Invitro Plantlet Regeneration in Red Delicious Cultivar of Apple (*Malus pumila* Mill.) Through Shoot Tip Culture

Azra N.Kamili ,Manzoor A.Bhat, Anees Fatima and A.M.Shah
Plant Tissue Culture Laboratory, Centre of Research for Development, University of Kashmir, Srinagar-190006, J&K.India

ABSTRACT

Shoot tips of Red Delicious Cultivar of apple were collected from juvenile branches of fruit bearing trees and inoculated on half strength MS medium supplemented with BAP (5µM) and PG (10µM) which resulted in prominent shoot growth and its multiplication. Adventitious microshoots elongated on the same medium which later on were individually subcultured on rooting medium containing IBA (2.5µM). Thin elongated adventitious roots were regenerated within one week of its subculture .After 90 days of initial culture more than 100 shoots were recovered from a single shoot tip which resulted in complete plantlet formation after one month of using rooting medium

Keywords: Shoot tips, adventitious shoots, apple, plantlet

Abbreviations: MS-Murashige and Skoog; BAP-6 benzylamino purine;IBA-indole butyric acid; PG-phloroglucinol

INTRODUCTION

Apple is the most important fruit crop of the north western Indian Himalayan region. In India the commercial cultivation of apple is largely confined to the states of Jammu& Kashmir, Himachal Pradesh and Uttar Pradesh which together accounts for 99% of the total production (Chadha and Awasthi,2005). While traditional methods of propagation are considererably time consuming, micropropagation or manipulation of plants have rapidly evolved as dynamic and important fields of endeavors during last few decades and stands out as logical method for large scale production of disease free and uniform sized plants in a limited time and space, independent of the season. It also enables an easy transshipment of germplasm at national and international level due to phytosanitary conditions.

Selection of proper variety is the most important for commercial production. The delicious cultivars and its strains have gained popularity all over the world. Present study describes a protocol for micropropagation of Red Delicious cultivar of Apple (*Malus pumila*

Mill.).Red delicious tree is vigorous, forms spur freely, fruit is large sized and oblong-conical in shape. Fruit is greenish yellow with red streaks. Flesh is juicy creamish, aromatic and sweet in taste, it ripens in 3rd week of August and can be stored for 3-4 months (Chadha and Awasthi,2005).

MATERIAL AND METHODS

Fresh green shoot apices (1.5 to 2.5 cm long) were collected from Red Delicious cultivar of apple. The selected explants were washed with 5% lab. detergent containing 2-4 drops of Tween 20 (wetting agent) and 2mls of 2% v/v of NaOCl solution for 5-7 min followed by rinsing with tap water for 30-40 min and then again rinsed with filtered and distilled water. These pre-washed shoot apices were transferred in 250 ml Erlenmeyer flasks containing 100 ml of 20μM kinetin solution. The material as such was kept over night (12-18 hours) in a refrigerator to leach out water soluble phelonic compounds. Kinetin was used to promote green colour of shoot apices. On the following day kinetin solution containing phenolic exudates was drained out from the flasks which were followed by three washes with double distilled water. The explants were then surface sterilized with 0.1% $HgCl_2$ for 2 min. and were finally rinsed 3-4 times with autoclaved double distilled water. Standard procedures for carrying out the inoculation under aseptic conditions onto nutrient medium were carried out in the inoculation room.

RESULTS AND DISCUSSION

Shoot apex culture of an orchid *Cymbidium* by Morel (1960) became a milestone in tissue culture science laying foundation for *in vitro* mass propagation of different plant species of importance in horticulture, agriculture, food industry and in silviculture.

In present studies aseptic shoot tips of Red Delicious apple were inoculated on MS(x1/2) medium augmented with BAP (5 μM) + PG (10μM) which resulted in the exudation of phenolic compounds within 12 hours and continued for a few days. Regular transfer of shoot tips to fresh medium of same composition after every 24 hours, for first 5 days and then on alternate days resulted in establishment of growth after 15 days (Fig.1). Prominent shoot growth besides the expansion of basal leaves was registered after 35 days (Fig.2).Table 1 depicts response of shoot tips after different subcultures. Many adventitious shoots initiated from the basal nodes of each shoot tip while the growth of the main shoot tip was rather slow .Besides, compact and nodular callus was formed at the basal end of each shoot tip after 70 days of its primary culture (Fig.3).The basal compact nodular callus was aseptically cut off and the rest of explants (individual shoot) showed abrupt proliferation of adventitious shoots within next 20 days (Fig. 4). While studying the effectiveness of different cytokinins in stimulating shoot proliferation, Lundergan and Janick (1980) reported BA to be most effective, 2ip to be least effective and Kn to be intermediate. Work done on similar lines

Table 1. Response of shoot tips in different subcultures

S.No	Response	No.of shoots	Period (days)
1.	Establishment of shoot tips on medium	-	15
2.	Prominent shoot growth besides expansion of basal leaves.(subculture I)	-	35
3.	Multiple adventitious shoots initiated from basal node.(subculture II)	10	70
4.	Abrupt proliferation of isolated adventitious shoots.(subculture III)	>100	90

by Jones (1967) on *M.26* cultivar has also shown BA (4.4μM) effective for the proliferation of shoots apices. Use of shoot apices as explants for clonal micro propagation of apple has been found effective by Jones (1967); Elliott (1972); Abbott (1976); Zimmerman (1984); Kumar and Kumar (1998) and others. In present work also shoot tip culture using BAP in apple proved to be highly effective in raising clonal (true to the type) plants. These results are in quite conformity with our results. Similar observation was made by Manzoor *et al.* (2005) in American cultivar of Apple.

In present studies elongation of microshoots was observed on the same medium which favoured shoot multiplication. Long adventitious shoots or cuttings individually sub cultured on rooting medium containing IBA (2.5 μM) resulted in formation of thin elongated adventitious roots within 1 month of its sub culture (Fig 5) which is confirmed earlier by Zimmerman and Broome (1981) who also suggested that IBA helped in vitro rooting of apple shoots. Similar results were also given by Cheema and Sharma (1983); Standardi (1985); Caboni *et al.*, (1992) on *M. domestica* , Delicious and M.9York cultivar of apple respectively.

Total number of shoots produced from single shoot tip after 90 days of its culture was more than 100.After 4 month period the microplantlets were ready for deflasking. It becomes quite evident from the present studies that shoot tip culture can be considered a viable method for mass clonal multiplication of Red Delicious cultivar of Apple after proper standardization of protocol. The presumption that more than 80,000 plants could be produced from single shoot tip in six months by Jones (1967) seems to be applicable in present case also after slight refinement of multiplication phase.

ISSN 0972-5407





Fig.1-5: *Invitro* response of shoot tips of Red Delicious cultivar of Apple(*Malus pumila* Mill.).

Fig.1.Establishment of shoot tip on MS(x1/2) +BAP (5μM) +PG (10μM) (after 15 days).
Fig.2.Growth of shoot on MS(x1/2) +BAP (5μM) +PG (10μM) (after 35 days).
Fig.3.Adventitious shoot development and formation of nodular callus at lower end on MS(x1/2) +BAP (5μM) +PG (10μM) (after 70days).
Fig.4.Growth and proliferation of adventitious shoots after excising basal callus portion on MS(x1/2) +BAP (5μM) +PG (10μM) (after 20 days).
Fig.5.Rooting of isolated shoots on MS(x1/2) +IBA (2.5μM) (after 30 days).

ACKNOWLEDGMENTS

The authors wish to thank Director, C.O.R.D for providing laboratory facilities to carry out these investigations. The study forms a part of M.Phil. work of the second author.

REFERENCES

Abbott, A.J. and Whitely, E.1976. Culture of *Malus* tissue *in vitro*:Multiplication of apple plants from isolated shoot apices. *Scientia Horticulture*.**4**:183-189.

Bhat,M.A., Fatima,A, Kamili,A.N. and Shah,A.M.2005. *invitro* plantlet regeneration in American cultivar of Apple (*Malus pumila Mill.*) through seed culture for rootstock purpose.*J.Res.Dev.***5**:121-128.

Caboni,E., Boumis,G. and Damiano,C.1992.Effect of phenols, gibberlic acid and carbohydrates on rooting of the apple root stock M.9 Jork. *Agronomie*.**12**(10):789-794.

Chada,K.L. and Awasthi,R.P.2005.*The Apple:Improvement,Production and Post harvest Management*.Malhotra Publishing House,New Delhi.

Cheema,G.S. and Sharma,D.1983.*In vitro* propagation of apple. p 309-317. In: *Plant Cell Culture in Crop improvement.* (S.K. Sen and K.L.Giles, eds) Plenum press, New York.

Elliot,R.F.1972. Axenic culture of shoot apices of apple. *New Zealand Journal of Botany*.**10**:254-258.

Jones,O.P.1967. Effect of benzyladenine on isolated apple shoots. *Nature* (London). **215**:1514-1515.

Kumar,A.and Kumar,V.1998. Culture of fruit crops-Apple. p.136-142. In: *Clonal Tissue Culture of Important Fruit Crops.* International Book Distributing Co.Lucknow

Liu Shih-Feng., Chen wei-Lun.,Wang Hang-Hsin. and Hang Shan Yung.1978.Shoot tip culture of Apple rootstocks and apple seedlings *invitro*. *Pract.Symp. Plant Tissue Culture*. Peking,Chinpp.pp.-485-490.

Lundergan,C. and Janick,J. 1979.,Regulation of Apple shoot proliferation and growth *in vitro* .*Hortic.Res*.**20**.19-24.

Morel, G.M.1960. Producing virus free *cymbidium. Am.Orchid Soc.Bull* **29**:495-97.

Standardi,A.1985. Effect of light,activated charcoal and IBA on *in vitro* propagation of apple. *Universita delgi studi di Perugia*(Italy)**39**:109-120.

Zimmerman,R.H. and Broome,O.C.1981.Phloroglucinol and rooting of apple cuttings.*J.Amer.Soc.Hort.Sci*.**106**:648-652.

Zimmerman,R.H. 1984.Apple.In :Hand Book of Plant Cell Culture.s Vol II (Sharp,W.R.;Evans,D.A.,Ammirato,P.V.and Yamada,Y.Eds.) Mac Millan Publishing Co.New York.