Micropropagation of two apricot × plum inter specific hybrid rootstocks (HS405 and HS706)

Elham Vaghari-Azar[1], Ali Vatanpour-Azghandi[2*], Eslam Majidi-Heravan[2], Jalil Dejampour[3], Ali Akbar Habashi[2]

[1]Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Agriculture Biotechnology Research Institute of Iran (ABRII), P. O. Box: 31535-1897, Karaj, Iran.
[3]Agriculture and Natural Resources Research Center, Sahand Horticulture Station, Tabriz, Iran.
**Corresponding author, E-mail: avazghandi@yahoo.com. Tel/Fax: +98 26 34656023.*

Abstract

Several new superior apricot × plum (*Prunus armenica* × *P. domestica*) inter specific hybrid rootstocks have been recently produced in Iran, but the propagation of these hybrids by conventional means has been shown to be very difficult. *In vitro* propagation could be a reliable method for mass clonal propagation of these new rootstocks. In this study, *in vitro* establishment, proliferation and rooting of two apricot × plum inter specific hybrid root stocks namely HS405 and HS706 were evaluated. For disinfection, different concentrations of ethanol, sodium hypochlorite and mercuric chloride were tested. The highest number of active buds was obtained from spring season nodal explants in hormone-free WPM medium. Addition of 200 mg/l Na-Cefotaxime or 100 mg/l nano silver had a positive effect on controlling indigenous contaminations. The greatest shoot proliferation was recorded by using 4 mg/l BAP + 0.5 mg/l GA_3 supplemented in the WPM medium. Addition of 2 mg/l GA_3 to the culture medium improved shoot elongation, significantly. The best results were obtained for rooting when micro shoots were dipped in a 1000 mg/l IBA solution for 20 seconds and cultured on hormone-free WPM.

***Keywords*:** Tissue culture, *Prunus*, Proliferation, Inter specific hybrid, Rootstocks.

INTRODUCTION

One of the most widely used clonal rootstocks for peach is GF 677 (*Prunus amygdalus* × *Prunus persica*) (Webster, 1995). However, it is quite sensitive to low temperatures during winter (Balla and Kirilla, 2006). This has been the reason for the breeding of new interspecific hybrid rootstocks. Researchers have selected new individual hybrids with more desirable characteristics as alternative to GF-677. For instance, the PR 204/84 rootstock which is a hybrid between peach and almond, selected in the Pomology Institute of Greece by Tsipouridis (2003) adapts well in low fertility soils, arid or semi arid regions, as well as in calcareous soils with a greater resistance to *Phytopthora*, *Verticillium dahliae* and *Agrobacterium tumefaciens,* in comparison to GF-677.

Several new superior inter specific hybrid rootstocks of apricot × plum, peach × almond and plum × almond were developed and selected by Dejampour and his co-workers in Sahand Horticulture Research Station, East Azerbaijan Agriculture and Natural Resources Research Center, Tabriz, Iran during 1999 to 2002 as alternative rootstocks to GF-677 (Dejampour, 2006). In spite of their desirable horticultural characteristics, the propagation of these hybrids using seeds is almost impossible and vegetative propagation by conventional methods of cutting or grafting often is associated with

many difficulties, due to some physiological disorders such as a low rooting ability (Dejampour *et al*., 2007). Therefore, developing protocols for *in vitro* clonal propagation of these hybrids has been focused as an efficient alternative method for their mass propagation.

The micropropagation of *Prunus* rootstocks and its various interspecific hybrids such as peach × almond, apricot × almond, plum × almond and apricot × plum has been reported previously (Fotopoulos and Sotiropoulos, 2005; Martinelli, 2005; Espinosa *et al*., 2006; Dejampour *et al*., 2007; Kalinina and Brown, 2007). Battistini and De Paoli (2002) reported that although their nursery produces all *Prunus* rootstocks by micropropagation but each clone needs specific experimentation to balance the components in the medium to optimize the shoot proliferation and rooting, and to improve the acclimatization process. Phytosanitary has also been a great concern in the micropropagation processes (Balla and Kirilla, 2006).

Although *in vitro* propagation can be a reliable method for mass clonal propagation of healthy materials from new rootstocks, it has not been attempted on the new desired interspecific hybrid rootstocks of apricot × plum, HS405 and HS706, so far. In this study, *in vitro* establishment, shoot proliferation and rooting of these two hybrids are reported.

MATERIALS AND METHODS

Plant materials

Plant materials were collected during dormant (December) and actively growing (April-July) seasons of 2007 and 2008, from 5-7 year-old trees grown in Sahand Horticulture Research Station, Tabriz, Iran. Experiments were conducted at the Department of Plant Biotechnology, Science and Research Branch, Islamic Azad University, Tehran, Iran, during 2008 and 2009. Axillary node explants were surface sterilized by immersion in 70% (v/v) ethanol for 30 seconds, followed by immersion in 2.5% (v/v) sodium hypochlorite for 7, 8, 10 and 12 min depending on the status of explant tissues and then rinsed three times with sterile distilled water. In some cases, hardwood explants were further sterilized for 5 min in mercuric chloride (0.8 g/l).

Culture establishment

In the culture establishment stage, the effects of sampling date (spring, summer and autumn), genotype (HS405 and HS706 hybrids), culture media (MS, Murashige and Skoog (1962) and WPM, McCown and Sellmer (1987) and different concentrations of BAP (0, 1.0 and 2 mg/l) and 0.05 mg/l NAA were evaluated on the percentage of active buds and bud growing quality index.

To break physiological bud dormancy, winter explants were either treated by GA_3 by two methods: 1) the addition of 1 mg/l GA_3 in the culture medium and 2) dipping the explants in 10 mg/l GA_3 solution for 5-7 min before culturing or a chilling treatment of the shoots for 2 weeks at 4ºC before the preparation of explants for culturing. After four weeks, the percentages of active buds were recorded and a scoring index system (1-4) was used for the determination of growth quality.

Suppressing internal contamination using antibiotic and nano silver

To suppress bacterial contaminations during culture establishment, addition of 200 or 250 mg/l Na-cefotaxime and 100 mg/l nano silver into the culture media were tested.

Shoot proliferation

WPM medium supplemented with BAP (0, 1.0, 2.0 and 4.0 mg/l) + 0.5 mg/l GA_3 was used in the proliferation stage. The effect of different concentrations of GA_3 (0, 1.0 and 2.0 mg/l) + 2.0 mg/l BAP added to this medium was also evaluated on shoot elongation. After four weeks, the number of shoots, shoot length, shoot quality index and leaf chlorosis index were recorded.

In vitro rooting

Micro shoots were cultured on the WPM medium with and without activated charcoal supplemented with IBA by two methods: 1) IBA in 5 concentrations (0, 1.0, 1.5, 2.0 and 4.0 mg/l) added into the culture medium, and 2) the bottom of micro shoots dipped in (0, 500, 750 and 1000 mg/l IBA) for 20 seconds and transferred to the hormone-free WPM supplemented with 3 gr/l active charcoal for the first 3 weeks and then transferred into the same medium without charcoal for the next 3 weeks.

All culture media were supplemented with 3% (w/v) sucrose and 0.7% (w/v) agar and pH was adjusted to 5.7 before the addition of agar and the autoclaving process. Cultures were maintained in a growth room at 24±1ºC and a 16-h photoperiod under illumination created by white fluorescent lights.

The experiments were set up as completely randomized designs (CRD) or separate factorials based CRD, with at least 5 replications each containing 4-5

explants. Data were analyzed using SAS software and means were compared by Duncan's multiple Range Test at alpha=0.05.

RESULTS

Culture establishment

The effects of sampling date, genotype, culture media and different concentrations of BAP were significantly different on the percentage of active buds and bud growing quality index (Table 1). The highest percentage of active buds and bud quality index were obtained from explants collected in the spring season. HS405 genotype had a higher number of active buds compared to HS706. WPM medium was superior to MS in the establishment stage. The highest percentage of active buds was obtained in the hormone-free medium (Figure 1). For breaking dormancy in winter-collected explants, the addition of 1 mg/l GA_3 in the culture medium was beneficial, (Table 2). Short term chilling of the shoots at 4ºC for 2 weeks was also effective in breaking buds dormancy (data are not shown).

Suppressing internal contamination

The genotype HS706 showed more indigenous bacterial contaminations compared to HS405 (Table 3). The addition of Na-cefotaxime or nano silver into the culture medium led to controlling contamination, completely (Table 3). Using 200 mg/l Na-cefotaxime in the culture medium led to higher growth quality index compared to 250 mg/l Na-cefotaxime.

Shoot proliferation

Shoot growth and proliferation were affected by BAP concentrations, significantly (Table 4). The best treatment for shoot proliferation was 4 mg/l BAP + 0.5 mg/l GA_3 (Figure 2). Genotype HS706 showed a higher shoot length and quality index compared to HS405 (Table 4). The addition of GA_3 to the culture medium influenced shoot length, quality and chlorosis indices, significantly (Table 5).

In vitro rooting

In rooting phase, micro shoots were first dipped in the rooting solution containing different concentrations of IBA and cultured in the WPM supplemented with active charcoal. After three weeks, micro shoots were transferred into the culture medium without activated charcoal. The best results were obtained in 1000 mg/l IBA treatment (Figure 3).

DISCUSSION

In the present study, sampling date, genotype, culture media and different concentrations of BAP affected culture establishment. Spring-season nodal explants produced a higher number of active buds which also grew better. This is in consistence with the results reported by Dejampour *et al*., (2007) on interspecific hybrids of peach × almond and apricot × plum. Winter-collected explants showed low responses to culture establishment, probably due to dormancy. For breaking their dormancy, the addition of 1 mg/l GA_3 in the cul-

Table 1. Effects of sampling dates, genotypes, culture media and BAP concentrations on culture establishment of apricot × plum hybrid rootstocks.

Treatment		Internal infection	Active buds (%)	Quality index*
Sampling date	Spring	0.25^{a}	81.30^{a}	2.36^{a}
	Summer	0.13^{b}	61.53^{b}	2.15^{a}
	Autumn	0.22^{ab}	47.57^{c}	1.71^{b}
Genotype	HS405	0.18^{a}	74.23^{a}	2.25^{a}
	HS706	0.23^{a}	52.31^{b}	1.89^{b}
Culture medium	MS	0.18^{a}	54.70^{b}	1.86^{b}
	WPM	0.23^{a}	74.30^{a}	2.33^{a}
BAP Concentration (mg/l)	0	0.22^{a}	69.23^{a}	2.14^{a}
	1	0.20^{a}	57.27^{b}	1.93^{b}
	2	0.19^{a}	65.48^{ab}	2.17^{a}

Means with similar characters in each column for each factor show no significant differences (Duncan's multiple range test, α=0.05). *Quality index 1: weak; 2: medium; 3: good and 4: excellent.

Table 2. Effect of GA_3 on breaking dormancy of winter apricot × plum hybrid rootstocks.

GA_3 (mg/l)	Active buds (%)
0.0	0.00[b]
1.0	40.62[a]
10.0	9.37[b]

Means with similar characters show no significant differences (Duncan's multiple range test, α=0.05).

Table 3. Effect of antibiotic and nano silver on *in vitro* growth of apricot × plum hybrid rootstocks.

Treatment		Internal infection	Quality index*	Shoot necrosis (%)
Genotype	HS405	0.00[b]	2.55[a]	15.62[a]
	HS706	0.25[a]	2.41[a]	24.59[a]
Antibiotic or Nano silver (mg/l)	0 (control)	0.09[a]	2.57[ab]	17.85[ab]
	nano silver 100	0.00[b]	2.61[a]	33.33[a]
	Na-cefotaxime 200	0.00[b]	2.56[ab]	8.33[b]
	Na-cefotaxime 250	0.00[b]	2.20[b]	23.52[ab]

Means with similar characters in each column for each factor show no significant differences (Duncan's multiple range test, α=0.05). *Quality index 1: weak; 2: medium; 3: good and 4: excellent.

Table 4. Effects of genotypes and BAP concentrations on shoot proliferation of apricot × plum hybrid rootstocks.

Treatment		Shoot number	Shoot length (mm)	Quality index*	Chlorosis index**
Genotype	HS405	1.44[a]	5.22[b]	2.49[b]	1.33[a]
	HS706	1.16[a]	7.71[a]	2.81[a]	1.14[a]
BAP (mg/l)	0	1.33[b]	5.15[b]	2.31[b]	1.08[b]
	1	1.16[b]	3.87[b]	2.39[b]	1.70[a]
	2	1.40[b]	6.35[ab]	2.65[b]	1.09[b]
	4	1.69[a]	9.69[a]	3.08[a]	1.08[b]

Means with similar characters in each column for each treatment show no significant differences (Duncan's multiple range test, α=0.05). *Quality index 1: weak; 2: medium; 3: good and 4: excellent. **Chlorosis index 1: no chlorosis; 2: mild chlorosis; 3: high chlorosis.

Table 5. Effect of different GA_3 concentrations on shoot elongation of apricot × plum hybrid rootstocks.

GA_3 (mg/l)	Shoot number	Shoot length (mm)	Quality index*	Chlorosis index**
0	1.25[a]	4.09[b]	2.06[c]	1.38[a]
1	1.10[a]	5.95[b]	2.45[b]	1.38[a]
2	1.39[a]	10.46[a]	3.04[a]	1.04[b]

Means with similar characters in each column for each treatment show no significant differences (Duncan's multiple range test, α=0.05). *Quality index 1: weak; 2: medium; 3: good and 4: excellent. **Chlorosis index 1: no chlorosis; 2: mild chlorosis; 3: high chlorosis.

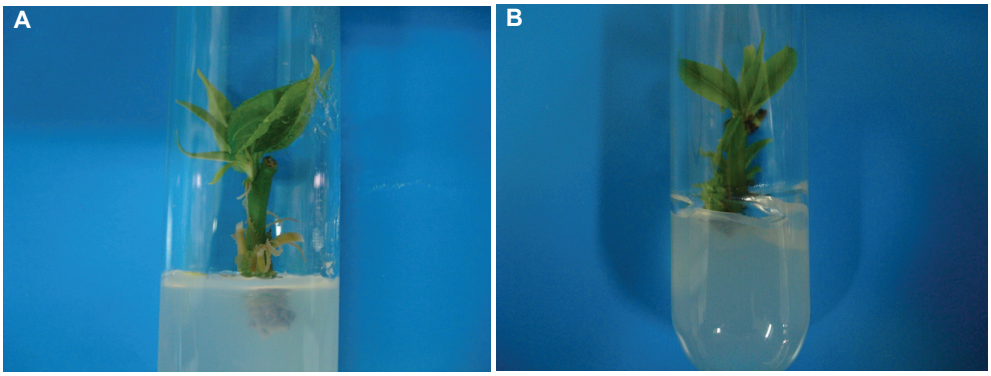

Figure 1. Establishment stage of two apricot × plum genotypes, HS706 **(A**) and HS405 **(B)** on WPM medium without BAP.

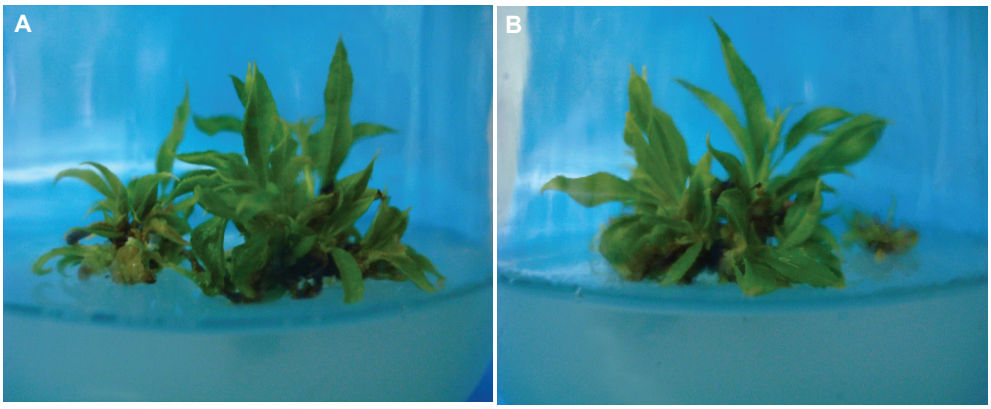

Figure 2. Shoot proliferation of two apricot × plum genotypes (HS405 and HS706) cultured on WPM containing 4 mg/l BAP + 0.5 mg/l GA_3. HS706 **(A)** and HS405 **(B)**.

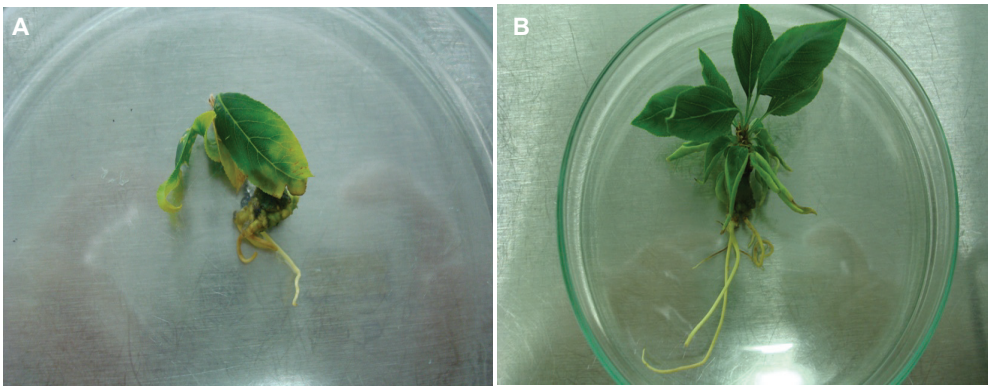

Figure 3. Shoot rooting of two apricot × plum genotypes cultured on woody plant medium. HS405 **(A)** and HS706 **(B)**.

ture medium or a short-term chilling period (2 weeks at 4ºC) before culture was beneficial but still their growth and development was less than spring season explants. Koubouris and Vasilakakis (2006), reported that chilling of explants for 300 h at 4ºC, significantly increased the number of new shoots in apricot.

In the present work, WPM medium was found superior in the establishment stage. Murai *et al*., (1997) reported that in propagation of apricot cultivars, using MS medium caused shoot chlorosis and a low explant survival. According to Perez-Tornero and Burgos (2000), MS medium was not a suitable medium for the propagation of apricot due to its high concentration of NH_4NO_3 compared to WPM and QL medium. However, for the establishment of almond, Gural and Gulsen (1998) used hormone-free MS.

The two genotypes studied showed different indigenous bacterial contamination rates. Na-cefotaxime or nano silver added into the culture media were able to control contamination completely. Koubouris and Vasilakakis (2006), reported that 250 mg/l Na-cefotaxime in the micropropagation of apricot reduced bacterial contamination significantly without any effect on the proliferation rate. Interestingly, in our study, when 100 mg/l nano silver was added into the culture medium, not only the contaminations were completely controlled but also it had a positive effect on shoot growth and quality. Similar to the findings reported previously by Orlikowska (1997), and Abdi *et al*., (2008), our results also showed that nano silver could have a good potential for disinfecting plant tissues from bacterial contaminations.

The best treatment for shoot proliferation was 4 mg/l BAP. Genotype HS706 had a higher shoot length and a quality index compared to HS405. Addition of GA_3 to the culture medium showed a positive influence on shoot length, quality and chlorosis indices, confirming the results reported by Dejampour *et al*., (2007) on peach × almond and apricot × plump inter specific hybrids. Morini and Perrone (2006), obtained the best result for the shoot proliferation of apple rootstock MM106 on MS medium supplemented with 3 mg/l BAP. Multiple shoot formation in nine ornamental *Prunus* species was obtained using 1 mg/l BAP (Kalinina and Brown, 2007).

In the present work, microshoots rooted only when dipped in a 1000 mg/l IBA solution and cultured in the WPM supplemented with 3 gr/l active charcoal for the first 3 weeks and re-cultured in the same medium without charcoal for another 3 weeks. This is in agreement with the results reported by Cos *et al*., (2004) on peach × almond interspecific hybrid cv. Mayor. According to Fotopoulos and Sotiropoulos (2005), the reduction of the mineral concentration of MS medium to half the normal value increased the rooting percentage of PR 204/84 explants (peach × almond hybrids). Increasing IBA concentration from 0 to 10 µM, increased the mean root number per shoot in both media (full and half strength). The mean length of roots was not significantly affected by IBA and mineral concentration of the culture media.

Kalinina and Brown (2007), reported that rooting and acclimatization conditions were improved for GF305 peach using a two-step protocol with a 4 day root induction in IBA-containing media with consequent 3-week root elongation in IBA-free media. One-month incubation of rooted shoots in a vermiculite-based medium resulted in additional shoot and root growth and provided better acclimatization and plant recovery.

Balla and Kirilla (2006), reported that following a nice growth during the multiplication and elongation phases, difficulties arose during the rooting phase and large differences were found in the nutrient demand of the rooting phase. In spite of the high percentage of rooting, widespread shoot tip necrosis was detected and optimal levels of different macroelements, iron and sugar had to be determined in a series of experiments for the clonal rootstock cultivars.

In conclusion, *in vitro* propagation of two apricot × plum interspecific hybrids; namely HS405 and HS706 were investigated for the first time. To achieve an efficient protocol for the mass propagation of these hybrids, further experiments especially in the rooting stage need to be done before any commercialization.

ACKNOWLEDGEMENT

Thanks are due to the Science and Research Branch, Islamic Azad University for their financial support and Department of Tissue Culture and Gene Transformation of Agricultural Biotechnology Research Institute of Iran (ABRII) for their help and cooperation.

REFERENCES

Abdi G., Salehi H., and Khosh-Khui M. (2008). Nano silver: a novel nano material for removal of bacterial contaminants valerian (*Valerian officinalis* L.) tissue culture. *Acta Physiologiae Plantarum,* 30: 709-714.

Balla I., and Kirilla Z. (2006). Micropropagation of peach rootstocks and cultivars. *Acta Horticultural.* (*ISHS)*, 725: 511-516. [Online] Available: http://www.actahort.org/books/725/725_74.htm

Battistini A., and De Paoli G. (2002). Large scale micropropagation of several peach rootstocks. *Acta Horticultural.* (*ISHS)*, 592: 29-33.

Cos J., Frutos D., Garcia R., Rodriguez J., and Garcia J. J. (2004). *In vitro* rooting study of the peach-almond hybrid Mayor. *Acta Horticultural.* (*ISHS)*, 658: 623-627.

Dejampour J. (2006). Evaluation of micropropagation ability and salinity tolerance of some *Prunus* interspecific hybrid rootstocks. PhD Thesis, College of Agriculture, Tabriz University, Iran. (Persian).

Dejampour J., Grigourian V., Majidi E., and Asgharzade N. (2007). Sterilization, establishment and proliferation of some *Prunus* interspecific hybrids for *in vitro* culture. *Journal of Horticultural Science Technology,* 8: 165-174. (Persian).

Espinosa A. C., Pijut P. M., and Michler C. H. (2006). Adventitious shoot regeneration and rooting of *Prunus serotina in vitro* cultures. *HortScience,* 4: 193-201.

Fotopoulos S., and Sotiropoulos T. E. (2005). *In vitro* rooting of PR 204/84 rootstock (*Prunus persica* × *P. amygdalus*) as influenced by mineral concentration of the culture medium and exposure to darkness for a period. *Agronomy Research,* 3: 3-8.

Gurel S., and Gulsen Y.(1998). The effect of IBA and BAP on *in vitro* shoot production of almond (*Amygdalus comminus* L.). Turkish Journal of Botany. 1998, 22: 375-37.

Kalinina A., and Brown D. (2007). Micropropagation of ornamental *Prunus* spp. and GF305 peach, a *Prunus* viral indicator. *Plant Cell Reports*, 26: 927-935.

Koubouris G., and Vasilakakis M. (2006). Improvement of *in vitro* propagation of apricot cultivar "Bebecou". *Plant Cell Tissue and Organ Culture*, 85: 173-180.

McCown B. H., and Sellmer J. C. (1987). General media and vessels suitable for woody plant culture. In: Bonga J.M., and Durzan D.J. (Eds.), *Cell and Tissue Culture in Forestry, Vol 1, General Principles and Biotechnology*, (Pp. 4-16). Martinus Nijhoff Publishers.

Martinelli A. (2005). Factors affecting *in vitro* propagation of the peach-almond hybrids "Hansen 2168" and "Hansen 538". *Acta Horticultural*, 173: 237-244.

Morini S., and Perrone S. (2006). Effect of short light-dark regimes on *in vitro* shoot rooting of some fruit tree rootstocks. *Biologia Plantarum*, 50: 429-432.

Murai Y., Harada H., and Yamashita H. (1997). *In vitro* propagation of apricot (*Prunus armeniaca* L.) cv. 'Bakuoh junkyou'. *Journal of the Japanese Society for Horticultural Science*, 66: 475-480.

Murashige T., and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum,* 15: 473-497.

Orlikowska T. (1997). Silver nitrate inhibits bacterial growth in plant tissue culture. *Agricultural cell Reports*, 29: 25-35.

Perez-Tornero O., and Burgos L. (2000). Different media requirement for micropropagation of apricot cultivars. *Plant Cell Tissue and Organ Culture*, 63: 133-14.

Tsipouridis K. (2003). Annual report of the Pomology Institute of Naoussa. Pomology Institute, Greece. Pp. 24-29

Webster A. D. (1995). Temperate fruit tree rootstock propagation. *New Zealand Journal of Crop and Horticultural Science*, 23: 355-372.