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

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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, Somalia, 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...
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 had potentials that were not appreciated early. Some
Fig. 1.

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

| Genus   | Species                |
|---------|------------------------|
| Moringa | Oleifera               |
| Moringa | Arborea                |
| Moringa | Borziana              |
| Moringa | Concannensis           |
| Moringa | Drouhardi              |
| Moringa | Hildebradini           |
| Moringa | Longiuba              |
| Moringa | Ovalifolia             |
| Moringa | Peregine               |
| Moringa | Pygmaea                |
| Moringa | Rivae                  |
| Moringa | Nuspoliana             |
| Moringa | Stenopetala            |

**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  | 239    | 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 D                | 0.2  | 0.8    | 8.2         |
| Vitamin E                | 120  | 220    | 17.3        |
| Arginine                 | 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        |
| Phenylalanine            | 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
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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.

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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 (Pterygosperminga) 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).

**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.

![Fig. 1. Moringa oleifera tree.](https://example.com/moringa_tree.png)
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 the generation 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 Moringa oleifera plant has 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.

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, hypocholesteremia, thyroid, hepatorenal, colitis/infection, diarrhea/dysentery, ulcer/gastritis, rheumatism/headache, antioxidant, carotene, helminthes/trypanosomases, external sores/ulcer/Heptic, anti-tumor, prostate, radioprotective, anemic, anti-hypertensive, diabetes/hypoglycemia, diuretic, hypocholesteremia, thyroid, hepatorenal, colitis/infection, diarrhea/dysentery, ulcer/gastritis, rheumatism/headache, antioxidant, carotene, helminthes/trypanosomases, external sores/ulcer | 40 |
| Barks       | Anti-tumor, digestive, epileptic, hysterical, headache, antinutritional factors, abortifacient, aphrodisiac, birth control, scurry, and dental caries/toothache. | 16 |
| Roots       | Dental caries/toothache, common cold, trypanosomases, external sores/ulcers, fever, asthma, cardiotoxic, diuretic, hepatorenal, diarrhea, flatulence, anti-spasmodic, epidemic, hysteria, headache, abortifacient, aphrodisiac, rubefacient, vesicant, gout, hepatomegaly, low back/kidney pain, scurry 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 arthritis pain. | 5 |
| Seeds       | For conditions such as worms, cancer, ulcers, rheumatism, arthritis, spams, 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, through 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.

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 Manihot esculenta ntonfaliussima), stem tubers (yam, Dioscorea spp); corms (Banana/plantain Musa spp), "life" trees; bamboo, Bambos vulgaris, Baphia nitida, Newbouldia laevis, Pterocarpus spp.; bulbs (Onions Alium cepd); suckers (Pineapple Ananas comosus); leaf cuttings (Cactus spp., Opuntia epithyphila); 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 naphthalenecarboxylic 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, when 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 bulk 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 coordinating 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 marcoting. 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, routine, 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 different woody plant species had conflicting results. Thiaman responses, respectively.

Puffy et al. [42] observed that at the same concentration, IBA was more effective than NAA and IAA in rooting the stem cuttings of fever tree and Lappia javanica. Badji et al [10] also worked on rooting the stem cuttings of gum Arabic (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 naturally 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 (BAP) and kept at 25 °C for 5 weeks. Multiple shoot induction was observed in stem explants grown in MS medium supplemented with BAP, TDZ and NAA at selected concentrations. 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
prevents 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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