Biological Forum – An International Journal **13(2): 381-385(2021)**

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

Influence of Antioxidants on *in vitro* Culture Establishment of Clonal Apple Rootstocks

Manmohan Lal*[1], Mahital Jamwal[1], Parshant Bakshi[1], Amit Jasrotia[1], Nirmal Sharma[1], Mamta Sharma[2], Prabhdeep Singh[1], Sakshi Sharma[1] and Sanjay Kumar[1]

[1]*Division of Fruit Science, SKUAST-Jammu, Chatha Campus, Jammu (J & K), India.*
[2]*Principal Investigator DST, School of Biotechnology, SKUAST-Jammu, Chatha Campus, Jammu (J & K), India.*

(Corresponding author: Manmohan Lal)*
(Received 02 April 2021, Accepted 14 June, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTARCT: The phenol exudation from explants of woody plant species increases the rate of oxidative browning that causes death of the majority of cultured explants. A study was carried out to identify the best antioxidant for *in vitro* culture establishment and to minimize the phenol exudation in explants of clonal apple rootstocks MM106 and MM111. The experiment was carried out at Plant Tissue Culture laboratory of School of Biotechnology and Tissue Culture laboratory of Advanced Centre for Horticulture Research, Udheywalla of SKUAST-Jammu during the year 2018. Results indicated that MS + 10 g PVP (AM4) resulted in highest culture establishment in MM111 and MM106 clonal apple rootstocks. The shoot tip and nodal segment explants of MM111 rootstock recorded highest culture establishment i.e. 47.88 per cent and 35.33 per cent, respectively, whereas 32.22 per cent and 27.27 per cent culture establishment was obtained in shoot tip and nodal segment, respectively in MM106 rootstock.

Keywords: Antioxidants, *in vitro*, culture establishment, MM106 and MM111.

INTRODUCTION

Apple clonal rootstocks are generally propagated through traditional methods like mould layering or trench layering but these traditional methods are very reliant on season, has lower multiplication rates, susceptible to many diseases, insects pests and viruses which limits the rate of output and makes the end product more expensive. For production of adequate quality planting material within a short period of time with less input costs, in the recent past years, *in-vitro* propagation techniques are observed quite reliable for several plants for restoration of vigor and yield, preservation of germplasm in less space over the traditional methods (Singh, 2002). (Amiri *et al*., 2011, Mir *et al*., 2010, Sedlak and Paprstein, 2008 and Adıyaman *et al*., 2004). Apple was one of the first woody plants to be successfully propagated under *in vitro* conditions. It was first micropropagated by Jones (1967) where it was found that growth of shoot tip could be stimulated by the synthetic plant growth regulator, benzyl aminopurine (BAP). Over the last two decades, several research groups purposed different *in vitro* conditions that were suitable for micropropagation of apple cultivars and rootstock material (Skirvin *et al*., 1986, Marin *et al*., 1983 and Quoirin and Lepoivre, 1977). The clonal apple rootstock MM106 is a cross of Northern Spy × M 1 and most popular rootstock in its category. It was released from East Malling Merton Research Station, England. It is resistant to wooly apple aphid, burr knot and ideal for high density planting. MM111 is a cross of Merton 793 × Northern Spy developed by John Innes Horticultural Institute and East Malling Research Station, England. It is resistant to wooly apple aphid and drought conditions but quite tolerant to fire blight and crown and root rots. Considering the climatic and topographical conditions of Jammu and Kashmir (J & K), clonal apple rootstocks MM106 and MM111 are very high in demand but still the availability of quality planting material is very low.

Phenols are the chemical compounds which posses in common, an aromatic ring bearing one or more hydroxyl constituents (Kumar and Goel, 2019 and Onuoha, 2011). Exudation of phenol have been reported to be the major problem during *in vitro* propagation of apple and some other woody plant species, which increases the rate of oxidative browning that causes death of the majority of cultured explants (Nishchal *et al*., 2018). Volz and McGhie (2011) reported that all *Malus* species contain high level of phenols that inhibits the extent of culture establishment. *In vitro* propagation of woody plants is recalcitrant for optimum growth because of phenol exudation from the excised portion of the explants during *in vitro* establishment of explants. Although, different

antioxidant/ absorbents *viz.* PVP, citric acid, ascorbic acid and activated charcoal have been used to reduce the problem of phenol exudation but the effectiveness of antioxidant/ absorbents varies from species to species as well as physiological conditions of the mother plants (Jakhar *et al*., 2019 and Quoirin and Lepoivre, 1977). The use of antioxidants and absorbents has been also demonstrated by several research workers in pear (Podyal *et al*., 2008), in pomegranate (Chaugule *et al*., 2007), in apple (Kaushal *et al*., 2005) and in mango (Chandra *et al*., 2003). According to them, production of phenolic compounds is indirectly stimulated by various factors such as physiological condition, size and age of the explants used. Therefore, the present study was carried out to identify the best antioxidant for *in vitro* culture establishment of apple clonal rootstocks MM106 and MM111.

MATERIALS AND METHODS

A. Collection of Plant Material

The experimental material for explants of clonal apple rootstock MM106 and MM111 were taken from newly grown flushes of selected 2-3 year-old plants grown at mother block 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. Finally, the explants were placed on the petridish lined with pre-autoclaved filter paper and finally cultured on conical flasks containing MS medium.

B. Culturing of Explants for Establishment

The explants were inoculated on MS basal or MS medium having adsorbent like PVP (2.5 g, 5 g and 10 g), citric acid (2.5 g, 5 g and 10 g) and ascorbic acid (2.5 g, 5 g and 10 g). The MS medium used was solidified with 0.8 per cent agar.

RESULTS AND DISCUSSION

The different treatments of antioxidants showed a significant influence on rate of culture establishment in clonal apple rootstock MM106 and MM111 as showed in Table 1, Fig. 1 and Table 2, Fig. 2, respectively. The clonal rootstock MM106 recorded maximum culture establishment (30.00 %) on MS media containing 10 g polyvinyl pyrrolidone (AM4) which was statistically at par with 28.88 per cent in MS media + 5 g polyvinyl pyrrolidone (AM3). The MS basal medium gave minimum culture establishment (0.00 %).

Table 1: Effect of antioxidants on per cent culture establishment of clonal apple MM106 rootstock.

Media Code	Treatment	MM106		
		Shoot tip	Nodal segment	Mean
AM1	Control: MS (Basal)	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
AM2	MS + PVP (2.5 g)	16.66 (24.01)	25.55 (30.34)	21.11 (27.17)
AM3	MS + PVP (5 g)	26.66 (31.07)	31.11 (33.85)	28.88 (32.46)
AM4	MS + PVP (10 g)	27.77 (31.78)	32.22 (34.54)	30.00 (33.16)
AM5	MS + Citric acid (2.5 g)	11.11 (19.42)	18.89 (25.73)	15.00 (22.57)
AM6	MS + Citric acid (5 g)	18.88 (25.67)	27.77 (31.78)	23.33 (28.72)
AM7	MS + Citric acid (10 g)	20.00 (26.50)	28.88 (32.46)	24.44 (29.48)
AM8	MS + Ascorbic acid (2.5 g)	15.55 (23.18)	24.44 (29.60)	20.00 (26.39)
AM9	MS + Ascorbic acid (5 g)	16.66 (24.08)	25.56 (30.34)	21.11 (27.12)
AM10	MS + Ascorbic acid (10 g)	17.77 (24.90)	26.67 (31.08)	22.22 (27.99)
	Mean	17.11 (23.06)	24.11 (27.97)	
	$CD_{(0.05)}$	Explant (E) = 0.86 Antioxidant (A) = 1.93 E × A = 2.72		

*PVP = Polyvinyl pyrrolidone

*Figures presented in parenthesis are arcsine transformed values

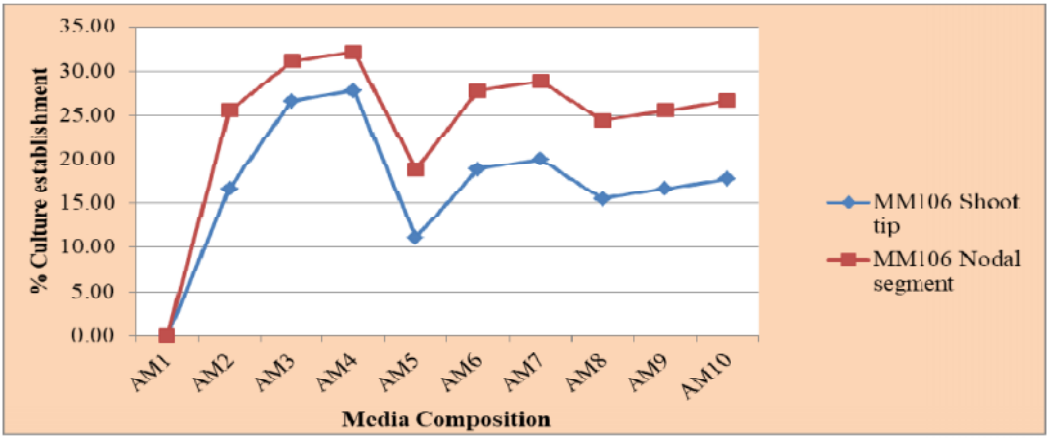

Fig. 1. Effect of antioxidants on per cent culture establishment of clonal apple MM106 rootstock.

Table 2: Effect of antioxidants on per cent culture establishment of clonal apple MM111 rootstock.

Media Code	Treatment	MM111		
		Shoot tip	Nodal segment	Mean
AM1	Control: MS (Basal)	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)
AM2	MS + PVP (2.5 g)	27.78 (31.78)	36.66 (37.24)	32.22 (34.51)
AM3	MS + PVP (5 g)	34.55 (35.98)	46.66 (43.06)	40.61 (39.52)
AM4	MS + PVP (10 g)	35.33 (36.45)	47.88 (43.76)	41.61 (40.11)
AM5	MS + Citric acid (2.5 g)	21.55 (27.64)	31.33 (34.01)	26.44 (30.82)
AM6	MS + Citric acid (5 g)	28.89 (32.49)	37.78 (37.90)	33.34 (35.20)
AM7	MS + Citric acid (10 g)	30.00 (33.19)	38.89 (38.56)	34.45 (35.88)
AM8	MS + Ascorbic acid (2.5 g)	27.77 (31.78)	34.44 (35.91)	31.11 (33.84)
AM9	MS + Ascorbic acid (5 g)	28.89 (32.49)	35.55 (36.58)	32.22 (34.53)
AM10	MS + Ascorbic acid (10 g)	30.00 (33.17)	36.66 (37.24)	33.33 (35.21)
	Mean	26.48 (29.49)	34.59 (34.43)	
	$CD_{(0.05)}$	Explant (E) = 0.54 Antioxidant (A) = 1.21 E × A = 1.72		

*PVP = Polyvinyl pyrrolidone
*Figures presented in parenthesis are arcsine transformed values

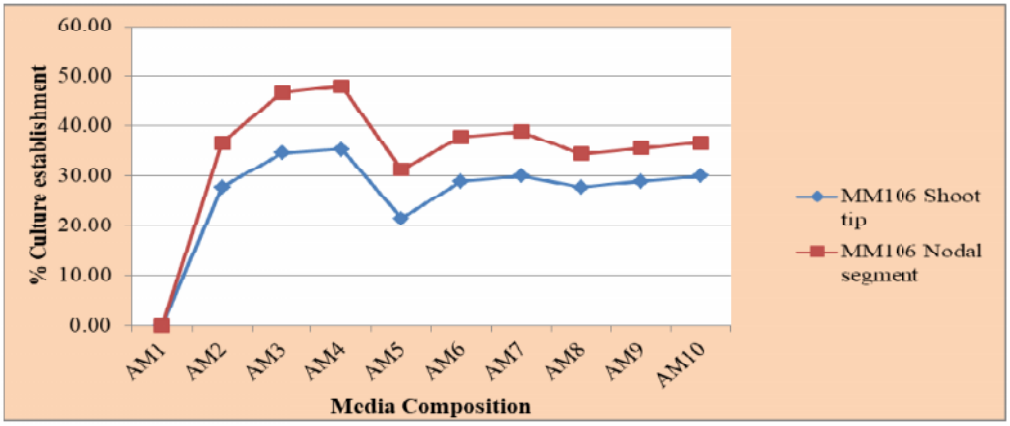

Fig. 2. Effect of antioxidants on per cent culture establishment of clonal apple MM111 rootstock.

This is might be due to the lack of antioxidants like polyvinyl pyrrolidone, citric acid and ascorbic acid which control the exudation of phenols from the excised portion of the explants in the medium (Kaushal *et al*., 2005). Between the explants used (shoot tip and nodal segment), nodal segment showed higher culture establishment (24.11 %) which was significantly higher than the shoot tip (17.11 %). In the interaction effect, media composition AM4 showed maximum culture establishment (32.22 %) for nodal segment followed by AM3 (31.11 %), while shoot tip showed 27.77 per cent culture establishment with AM4 followed by AM3 (26.66 %). However, for both explants, minimum culture establishment was recorded as 0.00 per cent under control.

In clonal apple rootstock MM111, maximum culture establishment (41.61 %) was recorded under MS media + 10 g polyvinyl pyrrolidone (AM4) which was statistically at par with MS media + 5 g polyvinyl pyrrolidone AM3 i.e. 40.61 per cent. However, minimum culture establishment was recorded as 0.00 per cent under control. Between the two explants, nodal segment explants gave maximum culture establishment of 34.59 per cent which was significantly higher than the culture establishment with shoot tip (26.48%). The interaction data revealed that nodal segment with AM4 gave maximum culture establishment of 47.88 per cent followed by 46.66 per cent in AM3, while shoot tip recorded 35.33 per cent culture establishment followed by 34.55 per cent with AM3. The MS basal medium recorded minimum culture establishment of 0.00 per cent for both the explants. The phenol exudation was the major problem during culture establishment, which increases the rate of oxidative browning that causes death of the majority of cultured explants (Nishchal *et al*., 2018). Volz and McGhie (2011) reported that all *Malus* species contain high level of phenols that inhibits the extent of culture establishment. In the present study, among different antioxidants, polyvinyl pyrrolidone was found to be more effective antioxidant to minimize the phenol exudation from the excised portion of the explants, while citric acid and ascorbic acid revealed comparatively lower effect on phenol exudation as compared to polyvinyl pyrrolidone. The effect of various antioxidants in controlling browning and phenolic excretion varies between plants and species (Jakhar *et al*., 2019). This could be due to the specificity of different antioxidants like polyvinyl pyrrolidone, citric acid, ascorbic acid and activated charcoal to certain plants and species (Prajapati *et al*., 2003) who found that culture browning can be successfully controlled by the addition of polyvinyl pyrrolidone (PVP) into the medium.

Regarding the season and type of explants, our results corroborate with the results of Modgil *et al*. (1999) who observed that explants of Tydeman's Early Worcester apple plants showed maximum sprouting (75.00 %) during spring and summer months in comparison with those collected during other seasons (30.00 %). Similarly, Webster and Jones (1991) and Hutchinson (1984) agreed that the rate of browning and phenol exudation of apple explants decreases considerably when they are collected in the summer months. It has been also noticed that nodal segment explants showed a better rate of culture establishment as compared to the shoot tip explants, this may be due to the reason that stem nodes (nodal segment) contained the adequate amount of cytokinins for adventitious shoot production (Singh and Patel, 2013). Our results are also in confirmity with the results obtained by Singh and Patel (2016), Tang *et al*., 005), Murkute *et al*., (2004), Singh *et al*., (2002) and Pandeliev *et al*., (1990).

The present study concluded that different antioxidants play an important role in controlling phenolic exudation from the excised portion of the explants and decreases the rate of oxidative browning which caused death of the majority of cultured explants. MS media + 10 g PVP gave maximum culture establishment of explants as compared to all other media compositions in clonal apple rootstocks MM106 and MM111.

REFERENCES

Adıyaman, A. F., Iıkalan, C., Kara, Y., & Baaran, D. (2004). The comparison on the proliferation of lateral buds of *Vitis vinifera* L. cv. Perle de Csaba during different periods of the year *in vitro* conditions. *International Journal of Agriculture and Biology*, *2*: 328-330.

Amiri, E. M., & Elahinia, A. (2011). Optimization of medium composition for apple rootstocks. *African Journal of Biotechnology*, *10*(18): 3594-3601.

Chandra, R., Padaria, J. C., & Srivastava, S. (2003). Factors influencing *in vitro* establishment of mango shoot buds. *Indian Journal of Plant Physiology*, *9*(2): 136-144.

Chaugule, R. R., More, T. A., Kamble, A, B., & Karale, A. R. (2007). Studies of micropropagation and callus induction in pomegranate (*Punica grantum* L.) cv. Mridula. In: Recent Trends in Horticultural Biotechnology, (EDs.). Ragunath Keshvachandran. pp. 195-199.

Hutchinson, J. F. (1984). Factors affecting shoot proliferation and root initiation in organ cultures of the apple 'Northern Spy'. *HortScience*, *22*: 347-358.

Jakhar, M. L., Verma, R., & Dixit, D. (2019). Effect of antioxidants on *in vitro* degree of browning and culture establishment of Guggul [*Commiphora wightii* (Arnott)]: A valuable desert medicinal plant. *Journal of Pharmacognosy and Phytochemistry*, *5*: 250-254.

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

Kausal, N., Modgil, M., Thakur, M., & Sharma, D. R. (2005). *In vitro* clonal multiplication of an apple rootstock by culture of shoot apices and axillary buds. *Indian Journal of Experimental Biology*, *43*: 561-565.

Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Report*, *24*: 1-10.

Marin, J. A., Jones, O. P., & Hadlow, W. C. C. (1983). Micropropagation of columnar apple trees. *Journal of Horticultural Science*, *68*(2): 289-297.

Mir, J. I., Ahmed, N., Verma, M. K., Muneer, A., & Lal, S. (2010). *In vitro* multiplication of cherry rootstocks. *Indian Journal of Horticulture*, *67*: 29-33.

Modgil, M., Sharma, D. R., & Bhardwaj, S. V. (1999). Micropropagation of apple cv. 'Tydeman's Early Worcestor'. *Scientia Horticulturae*, *81*: 179-188.

Murkute, M., Patil, S., & Singh, S. K. (2004). *In vitro* regeneration in pomegranate cv. Ganesh from mature plant. *Indian Journal of Horticulture*, *61*(3): 206-208.

Nishchal, N., Mir, H., Rani, R., & Pal, A. K. (2018). Effect of antioxidants in controlling phenol exudation in micropropagation of litchi cv. Purbi. *Current Journal of Applied Science and Technology*, *31*(4): 1-7.

Onuoha, C. I., Chinonye, J. E., & Unamba, C. I. N. (2011). *In vitro* prevention of browning in plantain culture. *Journal of Biological Science*, *11*(1): 13-17.

Pandeliev, S., Ruseva, R. M., & Georgieva, P. (1990). Degree of development of grape vine plants *in vitro* relation to the biology of the initial explants. *Rostniev dni-Nauki*, *27*(7): 79-83.

Poudyal, B. K., Du G., Zhang, Y., Liu, J., & Shi, Q. (2008). Studies on browning problem and phenols content on shoots of Yali Aikansui and Abbe Fetel pear for *in vitro* culture. *Frontier of Agriculture in China*, *2*(3): 321-330.

Prajapati, H. A., Mehta, S. R., Patel, D. H., & Subramanian, R. B. (2003). Direct *in vitro* regeneration of *Curculigo orchioides* Gaertn: An endangered anti-carcinogenic herb. *Current Science*, *84*(6): 747-749.

Quoirin, M., & Lepoivre, P. (1977). Improved media for *in vitro* culture of Prunus sp. *Acta Horticulturae*, *78*: 437-442.

Sedlak, J., & Paprstein, F. (2017). In vitro establishment and proliferation of apple cultivars. *Acta Horticulturae*, *1113*: 107-111.

Singh, P., & Patel, R. (2016). Factors affecting in vitro degree of browning and culture establishment of pomegranate. *African Journal of Plant Science*, *10*: 43-49.

Singh, S. K., Khawale, R. N., Vimlay, Y., & Singh, S. P. (2002). Effect of season, type of explants and pre-treatment to minimize polyphenolics exudation on *in vitro* culture establishment in grape. *Indian Journal of Horticulture*, *59*(3): 233-238.

Skirvin, R. M., Koudir, H., Joung, & Korban, S. S. (1986). The Tissue Culture of Apple (*Malus* × *domestica* Borkh.). In: Bajaj, Y.P.S (Ed.). *Biotechnology in Agriculture and Forestry*. Vol. *1*. Springer Verlag. p. 189.

Tang, W., & Newton, R. J. (2004). Increase of polyphenol oxidase and decrease of polyamines correlate with tissue browning in *Virginia* pine (*Pinus virginiana* Mill.). *Plant Science*, *167*: 621-628.

Volz, R. K., & Mcghie, T. (2011). Genetic variability in apple fruit polyphenol composition in *Malus* × *domestica* and *Malus sieversii* germplasm grown in New Zealand. *Journal of Agricultural and Food Chemistry*, *59*(21): 11509-11521.

Webster, C. A., & Jones, O. P. (1991). Micropropagation of some cold-hardy dwarfing rootstocks for apple. *Journal of Horticultural Science*, *66*: 1-6.

How to cite this article: Lal, M., Jamwal, M., Bakshi, P., Jasrotia, A. Sharma, N., Sharma, M., Singh, P., Sharma, S. and Kumar, S. (2021). Influence of Antioxidants on *in vitro* Culture Establishment of Clonal Apple Rootstocks. *Biological Forum – An International Journal*, *13*(2): 381-385.