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Influence of medium solidification and pH value on in vitro propagation of *Maranta leuconeura* cv. Kerchoviana

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Abstract

Aseptic cultures of *Maranta leuconeura* were established from shoot tip explants. They were initiated on Murashige and Skoog (MS)-basal medium supplemented with benzyladenine (BA, 5 mg/l) and agar (6 mg/l) (starting medium). Explants were transferred at 6 week intervals until the onset of proliferation (ca. 6 months). Thereafter, four subcultures were made in liquid multiplication medium containing BA (5 mg/1) and the produced shoots were cultured in media having different levels of gelling agents and pH. Superior growth and development were obtained in liquid media and significant differences among the mean values of most treatments were also obtained. Agar and Gelrite (gel) used at 5 and 1.5 g/l, respectively, supported the fastest growth and development of both root and shoot. Adding agar plus gel at the rate of 3+1 g/l led to the best growth and development of shoots, but rooting response was more pronounced at 5+0.5 g/l. A medium pH of 5.7 resulted in the maximum multiplication rate, shoot strength and leaves differentiation.

Maranta leuconeura can be successfully micropropagated at pH 5.7 whether in liquid or solid media, but in the solid we recommend the use of gel (1.5 g/l), agar plus gel (3+1 g/l) or agar (5 g/l) in the same order. Also, liquid medium is preferred for micropropagation until the third subculture. Thereafter, solid medium should be used to overcome production of vitrified shoots and to insure obtaining healthy vigorous plantlets with a higher chlorophyll content.

Hardening-off and acclimatization of the plantlets that were produced resulted in numerous plants used for indoor decoration.

Keywords: Agar; Gelrite; pH value; Growth and development; Vitrification; Chlorophyll

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1. Introduction

Maranta leuconeura is a shade-loving, monocotyledonous ornamental plant belonging to the Marantaceae (Bailey, 1949). It has an attractive foliage and is used for decoration of entrances, offices, rooms and windows. The plant can not be propagated by seeds, but can be cloned by meristems through tissue culture techniques. Liquid as well as semi-solid media used in tissue cultures can induce the physiological disorder known as vitrification. Vitrified plants have water-soaked translucent stems and leaves which are elongated and concave (Debergh, 1983).

The vitrification phenomenon could be minimized or avoided by adding agar or Gelrite at certain levels (Mackay and Kitto, 1987; Ibrahim, 1994). Agar and Gelrite are natural polysaccharides that form a gel at room temperature. Their gels can combine with water and absorb other compounds (Ibrahim, 1994). Both agents are the most expensive components of solid media. Agar is cheaper than Gelrite, but the latter is more clear and solidifies more rapidly. Gelrite also contains no contaminating materials, such as phenolic compounds, which may be toxic (Pierik, 1987).

Studies performed by Debergh (1983), Thentz and Moncousin (1984), Ibrahim (1994) and others showed that the recommended rates of gelling agents are dependent upon explant type, micropropagated plant and culture conditions.

In vitro growth and organogenesis are pH-dependent. The slightly acidic pH values, ranging from 6.3 to 5.7, seem to be the optimal especially for root formation of most species (Mellor and Stace-Smith, 1969; Vennerloo, 1976; Takayama and Misawa, 1979; Aydieh et al., 1999; Ebrahim et al., 1999).

The present study examined the concentration of gelling agent (agar, Gelrite and their combinations) and the best pH value for growth and development of the in vitro cultured *M. leuconeura* cv. Kerchoviana. Plantlets produced were hardened-off and acclimatized to obtain healthy vigorous plants which could then be exported and used for indoor decoration.

2. Material and methods

Greenhouse-grown plants of *M. leuconeura* cv. Kerchoviana obtained from the Agricultural Development Systems Project (Giza, Egypt) served as the source material for shoot tips during the study period (1997–1999).

Aseptic cultures were established from shoot tips which were surface-sterilized in 80% ethanol for 5 s, 1.5% NaOCl for 20 min and 0.1% $HgCl_2$ for 2 min, then rinsed three times in sterilized distilled water to remove all traces of chlorine. After removal of the outside tissues, apical meristems were cultured on Murashige and Skoog (1962) basal medium (pH 5.7) supplemented with benzyladenine (BA, 5 mg/l) and agar (6 g/l). Growing explants were recultured,

at 6 week intervals, on fresh medium until the onset of proliferation (ca. 6 months, starting stage, Plate 1A). In order to obtain sufficient number of plantlets, the adventitious shoots were subcultured four times in liquid MS-basal media (pH 5.7) supplemented with BA (5 mg/l).

To determine the influence of gelling agent concentration and pH on the in vitro growth and development of *M. leuconeura*; liquid media with pH values of 4.2, 4.7, 5.2, 5.7 and 6.2 were solidified by adding Difco-Bacto agar (0.0, 3, 5, 7 and 9 g/l), Gelrite (0.0, 1.0, 1.5, 2.0 and 2.5 g/l) and their combinations (0.0, 3+0.5, 3+1, 5+0.5 and 5+1 g/l) as gelling agents. Thereafter, the adventitious shoots were cultured for 6 weeks on the previous media, then the number of shootlets shoot length, shoot strength, number of leaves, and percentage of rooting were recorded. Shoot strength (viability and vigor) was estimated according to Pottino (1981) and presented as follows: (a) negative growth=1, (b) below average growth=2, (c) average growth=3, (d) above average growth=4 and (e) excellent growth=5.

Due to the development of vitrified plants during the fourth and fifth subculture of the liquid multiplication medium, a final in vitro experiment was constructed to investigate the influence of gelling agent on vitrification. The experiment included culturing the non-vitrified shootlets, produced from the fifth subculture of the liquid medium, on rooting media (no BA, pH 5.7) containing MS-basal medium supplemented with gelling agent as follows: (1) no gelling agent, (2) agar (5 g/l), (3) agar plus Gelrite (3+1 g/l) and (4) Gelrite (1.5 g/l). After 6 weeks, shoot strength, leaves number, percentage of rooting, number and length of roots, number of vitrified shoots (%), fresh to dry weight ratio of shoot and total chlorophyll content (Moran, 1982) as a ratio to control (no gelling agent) were determined.

In all cases, media were distributed in 200 ml Pyrex-glass jars (25 ml/jar), autoclaved for 20 min at 121°C and 1.2 kg/cm^2, then cooled and kept for 4–15 days before use. All cultures were incubated at 27±2°C and 2 klux (16 h per day) provided by cool white fluorescent lamps.

During the acclimatization stage, the non-vitrified plantlets were carefully cleaned of gelling agent, individually transplanted in plastic pots (6 cm diameter) containing peatmoss–sand mixture (3:1 v/v), then allowed to grow for 3 months under greenhouse conditions (25±2°C, 6 klux for 12 h daily and 75% relative humidity). Pots were irrigated with tap water every day and were covered with transparent polyethylene-bag which were gradually removed. All experiments were repeated twice, under controlled conditions, and conducted by using a completely randomized design in factorial arrangement with nine replicates.

All data were averaged and statistically analyzed by using one- and two-way analysis of variance. In case of percentages, the original data were firstly arcsine-transformed prior to statistical analysis. The least significant difference (L.S.D. at 5%) was used to compare between means (Steel and Torrie, 1980).

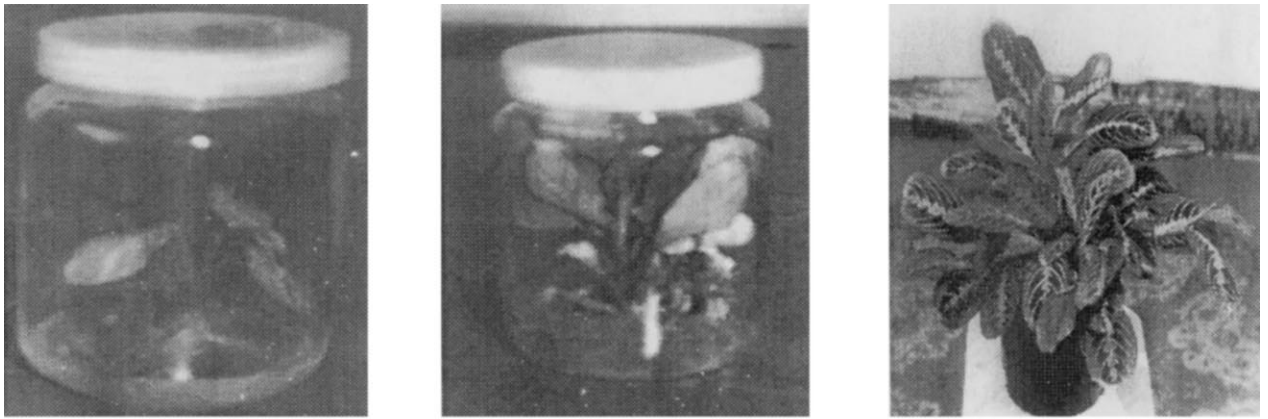

Plate 1. Different stages of *M. leuconeura* cv. Kerchoviana propagated through in vitro culture. Hardening-off and acclimatizing of plantlets that were produced, in peatmoss–sand mixture (3:1 v/v) and under greenhouse conditions, resulted in numerous plants used for indoor decoration. (A) Starting stage, (B) multiplication stage and (C) acclimatization stage.

3. Results and discussion

The effect of gelling agent (agar, Gelrite and their combinations) and pH value on the in vitro growth and organogenesis of *M. leuconeura*, cultured for 6 weeks in/on a multiplication medium, is shown in Tables 1–3. It is obvious that the liquid medium (control) had the superior values of all test parameters, compared

Table 1
Effect of agar concentration and pH value on growth and development of *M. leuconeura* (cv. Kerchoviana) cultured for 6 weeks on MS-basal medium supplemented with benzyladenine (5 mg/l)[a]

pH value	Agar concentration (g/l)				
	0.0 (control)	3.0	5.0	7.0	9.0
No. of shootlet per explant					
4.2	1.7 defg	1.7 defg	1.4 eff	1.2 fg	1.1 g
4.7	2.9 bc	1.7 defg	1.2 fg	1.3 efg	1.2 fg
5.2	3.1 b	1.8 defg	1.7 defg	1.6 defg	1.4 efg
5.7	3.9 a	2.1 de	2.3 cd	2.0 def	1.9 defg
6.2	2.0 def	1.4 efg	2.1 de	1.3 efg	1.3 efg
Shoot length (cm)					
4.2	6.6 a	3.1 b	3.6 b	3.1 b	2.9 b
4.7	5.9 a	3.0 b	3.2 b	3.0 b	2.8 b
5.2	6.3 a	3.3 b	3.3 b	3.0 b	2.9 b
5.7	5.9 a	3.0 b	3.4 b	3.1 b	2.8 b
6.2	6.7 a	3.8 b	3.7 b	3.3 b	3.0 b
Shoot strength					
4.2	4.0 a	1.1 d	1.0 d	1.0 d	1.0 d
4.7	3.0 b	1.0 d	1.0 d	1.0 d	1.0 d
5.2	4.0 a	1.0 d	3.0 b	3.0 b	1.0 d
5.7	4.0 a	2.0 c	3.0 b	3.0 b	2.0 c
6.2	4.0 a	1.0 d	1.0 d	1.0 d	1.0 d
No. of leaves per shootlet					
4.2	4.3 abc	3.6 bc	3.6 bc	3.1 c	3.1 c
4.7	4.5 ab	3.7 bc	3.8 bc	3.6 abc	3.2 c
5.2	5.2 a	3.7 bc	3.7 bc	4.0 abc	3.4 bc
5.7	5.6 a	4.0 abc	4.7 ab	4.2 abc	3.4 bc
6.2	4.5 ab	3.7 bc	3.7 bc	3.7 bc	3.0 c
Percentage of rooting					
4.2	44.4 a	11.1 a	33.3 a	11.1 a	11.1 a
4.7	44.4 a	22.2 a	22.2 a	22.2 a	11.1 a
5.2	22.2 a	11.1 a	11.1 a	33.3 a	11.1 a
5.7	22.2 a	22.2 a	22.2 a	22.2 a	11.1 a
6.2	55.6 a	22.2 a	33.3 a	33.3 a	22.2 a

[a] Means followed by different letters are significantly different at the 0.05 level according to L.S.D.

Table 2
Effect of Gelrite concentration and pH value on growth and development of *M. leuconeura* (cv. Kerchoviana) cultured for 6 weeks on MS-basal medium supplemented with benzyladenine (5 mg/l)[a]

pH value	Gelrite concentration (g/l)				
	0.0 (control)	1.0	1.5	2.0	2.5
No. of shootlet per explant					
4.2	1.7 f	1.8 f	1.6 f	1.8 f	1.3 f
4.7	2.9 bcde	2.2 cdef	2.1 def	1.8 f	1.6 f
5.2	3.1 abc	2.9 cdef	3.0 abcd	2.1 def	1.6 f
5.7	3.9 a	3.7 ab	3.8 ab	2.3 cdef	1.7 f
6.2	2.0 ef	1.9 f	2.1 def	1.3 f	1.5 f
Shoot length (cm)					
4.2	6.6 a	6.4 ab	5.6 abc	3.9 de	3.4 de
4.7	5.9 ab	5.8 ab	5.8 ab	3.8 de	3.3 e
5.2	6.3 ab	5.8 ab	5.9 ab	4.0 de	3.4 de
5.7	5.9 ab	5.2 bc	5.6 abc	3.6 de	3.7 de
6.2	6.7 a	6.1 ab	5.7 ab	4.6 cd	3.7 de
Shoot strength					
4.2	4.0 a	3.0 ab	3.0 ab	1.0 d	1.0 d
4.7	3.0 ab	2.6 bc	2.0 bcd	1.0 d	1.0 d
5.2	4.0 a	3.0 ab	3.0 ab	2.0 bcd	1.7 cd
5.7	4.2 a	3.0 ab	3.0 ab	2.0 bcd	2.0 bcd
6.2	4.0 a	2.0 bcd	2.6 bc	1.0 d	1.0 d
No. of leaves per shootlet					
4.2	4.3 bc	3.8 c	4.3 bc	3.9 c	3.9 c
4.7	4.5 abc	4.3 bc	4.2 bc	4.1 bc	4.0 bc
5.2	5.2 ab	4.7 abc	5.1 ab	4.6 abc	4.0 bc
5.7	5.6 a	4.9 abc	5.6 a	4.2 bc	4.1 bc
6.2	4.5 abc	4.2 bc	4.7 abc	3.9 c	3.9 c
Percentage of rooting					
4.2	44.4 a	33.3 a	33.3 a	44.4 a	22.2 a
4.7	44.4 a	44.4 a	33.3 a	33.3 a	33.3 a
5.2	22.2 a	22.2 a	44.4 a	22.2 a	22.2 a
5.7	22.2 a	11.1 a	33.3 a	11.1 a	22.2 a
6.2	55.6 a	55.6 a	44.4 a	55.6 a	33.3 a

[a] Means followed by different letters are significantly different at the 0.05 level according to L.S.D.

with solidified media. This is in accordance with results obtained by Debergh (1983), Ibrahim (1994) and El-Zifzafi (1998). This finding could be ascribed to: (1) a better contact between explants and liquid medium which increases the availability of cytokinin and the ability for nutrient uptake (Debergh, 1983), (2) dilution of any exudates from explants in the liquid medium (Ziv and Halevy, 1983) and/or (3) more adequate aeration in the liquid medium, which enhances growth and multiplication (Ibrahim, 1994).

Table 3
Effect of agar plus Gelrite concentration and pH value on growth and development of *M. leuconeura* (cv. Kerchoviana) cultured for 6 weeks on MS-basal medium supplemented with benzyladenine (5 mg/l)[a]

pH value	Agar plus Gelrite concentration (g/l)				
	0.0 (control)	3+0.5	3+1.0	5+0.5	5+0.1
No. of shootlet per explant					
4.2	1.7 edef	1.1 ef	1.3 cdef	1.2 def	1.1 ef
4.7	2.9 c	1.0 f	1.4 cdef	1.4 cdef	1.3 cdef
5.2	3.1 b	1.2 def	1.6 cdef	1.9 cd	1.7 cdef
5.7	3.9 a	1.4 cdef	3.3 b	3.0 b	1.8 cde
6.2	2.0 c	1.1 ef	1.4 cdef	1.3 cdef	1.4 cdef
Shoot length (cm)					
4.2	6.6 a	3.0 b	2.9 b	2.9 b	2.9 b
4.7	5.9 a	3.1 b	3.1 b	2.8 b	2.9 b
5.2	6.3 a	2.8 b	3.1 b	2.9 b	3.0 b
5.7	5.9 a	3.0 b	3.2 b	3.2 b	3.0 b
6.2	6.7 a	2.9 b	2.9 b	2.9 b	2.9 b
Shoot strength					
4.2	4.0 a	1.0 d	2.2 bc	2.1 bc	1.0 d
4.7	3.0 b	1.0 d	1.0 d	1.0 d	1.0 d
5.2	4.0 a	2.0 c	3.0 b	3.0 b	2.0 c
5.7	4.0 a	2.0 c	3.0 b	3.0 b	2.0 c
6.2	4.0 a	1.0 d	1.0 d	1.0 d	1.0 d
No. of leaves per shootlet					
4.2	4.3 abcd	3.0 de	4.1 abcd	3.4 cde	3.2 de
4.7	4.5 abc	2.9 de	3.9 abcde	3.9 abcde	3.3 de
5.2	5.2 ab	3.0 de	3.7 bcde	3.7 bcde	3.6 bcde
5.7	5.6 a	3.8 bcde	4.8 ab	4.8 ab	4.0 abcde
6.2	4.5 abc	2.8 e	3.7 bcde	3.7 bcde	3.2 de
Percentage of rooting					
4.2	44.4 a	22.2 a	22.2 a	33.3 a	22.2 a
4.7	44.4 a	22.2 a	11.1 a	33.3 a	33.3 a
5.2	22.2 a	33.3 a	33.3 a	22.2 a	11.1 a
5.7	22.2 a	22.2 a	22.2 a	22.2 a	22.2 a
6.2	55.6 a	33.3 a	22.2 a	33.3 a	11.1 a

[a] Means followed by different letters are significantly different at the 0.05 level according to L.S.D.

In all cases, the slightly acidic pH values appeared to be preferred for growth and development. Although pH 5.7 resulted in the maximal multiplication rate, shoot strength and leaves differentiation, neither shoot elongation nor rooting percentage were improved by changing the pH of the medium (Tables 1–3).

A compact callus was obtained from plumule tissue of *Hevea* seedlings at pH 6.2–6.8, but soft and spongy callus was produced at pH below 5.4 and above 8.0

(Chu, 1966). Root formation by excised potato buds was maximal at pH 5.7 and evidently inhibited at pH below 4.8 or above 6.2 (Mellor and Stace-Smith, 1969). *Nautilocalyx* leaves explants showed a rapid and good root formation at pH 5.0–6.3 (Vennerloo, 1976). Root and bulblet formation of *Lilium auratum* bulb scales occurred when pH was adjusted from 4.7 to 5.7, but the optimal value was about 6.0 (Takayama and Misawa, 1979). Somatic embryos were developed from excised zygotic embryos of carrot at pH 5.7 in an auxin-free medium (Smith and Krikorlan, 1990). Growth of *Platycerium* leaves sections, sugarcane suspension cells and pineapple stem-tip explants was optimized at pH 5.2, 5.5 and 5.7, respectively (El-Zifzafi, 1998; Aydieh et al., 1999; Ebrahim et al., 1999).

Agar and Gelrite are natural polysaccharides with high capability of gelation. Their gels combine with water and absorb other compounds. Agar is most frequently used for solidification of plant culture media because of its desirable characteristics such as clarity, stability, resistance to metabolism during use and its inertness (Ibrahim, 1994). Gelrite, the alternative gelling agent, is increasingly used because it forms clear gels and contains no contaminants (Pierik, 1987).

The present data demonstrated that proliferation rate, organogenesis and growth were influenced by the type and the concentration of gelling agent. Gelrite gave better values than agar plus Gelrite which in turn was better than agar (Tables 1–3). Similar results were reported by Zimmerman and Robacker (1988) on cotton culture and Klimaszeqska (1989) on immature zygotic embryos of hybrid Irach. This observation could be attributed to the difference between both gelling agents in most characteristics mentioned above. Moreover, the complete solidification of both agents were obtained at different conditions (5.0 g/l and pH 5.7 for agar, 1.5 g/l and pH 5.2 for Gelrite). Also, both agents were only semi-solidified at the lower values (data not shown). These differences not only affect the medium pH, but also the studied parameters.

Amongst the solidified media, it could be shown that lowering or raising the concentration of agar, Gelrite and agar plus Gelrite below or above 0.5, 0.15 and 0.3+0.1%, respectively, did not significantly affect most studied parameters, especially shoot length, rooting percentage and leaves number (Tables 1–3). Likewise, the in vitro growth and organogenesis were adversely affected when the gelling agent level was too high. In this regard, agar should be used at 0.6–0.8% (Debergh, 1983), while 0.2% Gelrite was recommended (Pierik, 1987). Using lower levels makes the medium sloppy especially when the pH is low, while the higher levels lead to more solidification making the inoculation too difficult (Debergh, 1983).

The data showed that *M. leuconeura* can be successfully micropropagated at pH 5.7 whether in liquid or solid media, but in the solid media we recommend the use of Gelrite (1.5 g/l), agar plus Gelrite (3+1 g/l) or agar (5 g/l) in that order (Plate 1B).

Table 4
Effect of some gelling agent levels on some growth parameters, number of vitrified shoots (%) and chlorophyll content (ratio to control) of leaves of *M. leuconeura* (cv. Kerchoviana) cultured for 6 weeks on rooting medium (no BA, pH 5.7)[a]

Gelling agent (g/l)	Shoot strength	Leaf no.	Rooting percentage	Root no.	Root length (cm)	Vitrified shootlet no.	FW/DW ratio	Chlorophyll content ratio
Control (0.0)	3.0 c	3.5 c	100 a	3.7 a	2.0 a	10.0 a	7.7 a	1.00 c
Agar (5.0)	3.7 b	4.1 b	100 a	2.7 a	1.7 b	1.0 b	6.8 b	1.07 c
Agar+Gelrite (3+1)	3.7 b	4.3 b	100 a	3.0 a	1.5 b	1.0 b	6.8 b	1.17 b
Gelrite (1.5)	4.0 a	5.0 a	100 a	3.0 a	1.5 b	2.0 b	7.0 b	1.27 a

[a] The non-vitrified shootlets, produced from the fifth subculture of the liquid medium, were cultured on rooting media contained the recommended rates of gelling agents beside control. Means followed by different letters are significantly different at the 0.05 level according to L.S.D.

Due to vitrification during the fourth and fifth subcultures of the liquid multiplication medium, a final experiment was performed on four rooting media (no BA, pH 5.7) containing the above-recommended rates of gelling agents beside control.

All media showed pronounced rooting response (100%) with no significant differences in root number, but significant decrease in root elongation was recorded under medium solidification (Table 4).

It is also indicated that adding gelling agent decreased vitrification and insured obtaining healthy vigorous plantlets with a higher chlorophyll content. In the liquid medium, vitrification was clearly shown and led to pronounced elongation followed by translucence (increase of FW/DW ratio) and eventually necrosis. Furthermore, the non-vitrified plantlets were dark green (higher chlorophyll content) and did not branch, while the vitrified ones were light green (low chlorophyll content). These results are in line with those obtained by Ibrahim (1994) on *Cordyline terminalis* and confirm our recommendation mentioned above.

When the non-vitrified plantlets were hardened-off and acclimatized for 3 months under greenhouse conditions, we obtained healthy vigorous plants used for indoor decoration (Plate 1C).

4. Concluding remarks

Maranta shoot tip explants can be successfully propagated when: (1) the surface is sterilized in solutions of 80% ethanol for 5 s, 1.5% NaOCl for 20 min and 0.1% $HgCl_2$ for 2 min, (2) it is multiplicated by culturing for 6 months on solid MS-basal medium supplemented with BA (5 mg/l) and freshly exchanged at 6 week intervals (3) it is subcultured four times in liquid medium, and then on

certain solid media to avoid vitrification. The rates of 1.5 g/l Gelrite, 3+1 g/l agar plus Gelrite or 5 g/l agar in the same order, and pH 5.7 are recommended for solid media, to decrease the production of vitrified shoots.

To obtain healthy vigorous plants with a higher chlorophyll content, the non-vitrified shoots should be cultured on solid rooting MS-basal media (no BA, pH 5.7). Thereafter, the plantlets that are produced must be transplanted in peatmoss–sand mixture (3:1 v/v), hardened-off and acclimatized for 3 months under greenhouse conditions (25±2°C, 6 klux for 12 h daily and 75% relative humidity).

References

Aydieh, A.A., Ebrahim, M.K.H., Ibrahim, I.A., 1999. Propagation and fruiting of pineapple (*Ananas comosus* L. Merr.) through tissue culture techniques. Egypt. J. Physiol. Sci. 23, 213–228.

Bailey, L.H., 1949. Manual of Cultivated Plants. Macmillan, New York.

Chu, S.E., 1966. Studies on tissue culture of *Hevea brasiliensis*. I. Role of osmotic concentration carbohydrate and pH value in induction of callus growth in plumule tissue from *Hevea* seedling. J. Rubber Res. Inst. Malya 19, 272–276.

Debergh, P.C., 1983. Effects of agar brand and concentration on the tissue culture medium. Physiol. Plant 59, 270–276.

Ebrahim, M.K.H., Zingsheim, O., Veith, R., Abo-Kassem, E.E.M., Komor, E., 1999. Sugar uptake and storage by sugarcane suspension cells at different temperatures and high sugar concentrations. J. Plant Physiol. 154, 610–616.

El-Zifzafi, M.M.A., 1998. Propagation of *Maranta leuconeura* and *Platycerium bifurcatum* through tissue culture techniques. M.Sc. Thesis. Faculty of Agriculture, Cairo University, Egypt.

Ibrahim, A.I., 1994. Effect of gelling agent and activated charcoal on the growth and development of *Cordyline terminalis* cultured in vitro. In: Proceedings of the First Conference of Ornamental Horticulture, Vol. 1, pp. 55–67.

Klimaszeqska, K., 1989. Plantlet development from immature zygotic embryos of hybrid Irach through somatic embryogenesis. Plant Sci.-Limerick 63, 95–103.

Mackay, W.A., Kitto, S.L., 1987. Rapid propagation of French taragon using in vitro techniques. Acta Hort. 208, 251–261.

Mellor, F.C., Stace-Smith, R., 1969. Development of excised potato buds in nutrient medium. Can. H. Bot. 47, 1617–1621.

Moran, R., 1982. Formula for determination of chlorophyllous pigments extracted with *N*,*N*-dimethylformamide. Plant Physiol. 69, 1370–1381.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15, 473–497.

Pierik, R.L.M., 1987. In Vitro Propagation of Higher Plants. Martinus, Boston.

Pottino, B.G., 1981. Methods in Plant Tissue Culture. Department of Horticulture, Agricultural College, Maryland University, College Park, MA, pp. 8–29.

Smith, D.L., Krikorlan, A.D., 1990. Somatic proembryo production from excised wounded zygotic carrot embryos on hormone-free medium. Evaluation of the effects of pH ethylene activated charcoal. Plant Cell Rep. 9, 34–37.

Steel, R.G.D., Torrie, J.A., 1980. Principals and Procedure of Statistics, 2nd Edition. McGraw-Hill, New York.

Takayama, S., Misawa, M., 1979. Differentiation in *Lilium auratum* bulb scales grown in vitro. Effect of various cultural conditions. Physiol. Plant. 46, 184–190.

Thentz, M., Moncousin, C., 1984. In vitro propagation of *Platycerium bifurcatum* (Cav). C. Ch. Rev. Hort. Sci. 57, 293–297.

Vennerloo, C., 1976. The formation of adventitious organs. III. A comparison of root and shoot formation on *Nautilocalyx* explants. Z. Pflanzen Physiol. 80, 310–322.

Zimmerman, T.W., Robacker, C.D., 1988. Media and gelling agent effect on cotton callus initiation. Plant Cell Tissue and Organ Culture 15 (3), 269–274.

Ziv, M., Halevy, A.H., 1983. Control of oxidative browning and in vitro propagation of *Strelitzia reginae*. Hort. Sci. 18 (4), 434–436.