DEVELOPMENT OF TISSUE CULTURE METHODS FOR THE RESCUE AND PROPAGATION OF ENDANGERED *MORINGA* SPP. GERMPLASM[1]

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Stephenson, Katherine K. *(The Lewis B. and Dorothy Cullman Cancer Chemoprotection Center, Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine), and* **Jed W. Fahey** *(The Lewis B. and Dorothy Cullman Cancer Chemoprotection Center, Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205 and Center for Human Nutrition, Johns Hopkins Bloomberg School of Public Health; e-mail jfahey@jhmi.edu).* DEVELOPMENT OF TISSUE CULTURE METHODS FOR THE RESCUE AND PROPAGATION OF ENDANGERED *MORINGA* SPP. GERMPLASM. *Economic Botany 58(Supplement):S116–S124, 2004.* *Moringa is an Old-World dry tropical plant genus with great food, horticultural, industrial, and pharmaceutical potential. Although many of the thirteen known* Moringa *species are in danger of extinction, one species,* M. oleifera *Lam., is now widely cultivated.* M. oleifera *was therefore utilized to develop micropropagation techniques that may be applicable to the more endangered members of this genus. Immature seeds were the most responsive tissue source, and greatest success was achieved using membrane rafts and a liquid growth medium. The success rate was 73%, but the multiplication rate averaged only 4.7 shoots per culture. Most vigorous plantlet development through the transplant stage was achieved using a commercial plant preservative formulation of isothiazolones following shoot proliferation. Although there was no evidence of contamination, treatment with this microbiocide prevented early tissue senescence and it increased culture survivability.*

Key Words: *Moringa*; plant tissue culture; horseradish tree; nutrition; drumstick tree.

Moringa oleifera Lam. (also known as the horseradish, drumstick, or ben oil tree) is the most widely cultivated species of an Old-World dry, tropical, monogeneric family, Moringaceae. It is a fast-growing perennial soft-wooded tree with a long history of traditional medicine and industrial uses that is native to the sub-Himalayan tracts of Northwestern India. *M. oleifera* (syn. *M. pterygosperma* Gaertn.) is becoming an important crop in India, the Philippines and the Sudan. It is being cultivated in West, East and South Africa, tropical Asia, Latin America, the Caribbean, and in the Pacific islands (Fuglie 1999; Morton 1991; Palada 1996; Verdcourt 1985). *M. stenopetala* Bak. f. Cuf. is an important crop in Kenya and Ethiopia (Verdcourt 1985). *Moringa peregrina* was known to the ancient Egyptians who utilized its seed oil. All of the other 10 species of this genus enjoy attention based primarily on their pharmacologic properties (Olson 2001), however some are in danger of extinction, and at least one (*M. hildebrandtii*) is now extinct in the wild (Olson and Razafimandimbison 2000).

Moringa oleifera has been used to combat malnutrition, especially among infants and nursing mothers. Non-governmental organizations such as Church World Service (CWS) and Educational Concerns for Hunger Organization (ECHO) have been advocating Moringa as ''natural nutrition for the tropics.'' Leaves can be eaten fresh, cooked or stored as dried powder for many months without refrigeration, and apparently without loss in nutritional value. *Moringa oleifera* is especially promising as a food source in the lowland dry tropics, because the tree is in full leaf at the end of the dry season when other foods are typically scarce (Fuglie 1999). Seeds are eaten green, roasted, powdered, steeped for tea or used in curries (Berger et al. 1984). Powdered seeds are highly effective for purifying drinking water by flocculation and sedimenta-

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tion of particulate contaminants (Berger et al. 1984; Gassenschmidt et al. 1995; Olsen 1987). *Moringa oleifera* seed oil (30–40% of seed weight), also known as ben oil, is a sweet, non-sticking, non-drying oil that resists rancidity and has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products (Tsaknis et al. 1999). *Moringa* species have long been recognized by folk medicine practitioners as a tumor therapeutic, and have recently been shown to contain potent inhibitors of TPA-induced Epstein-Barr virus-early antigen activation in lymphoblastoid (Burkitt's lymphoma) cells (Guevara et al. 1999; Murakami et al. 1998).

The preservation of the *Moringa* species is thus of great concern from biodiversity, ethnobotanical, dietary and pharmacological perspectives. There are tremendous potential opportunities with *M. oleifera* for sustainable agriculture, and the development of cash crops in semi-arid regions. Though less well studied, all of the *Moringa* species now in the wild have local medicinal uses (M. Olson pers. comm.) but could readily become casualties of the ongoing decline in biodiversity. Sexual propagation of some of these species would be tedious and would not even be possible without having enough individual plants for cross-pollination. Since flowering of a number of the large tree species of *Moringa* does not even commence until a critical size is attained—highly unlikely to occur with multiple trees in a greenhouse—tissue culture may be the only practical way to cultivate these trees outside the tropics. Additionally, there are only one or a few individuals of the other *Moringa* species (*M. concanensis*, *M. ruspoliana* and *M. arborea*) in cultivation in the United States (M. Olson pers. comm.). Amplification of these rare individuals by tissue culture propagation would make them more widely available and less likely to become lost to cultivation. Thus developing tissue culture methods for this genus is urgent.

We have used a wide variety of explants from accessions of *Moringa oleifera* and *M. stenopetala*—the two most accessible *Moringa* species—to develop protocols for clonal multiplication that could be employed for germplasm maintenance of some of the more endangered species of *Moringa.* Since *M. oleifera* is likely a highly genetically heterogeneous species (M. Olson pers. comm.), our efforts to establish cultures, to improve upon node culture and axillary node regeneration techniques, and to establish protocols for clonal multiplication using immature seed tissues could also directly benefit micropropagation programs for this species. The approach taken herein is to expand upon the earlier observations by Kantharajah and Dodd (1991), Mohan et al. (1995), and Iyer and Gopinath (1999) as a starting point for a systematic evaluation of basal culture media formulae. Additionally, we have subjected large numbers of immature *M. oleifera* seeds to a range of experimental conditions in an effort to improve upon reported rates of shoot proliferation, multiplication and long-term survival for field transplanting in *M. oleifera* and in *M. stenopetala,* a species for which in vitro propagation has not heretofore been reported. And finally, we have examined the issue of early shoot senescence that continues to restrict rapid culture proliferation beyond the initial shoot proliferation stage, and which would severely limit the scale-up of a micropropagation effort.

Materials and Methods

Explant Sources

Source material for cultures was provided by Dr. Manuel Palada (University of the Virgin Islands, St. Croix, U.S.V.I.), and was either collected from trees growing as ornamentals, or from trees being cultivated in the U.V.I. Agricultural Experiment Station tree nursery. Two separate harvests of seed-pods were made. After surface disinfestation, all tissue culture manipulations were carried out under sterile conditions in a laminar flow hood.

A) Axillary Nodes and Single Shoots from Nodes. Meristematic nodes were excised from *Moringa oleifera* seedlings that were grown in 4-inch pots of sterile Vermiculite™ under cool-white fluorescent lighting with a 16h light/8h dark photoperiod, at 25°C until a minimum of four fully expanded leaves were present. At about one week post-germination, the leaf axils were generally distinct and visible. Entire stems were excised from young seedlings and sterilized by immersion into 70% ethanol for two minutes followed by 15 minutes in 25% Clorox™ bleach and thrice rinsing in sterile distilled water. After surface-sterilization the stems were cut into segments containing single axillary meristems just prior to culture and the nodal tissue was oriented such that the cut surface(s) was in

direct contact with the culture medium. Single shoots that developed from the dormant axillary meristem were subsequently used to screen culture media for their capacity to support clonal multiplication.

B) Immature Seed. Seeds from seed pods representing a wide range of maturities of both *M. oleifera* and *M. stenopetala* were used. Whole seed pods were shipped directly from St. Croix to Baltimore, and processed as soon as possible to insure maximum viability. Pods were segregated into four size categories based on their diameter: # 1 = 3–5 mm, # 2 = 5–10 mm (8 mm seeds), # 3 = 10–18 mm (9–10 mm seeds) and # 4 = 18–25 mm (11–13 mm seeds). Harvests were made in the spring and summer of 2002. At first harvest we collected both *M. oleifera* and *M. stenopetala* pods, all of which were of size range #4, and the seeds ranged from dry and brown to green and fleshy. The second seed pod harvest was exclusively from *M. oleifera*, and it contained all four size categories. Seed pods were surface sterilized by wiping with 95% ethanol, then immersing them for 15 min in 50% Clorox™ bleach (aqueous) containing about 10 mg/L of Alconox™ detergent. Pods were then placed on a drying mat and a longitudinal incision was made with a sterile scalpel to separate the dehiscent valves and release the seeds. Prior to culture, the seed coats were either scarified, or removed to permit direct contact of seed tissue with the culture medium. Between one and five explants were placed on semi-solid medium in 10 cm Petri plates or on liquid medium rafts inside GA-7 culture vessels (Sigma/Aldrich Chemical Co., St. Louis, MO, USA).

Cultivation

All cultures were incubated under cool-white fluorescent lights with a 16:8 light/dark cycle at 25°C. Tissue culture medium components, culture vessels and membrane rafts were purchased from Sigma/Aldrich or Caisson Laboratories, Inc. (Rexburg, ID, USA) with the exception of PPM™ (Plant Cell Technologies, Inc., Washington, DC, USA). Cultures utilizing a semi-solid support matrix were prepared with autoclaved Gelrite (0.7% aqueous) (Schweizerhall Inc., South Plainfield, NJ) as the gelling agent and amended as indicated below. Liquid media were sterilized by filtration through a 0.22 μm filter. Transplants from tissue culture were made to sterile potting soil (Fafard brand; Maryland Plants & Supplies, Inc., Baltimore, MD, USA), and Oasis horticulture cubes (Smithers Oasis, Kent, OH, USA).

Media Preparation

(A) *Moringa oleifera* seedlings were used as an axillary meristem tissue source for preliminarily screening of basal salt formulations. Six basal salt formulations were prepared with and without the addition of 0.25% or 0.5% activated charcoal (AC): Murashige & Skoog (MS) (Murashige and Skoog 1962), half strength Murashige & Skoog (½ MS), Woody Plant Medium (WPM) (Lloyd and McCown 1980), Schenk & Hildebrandt (S&H) (Schenk and Hildebrandt 1972), Gamborg's B5 (B5) (Gamborg et al. 1968), and White's (White 1963). Gelrite™ (0.7%) alone was used as a control, both with and without activated charcoal (designated G and G+AC, respectively). None of the media used in the basal nutrient screen was amended with plant growth regulators. All solid media were prepared with 3% sucrose, and Murashige & Skoog vitamins (Murashige and Skoog 1962), and pH was adjusted to 5.8 prior to autoclaving at 121°C for 15 minutes. The molten autoclaved mixture was poured into pre-sterilized, disposable 10 cm diameter Petri plates.

(B) Explants from the 1st pod harvest were cultured on a semi-solid medium consisting of MS basal salts, 3% sucrose, 1 mg/L BAP (6-benzylaminopurine), 1 mg/L GA_3 (gibberellic acid-A_3), 0.25% activated charcoal, pH 5.8. Explants from the 2nd pod harvest were cultured using a liquid, charcoal-free variant of this medium, whereby explants were supported on a permeable membranous raft providing continuous contact with the medium components. Rafts were autoclaved inside a GA-7 culture box equipped with a gas permeable lid. About 30 ml of culture medium was added to each GA-7 beneath the raft and a single droplet of medium was placed on the raft surface to initiate proper contact with the explant.

(C) Single shoots resulting from seedling axillary nodes cultured on MS medium without the addition of exogenous plant hormones were plated across several MS-based media containing the plant growth regulator ancymidol and a combination of NAA and kinetin at various concentrations. Clonal multiplication medium was made with MS basal salts and vitamin stock, 3% (w/v) sucrose and 0.7% (w/v) Gelrite; pH was

adjusted to 5.8 prior to autoclaving, and it contained one of four levels of ancymidol (0.25 mg/L, 0.5 mg/L, 1.0 mg/L or 1.5 mg/L). A constant level (1 mg/L of kinetin) was also combined with either 0.25, 0.5 or 1 mg/L of the auxin NAA. An MS-based control medium contained no plant growth regulators. Additional shoot proliferation formulae adapted from reports of the successful cultivation of tissues from *Moringa* spp., from papaya, a member of the sister family Caricaceae and thus a phylogenetically rational starting point for culture medium formulations (Cohen and Cooper 1982), or from other woody plant species (Lloyd and McCown 1980) were also evaluated, these MS-based media were amended with the following plant growth regulators: BAP [1 mg/L]/ ADS [80 mg/L], BAP [0.5 mg/L]/ NAA [0.2 mg/L), TDZ [5 mg/L]/ BAP [2 mg/L], BAP [5 mg/L]/ NAA [0.1 mg/l], BAP [1 mg/L], and in ½ strength MS with BAP [1 mg/L]/ NAA [0.05 mg/L].

Results and Discussion

Axillary Meristem Culture

Initial in vitro studies revealed that dormant axillary node explants from young, growth chamber-germinated *M. oleifera* seedlings could be readily cultured. The widely used MS medium was suitable for single shoot induction, producing healthy green shoots which developed leaves indistinguishable from those of seed-derived plantlets. A single axillary shoot was invariably produced with moderate friable callusing at the cut tissue surface in direct contact with the medium. Within three weeks of shoot emergence, however, cultures abruptly began to show signs of senescence and subsequent growth was comprised of only very small, narrow leaves and shortened internodes. Further optimization of the basal medium formulation was thus pursued for micropropagation of these dormant axillary nodes.

Several of the six basal salt formulations examined, with and without the addition of two levels of activated charcoal, supported nodal-derived shoot growth. Only MS and half-strength MS medium supported both shoot growth and good shoot quality (Table 1). The addition of charcoal at the high rate had no effect on shoot development, and curiously, there was a marked reduction in shoot production at the low rate.

The following plant growth regulators were evaluated both alone and in combination (ancymidol, NAA, GA_3, 6-BAP, IAA, kinetin, adenine sulfate, and TDZ) in an attempt to stimulate clonal shoot multiplication, shoot development and root establishment. Similar protocols have been employed successfully for many herbaceous plant species as well as for woody or tree species (Goyal and Arya 1985; Magdalita 1997; Murashige and Skoog 1962; Pandey 1987). Randomly selected shoots derived from node cultures were transferred to five plates of each of the eight media. After three weeks on these media there were no signs of additional shoot formation and the explanted shoot vigor and quality declined rapidly on all but the control (plant growth regulator-free) medium. The shoots that were transferred to this control medium remained healthy and green and produced a primary root. Mohan et al. (1995) reported a similar phenomenon which they attributed to the high auxin levels in the culture medium. Their solution to this problem was to transfer the cul-

TABLE 1. EFFICACY OF BASAL SALT FORMULATIONS TO SUPPORT THE GENERATION OF SHOOTS FROM *MORINGA OLEIFERA* LAM. AXILLARY MERISTEMS.

Basal medium Formulation	Total # shoots produced[1]	Presence of callus	Shoot quality[2]
MS	9	yes	+++
½ MS	12	yes	++
WPM	2	yes	++
S&H	9	yes	+
B5	1	yes	−
White's	0	yes	
G	0	no	
MS + 0.5% AC	7	yes	+++
½ MS + 0.5% AC	6	yes	−
WPM + 0.5% AC	3	yes	++
S&H + 0.5% AC	4	yes	−
B5 + 0.5% AC	1	yes	−
White's + 0.5% AC	0	yes	
G + 0.5% AC	0	no	
MS + 0.2% AC	3	yes	−
½ MS + 0.2% AC	0	yes	
WPM + 0.2% AC	0	yes	
S&H + 0.2% AC	0	yes	
B5 + 0.2% AC	0	yes	
White's + 0.2% AC	0	yes	
G + 0.2% AC	0	no	

[1] 15 nodes explanted per medium formulation.

[2] +++ = very good, ++ = good, + = moderately good and − = poor.

tures to fresh basal medium without hormones in order to revive the tissues. In our hands, this approach was not successful with shoots generated from cultured axillary nodes. The formation of de-differentiated callus was observed in some cultures (e.g., those cultured on White's basal formula and MS with NAA/kinetin) when the medium contained an auxin with subsequent additional shoot and root formation from a common node-based-meristematic tissue. However, this occurred randomly across treatments and was not uniquely associated with an experimental protocol. In a parallel effort, colleagues in Kenya report success culturing meristematic tissues of *Moringa oleifera* seedlings (D. Odee pers. comm.).

Premature tissue senescence after about the third week of culture continues to be a problem. In other tissue culture systems, accumulation of ethylene in the vessel has been identified, and solved by the addition of low levels of $AgNO_3$ to bind to the tissue ethylene receptors (Leonhardt 1987; Mohiuddin 1997) or passage through multiple hormone-free media (Kantharajah and Dodd 1991). We thus transferred young, healthy shoots from their original medium to a fresh medium containing 5 mg/L $AgNO_3$ with and without the addition of the plant growth regulators. After cultivation on these media for several days the apical leaves of most cultures appeared green and healthier than the controls, but then succumbed to rapid necrosis after about one week. Latent or low-level bacterial or fungal contamination of explants and/or cultures has previously been shown to generate excessive ethylene gas leading to rapid tissue ripening, leaf narrowing and abscission (Jackson 1991; Marino 1996; Weingart 1997). In our hands, treating asymptomatic, not obviously contaminated shoot cultures with a combination of amphotericin B (2.5 mg/L) and gentamicin sulfate (50 mg/L) had no beneficial effect. However, the addition of an isothiazolone mixture (PPM™) by either pre-soak or addition to the culture medium improved plantlet survival and vigor, but will require further investigation before the nature of any beneficial effects on *Moringa* cultures can be described adequately.

Clonal Shoot Proliferation from Immature Seeds

Many *Moringa* species are prolific seed producers, and thus green seeds were evaluated for their potential to undergo epicotyl elongation followed by subsequent clonal shoot proliferation. Immature seeds with and without their investing seed coats were taken from surface sterilized seed pods and cultured on either solid or liquid MS medium containing 3% sucrose, and amended with 1 mg/L BAP and 1 mg/L GA_3. Activated charcoal (0.5% and 0.25%), which reportedly enhanced seedling development (Iyer and Gopinath 1999), was also examined. Seed pods were segregated into four groups based on their diameter and processed immediately upon receipt in Baltimore from St. Croix (by overnight courier). A total of 500 (408 *M. oleifera* and 92 *M. stenopetala*) seeds were cultured in two separate rounds. In the first group, 20% of *M. oleifera* immature seeds germinated, with subsequent shoot proliferation from the epicotyl meristematic tissue, compared to only 1 (0.8%) of the *M. stenopetala* seeds (Table 2). Most of the *M. stenopetala* explants produced a very friable white callus directly on the seed coat and wing tissue. The regenerating *M. oleifera* cultures produced an average of 4.7 shoots per cultured seed (Table 2A).

Table 2. Clonal shoot proliferation from germinated immature *Moringa oleifera* Lam. and *M. stenopetala* Bak. f. Cuf. seeds.

A. *M. oleifera* and *M. stenopetala* seeds explained to semi-solid medium in Petri-plates[1]

Moringa sp.	Seeds explanted	Immature seed cultures	Success rate[2]	Total shoot no.
M. oleifera	176	35	20%	164
M. stenopetala	128	1	0.78%	8

[1] Two seeds were removed from the *M. oleifera* treatment due to fungal contamination. All *M. oleifera* seeds were ~11–13 mm in diameter (spherical seeds) and all *M. stenopetala* seeds were ~25 mm in length and ~10 mm width (elongate seeds). Culture medium was MS basal medium amended with 1 mg/L BAP, 1 mg/L GA_3, 0.25% activated charcoal, 0.7% Gelrite and pH adjusted to 5.8 prior to autoclaving.

[2] Success was scored as formation of clonal shoots on the epicotyl end of the germinating seedling.

B. *M. oleifera* Immature Seeds Cultured on Rafts Supplied by Liquid Medium[1]

Seed size	# Seeds cultured	# of successes
#1 (no seed)[2]	0	0
#2 (~8 mm)	232	0
#3 (~9–10 mm)	163	0
#4 (11–13 mm)	41	30 (73%)

[1] Liquid culture medium was MS basal medium amended with 1 mg/L BAP, 1 mg/L GA_3, pH 5.8.

[2] Seed size #1 was too immature and seed embryos were not fully formed and could not be plated.

Fig. 1. *Moringa oleifera* Lam. shoots, clonally propagated from immature embryos.

MORINGA OLEIFERA

M. oleifera shoot development was dramatically improved for the #4 (largest) size class when cultured upon a membrane raft (73% success rate) rather than more traditional semi-solid medium (20% success rate), and shoot number was roughly comparable (4.7 and 3.6 shoots per culture, respectively; Fig. 1 and 2). The smallest useable seeds had jelly-like endosperms, and none of the 232 explants plated from these produced shoots. The larger seed sizes had a firm, well-formed embryo and endosperm that could easily be removed from the seed coat and cultured (Table 2B). The use of liquid culture medium and membrane rafts provides the cultured tissue with more intimate contact with medium and more rapid dilution of phenolic compounds and stress metabolites. The use of membrane rafts also facilitates aerobic growth and permits replacement of medium without disturbance of the cultured tissue. Since *Moringa* seedlings are very susceptible to water-logging these membrane rafts are thought to provide the best oxygen availability to the non-aerial portions of the developing plantlet.

Fig. 2. *Moringa oleifera* Lam. shoots, clonally propagated from immature embryos.

MORINGA STENOPETALA

To our knowledge, this is the first report of in vitro *Moringa stenopetala* cultivation. It is also the first report of clonal multiplication from whole germinating seedlings and their meristematic epicotyl tissues, and of the use of membrane rafts with *Moringa spp.* However, success was achieved with only a single explant out of a total of 128 (Table 2A), and examination of a wider range of seed sizes and conditions is indicated.

Root Development on Clonal Shoots. Shoot aggregates resulting from clonal shoot proliferation in the presence of exogenous plant growth regulators were transferred to a culture medium designed for root initiation (½-strength MS basal medium supplemented with 0.5 mg/L NAA; 93.1 mM) (Iyer and Gopinath 1999). This medium supported the development of robust primary and secondary roots (Fig. 3). Shoot aggregates which were not rooted, but did possess seedling radicle tissue, were transferred to sterile potting soil designed for seedlings, and to sterile Oasis horticulture cubes maintained in sterile GA-7 containers to evaluate their ability to root directly. The seed radicle remained intact in all cultures and appeared viable. Soil and the horticulture cubes were wetted with sterile water, shoot clumps were planted into either sterile soil or Oasis cubes, and the lids were loosely positioned on the GA-7 containers. For three days the shoots remained vigorous in appearance and then began to wilt and become necrotic. No root

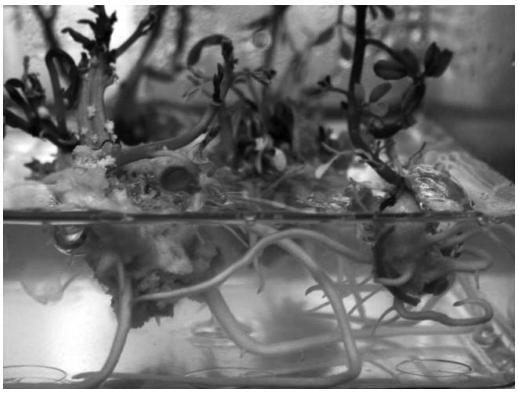

Fig. 3. Robust primary and secondary rooting from immature seed-derived *Moringa oleifera* Lam. plantlets.

initials formed and the cultures quickly declined and were terminated.

Transplantation of Clonal Shoots to Field

Prior to premature senescence, approximately 30 clonally propagated *Moringa oleifera* shoots with extensive root systems were maintained using an MS basal medium supplemented with 0.5 mg/L NAA and 0.1% (v/v) PPM™ isothiazolone mixture (final concentration: 5-chloro-2methyl-3(2H)-isothiazolone (1.35 mg/L) and 2-methyl-3(2H)-isothiazolone (0.41 mg/L). PPM amended culture medium induced substantially more vigorous growth and plantlet development than those without PPM. These plantlets formed substantial root systems and leaves of normal shape and size (Fig. 4). Established plantlets were then transplanted to a sterile potting soil under laboratory conditions for further root development and hardening prior to shipment for field planting. Similar results with PPM have been reported in the Brassicas (Fuller and Pizzey 2002). Only two of the thirty rooted plants survived transplanting to soil under laboratory conditions. The surviving plants were shipped overnight to a greenhouse at the University of the Virgin Islands, St. Croix, U.S.V.I. (Fig. 5). These plants have now been transplanted from greenhouse to field and continue to thrive.

Fig. 4. Immature embryo-derived *Moringa oleifera* Lam. plantlets in rooting medium containing 0.1% (v/v) PPM (isothiazolone mixture), ready for transplantation.

Conclusions

We have used a wide variety of explants from accessions of *Moringa oleifera* to establish cultures, to improve upon node culture and axillary node regeneration techniques, and to validate protocols for embryogenesis. While only modest gains were made, we determined that *M. oleifera* tissues respond well to the early stages of in vitro cultivation. Our initial approach to identify a superior in vitro protocol was to explant seedling meristematic axillary nodes across a range of basal culture media formulations. Evaluation of 21 media resulted in the identification of superior media, but also resulted in the implication of a senescence factor which arrested plantlet development at a critical stage of growth. While this is not an uncommon problem, it is unusual in as robust and easily cultivated a species as *M. oleifera.* Good root development was induced, but early culture senescence continues to limit the transfer of this technology to an applied micropropagation setting. We have attempted to rule out ethylene overproduction and latent microbial contamination as two of the most likely causes of early senescence in our cultures. Shoot formation and multiplication was obtained from leaf axils of germinating immature seedlings on both semi-solid and liquid culture media and regeneration frequency was dramatically improved when tissue explants were placed upon semi-

Fig. 5. Tissue culture derived *Moringa oleifera* Lam. plants in greenhouse.

permeable membrane rafts floating on a liquid medium. The regeneration success rate was good with *M. oleifera,* but poor with *M. stenopetala,* and this may be due to the limited size range of *M. stenopetala* seeds used in this study. Rooted plantlet survival has been enhanced by the use of isothiazolones, but it is unclear if this is due to their putative plant growth regulatory activity as suggested in the patent literature, or to their antimicrobial activity. More extensive evaluation is now planned with the ultimate objective of extending these observations to some of the endangered *Moringa* species.

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List of Abbreviations

ADS, adenine sulfate; **BAP,** 6-benzylaminopurine; **GA_3,** gibberellic acid-A_3; **IAA,** indole-3-acetic acid; **IBA,** indole-3-butyric acid; **NAA,** -naphthaleneacetic acid; **PPM,** a proprietary mixture of 5-chloro-2-methyl-3(2H)-isothiazolone (0.1350%) and 2-methyl-3(2*H*)-isothiazolone (0.0412%); **TDZ,** 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea.

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