

Variability of Phenolic Exudation in Cultured Shoot Apices of Apple Cv. American

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ABSTRACT

Shoot apices of *Malus pumila* Cv. American were collected from two mature trees growing in two adjacent orchards of Zakura (irrigated and unirrigated orchards) and cultured on MS medium (1962) with half salt strength supplemented with BA-5 μM . A comparative study of *in vitro* culture of juvenile and mature shoot apices of irrigated and unirrigated trees was made with regard to variability of phenolic exudation in the medium and its effect on survival and establishment of explants. Juvenile and mature explants from irrigated trees exuded less phenolic compounds than explants from unirrigated trees. Frequent transfer of explants to fresh medium in the first week of culture period helped in overcoming the problem of necrosis and death of explants due to browning of the medium.

Keywords: *Malus pumila*, shoot apex, tissue culture, phenolic exudation, variability

Abbreviations: MS - Murashige & Skoog; BA - Benzyl adenine

INTRODUCTION

Compared to herbaceous plant species the number of woody trees that have been successfully propagated in an *in vitro* culture system is less (Pareek and Mathur, 1977). Establishment of explants of woody trees in cultures pose a great difficulty due to browning of explants and culture medium. Browning is generally considered to result from the oxidation of phenolic compounds, released from the cut ends of the explants, by polyphenoloxidases, peroxidases or air (Bhat and Chandel, 1991). *In vitro* studies on apple and many other members of family Rosaceae are frequently hampered by browning and necrosis of tissues, the most critical period being first 24-48 hours in culture medium (Machado *et al.*, 1991).

Therefore, the biggest problem in establishing the apple cultures is the phenolic exudation of explants in the medium. Present communication deals with *in vitro* culture of mature and juvenile shoot apices of apple Cv. American, collected from irrigated and unirrigated trees, to see their effect on concentration of exudation in culture medium and establishment of cultures thereof.

MATERIAL AND METHODS

Two apple trees of American cultivar growing in the two adjacent orchards at Zakura, were selected for the collection of young and mature shoot apices. The physiological condition of these orchards revealed that the trees of one orchard were not irrigated for complete season of 1998 while the adjacent orchard, which has water channel in it, was watered twice in the growing season. The basic purpose of irrigating the trees was to facilitate a good colour in the fruit.

Fresh green, young and mature shoot apices (1.5 – 2.5 cms long) were collected from the irrigated and unirrigated trees. The explants were washed with 5% Lab detergent (Labolene) containing 2-4 drops of Tween -20 (wetting agent) and 2 mls (2% v/v) of sodium hypochlorite solution for 5 -7 minutes and then washed thoroughly in running water for 5-10 min., followed by 3-4 rinses in distilled water. Further, surface sterilization was carried out with 0.1% $HgCl_2$ for 2 min., and finally rinsed 3-4 times with autoclaved double distilled water.

Sterile shoot apices (1- 1.5 cms long) were excised and cultured on half strength MS medium (1962) supplemented with 3% sucrose and BA $-5 \mu M$. The pH of the medium was adjusted to 5.8 before gelling the medium with 0.8% agar. The medium was autoclaved at $121^\circ C$ for 17-20 min. All cultures were maintained at $23 \pm 2^\circ C$ with 55-65% RH and exposed to 16 hr. photoperiod provided by cool fluorescent tubes (2500 lux).

RESULTS

A comparison was made between the culture of juvenile and mature shoot apices of irrigated and unirrigated tree with regard to variability of phenolic exudation in the medium and its effect on survival and establishment of explants (Table 1). Juvenile shoot apices from irrigated tree exuded very less phenolic compounds in the medium and hence their rate of survival and establishment was higher than juvenile shoot apices from unirrigated tree which released more phenolic compounds in the medium. Mature shoot apices from unirrigated trees release a high concentration of metabolites than shoots apices from irrigated trees

which was evidenced by the colour of the medium being brick brown in former case and pale green to light brown in latter case. Survival and establishment of cultures was found to be adversely affected by the concentration of metabolites in the medium. Lesser the browning of the medium greater was the survival rate of explants on the medium. The inhibitory action of the phenolic compounds on overall physiological and metabolic activities of the explant leads to their immediate death. The results from irrigated trees reveal that perhaps irrigation helps in leaching out the excessive secondary metabolites and hence lesser browning occurs in the medium.

To combat the problem of phenolic exudation for survival and establishment of cultures, the explants were regularly transferred to fresh medium after every 24 hours in the first week of culture or till there was no more browning in the medium. The concentration of phenolic exudation decreased after every transfer of explants to fresh medium which increased the survival rate of the explants on the medium to a great extent (Table 1).

Table 1. Variation of phenolic levels in the medium after culturing shoot apices from irrigated and unirrigated trees of apple Cv. American and its effect on survival rate of cultures*.

Physiological condition of the tree	Developmental stage of shoot apices	Colouration of culture medium due to phenolic compounds	Survival rate* (%)	No. of transfers to fresh medium	Survival rate after transfers ^b (%)
Irrigated (Twice in the season)	Juvenile shoot apices	Slightly pale green	90	1	100
	Mature shoot apices	Pale green to light brown	60	2-3	75
Unirrigated	Juvenile shoot apices	Light brown	40	4-5	55
	Mature shoot apices	Brick brown	5	6-7	30

* 20 cultures / treatment; a. data scored after 1 week; b. data scored after 2 weeks

DISCUSSION

The study demonstrates that explants can survive and get established on the nutrient media for various studies if the juvenile shoot apices are obtained from irrigated mature apple tree which release less phenolic metabolites in the medium thereby reducing the possibility of necrosis and death of the explants thereof. These results are favoured by the reports of Chen and Evans (1990) who laid stress on the appropriate selection of the explant for *in vitro* propagation of

apple. Mature shoot apices from unirrigated tree exudates maximum phenolic compounds in the medium followed by juvenile shoot apices from the same tree and comparatively lesser phenolic compounds are released by mature explants from irrigated tree. Hence survival rate of these explants and their establishment was higher than others. Frequent transfers of explants to fresh medium in the first week of culture reduced the death of the explant to a great extent thereby increasing the rate of establishment of explants. These findings are in agreement with those of Broome and Zimmerman (1978) and Llyod and McCown (1980).

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