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IN VITRO PROPAGATION OF *MORINGA OLEIFERA* L. UNDER SALINITY AND VENTILATION CONDITIONS

Salem J. M.

Central Laboratory of Genetic Engineering, Sohag University, 82524 Sohag, Sohag, Egypt

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Summary: *Moringa oleifera* is a fast growing tree under field conditions. A micropropagation protocol of *M. oleifera* was established, and effects of salinity and ventilation on fast growing cultured plant materials were studied. Efficient shoot formation and multiplication were obtained when nodal or shoot tip explants were cultured on MS medium supplemented with 2.5 μM 6-benzylaminopurine (BAP) at mild ventilation conditions. Adventitious root formation was obtained when shoot cuttings were cultured on MS medium supplemented with 4.92 μM indole-3-butyric acid (IBA). Under salinity, *M. oleifera* shoots showed symptoms of vitrification such as retardation in shoot multiplication and growth, stimulated callus formation on the basal part of the cultured explants and increased shoot thickness. *M. oleifera* shoot multiplication and growth were negatively affected by ventilation deficiency.

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Keywords: *Moringa oleifera* L., micropropagation, salinity, ventilation, vitrification.

Abbreviations: BAP – 6-benzylaminopurine; IBA – indole-3-butyric acid; MS medium – Murashige and Skoog nutrient medium.

INTRODUCTION

Mediterranean basin suffers from water scarcity; which is one of the main factors limiting the expansion in the cultivation of new areas and food production particularly between 2000 and 2025 (Chartzoulakis et al., 2000). To control water shortage, the use of water of marginal quality, such as saline water, will be an essential prerequisite to produce enough food for the region's population increase. Application of saline water in agriculture requires deep understanding of how to overcome the negative effects of salinity. Tissue culture technique supplements us with artificial tools to study the effect of salinity on plants under controlled conditions and production of genetically engineered plants. However, this technique has been successfully applied to a wide range of plant species all over the world (Gayathri et al. 2015).

Vitrification, also known as

*Corresponding author: hashsalem@yahoo.com

hyperhydricity, is a morphological, anatomical and physiological malformation that causes water-swollen of the *in vitro* cultured plants (Paques and Boxus, 1987). High concentrations of ethylene and CO_2 were found to accumulate in tightly closed vessels leading to shoot vitrification, and it could be prevented by sufficient gas exchange during *in vitro* shoot culture (Lai et al., 2005). Increased ethylene production in several plants under conditions of salt stress was reported (Zapata et al., 2004; Cordovilla et al., 2014). The relation between salinity and ventilation in tomato seedlings grown *in vitro* was studied (Mills and Tal, 2004).

Among twelve species of genus *Moringa*, the most commonly cultivated and widespread one is *Moringa oleifera* (Olson, 2002). *M. oleifera* belongs to family Moringoryaceae. It is a drought tolerant plant species, which grows in almost all types of well-drained soil. *M. oleifera* was reported to provide nutritional benefits to human and livestock (Fuglie, 2001). All parts of *M. oleifera* are consumed by humans in different ways. The leaves of *M. oleifera* are a source of digestible proteins, calcium, iron, vitamin C, vitamin A and antioxidant compounds such as flavonoids, phenolics, carotenoids, and vitamin E (Sultana and Anwar, 2008). Seeds are eaten green or dry (Berger et al., 1984) and their powder is used for water purification (Anwar et al., 2007; Kardam et al., 2010). In addition, seeds are used as a source of sweet oil (30-40% of seed weight). *Moringa* oil resists rancidity and it can be used for fine machine lubrication, and manufacture of perfume and hair care products (Tsaknis et al., 1999). Plant extracts, oils, salves, pulps, and powders have been prepared from *M. oleifera* to treat inflammations, hypotensive, cardiovascular problems, anti cancerogenic effects, gastrointestinal illnesses, nervous disorders, infectious diseases, cold, fever, common micronutrient disorders (such as vitamin A, iron and iodine deficiencies) and excessive pain (Caceres et la., 1992; Nikkon et al., 2003).

M. oleifera is traditionally propagated by seeds. Plants obtained from seeds vary in genotype and phenotype thus leading to variations in quality and quantity of the yield. Therefore, propagation of elite genotype can be faithfully fulfilled via micropropagation techniques. In general, explant type, culture conditions and plant growth regulator regimes must be defined for each genotype in order to develop a successful micropropagation protocol (Stephenson and Fahey, 2004; Marfori, 2010; Saini et al., 2012).

M. oleifera is a fast growing tree under field conditions (Marfori, 2010; Saini et al., 2012), and its *in vitro* growth is much faster than in the greenhouse (Förster et al., 2013). Under insufficient aeration in tight sealed containers, *M. oleifera* shoots as well as other plant species show a degree of vitrification and callus formation (Riyathong et al., 2010; Saini et al., 2012; Förster et al., 2013), which may be due to accumulation of CO_2 and ethylene (Paques and Boxus, 1987; Ziv, 1991; Zobayed et al., 2001). Also, salinity induces vitrification when plants are grown in closed containers (Mills and Tal, 2004). Under these conditions, callus formation is commonly associated with vitrification (Paques and Boxus, 1987; Ziv, 1991; Zobayed et al., 2001).The

vitrification phenomenon including callus formation on the base of explants should be excluded during clonal multiplication.

The aim of this work was to establish a protocol for *in vitro* clonal propagation of *M. oleifera*, and to study how *in vitro* multiplication of fast growing tissue of *M. oleifera* was influenced by salinity and ventilation.

MATERIAL AND METHODS

Micropropagation of *M. oleifera*

Nodal segment culture

Under room conditions, *Moringa* seeds were cultured in plastic pots containing 2 kg of clay soil. The soil was watered every three days to reach full water capacity in each time point. Four week-old-plants (50-60 cm tall) were harvested, and their nodes (8-10 nodes per plant) were parted into upper, middle and lower nodes. Nodes were surface sterilized inside the laminar flow hood by immersion in 70% ethyl alcohol (v/v) for 30 sec and 20% sodium hypochlorite (v/v) for 3 min, followed by rinsing three times in sterile distilled water, 10 min each. The nodal segment ends were discarded and 1.2-1.5 cm long nodes were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and different concentrations of BAP (0.0, 1.25, 2.5, or 5.0 µM). The medium was solidified with 8 g L^{-1} agar at pH 5.8. The following vitamins (mg L^{-1}) were added: myo-inositol (100), vitamin B_1-hydrochloride (4), nicotinic acid (4), pyridoxal hydrochloride (0.7), biotin (0.04) and folic acid (0.5). Nodal cultures were incubated at 25±2°C and 16-h photoperiod (tissue culture room conditions) for 25 days. Growth parameters such as number and size of formed shoots, number of formed nodes per shoot, and shoot cluster fresh weight (FW) were recorded.

Shoot tip culture

Healthy uniform seeds of *M. oleifera* were surface sterilized by immersion in 0.1% mercuric chloride (w/v) for 4 min and 20% sodium hypochlorite (v/v) for 7 min, followed by rinsing three times in sterile distilled water, 10 min each. Seeds were germinated on MS medium supplemented with 3% sucrose and 2.5 µM BAP under tissue culture room conditions. Shoot tip cutting of seedlings containing shoot apical meristem (2-3 mm) was obtained and cultured on MS medium supplemented with 2.5 or 5 µM BAP. After ten weeks, the obtained mass was subcultured on MS medium without BAP for two weeks to stimulate shoot formation. The number of formed buds and FWof the obtained mass/ shoot tip were determined.

Rooting

For root induction, microshoots (2-3 cm long) were subcultured on full strength MS medium supplemented with or without 4.92 µM IBA for two weeks. The number of the formed roots per shoot and their length were determined. The plantlets were transferred to soil after hardening for four weeks in a tissue culture room.

Effect of salinity and ventilation on multiplication and growth of *M. oleifera*

Nodal segments (1.2-1.5 cm long) were cultured on MS medium supplemented with 2.5 µM BAP and different concentrations of NaCl (1, 2, 4, or 8 g L^{-1}), or incubated in tight-, mild-

or loose-sealed lid glass jars, to study the effect of salinity and ventilation rate on rapid clonal propagation of *M. oleifera*, respectively. Parafilm was used to avoid contamination in loose treatment. Cultures were incubated under tissue culture room conditions for 25 days. Shoot multiplication and shoot growth were determined.

Data analysis

Six replicates with thirty explants for each treatment were done in all experiments. Data are presented as means ± standard deviation (SD) according to the method described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Micropropagation of *M. oleifera*

The explants for cultivation were collected from young (four-week-old) plants grown under laboratory conditions as was previously recommended (Stephenson and Fahey, 2004; Förster et al., 2013); thus, healthy plant material for *in vitro* culture was available regardless of the season.

High shoot multiplication was obtained over a cultivation period of 25 days because the nodal explants were collected from young plants, and all explants formed shoots regardless of node position or cytokinin (BAP) concentration (Table 1). The induction of shoot multiplication on growth regulators-free MS basal medium indicated that endogenous hormonal content in the nodal segments was sufficient to trigger shoot formation and it was improved by application of exogenous cytokinin in *M. oleifera* as it was previously noticed in other plants (Hassanein, 2004; Salem and Hassanein, 2013). Multiple shoot formation on cotyledonary node and single node segments of *M. oleifera* on basal MS medium without growth regulators was reported by Steinitz et al. (2009). There was no clear indication of the source of obtained shoots because they resulted from pre-existing axillary meristems or through induction of adventitious meristems (Steinitz et al., 2009). Formation of additional axillary meristems was reported by Graham et al. (1998).

When nodal segments were obtained from different parts of *ex vitro* grown plants, and cultured on MS medium with different BAP concentrations, the upper nodal segments expressed the highest shoot multiplication regardless of the BAP concentration; however, 2.5 μM BAP was the best one as it stimulated the production of the highest number of shoots (Table 1, Fig. 1a). On the other hand, the highest fresh shoot cluster mass was obtained in the presence of 5 μM BAP (Table 1), however it was due to the large callus mass formed in shoot cluster base. Therefore, in the current experimental scheme, the concentration of 2.5 μM BAP is recommendable for multiplication of *M. oleifera*.

In another study (Saini et al., 2012), the highest shoot multiplication was obtained from basal parts of *in vitro* grown shoots. The established ratio between exogenous BAP and endogenous hormones determined the number of shoots per nodal segment. Other reports indicated that induction of specific type of organogenesis needed specific endogenous hormonal content and some modifications in the hormonal part of the

Table 1. Effects of nodal segment position and BAP concentration on the formation and growth of shoots of *M. oleifera* explants.

Node position	BAP concentration [μM]	Number of shoots/explant	Shoot length [cm]	Number of nodes/shoot	Shoot cluster FW/explant [g]
Lower	0	2.33 ± 0.57	4.50 ± 0.14	5.33 ± 0.58	0.28 ± 0.05
Middle		3.00 ± 0.30	4.00 ± 0.20	5.00 ± 1.00	0.18 ± 0.01
Upper		4.33 ± 0.57	4.00 ± 0.50	4.67 ± 0.58	0.20 ± 0.01
Lower	1.25	2.67 ± 1.15	4.40 ± 0.26	6.00 ± 0.00	0.39 ± 0.04
Middle		4.00 ± 1.00	4.60 ± 0.10	6.33 ± 0.58	0.34 ± 0.01
Upper		4.33 ± 0.58	6.06 ± 0.15	7.33 ± 0.58	0.41 ± 0.12
Lower	2.5	5.67 ± 0.57	2.73 ± 0.30	5.00 ± 1.00	0.68 ± 0.05
Middle		7.33 ± 0.57	1.47 ± 0.20	4.67 ± 0.58	0.89 ± 0.16
Upper		9.00 ± 1.00	1.45 ± 0.21	5.33 ± 0.58	0.39 ± 0.03
Lower	5	4.67 ± 0.58	4.80 ± 0.20	9.67 ± 0.58	0.91 ± 0.04
Middle		6.33 ± 0.58	4.77 ± 0.15	10.00 ± 1.00	0.92 ± 0.04
Upper		6.67 ± 1.15	4.80 ± 0.20	9.67 ± 1.53	0.95 ± 0.01

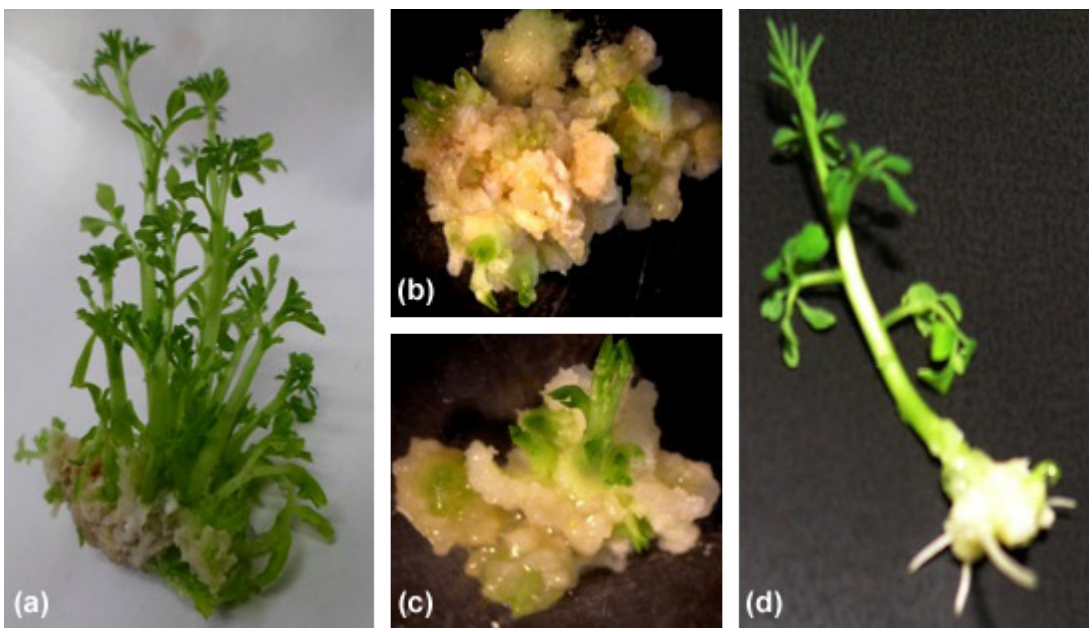

Figure 1. Different regeneration pathways of *M. oleifera:* using nodal segment cultured on MS medium with (a) 2.5 μM BAP for 25 days resulted in best shoot multiplication; using shoot tip cultured on MS medium with (b) 5 μM, or (c) 2.5 μM BAP for ten weeks formed callus with adventitious green buds; using microshoot cultured on MS medium with (d) 4.92 μM IBA for two weeks improved root formation.

medium (Hassanein, 2004; Salem and Hassanein, 2013).

Shoot tips obtained from aseptically grown seedlings were cultured on MS medium supplemented with 2.5 or 5 µM BAP for 10 weeks. Callus mass with numerous adventitious green buds was formed, and 5 µM BAP was the better concentration (Table 2, Fig. 1b, c). The initiated masses were subcultured for further two weeks on MS basal medium without BAP to avoid callus formation and stimulate further shoot growth. Shoot tips obtained from seedlings were better explants because they formed a higher number of buds in comparison to nodal segments obtained from seedling or *ex vitro* grown plants (Tables 1, 2). Similar results were reported by Rani et al. (2006). However, compared to shoot tips, nodal segments produced more elongated shoots with more nodes (Fig. 1a, c). Therefore, according to our results, nodal segments cultured on MS medium containing 2.5 µM BAP can be defined as the most efficient approach for *M. oleifera* micropropagation.

Spontaneous root formation was noticed on hormone-free MS medium (Table 2); which means that root development in *M. oleifera* plants can be easily initiated. Root formation was improved when microshoots were cultured on MS medium with 4.92 µM IBA (Fig. 1d). Addition of a root inducing growth regulator such as IBA increased the number of the formed roots in short time (two weeks) but affected negatively root system enlargement.

Effect of salinity and ventilation on multiplication and growth of *M. oleifera*

When nodal segments were subjected to salinity, even at low concentrations, a reduction in shoot number per explant and shoot growth was noticed (Table 3 and Fig. 2) that was associated with increased enlargement of explants. Fresh mass of explants increased in the presence of 2 or 4 g L^{-1} NaCl, as well as large callus mass was formed (Table 3, Fig. 2c, d). The relatively high concentration of NaCl led to shoot death (Fig. 2e).

Typically, the vitrified shoots are larger compared to normally growing

Table 2. Effect of growth regulators on shoot and root formation and growth of *M. oleifera* L.: (A) shoot tip explants were cultured on MS medium with 0, 2.5, or 5 µM BAP for 10 weeks to form shoots; (B) microshoots were cultured on MS medium without and with 4.92 µM IBA for 2 weeks to form adventitious roots.

(A) BAP concentration [µM]	Number of buds/explant	FW/bud cluster [g]
0	14.67 ± 0.57	1.75 ± 0.04
2.5	17.33 ± 0.57	2.73 ± 0.30
5	21.33 ± 1.53	3.18 ± 0.18
(B) IBA concentration [µM]	Number of roots/shoot	Root length [cm]
0	2.00 ± 0.00	2.4 ± 0.20
4.92	3.67 ± 0.58	2.3 ± 0.35

Table 3. Effect of salt concentration on shoot proliferation and growth of *M. oleifera* L nodal explants.

NaCl concentration [g L^{-1}]	Number of shoots/explant	Length of shoots [cm]	FW/shoot cluster [g]
0 (control)	8.67 ± 0.57	4.33 ± 0.57	0.7971 ± 0.080
1	6.67 ± 0.57	2.76 ± 0.20	0.6904 ± 0.006
2	5.33 ± 0.57	1.97 ± 0.25	1.1792 ± 0.269
4	4.33 ± 0.57	1.46 ± 0.05	1.0825 ± 0.249
8	3.67 ± 0.57	1.13 ± 0.05	0.3768 ± 0.049

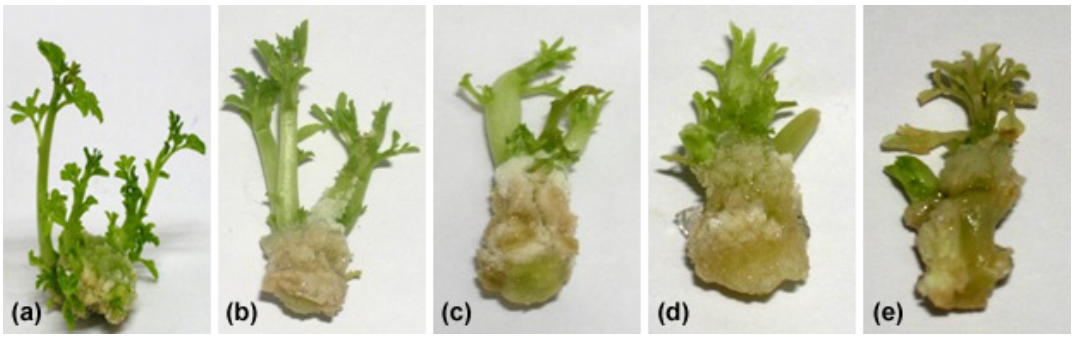

Figure 2. Effect of salinity on shoot formation and vitrification in *M. oleifera* L nodal explants cultured on MS medium with 2.5 μM BAP and (a) 0 (control), (b) 1, (c) 2, (d) 4, and (e) 8 g L^{-1} NaCl.

ones; besides they are thick with short stems and dense leaves (Sharma and Mohan, 2006). Usually, vitrification is accompanied by enlargement and callus formation (Paques and Boxus, 1987; Ziv, 1991). Interaction between salinity and ventilation was studied by Mills and Tal (2004). They reported that while tomato seedlings were relatively tolerant to salinity in unventilated conditions, they were more susceptible in ventilated ones.

In the present work, the number of formed shoots, shoot growth and formation of callus on the base of explants were influenced by the ventilation rate in the jars (Table 4 and Fig. 3). In tightly sealed jars with low ventilation, shoot enlargement and callus formation on the base of *M. oleifera* shoots were accompanied by retardation of shoot multiplication and shoot growth (Table 4). This may be due to vitrification and ethylene accumulation (Zobayed et al., 2001), accumulation of carbon dioxide and depletion of oxygen (Jackson et al., 1991), as well as anatomical variability (Majada et al., 2000). Sharma and Mohan (2006) reported that vitrification of *in vitro* raised shoots of *Chlorophytum borivilianum* was minimized when BAP was omitted from the cultural medium. In tightly sealed jars, abscission of plant leaves, enlargement of the cultured

Table 4. Effect of ventilation rate on shoot proliferation and growth of *M. oleifera* L nodal explants.

Rate of ventilation	Number of shoots/explant	Length of shoot [cm]	FW/shoot cluster [g]	Callus formation
Mild	7.00 ± 1.00	2.50 ± 0.28	1.2503 ± 0.18	+
High	4.67 ± 0.57	3.00 ± 0.80	0.5501 ± 0.07	–
Low	5.67 ± 0.57	1.67 ± 0.05	1.2523 ± 0.25	+

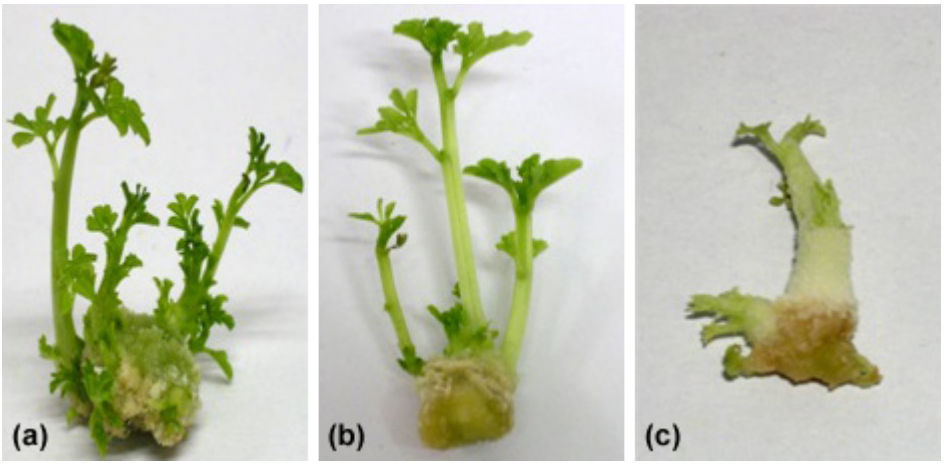

Figure 3. Effect of ventilation rate on shoot proliferation from *M. oleifera* L nodal segments: (a) mild ventilation; (b) high ventilation; (c) low ventilation.

explants, and formation of callus on the base of the cultured shoots were detected in *M. oleifera* as well as in other plant species (Paques and Boxus, 1987; Ziv, 1991; Zobayed et al., 2001).

In the present work, cone shape shoots due to tissue enlargement were formed (Fig. 3c). This abnormality was accompanied by a reduction of shoot growth and stimulated vitrification (Park et al., 2004; Lai et al., 2005). Also, net photosynthetic rate was changed which affected negatively plant growth (Zobayed et al., 2002). The unventilated container established atmosphere with relatively high concentrations of CO_2, ethylene and water vapors (Smith et al., 1990; Jackson et al., 1991) that caused hyperhydricity of *in vitro* propagated plant organs (Debergh et al., 1992; Mills and Tal, 2004). Thus, prolonged cultivation under low ventilation rate (or insufficient ventilation) resulted in a loss of cultured plant material of *M. oleifera* in two months (Fig. 3c).

High ventilation established in loosely sealed jars stimulated shoot formation and growth without formation of callus on the base of explants (Fig. 3b). Our results were in agreement with another study showing that sufficiently ventilated *in vitro* grown plants exhibited improved shoot growth compared to plants cultivated in low ventilation conditions (Sharma and Mohan, 2006).

In gently sealed jars (Fig. 3a) where

mild ventilation was achieved, the values of growth parameters (Table 4) were improved compared to growth in loosely sealed jars (Fig. 3b); callus mass was also formed on the explant base, however, the number of initiated shoots was increased.

In conclusion, upper nodal explants cultured on MS medium supplemented with 2.5 µM BAP and mild ventilation can be recommended for efficient *M. oleifera* multiplication. *M. oleifera* shoot multiplication and growth were negatively influenced by ventilation deficiency and salinity stress.

REFERENCES

Anwar F, S Latif, M Ashraf, AH Gilani, 2007. Review article. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother Res, 21: 17–25.

Berger MR, M Habs, SAA Jahn, D Schmahl, 1984. Toxicological assessment of seeds from *Moringa oleifera* and *Moringa stenopetala*, two highly efficient primary coagulants for domestic water treatment of tropical raw waters. East Afr Med J, 61: 712–716.

Caceres A, A Saravia, S Rizzo, L Zabala, E De Leon, F Nave, 1992. Pharmacologic properties of *Moringa oleifera*. 2: screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol, 36: 233–237.

Chartzoulakis K, M Loupassaki, M Bertaki, I Androulakis, 2002. Effect of NaCl salinity on growth, ion content and CO_2 assimilation rate of six olive cultivars. Sci Hortic, 96: 235–247.

Cordovilla MP, M Bueno, C Aparicio, M Urrestarazu, 2014. Effects of salinity and the interaction between *Thymus vulgaris* and *Lavandula angustifolia* on growth, ethylene production and essential oil contents. J Plant Nutr, 37: 875–888

Debergh PC, J Aitken-Christie, D Cohen, B Grout, S Von Arnold, R Zimmerman, M Ziv, 1992. Reconsideration of the term 'vitrification' as used in micropropagation. Plant Cell Tissue Organ Cult, 30: 135–140.

Förster N, I Mewis, C Ulrichs, 2013. *Moringa oleifera* - establishment and multiplication of different ecotypes *in vitro*. Ges Pfl, 65: 21–31.

Fuglie LJ, 2001. The Miracle Tree: The Multiple Attributes of Moringa. Church World Service, West Africa Regional Office, Dakar.

Gayathri M, PS Kumar, AML Prabha, G Muralitharan, 2015. *In vitro* regeneration of *Arachis hypogaea* L. and *Moringa* oleifera Lam. using extracellular phytohormones from Aphanothece sp. MBDU 515. Alg Res, 7: 100–105.

Graham AW, MA Wallwork, M Sedgley, 1998. Lignotuber bud development in *Eucalyptus cinerea* (F. Muell. ex benth). Int J Plant Sci, 159: 979–988.

Hassanein AM, 2004. Hormonal requirements trigger different regeneration pathways in *Alhagi graecorum*. J Plant Biotech, 6: 171–179.

Jackson MB, AJ Abbott, AR Belcher, KC Hall, R Butler, J Cameron, 1991. Ventilation in Plant Tissue Cultures and Effects of Poor Aeration on Ethylene and Carbon Dioxide Accumulation, Oxygen Depletion and Explant Development. Ann Bot, 67: 229–237.

Kardam A, KR Raj, JK Arora, MM Srivastava, S Srivastava, 2010. Artificial Neural Network Modeling for Sorption of Cadmium from Aqueous System by Shelled *Moringa oleifera* Seed Powder as an Agricultural Waste. J Water Res Prot, 2: 339–344.

Lai C, H Lin, SM Nalawade, W Fang, H Tsay, 2005. Hyperhydricity in shoot cultures of Scrophularia yoshimurae can be effectively reduced by ventilation of culture vessels. J Plant Physiol, 162: 355–361.

Majada JP, F Tadeo, MA Fal, R Sánchez-Tamés, 2000. Impact of culture vessel ventilation on the anatomy and morphology of micropropagated carnation. Plant Cell Tissue Organ Cult, 63 (3): 207-214.

Marfori EC, 2010. Clonal micropropagation of *Moringa oleifera* L. Philipp Agric Sci, 93: 454–457.

Mills D, M Tal, 2004. The effect of ventilation on the *in vitro* response of seedling of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt stress. Plant Cell Tissue Organ Cult, 78: 209–216.

Murashige T, FA Skoog, 1962. Revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant, 15: 473–497.

Nikkon F, ZA Saud, MH Rahman, MDE Haque, 2003. *In vitro* antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. Pak J Biol Sci, 6: 1888–1890.

Olson ME, 2002. Combining data from DNA sequences and morphology for a phylogeny of *Moringaceae* (*Brassicales*). Sys Bot, 27: 55–73.

Paques M, P Boxus, 1987. Vitrification: review of literature. Acta Hort, 212: 155–66.

Park SW, JH Jeon, SH Kim, YM Park, C Aswath, H Joung, 2004. Effect of sealed and vented gaseous microenvironment on hyperhydricity of potato shoots *in vitro*. Sci Hort, 99: 199–205.

Rani G, D Talwar, A Nagpal, GS Virk, 2006. Micropropagation of *Coleus blumei* from nadal segments and shoot tips. Biol Plant, 50 (4): 496–500.

Riyathong T, S Dheeranupattana, J Palee, L Shank, 2010. Shoot Multiplication and Plant Regeneration from *In Vitro* Cultures of Drumstick Tree (*Moringa oleifera* Lam.). In: Proceedings of the 8th International Symposium on Biocontrol and Biotechnology. King Mongkut's Institute of Technology Ladkrabang and Khon Kaen University, Nongkhai Campus, Thailand, 99–104.

Saini RK, NP Shetty, P Giridhar, GA Ravishankar, 2012. Rapid *in vitro* regeneration method for *Moringa oleifera* and performance evaluation of field grown nutritionally enriched tissue cultured plants. Biotech, 2: 187–192.

Salem J, AM Hassanein, 2013. Hormonal requirements trigger different organogenic pathways on tomato nodal explants. Amer J Plant Sci, 4: 2118–2125.

Sharma U, JSS Mohan, 2006. Reduction of vitrification in *in vitro* raised shoots of *Chlorophytum borivilianum* Sant. & Fernand, a rare potent medicinal herb. Ind J Exp Biol, 44: 499–505.

Smith EF, AV Roberts, J Mottley, 1990. The

preparation *in vitro* of chrysanthemum for transplantation to soil. 3. Improved resistance to desiccation conferred by reduced humidity. Plant Cell Tissue Organ Cult, 21: 141–145.

Snedecor GW, WG Cochran, 1980. Statistical Methods, 7th Edition. The Iowa State University Press, Ames, 166–190.

Steinitz B, Y Tabib, V Gaba, T Gefen, Y Vaknin, 2009. Vegetative micro-cloning to sustain biodiversity of threatened *Moringa* species. *In Vitro* Cell Dev Biol Plant, 45: 65–71.

Stephenson KK, JW Fahey, 2004. Development of tissue culture methods for the rescue and propagation of endangered *Moringa* spp. germplasm. Econ Bot, 58: 116–124.

Sultana B, F Anwar, 2008. Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chem, 108: 879–884.

Tsaknis J, S Laias, V Gergis, V Douroglou, V Spiliotis, 1999. Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya. J Agric Food Chem, 47: 4495–4499.

Zapata PJ, M Serrano, T Pretel, A Amoros, MA Botella, 2004. Polyamines and ethylene changes during germination of different plant species under salinity. Plant Sci, 167: 781–788.

Ziv M, 1991. Vitrification: morphological and physiological disorders of *in vitro* plants. In: Debergh PC, Zimmerman RH (eds.) Micropropagation. The Netherlands: Kluwer Academic Publishers, 45–69.

Zobayed SMA, J Armstrong, W Armstrong, 2001. Micropropagation of potato: evaluation of closed, diffusive and forced ventilation on growth and tuberization. Ann Bot, 87: 53–59.

Zobayed SMA, J Armstrong, W Armstrong, 2002. Multiple shoot induction and leaf and flower bud abscission of Annona cultures as affected by types of ventilation. Plant Cell Tiss Org Cult, 69:155–165.