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STUDIES ON DIRECT CAULOGENESIS CULTURE OF *MORINGA OLEIFERA*, AN EXOTIC MULTIPURPOSE TREE SPECIES FOR ROMANIA

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Abstract
In Romania, M. oleifera Lam. species caught the attention of growers and consumers only with five years ago. Because planting material for commercial cultivation is now very low accesible, as for the culture establishment, like many other species of cultivated plants M.oleifera Lam., tehniques "in vitro" culture applications have the outstanding contributions to overcoming of the limits facing culture technology of this species. First, the attention is given to rapid multiplication of biological material genetically pure and at the same time is free from diseases and pests. The method of micropropagation by axillary branching method using apical buds as explants and nodal explants from seedlings was initiated in this study. Cultures were initiated by multiple shoots on MS medium containing variable concentrations of BA 1.0-2.5 mg/l and IBA 0.2 mg/l. Maximum shoot proliferation was achieved on medium containing BA more than 2.0 mg /l and 0.2 mg/l IBA within 3 subcultures. The proliferation of shoots was improved in the medium of the same composition after the addition of citric acid as an antioxidant in reducing the emission of polyphenols. A maximum rate of 5 copies of shoots was performed on the addition of citric acid (10 mg/l) in the environment. Leaves were excised from the M.oleifera Lam. plants propagated through "in vitro", for callus induction.

Keywords*: culture technology*, direct caulogenesis, *Moringa oleifera*

1. INTRODUCTION

Moringa is a perennial softwood tree of many uses. It is known by several names, including horseradish tree, ben oil tree, drumstick, and sohnja in India; shagarat al rauwag in Sudan; and murunga-kai, balunggay, or malunggay in the Philippines (Benge, 1987; Jahn, 1996; Ramachandran, 1980).

Although not known for a long time, ancestral *Moringa oleifera* Lam. tree belonging to one genus of *Moringaceae* family, originating from the southern foothills of the Himalayas in northwest India, has demonstrated these qualities.

The leaves, shoots and young pods have a high vitamin content (A, B, C, E), calcium, iron, magnesium, phosphorus and highly beneficial for many other body substances. In addition, the values of Omega-3 acids are very high, and antioxidants represented by ORAC values, recorded the highest amount of all the known plants.

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The large properties that have vegetative parts (leaves, shoots, roots, pods and seeds) of that plant and multiple fields of use such as vegetable food (Peter, 1979; Girija, 1982; Price, 1985), medicine through numerous compounds that act as antimicrobial agents, antifungal and antibacterial (Eilert, 1981; Caceres et al., 1991a, 1992; Anwar et al., 2007), some with antitumor (Guevara, et.al., 1999) and anti-carcinogenic properties (Hartwell, 1982), a source of edible oil (30-35% in seeds), possible source of biodiesel and lubricant (Rashid, et al, 2008), water purifier in counties with tropical climate (Berger et al. 1984; Jahn et al., 1986; Duke, 1987; Bensimon, 1992; Mayer and Stelz, 1993), and also in soil phytoremediation (Palada, 1996).

Moringa originated in India and Arabia. It is widely cultivated or naturalized in several countries in the tropics. It is a slender, deciduous tree with drooping leaves, and it grows to about 10 m tall. The branches and stems are brittle, with corky bark. The leaves are feathery, pale green, compound, tripinnate, 30 to 60 cm long, with 3 to 9 leaflets on the ultimate pinnules (Caceres, 1991b). Each leaflet is 1.3 to 2.0 cm long and 0.6 to 0.3 cm wide. Lateral leaves are somewhat elliptical, while the terminal ones are obovate and slightly larger than the lateral ones (Dao and Hema-Kabore, 2015).

The flowers (Figure 1) are fragrant, white or creamy-white with yellow stamens; 2.5 cm in diameter, borne in sprays.

Figure 1. Aspects of *Moringa oleifera* Lam. flowers
(http://www.starrenvironmental.com/images/search/?q=*Moringa*+*oleifera*)

The pods are pendulous, brown, triangular, tapering at both ends, 30 to 120 cm long and 1.8 cm wide (Figure 2), and contain about 20 seeds embedded in the pith. Pods split lengthwise into three parts when dry. Seeds are dark brown with three papery wings.

Figure 2. Aspects of *Moringa oleifera* Lam. pods
(http://www.starrenvironmental.com/images/search/?q=Moringa+oleifera)

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The main root is thick. The tree flowers and produces pods and seeds throughout the year (Ramachandran et al., 1980).
In Romania, *Moringa oleifera*, known worldwide as the "Tree of Life", is beging of last 3 years to acclimatised, at Buzău county, to the Vegetable Research and Development Station by researcher Vânătoru Costel on the grounds that it can replace meat in the human diet, by eating beans and young leaves, rich in nutritional properties *(http://www.scdlbuzau.ro/).*Through its research, engineer Vânătoru C. aims to transform this plant into a perennial autochthonous culture that would open new roads in many areas in our country.
The unconventional method used in the present study, applied to *M. oleifera* Lam. was represented by the culture of meristematic apexes, namely the culture consisting of apical meristem accompanied by the first pair of foliar primaries, taken from the peaks of the lateral branches and from the main stem and also, as well as by the use leaf to induce morphogenetic structure, prelevated from the youngest part of the seedlings stem.

2. MATERIALS AND METHODS

Biological material submitted explant type used new species *Moringa oleifera* micropropagation consisted of meristematic apexes, nodal and mesophilic leaf explants taken from the seedlings resulting from seed germination in the greenhouse and laboratory. The experiment was conducted at the aclimatized culture laboratory of the Department of Biotechnology (Figure 3.a), from Faculty of Biotehnology and in the greenhouse automatized unit (Figure 3.b) from HORTINVEST Research Centre, of UASMV Bucharest, during the period from March to June 2016.

a.

b.

Figure 3. Biological material from *Moringa oleifera* Lam. obtained *in controlled conditions:* ***a.)***aclimatized culture laboratory ***condition; b.)*** greenhouse automatized unit.

Characteristics of the compartment distributed to our experiment, with the scope to obtain seedlings from HORTINVEST Research Centre, of UASMV Bucharest, offer facilities automatizeted: heating, shielding, air-conditioning, electric set up, tide type irrigation, microaspersion, 4 assimilation lamps.
Rosu (1999, 2006) appreciate that the age of the donor plant is of particular importance for the induction of morphogenetic processes and it is therefore recommended, especially in the case of

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wood species, the initiation of cultures by extraction of explants from the physiologically youngest areas (Rosu, 2006, 1999). Given this consideration, the explants prelevated from *Moringa oleifera* Lam. seedlings were used as biological material obtained after 2 months of cultivation in the two growing areas of culture. The first inoculation of biological material it was made in controlled conditions, on Murashige-Skoog (1962) basic medium (MS), half strength for the macro and microelements, without hormones, at ph=5.8, containing 3% sucrose and 0.8 % Agar Noble, for realised the selection of healthy viable explants. Culture media were autoclaved at 1.1 kg.cm^{-2}, 121^{o} C for 20 min. The experiments were in two replicates.

The surface sterilization of the explants: an aqueous solutions of sodium hypochlorite (0.5%) for 20 min. followed by 3 rinses with sterile distilled water. After surface sterilization of the explants, the leaf explants and apexes of Moringa were transferred into petri dishes with sterile culture medium prepared in advance for inoculation.

Incubation of the cultures was performed in the growth chamber at temperatures gives 25°C, light intensity of 3000 lux, and 16 hours fotoperiod alternating 8-hour dark light whatever.

For the extension of the initial primordial, nodal explants and meristematic apexes as well as for the development of morphogenetic structures continuously at *Moringa oleifera* Lam., experiments were initiated under conditions of observing *in vitro* cultures procedures on MS (1962) medium supplemented with phytoregulators in various combinations and concentrations were used (Table 1) while the content of Agar Noble was constant (7.0 g/l). Response of 30 to 50 explants was examined for each medium variant and the transplantation of explants on fresh culture medium were performed at 3 week intervals.

Table 1. Composition of culture media for caulogenesis induction to *Moringa oleifera* explants.

Media variant	MS (1962)	BAP (mg/l)	IBA (mg/l)	Other
VC-MS Control	Full strength medium without hormones	-	-	3% sucrose and 0.8 % Agar Noble
V1	Full strength medium with hormones	1.0	-	3% sucrose and 0.8 % Agar Noble
V2	Full strength medium with hormones	1.5	-	3% sucrose and 0.8 % Agar Noble
V3	Full strength medium with hormones	2.0	0.2	3% sucrose, 0.8 % Agar Noble and citric acid (10 mg/l)
V4	Full strength medium with hormones	2.5	0.2	3% sucrose and 0.8 % Agar Noble and citric acid (10 mg/l)

Legend: Murashige-Skoog (1962) basic medium MS (1962); benzyl-aminopurine (BAP); ß indolilaceticacid (IBA)

3. RESULTS AND DISCUSSIONS

The ability apex meristematic Moringa to survive in artificial conditions, to multiply and regenerate independent plants depend upon a variety of factors, including the concentrations of the incretin hormones and general conditions of the culture (phytohormones exogenous light and temperature) plays an vital role. By using the inoculum of shoots relatively large (0.5-1 cm) is ensured a 100% survival rate of the culture, because the leaves and adjacent tissue serve as sources caulinar endogenous hormone and provides several important factors in the initiation stage of culture.

After restoration of sterile cultures observations were made on the proliferative capacity of *M. oleifera* cultures. Thus, it was found to be induced progressively after the restoration of cell divisions and regenerative capacity, starting with the 2nd and 3rd transfer respectively, these processes being supported by the composition of the culture medium and the concentration of applied phytohormones (Figure 4).

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Figure 4. Regenerative capacity *Moringa oleifera* Lam., explants cultivated under *in vitro* conditions, after the 2nd transfer on variant V1 (basic medium MS supplemented with *BA 1.0mg/l*)

At this research study, the main purpose was to increase the rate of multiplication while maintaining the genetic stability of the material. This goal was achieved by increasing the amount of cytokines in the culture medium and by creating a hormonal balance inclined in favor of cytokinins when using a combination with other phytohormones (Figure 5).

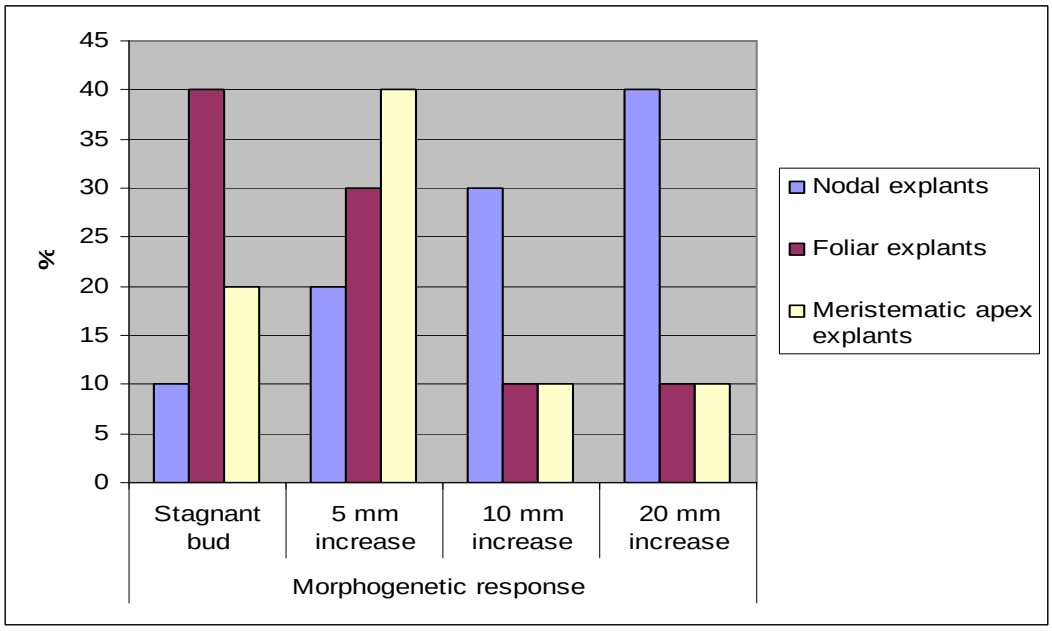

Figure 5. Results of monitoring the morphogenetic response of *Moringa oleifera* Lam., explants cultivated under *in vitro* conditions

From the analysis of the results represented in Figure 5, we find that the percentage of morphogenetic cultures obtained from the meristematic apex obtained was on average 40%.
In Figure 6, can see the elongation of the adventitious shoots regenerated on hormonal variant V3, from the nodal *Moringa oleifera* Lam., explants after the 5nd transfer.

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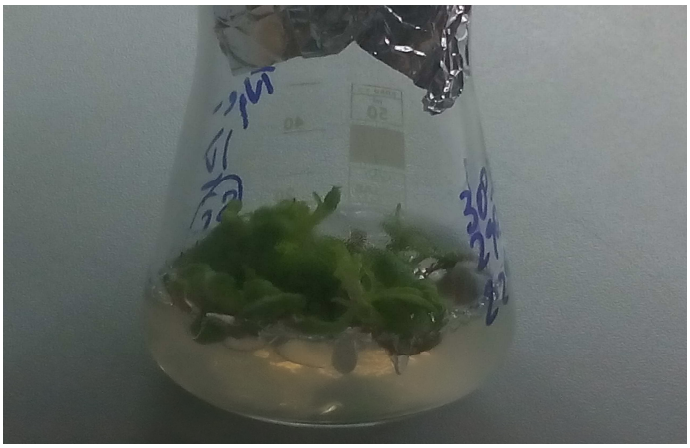

Figure 6. Regenerative capacity *Moringa oleifera* Lam., explants cultivated under *in vitro* conditions, after the 5nd transfer on variant V3

4. CONCLUSIONS

Moringa oleifera Lam. is a multipurpose tree with agronomic, horticultural, medicinal, nutritional, and industrial uses. It has been the subject of great interest in our days in condition of climat changes. Many developing countries have benefited from its multiple uses. As an exotic crop, the Romania can also benefit by cultivating Moringa in south regions or in controlled environment condition and processing its by-products into various goods for vegetarian and vegan people. We consider after take in consideration the multiple values of this specie, research and development efforts are needed to explore the potential of Moringa in our country. Our observations on the effect of phytohormones on development of *Moringa oleifera* Lam. explants cultured *in vitro* have led to the conclusion as benzilaminopurine (BAP) at concentrations of 2 mg/l in the presence of low concentrations (0.2mg/l) of auxine ß indolilacetic acid hormonal supplement stimulate the initial bud preformed but also for the efficient induction to development of multiple shoots on long-term, to be included in later phases of the micropropagation process

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