential of the plant was observed by culturing seeds on medium supplemented with BAP+IAA. These results are in accordance with those of Rubos and Pryke (1984) but are not in consonance with the results of Stany *et al.* (1992) who observed caulogenesis from seeds on MS basal alone. Orlikowska (1988) reported rooting of shoot tips of crab apples on medium supplemented with IBA. Likewise Correa *et al.* (1990) also used the same auxin for root induction from shoot tips of M.7 cultivar of Apple. Present observations are in conformity with these findings but contradict the results of Lankes and Zimmerman (1990) who observed rooting on MS basal medium. Present studies reveal that this technique of propapagation is quite viable for raising multiple plantlets from embryonic shoots through seed culture for rootstock production on large scale in quicker time.

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