

IN VITRO PRODUCTION OF CITRUS FRUIT BY USING EXPLANT OF MANDARINAyesha Ameen*¹ and Shahid Raza¹¹University of South Asia, Lahore Pakistan.

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ABSTRACT

Citrus fruit is extensively produced worldwide. This fruit has a commercial importance and a large nutritional value. This study was designed for the production of citrus fruit Mandarin *in vitro*. The MS media was prepared with addition of IAA, Kinetin and BAP to induce appropriate root and shoot formation. The explant was taken from shoot tips and it was 2mm long. It was concluded from this study that root formation was started after 3 weeks of explant inoculation in MS media. The plantlets were established in soil for further growth and maturity.

KEYWORDS: IAA, Kinetin and BAP.**INTRODUCTION**

Citrus fruit is ranked first for the higher production among all fruits (Ladanyia, 2010). Its popular fruit worldwide because of its nutritional value and production (Baldwin, 1993). These fruits are grown in almost 50 countries. Citrus industry contributes to world economy by giving jobs to millions of people in harvesting, storage, handling and transportation. The maximum demand of citrus in a growing population accelerated research and development of technologies. Knowledge of fruit physiology, biochemistry and morphology is important for the understanding of production and post harvesting. Scientist have a considerable interest in eliminating the pre harvest and post-harvest diseases. There is an extensive scope of using biotechnological techniques for citrus fruit production.

Citrus fruit, belongs to genus Citrus of family Rutaceae, they are well known of providing Vitamin C, and they contains a variety of phytochemicals. Modern biotechnological techniques include *in vitro* cell, protoplast, tissue and organ culture and the introduction of foreign genes. The quality of citrus fruit is varied according to the agro-climate variation (Ladanyia, 2010). Tangerine and mandarin are made by these tissue culturing techniques.

Seasonal changes in enzyme activities and some components of Satsuma mandarin and sweet lime has been studied, although the main acid in mature Satsuma mandarin fruit is citrate, malate was predominantly accumulated in the very early stage of fruit development. In sweet lime, malate was chiefly accumulated throughout fruit development.

Juice vesicle tissue in Satsuma mandarin fruit developed in four distinctive stages. In the first stage, enzyme activities and the contents of protein and nucleic acid increased. The activity of phosphoenolpyruvate carboxylase increased most rapidly. Cell division was observed in the first half of this stage. In the second stage, acids accumulated remarkably but enzyme activities and RNA content did not change. In the third, maturation stage, the content of RNA increased again. In the fourth stage, the contents of citrate and RNA decreased, whereas the activity of NAD-dependent isocitrate dehydrogenase increased (Hirai and ueno, 1977).

MATERIAL AND METHODS

The Murishage and skoog media for growing root and shoot was prepared according to the composition of macro and micronutrients mentioned in table 1 (Gamborg *et al.*, 1976). The media was autoclaved at 121⁰C for 15 minutes. The MS media was poured in 4 sterilized test tubes and left at room temperature to solidify. The shoots were obtained from shoot tips, 2cm length. The tubes were inoculated with citrus shoot tips under sterilized conditions to induce root and shoot formation. The tests tubes containing MS media were placed in tissue culture room at optimized conditions for many days. 1.0 BAP, 0.5 kinetin, and 0.5 NAA was also used for root and shoot formation *in vitro* (Singh *et al.*, 1994).

Table No: 1 Composition of MS media for citrus growth.

Micronutrients	Mg/L
NH ₄ NO ₃	1650
KNO ₃	1900
CaCl ₂ . 7H ₂ O	440
KH ₂ PO ₃	170
Macronutrients	
KI	0.83
H ₃ BO ₃	6.2
MnSO ₄ .4H ₂ O	22.3
ZnSO ₄ . 7H ₂ O	8.6
Na ₂ MoO ₄ . 2H ₂ O	0.25
CuSO ₄ .5H ₂ O	0.025
CoCl ₂ .6H ₂ O	0.025
Na ₂ .EDTA	37.3
FeSO ₄ .7H ₂ O	27.8
Sucrose (g)	30
pH	5.7

RESULTS AND DISCUSSION

The shoots of 2mm length were developed in to an undifferentiated mass of cells called callus after 20-25 days. These tubes were further placed for root and shoot formation under optimized conditions in tissue culture room.



The most important group in fresh citrus fruit market are mandarin. These fruits are produced by using various tissue culture techniques. The difference between tangerines and mandarins is only the size, the size of tangerine is smaller than mandarin (Ladanyia, 2010). Citrus flowering remains poorly understood with many contradictions and conflicts existing between reports (Krajewski and Rabe, 1995). The root formation was observed after 3 weeks. These plantlets were successfully introduced in soil for further growth in green house (Singh *et al.*, 1994). Numerous reviews are available on various aspects of citrus fruit, including information on industry, production and harvesting, citrus products, postharvest, nutrition, chemistry and biochemistry (Kefford, 1960).

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