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Callus Iduction and Differentiation for Value Medicinal Plant (*Moringa oleifera*) in Response to Different Explant Types and Growth Regulators

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ABSTRACT

The laboratory experiments aims to investigate the response of different explant types obtained from a very value medicinal plant, Moringa oleifera for callus induction and its plant regeneration. Four different explant types (epicotyls, stems, leaves and shoot tips) were cultured in Murashige and Skoog (MS) Medium fortified with various auxins 2,4-Dichlorophenoxyacetic acid (2,4-D) and a-naphthalene acetic acid (NAA) at different concentrations of 0.5, 1, 1.5 and 2 mg/L singly or in combinations with different cytokinins (BA and Kin) to induce callus formation. The results obtained indicated that callus can be generated from all the tested plant parts, but there were very big differences among the characters of callus induced from the different explants. Shoots tips cultured on MS medium supplemented with 2,4-D at 2.0 mg/L in combination with BA at 0.5 mg/L induced Maximum callus formation percentage of 100 % coupled with the highest callus fresh weight of 9.99 g. For Callus differentiation, callus derived from shoot tip and cultured on MS media fortified with BA at 1.5 mg/L produced 100 % callus differentiation percentage and significantly the highest value of shoots number (10.36 shoots).

INTRODUCTION

There are about 14 species of moringa trees in the family Moringaceae. Of these species, Moringa oleifera is the most widely known cultivated and utilized species (Morton, 1991). Moringa oleifera is a small to medium-sized fast-growing deciduous tree that usually grows up to 10 or 12 m in height. It is a native to northern India, Pakistan and Nepal. But, It is now cultivated and has become naturalized well beyond its native range, including throughout South Asia, and in many countries of Southeast Asia, tropical Africa, the Arabian Peninsula, Central America, the Caribbean and tropical South America (Iqbal and Bhanger, 2006). It is commonly known by different regional names such as drumstick trees, horseradish tree, ben tree, miracle tree and mother's best friend (Mishra *et al*., 2012).

Almost every part of moringa tree has a value, from the seeds to the leaves and the bark, down to the roots has beneficial properties. It is a multipurpose plant with numerous medicinal (Shank *et al*., 2013) and nutritional (Jed, 2005; Mishra *et al*., 2012) benefits. The leaves are known to be a great source of minerals and vitamins according to Jed (2005) who found that M. oleifera leaves provides 2 times the Vitamin A in carrot, 14 times the Calcium in milk, 9 times the Iron in Spinach, 4 times the Potassium in bananas, 2 times the protein in yogurt and 4 times the fiber in oats. Also, Elkhalifa *et al*. (2007) studied the nutritive values of the leaves of M. oleifera tree and found that the moisture content was 74.42 %, protein 16.7 %, fiber 3.5 %, ash 8%, and 1.7 % oil. In medicinal field, The extracts of M. oleifera leaves show various biological activities, including hypolipidemic, antimicrobial, antioxidant and antifungal activity in addition to cancer preventive effect (Siddhuraju and Becker, 2003; Devendra *et al*., 2011). Based on ethanobotanical studies its seeds, flowers and roots are extensively used for curing colic, bronchitis, inflammations, liver diseases, splenomegaly, epilepsy and regulate cholesterol (Jed, 2005; Nepolean *et al*., 2009).

Due to the nutritional and medicinal values, the plant has found wide uses in industries (Shank *et al*., 2013). So, this plant has to be conserved and multiplied to reach commercial requirement. The conventional approaches methods of plant propagation and improvement, like seed planting and stem cutting have limited applicability (Yadav *et al*., 2012). In respect of facing this large requirements, plant tissue culture technique has been extensively used for M. oleifera using direct micro propagation pathway (Shahina *et al*., 2005; Eufrocinio, 2010; Saini *et al*., 2012). Also, using indirect micro propagation technique have been described in a few reports by Devendra *et al*., 2012; Shittu and Igiehon, 2016 and Shittu *et al*., 2017. This study is aimed to compare the type of morphogenetic callus and plant regeneration that could be obtained from using different explant types of Moringa oleifera in medium containing different combinations of growth regulators.

MATERIALS AND METHODS

The current research was conducted at the experimental station and tissue culture laboratory of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University from 2017 to 2018.

Plant material:

Four different explants types (epicotyls, stems, leaves and shoot tips) of Moringa oleifera plants were used as explants for callus induction and its plant regeneration. For epicotyls explants, seeds of Moringa oleifera collected in October from the farm of Medicinal and Aromatic plant, Faculty of Agriculture, Mansoura University. The disinfestation process was done by soaking the seeds in a 3 % commercial bleach (sodium hypochlorite) solution for 20 min followed by a series of sterile water rinses. The seeds were cultured onto gars containing sterilized wet cotton. Epicotyls were excised after 15 days from germinated sterilized seedlings.

For the other three explants (stem, leaf and shoot tip), usually 8-10 cm and about 25 days old soft, active healthy terminal shoots of Moringa oleifera were snipped early in the morning in the month of April from an adult plant growing in the farm of Medicinal and Aromatic plant. The clean-up process for shoots involves several cold soapy water washes for one hour and then cut into three explant parts (stem, shoot tip and leaf) with length of 1-1.5 cm for the first two explants and the 3rd leaf from the shoot tip for the third explant. In the laminar flow hood cabinet the disinfestation was done by soaking the explants in 3 % commercial bleach (sodium hypochlorite) solution along

with a surfactant such as Tween 20 for 10 min for the leaves and 15 min for both stems and shoots tips. Finally all explants were washed 3 times with sterile distilled water for 3 min each.

Media and culture conditions:

Murashig and Skoog medium (1962) nutrient medium was used in all experiments supplemented with 3% (w/v) sucrose. The medium was solidified with 7g agar /l (w/v) and the pH of the medium was adjusted to 5.8 before autoclaving at 121° C for 20 min. All the cultured jars (250 ml) contained 30 ml of medium were incubated in plant growth room at 25 ± 2°C under constant fluorescent light of 2500 Lux for 16/8 h (light/ dark) photoperiod.

Callus induction:

Initially, the four different explants types (epicotyl, stem, leaf and shoot tip) were cultured on MS basal medium fortified with various auxins 2,4-Dichlorophenoxyacetic acid (2,4-D) and a-naphthalene acetic acid (NAA) at different concentrations of 0.5, 1, 1.5 and 2 mg/L in order to study the effects of various auxins on callus induction from different explant types. Also, the study investigated the effect of combinations between auxin (2,4-D at concentration of 2 mg/L) and three cytokinins; Kinetin (Kin), 6-benzyladenine (BA) and thidiazuron (TDZ) at two concentrations of 0.25 and 0.5 mg/L on the callus induction from the four explant types. Days to start callus initiation were recorded and after 30 days of culture callus formation percentage, fresh weight of callus, shoot formation percentage, shoots number and shoot length data were recorded.

Callus differentiation:

For plant regeneration by using callus as explant, different friable callus resources (callus from epicotyl, stem, leaf and shoot tip) were cut into pieces of 1 × 1 cm^2 and inoculated on MS medium supplemented with two types of cytokinins (BA and Kin) each alone at different concentrations (0.5, 1.0, 1.5 and 2.0 mg/L). 30 days later, data of callus percentage forming shoots, shoots number and shoot length were registered.

Statistical analysis:

A factorial experiment based on completely randomized design with 4 replicates included 12 jars for each treatment was used with all the designed experiments. Data subjected to analysis of variance (ANOVA) by using COSTAT v. 63 statistical software. Mean comparisons were performed using the least significant difference (LSD) at level of 5 % method according to (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

I- Callus induction stage:

Callus characteristics including days to start callus initiation, callus formation percentage, fresh weight of callus, shoot formation percentage, shoots number and shoot length as affected by the interaction between four different explant types (epicotyl, stem, leaf and shoot tip) and different plant growth regulators at various concentrations were shown in Table (1). It was cleared that characteristics of callus varied depending on explant type used as well as type and level of growth regulators used.

Dealing with the number of days to callus initiation, which were recorded from the first sighting of callus induction after inoculation in culture media Data presented in Table (1) showed that among all the treatments there were significantly differences in the number of days to initiate callus. The number of days from culturing the different explants to callus initiation was ranged from the 4.25 to 22.5 days. It was a matter of importance to notice that culturing shoot tip explant on nutrient media (MS) supplemented with different growth regulators at various concentrations always had the superior effect. The most efficient treatment for the days to initiate callus (4.25 days) achieved when shoot tip explant was cultured on MS nutrient media supplemented with combination of 2,4-D at 2.0 and BA at 0.5 mg/ L. While, the lateness in callus initiation days was always linked to stem explant cultured at different media and the longest time to start in initiation callus (22.5 days) was obtained with MS media augmented with NAA at 2 mg/L.

Table 1. Callus characteristics of *Moringa oleifera* as affected by the interaction between explant types and different plant growth regulators at various concentrations.

Treatments					Days to initiate callus				callus formation %				callus fresh weight (g)			
Auxin type conc. mg/L		Cytokinin type conc. mg/L			Epicotyl	Stem	Leaf	Shoot tip	Epicotyl	Stem	Leaf	Shoot tip	Epic tyl	Stem	Leaf	Shoot tip
2,4-D	NAA	BA	TDZ	Kin												
1.0					15.25	0.00	11.13	7.38	50.0	0.0	66.7	75.0	3.66	0.00	4.27	5.47
1.5					13.50	19.50	9.33	6.13	66.7	16.7	75.0	83.3	4.35	1.68	4.74	6.18
2.0					11.25	17.20	8.50	5.25	75.0	25.0	91.7	91.7	5.74	2.41	6.39	7.23
2.5					10.25	16.00	7.33	5.13	58.3	16.7	83.3	83.3	5.18	1.02	5.49	6.85
	1.0				17.38	0.00	15.38	14.13	41.7	0.0	50.0	66.7	3.69	0.00	4.34	4.96
	1.5				15.13	0.00	12.25	11.25	50.0	0.0	66.7	75.0	3.98	0.00	4.84	5.07
	2.0				13.25	22.50	11.25	9.25	33.3	16.7	58.3	83.3	3.48	0.67	4.18	5.63
	2.5				12.00	20.50	10.13	8.38	16.7	25.0	41.7	75.0	1.89	1.28	2.98	4.56
2.0		0.25			10.25	15.25	7.42	6.12	91.7	33.3	91.7	91.7	6.86	3.19	8.28	8.67
2.0		0.5			9.25	13.38	6.38	4.25	100	41.7	100	100	7.78	3.26	9.13	9.99
2.0			0.25		11.38	15.25	9.00	6.25	75.0	25.0	91.7	91.7	6.38	1.95	6.87	7.54
2.0			0.5		10.12	14.33	9.25	5.13	83.3	33.3	91.7	100	6.48	3.28	7.11	7.67
2.0				0.25	11.12	16.00	9.75	9.25	83.3	16.7	83.3	83.3	5.77	1.34	5.97	6.47
2.0				0.5	9.25	15.00	8.38	7.13	75.0	16.7	75.0	91.7	5.26	1.47	5.39	5.55
L.S.D. at 5 %					0.56				21. 47				0.72			

Table 1. Continued….

Treatments					Shoot formation %				Shoots number				Shoot length			
Auxin type conc. mg/L		Cytokinin type conc. mg/L			Epicotyl	Stem	Leaf	Shoot tip	Epicotyl	Stem	Leaf	Shoot tip	Epicotyl	Stem	Leaf	Shoot tip
2,4-D	NAA	BA	TDZ	Kin												
1.0					0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5					0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0					0.00	0.00	33.33	49.99	0.00	0.00	1.13	2.63	0.00	0.00	0.83	1.25
2.5					0.00	0.00	49.99	49.99	0.00	0.00	2.38	3.38	0.00	0.00	1.10	1.63
	1.0				0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.5				0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2.0				0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2.5				0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0		0.25			0.00	0.00	49.99	75.00	0.00	0.00	2.13	3.75	0.00	0.00	2.13	2.62
2.0		0.5			0.00	0.00	58.33	83.33	0.00	0.00	3.38	4.63	0.00	0.00	2.42	2.83
2.0			0.25		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0			0.5		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0				0.25	0.00	0.00	41.66	49.99	0.00	0.00	2.00	1.75	0.00	0.00	3.13	3.25
2.0				0.5	0.00	0.00	41.66	66.66	0.00	0.00	2.25	3.25	0.00	0.00	3.35	3.50
L.S.D. at 5 %							11.91				0. 35				0.07	

The recorded results in the same table showed that single supplementation of auxin (2,4-D and NAA) at different concentrations in the nutrient medium affected quite well the percentage of callus formation for the four different explant types. The results clarified that single addition of 2,4-D always gave the highest percentage of callus formation when compared to NAA at the same concentration and cultured with same explant type. In this concern, the single addition of 2,4-D at the concentration of 2 mg/L gave the highest percentage of callus formation for the all explant types. Also, it was clearly noticed that the combination between different growth regulators (auxin and cytokinin) had a strong efficacy in this regard. Culturing of epicotyl, leaf and shoot tip on MS nutrient medium fortified with 2,4-D at 2 mg/L and BA at 0.5 mg/L helped to achieve the maximum percentage of callus formation (100 %). Also, shoot tip cultured on MS medium fortified with 2,4-D at 2 mg/L and TDZ at 0.5 mg/L recorded 100 % of callus formation percentage. On the other hand, using stem cut as explant significantly tabulated the lowest value of callus formation percentage and sometimes failed to induce callus when compared with the other explants at the same medium.

Before regarding the effect of this interaction on callus fresh weight, it is worth to mention to the texture and degree of callus formation which was measured based on visible rating using three scales slight, moderate and profuse for the degree of callus and friable or compact for texture of callus. Because sometimes the callus looked very big in size but its weight gave very small value. As it appeared from the results, culturing epicotyl explants in MS medium augmented with 2.5 mg/L of NAA gave very big profuse white loose friable callus which tabulated very low value of callus fresh weight (1.89 g). While, culturing shoot tip in the same medium gave a moderate compact green callus which significantly resulted high callus fresh weight (4.56 g) as shown in Fig. 1A&B. Generally, shoot tip explant had the upper hand with all culture media tested in the character of callus fresh weight when compared with all explant types. The highest significant green compact callus fresh weight (9.99 g) was achieved when shoot tip was culture in nutrient medium augmented with a combination of 2,4-D at 2 mg/L and BA at 0.5 mg/L as shown in Fig. 1C. The next positive results were obtained with the same medium cultured with leaf and epicotyl explants, as they were 9.13 and 7.78 g, respectively. In contrast, stem explant always tabulated the lowest value of callus fresh weight and the callus tended to be brown slight friable as shown in Fig. 1D.

In addition, it was noticed from the data in Table (1) that only compact green callus obtained from shoot tip and leaf explants at varied treatments success to give a percentage of callus regeneration and number of shoots in the same stage of callus induction. Comparing between the two auxins used (2,4-D and NAA) it was cleared that the two explants (shoot tip and leaf) success to give a percentage of shoot regeneration with 2,4-D at the high level of concentrations 2 and 2.5 mg/ L. Looking for the combination between auxin and cytokinin in this regard, the results provide that 2,4-D at 2 mg/ L in combination with BA and Kin at the two different concentrations gave percentage of shoot formation while TDZ failed to induce shoots. The highest shoot formation percentage of 83.33 % and the highest significant shoots number of 4.63 shoot with shoot length about 2.83 cm obtained when shoot tip was cultured on MS medium fortified with combination of 2,4-D at 2 and BA at 0.5 mg/L. While, The lowest shoot formation percentage of 33.33 % linked to the lowest significant shoots number of 1.13 shoot and the shortest shoot length of 0.83 cm was obtained when leaf was cultured on MS medium fortified with 2,4-D at 2 mg/L.

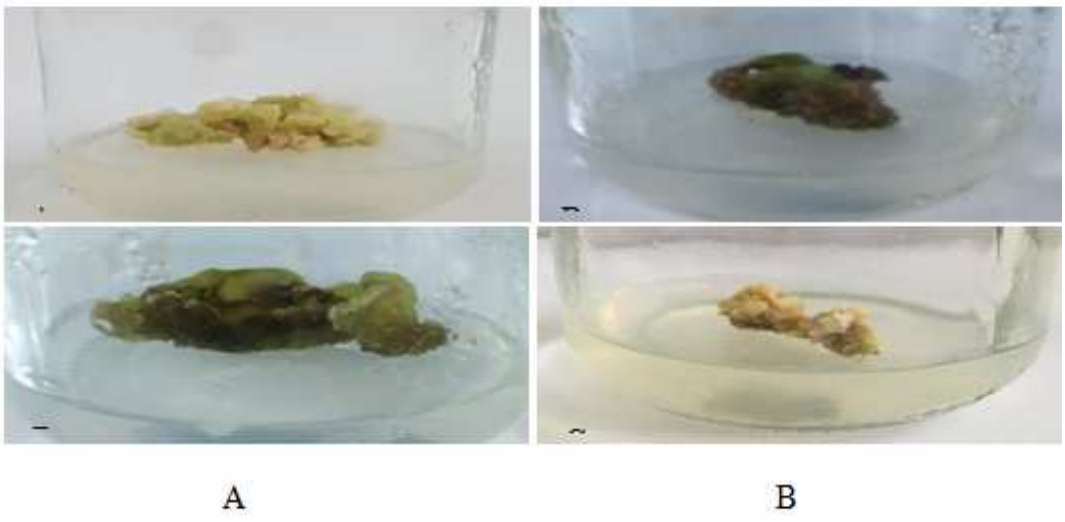

Figure 1. Callus formation of *Moringa oleifera*. A) By culturing hypo B) By culturing shoot tip in MS medium augmented with 2.5 mg/L of NAA. C) By culturing shoot tip in MS medium augmented with combination of 2,4-D at 2 mg/L and BA at 0.5 mg/L. D) By culturing stem explant.

Generally callus is a relatively undifferentiated tissue consisting primarily of parenchymatous cells. Callus tissue can serve as an experimental system to investigate and solve a broad range of basic research problems in plant cytology, physiology, morphology, anatomy, biochemistry, pathology and genetics. It can also be used to resolve applied research problems in organogenesis and embryogenesis related to the propagation of horticultural and agronomic plants. Tissue from various organs from many species of plants can be induced from callus. However, the ability of a specific tissue to form callus may determine by many seemingly unrelated factors. Among these factors are mineral nutrition and plant growth regulators, environmental factors and the genetic constitution or the genotype of the plant (Trigiano and Gray, 2011). So, different tissue from plant species can respond differently from one another under various conditions of medium nutritional ingredients, plant growth regulator and environmental factors.

The previous laboratory experiments illustrated the technique to obtain callus from different explants from Moringa oleifera using various plant growth regulators at various concentrations and compare the callus growth. It was quite clear that the type of explant has a significant role in callus growth and this was in agreement with Passey *et al*. (2003) who reported that callus induction was dependent on the explants source and types of tissue used. Such variations can be indicative of the physiological condition of the explant, which is determined by genetic factors (Nagarathna *et al*., 1991). Also Trigiano and Gray (2011) stated that the differential response seen between tissue sources maybe due to tissue competency, which in turn could be due to different levels of inductive factors (morphogens) preexisting in the tissue. Furthermore, it was observed that the combination between auxin and cytokinin promoted the callus growth and this was in agreement with Trigiano and Gray (2011) who stated that some ingredients in a medium are more vital for callus tissue growth when provided in combination rather than separately. They also mentioned that many different facets of the in vitro environment and the explants affect success in vitro callus induction, but the type, concentration, and duration of exposure to plant growth regulator typically have the most profound effect.

II- Callus differentiation stage:

Data presented in Table (2) indicated that shoot tip derived callus which cultured on media fortified with BA at concentrations of 1.5 and 2.0 mg/L induced spectacularly the highest callus redifferentiation percentage value of 100 %, when compared with all other cases. The next positive effect of 91.67 % was recorded for callus derived from also shoot tip explant and cultured on the media supplemented with BAP at 1.0 mg/L or Kin at the highest concentration of 2.0 mg/L. Clearly, it was obvious that the callus source had the upper hand in that concern comparing with the other two factors, because no callus redifferentiation was obtained when callus derived from stem explant cultured on all media tested. Furthermore, callus derived from epicotyl cultured on media supplemented with low concentrations of BA or Kin (0.5 and 1.0 mg/L) failed to induce callus and at the high concentrations 1.5 and 2.0 mg/L resulted in the lowest values in these respects, as they ranged from 25.0 to 33.33 % and 16.7 to 25.0 %, respectively.

Looking for shoot number character, it was obvious from data illustrated in the same table that callus of shoot tip cultured on nutrient media supplemented with BA 1.5 mg/L attained significantly the outstanding value of 10.36 shoot (Fig. 2A), when compared with all of the other cases. The next positive value of 9.15 shoots was obtained when the callus of leaf was cultured on the same medium. On the other hand, the callus of epicotyl cultured on medium supplemented with Kin at 1.5 mg/L significantly recorded drastically the lowest shoots number of 1.25 shoots (Fig. 2B).

Table 2. Effect of callus source and cytokinin type at different concenterations on callus redifferentiation of Moringa oleifera.

Treatments		Callus redifferentiation (%)				Shoots number/ explant				Shoot length (cm)			
cytokinin type	cytokinin conc. mg/L	Epicotyl	Stem	Leaf	Shoot tip	Epicotyl	Stem	Leaf	Shoot tip	Epicotyl	Stem	Leaf	Shoot tip
BA	0.5	0.00	0.00	58.33	83.33	0.00	0.00	5.47	6.23	0.00	0.00	3.24	2.83
	1.0	0.00	0.00	66.66	91.67	0.00	0.00	6.71	7.45	0.00	0.00	3.22	3.15
	1.5	25.0	0.00	74.99	100	2.75	0.00	9.15	10.36	2.73	0.00	2.99	2.45
	2.0	33.33	0.00	66.66	100	3.00	0.00	7.28	8.44	3.14	0.00	2.93	2.65
Kin	0.5	0.00	0.00	41.66	50.0	0.00	0.00	3.37	3.85	0.00	0.00	3.33	3.42
	1.0	0.00	0.00	66.66	75.0	0.00	0.00	4.22	4.90	0.00	0.00	3.43	3.43
	1.5	16.66	0.00	74.99	83.33	1.25	0.00	5.36	6.25	3.65	0.00	3.53	3.34
	2.0	25.00	0.00	58.33	91.67	2.25	0.00	6.31	7.37	3.83	0.00	3.64	3.15
L.S.D. at 5 %		16.19				0.61				0.13			

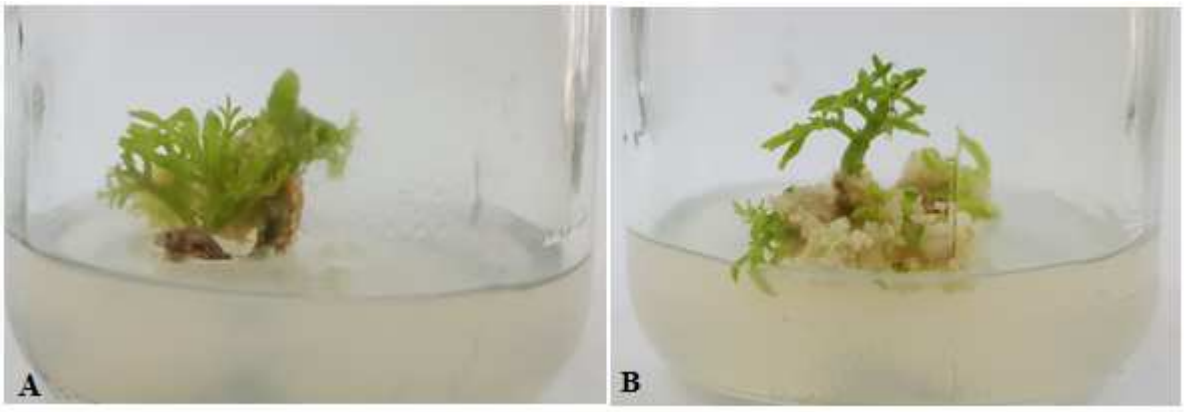

Figure 2. Shoot regeneration from callus of *Moringa oleifera*. A) By culturing callus obtained from shoot tip on MS medium supplemented with BA 1.5 mg/L. B) By culturing callus obtained from epicotyl on MS medium supplemented with Kin at 1.5 mg/L.

Results in the same table cleared that in vitro culturing of callus obtained from epicotyl on nutrient medium fortified with 2.0 mg/L of Kin significantly resulted the highest values of shoot length (3.83 cm), followed by 3.65 cm for nutrient medium fortified with 1.5 mg/L of Kin with the same explant type. But, it was quite clear that a significant difference was detected between them. On the other hand, callus of shoot tip cultured on nutrient media fortified with 1.5 mg/L of BA significantly measured the shortest value of shoots length, as it recorded 2.45 cm.

In general, the organogenic process begins with changes in a single or small group of parenchyma cells, which then divide to produce a globular mass of cells or meristemoid, which is plastic and can give rise to either a shoot or root primordium. These events can occur directly in the explant or indirectly after callus formation (Thorpe, 1980). In vitro organogenesis has been achieved in over 1000 plant species through empirical selection of the explant, the medium composition and control of the physical environment (Brown and Thorpe, 1986). Hence, in this study the organogenic process had been tested by using different source of callus and different cytokinin and it was clear that explant type substantially affected this process (shoot differentiation) and this results support the fact which was reported by Bhojwani (1990) who reported that the degree of cell sensitivity towards growth regulators depending upon the tissue type of the inoculum, and upon the physiological state of the donor plant and of the donor organ and all of this are factors influence the organogenic differentiation. Also, Trigiano and Gray (2011) reported that in addition to hormone signals tissue maturity of the explant donor can be critical to the explant's competency to respond to morphogenetic signals to form adventitious organs.

As well as the explants affect the success of in vitro culture the choice of plant growth regulators type and concentration have a profound effect. In this study cytokinin play a vital role in differentiation and this was in agreement with Trigiano and Gray (2011) whom illustrated that cytokinins play a role in a variety of processes including cell division and differentiation, delay of senescence, development of chloroplasts, resource uptake and allocation, nodulation in leguminous species, vascular development, as well as initiation and development of shoots.

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إنتاج وتمايز الكالس لنبات طبي هام (المورينجا) بالاستجابة لأنواع مختلفة من الأجزاء النباتية و منظمات النمو

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تهدف التجارب المعملية لدراسة استجابة أجزاء نباتية مختلفة من نبات طبي هام جدا (المورينجا) لانتاج الكالس و تخليق النباتات منها. باستخدام أربعة أجزاء نباتية مختلفة (السويقة الجنينية العليا- الساق- الورقة- القمة النامية) تم زراعتها على بيئة موراشيج و سكوج محتوية على تركيزات مختلفة من الاوكسينات (بنفثالين حامض الخليك و 2،4 داى كلوروفينوكسى حامض الخليك منفرده او في توليفات مع سيتوكينينات مختلفة (البنزيل ادينين و الكينيتين) لانتاج الكالس. أوضحت النتائج المتحصل عليها ان كل الأجزاء النباتية المختبرة يمكن ان تعطي كالس لكن هناك اختلافات كبيرة في صفات الكالس المنتج من أجزاء نباتية مختلفة. زراعة القمم النامية على بيئة موراشيج و سكوج محتوية علي 2،4 داى كلوروفينوكسى حامض الخليك بتركيز 2 مللجم / لتر في تداخل مع البنزيل ادينين بتركيز 0.5 مللجم / لتر اعطت اعلى نسبة تكون للكالس 100 % مرتبطة باكبر وزن طازج للكالس 9.99 جرام. لإعادة تكشف الكالس, زراعة الكالس الناتج من القمم النامية علي بيئة موراشيج و سكوج محتوية علي البنزيل ادينين بتركيز 1.5 مللجم / لتر سجلت أعلى نسبة مئوية لإعادة التكشف 100 % و اعلى عدد افرع 10.36 فرع.