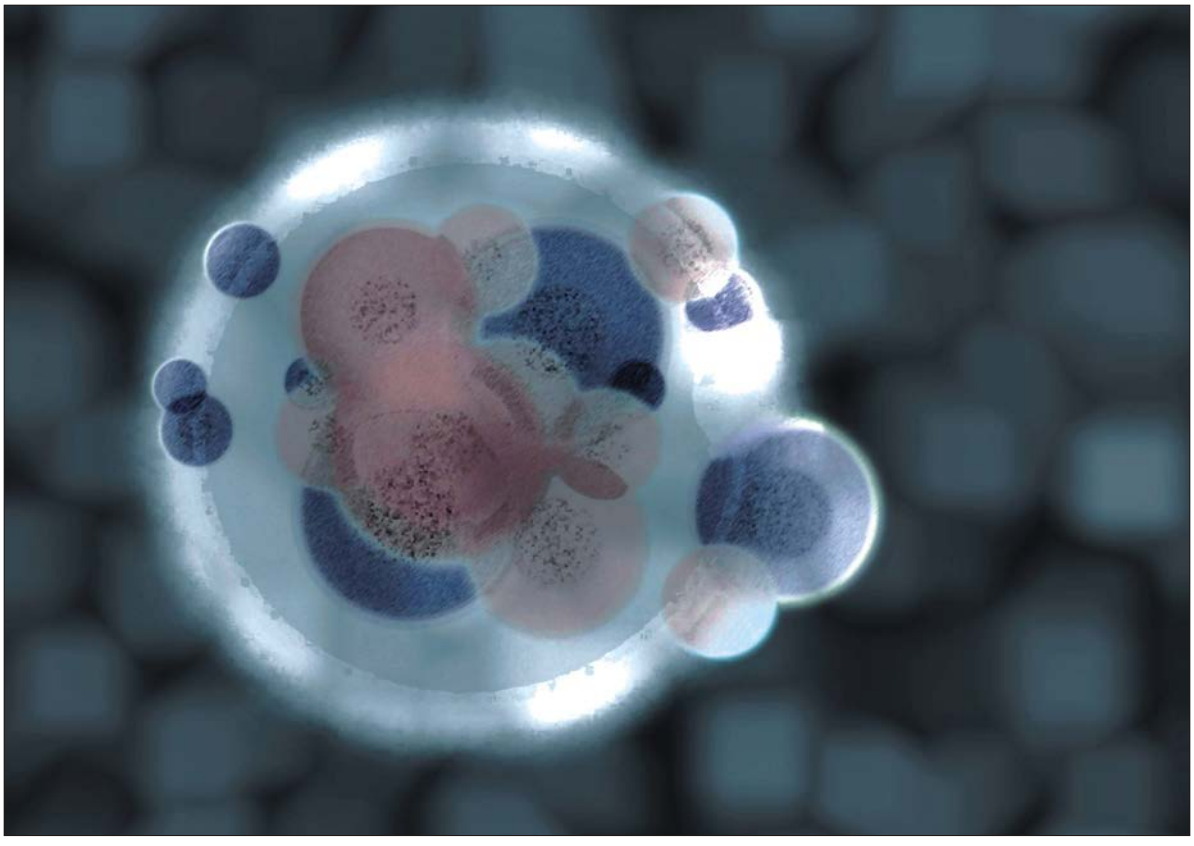

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In vitro Regeneration and Multiplication of Year-round Fruit Bearing *Moringa oleifera* L.

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Abstract: The shoot multiplication rate is most significant in terms of shoot production. Cytokinins are usually added to tissue culture media to stimulate axillary or adventitious shoot development. The type and concentration of cytokinin used have profound effects on shoot multiplication. Rates of shoot induction of *Moringa* nodes in presence of Benzylaminopurine (BAP) were observed. Hundred percent explants induced shoots in presence of 1.0 and 1.5 mg L^{-1} BAP concentrations. But in terms of highest shoot production, the 1.0 mg L^{-1} BAP conc. was best. Highest shoot multiplication rate was found in the 3rd subculture on it. The regenerated shoots were rooted on MS (Murashiege and Skoog) medium alone and fortified with different concentrations of NAA, IBA and IAA. The maximum percentage of rooting was obtained on MS medium alone. The rooted plantlets were hardened and successfully established in the soil.

Key words: *Moringa oleifera,* node, explants, growth regulators

INTRODUCTION

Moringa tree is adapted to a wide range of soil types but it does best in well-drained loam to clay-loam soil. It does not withstand prolonged water logging. *Moringa* is not a nitrogen fixing tree, but its fruits, flowers and leaves contain 5 to 10% proteins on average[1]. All of these parts are eaten widely as vegetables, providing excellent food for humans.

The pods are often cooked and eaten like green beans and is reported to contain 2.5 g protein/100 g and the same mass of *Moringa* leaves contain 6.7 g of protein. The leaves also contain 440 mg Ca, 70 mg P, 7 mg Fe, 110 μg Cu, 5.1 μg I, 11300 IU Vit. A, 120 μg Vit. B and 0.8 mg nicotinic acid and 220 mg ascorbic acid/100 g[2]. It has a density of 0.5 to 0.7 and yields approximately 4,600 kcal/kg[1]. According to Burkill[3], the seeds yield a clear inodorous oil to the extent of 22 to 38.5%. This oil, sweet and non-sticking, is often extracted for lubricating watches and other delicate machinery. It is excellent in salads, can be used for soap making and burns with a clear light and without smoke[4]. The bark contains a gum that is used as a seasoning and in treating some stomach ailments.

Pterygospermin is a bactericidal and fungicidal compound isolated from *Moringa*. Root-bark yields two alkaloids, moringine and moringinine. Moringinine acts as cardiac stimulant, produces rise of blood-pressure, acts on sympathetic nerve endings as well as smooth muscles all over the body. The flowers, leaves, roots, barks are used in folk remedies for tumors, abdominal discomfort, boils, cold, conjunctivitis, high blood pressure, hysteria, relapsing fever, skin diseases, Rheumatism etc.[5].

A report also mentioned trials by pharmacologists at Gadja Mada University in Indonesia that showed that one crushed *Moringa* seed could clear 90% of the total coli form bacteria in a liter of river water within 20 min[6].

In Bangladesh *Moringa* trees are distributed all over the country. There are two types of *Moringa* trees found. One produces pods in March and April and another produces pods round the year. Planting limb cuttings of 1.5-2 m long propagates this valuable plant. Although plantation of *Moringa* by cuttings is good for bearing the same characters as mother plants have but the problem is that this reduces the mother plants growth, yields and sometimes the mother plants even die. So the main object of the research is how *Moringa*, a common seasonal vegetable, could be brought to human diet as a nutritious vegetable bearing medicinal properties by massive plantation through tissue culture. To make it available throughout the year, an attempt has been taken for its regeneration and multiplication from the tissue of the year round *Moringa* plants.

MATERIALS AND METHODS

Juvenile shoots were obtained from mature year-round fruit bearing plants of *Moringa oleifera* L.

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growing in the BCSIR campus, Dhaka, Bangladesh. Shoots were cut into nodal explants each bearing one or two axillary buds. The explants were thoroughly washed with household bleach for five minutes and then kept under running tap water for ½ an h. They were surface sterilized with 0.1% $HgCl_2$ solution (w/v) for 15 min followed by several washes with autoclaved distilled water under aseptic condition in the laminar airflow cabinet. For shoot initiation the individual nodal explants were inoculated aseptically on sterilized MS[7] nutrient media containing 3% sucrose, 0.8% agar and pH adjusted to 5.8 before autoclaving. The cytokinin BAP (Benzylaminopurine) alone at a range of concentrations, 0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1} were used in the nutrient media. The regenerated numbers of shoots and the highest proportional shooting response were recorded after four weeks of culture. Regenerated shoots were subcultured every four weeks onto the freshly prepared same medium that was subjected to produce the highest proportional shooting response. Numbers of shoots were recorded at the end of each four week culture period for a total of five generations. Rooting media consisted of MS medium alone and supplemented with auxins like NAA, IBA and IAA with concentrations 0.05, 1.0 and 2 mg L^{-1} were used.

The plantlets obtained after root initiation were carefully separated from the medium to avoid damaging and washed with tap water to remove the agar adhering to them. The plantlets were then transplanted into 10 cm. diameter nursery pots containing the mixture of biogas slurry and soil (1:1). The plantlets were kept in a mist chamber for one week with normal temperature, in 90-100% humidity and with no additional lighting. From the plantlets new leaves emerged after 10-12 days in the mist chamber, they were then placed in the net house initially for 1 h and assessed for signs of leaf wilting. The exposure time was increased daily until the plants were fully acclimatized to the normal environment. When the plants were established fully under normal environment conditions, they were then transferred into the field for their growth.

RESULTS AND DISCUSSION

The explants initiated shooting after 2-3 weeks of incubation in all concentrations of BAP (Table 1). Among them 100% explants produced shoots in BAP 1.0 and 1.5 mg L^{-1}. Both the conc. of BAP 1.0 and 1.5 mg L^{-1} initiated shoots with some callus but the maximum number of shoots were observed to the 1.0 mg L^{-1} BAP. Number of shoots were much more increased when repeatedly subculture on the same BAP conc. (Table 2). Results of this study indicate that large-scale propagation of *M. oleifera* by tissue culture methods is feasible and several plantlets can be regenerated from one nodal explant. BAP was the effective cytokinin for shoot induction as well as shoot proliferation in *M. oleifera*. One of the main functions of exogenous cytokinins in tissue culture is induction of adventitious shoots. They are also used to release axillary buds from apical dominance thus initiating shoot proliferation. Wareing and Phillips[8] showed that the cytokinin BAP was more active than other cytokinins in shoot proliferation and BAP is the only one that can be autoclaved. So in commercial micropropagation establishments, where lowering costs and ease of handling are major considerations, BAP is the most suitable cytokinin.

Initially the node explants in BAP containing media produced some callus after one week and within four weeks emerged small shoots (Fig. 1A). Large number of *Moringa* shoots was found when repeated subculture performed in 1.0 mg L^{-1} BAP containing media (Fig. 1B). Highest number of shoot regeneration was recorded at third subculture (Table 2). For root initiation of the *in vitro* regenerated shoots were placed into NAA, IBA,

Table 1: *In vitro* shoot regeneration rate of *Moringa oleifera*, in presence of different Benzylaminopurine concentrations

Medium	% of responded explants	Number of shoots/ explantsx ± SE*
MS + BAP (0.5 mg L^{-1})	60	1.8±0.55
MS + BAP (1.0 mg L^{-1})	100	4.0±0.29
MS + BAP (1.5 mg L^{-1})	100	3.1±0.23
MS + BAP (2.0 mg L^{-1})	60	1.1±0.31
MS + BAP (2.5 mg L^{-1})	20	0.4±0.27

* Value indicates the number of shoots ±SE of three independent determinations, involving ten replicates

Table 2: Shoot multiplication rate of *Moringa oleifera, in vitro*. Data expressed as the increase in shoot number over a 4-week passage

Subcultures	Shoot yield	Average shoot*	Multiplicationrate
1st	120	12	3 times
2nd	120	12	3 times
3rd	250	25	6.25 times
4th	200	20	5 times
5th	200	20	5 times

*4 shoots were transferred in a conical flask with 10 replicates of each subculture

Table 3: Data on the development of roots in individual *Moringa oleifera* shoots when cultured on rooting media

Growth regulators	Concentrations (mg L^{-1})	Percentage of shoots rooting	Average number of roots/plant
NAA	0.05	0	0
	1.0	0	0
	2.0	0	0
IBA	0.05	0	0
	1.0	10	2.0
	2.0	10	2.8
IAA	0.05	40	3.70
	2.0	20	3.5
	1.0	10	2.2
MS	0	100	4.0

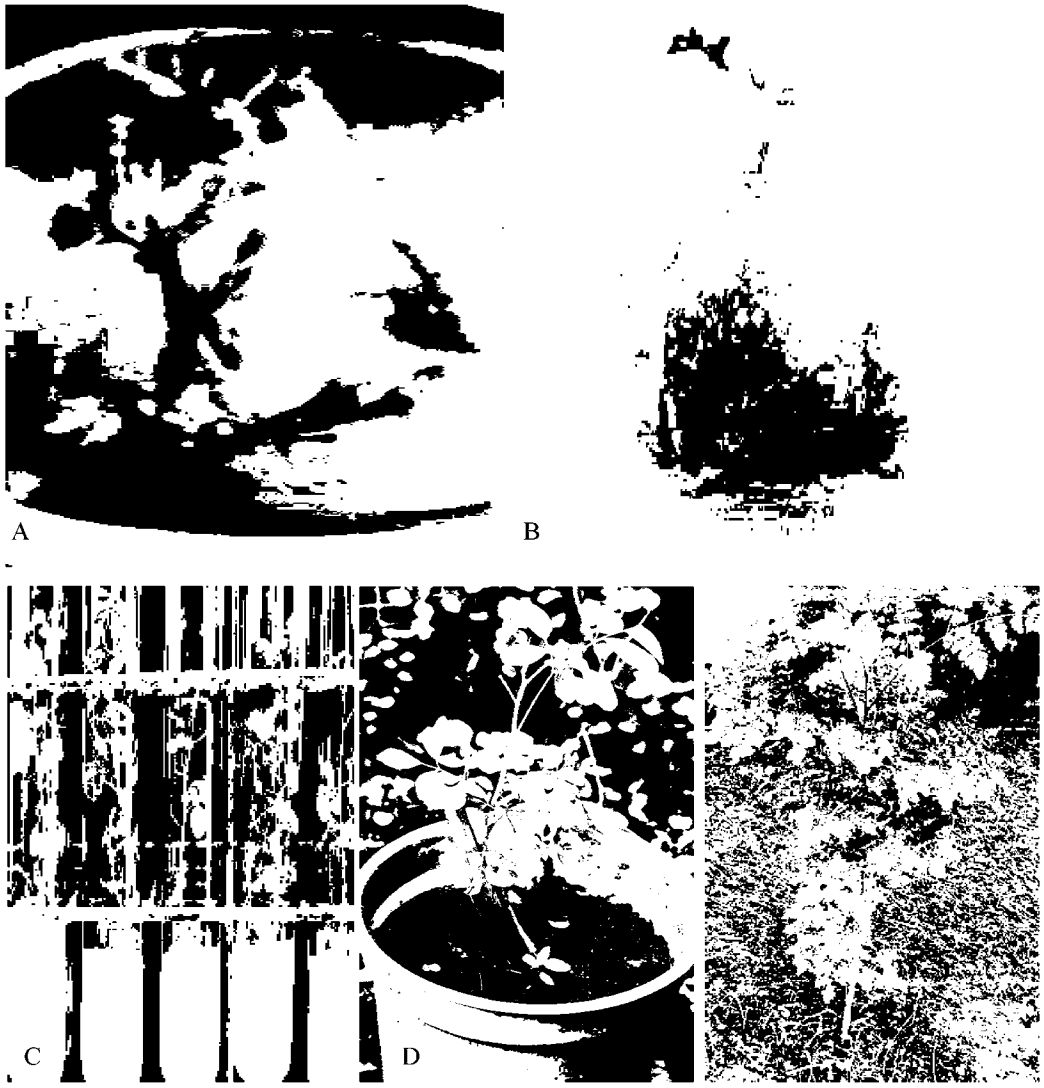

Fig. 1A-E: Regeneration of plants *in vitro* from node explants of *Moringa oleifera*. A: Developing shoots, B: Multiple shoots, C: Root induction from isolated shoots, D: Acclimatized plant, E: *M. oleifera* in the field

IAA and in MS alone (Table 3). In presence of NAA no shoots were induced in any concentrations. Lower concentration of IBA (0.05 mg L^{-1}) did not produce any shoots and the rooting percentage was very low in other two concentrations. In presence of IAA the shoots produced root in all three concentrations. Here the lower concentration of IAA induced more roots than the other two concentrations. But the best rooting was observed (Fig. 1C) on MS medium without any growth regulators. The *in vitro* regenerated plantlets were successfully established on the soil (Fig. 1D and E). The pattern of growth of all tissue-cultured plants was normal and the plants grew quickly in the soil.

The *in vitro* regeneration of shoots, rooting and soil establishment protocol in this study suggests that there is possibility of adapting tissue culture technique to mass propagation of *Moringa oleifera* tree. Several plantlets could be regenerated and the numbers would increase with repeated subcultures.

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