AgEcon SEARCH
RESEARCH IN AGRICULTURAL & APPLIED ECONOMICS

The World's Largest Open Access Agricultural & Applied Economics Digital Library

This document is discoverable and free to researchers across the globe due to the work of AgEcon Search.

Help ensure our sustainability.

Give to AgEcon Search

AgEcon Search
http://ageconsearch.umn.edu
aesearch@umn.edu

*Papers downloaded from **AgEcon Search** may be used for non-commercial purposes and personal study only. No other use, including posting to another Internet site, is permitted without permission from the copyright owner (not AgEcon Search), or as allowed under the provisions of Fair Use, U.S. Copyright Act, Title 17 U.S.C.*

PROCEEDINGS

OF THE

26th ANNUAL MEETING

July 29 to August 4, 1990

Mayaguez, Puerto Rico

Published by:

Caribbean Food Crops Society

with the cooperation of the USDA-ARS-TARS

Mayaguez, Puerto Rico

IRRADIANCE EFFECTS ON ACCLIMATIZATION OF MICROPROPAGATED *Aronia arbutifolia* (L.) Ell

W. Colón, D.L. Ingram and M.E. Kane

Department of Environmental Horticulture, IFAS
University of Florida, Gainesville, FL 32611

ABSTRACT

Rooted micropropagated plantlets of *Aronia arbutifolia* were transplanted into sterile rootcubes and placed into a growth room under three irradiance levels. The irradiance levels consisted of 350 (low), 550 (medium) and 700 (high) µmol m^{-2} s^{-1}. Plants were harvested from day one and at 10-day intervals. Shoot growth, number of leaves and leaf area were significantly affected by irradiance treatments. Plants grown at the lowest irradiance level developed longer shoots, larger leaf area and more roots. Repeated measurements analysis on shoot length determined that the response to low irradiance was significantly greater from the other two treatments. Seventy percent of the plants in the highest irradiance treatment died before 30 days. Photosynthetic rate was greater in plants grown under high irradiance. Transmission electron micrographs were taken to determine chloroplast development, including grana stacking under the three irradiance levels.

INTRODUCTION

Improper acclimatization is the main limitation of expansion of micropropagation techniques encountered with transplanting *in vitro* propagules to the greenhouse environment (Sutter, 1981; Poole and Conover, 1983). Acclimatization has been defined as the process by which organisms adapt to man-made environments (Brainerd and Fuchigami, 1981). Micropropagules must be acclimatized and adapted to changes in light intensity temperature and relative humidity.

Light intensity can enhance acclimatization of micropropagules (Capellades et al., 1990; Wetzstein and Sommer, 1983; Desjardins et al., 1990) showed that supplemental radiation of 80 µmol m^{-2} s^{-1} increased dry weight of micropropagated asparagus after 22 days. Lee et al. (1988) grew sweetgum micropropagules under irradiance levels of 50, 155 and 315 µmol m^{-2} s^{-1}. Plants grown under high irradiance had thicker leaves with greater mesophyll differentiation and larger cells than plants grown under lower irradiance levels. Plants grown under different irradiance levels had similar leaf areas. Desjardins et al. (1987) obtained higher leaf and root dry weight when strawberry micropropagules were placed in a greenhouse environment and exposed to supplemental lighting of 150 µmol m^{-2} s^{-1}. The supplemental lighting also increased total leaf area after 10, 20 and 30 days of treatments.

Another aspect of the acclimatization process is the change in the mode of carbon nutrition of micropropagules out of culture. Micropropagules are produced under heterotrophic conditions but once transplanted plantlets must acclimatize to autotrophic conditions (Grout and Aston, 1978). Low photosynthetic rates were obtained from in vitro cultured birch (Smith et al., 1986). Plantlets kept for long period of time under in vitro conditions had reduced photosynthetic rates. Yet, once plantlets were removed from in vitro environment, they resumed normal rates of photosynthesis.

Lee et al. (1985) studied the effects of quantum flux density on photosynthesis and chloroplast ultrastructure in tissue-cultured plantlets and seedlings of Liquidambar styraciflua L. Photosynthesis of in vitro grown plantlets was light saturated at 350 to 450 µmol m0-2 s0-1. Tissue-cultured plantlet chloroplast had more grana development in medium and high light pretreatment than under low light. Also in vitro plantlets of Liquidambar styraciflua contained chloroplasts with irregularly arranged internal thylakoid membranes.

The objective of this study was to identify the optimum irradiance level necessary to achieve successful acclimatization of micropropagules of Aronia arbutifolia in the shortest period of time.

MATERIALS AND METHODS

Aronia arbutifolia (L.) Ell. is a native Florida woody plant, with potential for use in revegetation efforts (Dehgan et al., 1989). The micropropagation protocol is an adaptation of Kane et al. (1987). Stems with lateral buds were cut from actively growing and sexually mature plants. Stems were divided into 15 mm length with various lateral buds attached. These nodal explants were then rinsed in tap water for 1 hr and surface sterilized by repeated immersion in 50% (v/v) ethanol for 1 min and in 1.05% (v/v) sodium hypochlorite for 12 min, followed by three 5 min rinses in sterile deionized water.

Explants were transferred into 25 x 150 mm culture tubes containing 15 ml medium consisting of Woody Plant Medium (WPM) salts and vitamins (Lloyd and McCown, 1980), 3% sucrose , 1 mg/L N06-benzylaminopurine and solidified with 1.0% (w/v) TC0TM agar (Hazleton Research Products, Inc., Lenexa, KS). The pH in the medium was adjusted to 5.5 with 0.1 N KOH before autoclaving at 1.2 Kg cm0-2 for 20 min at 1210oC. All cultures were then placed under a 16-hr photoperiod provided by cool-white fluorescent lamps at 45 µmol m0-2 s0-1. Air temperature was maintained at 25 $\pm$ 20oC. Stock cultures were maintained by subdividing and transferring onto fresh medium every 5-7 weeks.

Stage III rooted micropropagules were prepared by cutting 5-week old shoots into 10 mm stem segments consisting of 2-3

nodes. Fifteen microcuttings were transferred into 473 ml clear polypropylene culture vessels (Better Plastics, Kissimmee, Fla.) containing 100 ml of WPM supplemented with 1 mg/L of indole-3-butyric acid (IBA). At three weeks, stage III micropropagules of A. arbutifolia were transplanted into sterile rootcubes containing 50 micropropagules/tray. Micropropagules were fertilized with a dilute solution of a commercial 20-20-20 formula with micronutrients (Peter's, Folgelville, Pa.). All trays of micropropagules were placed in a walk-in growth room at 24 $\pm$ 20oC under Sylvania (GTE Products Corp., Manchester, N.H.) metal arc High Intensity Discharge (HID) lamps for a 16-hour photoperiod.

Propagules were placed under three irradiance levels (low: 350 umol m0-2 s0-1; medium: 550 umol m0-2 s0-1; high: 700 µmol m0-2 s0-1) provided by shade cloths. Growth was measured every 10 days for 30 days. Treatment effects on shoot length, number of leaves and roots produced, dry weights and leaf area were taken at 10-day intervals. The experimental design was a randomized complete block with 5 blocks. The data obtained were analyzed using the General Linear Model (GLM) procedures developed by the Statistical Analysis System (SAS, 1985).

Cytological examination of chloroplast development was made after 30 days post-transplant. Leaf samples were collected from each treatment and fixed in 6% (v/v) glutaraldehyde, 6% (v/v) paraformaldehyde, 4% (v/v) osmium tetroxide, passed through ethanol dehydration series (75-100%) and embedded in Spurr's resin (Spurr, 1969). Resin blocks were heat polymerized and sectioned on a conventional ultramicrotome with glass knives. Samples were observed under a Jeol 100-CX at 65 kV.

Photosynthetic rates were determined at the end of 30 days with the Hansatech D.W., Clark-type 012 electrode (Hanastech Limited, Norfolk, England).

RESULTS

Analysis of repeated measurement (Littell, 1989) conducted on shoot growth through time showed significant linear differences between the irradiance treatments (Fig. 1). Micropropagules maintained for 20 days at 350 µmol m0-2 s0-1 produced the significantly longest shoots while micropropagules maintained at 550 µmol m0-2 s0=1 produced significantly longer shoots than 700 µmol m0-2 s0-1. Similar significant differences between shoot lengths due to irradiance treatments were also obtained from data collected at 20 and 30 days using destructive sampling (Fig. 2). Significant differences in shoot growth were apparent at 20 days after transplanting.

Transplanted micropropagules produced more roots and significantly more leaves at the lower irradiance treatment (data not shown). Total leaf area increased significantly with

decreasing irradiance (Fig. 3). No significant difference in shoot dry weights were obtained between the three irradiance treatments. The highest irradiance level was detrimental and caused a 70% mortality rate.

Photosynthetic rates were significantly greater for plantlets maintained at the highest irradiance level (Fig. 4). Chloroplasts present in plantlets cultured *in vitro* had disorganized thylakoid membranes with prominent starch granules (Fig. 5a). At 350 µmol m^{-2} s^{-1} thylakoid membranes were arranged in grana (Fig. 5b). As the irradiance level increased from 350 µmol m^{-2} s^{-1} to 700 µmol m^{-2} s^{-1} grana stacking decreased (Fig. 5c,d).

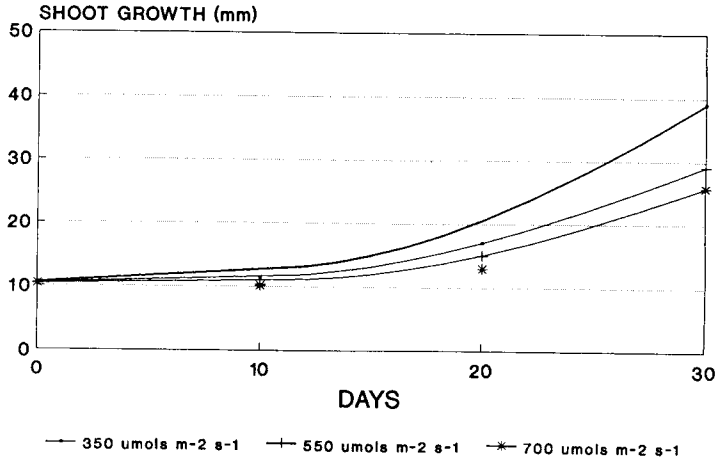

Figure 1. Shoot growth (mm) of transplanted *A. Arbutifolia* micropropagules in response to 3 irradiance levels.

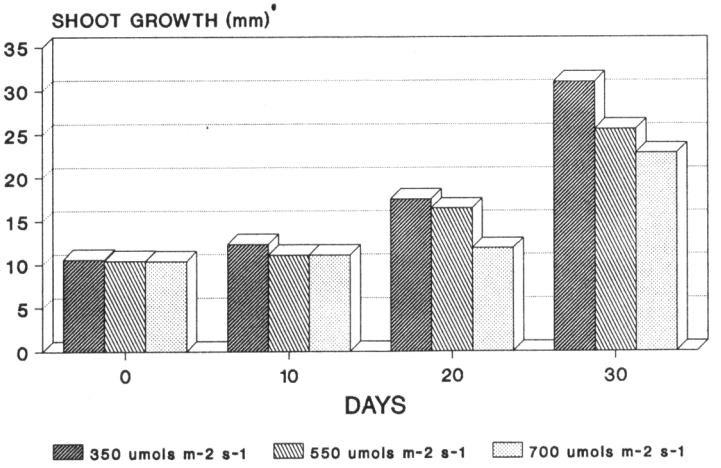

Figure 2. Shoot growth (mm) of transplanted A. Arbutifolia micropropagules to 3 irradiance levels.

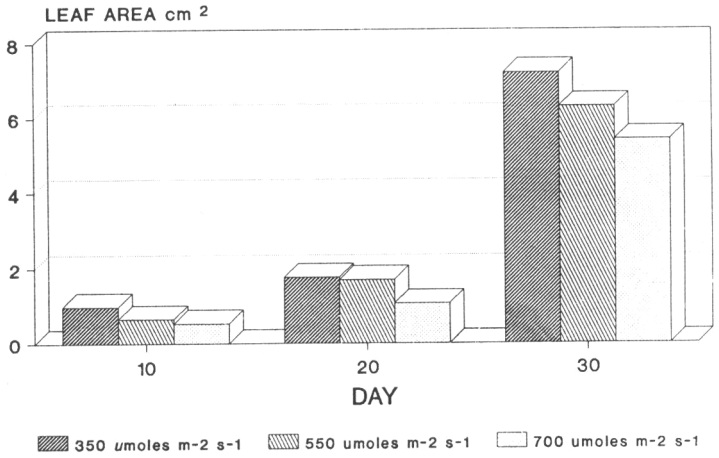

Figure 3. Total leaf area (cm^2) of transplanted A. Arbutifolia micropropagules in response to 3 irradiance levels.

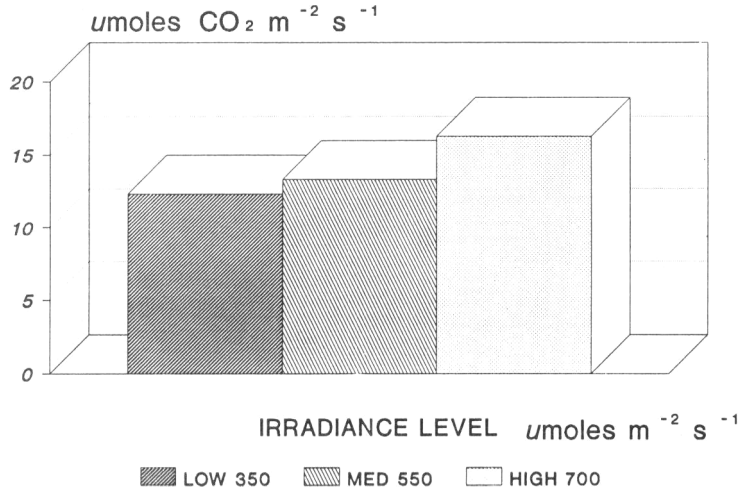

Figure 4. Photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) of transplanted A. Arbutifolia micropropagules maintained for 30 days under three irradiance levels.

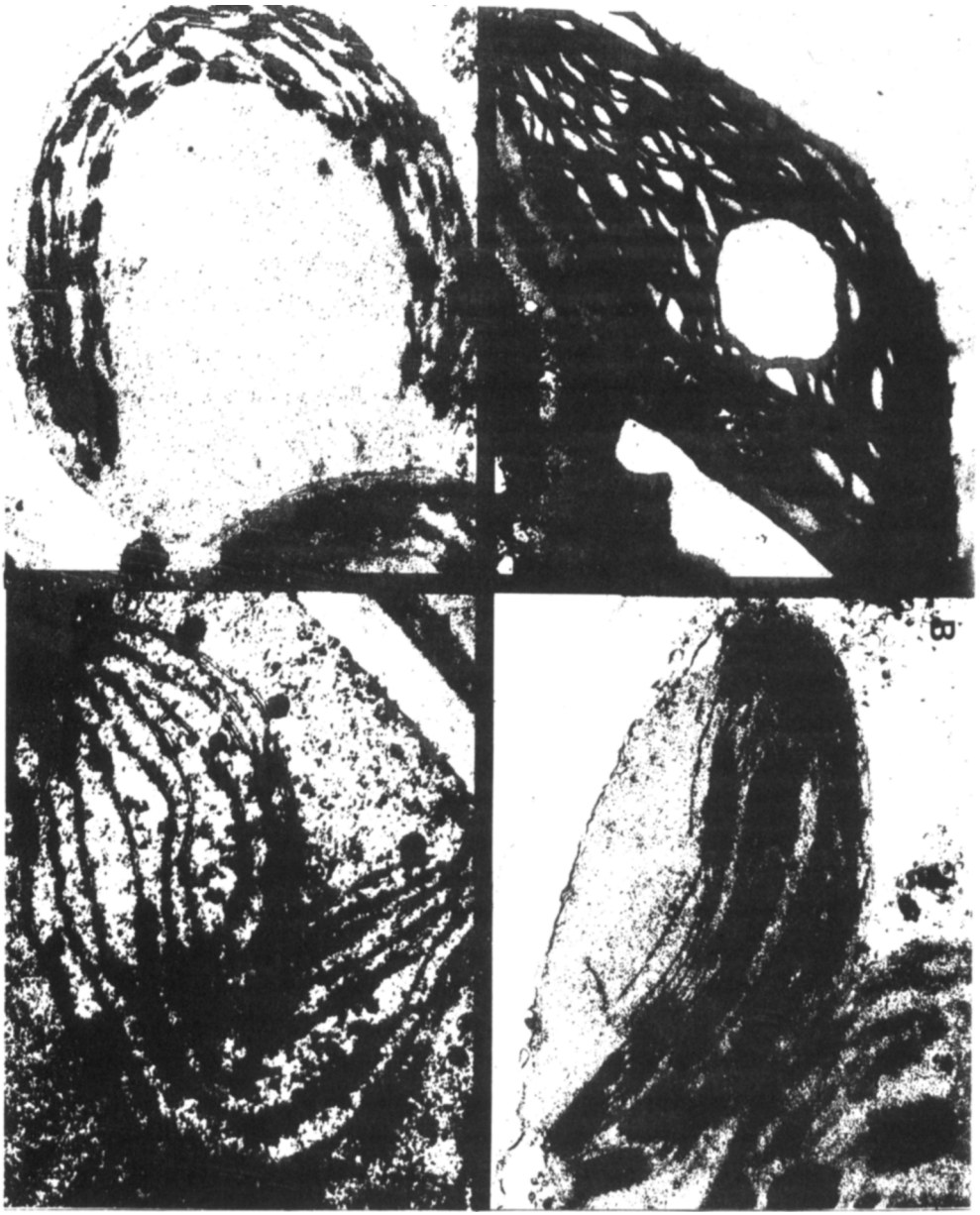

Figure 5. Chloroplast ultrastructure of transplanted A. Arbutifolia micropropagules (A. in vitro 45 μmol m^{-2} s^{-1}, 27,000X; B. 350μmol m^{-2} s^{-1}, 29,000X; C. 550 μmol m^{-2} s^{-1}, 20,000X; D. 700 μmol m^{-2} s^{-1}, 29,000X).

DISCUSSION

Various researchers have shown that growth of tissue-cultured plantlets increases with increasing irradiance from in vitro levels (25 to 45 µmol m^{-2} s^{-1}) to 120-150 µmol m^{-2} s^{-1} (Donnelly et al., 1985; Desjardins et al., 1987). In this experiment, growth and development of transplanted micropropagules were altered by light intensity. The best survival rate and growth response was obtained when transplanted micropropagules were subjected to an irradiance level of 350 µmol m^{-2} s^{-1}. This irradiance level proved to be the upper threshold of increasing light intensity for transplanting *A. arbutifolia* micropropagules. The increase in leaf area with decreased light intensity obtained is in agreement with the behavior of sun and shade plants (Boardman, 1977). In this experiment shoot dry weights were not significantly different between treatments even though leaf area was, the reason being sun leaves have thicker leaves and higher fresh weight per leaf area than shade leaves (Lonstrech et al., 1985; Boardman, 1977).

In vitro cultured plants have been shown to have a negative carbon balance when maintained in culture (Grout and Millan, 1985). While at 7 days after transplanting to the *ex vitro* environment, normal photosynthetic rates are recorded. Measurements also indicated negative photosynthetic rates of *in vitro* cultured *A. arbutifolia* (Colón, unpublished data, 1990). In this study at 30 days, all transplanted micropropagules had positive photosynthetic rates at all irradiance levels. The highest irradiance treatment had the highest photosynthetic rates and was probably due to modification of chloroplast development. Chloroplasts developed under higher light intensity had less grana stacking and more stroma area partitioned for protein synthesis. Thus irradiance energy was efficiently used. Chloroplasts developed under lower light intensity had more area partitioned for grana stacking which increases chlorophyll content for capture of incoming irradiance (Anderson, 1986).

Micropropagated Asian White Birch, exhibited increased photosynthesis when light intensity was increased from 200 to 600 µmol m^{-2} s^{-1} (Smith et al., 1986). However, Lee et al. (1985) obtained similar results with noncultured seedlings of *Liquidambar styraciflua* but opposing results when tissue-cultured plantlets were used. These investigators used lower irradiance levels (315, 155 and 50 µmol m^{-2} s^{-1}) in addition plantlets were maintained under *in vitro* culture environment. Similar to Lee et al. (1985), our study indicated that in *A. arbutifolia*, chloroplasts with disorganized thylakoid developed in tissue-cultured plantlets maintained under *in vitro* light intensity of 45 µmol m^{-2} s^{-1}. This anomaly could be due to the limited gas exchange and the heterotrophic mode of nutrition typical *in vitro* conditions.

The results obtained from this study illustrate that an irradiance level of 350-550 µmol m0-2 s0-1 was significantly effective in reducing the time period for acclimatization or A. arbutifolia micropropagules.

REFERENCES

Anderson, J.M. 1986. Photoregulation of the composition, function, and structure of thylakoid membranes. Ann. Rev. Plant Physiol. 37:93-136.

Brainerd, K.E., and Fuchigami, L.H. 1981. Acclimatization of aseptically cultured apple plants to low relative humidity. J. Amer. Soc. Hort. Sci. 106:515-518.

Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. 28:355-377.

Capellades, M., Fontarnau, R., Carulla, C., and Debergh, P. 1990. Environment influences anatomy of stomata and epidermal cells in tissue-cultured Rosa multiflora. J. Amer. Soc. Hort. Sci. 115:141-145.

Dehgan, B., Gooch, M., Almira, F., and Kane, M. 1989. Vegetative propagation of Florida native plants: III. Shrubs. Proc. Fla. State Hort. Soc. 102:254-260.

Desjardins, Y., Gosselin, A., and Lamarre, M. 1990. Growth of transplants and in vitro-cultured clones of asparagus in response to C012 enrichment and supplemental lighting. J. Amer. Soc. Hort. Sci. 115:364-368.

Desjardins, Y., Gosselin, A., and Yelle, S. 1987. Acclimatization of ex vitro strawberry plantlets in C012-enriched environments and supplementary lighting. J. Amer. Soc. Hort. Sci. 112:846-851.

Donnelly, D.J., Vidaver, W.E., and Lee, K.Y. 1985. The anatomy of tissue cultured red raspberry prior to and after transfer to soil. Plant Cell Tissue Organ Culture 4:43-50.

Grout, B.W.W., and Aston, M.J. 1978. Transplanting of cauliflower plants regenerated from meristem culture. II. Carbon dioxide fixation and the development of photosynthetic ability. Hort. Res. 17:65-71.

Grout, B.W.W., and Millan, S. 1985. Photosynthetic development of micropropagated strawberry plantlets following transplanting. Annals Bot. 55:129-131.

Kane, M.E., Sheehan, T.J., and Philman, N.L. 1987. A micropropagation protocol using Fraser Photinia for mutation induction and new cultivar selection. Proc. Fla. State Hort. Soc. 100:334-337.

Lee, N., Wetzstein, H.Y., and Sommer, H.E. 1985. Effect of quantum flux density on photosynthesis and chloroplast ultrastructure in tissue-cultured plantlets and seedlings of Liquidambar styraciflua L. towards improved acclimatization and field survival. Plant Physiol. 78:637-641.

Lee, N., Wetzstein, H.Y., and Sommer, H.E. 1988. Quantum flux density effects on the anatomy and surface morphology of in vitro- and in vivo-developed sweetgum leaves. J. Amer. Soc. Hort. Sci. 113:167-171.

Littell, R.C. 1989. Statistical analysis of experiments with repeated measurements. HortScience. 24:37-40.

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

Longstreth, D.J., Bola|os, J.A., and Goddard, R.H. 1985. Photosynthetic rate and mesophyll surface area in expanding leaves of Alternanthera philoxeroides grown at two light levels. Amer. J. Bot. 72:14-19.

Poole, R.T., and Canover, C.A. 1983. Establishment and growth of in vitro-cultured Dieffenbachia. HortScience. 18:185-187.

SAS Institute, Inc. 1985. SAS user's guide: Statistics, Version 6 ed. SAS Institute, Inc., Cary, N.C.

Smith, M.A.L., Palta, J.P., and McCown, B.H. 1986. Comparative anatomy and physiology of microcultured seedlings and greenhouse-grown Asian white birch. J. Amer. Soc. Hort. Sci. 111:437-442.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

Sutter, E. 1981. Problems posed by microplant morphology. Proc. Inter. Plant Prop. Soc. 31:563-566.

Wetzstein, H.Y., and Sommer, H.E. 1983. Scanning electron microscopy of in vitro cultured Liquidambar stryraciflua plantlets during acclimatization. J. Amer. Soc. Hort. Sci. 108:475-480.