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In Vitro Morphogenetic Response of *Moringa oleifera* Lam. Leaf Petiole Explant to Cytokinin and Auxin Concentrations

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Abstract

The study investigated the morphogenetic response that can be obtained from *M. oleifera* leaf petiole explant *in vitro*. Murashige and Skoog (MS) medium was supplemented with different concentrations and combinations of plant growth regulators. The concentrations (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 mg/l) of Indole acetic acid (IAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D) were used singly and in combination with 0.1 mg/l Benzyl amino purine (BAP) for media supplementation. The results obtained seven days after culture initiation indicated that different morphogenetic responses can be obtained from *M. oleifera* leaf petiole culture. When plant growth regulators were used singly for media supplementation, IAA favored callus and root formation, while 2,4-D supported callus and shoot development, Cultures containing combinations of both IAA and 2,4-D hormones resulted in the formation of calli, roots and shoots.. A plantlet was obtained from the culture supplemented with 7.0 mg/l IAA and 0.1 mg/l BAP after 30 days of culture initiation.

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Introduction

Moringa oleifera Lam. is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is now widely distributed in many countries of the tropic and sub-tropic regions of Africa, Latin America, Caribbean and the Pacific Island (Muhammad *et al.,* 2013). It is commonly known by different regional names such as drumstick trees, horse-radish, benzolive, kelor, morango, murungiakaai, mulanlangay, saijhan, sajna, moorangai, nebeday (Mishra *et al.,* 2012). *M. oleifera* is a multipurpose plant with numerous medicinal (Saini *et al*., 2012; Shank *et al.,* 2013) and nutritional (Fahey, 2010; Mishra *et al.,* 2012) benefits. In some part of the world, it is used as a food supplement in combating malnutrition in children and nursing mothers, in improving nutritional quality of diets in the tropics (Mishra *et al.,* 2012). The plant has found wide uses in industries (Islam *et al.,* 2005; Shank *et al.,* 2013). *M. oleifera* has been mainly propagated by conventional methods of seed planting, stem cuttings and transplanting. However, these conventional approaches of plant propagation and improvement have limited applicability (Yadev *et al.,* 2012). Conventional methods of propagating *M. oleifera* are time consuming, labor intensive, less productive, lack of fresh viable seeds, pathogen infected materials for planting and death of the mother plants (Islam *et al.,* 2005; Mishra *et al.,* 2012; Kataria *et al.,* 2013). These limitations necessitated clonal propagation of *M. oleifera* by *in vitro* technique to surmount these challenges.

Plant tissue culture procedure has been extensively used to produce high quality, disease free planting materials, increase number of desirable germplasm, especially for vegetative crops instead of the traditional methods of seed and stem cutting propagation. In plant tissue culture technique, plant regeneration from an explant follows either of two pathways: the indirect regeneration and direct regeneration pathways. The indirect regeneration pathway involves an explant in culture medium forming unspecialized, unorganized dedifferentiated mass of cells, known as callus. The direct pathway involves a process where an explant in culture medium undergoes organogenesis (formation of organ such as root and shoot) without an intermediate callus stage. The morphogenetic response obtained from an explant in cultures depends on the type and nature of explants as well as the type, concentration and combination of growth regulators. The action of the different hormones/regulators is not consistent: Different plants will respond to the same chemical differently and also, different plant parts from the same plant can respond differently (Isikhuemen, 2015). In many cases, different genotypes within a species will have variable responses in culture.

This study is aimed at investigating the type of morphogenetic response that could be obtained when *M. oleifera* leaf petiole is used as explant in medium containing different combinations of growth regulators.

Materials and methods

Plant materials

Leaf petiole of *M. oleifera* Lam. used as explant for this study was collected from the Physiology Division of the Nigeria Institute for Oil Palm Research (NIFOR), Benin City, Edo State.

Media preparation and phytohormone supplementation

Full strength Murashige and Skoog (MS) medium (1962) containing 3% sucrose was used for the study. In a medium containing single phytohormone, either IAA or 2,4-D was used for media supplementation, each at concentrations of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 mg/l. The medium containing combinations of growth regulators, IAA (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 mg/l) and BAP (0.1 mg/l) were used. The pH of each medium was adjusted to 5.8 ± 0.02 with 1 N NaOH or 1 N HCl and 0.4 % agar was added. The medium was dispensed into MacCartney bottles and autoclaved at 121^{o}C for 30 minutes and allowed to cool before culture initiation.

Preparation of explants

Juvenile healthy explant materials were collected from fully matured *M. oleifera* tree. The collected materials were brought to the laboratory and washed thoroughly with running tap water for 10 minutes and distilled water for 2 minutes to remove all forms of debris. Portions of the explants were then excised and transferred into the laminar flow hood. The excised explants were sterilized with 3.5 % solution of sodium hypochlorite (NaOCl) for 5 minutes. The explants were rinsed with double distilled water (ddH_2O) three times, so as to lower the toxic effect of the sterilizing agent.

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Inoculation of explants and incubation of cultures

All the equipment used for the process of inoculation was wrapped in aluminum foil and first sterilized by autoclaving. Sharp sterile surgical blade and scalpel were used to cut the sterile explants into tiny pieces of about 1 mm^2. With sterile forceps, each tiny piece of the explant was transferred onto the various solidified MS medium supplemented with different type and concentrations of plant growth regulators. The various cultures were incubated in the dark room at 25 ± 0.02 °C with 70 % relative humidity at 16 hours of photoperiod (light and dark regime). Cultures were observed and readings were taken at 7 days interval for a period of one month. The cultures that gave rise to shoots and roots were transferred to light room for plantlet formation.

Results

Supplementation of MS medium with various concentrations of IAA alone at the range of 1.0 - 9.0 mg/l stimulated the formation and proliferation of callus, rooting and shooting as shown in Table 1 and Figures 1a-e. It was observed that low concentrations of 1.0 mg/l, 2.0 mg/l, 3.0 mg/l and 4.0 mg/l of IAA generated calli and roots; the concentrations of 6.0 mg/l and 9.0 mg/l produced only callus. While the concentrations of 5.0 mg/1 and 8.0 mg/1 gave rise to callus, shoot and root formations after seven day of culture initiation. The concentrations that produced callus, shoots and roots were transferred from the dark room after two weeks to the light room for a period of another three weeks in the same culture media. Eventually, only the concentration of 8.0mg/l gave rise to plantlet (Figure 1).

Table 2 and Figures 2a - 2e show that when different concentrations of 2,4-D were used alone at the range of 1.0 - 9.0 mg/l, it was observed that low concentrations of 1.0 mg/1, 2.0 mg/1 and 3.0 mg/1 induced shoots and some callus formation; higher concentrations from 4.0 mg/l to 9.0 mg/l stimulated only callus formation, with 8.0 mg/1 producing largest callus intensity within a month after culture initiation. The combination of 0.1 mg/1 concentration of BAP with varying concentrations of (1.0-9.0 mg/1) IAA (Figures 3a-d) stimulated responses of callus formation and shooting after two weeks of culture initiation. Treatment concentrations of 0.1 mg/l BAP with 3.0, 4.0 and 7.0 mg/l IAA generated callus shoots and roots, while 1.0, 6.0, 8.0, 9.0 mg/l IAA with 0.1mg/l BAP stimulated only callus formation. The culture that generated callus and shoots were transferred from the dark room to light room. After further observation for two weeks, eventually 7.0 mg/1 of IAA and 0.1 mg/1 of BAP gave rise to plantlet formation.

Table 1: Effect of IAA alone on the morphogenetic response of *Moringa oleifera* leaf petiole explant

Concentration of IAA (mg/l)	Response observed	Callus intensity	Number of root formed	Number of shoot formed	Time of initiation of response (days)
1.0	Callus, roots	+	3	--	7
2.0	Callus, roots	+	4	--	7
3.0	Callus, roots	++	6	--	7
4.0	Callus, roots	++	5	--	7
5.0	Callus, shoots, roots	+	5	9	7
6.0	Callus	+++	--	--	7
7.0	--	--	--	--	--
8.0	Callus, shoots roots	++	6	8	7
9.0	Callus	++	--	--	7

Legend: (-) = No callus formation, (+) = Not profuse, (++) = Slightly profuse, (+++) = Profuse, IAA = Indole-3-acetic acid

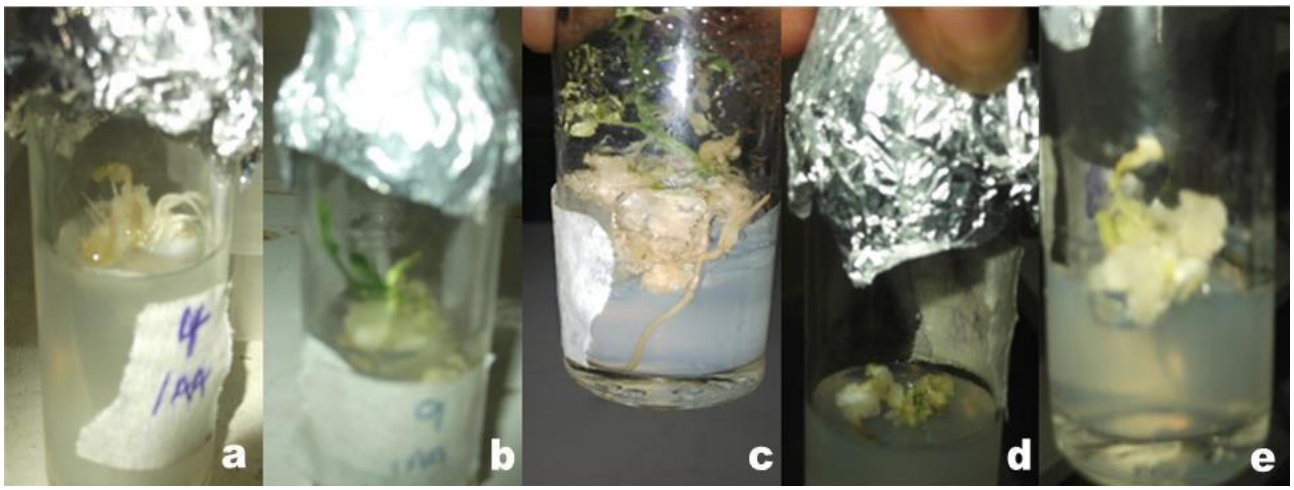

Figure 1: Various morphogenetic responses observed with M. oleifera leaf petiole explant cultured in MS medium supplemented with IAA alone

a) Callus and roots formed with 3.0mg/l IAA after 14 dci,
b) Shoot and callus formed with 8.0mg/l IAA after 14 dci,
c) Callus, shoot and root formed with 8.0mg/l IAA after 30 dci,
d) Callus and roots formed with 2.0mg/l IAA after14 dci,
e) Callus and shoot formed with 5.0mg/l IAA after 14 dci.

MS = Murashige and Skoog, IAA = Indole-3-acetic acid, dci = days of culture initiation

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Table 2: Effect of 2,4-D alone on the morphogenetic response of *Moringa oleifera* leaf petiole explant

Concentration of 2,4-D (mg/l)	Response observed	Callus intensity	Number of root formed	Number of shoot formed	Time of response (days)
1.0	Shoots, callus	+	--	4	14
2.0	Shoots	+	--	2	14
3.0	Shoots, callus	+	--	3	14
4.0	Callus	++	--	--	14
5.0	Callus	++	--	--	14
6.0	Callus	+++	--	--	14
7.0	Callus	++++	--	--	14
8.0	Callus	+++++	--	--	14
9.0	Callus	+++	--	--	14

Legend: (-) = No callus formation, (+) = Not profuse, (++) = Slightly profuse, (+++) = Profuse, (++++) = Very profuse, (+++++) = Highly profuse, 2,4-D = 2,4-Dichlorophenoxyacetic acid

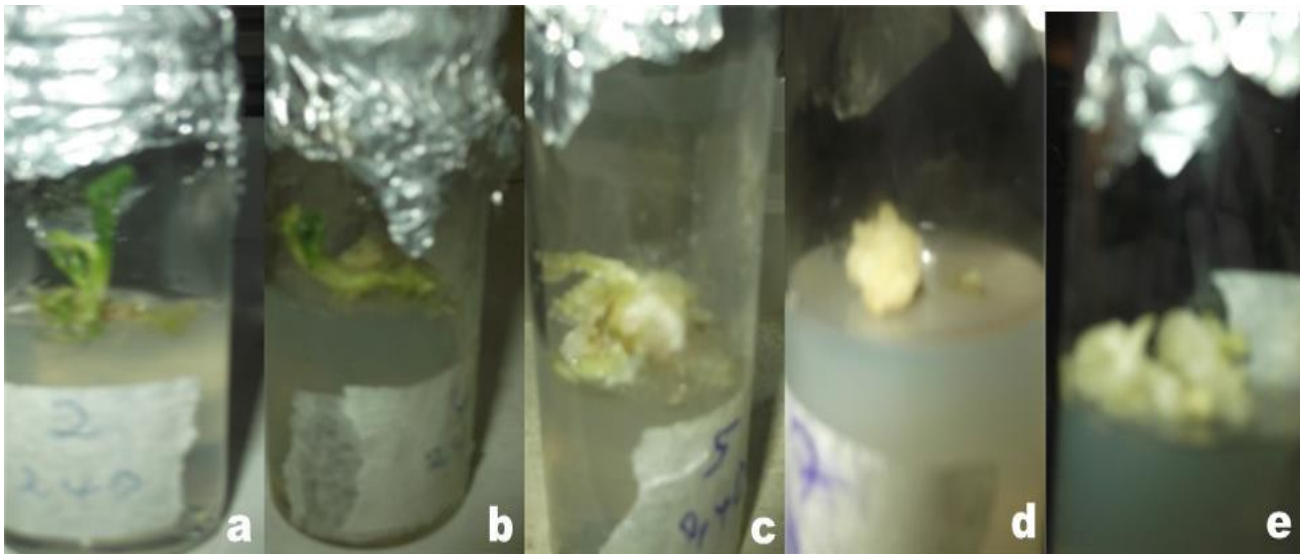

Figure 2: Shoots and calli formed from *M. oleifera* leaf petiole explant cultured in MS medium supplemented with 2,4-D alone 14 days after culture initiation

a) 1.0 mg/l 2,4-D, b) 3.0 mg/l 2,4-D, c) 4.0 mg/l 2,4-D, d) 6.0mg/l, e) 8.0mg/l 2,4-D.

Table 3: Effect of BAP and IAA on the morphogenetic response of *Moringa oleifera* leaf petiole explant

Concentration of Phytohormone		Response observed	Callus intensity	Number of root formed	Number of shoot formed	Time of initiation of response (days)
IAA (mg/l)	BAP (mg/l)					
1.0	**0.1**	Callus	+	--	--	14
2.0	**0.1**	--	--	--	--	14
3.0	**0.1**	Callus, Shoots Roots	++++	3	8	14
4.0	**0.1**	Callus, Shoots Roots	++	5	6	14
5.0	**0.1**	--	--	--	--	14
6.0	**0.1**	Callus	+++	--	--	14
7.0	**0.1**	Callus, Shoots Roots	++	8	7	14
8.0	**0.1**	Callus	++++	--	--	14
9.0	**0.1**	Callus	++++	--	--	14

Legend: (-) = No callus formation, (+) = Not profuse, (++) = Slightly profuse, (+++) = Profuse, (++++) = Very profuse, (+++++) = Highly profuse, IAA = Indole-3-acetic acid, BAP = Benzyl amino purine

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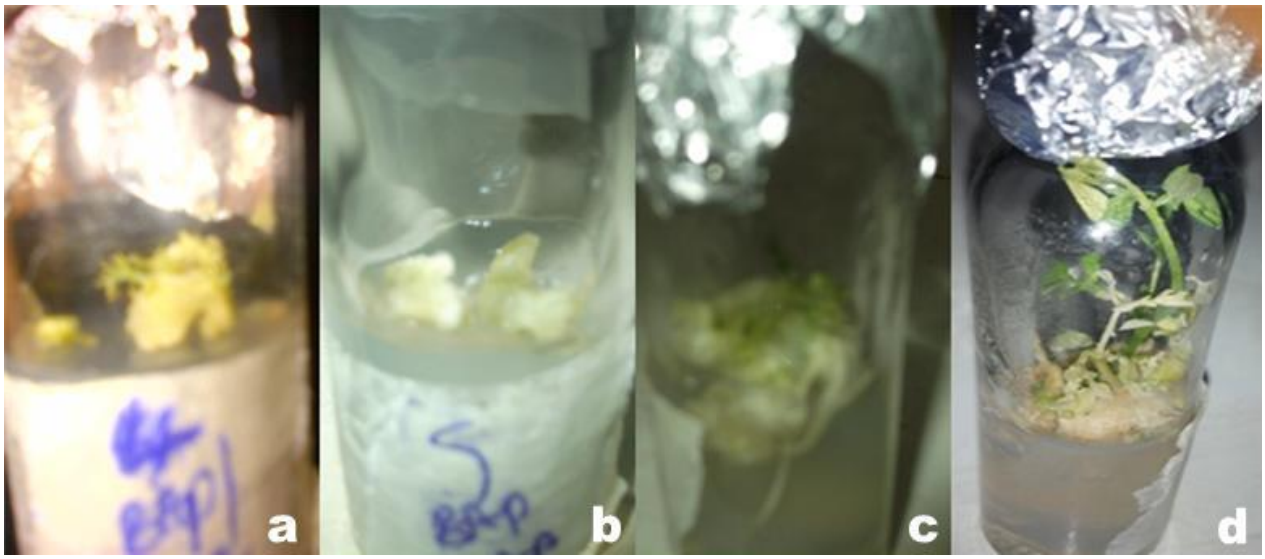

Figure 3: Various morphogenetic responses observed with *M. oleifera* leaf petiole explant cultured in MS medium supplemented with IAA and BAP

a) Callus and shoots formed with 3.0 mg/l IAA and 0.1mg/l BAP after 7days of culture initiation,
b) Callus formed with 8.0 mg/l IAA and 0.1 mg/l BAP after 7 days of culture initiation,
c) Callus, shoot and root formed with 4.0 mg/l IAA and 0.1 mg/l BAP after 14 days of culture initiation,
d) Plantlet formed with 7.0 mg/l IAA and 0.1 mg/l BAP after 30 days of culture initiation.

MS: Murashige and Skoog, IAA: Indole-3-acetic acid, BAP: Benzyl amino purine

Discussion

Studies have shown that morphogenetic responses (callogenesis, embryogenesis and organogenesis) can be obtained from different plant parts such as leaf, petiole, stem, flower, root, hypocotyls, seed, seedlings, embryo, cotyledon, pollen, anther, ovule, ovary and inflorescence (Bajaj *et al.,* 1992; Ajenifusan-Solebo *et al.,* 2013). In the present study, leaf petiole explant from *M. oleifera* showed different *in vitro* morphogenetic responses in MS medium supplemented with different concentrations of growth regulators. Media supplementation with 1.0 mg/l, 2.0 mg/l, 3.0 mg/l and 4.0 mg/l IAA supported both callus and root formation. Higher concentrations of 5.0 mg/l and 8.0 mg/l favor the production of callus, shoots and roots. It was observed that some leaf petiole explants developed into shoots while others into calli in MS medium treated with 2,4-D at a lower concentrations of 1.0 mg/l, 2.0 mg/l and 3.0 mg/l, 7 days after culture initiation. While the higher concentrations of 2,4-D at concentrations of 4.0 mg/l to 9.0 mg/l gave rise only to callus formation and proliferation. This study also showed that the concentration of 0.1 mg/l of BAP combined with the concentrations of 1.0 mg/l – 9.0 mg/l IAA stimulated callus, shoots and roots. Concentrations of 3.0 mg/l, 4.0 mg/l and 7.0 mg/l IAA with 0.1 mg/l of BAP produced callus with shoots and roots within a month after culture initiation. The cultures that generated callus, shoots and roots which were transferred to light room were observed to have given rise to plantlet formation from the media treated with 8.0 mg/l of IAA alone; 0.1 mg/l of BAP and 7.0 mg/l. Previous works have shown that petiole section explant of *Oxalis tuberose* gave rise to direct regeneration of numerous adventitious shoots in media treated with 3 mg/l NAA and 3 mg/l BAP (Khan *et al.,* 1988). Brown *et al.* (1987) demonstrated that there was direct regeneration of roots and shoots formations from cotyledon explant of *Pyrus malus* seed. These observations agreed with the findings of this research that direct regeneration of plantlet from leaf petiole explant of *M. oleifera* is feasible.

Conclusion

This study has demonstrated that different morphogenetic responses (callus, shoot and root formation) can be induced from leaf petiole explant of *M. oleifera* in MS media supplemented with growth regulators. The findings from the study suggest that plant regeneration from *M. oleifera* was favored by higher concentration (8.0 mg/l) of IAA alone and a combination of 0.1 mg/l BAP and 7.0 mg/l IAA.

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