In Vitro Cell. Dev. Biol.—Plant 34:244–248, July–September 1998

1054-5476/98 $05.00+0.00

CONSERVATION OF *HYPERICUM FOLIOSUM* AITON, AN ENDEMIC AZOREAN SPECIES, BY MICROPROPAGATION

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(Received 15 April 1997; accepted 20 March 1998; editor M. R. Becwar)

SUMMARY

One of the first Azorean endemic vascular taxa chosen for the development of *in vitro* multiplication techniques was *Hypericum foliosum* Aiton, due to its colonizing ability (Sjögren, 1984), a loss of seed germination capacity after only 1 yr of storage (Maciel, 1994), and the populations' generally low number of individuals. The following culture media were tested using *Hypericum foliosum*'s single node cuttings: Murashige and Skoog (1962), Roest and Bockelmman (1973), Lloyd and McCown (1980), Côrte and Mendonça (1985), and Cellárová et al. (1992). Further experiments were performed on CM medium supplemented with four different growth regulators: α-naphthaleneacetic acid (NAA), N^6-benzyladenine (BA), γ,γ-(dimethylallyl) aminopurine (2iP), and kinetin (KIN). The acclimatization stage was carried out in Jiffy 7® pots and in a 2:1 or 1:1 peat/perlite mixture. We found that micropropagation of *Hypericum foliosum* is possible on CM medium and that the best results with growth regulators were achieved with the following supplements: 0.1 mg/l (0.4 μ*M*) BA and 0.5 mg/l (2.6 μ*M*) NAA + 1.0 mg/l (4.4 μ*M*) BA (in the initiation stage), and 0.1 mg/l (0.4 μ*M*) BA (in the elongation stage). As for culture multiplication, 0.1 mg/l (0.4 μ*M*) BA (in the initiation stage) and 0.5 mg/l (2.6 μ*M*) NAA + 1.0 mg/l (4.4 μ*M*) BA (both in the initiation and elongation stages), proved to be the most efficient concentrations. The acclimatization stage was successfully performed in Jiffy 7® pellets.

Key words: micropropagation; *Hypericum foliosum* Aiton; conservation; endemic; nodal explants.

INTRODUCTION

The Azores, with its archipelago status and an increasing human pressure to which the natural vegetation is subjected, has become a priority region for the development of conservation methodologies. Currently, various endemic Azorean taxa appear only in small dispersed populations or are restricted to a single locality in one or more islands. As part of an autoecological conservationist plan, a micropropagation unit was established in the Biology Department of the University of Azores, and is fully working since late 1993.

One of the first Azorean endemic vascular taxa chosen for the development of *in vitro* multiplication techniques was *Hypericum foliosum* Aiton (*Guttiferae* family). This plant (Fig. 1 *A*), commonly known as "Furalha" or "Malfurada," is an Azorean endemic nanophanerophyte (Franco, 1971), occurring on all islands of the archipelago (Dias, 1989). It is a woody plant that, according to Sjögren (1984), grows preferentially above 400 m and occasionally below 100 m, on both sheltered and exposed places. On São Miguel, the most developed populations were found on the northeast part of the island (at altitudes ranging from 570 to 887 m), in open, treeless sites. The plants colonizing ability (Sjögren, 1984) was verified by our study, as several individuals were observed in recent landslide areas and man-made glades.

It is also known that the seeds of this species lose the ability to germinate after only 1 yr of storage (Maciel, 1994) and its populations are usually formed by a small number of individuals. Micropropagation will overcome such problems and was made a component of the LIFE project, whose major objective is the recuperation of several altered vegetation patches, located in the feeding areas of an Azorean endemic bird known as "Priôlo" (*Pyrrhula murina*).

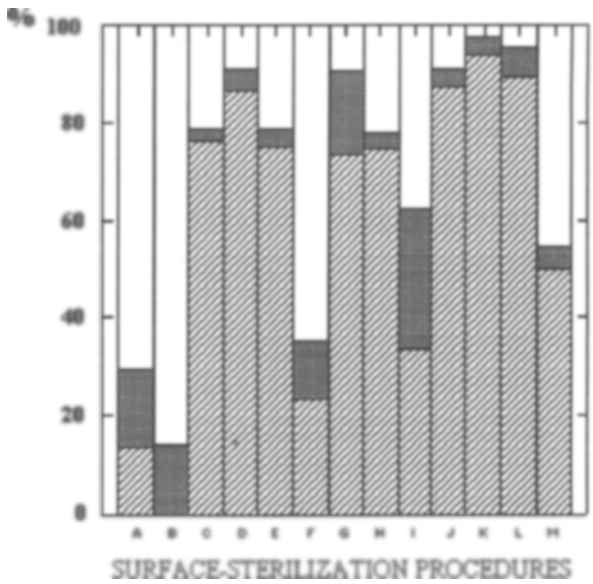
As to previous *in vitro* cultures of *Hypericum* spp., the only available information referred to the shoot production and rooting of *Hypericum perforatum* (Roest and Bockelmann, 1973; Cellárová et al., 1992) and *Hypericum canariense* (Mederos, 1991). Roest and Bockelman obtained adventitious shoots from capitula, in RB medium supplemented with 1 mg/l of N^6-benzyladenine (BA) and 5 mg/l sucrose. The media was solidified with 6 g/l agar. Rooting was accomplished *ex vitro*, after previously dipping the shoots in 1% indole-3-acetic acid (IAA) talc. Mederos (1991) initiated the culture of *Hypericum canariense* from apical and axillary buds in an Almacigo medium (Mederos and Rodríguez, 1990). The author considers the MS macronutrient solution, [Murashige and Skoog 1962], with BA and α-naphthaleneacetic acid (NAA) added, as the most effective to propagate the species. Rooting was obtained in a 1/2 diluted MS macronutrient solution to which was added indole-3-butyric acid (IBA) or NAA and the other remaining regular Almacigo additives. The author also refers to the importance of adding rosmanol to prevent explant and media darkening during the culture initiation stage, as the only condition to allow *in vitro* production of shoots. Cellárova et al. (1992) obtained several adventitious shoots from whole *Hypericum perforatum* plantlets, excised leaves, shoots, and roots, using MS mineral salts, PRL-4-C Gamborg vitamin solution (Gamborg et al., 1968), and Skoog amino acids. The medium was supplemented with 87.6×10^{-3} *M* of sucrose, 0.5 or 0.91 mg/l BA, and solidified with 0.6% agar. The pH was set to 5.6 before autoclaving. The shoots

A

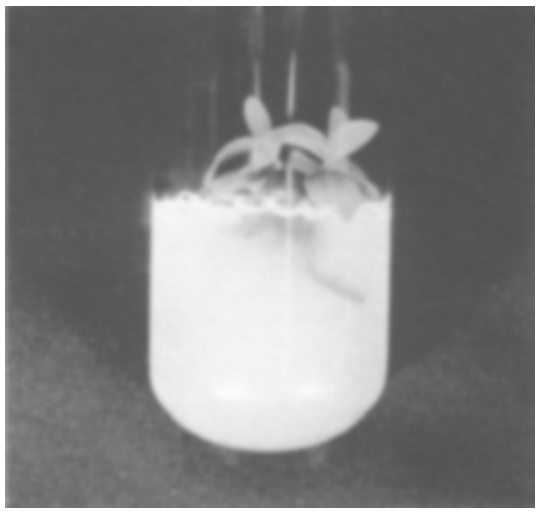
B

C

D



E



F

FIG. 1. Stages in the micropropagation of *Hypericum foliosum* Aiton from single node explants. *A*, Field-established plant. *B*, Germinated seedling. *C*, Single node stem cuttings (each pair of leaves will be excised before placing the explant in the culture vessel). *D*, Visible contamination percentages for the surface-sterilization procedures tested. The values were taken after 4 wk in culture. □ Without visible contamination. ■ Bacteria + yeasts. ▨ Fungi. *E*, Single node explant in CM medium (Côrte and Mendonça, 1985), with differentiated shoots and roots. *F*, Acclimatized young plant.

TABLE 1

SURFACE-STERILIZATION PROCEDURES TESTED FOR *HYPERICUM FOLIOSUM*

Plant Material	Pr.	Alcohol (%)	Time (min.)	Bleach (%)	Time (min.)	HgCl2 (%)	Time (min.)
Whole	A	—	—	10	15	—	—
Plants	B	—	—	15	15	—	—
	C	70	1	20	15	—	—
	D	70	1	10	10	0.1	5
	E	70	1	10	10	0.2	5
4–6 cm Stem	F	70	1	10	10	0.3	5
Portions	G	70	1	10	15	0.1	5
Without	H	70	1	10	20	0.1	5
Leaves	I	70	1	10	20	0.2	5
	J	70	1	15	10	0.1	5
	K	70	1	15	15	0.1	5
	L	70	1	15	20	0.1	5
	M	96	1	10	10	0.1	5

were rooted in the same medium, without growth regulators. Though there were no references to the use of Lloyd and McCown (1980) and Côrte and Mendonça (CM, 1985) media for micropropagating this specific genus, it was decided to also include them in the experiment, because these media are known to have produced good results with other woody plants (Côrte and Mendonça, 1985; George et al., 1987; Matos, 1992).

Materials and Methods

Plant material. All plant material came from São Miguel island. The explant sources were young seedlings (Fig. 1 *B* and *C*), by *in vitro* culture and this year's growth from field established plants. The whole plants, produced by seed germination on petri dishes with moist Whatman® No. 1 paper, or 4–6 cm stem sections taken from field-established specimens, without leaves nor apical portions (including the last three to four nodes), were previously washed in running tap water for 3 h. The material was then surface-sterilized and several procedures were tested (Table 1). 0.01% of Tween 20 was added to all treatments. The last stage was also identical in all the experiments and consisted of three rinses with sterile distilled water.

In the initiation stage, the explants used were single node cuttings without leaves and in the elongation and rooting stage, axillary shoots produced *in vitro*. The explants obtained from seed-produced plants were solely used in the media experiments, while the field-collected material was uniquely used in the growth regulator experiments.

Culture conditions. The following basal media were tested: MS (Murashige and Skoog, 1962), RB (Roest and Bockelmman, 1973), WPM (Lloyd and McCown, 1980), CM (Côrte and Mendonça, 1985), and CK (Cellárová et al., 1992).

CM medium consists of a 2/5 dilution of the MS macronutrient solution, the MS micronutrient solution, 15 mg/l (40.9 μ*M*) of NaFeEDTA, the Rose Galzy (1964) vitamin formulation, and 1 mg/l (2.6 μ*M*) of vitamin D3. In the CM medium, vitamin D3 was omitted. All the media were supplemented with 20 g of sucrose, solidified with 7 g of Bacto Agar Difco®, and autoclaved at pH 5.8. Further experiments were performed using CM medium supplemented with four different growth regulators: NAA, BA, γ,γ-(dimethylallyl) aminopurine (2iP), and kinetin (KIN).

In the initiation stage, the effect of the four growth regulators, in the following concentrations, was tested: 0.1, 0.5, and 1.0 mg/l. The matrix had all possible combinations between auxin and cytokinins. In the elongation and rooting stage, the medium was supplemented with 0.1 mg/l (0.4 μ*M*) BA, 0.1 mg/l (0.5 μ*M*) NAA + 1.0 mg/l (4.4 μ*M*) BA, 0.5 mg/l (2.6 μ*M*) NAA + 1.0 mg/l (4.4 μ*M*) BA, and 1.0 mg/l (4.9 μ*M*) 2iP. To specifically induce rooting, two auxins were added to the basal medium: 0.1 (0.5 μ*M*) and 1.0 mg/l (5.3 μ*M*) NAA, 0.1 (0.4 μ*M*) and 0.5 mg/l (2.4 μ*M*) IBA.

The culture vessels used were 125 × 25 mm Pyrex® test tubes with Kaput® translucent closures. All cultures were placed in a growing chamber at 21 ± 1° C, with a light intensity of approximately 56 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a photoperiod of 16 h.

The acclimatization stage was carried out in Jiffy 7® pots and in a 2:1 or 1:1 peat/perlite mixture. Single plants were kept inside 330 ml plastic greenhouses and, after 6 to 8 wk, were transplanted to pots with humus-enriched soil (Fig. 1 *E*).

The experiments conducted with seed-produced explants were carried out in 22 to 24 test tubes, with a single explant each and repeated twice. For the growth regulator tests and due to the plants reduced number of individuals, each test tube with one single explant, was considered the experimental unit and was replicated 48 times. The values used in the statistical analysis were taken after 4 wk in culture.

The multiplication rates (i.e., the number of single node cuttings produced *in vitro*), were determined in the initiation stage by the product between the number of nodes per normal shoot and the number of normal shoots per explant. In the elongation stage, the multiplication rates were determined by the number of nodes obtained per normal shoot.

When evaluating the contamination rates, in the few cases where both fungi and bacteria/yeast contaminants were visible, the contamination was considered fungal in the statistical analysis, because fungi development inside the test tubes overcame those of the other contaminants.

A single factor analysis of variance (ANOVA) was performed whenever there were sufficient amounts of data to justify it. Verification of the homogeneity of variances, for each parameter analyzed, was accomplished through a graphical representation of residuals. When homoscedasticity was not observed, the data was normalized with one the following transformations: $x' = \text{arcsen}\sqrt{x}$, $x' = \log x$ or $x' = \log (x + 1)$. For a multiple comparison of means, Tukey's test was used at the 5% significance level. The statistical analysis and graphics were done with SYSTAT 5.2, running in a Macintosh LCIII.

Results and Discussion

Surface sterilization. The contamination (Fig. 1 *D*) in the tests performed with laboratory seed-germinated plants (Table 1, procedures *A* and *B*) was significantly different from those observed in field-obtained stem cuttings (by Tukey's test, at the 5% level). In *B*, no visible fungal contamination was detected. The best results using field material was with procedures *F*, *I*, and *M*, with *I* being the most efficient one, relative to necrosis (data not shown).

In vitro *culture.* CM medium gave the best results (Table 2, Fig. 1 *E*) in the initiation stage, with a multiplication rate of 2.8 nodes per explant, against 2.2 for WPM, 1.8 for MS, 0.9 for CK, and 0.8 for RB. In the elongation stage, CM also produced the longest shoots, the highest number of rooted explants, and the longest roots, though these values were not significantly different by Tukey's test.

Although CM and CK produced the highest number of nodes per normal shoot in the elongation stage (respectively, 2.8 and 3.0 nodes), CK also produced the highest percentages of shoot necrosis (approximately 15% in the initiation and 23% in the elongation stages).

CM's performance can most likely be explained by its two-fifth's dilution of the MS macronutrient solution, for it has already been previously stated that a high ionic concentration (which is the case with MS and CK media) has growth-inhibitory effects in several woody species (McCown and Sellmer, 1987).

The complete lack of vitamins in RB's formulation can be pointed as a possible explanation for its low efficiency, being referred by several authors the importance of these substances, particularly thiamine, for *in vitro* cultures (Gamborg and Shyluck, 1981; Bhojwani and Razdan, 1983; Boccon-Gibod, 1989).

The positive results obtained in the initiation stage in CM media with added growth regulators are shown in Table 3. From these various treatments tested in the elongation stage, only two produced posi-

TABLE 2

EFFECT OF BASAL MEDIUM ON SHOOT AND ROOT GROWTH OF *HYPERICUM FOLIOSUM* EXPLANTS

Media	Normal Shoots/Expl.	Nodes/Normal Shoot	Nodes/Normal Shoot	Shoot Length (mm)	Rooted Shoots (%)	Longest Root Length (mm)
	Initiation			Elongation and Rooting		
CK	0.3 a**	3.0 a	3.0 a	5.5 a	8 a	2.5 a
CM	1.2 b	2.3 a	2.8 a	6.9 a	39 a	5.6 a
MS	0.7 ab	2.5 a	2.2 ab	4.8 a	0 a	—
RB	0.6 a	1.4 b	1.1 b	4.5 a	26 a	2.2 a
WPM	0.9 ab	2.4 a	2.0 ab	4.4 a	18 a	3.8 a

*CK = Cellárová et al., 1992; CM = Côrte and Mendonça, 1985; MS = Murashige and Skoog, 1962; RB = Roest and Bockelmann, 1973; WPM = Lloyd and McCown, 1981.

**Significantly different from b, by Tukey test, at the 5% level.

TABLE 3

EFFECT OF DIFFERENT AUXIN AND CYTOKININ CONCENTRATIONS ON CULTURE INITIATION OF *HYPERICUM FOLIOSUM*

Growth Regulators (mg/l)*	Normal Shoots/Explant	Nodes/Normal Shoot
	Initiation	
0.1 BA	1.0	2.0
0.5 BA	0.1	3.0
1.0 BA	0.2	1.5
0.1 2iP	0.1	4.0
1.0 2iP	0.4	2.1
0.1NAA + 0.5BA	0.2	2.6
0.1NAA + 1.0BA	0.5	2.4
0.1NAA + 0.52iP	0.1	1.0
0.5NAA + 0.1BA	0.1	1.7
0.5NAA + 0.5BA	0.0	1.0
0.5NAA + 1.0BA	0.7	3.1
0.5NAA + 1.02iP	0.1	1.5

*BA = N^6-benzyladenine; 2iP = Y,Y-(dimethylallyl) aminopurine; NAA = α-naphthaleneacetic acid.

tive results: 2.9 nodes per normal shoot with a mean shoot length of 7.0 mm in the media supplemented with 0.1 mg/l BA, and 4.4 nodes per normal shoot with a mean shoot length of 4.7 mm in 0.5 mg/l NAA + 1.0 mg/l BA. Neither one of the treatments promoted rooting.

Considering the various growth regulators, when added singly to the medium, BA was the most efficient in promoting shoot differentiation and proliferation, as stated by Cellárová et al. (1992) and Roest and Bockelmann (1973) for *Hypericum perforatum*. The best results using an auxin and a cytokinin simultaneously happened in an NAA + BA supplemented media. This was also stated by Mederos (1991) for *Hypericum canariense*. In contrast to what was said by Cellárová et al. (1992) for *Hypericum perforatum*, high BA concentrations did not improve results. The absence of rooting in the presence of NAA and IBA, as mentioned by the same authors, was also confirmed for *Hypericum foliosum*.

An increase in NAA, generally proved to be inversely proportional to shoot differentiation. Its inhibitory effect was only counterbalanced by BA's concentrations of 1 mg/l. It was observed that even with the highest concentrations of BA, the results were very poor when in the presence of 1.0 mg/l NAA. The inhibitory effect of NAA was further confirmed by a total lack of shoot differentiation on the modalities supplemented only with this auxin.

The results obtained with NAA in the initiation stage only partially agrees with Skoog and Miller's theory (1957), which states that shoot versus root organogenesis is dependent on the proportion between the exogenous auxin/cytokinin levels in the culture media. According to these authors, a relatively high auxin concentration will promote root differentiation and inhibit shoot development. The fact that the results with NAA in this stage are so extreme, even when used in low concentrations, suggest a possible high endogenous auxin level present in the field-collected plant material (Tran Thanh Van and Trinh, 1990).

During elongation, the inefficiency of 1 mg/l 2iP and of 0.1 mg/l NAA + 1 mg/l BA, was obvious. With 0.1 mg/l of BA, an auxin source did not seem to be needed, whereas it was required when 1.0 mg/l of BA was added. This suggests the need of a compensatory effect from an exogenous auxin source, which may be a sign of a weak apical dominance in the *in vitro* produced *Hypericum foliosum* shoots.

Tamas (1987), summarizing various work done in apical dominance, indicates that the lack of apical dominance may lead to the channelling of the available cytokinin mainly to the axillary buds in detriment of the apex, thus implementing an axillary shoot development instead of the main shoot growth. On the other hand, and according to the same author, the accumulation of cytokinin in the apical bud, due to a strong apical dominance, increases the production of endogenous auxins in this structure, which have a suppressive effect on the axillary caulogenesis.

NAA did not promote rooting, as neither did any of the cytokinins tested alone or combined with this auxin. The results obtained with NAA seem to indicate that the caulogenesis suppression effect attributed to this growth regulator is, in this plant, dissociated from its rooting effect. In fact, having been observed the first in a remarkable way during initiation, the later was never verified.

Rooting also was not promoted by the addition of IBA and, in a last instance, the absence of rooting in all the growth regulator tests may be explained by the fact that these were conducted with material obtained from field-established plants. Though the year's growth was used as the preferred explant source, its degree of differentiation is higher than the one expected in a seedling or in a plantlet, increasing thus the difficulty of *in vitro* rooting (Debergh, 1988).

Considering that the number of nodes per explant obtained in the initiation stage, in a nonsupplemented CM, was slightly higher than the one obtained with growth regulators, and that the shoot length and number of nodes per normal shoot in the elongation stage were

not very different from the values obtained in supplemented media, it seems that the micropropagation of *Hypericum foliosum* could be efficiently done in a growth-regulator-free CM medium. However, because the seed-produced explants used in the media tests and the field-established explants used in the growth regulators tests were not interchangeable, it is possible that the results obtained in a simple CM could be improved using growth regulators with seed-produced explants.

After 4 wk of acclimatization to progressively lower humidity levels, there were no differences in the shoot length and number of nodes observed between the plantlets potted in the three media used. However, the plantlets rooted in Jiffy 7® pellets originated new shoots. After 8 wk of acclimatization, these new shoots looked normal and the plantlets had a well-developed root system. The plantlets rooted in peat/perlite mixtures not only did not develop any shoots, but the plantlets showed signs of necrosis.

Conclusions

Culture initiation from laboratory seed-produced plants was the preferable technique for *Hypericum foliosum*'s micropropagation. The most efficient surface-sterilization technique tested with seed-produced plants was *B* (15% sodium hypochlorite + 0.01% Tween 20 for 15 min) and the procedure labeled as *I* was the best for field material (70% alcohol for 1 min + 10% sodium hypochlorite + 0.01% of Tween 20 for 20 min + 0.2% mercuric chloride for 5 min).

We also conclude that CM was the most efficient medium for all culture stages and that the best results with growth regulators are achieved in the following supplements: 0.1 mg/l (0.4 μ*M*) BA and 0.5 mg/l (2.6 μ*M*) NAA + 1.0 mg/l (4.4 μ*M*) BA, in the initiation stage, and 0.1 mg/l (0.4 μ*M*) BA in the elongation stage. For culture multiplication, 0.1 mg/l (0.4 μ*M*) BA (in the initiation stage) and 0.5 mg/l (2.6 μ*M*) NAA + 1.0 mg/l (4.4 μ*M*) BA (in the initiation and elongation stages) are the most efficient supplements.

The acclimatization stage was successfully performed in Jiffy 7® pellets. Using these conditions, it would be possible to produce two to four plantlets from single nodal explants in approximately 4 mo.

Acknowledgments

This study was cofinanced by the European Community "CIÊNCIA" program and by the "Associaç;ão de Municípios da Região Autónoma dos Açores."

References

Bhojwani, S. S.; Razdan, M. K. Developments in crop science. Vol. 5 Plant tissue culture: theory and practice. Netherlands: Elsevier Science Publishers B.V.; 1983.

Boccon-Gibod, J. Les besoins nutritifs des tissus cultivés en conditions aséptiques. In: Vidalie, H., ed. La culture in vitro et ses applications horticoles. Paris: Lavoisier Abonnements; 1989:31–36.

Cellárová, E.; Kimáková, K.; Brutovská, R. Multiple shoot formation and phenotypic changes of R_0 regenerants in *Hypericum perforatum* L. Acta Biotechnol. 12:445–452; 1992.

Côrte, G.; Mendonça, A. Importance de la culture de méristèmes pour la multiplication accélérée de clones de vigne exempts de virus. Bull. de L'O. I. V. 650–651: 397–402; 1985.

Debergh, P. C. Micropropagation of woody species—state of the art on *in vitro* aspects. Acta Hortic. 227:287–295; 1988.

Dias, E. Flora e vegetação endémica na ilha Terceira. Trabalho de síntese apresentado para provas de A. P. C. C. Departamento de Ciências Agrárias, Universidade dos Açores; 1989.

Franco, J. A. Nova flora de Portugal (Continente e Açores). Vol. I. *Lycopodiaceae-Umbelliferae*. Lisboa, Portugal; 1971.

Galzy, R. Technique de thermothérapie des viroses de la vigne. Ann. Épiphyties. 15:245–256; 1964.

Gamborg, O. L.; Miller, R. A.; Ojima, K. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151–158; 1968.

Gamborg, O. L.; Shyluck, J. P. Nutrition and characteristics of plant cell and tissue cultures. In: Thorpe, T. A., ed. Plant tissue culture, methods and applications in agriculture. London: Academic Press; 1981:21–44.

George, E. F.; Puttock, D. J. M.; George, H. J. Plant culture media. Vol. 1. Formulations and uses. Edington, England: Exegetics Ltd.; 1987.

Lloyd, G.; McCown, B. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb. Proc. Int. Plant Prop. Soc. Proc. 30:421–427; 1980.

Maciel, M. G. B. Ecofisiologia da germinação de sementes de plantas vasculares endémicas dos Açores. Trabalho de síntese apresentado para provas de A. P. C. C. Departamento de Biologia, Universidade dos Açores; 1994.

Matos, C. Micropropagação vegetativa *in vitro* de: *Brassica oleracea* L., *Lippia citriodora* Ortega *e Punica granatum* L. Relatório do trabalho final de curso de bacharelato em engenharia de produção agrícola. Instituto Politécnico de Santarém; 1992.

McCown, B.; Sellmer, J. C. General media and vessels suitable for woody plant culture. In: Bonga, J. M.; Durzan, D. J., ed. Cell and tissue culture in forestry. Vol. 1. Netherlands: Martinus Nijhoff Publishers; 1987:4–16.

Mederos, M. S. In vitro growth and multiplication of *Hypericum canariense* L. Acta Hortic. 289:133–135; 1991.

Mederos M. S.; Rodríguez E. M. J. Physiologia Plantarum. 79:A3, 17; 1990.

Murashige, T. Plant propagation through tissue cultures. Annu. Rev. Plant Physiol. 25:135–166; 1974.

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

Roest, S.; Bokelmann G. S. Vegetative propagation of *Chrysanthemum cinerariaefolium in vitro*. Sci. Hortic. 1:120–122; 1973.

Sjögren, E. Açores, Flores. Horta: Direcção Regional de Turismo; 1984.

Skoog, F. Growth and organ formation in tobacco tissue cultures. Am. J. Bot. 31:19–24; 1944.

Skoog, F.; Miller C. O. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Exp. Biol. 11:118–131; 1957.

Tamas, I. A. Hormonal regulation of apical dominance. In: Davies, P. J., ed. Plant hormones and their role in plant growth and development. Dodrecht, Netherlands: Martinus Nijhoff Publishers; 1987:393–410.

Tran Thanh Van, K.; Trinh, T. H. Organogenic differentiation. In: Bhojwani, S. S., ed. Plant tissue culture: applications and limitations. Netherlands: Elsevier Science Publishers B.V.; 1990:34–53.