African Journal of Biotechnology Vol. 10(24), pp. 4851-4859, 6 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1456
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

The effects of paclobutrazol and daminozide on *in vitro* micropropagation of some apple (*Malus domestica*) cultivars and M9-rootstock

Kahraman Kepenek* and Zuhal Karoğlu

Department of Agricultural Biotechnology, Faculty of Agriculture, Süleyman Demirel University, Isparta-Turkey.

Accepted 4 May, 2011

This study was carried out to determine the effects of PP 333 and Alar-85 containing 0.0, 0.5, 1.0, 2.5, and 5.0 ppm on the shoot image density, root image density, primer root number, rooting percentages, adventitious shoot numbers, shoot length and primer root length of apple rootstock-M9 and two apple cultivars (Starking Delicious and Amasya) in *in vitro* conditions. *In vitro* propagation of M9, Starking Delicious and Amasya was made on modified MS (Murashige and Skoog) medium supplemented with growth regulators either in a multiplication medium containing agar solidified MS basal composition and 0.5 ppm BAP with 0.1 ppm IBA, or in rooting medium: rotated liquid 1/2 MS and 0.5 ppm IAA, and PP 333 or Alar-85. Subculturing the shoots on modified MS medium supplemented with PP 333 (0.5 to 1.0 ppm) or Alar-85 (0.5 to 2.5 ppm) induced shoot image density, root image density, primer root number, rooting percentages, and adventitious shoot numbers while inhibiting the length of shoots and primer root, in every 5 weeks from a single shoot tip or adventitious bud, with best shoot extension and leaf growth. One ppm PP 333 and 2.5 ppm Alar-85 favoured the development of adventitious shoots or buds, and rooting in isolated internodes of the development shoots. Especially, it appears that rooting and adventitious shoot numbers of Starking Delicious and Amasya were more than M9. The PP 333 and Alar-85 threshold values were low at < 1.0 and < 2.5 ppm, respectively. The thresholds of PP 333 at 1.0 ppm were 59.2% (Starking Delicious), 48.6% (Amasya) and 32.5% (M9), and thresholds of Alar-85 at 2.5 ppm were 50.4% (Starking Delicious), 41.3% (Amasya), and 35.7% (M9) for rooting percentages. The thresholds of the proliferation and rooting coefficients of M9 were significantly lower than those of other cultivars. M9 gave the poorest response to the treatments.

Key words: *Malus* spp., micropropagation, plant growth regulators, tissue culture.

INTRODUCTION

Many woody plants, economically important for timber and/or fruit production, are often difficult to regenerate shoots and roots, both with conventional and *in vitro* propagation techniques. In some cases, it is possible to improve *in vitro* shoot multiplication and rooting with growth regulator applications, etiolation, and use of polyamines (Ziv and Ariel, 1992) or *Agrobacterium rhizogenes* (Patena et al., 1988; Masuda et al., 1993).

The traditional method of propagation of apple consists of budding or grafting the apple scion cultivar onto clonal rootstocks raised by stooling or layering. But, there is a considerable potential for *in vitro* propagation of fruit trees for nursery tree production. Apple rootstocks and well-known cultivars of fruitting apples, can all be propagated rapidly by tissue culture. However, the reported results (Jones, 1976; Boxus and Quorin, 1977; Lane, 1978; Boxus and Druart, 1980; Zimmerman, 1980; Cheema and Sharma, 1981) with tissue culture initiation of apple cultivars present major difficulties. The most frequent problem is the shoot and root induction (Skirvin et al., 1986; Damiano et al., 1991). As it has been difficult to

*Corresponding author. E-mail: kkepenek@ziraat.sdu.edu.tr. Tel: + 90(246)2114660. Fax: +90(246) 2371693.

Abrrevations: 2,4-D, 2,4-Dichlorophenoxyacetic acid.

obtain a high proportion of rooted and established plantlets from many *Malus* genotypes, a great deal of the research on the micropropagation of this genus has been concentrated on rooting and its physiology. Chen et al. (1979) reported the induction of organogenesis in M9 callus, but calli subcultured, once failed to produce any shoot; an observation which has been repeatedly confirmed in short term experiments with rootstock-M9 cultures. A dependable system of inducing organization of shoots in basal internodes and/or their callus in apple has been reported by Cheema and Sharma (1981). All the optimisation work of culture media and conditions should be carried out with about one hundred cultivars of *Malus* spp. (Hanzer et al., 1993a and 1993b). Several authors reported successful shoot regeneration and rooting using growth regulators in fruit trees (George, 1996), such as kiwi (Pedroso et al., 1992), walnut (Grusella and Boxus, 1990), almond (Weiss et al., 1993), apple (Laimer et al., 1988a, b, 1991; Marin et al., 1993). Patena et al. (1988) reported that, at first only 8% of shoots produced from *Malus domestica* cv. 'Jonathan' shoot cultures formed roots, but after 9 subcultures, the rooting arose to 95%. By contrast, cv. 'Golden Delicious' shoots produced during the first 18 months of shoot culture rooted well on LS basal medium plus 1 ppm IBA, but then became difficult to root by any method.

Hyperhydric shoots survive very poorly in outside environment. Hyperhydricity is caused by the culture of leafy tissue in liquid media. Ziv (1992) found that the addition of growth retardants to a culture medium can reduce or prevent leaf development, and cause the induction of compact growth. But aggregates or meristemmoides are not vitrescent. The normal inhibitory effect of gibberellins on rooting is emphasized by root promoting activity of 'anti-gibberellin' growth retardants on some plants. Root formation on explants or microcuttings *in vitro* can also be promoted by 'anti-gibberellins', and the effect is additional to that produced by auxins (George, 1996). Researches into growth retardants like paclobutrazol could prove useful to the horticultural industry by enabling the production of previously unavailable and/or unusual species such as ornamental plants or fruit trees. Milandri et al. (2008) found that *Gladiolus tristis* was tested for its potential as a flowering plant, using the growth retardant paclobutrazol. All treatments reduced perpendicular leaf height. The flower spikes of plants treated with 2, 4 and 8 mg active ingredient were only marginally shorter than the control, while the height of plants treated with 16 mg was significantly reduced. El-Sherbini et al. (1988) found that blackberry (*Rubus* sp.) shoots dipped into 10 ppm paclobutrazol subsequently produced greater numbers of roots *in vitro* while *Euonymus kiautschovica* cuttings dipped into 50 or 200 ppm paclobutrazol solutions produced more roots than the control when placed in mist beds. Smith et al. (1990) found that *Chrysanthemum* sp. plantlets rooted in a medium containing 0.5 to 2.0 ppm paclobutrazol were less liable to wilt on being transplanted to soil than the control. This was attributed to plants having leaves with more epicuticular wax, chlorophyll, and smaller stomata with an improved capacity for closure; and roots with an increased thickness if 1 to 2 ppm paclobutrazol had been used, a reduced stem length, root length and leaf area. Nowello et al. (1992) observed that 1 ppm paclobutrazol applied in the same way to cultured shoots of *Vitis vinifera* caused plantlets to have leaves with a reduced area and bearing smaller stomata than usual. Stem length was also reduced. When roots were formed, they were thicker and more numerous than those on the control shoots. The conductivity of water from the paclobutrazol-treated plants were less than that from untreated plantlets grown under 94% relative humidity. It therefore appeared that paclobutrazol treatment would make plantlets be able to withstand acclimatization better. Ziv (1992) also discovered that adding paclobutrazol to liquid media to induce the proliferation of compact bud clusters or meristemmoides results ultimately to enhance wax formation and normal stomatal function in the plantlets during the hardening stage (Finnie and Van Staden, 1989). Corms were induced to form reliably on shoots of *Gladiolus* sp. by adding paclobutrazol to a shaking liquid medium (Steinitz et al., 1996). By culturing buds of *Gladiolus* sp., *Nerine* sp., *Brodiaea* sp., and *Philodendron* sp. in a liquid medium containing a growth retardant, the explants proliferated and produced large bud aggregates, protocorm-like bodies, or organogenic cell clusters without leaves. Subculture without growth retardants resulted in further proliferation of the bud aggregates, production of plantlets, cormlets, or bulblets. The addition of paclobutrazol or daminozide to the culture medium could increase the rate of shoot multiplication, but all genotypes did not respond to it positively. Ziv (1989) transferred shoot buds of *Gladiolus* cv. 'Eurovision' directly to an agitated liquid version of the Stage I medium supplemented with daminozide (2.5 ppm) or paclobutrazol (1.0 ppm). The explants proliferated into massive bud aggregates or protocorms (with paclobutrazol) under these conditions. The preferred growth retardants were 1.0 to 2.5 ppm paclobutrazol, though similar results were also obtained by Ziv (1992) with 2.5 ppm daminozide. Daminozide however tends to stimulate callus formation. Gmitter and Moore (1986) found that by culturing undeveloped ovules and embryogenic calli of citrus in a medium containing a 0.01 ppm 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1 ppm daminozide (Alar-85) on the embryo formation and plant growth, the addition of 2,4-D and daminozide to the culture medium can increase the rate of embryo production, germination, and plant survival. Kofidis et al. (2008) found that by culturing *Coriandrum sativum* L. in a medium containing a prohexadione-Ca and daminozide were found effective in reducing stem elongation and apparently affected leaf and stem anatomy; the response varied with the concentration used. Generally, retardant-

Table 1. Cultural media used.

Media	Basal composition (ppm)	Growth regulator	Growth regulator composition (ppm)
Beginning medium			
Agar	0.4 %	BAP	1.0
Activated charcoal	1 %	Glutathione	500.0
Sucrose	3 %		
MS*			
Microplant Multiplication Medium			
Multiplication	**Rooting**	**Vitamin (ppm)**	**Multiplication** / **Rooting**
MS (agar solidified)	1/2 MS (in rotated liquid medium) Sucrose: 2 % Activated charcoal: 1%	Nicotinic acid 0.5 Pyiridoxine 0.5 Thiamine 1.0 Inositol 100.0	BAP 0.5 IBA 0.1 / IAA 0.5

MS*, Murashige and Skoog (1974).

treated plants possessed thicker leaves, wider stems with more collenchyma tissue, and more vessels in the vascular bundles.

The aim of this work was to determine the ability of PP333 and Alar-85 concentrations *in vitro* conditions to induce adventitious shoot (shooting) and root (rooting) formation on M9 rootstock, Amasya and Starking Delicious cultivars, both recalcitrant and easy-to-root, in the presence of rooting substances; and to evaluate the nature of these adventitious shoots and roots to achieve a successful transfer to the field.

MATERIALS AND METHODS

Conventional propagated plants were used for the experiments on the M9 apple rootstocks, and the Amasya and Starking Delicious cultivars. Actively growing apical domes were taken in mid-spring or in the beginning of summer. These explants were placed in running water for 1 h before surface sterilisation. After they were cleaned and disinfected (sterilisation: 30 min, 10% NaOCl), the explants were dissected to 2 to 3 mm and placed the upper and lateral position on a MS medium (Hutchinson, 1985). One ppm BA and 500 ppm glutathione was added to the medium. Explants were covered for 24 h with 200 µl 8-HQS, and then moved to a fresh medium. After 2 weeks, the explants were transferred to a new medium containing both growth regulators (BAP, IBA, IAA) and Paclobutrazol (PP333) or Daminozide (Alar-85). The microplants were multiplied on MS supplemented with growth regulators (for multiplication medium: with agar solidified MS basal composition with 0.5 ppm BAP and 0.1 ppm IBA; for rooting medium: in rotated liquid 1/2 MS, 2% sucrose, 1% activated charcoal (AC), 0.5 ppm IAA, and growth retardants (PP333 or Alar-85)) as reported in Table 1. The pH of the medium was adjusted to 5.8 with 1N HCl or 1N NaOH before adding agar (0.4%) and autoclaving. The medium was sterilized at 120°C and 1.1 kg/cm^2 pressure for 15 min. 25 ml medium was dispensed into 25 x 150 mm test tubes.

Green healthy apple microcuttings were prepared after subculturing for five weeks. The most homogeneous shoots were selected, and their base was neatly cut. The test propagules were selected for uniform size and single shoots. After explants were dissected to 2 to 3 mm and placed the upper position on a MS medium, and covered for 24 h with 200 µl of a 0.1% solution of 8-HQS, the explants were transferred to the appropriate co-cultivation shoot and root induction medium containing growth regulators. Finally, the microcuttings were transferred to the same medium containing PP333 (0.0, 0.5, 1.0, 2.5, 5.0) or Alar-85 (0.0, 0.5, 1.0, 2.5, 5.0 ppm). The microcuttings (shoots 2.0 to 2.5 cm in length) were cultured in darkness (Norton and Boe, 1982; Zimmerman and Fordham, 1985; Webster and Jones, 1989a, b) for ten days before being exposed to light. Some M9, Amasya and Starking Delicious explants were maintained with the basal part in darkness before the exposure to light.

All the cultures were kept in an environmentally controlled growing room maintained at 21 ± 2°C. The source of light consisted of four 120 cm white fluorescent tubes (40 W) and an incandescent lamp (25 W, 100 W). The intensity of light at the level of cultures was 3000 to 3500 lux. The light regime consisted of 16 h light/8 h darkness. The apple microcuttings were incubated in the dark during stage-II culture for two weeks on shoot multiplication medium. The shoot microcutting cultures were placed in polyethylene bags (covered with aluminium foil) for two weeks after which the cultures were kept for two weeks in the light so that the shoots became green. After three weeks on the rooting medium, the plantlets were transferred from the propagation temperatures of 21 ± 2°C to a low temperature condition at 10 ± 2°C for another 8 weeks on the same medium.

Rooted plantlets with shoots 2.5 cm in length and with dormant bud like structure characteristics were carefully washed off, all adherents and potted (plastic pots: Ø 8 cm) in sterilized soil mixture composed of 1:2 (v:v) peat (Yeniçağ, Bolu, Turkey): agro perlite (Kunneman and Albert, 1992) and were continuously irrigated to supply urea or ammonium nitrate, potassium dihydroxide orthophosphate (200 ppm of NPK), and minor and trace elements of MS (Murashige, 1974) and was then transferred in tunnelled mist beds under glasshouse.

The subjective image density of apple shoot microcutting categories were established using root and shoot image density scores obtained previously. Scores were generated as 1.0 to 2.0 for relatively poor, 3.0 for intermediate, 4.0 for good, and 5.0 for a very good with a possible range of 1.0 to 5.0. All determinations were conducted as triplicates.

The rate of shoot multiplication was determined by counting the number of shoots produced having a medium shoot length between 2 mm and 2.0 to 2.5 cm. There were three replicates with 16 shoots per plot for each cultivar. The responses of a minimum of three subcultures on the same medium were taken into consideration. Data were recorded after 35 days from the start of the experiment.

Table 2. Effect of different Alar-85 and PP333 doses on rooting percentage (%) of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking delicious (% ± S.E.)		Amasya (% ± S.E.)		M9 (% ± S.E.)		Average of growth retardant (% ± S.E.)	
	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333
0.0	47.7 ± 0.9 (0.00)	56.4 ± 1.1 (0.00)	42.6 ± 1.1 (0.00)	47.5 ± 1.0 (0.00)	34.7 ± 1.1 (0.00)	30.7 ± 0.9 (0.00)	41.6 ± 6.5 (0.00)	44.8 ± 11.1 (0.00)
0.5	47.8 ± 0.90 (+0.20)	58.4 ± 1.3 (+3.54)	43.4 ± 0.8 (+1.87)	48.4 ± 1.3 (+1.89)	35.4 ± 1.3 (+2.01)	31.3 ± 1.1 (+1.95)	42.2 ± 6.4 (+1.44)	46.0 ± 11.6 (+2.67)
1.0	49.3 ± 1.1 (+3.35)	59.2 ± 1.8 (+4.96)	43.7 ± 1.1 (+2.58)	48.6 ± 1.1 (+2.31)	35.5 ± 1.3 (+2.30)	32.5 ± 1.0 (+5.86)	42.8 ± 6.7 (+2.88)	46.7 ± 11.6 (+4.24)
2.5	50.4 ± 1.0 (+5.66)	54.6 ± 1.3 (-3.19)	41.3 ± 0.8 (-3.05)	48.2 ± 1.6 (+1.47)	35.7 ± 1.2 (+2.88)	30.7 ± 0.9 (0.00)	42.4 ± 6.9 (+1.92)	44.5 ± 10.4 (-0.66)
5.0	45.6 ± 1.3 (-4.40)	53.5 ± 1.2 (-5.14)	38.6 ± 0.9 (-9.38)	40.6 ± 0.8 (-14.52)	33.6 ± 0.9 (-3.17)	28.7 ± 1.2 (-6.51)	39.2 ± 5.2 (-5.76)	40.3 ± 10.8 (-10.04)
Average of cultivar	48.1 ± 1.9 (+0.83)	56.4 ± 2.5 (0.00)	41.9 ± 2.1 (-1.64)	46.6 ± 3.3 (-1.89)	35.0 ± 1.2 (+0.86)	30.8 ± 1.58 (+0.32)	41.6[b] (0.00)	44.4[a] (-0.89)

Mean separation within columns was by Duncan's multiple range at 0.05 levels. (±), value % compared to the control. treatment.

Table 3. Effect of different Alar-85 and PP333 doses on length of shoots (mm/shoot) of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking Delicious (mm/shoot ± S.E.)		Amasya (mm/shoot ± S.E.)		M9 (mm/shoot ± S.E.)		Average of growth retardant (mm/shoot ± S.E.)	
	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333
0.0	27 ± 1 (0.00)	24 ± 1 (0.00)	17 ± 1 (0.00)	19 ± 1 (0.00)	14 ± 1 (0.00)	13 ± 1 (0.00)	19.3 ± 5.7 (0.00)	18.6 ± 4.8 (0.00)
0.5	25 ± 1 (-7.40)	22 ± 1 (-8.33)	15 ± 1 (-11.76)	16 ± 1 (-15.78)	12 ± 1 (-14.28)	12 ± 1 (-7.69)	17.3 ± 5.8 (-10.36)	16.6 ± 4.5 (-10.75)
1.0	21 ± 1 (-22.22)	16 ± 1 (-33.33)	10 ± 1 (-41.17)	9 ± 1 (-52.63)	9 ± 1 (-35.71)	10 ± 1 (-23.07)	13.3 ± 6.0 (-31.08)	11.6 ± 3.12 (-37.63)
2.5	17 ± 1 (-37.07)	10 ± 1.5 (-58.33)	7 ± 1 (-58.82)	5 ± 1 (-73.68)	5 ± 1 (-64.28)	6 ± 1 (-53.84)	9.6 ± 6.0 (-50.25)	7.0 ± 2.3 (-62,36)
5.0	9 ± 1 (-66.66)	6 ± 1 (-75.00)	4 ± 1 (-76.47)	3 ± 1 (-84.21)	2 ± 1 (-85.71)	3 ± 1 (-76.92)	5.0 ± 3.3 (-74.09)	4.0 ± 1.5 (-78.49)
Average of cultivar	19.8 ± 6.6 (-26.66)	15.7 ± 7.0 (-34.58)	10.6 ± 5.0 (-37.64)	10.4 ± 6.4 (-45.26)	8.4 ± 4.6 (-68.57)	8.8 ± 3.9 (-32.30)	12.9[a] (-33.16)	11.5[b] (-38.17)

Mean separation within columns was by Duncan's multiple range at 0.05 levels. (±), Value % compared to the control treatment

Data collected were the number of shoots, the number of rooted plants (rooting percentages), the number of primer roots per plant, and lenght of primer roots. Results, expressed as percentages, were normalised according to the formula arcsin before statistical analysis. Data were analysed by the analysis of variance with SAS statistical package programme.

RESULTS AND DISCUSSION

PP333 or Alar-85 applications had effect on shoot image density, root image density, primer root number, rooting percentage, adventitious shoots number, and length of shoots of apple rootstock-M9 and two apple cultivars (Starking Delicious and Amasya) in *in vitro* condition (Tables 2, 3, 4, 5, 6, 7, 8 and 9). 0.5 to 1.0 ppm PP333 or 0.5 to 2.5 ppm Alar-85 increased rooting percentage, adventitious shoot numbers, shoot image density, root image density, and primer root number were more than control (0.0 ppm). The results indicated that rooting percentage, and adventitious shoots number increase depends on cultivars and PP333 or Alar-85 concentrations. According to the scoring of the applications of PP333 or Alar-85, in high PP333 (> 1.0 ppm) or Alar-85 (> 2.5 ppm) concentrations negative correlations were found between PP333 and Alar-85 concentrations. The results were quite similar in M9, Starking Delicious, and

Table 4. Effect of different Alar-85 and PP333 doses on primer root length (mm/shoot) of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking Delicious (mm/shoot ± S.E)		Amasya (mm/shoot ± S.E.)		M9 (mm/shoot ± S.E.)		Average of growth retardant (mm/shoot ± S.E.)	
	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333
0.0	21 ± 1 (0.00)	24 ± 1 (0.00)	28 ± 1 (0.00)	27 ± 1 (0.00)	5 ± 1 (0.00)	7 ± 1 (0.00)	18.0 ± 3.4 (0.00)	18.6 ± 3.1 (0.00)
0.5	18 ± 1 (-14.28)	21 ± 1 (-12.50)	25 ± 1 (-10.71)	22 ± 1 (-18.51)	5 ± 1 (0.00)	6 ± 1 (-14.28)	16.0 ± 2.9 (-11.11)	16.3 ± 2.6 (-12.36)
1.0	13 ± 1 (-38.09)	16 ± 1 (-33.33)	21 ± 1 (-25.00)	18 ± 1 (-33.33)	4 ± 1 (-20.00)	6 ± 1 (-14.28)	12.6 ± 2.4 (-30.00)	13.1 ± 1.8 (-29.56)
2.5	9 ± 1 (-57.14)	7 ± 1 (-70.83)	15 ± 1 (-46.42)	8 ± 1 (-70.37)	3 ± 1 (-40.00)	4 ± 1 (-42.85)	9.0 ± 1.7 (-50.00)	6.3 ± 0.7 (-66.12)
5.0	6 ± 1 (-71.42)	5 ± 1 (-79.16)	7 ± 1 (-75.00)	5 ± 1 (-81.48)	2 ± 1 (-60.00)	3 ± 1 (-57.14)	5.0 ± 0.8 (-72.22)	4.3 ± 0.4 (-76.88)
Average of cultivar	13.4 ± 5.7 (-36.19)	14.6 ± 7.8 (-39.16)	19.2 ± 7.8 (-31.42)	16.0 ± 8.6 (-40.74)	3.8 ± 1.6 (-24.00)	5.2 ± 1.8 (-25.71)	12.1 a (-32.77)	11.7 b (-37.09)

Mean separation within columns was by Duncan's multiple range at 0.05 levels. (±), value % compared to the control treatment.

Table 5. Effect of different Alar-85 and PP333 doses on primer root number (number/shoot) of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking Delicious (number/shoot ± S.E.)		Amasya (number/shoot ± S.E.)		M9 (number/shoot ± S.E.)		Average of growth retardant (number/shoot ± S.E.)	
	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333
0.0	8.4 ± 0.8 (0.00)	8.6 ± 1.2 (0.00)	10.4 ± 0.8 (0.00)	10.5 ± 0.9 (0.00)	4.7 ± 0.8 (0.00)	4.6 ± 1.0 (0.00)	7.8 ± 2.6 (0.00)	7.9 ± 2.7 (0.00)
0.5	8.5 ± 0.8 (+1.19)	8.6 ± 1.0 (0.00)	10.5 ± 0.8 (+0.96)	10.4 ± 1.2 (-0.95)	5.5 ± 1.1 (+17.02)	5.6 ± 0.7 (+21.73)	8.1 ± 2.3 (+3.84)	8.2 ± 2.2 (+3.79)
1.0	8.7 ± 0.8 (+3.57)	9.4 ± 1.2 (+9.30)	11.6 ± 1.1 (+11.53)	11.6 ± 0.8 (+10.47)	5.6 ± 1.3 (+19.14)	6.5 ± 1.0 (+41.30)	8.6 ± 2.7 (+10.25)	9.1 ± 2.4 (+15.18)
2.5	9.3 ± 0.7 (+10.71)	9.3 ± 1.0 (+8.13)	11.4 ± 0.6 (+9.61)	11.6 ± 1.1 (+10.47)	6.4 ± 1.3 (+36.17)	6.2 ± 0.8 (+34.78)	9.0 ± 2.4 (+15.38)	9.0 ± 2.4 (+13.92)
5.0	8.6 ± 1.1 (+2.38)	8.4 ± 1.1 (-2.32)	10.5 ± 1.1 (+0.96)	10.5 ± 1.1 (0.00)	5.6 ± 1.1 (+19.14)	5.6 ± 0.7 (+21.73)	8.2 ± 2.3 (+5.12)	8.1 ± 2.2 (+2.53)
Average of cultivar	8.7 ± 0.8 (+3.57)	8.8 ± 1.0 (+2.32)	10.9 ± 0.9 (+4.80)	10.9 ± 1.0 (+3.80)	5.5 ± 1.1 (+17.02)	5.7 ± 1.0 (+23.91)	8.3 a (+6.41)	8.4 a (+6.32)

Mean separation within columns was by Duncan's multiple range at 0.05 levels. (±), value % compared to the control treatment.

Amasya. These results were supported by many other investigators (Davis et al., 1986; Smith et al., 1990; Nowello et al., 1992). In this respect, our results were in agreement with these investigators. Tables 2, 3, 4, 5, 6, 7, 8, and 9 also allow an evaluation on the response of three cultivars to the tissue culture method. While Starking Delicious and Amasya gave responses of the same magnitude, M9 appeared less sensitive to PP333 or Alar-85 treatments, and was more demanding as far as the composition of basal medium was concerned.

The effects of PP333 or Alar-85 on the rooting percentage, adventitious shoot number, shoot length and primer root length of M9, Starking Delicious, and Amasya were found significant. When PP333 concentration in the rooting medium was 1.0 ppm, rooting percentage of M9 was +5.86%. Without 0.5 to 1.0 ppm PP333 or 0.5 to 2.5 ppm Alar-85, rooting ability was reduced. In terms of PP333 concentrations, the rooting percentage decreased and varied from -3.19% at 2.5 ppm to -5.14% at 5.0 ppm for Starking Delicious. One ppm PP333 or 2.5 ppm Alar-85 concentrations were found to be optimal for root initiation, but again this probably relates to differences in media, culture conditions, and the status of the cultures. In this respect, our results are in agreement with Davis et

Table 6. Effect of different Alar-85 and PP333 doses on adventitious shoots number (number/explant) of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking Delicious (number/shoot ± S.E.)		Amasya (number/shoot ± S.E.)		M9 (number/shoot ± S.E.)		Average of growth retardant (number/shoot ± S.E.)	
	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333
0.0	2.5 ± 1.0 (0.00)	2.7 ± 0.9 (0.00)	3.5 ± 0.6 (0.00)	3.7 ± 1.0 (0.00)	2.7 ± 1.1 (0.00)	2.7 ± 1.1 (0.00)	2.9 ± 1.0 (0.00)	3.0 ± 0.8 (0.00)
0.5	2.5 ± 0.6 (0.00)	4.4 ± 0.87 (+62.96)	3.7 ± 0.9 (+5.71)	5.3 ± 1.3 (+43.24)	2.7 ± 0.9 (0.00)	3.5 ± 1.06 (+29.62)	3.0 ± 1.6 (+3.44)	4.4 ± 0.9 (+46.66)
1.0	3.5 ± 0.9 (+40.00)	4.7 ± 1.0 (+74.07)	4.5 ± 0.8 (+28.57)	6.5 ± 1.3 (+75.67)	3.6 ± 0.8 (+33.33)	4.3 ± 1.4 (+59.25)	3.8 ± 1.7 (+31.03)	5.1 ± 1.0 (+70.00)
2.5	3.6 ± 1.1 (+44.00)	4.6 ± 1.0 (+70.37)	5.4 ± 1.0 (+54.28)	5.4 ± 1.2 (+45.94)	4.5 ± 0.8 (+66.66)	4.3 ± 0.8 (+59.25)	4.5 ± 1.2 (+55.17)	4.7 ± 0.9 (+56.66)
5.0	3.5 ± 0.8 (+40.00)	3.7 ± 1.2 (+37.03)	4.5 ± 0.7 (+28.57)	5.4 ± 0.9 (+45.94)	3.5 ± 0.9 (+29.62)	3.4 ± 0.9 (+25.92)	3.8 ± 1.2 (+31.03)	4.1 ± 0.9 (+36.66)
Average of cultivar	3.1 ± 0.9 (+24.00)	4.0 ± 1.1 (+48.14)	4.3 ± 0.9 (+22.85)	5.3 ± 1.3 (+43.24)	3.4 ± 1.0 (+25.92)	3.6 ± 1.1 (+33.33)	3.6 b (+24.13)	4.2 a (+40.00)

Mean separation within columns was by Duncan's multiple range at 0.05 levels. (±), value % compared to the control treatment.

Table 7. Effect of different Alar-85 and PP333 doses on shoot image density of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking Delicious (± S.E)		Amasya (± S.E)		M9 (± S.E)		Average of growth retardant (± S.E)	
	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333
0.0	3.5 ± 0.9	3.7 ± 1.2	4.5 ± 0.9	3.7 ± 0.7	2.6 ± 0.8	2.7 ± 0.8	3.5 ± 0.8	3.3 ± 1.2
0.5	3.7 ± 1.0	4.5 ± 1.0	4.3 ± 1.1	4.4 ± 1.2	2.6 ± 0.8	2.8 ± 1.0	3.5 ± 1.2	3.9 ± 1.2
1.0	4.4 ± 0.8	4.5 ± 1.4	4.5 ± 1.1	4.6 ± 0.7	2.6 ± 0.8	3.3 ± 1.1	3.8 ± 1.1	4.1 ± 1.2
2.5	4.4 ± 0.8	4.6 ± 0.9	4.4 ± 0.8	4.6 ± 0.9	3.4 ± 0.98	3.3 ± 0.9	4.0 ± 0.9	4.1 ± 0.9
5.0	4.3 ± 0.7	4.4 ± 1.2	4.4 ± 1.3	4.6 ± 0.9	2.6 ± 0.9	2.5 ± 0.6	3.7 ± 1.2	3.8 ± 1.3
Average of cultivar	4.1 ± 0.8	4.3 ± 1.0	4.4 ± 0.9	4.4 ± 0.8	2.7 ± 0.8	2.9 ± 0.8	3.7 a	3.8 a

Mean separation within columns was by Duncan's multiple range at 0.05 levels. (±), value % compared to the control treatment.

Table 8. Effect of different Alar-85 and PP333 doses on root image density of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking Delicious (± S.E)		Amasya (± S.E)		M9 (± S.E)		Average of growth retardant (± S.E)	
	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333	Alar-85	PP333
0.0	4.4 ± 1.0	4.3 ± 0.8	3.4 ± 1.0	3.5 ± 1.1	2.5 ± 0.8	2.5 ± 0.9	3.4 ± 1.2	3.4 ± 1.1
0.5	4.5 ± 1.1	4.5 ± 0.9	3.6 ± 0.9	4.3 ± 0.9	2.5 ± 0.8	2.6 ± 1.0	3.5 ± 1.2	3.8 ± 1.1
1.0	4.6 ± 0.9	4.5 ± 0.6	3.6 ± 0.9	4.5 ± 1.0	2.6 ± 0.9	2.7 ± 0.8	3.6 ± 1.2	3.9 ± 1.0
2.5	4.6 ± 0.8	4.5 ± 0.7	3.6 ± 0.8	4.2 ± 0.8	2.7 ± 0.9	2.6 ± 0.8	3.6 ± 1.2	3.7 ± 1.1
5.0	4.1 ± 0.8	4.3 ± 0.7	3.3 ± 0.9	3.5 ± 1.0	2.3 ± 0.9	2.4 ± 1.0	3.2 ± 1.1	3.4 ± 1.1
Average of cultivar	4.4 ± 0.8	4.4 ± 0.6	3.5 ± 0.8	4.0 ± 0.9	2.5 ± 0.7	2.5 ± 0.8	3.4 a	3.6 a

Mean separation within columns was by Duncan's multiple range at 0.05 levels. (±), value % compared to the control treatment.

al. (1986). This experiment on the rooting of shoots in culture indicated that dependable high frequency rooting of shoots can be achieved rapidly in reduced concentration of PP333 or Alar-85. PP333 concentrations were found average in +5.86% (M9), +4.96% (Starking Delicious), +2.35% (Amasya) at 1.0 ppm. It varied from +2.67% at 0.5 ppm to -10.04% at 5.0 ppm depending on the PP333 concentrations. However, at Alar-85

Table 9. Effect of different Alar-85 and PP333 doses on callus image of apple shoot microcuttings for different apple cultivars.

Treatment (ppm)	Starking Delicious				Amasya				M9			
	Alar-85		PP333		Alar-85		PP333		Alar-85		PP333	
	Call. Dev.	Remark	Call. Dev.	Remark	Call. Dev.	Remark	Call. Dev.	Remark	Call. Dev.	Remark	Call. Dev.	Remark
0.0	-	Shoots remain healthy	-	Shoots remain healthy	-	Shoots remain healthy	-	Shoots remain healthy	-	Shoots remain healthy	-	Shoots remain healthy
0.5	-	Same as above	+	Same as above	-	Same as above	-	Same as above	+	Same as above	+	Same as above
1.0	++	25% shoots senesence	+	25% shoots senesence	+	25% shoots senesence	+	25% shoots senesence	+	50% shoots senesence	++	50% shoots senesence
2.5	+++	50% shoots senesence	++	75% shoots senesence	++	50% shoots senesence	+++	50% shoots senesence	++++	75% shoots senesence	+++	75% shoots senesence
5.0	++++	Develop dormant bud like structure on plonged culture	+++	Develop dormant bud like structure on plonged culture	+++	Develop dormant bud like structure on plonged culture	++++	Develop dormant bud like structure on plonged culture	++++	Develop dormant bud like structure on plonged culture	++++	Develop dormant bud like structure on plonged culture

-, nil; + negligible to scarce; ++, good callus; +++, moderate callus; ++++, profuse; Call. Dev., callus development.

concentrations, the root rate were found average at -2.88% (M9), +5.66% (Starking Delicious), and -3.05% (Amasya) at 2.5 ppm, and depending on the Alar-85 concentrations which varied from +1.44% at 0.5 ppm to-5.76% at 5.0 ppm (Table 2).

The behaviour of shoot-tips and the axillary buds was similar to each other. The best proliferative response of multiplication was achieved on microplant multiplication medium [MS (agar solidified), containing 0.5 ppm BAP, 0.1 ppm IBA] and 1 ppm PP333 or 2.5 ppm Alar-85]. The explants obtained with the PP333 or Alar-85 were shorter as shoot length and internode length were sufficiently constant for each cultivar. The linear growth velocity was reduced. The shoot length decrease was observed on microplant multiplication medium. As seen in Table 3, the length of shoots decreased and varied depending on PP333 or Alar-85 concentrations. Significant differences in PP333 or Alar-85 concentrations in the medium were evident for highest concentrations. Higher PP333 or Alar-85 levels inhibited length of shoots more than control. These results were supported by many other investi-gators (Smith et al., 1990; Nowello et al., 1992). Nowello et al. (1992) stated that 1.0 ppm PP333 could result in measurable decrease in shoot length (Table 6).

The primer root length decrease was observed on microplant rooting medium [1/2 MS (in rotated liquid medium), 2% sucrose, 1% activated charcoal containing 0.5 ppm IAA, and 1 ppm PP333 or 2.5 ppm Alar-85]. The primer root length decreased and varied depending on PP333 or Alar-85 concentrations (Table 4). There was a characteristic difference in the quality of rooting and rooted shoots. Significant differences in PP333 or Alar-85 concentrations in the medium were evident for the highest concentrations. Higher PP333 or Alar-85 levels inhibited primer root length more than control. In this respect, our results are parellel with Smith et al. (1990).

In this work, at PP333 or Alar-85 concentrations, primer root number increased. On average, PP333 concen-trations was found as +41.30% (M9), +9.30% (Starking Delicious), and +10.47% (Amasya) at 1.0 ppm. At PP333 concentrations, the primer root number varied from +3.79% at 0.5 ppm to +2.51% at 5.0 ppm. However, on average at Alar-85 concentrations, it was found as +36.17% (M9), +10.71% (Starking Delicious), and +9.61% (Amasya) at 2.5 ppm, and depending on Alar-85 concen-trations, it varied from +3.84% at 0.5 ppm to +5.12% at 5.0 ppm. At all PP333 concentrations, an average of the primer root per cutting for M9 6.5, for Starking Delicious 9.3, and for Amasya 11.6 were initiated. These results are supported by many other investigators (El-Sherbini et al., 1988; Nowello et al., 1992).

In this work, different PP333 or Alar-85 concen-

trations increased adventitious shoot number. On average, PP333 concentrations was found as +59.25% (M9), +74.07% (Starking Delicious), and +75.67% (Amasya) at 1.0 ppm. The adventitious shoot number varied from +46.66% at 0.5 ppm to +36.66% at 5.0 ppm. However, on average, at Alar-85 concentrations was found as +66.66% (M9), +44.00% (Starking Delicious), and +54.28% (Amasya) at 2.5 ppm. Depending on the Alar-85 concentrations, it varied from +3.44% at 0.5 ppm to +31.03% at 5.0 ppm (Table 6). PP333 or Alar-85 at different concentrations supported only 2 to 4 multiplications at lower concen-trations (0.5 ppm). But the shoots were very stunted at higher concentration (5.0 ppm). In this respect, our results are parellel with other investigators. Gmitter and Moore (1986) stated that in a medium containing 0.01 ppm 2,4-D and 0.1 ppm daminozide could result to increase in plant regeneration, embryo production, and growth from undeveloped ovules and embryogenic calli of citrus.

Alar-85 was proved less effective than PP333. Nevertheless, Alar-85 supported callus formation more than that of PP333 in the medium (Table 9). A pre-culture of the apical domes in the beginning of the culture for a period of 6 weeks and later the transfer to the microplant multiplication medium for a period of 8 weeks resulted in about +59.25% (M9) and +75.67% (Amasya) regeneration of shoots from the incipient callus of the apical domes at 1.0 ppm PP333.

The results presented here confirm the feasibility of apple cultivar propagation *in vitro* and also showed that cultivars greatly differed in their responsiveness to the treatments utilized. These differences could result from several factors. One was that the trees used as sources for explants were treated differently before the explants were taken. Jones (1976) used actively growing shoots from the first flush of growth on cold-stored trees whereas the explants used in this study came from actively growing shoots in the orchard. Second, the time elapsed between initiating the cultures and taking shoots for rooting differed considerably. Jones (1976) usually started rooting trials within 6 months of culture establishment. In this experiment, shoots for rooting were taken only from cultures established at least 6 months and often established more than 1 year. Patena et al. (1988) reported that as in other woody plants established from mature tissues, shoots of *Malus* sp. produced from initial subcultures may not root well. The capacity to root will usually improve as subculturing is continued. The addition PP333 or Alar-85 to the medium has been found to improve the efficiency of morphogenetic response in several tissue culture systems (Ziv, 1992; Finnie and Van Staden, 1989; Steinitz et al., 1996). The influence of PP333 or Alar-85 in our system is similar to Ziv (1992) who specifically observed increased massive bud aggregates or protocorms.

From Tables 7 and 8, the values of the test on shoot image density and root image density at 1.0 ppm PP333 or at 2.5 ppm Alar-85 seems to positively influence the linear growth of the explants. The dark treatment increased rooting of all cultivars on which it was tried. However, the etiolated shoots produced weak plants that were particularly difficult to establish, a problem that Anderson (1981) also reported.

Conclusion

In conclusion, it appears that in two apple cultivars (Starking Delicious and Amasya) and rootstock-M9 are highly productive of adventitious shoot number at 1.0 ppm of PP333 or at 2.5 ppm of Alar-85 in this *in vitro* condition. The relatively less effectivity of Alar-85 than PP333 in promoting shoot proliferation was observed. These results indicate that this procedure can be an effective way for adventitious shoot production. According to these results, the effect of PP333 or Alar-85 application on the adventitious shoot production was found advantageous. Although there were variations on cultivars, generally PP333 or Alar-85 caused adventitious shoots development. These results were aslo supported by other investigators (El-Sherbini et al., 1988; Nowello et al., 1992).

REFERENCES

Anderson WC (1981). Etiolation as an aid to rooting. Comb. Proc.Int. Plant Prop. Soc. 31: 138-141.

Boxus PH, Quorin M (1977). Comportment en pepiniere d'arbres fruitieres issus de culture *in vitro*. Acta Hort. 78: 373-378.

Boxus PH, Druart PH (1980). Micropropagation and industrial propagation methods of quality plants true to type and a reasonable price. In: Plant Cell Cultures: Results and Perspectives. Elsevier, Amsterdam. pp. 265-269.

Cheema GS, Sharma DP (1981). *In vitro* propagation of apple. In: International Symposium Plant Cell Culture in Crop Improvement. Bose Institute, Calcutta. Plenum Publ. Co., New York. pp. 309-318.

Chen WL, Yang, Wang HX, Chang C (1979). Organogenesis of Malus (Rootstock M9) callus *in vitro*. Acta Bot. Sinica: 21: 191-194.

Damiano C, Chariotta A, Cabonie E, Quarta R, Boumis G (1991). Some factors affecting the induction and expression of rooting in different fruit species in vitro. Acta Hort. 300: 211-224.

Davis TD, Walser RH, Soerenson K, Sankhla N (1986). Rooting and subsequent growth of cuttings treated with paclobutrazol. Plant Prop. 32(1): 7-9.

El-Sherbini N, Swartz HJ, Gouin F, Stutte G, Bors R (1988). Paclobutrazol-assisted rooting. HortScience, 23: 756.

Finnie , Van Staden J (1989). *In vitro* propagation of Sandersonia and Gloriosa. Plant Cell Tissue Organ Cult. 19: 151-158.

George EF (1996). Plant propagation by tissue culture. In practice. Part: 2. Exegetics Ltd. Edington, Wilts, BA13 4QG, England.

Gmitter FG Jr, GA Moore (1986). Plant regeneration from undeveloped ovules and embryogenic calli of Citrus: embryo production, germination and plant survival. Plant Cell Tissue Organ Cult. 6: 139-147.

Grusella R, Boxus PH (1990). Walnut micropropagation. Acta Hort. 284:45-52.

Hanzer V, Weiss H, Knapp E, Da Câmara Machado A, Katinger H, Laimer da Câmara Machado M (1993a). *In vitro* Methoden zur Erhaltung und Virusfreimachung von Apfelsorten.Tagungsbericht Nutzbarmachung genetischer Resourcen für Züchtung und Landschaftsgestaltung. Pillnitz, pp. 322-326.

Hanzer V, Weiss H, Weiss B, Da Câmara Machado B, Knapp E, Pühringer H, Katinger H, Laimer da Câmara Machado M (1993b). Revitalisation of old Austrian fruit tree cultivars.Institute of Applied Microbiology, University of Agriculture, Vienna/Austria.

Hutchinson JF (1985). Micropropagation of 'Northern Spy' apple rootstock. Comb. Proc. Int. Plant Prop. Soc. 34: 38-48.

Jones OP (1976). Effect of phloridzin and phloroglucinol on apple shoots. Nature, 262: 392-393.

Kofidis G, Giannakoula AF, Ilias I (2008). Growth, anatomy and chlorophyll fluorescence of coriander plants (*Coriandrum sativum* L.) treated with prohexadione-calcium and daminozide. Acta Biologica Cracoviensia Series Botanica 50(2): 55-62.

Kunneman BPAM, Albert MJR (1992). Effect of tissue culture and acclimatization conditions on the survival and growth of rooted and unrooted Malus and Pyrus microcuttings. Acta Hort. 314: 147-154.

Lane DW (1978). Regeneration of apple plants from shoot meristem tips. Plant Sci. Lett. 13: 281-285.

Laimer M, Da Câmara Machado A, Hanzer V, Weiss H, Mattanovich D, Himmler G, Katinger H (1988a). *In vitro* Vermehrung der alten Lokalsorte Malus domestica "Graf Uhlhorns Augustkalvill". Mitt. Klosterneuburg, 38: 105-107.

Laimer M, Da Câmara Machado A, Hanzer V, Weiss H, Mattanovich D, Himmler G, Katinger H (1988b). *In vitro* Kultur zur Virusfreimachung alter Apfelsorten. Mitt. Klosterneuburg, 38: 247-250.

Laimer M, Da Câmara Machado A, Hanzer V, Kalthoff B, Weiss H, Mattanovich D, Regner F, Katinger H (1991). A new, efficient method for the initiation and establishment of tissue cultures of apple from adult material. PCTOC, 27: 155-160.

Marin JA, Jones OP, Hadlow WCC (1993). Micropropagation of columnar apple trees. J. Hort. Sci. 68: 289-297.

Masuda T, Komori S, Bessho H, Ito Y, Soejima J, Tsuchiya S (1993). Transformation in Malus prunifolia by *Agrobacterium rhizogenes*. Techniques on Gene Diagnosis and Breeding in Fruit Trees Ed. T.Hayashi et al.). Fruit Tree Research Station. Japan. pp. 213-218.

Milandri SG, Laubscher CP, Ndakidemi PA (2008). Hydroponic culture of Gladiolus tristis: Application of paclobutrazol for flowering and height control. 5 February. ISSN 1684–5315. Academic Journals. Afr. J. Biotechnol. 7(3):239-243.

Murashige T (1974). Plant propagation through tissue culture. Ann. Rev. Plant Physiol. 25: 135-166.

Murashige T, Tucker DPH (1969). Growth factor requirements of citrus tissue culture. In: Chapman H.D. (ed). Proc. 1st Int. Citrus Symp. Univ. Calif., Riverside Publication. 3: 1155-1161.

Norton ME, Boe AA (1982). *In vitro* propagation of ornemantal Rosaceous plants. Hort. Sci. 17:190-191.

Nowello V, Grimaudo I, Robers AV (1992). Effects of paclobutrazol and reduced humidity on stomatal conductance of micropropagated grapevines. Acta Hort. 319: 65-70.

Patena L, Sutter EG, Dandeker AM (1988). Root induction by *Agrobacterium rhizogenes* in difficult to root species. Acta Hort. 227: 324-329.

Pedroso C, Oliveira M, Pais S (1992). Micropropagation and simultaneous rooting of Actinidia deliciosa var. 'Hayward' Hort. Sci. 27: 443-445.

Skirvin RM, Kouider M, Joung A, Korban SS (1986). The tissue culture of apple Malus domestica Borkh. In: Bajaj Y. P. S. (ed.) Biotechnol. Agric. For. 1: 183-197.

Smith EF, Roberts AV, Mottley J (1990). The preparation in vitro of Chrysanthemum for transplantation to soil: 2. Improved resistance to desication conferred by paclobutrazol. Plant Cell Tissue Organ Cult. 21: 133-140.

Steinitz B, Cohen A, Goldberg Z, Kochba M (1996). Precocious gladiolus corm formation in liquid shake culture. Plant Cell Tissue Organ Cult. 26: 63-70.

Webster CA, Jones OP (1989a). Effects of sustained subculture on apparent rejuvenation of the apple rootstock M9 *in vitro* and *in vivo*. Ann. Sci. For. 46: 187-189.

Webster CA, Jones OP (1989b). Micropropagation of the apple rootstock 'M9': effect of substained subculture on apparent rejuvenation *in vitro*. J. Hort Sci. 64: 421-428.

Weiss H, Hanzer V, Knapp E, Da Câmara Machado A, Katinger H, Laimer Da Câmar, Machado M (1993). *In vitro* Vermehrung von *Prunus armeniaca*. Tagungsbericht Nutzbarmachung genetischer Resourcen für Züchtung und Landschaftsgestaltung. Pillnitz, pp. 322-326.

Zimmerman RH (1980). Nursery production of fruit plants through tissue culture application and feasibility. Agricultural Research, USDA, SEA Beltsville, Maryland USA. p.119.

Zimmerman RH, Fordham I (1985). Simplified method for rooting apple cultivars in *vitro*. J. Am. Soc. Hort. Sci. 110(1): 34-38.

Ziv M (1989). Enhanced shoot and cormlet proliferation in liquid cultured gladiolus buds by growth retardants. Plant Cell Tissue Organ Cult. 17: 101-110.

Ziv M (1992). Morphogenic control of plants micropropagated in bioreactor cultures and its possible impact on accilimatization. Act. Hort. 319: 119-124.

Ziv M, Ariel T (1992). On the relation between vitrification and somatal cell wall deformity in carnation. Acta Hort. 314: 121-129.