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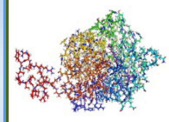

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Macro- and Micropropagation of *Moringa oleifera* Lam (Moringaceae): A Mini Review

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ABSTRACT

The most popular member of the family Moringaceae is *Moringa oleifera* Lam. While originally from the western sub-Himalayas, India, Pakistan, Asia, and Africa, this plant has since found its way around the world. Growth of the plant is slow in areas with harsh climates, despite evidence of its excellent adaptability in tropical regions. *Moringa oleifera* has been studied for its potential as a biodiesel source, as well as its usage as a natural coagulant for water purification. The maturation period of *Moringa oleifera* is rather long because it is frequently cultivated from seeds. Therefore, it is currently unable to meet the growing demand for this species to fulfil its many household, nutritional, economic, and medical purposes. This review covers the uses and the establishment, maintenance, and development of mass propagation of the plant. The review also covers extensive parts of the plant, from a single cell to an entire plant, under artificial and axenic conditions is the focus of plant tissue culture (PTC), which is an integral part of Plant Biotechnology due to the many benefits it offers over traditional methods of propagation. Plant hormones have been shown to improve the success of vegetative propagation from cuttings, but in order to obtain uniform planting materials, tissue culture techniques involving plant materials like nodal segments, indirect organogenesis, multiplication using immature seeds, and regeneration of axillary cotyledons and buds are preferable.

INTRODUCTION

Man's reliance, directly or indirectly on plants cannot be overemphasized [1]. Since decades, man obtained food, medicine, spices, industrial raw materials, etc from plants. Man also generated substantial revenue from the sale of plant products [2–5]. Numerous reports have shown that most plant species are multipotential confirming the multipotential nature of some plant species, listed the following plant species that served as food as well as medicine when consumed; *Moringa oleifera.* Lam., *Brillantasia lamium.* Pal, *Carica papaya* £., *Myrianthus arborus, Pleukenetum coneophorum* Mull. Arg., *Xylopia aethiopica* Duna, *Tetrapleura tetraptera Benth.* Etc [2–5]. Consequent upon the benefits of plants in man's socio-economy, a lot of economic plants have been identified, cultivated, conserved/protected on large scale (Plantations) and small scale (Home stead farms) [6]. In the developed countries like the United States of America, Britain, Canada, Italy, India, China etc. where values of economic plants were recognized earlier, most of their economic plants have been identified, documented, conserved and are currently being exploited biotechnologically for the production of acceptable, accessible and affordable products that not only improve man's socio-economy, but serve the dual purpose of job creation and generation of revenue [4,7]. Apart from biotechnological exploitation of the plant resources, the developed countries have further genetically modified plants for increased and improved productivity [6].

Contrary to the status of economic plants in the developed countries, some indigenous or introduced plant species in developing countries like Nigeria, Gabon, Ghana, Togo, Zambia, Uganda, Somali, Malawi, etc. have not been either cultivated on large scale, conserved, biotechnologically exploited or genetically modified [4,5]. Forests in the developing countries, particularly the rainforests are very rich in phytobiota [8,9]. Several reports have shown that a lot of the useful plants in the forests of the developing nations are capable of contributing significantly to national economic growth had these plants been

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recognized early, conserved and biotechnologically exploited [2,3,10]. At present, most of the economic plants in the developing countries' forests are rapidly going extinct because, these plants had potentials that were not appreciated early. Some of their resources were considered fit for the rural dwellers, hence no priority attention was directed to their conservation. Above all, man's activities, such as depletion of the forest through agricultural activities have seriously forced the economic plant species to an endangered status [4,5]; hence the resources of these plants are mainly obtained from the wild trees. Okafor [2] pointed out that full biotechnological exploitation of the resources of plants in Nigeria can be achieved when the plant species of economic value are conserved. Okigbo [3] reported that one of the factors limiting food security in Africa is lack of priority attention in the national agricultural programme of most African countries. Mbakwe and Nzekwe [11] pointed out that large scale establishment of species of economic value can help conserve plant species that are expected to sustain industrial activities. The authors further reported that for plants to be conserved, a large quantity of uniform seedlings should be produced. The authors also observed that for the production of a large quantity of uniform seedlings, proper knowledge of the techniques for propagating the desired plant species is necessary. Of all the plant species that can be relied upon for sustainable biotechnological exploitation, *Moringa oleifera* is one of them.

Moringa oleifera

The genus *Moringa* is made up of thirteen (13) distinct species which developed as the genus spread from India to other tropical and sub-tropical countries where it is adapted [12]. Of the thirteen (13) species (**Table 1**), *Moringa oleifera* (**Fig. 1**) is the most popularly known.

Table 1. Species of *Moringa* (from [12]).

Genus	Species
Moringa	*Oleifera*
Moringa	*Arborea*
Moringa	*Borziana*
Moringa	*Concanensis*
Moringa	*Drouhardi*
Moringa	*Hildebradtii*
Moringa	*Longituba*
Moringa	*Ovalifolia*
Moringa	*Peregrine*
Moringa	*Pygmaea*
Moringa	*Rivae*
Moringa	*Nuspoliana*
Moringa	*Stenopetala*

Fig. 1. *Moringa oleifera* tree.

Uses *of Moringa oleifera*

Moringa oleifera tree contained chemical nutrients that are required for man's healthy growth, development and prevention of diseases. A detailed account of the nutritional and chemical contents *of Moringa* leaves and pods is shown in **Table 2**.

Table 2. Nutritional value of leaves and pods of *Moringa oleifera* (from [12]).

	% per 10g edible Portion		
	pods	leaves	leaf powder
Moisture	86.9	75.0	7.5
Calories	26	92	205
Protein	2.5	6.7	27.1
Fat	0.1	1.7	2.3
Carbohydrate	3.7	13.4	38.2
Fiber	4.8	0.9	19.2
Mineral	2.0	2.3	
Ca	30	440	2,003
Mg	24	24	368
P	110	70	204
K	259	259	1,324
Cu	3.1	1.1	0.57
Fe	5.3	7	28.2
S	137	137	870
Oxalic acid	10	101	1.6
Vitamin A	0.11	6.8	16.3
Vitamin bi	423	423	
Vitamin Bn	0.05	0.21	2.64
Vitamin C	0.07	0.05	20.5
Vitamin 62	0.2	0.8	8.2
Vitamin D	120	220	17.3
Vitamin E	-	-	113
Arginne	3.6	6.0	1.33
Histidine	1.1	2.1	0.61
Lysine	1.5	4.3	1.32
Tryptophan	0.8	1.9	0.43
Phenylanaline	4.3	6.4	1.39
Methionine	1.4	2.0	0.35
Threonine	3.9	4.9	1.19
Leucine	6.5	9.3-	1.95
Isoleucine	4.4	6.3	0.83
Valine	5.4	7.1	1.36

In total, *Moringa oleifera* contains 32 chemical substances with nutritional value, as reported by Price [12]. The leaves and pods of the Moringa plant are of great interest due to their high protein content; by eating these parts of the plant, one can fight malnutrition at a low cost. The *Moringa oleifera* plant, specifically its leaves, have been found to contain cytokinin, a plant growth hormone. In one study, the yield of several crops was greatly improved after Moringa hormone was sprayed on the seedlings. These crops included maize, bell peppers, onions, sorghum, coffee, and chili melons. Additional research by the same author found that feeding cattle a formula made up of 40-50% Moringa leaves increased milk yield by 30% and increased daily weight gain by 10% [12].

Moringa leaves, pods, and seed powder can be used to disinfect and purify water (After oil extraction). Polyelectrolytes isolated from Moringa leaves, pods, and seed powder, are the active ingredient responsible for water treatment in the species [12]. An antibiotic (Pterygospermina) with potent antibacterial and antifungal activities was discovered, as reported by Rajangam et al. [13]. This substance was extracted from the *Moringa oleifera* plant's flower and bark. The widespread demand for *Moringa oleifera* can be attributed to the species' high multipotential. Cash is made by selling raw or canned pods, leaves, and seeds, and the product is widely distributed and used [13]. Moringa leaves, leaf powder, and the seed powder (obtained after the seed oil has been extracted) are used in herbal medicine, and Rajangam et al. [13] and Fahey [14] provide an in-depth account of their applications (**Table 3**).

Table 3. Medicinal use of *Moringa oleifera* in folk medicine.

Plant parts	Traditional use condition/Effect	No. of diseases cured
Leaves	Hepatic, anti-tumor, prostate, radioprotective, anemic, anti-hypertensive, diabetes/hypoglycemia,diuretic, hypocholestemia, thyroid, hepatorenal, colitis/infection, diarrhea/dysentery, ulcer/gastritis, rheumatism/headache, antioxidant, carotene, helminthes/trypanospsnes, external sores/ulcerHepatic, anti-tumor, prostate, radioprotective, anemic, anti-hypertensive, diabetes/hypoglycemia,diuretic, hypocholestemia, thyroid, hepatorenal, colitis/infection, diarrhea/dysentery, ulcer/gastritis, rheumatism/headache, antioxidant, carotene, helminthes/trypanospsnes, external sores/ulcer	40
Barks	Anti-tumor, digestive, epileptic, hysterical, headache, antinutritional factors, abortifacient, aphrodisiac, birth control, scurvy, and dental caries/toothache.	16
Roots	Dental caries/toothache, common cold, trypanosomes, external sores/ulcers,-fever, asthma, cardiotonic, diuretic, hepatorenal, diarrhea, flatulence, anti-spasmodic, epilepsy, hysteria, headache, abortifacient, aphrodisiac, rubefacient, vesicant, gout, hepatamegaly, low back/kidney pain, scurvy and splenomegaly.	25
Exudates	Abortifacient and rubefacient properties; treatment for dental caries/toothache; treatment for syphilis; treatment for typhoid; treatment for earache; treatment for fever; treatment for asthma; treatment for dysentery; treatment for rheumatism; treatment for headache; and more.	12
Flowers	Useful for: sore throat, cold, anthelmintic, anti-tumor, rheumatism, diuretic, tonic, hysteria, and abortion.	9
Pods	For treating worms, skin cancer, high blood pressure, diabetes, and arthritic pain.	5
Seeds	For conditions such as worms, cancer, ulcers, rheumatism, arthritis, spasms, gout, goiter, and vitamin/mineral deficiencies.	9

In summary, many reports have shown that *Moringa oleifera* has innumerable economic values ranging from source of food/food condiment, water purification, feed, hormonal effects, [trough herbal medicine production to revenue generation. In Europe and the United States of America, intensive research is currently on-going for the production *of Moringa* tablets for use is nutritional supplement. However, detailed reports on how best large quantity of the species seedlings can be produced, especially when the seeds are out of season appear lacking.

Plant propagation of *Moringa oleifera*

Plant propagation is one of man's earliest known activities (Singh, 2004). According to Hartmann and Kerster (1983), there are two main ways to generate new planting materials: sexually, through the use of seeds, and asexually, through the use of vegetative parts of plants. Because plant products can support biotechnological exploitation, it is crucial that a large number of uniformly growing seedlings be available for large-scale farm establishment in the current context of conservation of useful plant species (Puri,1990).

Sexual Propagation of Plant Species

Naturally, some plant species germinate readily when conditions required for seed germination are provided; that is moistures, air (oxygen), adequate temperature, healthy seeds, good germination media [15]. However, seeds of many plant species have adaptive ecological strategies that allow them to germinate and develop in nature but pose problems in their domestication; by being recalcitrant to germinate despite the provision of conditions necessary for seed germination [16,17]. Series of research reports have revealed that the failure of such seeds to germinate could be caused by factors like: hard seed coat [18,19]; chemical contents of the seed [20]; mechanical resistance of seed coat and endosperm [21]; and effects of nursery media [15,22,23]. Following the discovery of the factors that delay or hinder seed germination, numerous solutions have been proffered by researchers: pre-soaking seeds before sowing [20]; priming [24]; scarification [19,25].

Seeds of the *Moringa oleifera* plant have been shown to germinate successfully in multiple studies, but it takes the seedlings a long time to reach reproductive phase, extending the amount of time it takes for the plants to produce resources/raw materials that can sustain large-scale biotechnological exploitation [13,26]. According to Price [12], this species only flowers and bears fruit once a year, so it's important to find other ways to propagate it when the fruits and pods aren't available.

Vegetative Propagation

Hartmann and Kerster [27], Okafor [2] and several other authors have listed various techniques by which plant species can be propagated vegetatively. These methods include stem cuttings (Cassava *Manihort escule ntumfutilissima),* stem tubers (yam, *Dioscorea spp);* corms (Banana/plantain *Musa* spp), "life" trees; bamboo, *Bambosa vulgari, Baphia nitida, Newbouldia laevis, Pterocarpus* spp.; bulbs (Onions *Alium cepd);* suckers (Pineapple *Ananas comosus);* leaf cuttings *(Cactus* spp., *Opuntia epiphylla);* root cuttings (oranges *Citrus* spp). These groups of plants have been reported to be propagated without application of rooting hormones [2,11,27,28]. They are referred to as "Easy-to-root Plant Species". Other methods of propagating plant species without involving the use of hormones have been reported such as grafting/budding [2,11,27–29].

In one study, the hardwood and semi-hardwood cuttings of *Moringa oleifera*, 30 cm in length, planted in a light, sandy soil performed best and produced the longest shoots. The softwood cuttings that resulted in the shortest shoots were only 15 centimeters long. Planting at least 30-centimeter-long hardwood or semi-hardwood cuttings in light sandy soil treated with naphthaleneacetic acid [NAA]-talc formula or in a 1:1 (w/w) mix of coconut coir and teak sawdust without naphthaleneacetic acid [NAA]-talc formula treatment is the best method for achieving successful vegetative propagation of *Moringa oleifera* [30]. In another study in South Africa, experiments were done in the spring and summer and then repeated the following year. Each variety had 45 cm stem cuttings taken from it and planted in a randomized complete block design with three replicates. The survival rate for cuttings planted in the spring was 72%, while it was only 35% for those planted in the summer [31]. These reports indicate that vegetative propagation of *Moringa oleifera* is possible and may yield high quantity of planting materials.

Hard-to-root Plant Species

Several authors have reported that many woody plant species are hard-to-root; that is, then- stem cuttings cannot root when planted unless the stem cuttings were treated with the appropriate concentration of hormones [10,27,32–34]. Of all the various methods of vegetative propagation of plant species, rooting the stem cuttings has been reported to be most likely to succeed on a large scale [35]. Puri [34] reported that vegetative propagation of forest trees is potentially useful for replicating clonal materials, as well as for rapid multiplication of stock. Menzie *et al.* [36] observed that vegetative propagation of plants by rooting the stem cuttings bulks up seedlings. Nzekwe [37] also reported that juvenile stem cuttings roots faster than mature stem cuttings.

Auxins popularly used in vegetative propagation of plants
Auxins, a group of growth regulating substances are recognized as phyto-hormones. These play very important role in co-ordinating many growth and developmental processes in plant life cycles [38]. Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA) have been reported to be typically the principal auxins popularly used in vegetative propagation of plant by rooting their stem cuttings or marcorting. Other growth regulating substances used in rooting stem cuttings include: phenoxy compounds. These growth regulating substances (hormones) have been variously prepared commercially and these include: hormodin, hormex, routone, hormo-root. They (commercially prepared hormones) can be in the form of powder or paste; examples are rooter; IAA in lanolin [27].

Effectiveness of the rooting hormones and conditions favourable for rooting stem cuttings
Very few studies have compared rooting activities of the most popularly used auxins, IB A, NAA and IAA or other auxins thoroughly in a plant species or more than one species [33]. Most previous works had conflicting reports on the order of the effectiveness of the auxins, IBA, NAA and IAA on rooting the stem cuttings of woody plant species. Some reports were of the view that the most active of the three hormones is IBA, followed by NAA and IAA; while others report that NAA is more active than IAA and IBA in that order. Zimmerman and Wilcoxon [39] reported that IBA is used more frequently than NAA and IAA because IBA has higher activity, broader range of effectiveness on rooting woody plant species without toxicity. Griffith (1940) worked on rooting the stem cuttings of Douglas-fir and reported that IAA was more active in stimulating bud burst and rooting than IBA. Similar studies, by Proebsting [33] showed that IBA was more active in stimulating bud burst and rooting in Douglas-fir than IAA; the author reported that at the same concentration and dip period, IBA stimulated 67% and IAA 54% rooting responses, respectively.

Several other reports that compared the effects of IBA, NAA and IAA on stimulating rooting on the stem cuttings of different woody plant species had conflicting results. Thiaman and Rogers [40] and, Hilnesley and Biazichi [41] working on rooting the stem cuttings of *Abies fraseri* reported that IBA was more effective in rooting the species stem cuttings than NAA. Puffy *et al.* [42] observed that at the same concentration, IBA was more effective than NAA and IAA in rooting the stem cuttings of fevere tree and *Lappia javanica.* Badji *et al* [10] also worked on rooting the stem cuttings of gum Arabica *(Acacia arabica)* and reported that IBA was more effective (50%) than NAA (10%) in rooting the species' stem cuttings. Tiwari and Das [43] reported that powdered formulations of NAA was more active in rooting the stem cuttings of *Embelia* and *Caesalpinia bonduc* respectively than IBA and IAA in that order.

Despite the extensive reports on the vegetative propagation of some woody plant species by rooting their hormone-treated stem cuttings, similar studies on *Moringa oleifera* appear lacking with the above use of NAA-talc formula [30] for propagation of *Moringa oleifera* stem cutting being one of the few examples. Price *et al* [12] reported that the species, *Moringa oleifera* has no seed germination problems. However, Okafor (1983) pointed out that the seedlings produced sexually have long juvenile period, hence, justifying the need for other faster propagation methods. Several authors have reported that seedlings produced vegetatively by rooting stem cuttings, budding, marcorting/air layering and grafting have reduced phenophase and reduced growth in height [2,11].

Plant tissue culture of *Moringa oleifera*
Moringa oleifera grows slowly because it is typically propagated from seeds. This means that it is currently impossible to meet the growing demands for this species in order to fulfill its various domestic, nutritional, commercial, and medicinal uses. The establishment of an in vitro tissue culture offers many benefits over the conventional methods of propagation, and is therefore an important part of Plant Biotechnology, which is based on the plant tissue culture (PTC) set of techniques that enables the establishment, maintenance, and development of any part of the plant, from a single cell to an entire plant, under artificial and axenic conditions [44]. Furthermore, the tissue culture technique is an effective method for producing GM plants. Some research has been done on in vitro propagation of Moringa using various explants, including nodal segments [45,46], indirect organogenesis [47], multiplication using immature seeds [48], and regeneration of axillary cotyledons and buds [49].

Some of the conditions used in the various methods of in vitro cultivation can be tweaked to achieve a higher yield. Thus, modifications to factors such as phytohormone composition and concentration, or variations in light intensity and temperature, can result in healthier, more robust plant growth [50]. Some genetic variation may occur during regeneration as a result of the use of varying hormone types and concentrations to aid in the development of callus during dedifferentiation and differentiation [51,52]. Because of this, uniformity among the plants produced via clonal or regeneration techniques is crucial to the success of these methods compared to that of conventionally propagated plants.

In one study, with the use of the Random Amplified Microsatellite Polymorphism (RAMP) marker, a system was put into place that allows for the rapid spread of *Moringa oleifera* while maintaining its genetic integrity. Bud and cotyledon apex explants were employed in MS media devoid of plant growth regulators for the propagation (PGR). Maximum bud formation per explant was achieved with 1 mg L 1 BA and 0.2 mg L 1 AIA in indirect regeneration, while massive root creation was achieved with the same treatment but employing leaves as explants. Plants that were acclimatized and then transplanted into the soil had a 95% success rate. The dendrogram of a study that employed DNA from leaf-propagated, -regenerated, and -ex vitro plants to examine genetic variability revealed no significant differences across the plants [44].

Among the most recent investigations involves *Moringa oleifera* stem explants cultured on Murashige and Skoog (MS) agar medium with various concentrations of 6-benzyl adenine purine (BAP) and kept at 25 2°C for 5 weeks. Multiple shoot induction was observed in stem explants grown in MS medium supplemented with BAP, TDZ and NAA at selected combinations. The highest shoot formation percentage (95.3%), as well as the highest average number of 8.4 shoots per explants, were both observed in the MS medium containing BAP at 1.5 mg/l. To promote plant regeneration, root inducing hormones IBA and IAA were added to the 12 MS + 7 g/l agar + 15 g/l sucrose medium in which the shoot explants were cultured. Half MS + 7 g/l agar + 15 g/l sucrose supplemented with 0.3 mg/l and 0.2 mg/l IBA and 0.2 mg/l IAA resulted in 100% root formation, with an average of 4.2 roots per explant and a length of 3.5 cm. After 5 weeks in a greenhouse, the average survival rate was 88.9 percent in a medium made up of (40 percent soil, 50 percent sawdust, and 10 percent worm compost) [23]. This research

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presents a new method for micropropagating uniform genotypes of *Moringa oleifera* plantlets for use in breeding selection and field production using a tissue culture protocol.

CONCLUSION

Given that *Moringa oleifera* is usually grown from seeds, its development time is lengthy. As a result, it is currently impossible to satisfy the increasing demand for this species to fulfill its numerous domestic, nutritional, commercial, and medicinal uses. Plant tissue culture (PTC) is a set of techniques that allows the establishment, maintenance, and development of any part of the plant, from a single cell to an entire plant, under artificial and axenic conditions; this is an important part of Plant Biotechnology because it offers many advantages over the conventional methods of propagation. It has been shown here that vegetative propagation of the plant can be enhanced by the addition of plant hormones to plant cuttings but to obtain uniform planting materials the use of tissue culture methods using plant materials such nodal segments, indirect organogenesis, multiplication using immature seeds and regeneration of axillary cotyledons and buds has proven to be effective and relatively uniform in genetic composition.

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