Biological Forum – An International Journal **13(2): 332-335(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Economics of *In Vitro* Grown Plantlets of Clonal Apple MM-106 Rootstock

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(Received 06 April 2021, Accepted 12 June, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: ***Lack of information is available on practicality and profitability of in vitro multiplied* plants of clonal apple rootstocks as compared to field multiplied plants through conventional methods. To develop a best economical and suitable protocol through *in vitro* techniques of apple clonal rootstock MM106, the present investigation was carried at Plant Tissue Culture laboratory of School of Biotechnology (SBT) and Tissue Culture laboratory of Advanced Centre for Horticulture Research (ACHR), Udheywalla campus, SKUAST-Jammu during the year 2018. Results from the investigations indicated that the unit cost of one *in vitro* raised plantlet from explant which were excised from 2-3 year-old clonal rootstock of apple was estimated to be Rs. 44.56, whereas through conventional methods the estimated production cost of *ex vitro* seedling is Rs. 80.00.**

Keywords: Apple, MM106, economics, *in vitro* and *ex vitro.*

INTRODUCTION

The cultivated apple (*Malus* × *domestica* Borkh.) is native to South-West Asia (Korban and Skirvin, 1994), belongs to sub family Pomoideae and family Rosaceae having basic chromosome number 17 (Brown, 1992). Apple, often considered as "King of temperate fruit" is an important temperate fruit crop grown throughout the world (Ghanbari, 2014). In India, UT of Jammu and Kashmir is the largest apple producer with an annual production of 1882319 MT from an area of 164742 ha (Anonymous, 2019). Considering the soil and climatic conditions, topography and rainfed conditions of apple growing temperate areas, the apple plants are mainly raised on seedling rootstocks. The seedling plants are more vigorous and show variability in their performance. Now-a-day's many clonal rootstocks have been developed which are considered better than seedling rootstocks due to their wide adaptability, precocious nature, disease and insect pests resistance. Among them one of the suitable clonal rootstock of apple is MM106. It is a semi dwarf, precocious rootstock released from East Malling Merton Research Station, England. It has a tree size of standard non spur type (80 %) and spur type (70 %), resistant to wooly apple aphid, burr knot and ideal for high density planting. From the past two decades there is a huge demand of MM106 clonal apple rootstock for various scion cultivars which is mainly multiplied through conventional methods like mould layering or trench layering but these methods are very reliant on season, has lower multiplication rates, which limits the rate of output and makes the end product more expensive. For production of adequate planting material within a shorter period of time with less input costs, in the recent past years, *in-vitro* propagation techniques are quite reliable for several economic plants, restoration of vigor and yield, preservation of germplasm in less space over conventional method (Singh, 2002). Thus, an effort was made with *in-vitro* propagation of apple clonal rootstocks MM106 in order to identify the economical methods for production of quality planting material for the benefits of orchardists.

MATERIALS AND METHODS

The experimental material for explants of clonal apple rootstock MM106 for *in vitro* propagation was taken from newly grown flushes of selected 2-3 year-old plants grown at mother block rootstock of Regional Horticulture Research Sub-Station (RHRSS), Bhaderwah of SKUAST-Jammu during 2018. The shoot tip and nodal segment of 0.5 cm to 0.75 cm were used as experimental material. The collected shoots were kept in plastic bags to prevent wilting till their use in the laboratory. The explants were treated with bavistin solution of 0.2 per cent for 20 minutes followed by mercuric chloride (0.05%, 0.1% and 0.2%) and sodium hypochlorite (3 %, 8 % and 12 %) at different durations i.e. 2, 4 and 6 minutes and 10, 20 and 30 minutes, respectively. After surface sterilization, explants were rinsed in sterile distilled water 3-4 times in order to remove the traces of sterilants.

The establishment media consists of MS basal and MS supplemented with (1.0-3.0 mg/l) IBA and (1.0-3.0

mg/l) GA_3 alone as well as in combination. The establishment media further contained antioxidants such as PVP (2.5-10.0 g/l), citric acid (2.5-10.0 g/l) and ascorbic acid (2.5-10.0 g/l) to minimize the phenol exudation from the excised portion of the explants. After six weeks, growing shoot tips and nodal segments with small shoots were transferred to the shoot proliferation media supplemented with MS basal or MS media containing (1.0-3.0 mg/l) BAP and (1.0-3.0 mg/l) GA_3 alone as well as in combination along with 0.2 percent NAA to facilitate further growth and elongation. To achieve rooting, half-strength MS medium containing (1.0-3.0 mg/l) IBA and (1.0-3.0 mg/l) NAA alone and in combination along with 0.2 percent activated charcoal (AC). Rooted cuttings were subjected to primary hardening for three weeks under laboratory conditions and then three weeks under greenhouse conditions and finally successfully established in potting mixture compositions. The data on the rate of culture establishment, rate of proliferation, rooting response of shoots, survival of plantlets, maximum capacity of the laboratory and the number of initial explant capable of producing 12288 plantlets was calculated. The total cost involved per cycle was worked out, which involves the cost of building, equipment, glassware, chemicals and miscellaneous items, having been distributed over the years according to their potential durability.

RESULTS AND DISCUSSION

The highest aseptic and survival percentage was noticed with 0.1 per ent $HgCl_2$ for 4 minutes, maximum culture establishment took place in MS medium supplemented with 1.0 mg/l BAP + 1.0 mg/l GA_3. Highest shoot proliferation took place in MS medium augmented 1.0 mg/l BAP + 1.0 mg/l + 0.2 per cent NAA. 1.0 mg/l NAA + 0.2 per cent activated charcoal (AC) resulted in maximum rooting percentage. As per protocol developed, the highest aseptic and survival cultures (83.33 % and 64.00 %), respectively was recorded during the month of April as compared to other months. The culture establishment resulted in 52.77 per cent success, highest proliferation rate was 3.58 shoots per explant while highest percentage of rooting was recorded to be 60.00 per cent. To calculate the economics, it was assumed that the production started with 300 explants initially (Fig. 1). They were inoculated on the establishment media (stage - I). The established explants were continuously multiplied to produce a total of 12288 shoots (stage - II) and out of 12288 shoots, only 9831 shoots of optimum size were selected and transferred to the rooting stage (stage - III). Only 60.00 per cent rooting was recorded in *in vitro* shoots in the rooting medium. The plantlets were transferred to pots containing a mixture of sand, soil, vermiculite and FYM alone as well as in combination and were kept in air conditioned room for hardening and then shifted to greenhouse (stage - IV). The cost of produce of one plantlet was calculated as per standardized protocol. It was estimated that 16 ml and 30 ml of nutrient medium was required per conical flask and glass jar, respectively in stage I, stage II and stage - III with 1 explant per flask and 3-5 explants per jar.

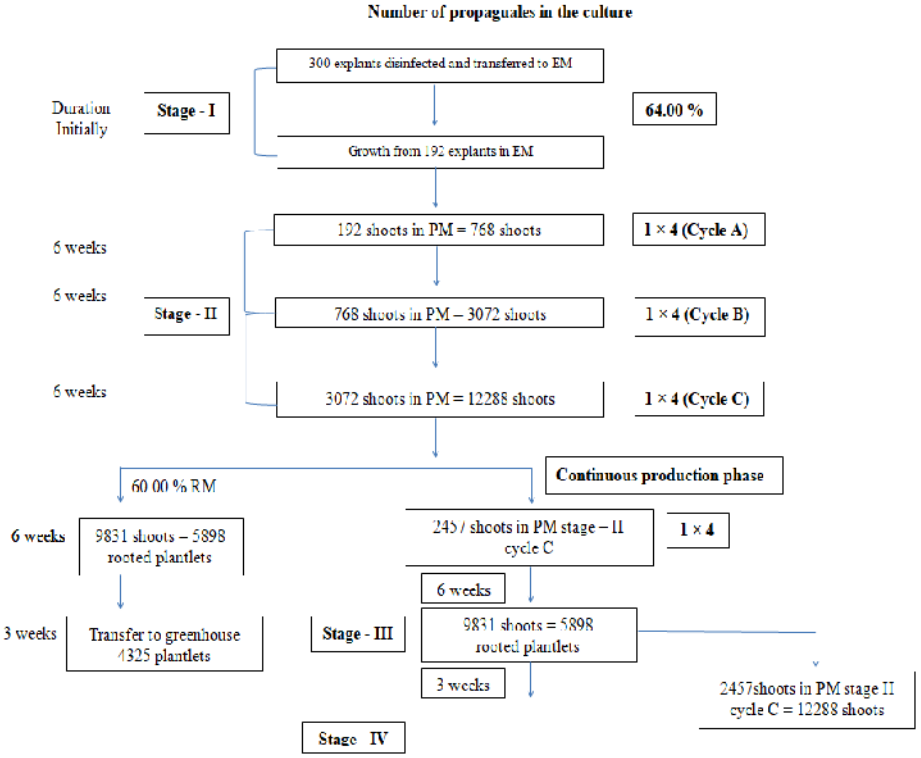

Fig. 1. Estimated cost for *in vitro* propagation of MM-106 rootstock.

Table 1: Estimated cost for *in vitro* propagation of clonal apple rootstock MMM106 through tissue culture on the basis of various inputs required.

A. Tissue culture production phase

S. No.	Items	Cost (Rs.)
1.	Media cost (calculated on the basis of cost of MS media vials and chemical required for its supplementation at each stage)	
	(a) Stage – I (300 explants in 300 test tubes × 16 ml) = 4.80 litre	**326.40**
	[192 shoots in 192 test tubes (capacity 50 ml) × 16 ml] = 3.07 litre	**208.76**
	(b) Stage – II Cycle – A [768 shoots in 154 glass jar (capacity 150 ml) × 30 ml/ glass jars] = 4.62 litre	**314.16**
	Cycle – B [3072 shoots in 615 glass jars (capacity 150 ml) × 30 ml] = 18.45 litre	**1254.60**
	Cycle – C [12288 shoots in 2458 glass jars (capacity 150 ml) × 30 ml] = 73.74 litre	**5014.32**
	(C) Stage – III [9831 shoots in 9831 test tubes (capacity 50 ml) × 16 ml = 157.29 litre	**10695.72**
Total media cost		**17813.96**
2.	**Glassware cost:**	
	(a) Culture vessels: (i) 3227 glass jars capacity 150 ml @ Rs. 150 per glass jar allocated over 50 monthly cycles (ii) 50 ml culture tubes (10323 @ Rs. 16.00 per tube allocated over 50 monthly cycles	**9681.00** **3303.36**
	(b) Other glassware of media preparation (Rs. 10,000 allocated over 50 monthly cycles	**200.00**
	Total glassware cost	**13184.36**
3.	**Equipment and laboratory facility:**	
	(a) Equipment (Laminar air flow chamber, air conditioners, autoclaves, weight balance, pH meter, refrigerator, hot air oven etc.) Rs. 6,00,000/- allocated over 90 months	**6666.66**
	(b) Laboratory facility (Building with growth room) Rs. 10,00,000 allocated over 300 months	**3333.33**
	Total equipment and laboratory cost	**9999.99**
4.	**Wages and salary:**	
	(a) 1 Technician with total emoluments Rs. 8000 per month	**48000.00**
	(b) 1 Supervisor with total emoluments Rs. 10000 per month	**60000.00**
	Total wages and salary	**108000.00**
5.	**Miscellaneous costs:**	
	(Electricity, water etc.) Rs. 5000 per month	**30000.00**
	Total tissue culture production phase cost	**178998.31**
	Unit cost of producing clonal apple rootstock (on the basis of 4325 transplants per month	**41.38**

B. Green House Production Phase

S. No.	Item	Cost (Rs.)
1.	Labour charges:	
	(a) 1 skilled labour @ Rs. 6000 per month	4200.00
	(b) 1 skilled greenhouse technician @ Rs. 8000 per month	5600.00
2.	Material cost:	
	(a) Pots (4325 @ Rs. 7 per pot allocated over 30 monthly cycles)	1009.16
	(b) Soil mixture (Soil + Sand + Vermiculite + FYM) all in equal proportions by v/v 100 cu ft @ Rs. 6 per cu ft.	600.00
	(c) Greenhouse implements (Rs. 10000 allocated over 20 cycles) each of 2 months duration	
3.	Greenhouse (size approximately 500 sq. ft.) facility Rs. 80000 allocated over 10 years (60 cycles) each of two month duration)	1333.33
4.	Miscellaneous cost (water, electricity, and other expenses) Rs. 1500 per month	1050.00
	Total greenhouse production phase cost	13792.49
	Unit cost during greenhouse production phase (On the basis of 4325 transplant per month)	3.18
	Total per unit cost A + B	**41.38 + 3.18**
	Total cost per tissue culturally raised plants	**44.56**

Thus, it was calculated that approximately 7.87 litres of nutrient medium was required for stage - I, 96.81 litre for stage - II and 157.29 litre for stage - III. Thus, a total of 261.97 litre nutrient medium was required for producing 12288 *in vitro* plantlets. The cost of nutrient medium for stage - I was Rs. 535.16, Rs. 6583.08 for stage - II and Rs. 10695.72 for stage - III. The cost of nutrient medium was estimated about Rs. 68 per litre and overall cost of nutrient medium required for plantlets production was estimated about Rs. 17813.96. The glassware costs were calculated for the approximate number required and was amortized over the normal life i.e. 50 monthly cycles. The overall equipments and laboratory cost was estimated to be about Rs. 9999.99. For technical support, one-laboratory technician with total emoluments Rs. 8000 per month and one supervisor with total emoluments Rs. 10000 per month for the technical support and supervision of an experiment were considered. The miscellaneous costs (i.e. electricity, water was Rs. 5000 per month). Thus, the total tissue culture production phase cost was estimated to be about Rs. 178840.87 and cost of *in vitro* raised plantlets of MM106 at tissue culture production phase was Rs. 41.38 on the basis of 4325 plantlets. In greenhouse production phase, total cost for production of 4325 plantlets of MM106 rootstock was estimated about Rs. 3.18, which includes cost of one skilled labour, one skilled greenhouse technician, cost of pots, cost of soil mixture (potting mixture), cost of greenhouse implements, miscellaneous cost (electricity, water and other expenses). So the overall cost per tissue culture raised plant of clonal apple rootstock MM106 is was Rs. 44.56 (Table 1).

Parul (2016) reported that cost of tissue cultured banana (TCB) after economics analysis was Rs. 35.57 per plantlet with survival rate 94.17 per cent. Kour *et al*. (2009) pointed out that *in vitro* rooting was the most labour intensive part, which consumes larger amount of total nutrient medium requirements for *in vitro* propagation system. Kour *et al*., (2009) also revealed that the unit cost of production of *Citrus jambhiri* upto greenhouse stage was estimated to be Rs. 2.54. Sharma *et al*., (2009) also reported that the unit cost of production of one plantlet of strawberry including three weeks of hardening and one week under polyhouse was estimated to be Rs. 3.56. Alagumani (2005) revealed that gross income was higher by 35.35 per cent in tissue cultured banana (TCB) plantlets than sucker propagated (SPB) plantlets, which worked out to Rs 253302 and Rs 187149 per hectare, respectively. They also noticed that the net income was higher by 42.37 per cent in TCB than in SPB. Babylatha (1994) estimated the unit cost of banana plantlet as Rs. 2.22 including 4 weeks greenhouse stage. Wali *et al*., (1996) reported that the unit cost of production of guava cv. Sardar upto greenhouse stage was estimated to be Rs. 1.95 in case of seedling explant and Rs. 2.05 in case of mature tree explant. Rajmohan (1985) also reported that the unit cost of one jackfruit plantlet including one month hardening was estimated to be Rs. 9.09.

The present investigation concluded that tissue-cultured MM106 plantlets were more profitable than plantlets raised through conventional methods as the resources could be used more efficiently in tissue culture techniques. However, 0.1% $HgCl_2$ for 4 minutes recorded maximum percentage of aseptic and survival of cultures. MS medium supplemented with 1.0 mg/l BAP + 1.0 mg/l GA_3 recorded maximum culture establishment, while shoot proliferation rate recorded maximum in 1.0 mg/l BAP + 1.0 mg/l GA_3 + 0.2% NAA. The highest rooting percentage was achieved with 1.0 mg/l NAA + 0.2% activated charcoal. Further, at hardening stage, maximum survival percentage was recorded under soil + sand + vermiculite + FYM (1:1:1:1 v/v/v/v).

REFERENCES

Anonymous (2018). Area and Production of Fruit Crops. Directorate of Horticulture, Government of Jammu and Kashmir. pp. 1-2.

Alagumani, T. (2005). Economic analysis of tissue-cultured banana and sucker-propagated banana. *Agricultural Economics Research Review*, *18*: 81-89.

Babylatha, A. K. (1994). *In vitro* propagation studies of banana: In: Proceedings of *2nd Asia Pacific Conference on Agricultural Biotechnology*, Abstract, pp. 211.

Brown, S. (1992). *Genetics of Apple*. *Plant Breeding Reviews*, *9*: 333-366.

Ghanbari, A. (2014). Impacts of plant growth regulators and culture media on *in vitro* propagation of three apple (*Malus x domestica* Borkh.) rootstocks. *Iranian Journal of Genetics and Plant Breeding*, *3*(1): 11-20.

Korban, S.S. and Skirvin, M. R. (1994). Nomenclature of the cultivated apple. *Hort Science*, *19*: 177-180.

Kour, K., Parshant, B., Singh, S.K. and Kher, R. (2009). Economics of *in vitro* and *ex vitro* grown plantlets of *Citrus jambhiri*. *Indian Journal of Horticulture*, *66*(3): 7-11.

Parul, K.R. (2016). *Economic analysis of tissue culture lab and it's product*. MBA Thesis, Junagadh Agricultural University, Gujarat. India.

Rajmohan, K. (1985). Standardization of tissue culture techniques for important horticultural crops. Ph.D. Thesis, Kerala Agricultural University, Vellanikkara, India.

Singh, I. P. (2002). Micropropagation in citrus. A review. *Agril. Rev*., *23*: 1-13.

Spengler, R.N. (2019). *Origins of the Apple*: The Role of Megafaunal Mutualism in the Domestication of *Malus* and Rosaceous Trees. *Frontiers in Plant Science*, pp. 36-67.

Wali, V.K., Patel, B. M. and Shah, R. R. (1996). *Micropropagation of guava*: Commercial feasibility. Nat. Sem. Biodiversity, Plant growth Regulators and Biotech. 22nd-24th February, Gujarat Agricultural University, Ahmedabad. Abstact: 50.

How to cite this article: Lal, M., Jamwal, M., Sharma, M., Bakshi, P., Jasrotia, A., Kumar, A., and Deep, A. (2021). Economics of *In Vitro* Grown Plantlets of Clonal Apple MM-106 Rootstock. *Biological Forum – An International Journal*, *13*(2): 332-335.