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In vitro culture and cytological features of two *Moringa* species cultivated in Egypt

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Abstract

The significance of *Moringa* lies in its diverse advantages, encompassing nutritional richness, medicinal properties, and potential environmental sustainability. The current study sheds light on the impact of explant type and medium composition on callus and shoot induction of two *Moringa* species cultivated in Egypt. Additionally, it investigates certain cytological features in root tip cells of those plants as well as cells of induced calli in vitro, such as cell shape, size and mitotic activity. The results revealed notable differences in callus induction and its fresh and dry weights between the two *Moringa* species (*M. oleifera* and *M. stenopetala*), as well as among MS media used. Furthermore, the study demonstrated significant variations in response of two tested explants (leaves and hypocotyls). Active meristematic cells were classified into three types: Type I includes dividing cells, Type II consists of meristematic cells with diffused chromatin, and Type III represents cylindrical cells with diffused chromatin. Interestingly, the percentages of dividing cells may suggest the mitotic index (MI), with the MI being considerably higher in cells of M. oleifera compared to M. stenopetala.
Abbreviations: 2, 4-D: 2, 4-Dichloro-phenoxyacetic acid; BAP: Benzyl amino purine, IBA: Indole butyric acid; NAA: Naphthalene acetic acid.

1. Introduction

Moringa oleifera Lam., generally known as drumstick tree, belongs to the Moringaceae family is a perennial species, native of India. It is usually cultivated in tropical and subtropical countries, and it was introduced to Brazil during the 1950s. Other species of genus *Moringa* is M. stenopetala which is a vital crop in Kenya and Ethiopia [1]. Likewise, M. peregrina was famous to the ancient Egyptians who used its seed oil [2]. All the other 10 species of this genus are described to have pharmacologic properties [3 - 4]. But some other species are in danger of extinction, particularly M. hildebrandtii and M. peregrina (Forssk.) Fiori [2 and 5]. *Moringa* has been evidenced to be an important food source containing proteins, vitamins, minerals, and antioxidants, where it supports overall wellness [6]. In the Philippines, this vegetable tree is quite common due to its medicinal properties and high nutritional value. One of its important uses is galactagogue property which encourages the secretion of milk, thus helping to increase milk production in lactating mothers [7]. It is also utilized in feeding programs to fight malnutrition in many third-world countries [8].

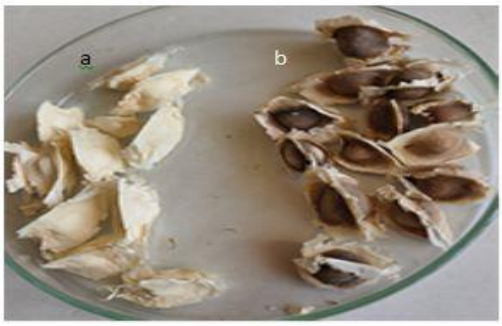
Islam et al., [9] reported that *Moringa* is propagated by stem cuttings; however, this method reduces the mother plants growth, affects yields and sometimes causes death of the mother plant. Therefore, an approach to solve this problem is through plant biotechnology, based on plant tissue culture. Consequently, the establishment of an in vitro tissue culture offers many advantages over the conventional methods of propagation [10]. In addition, tissue culture technique is a powerful tool for the generation of genetically modified plants [11]. There are many different explants such as: nodal segments [12 - 13], immature seeds [7] and axillary cotyledons and buds [14] used in in vitro propagation studies for *Moringa*. Al Khateeb et al., [15] described that, among the different paths of in vitro cultivation, some of the in vitro conditions can be modified to obtain better results. Changes in these conditions, such as phytohormone composition and concentration or light intensity and temperature, can change explant response, higher callus induction and produce more vigorous plants. Direct and indirect micropropagation techniques of M. oleifera have been described in a few reports [16 - 21]. Stephenson and Fahey, [7] reported 20% success rate of germination of immature seeds with subsequent shoot development from the epicotyl meristematic tissues of M. oleifera cultured on semi-solid MS medium (Murashige and Skoog),[22] amended with 1 mg /l BAP and 1 mg /l GA3.

Moringa oleifera plants have 2c genome size of 1.2 pg [23]. The diploid number (2N) of M. oleifera is 28 chromosomes as reported by Mendioro et al., [24]. The meiotic behavior of M. oleifera plants revealed 14 bivalents in diakinesis of all studied plants and the meiotic abnormalities were rare and metaphase I was the most affected phase [25]. Anwar, [26] investigated the cytological features of M. oleifera and M. stenopetala (such as cell shape and size, mitotic chromosomes and nucleolus appearance). Her study reported that active meristematic cells could be categorized into three types: Type I (including dividing cells), Type II (meristematic cells with diffused chromatin) and Type III (cylindric cells with diffused chromatin). Also, that mitotic index was considerably higher at cells of M. oleifera than that of M. stenopetala ones.

The present work aimed to study the effect of 2 different explant types and different plant growth regulators and their different concentrations and combinations in MS medium on callus induction and shoot regeneration of two cultivated *Moringa* species (M. oleifera and M. stenopetala). Likewise study cytological behavior of the two cultivated *Moringa* plant species and their induced calli.

2 . Materials and methods

Two species of *Moringa* (*M. oleifera* and *M. stenopetala*) were used in the present study. Seeds of the two species (Fig. 1) were kindly provided by the Ornamental Branch, Horticulture Department, Faculty of Agriculture, Minia University, El-Minia, Egypt; they have great thanks and gratitude.



Figure(1) : Seeds of the two *Moringa* genotypes, a: white seeds of M. *stenopetala* (left) and b: brown seeds of *M. oleifera* (right).

2.1. Seeds sterilization and germination

Seeds were washed sue with the culture medium, seeds were surface sterilized inside the laminar flow hood by immerunder running tap water for five minutes. After removing the seed coats to permit direct contact of seed tission in 70% ethanol (v/v) for 2 min, then 20% (v/v) Clorox® commercial bleach (5% sodium hypochlorite) for 20 minutes, followed by rinsing with sterile distilled water (3-6 times) before culture. The sterilized seeds were cultured (5 seeds/vial) on half-strength MS plant growth regulator-free medium [22] supplemented with 30 g sucrose/l and solidified with 0.8% agar. The vials were incubated in darkness at 25 ± 2ºC for four days (initiation of germination) and then transferred into 16/8 h (light/dark cycle) photoperiod at a light intensity of 1000 lux at 25 ± 2°C. Percentage of germinated seeds was recorded after two weeks of incubation for M. Oleifera and three weeks for M. stenopetala.

2.2. Callus induction and shoot regeneration

Six modified MS media were used; MS1, MS2, MS3, MS4, MS5 and MS6 which varied according to their type, concentration and combinations of plant growth regulators (NAA, 2, 4-D, BAP, IBA; Table 1). All cultured media were supplied with 30 g/l sucrose, 8 g/l agar and pH 5.8. Media were dispensed in vials (25 ml/vial) and were autoclaved at 121°C for 20 min.

Two explants' types (leaves or hypocotyls) were used. Leaves and hypocotyls of germinated seedling were transferred into Petri dish and cut into slices (0.5 - 1 cm long) under aseptic conditions and cultured on 6 examined MS modified media. Each vial contained 4 leaves explants or 3 hypocotyls explants. Each treatment was repeated five times. All cultures were incubated at 25°C in darkness. Numbers and percentages of induced calli were recorded after 4-week and data were analyzed statistically using MSTAT program (version 4.0) edited in 1985 by the MSTAT development team, Michigan University and Agricultural University of Norway. Cultures/vials with calli induced shoots were moved to incubation under the same conditions with using 16/8-h light/dark photoperiodic regime, some shoots were subcultured onto root induction MS media for further development.

Table (1): The composition of different MS media employed in the initiation of callus and the regeneration of *Moringa* plants.

Medium code	Composition
MS1	MS+ 0.25 mg/l NAA
MS2	MS+ 0.25 mg/l NAA+ 0.25 mg/l 2,4-D
MS3	MS+ 0.25 mg/l NAA +0.50 mg/l BAP
MS4	MS+ 1.50 mg/l BAP + 1.50 mg/l IBA
MS5	MS+ 1.00 mg/l BAP
MS6	MS+ 0.60 mg/l NAA +1.00 mg/l BAP

2.3. Callus fresh and dry weights

Fresh and dry weights of obtained calli were assessed. After four weeks in culture, fresh weights of calli tissue were measured (4 calli/ replicate). For measuring dry weight, 4 calli of each replicate were packaged in aluminum foil and incubated overnight in oven at 70ºC, then weight estimation. Fresh and dry weight data were analyzed also using MSTAT program (version 4.0).

2.4. Cytological studies

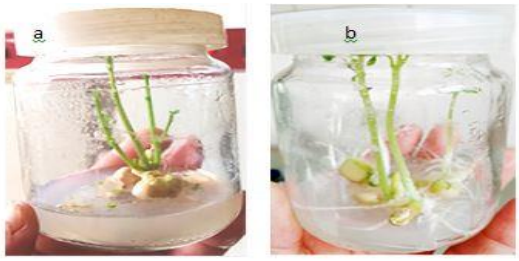
The cell types, frequency and percentages of root apex cells of the original plants of two *Moringa* species (as control) and callus tissue cells derived from 2 different explants were examined. The number of dividing cells (frequency of dividing cells or mitotic index) was also examined and monitored as an expression of the rate of mitotic division for meristematic root cells as well as callus tissue cells. Mitotic chromosomal preparation was done using the method reported by Anwar, [26].

3. Results:

3.1. Seeds germination

Following a two-week germination period for M. oleifera and a three-week period for M. stenopetala, the germination rate reached 100% for both species (Fig. 2). These results indicate that the conditions used to germinate the seeds of both species of *Moringa* were appropriate, in terms of the chemical materials and times used in the surface sterilization processes, as well as the germination conditions of temperature, light and humidity and also the suitability of the nutritional media. However, it was noted that the seeds of M. oleifera germinate faster than the

other species (M. stenopetala) and this may be due to the genetic background of both types.



Figure(2) : Germinated seeds of *Moringa oleifera* (a, left) and *M. stenopetala* (b, right).

3.2. Callus induction and shoot regeneration:

In our study here, we checked the 3 outmost important elements. Two important *Moringa* species: M. oleifera and M. stenopetala; two very famous explants (leaves and hypocotyls) as well as 6 different MS media.

Analysis of variance indicated a wide range of variation among these three factors and most of them had significant differences (Table 2). Explants (leaves and hypocotyls) of M. oleifera showed higher response and induced calli with high frequency (97.9%; Table 3) than the same explants of M. stenopetala which produce only 77.5% calli. As general, hypocotyls showed higher response (95%) than leaves (80.42%) through all treatments. Wide range and significant variations observed between 6 tested media, MS3, MS4, MS5 and MS6 induced 100% calli from cultured explants of M. oleifera, while only MS6 produced 100% calli from explants of M. stenopetala. Other tested MS media produce calli in percentage ranged from 57.5% (MS4 with M. stenopetala) to 95% (MS1 with M. oleifera). Hypocotyls of M. oleifera recorded higher response (99.17%) to produce calli than its leaves (96.6%). The same trend was observed for hypocotyl of M. stenopetala (95%) compare to their leaves (80.42%).

Our results clarified that hypocotyls of M. oleifera were the best explant to produce calli on the following 4 MS media (MS3, MS4, MS5 and MS6) which supplied with BAP with or without NAA or IBA.

Table (2): Analysis of variance of the number of the induced callus from two explant types (leaves and hypocotyls) of two *moringa* species (*M. oleifera* and *M. stenopetala*) cultured on six different MS callus media. Five replicates were used for each treatment; replicate contain 4 leaves or 3 hypocotyls explants cultured on different 6 MS media.

S. O. V.*	D. F.	M. S.	F. value
A	1	20.008	** 66.694
B	5	1.668	** 5.561
c	1	10.208	** 34.028
AB	5	2.188	** 7.294
AC	1	7.008	** 23.361
BC	5	0.508	N.S 1.694
ABC	5	0.428	N.S 1.428
Error	96	0.3	
Total	119		

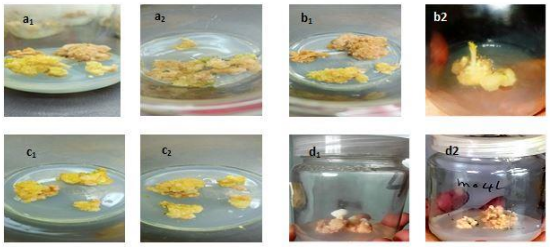
* A: *Moringa* genotypes; B: MS media; C: Explant types. **: highly significant; N.S.: not significant; Probability at 5%.

Table (3): Explants culture (leaves and hypocotyls) and its response to induce calli from 2 *Moringa* species (*M. oleifera* and *M. stenopetala*) cultured on 6 different MS callus induction media.

Species	Media	Calli induction								
		Explant type								
		Leaves			Hypocotyls			Total		
		Explant No.	Calli No.	Calli %	Explant No.	Calli No.	Calli %	Explant No.	Calli No.	Calli %
M. oleifera	MS1	20	18	90	20	20	100	40	38	95.0
	MS2	20	18	90	20	19	95	40	37	92.5
	MS3	20	20	100	20	20	100	40	40	100
	MS4	20	20	100	20	20	100	40	40	100
	MS5	20	20	100	20	20	100	40	40	100
	MS6	20	20	100	20	20	100	40	40	100
Total		120	116	96.6	120	119	99.1	240	235	97.9
Mean		20	19.3	96.6	20	19.8	99.1	40	39.17	97.9
M. stenopetala	MS1	20	13	65	20	20	100	40	33	82.5
	MS2	20	15	75	20	19	95	40	34	85.0
	MS3	20	10	50	20	16	80	40	26	65.0
	MS4	20	8	40	20	15	75	40	23	57.5
	MS5	20	11	55	20	19	95	40	30	75.0
	MS6	20	20	100	20	20	100	40	40	100
Total		120	77	64.17	120	109	90.83	240	186	77.5
Mean		20	12.8	64.0	20	18.17	90.83	40	31	77.5
Gross Total		240	193	80.42	240	228	95.0	480	421	87.71
Mean		20	16.1	80.42	20	19	95.0	40	35.1	87.71

3.3. Callus fresh and dry weights

The results indicated significant differences in callus fresh and dry weights between the two species (M. oleifera and M. stenopetala), as well as among the six used media. Furthermore, the results demonstrated significant differences between two explants (leaves and hypocotyls), as illustrated in Table (4) and Figures (3 and 4).



Figure(3)

Figure (3) : Calli and shoots developed after 28 days on MS3 (a1, a2, b1 and b2) and MS4 (c1, c2, d1 and d2) media from leaves (a1, b1, c1 and d1) and hypocotyl (a2, b2, c2 and d2) of *M. oleifera* (a1, a2, c1 and c2) and *M. stenopetala* (b1, b2, d1 and d2).

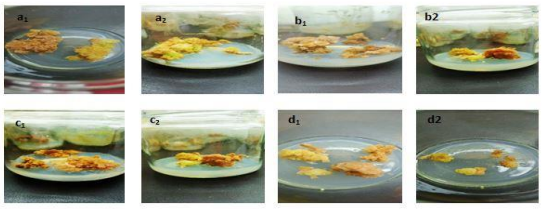


Figure (4) Calli and shoots developed after 28 days on MS2 (a1, a2, b1 and b2) and MS5 (c1, c2, d1 and d2) media from leaves (a1, b1, c1 and d1) and hypocotyl (a2, b2, c2 and d2) of *M. oleifera* (a1, a2, c1 and c2) and *M. stenopetala* (b1, b2, d1 and d2).

The data presented in Table (4) for the M. oleifera showed that the highest average fresh weight of 4 calli derived from leaf explants was observed on MS4 (3.13 mg) and MS6 (2.81 mg), respectively and for hypocotyl one was observed on MS4 (5.57 mg) and MS3 (5.29 mg), respectively. In contrast, calli grown in MS5 exhibited the lowest average fresh weight (0.6 mg and

1.39 mg) for callus in both explants (leaves and hypocotyl), respectively.

The results also indicated that the highest average of dry weight for calli was found on MS4 for both explants (1.99 mg and 3.17 mg, respectively). Conversely, MS2 had the lowest average of dry weight for calli derived from leaves (0.06 mg) and hypocotyls (0.13 mg), respectively.

As general means of fresh weight of calli derived from 2 explants (leaf and hypocotyl) grown on different MS media, MS4 showed the highest weight (4.35 mg) than other 5 media, while, MS5 showed the lowest weight (0.99 mg). Respect to dry weight, calli of MS4 medium also showed the highest weight (2.6 mg), while, MS2 showed the lowest weight (0.08 mg). Based on LSD test, significant differences between most treatments (species, media, explants) were observed (Table 4).

Table (4): Effect of different MS media and type of explants on developed calli fresh and dry weights of two *moringa* species (*M. oleifera* and *M. stenopetala*). Mean fresh and dry weight (mg) of calli/replicate developed on 6 MS different media (MS1 to MS6 media) from the two explants (leaves and hypocotyl) of both *Moringa* species.

Species (A)	Media (B)	Fresh weight (mg)		Means	Dry weight (mg)		Means
		Leaves (C)	Hypocotyls (C)		Leaves (C)	Hypocotyls (C)	
M. oleifera	MS_1	0.80	2.34	1.57	0.26	1.19	0.72
	MS_2	1.32	1.65	1.49	0.06	0.13	0.08
	MS_3	2.22	5.29	3.76	1.28	1.23	1.26
	MS_4	3.13	5.57	4.35	1.99	3.17	2.6
	MS_5	0.6	1.39	0.99	0.33	0.62	0.48
	MS_6	2.81	3.27	3.04	1.45	1.01	1.23
Means		1.81	3.25	2.53	0.90	1.23	1.07
M. stenopetala	MS_1	0.23	2.33	1.28	0.07	1.91	0.99
	MS_2	0.87	3.04	1.96	0.37	1.16	0.77
	MS_3	0.15	0.85	0.5	0.11	0.61	0.36
	MS_4	0.29	0.48	0.39	0.02	0.03	0.03
	MS_5	0.78	1.31	1.05	0.32	0.47	0.39
	MS_6	3.26	2.32	2.8	0.92	1.59	1.26
Means		0.93	1.72	1.33	0.30	0.96	0.63
L.S.D at alpha 0.05							
Factors	A	B	C	AB	AC	BC	ABC
Fresh weight	0.05	0.16	0.05	0.32	0.11	0.32	0.65
Dry weight	0.04	0.11	0.04	0.21	0.07	0.21	0.42

The results for M. stenopetala revealed that the highest average fresh weight of calli derived from leaf explants was on MS6 medium (3.26 mg), while MS2 medium showed the highest fresh weight from hypocotyl explants (3.04 mg). Also, calli grown on MS6 medium showed the highest dry weight for calli resulting from leaf explants (0.92 mg) and for hypocotyl one was observed on MS1 (1.91 mg) Additionally, results showed that leaf-derived calli grown on MS3 had the lowest average of fresh weight (0.15 mg), while calli of MS4 showed lowest dry weights (0.02 mg). In other hand, calli derived from hypocotyls showed the lowest fresh and dry weight when grow on MS4 (0.48 mg and 0.03 mg, respectively). Looking to general means of tested 6 MS media, calli of MS6 present the highest fresh weight (2.8 mg), while calli of MS4 showed the lowest weight (0.39 mg). In harmony with fresh weight, MS6 also showed the highest dry weight (1.26 mg) while, MS4 present the lowest weight (0.03 mg). Wide range of response and different fresh and dry weight were detected; therefore, significant differences were observed between most tested treatments (Table 4). Figure (5) demonstrates the swift development of shoots in MS3 and MS6 for both species, as well as for both explant types.

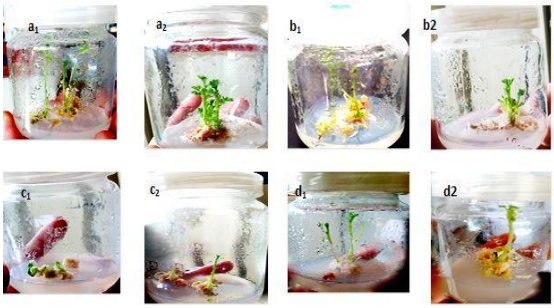


Figure (5) : Shoots developed after 28 days on MS3 (a1, a2, b1 and b2) and MS6 (c1, c2, d1 and d2) media from leaves (a1, b1, c1 and d1) and hypocotyl (a2, b2, c2 and d2) of *M. oleifera* (a1, a2, c1 and c2) and *M. stenopetala* (b1, b2, d1 and d2).

3.4. Cytological studies

Cytological studies of the 2 *Moringa* species faced significant challenges due to the high number of small-sized chromosomes in their genomes [26]. The current investigation reveals that active meristematic cells in the root tips (control) and callus tissues are also small-sized. Obtained results showed that the examined somatic cells were categorized into three types. Data in Table (5 & 6) showed the number and percentages of examined cells per category and Fig. (6) showed samples of these types. Type I includes dividing cells (Fig. 6 a1 & b1), type II meristematic cells with diffused chromatin (Fig. 6 a2 & b2) and those of type III that are representing cylindric cells with diffused chromatin (Fig. 6 a3 & b3). Interestingly, chromatins like bodies of interphase nuclei in non- dividing cells appeared especially in those of cylindrical and some of meristematic cells.

As shown in Table (5) the mitotic index (or frequency of dividing cells), is considerably higher (8.26%) in M. oleifera compared to M. stenopetala (6.73%), suggesting differences in the genetic control of the cell cycle between these congeneric species.

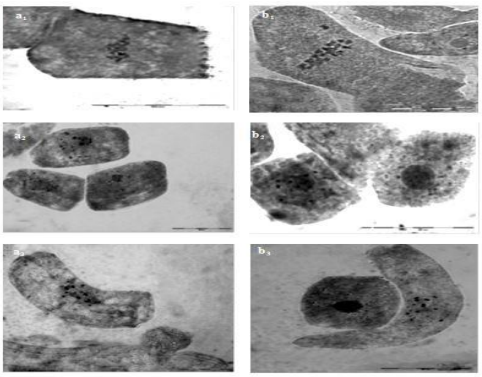


Figure (6) : Samples of the three cell types observed in tissues of the two *Moringa* species root tips and callus (*a1, a2* and *a3 M. oleifera and b1, b2* and *b3 M. stenopetala*), a1 and b1: type I (mitotic dividing cells), a2 and b2: type II (meristematic with diffused chromatin) and a3 and b3: type III (Cylinderic cells with diffused chromatin). The scal bar = 20 microns.

Table (5): Frequency and percentages of the cell types observed in tissues of the root tips of the two *Moringa* species plants which categorized according to cell size and shape (Type I, mitotic dividing cells; Type II, meristematic with diffused chromatin and Type III, cylindric cells with diffused chromatin).

Species	Total examined cells	Type I		Type II		Type III	
		No.	%	No.	%	No.	%
M. oleifera	4745	392	8.26	855	18.02	3498	73.72
M. stenopetala	951	64	6.73	299	31.44	588	61.83
Gross total	5696	456	8.0	1154	20.2	4086	71.7

Table (6): Frequencies and percentages of observed cell types in calli of leaves and hypocotyls induced on 6 MS callus induction media of the two *Moringa* species (Type I, mitotic dividing cells; Type II, meristematic with diffused chromatin and Type III, cylindric cells with diffused chromatin).

Species	Media	Leaves						Hypocotyls						Total gross number and means %					
														Type I		Type II		Type III	
		Type I	%	Type II	%	Type III	%	Type I	%	Type II	%	Type III	%	No.	%	No.	%	No.	%
M. oleifera	MS_1	10	1	56	5.6	934	93.4	15	1.5	0	0	985	98.5	25	1.25	56	2.8	1919	95.95
	MS_2	11	1.1	16	1.6	973	97.3	20	2	216	21.6	764	76.4	31	1.55	232	11.6	1737	86.85
	MS_3	5	0.5	10	1	995	99.5	10	1	32	3.2	958	95.8	15	0.75	42	2.1	1953	97.65
	MS_4	15	1.5	89	8.9	896	89.6	10	1	28	2.8	962	96.2	25	1.25	117	5.85	1858	92.9
	MS_5	10	1	60	6	930	93	10	1	16	1.6	974	97.4	20	1	76	3.8	1904	95.2
	MS_6	10	1	32	3.2	958	95.8	30	3	66	6.6	904	90.4	40	2	98	4.9	1862	93.1
	Total/mean	61	1.0	263	4.3	5686	15.8	95	1.6	358	6.0	5547	15.4	156	1.3	621	5.2	11233	15.5
M. stenopetala	MS_1	25	2.5	12	1.2	963	96.3	19	1.9	12	1.2	969	96.9	44	2.2	24	1.2	1932	96.6
	MS2	26	2.6	108	10.8	866	86.6	10	1	5	0.5	985	98.5	36	1.8	113	5.65	1851	92.55
	MS3	8	0.8	12	1.2	980	98	15	1.5	49	4.9	936	93.6	23	1.15	61	3.05	1916	95.8
	MS4	10	1	114	11.4	876	87.6	12	1.2	17	1.7	971	97.1	22	1.1	131	6.55	1847	92.35
	MS5	25	2.5	0	0	975	97.5	8	0.8	3	0.3	989	98.9	33	1.65	3	0.15	1964	98.2
	MS6	20	0.3	0	0	980	16.3	16	0.27	92	1.5	892	14.9	36	0.3	92	0.77	1872	15.6
	Total/mean	114	11.4	246	24.6	5640	94	80	8	178	17.8	5742	95.7	194	9.7	424	21.2	11382	94.85
Gross total	Mean/ %	175	1.46	509	4.24	11326	7.9	175	1.46	536	4.47	11289	7.84	350	1.46	1045	4.36	22615	7.9

Note: 1000 divided cells were examined per each callus induction medium.

The results presented in Table (6) indicated a significant decrease in the percentage of Type I (mitotic dividing cells) in the callus tissue (1.46%) compared to the percentage of this type in the tissues of root tips in plants of both 2 species (8%; Table 5). The results found in Table (6) clarified that the percentage of dividing cells (Type I) in calli derived from leaves and hypocotyls tissue is higher in M. stenopetala (11.4%; 8%) compared to M. oleifera (1%, 1.6%, respectively). Generally, type I cells of calli of M. oleifera (1.3%) was considerably less than that recorded (9.7%) for M. stenopetala. Beside the *Moringa* species effect on cells types and function, callus induction medium was also had effects on cells types. MS2 medium showed highest percentage of type I cells for M. oleifera (1.55%); while MS1 showed highest cells type I (2.2%) for M. stenopetala. Also, explant type had considerable effects on cell types of induced calli. It was noticed that calli derived from leaves of M. oleifera showed (1%) type I cells while hypocotyls induced calli with (1.6%) belong to type I cells. In contrast, leaves calli of M. stenopetala showed higher (11.4%) cells type I compared to only (8%) cells type I obtained from hypocotyls calli of the same *Moringa* species (M. stenopetala).

4. Discussion:

4.1. Seed germination

The germination process of seeds represents an important vital process in the life of the plant, as well as for obtaining explants to start any program in plant tissue culture technology. We were very successful in choosing the conditions for sterilizing the seeds and also the conditions for helping the seeds of the two species of *Moringa* germinate in this experiment, and we obtained the final percentage of seed germination for both species. But it was noted that M. oleifera was faster than M. stenopetala in terms of germination, as it was a whole week earlier.

4.2. Callus induction and shoot regeneration:

Callus induction is the corner stone in in vitro plant culture. However, many factors greatly affect this response; many published articles studied these factors in many different plant species. However, *Moringa* still needs further examination to reach the optimum in vitro culture protocol. Our results indicated that explant types, media components and *Moringa* species strongly affects the callus induction response. Hypocotyls induced calli more than leaves, MS medium with BAP with or without NAA or IBA produced highest percentage of calli than other tested media; moreover, M. oleifera recorded higher response than its counterpart M. stenopetala. Many green viable shoots were regenerated form obtained calli on the media of callus induction after 28-day of in vitro culture.

4.3. Callus fresh and dry weights

It was also noted that the same factors that affect the response of plant explants to callus production also affect the characteristics of calli in terms of fresh and dry weight of calli. Therefore, it was noted that the species of *Moringa*, the explant type and also the cultured medium content of growth regulators play a pivotal role in the characteristics of callus.

The adoption of an in vitro tissue culture method provides several advantages over traditional propagation techniques [27]. Additionally, this technique proves efficient generating genetically modified plants. Various approaches have been explored for in vitro propagation of *Moringa*, including nodal segments [13 and 28], indirect organogenesis [29], multiplication with immature seeds [7], and regeneration of axillary cotyledons and buds [30]. Passey et al., [31] noted that callus induction in strawberry cultivars depended on both the source of explants and the types of utilized tissue. The study by Shittu et al., [32], indicating that callus induction is enhanced in media with a high level of auxin without cytokinin. Multiple studies have reported that 2, 4-D induced callus formation in various species, including [33 - 34]. Adjusting conditions within these in vitro cultivation methods can enhance the response by modifying factors like phytohormone composition, concentration, light intensity, and temperature [35]. The growth regulators types and concentrations during dedifferentiation and differentiation processes may lead to genetic variations during regeneration [36]. Bud and cotyledon apex explants, cultivated in plant growth regulators-MS media, were employed for propagation. Optimal bud formation per explant was achieved with 1 mg/l BA and 0.2 mg/l IAA in indirect regeneration, while significant root development resulted from the same treatment using leaves as explants [27].

4.4. Cytological studies

Our results coincided with those obtained by previous researchers (e.g. [26]) in terms of the difficulty of monitoring *Moringa* chromosomes, which are often very small in size. We also noticed three types of root apex cells, as well as callus cells formed (Type I includes dividing cells, Type II meristematic cells with diffused chromatin and those of type III that are

representing cylindric cells with diffused chromatin). Perhaps this is related to the genetic material of *Moringa* species.

Deysson, [37] and Shehata et al., [38] indicated that the inhibition of mitotic division has been linked to numerous factors, including the inhibition of protein synthesis [39] and DNA amount and replication [40 - 41]. Also, there is a negative correlation between DNA content and the mitotic index in the root meristem. The second type of meristematic cells consists of non-dividing cells with condensed chromatin regions dispersed within the interphase nucleus, possibly due to the aggregation of heterochromatic regions. The third type of active cells is the largest and cylindrical shape, containing sharply visible condensed chromatin bodies embedded in the interphase nucleus. The interpretation of these chromatin bodies' aggregation aligns with Silva et al., [42] findings. The concepts and recent additions regarding how cells construct cell walls and underlying processes are summarized by Ivakove et al., [43]. These processes involve cell wall synthesis, the activity of actions and microtubule cytoskeletons, regulation by microtubule-associated proteins, actin-related proteins, GTPases, and their effectors, as well as the roles of plant growth regulators signaling and vesicular membrane trafficking. Morphological and genetic variation found in the in vitro regenerated plants is described as somaclonal variation [44]. Genomic changes, including endo-polyploidy, amplification, or deletion of DNA sequences, and polyteny, are noted during somatic differentiation [45]. These alterations may be influenced by factors such as prolonged exposure to plant growth regulators (PGR), the age of the culture, and the adaptive stress of the in vitro system [46 - 47].

5. Conclusion:

The present study sightsees the impact of explant type and medium composition on callus and shoot induction in two *Moringa* species cultivated in Egypt, revealing cytological features in root tip cells and induced calli. Variations in callus induction, fresh and dry weights between M. oleifera and M. stenopetala underscore species-specific considerations in tissue culture. Diverse outcomes among six MS media highlight nuanced influences on callus indication and shoot regeneration. Variations in the response of leaf and hypocotyl explants provide insights for optimizing tissue culture protocols, while a higher mitotic index in M. oleifera suggests differing regenerative capacities. These findings enrich our understanding of *Moringa* tissue culture, offering targeted approaches for harnessing its regenerative potential and contributing to sustainable agriculture.

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