In vitro production of somatic embryos from nucellus of mango (*Mangifera indica* L.)

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Abstract: For the first time, production of somatic embryogenesis were obtained from nucellus of polyembryonic *Mangifera indica* L cultivars (Zebda, Sedeek and Hindi) through direct and indirect somatic embryogenesis in Egypt using tissue culture techniques. Callus formation was affected by the cultivar and age of each. As the callus were obtained on the embryo of Zebda and Sedeek cultivar with ages 45, 60 and 75-day-old. Direct somatic embryos were induced on embryos of age 45 and 60 –day-old of Zebda and 45, 60 and 75 day –old of Sedeek. Callus formation of immature embryo cv Sedeek (age 45–day-old) was obtained on B5 medium supplemented with 1 mgl^{-1} 2, 4-D. The highest number of direct somatic embryos was produced from immature embryo of cv Sedeek cultured on B5 medium free 2, 4-D. The addition of 2, 4-D to the medium was effective in enhancement the somatic embryos formation as the media contained 1.0, 1.5 and 2mg l^{-1} 2, 4-D of cv Zebda and Sedeek. The highest somatic embryogenesis number and percentage of cv Zebda and Sedeek was obtained on the medium without 2, 4-D. The embryos were developed of somatic embryos during cotyledonary stage when somatic embryos of Zebda (40 and 66.67%) and Sedeek on half-strength MS + half- strength B5 medium with 50 gl^{-1} sucrose. At cotyledonary stage, embryos cultivars of Zebda, Sedeek were germinated (embryos with developed root and visible plumule) on half-strength MS + half- strength B5 medium with 30 g l^{-1} sucrose and mango plantlets were produced.
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1. Introduction

Mango (*Mangifera indica*, L.) is a diploid fruit tree (2n = 40). The mango is considered as one of the oldest cultivated trees in the world. Mango belongs to the dicotyledonous family Anacardiaceae (Cany et al., 1981). Mango malformation is well known in India and has also been confirmed in most mango growing countries as Pakistan, Bangladesh, Egypt, Sudan, South Africa, Mexico, Cuba, Brazil, Central America, USA, and recently, the United Arab emirates (Kumar et al., 1993)**.** Mango, often referred to as the king of tropical fruits, is an important fruit crop cultivated in tropical regions (Boghrma, 2000). Mango is an important fruit crop of the tropical and subtropical regions. Also, it is one of the most common and popular fruits since, it considered the king of fruits having delicious taste, captivating flavor with multifarious color and excellent source of nutritive values (Alam et al., 2006).

In Egypt, mangos (*Mangifera indica*, L) are the most popular fruits and are cultivated almost in the whole of the Nile valley and around the desert. There are several varieties grown in Egypt, the better known cultivars are Alphonso, Pairi, Zebda, Mabroka, Balady, and Succary (El-Soukkary et al., 2000). Mangoes are an important fruit crop in Egypt. According to the latest statistics provided by the Ministry of Agriculture (2007) indicated that, a total of 184204 Feddan are planted by mangoes.

In vitro induction of nuceller polyembryony in mango, proliferation of nuceller embryos and their development have been investigated in detail, but their predictable convertibility into plantlets and *ex vitro* survival of plantlets have eluded success to a great extent (Litz et al., 1993 and Litz et al., 1995)**.** Somatic embryogenesis has been reported in several mango cultivars (most of which are polyembryonic); yet reports on the development of plantlets from somatic embryos are limited to a few cultivars. As the responses are cultivar-dependent, the methods may not be applicable to other varieties (Litz et al., 1993 and Litz and Lavi 1997). Polyembryonic seeds contain many embryos, most of which are asexual (nucellar) in origin and genetically identical to the maternal parent. Polyembryonic seeds also contain a zygotic embryo that is the result of cross-pollination (Bally, 2006).

The definition of indirect somatic embryogenesis (SE) is that explants dedifferentiate to form callus, from which cells differentiate to form the somatic embryos of mango (Rivera-Domínguez et al., 2004)**.** The protocol for *in vitro* nucellar embryogenesis in mango using a high concentration of 5 mg/l of 2,4-diclorophenoxyacetic (2, 4-D), for a short duration of 7 days only, when the nucellar tissue proliferation had not ensued, is in contrast with the works of other investigators, who used its high concentrations from 2.5 to 5 mg/l throughout the process of nucellar tissue proliferation and

differentiation of embryos in suspension culture (Ara et al., 2000 and Kidwai, 2009). The effect of plant growth regulators (PGR) is an important factor impacting SE (somatic embryogenesis) and plant regeneration. In most cases, successful plant SE needs a mixture of the different concentration ratios of auxin and cytokinin, both of which are necessary for plant culture *in vitro*. In general, 2, 4-D which is one of the most important hormones inducing somatic embryogenesis (SE), has been widely used in horticultural plants (Aiqing et al., 2011).

The aim of the present study was to establish an effective protocol for direct and indirect (through callus) somatic embryogenesis regeneration from nucellus of some highly prized polyembryonic mango cultivars (Zebda, Sedeek and Hindi) in Egypt using tissue culture techniques.

2. Material and Methods

This study was carried out during the period from 2008 to 2012 in the Laboratory of Plant Cell and Tissue Culture, Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia University, Egypt.

Plant material

Certified fruits of eight developmental stages were collected, after 15, 30, 45, 60, 75, 90, 105 and 120 days of pollination, from a tree of Mango (*Mangifera indica*, L.) cultivars Zebda, Sedeek and Hindi mango cultivars of this study were obtained from the farm as sources of germplasm at Genetic Engineering and Biotechnology Research Institute (GEBRI), Menoufia University, Egypt.

Explants and surface sterilization

Mango fruits were surface-sterilized by soaking in 10% commercial bleach (containing 5 % active chloride) for 20 min, followed by three rinses with sterile distilled water. Fruits were then dipped in 75% ethanol and flamed for a few seconds (Xiao et al., 2004). The embryos were discarded from each ovule and the intact ovular halves containing nucellar tissue were placed in a nutrient medium such that the embryos was quickly placed in the B5 (Gamborg et al., 1966) medium supplemented with 400 mg l^{-1} L-glutamine, 100 mg/l ascorbic acid, 6% (w/v) sucrose and 0.8% (w/v) agar.

Experiment 1. Effect of developmental stages of embryo on its responses *in vitro*

Primary embryos were excised from 15, 30, 45, 60, 75, 90, 105 and 120 -day–old of fruits age (days were counted after pollination). Then, they were cultured on B5 medium for morphological response and callus induction. The explants were recorded to response and callus induction after 8 weeks.

Experiment 2. Induction of callus and somatic embryogenesis from immature embryo

According to the results of experiment (1), the age of 45-day–old after pollination was choose to run the rest of experiments of this study. Then, the embryos were removed aseptically from the ovule of immature fruits of *Mangifera indica* L. cv (Zebda, Sedeek and Hindi) and were cultured on B5 medium with various concentrations (0.0, 0.5, 1.0, 1.5and 2.0mg l^{-1}) of 2,4-D for initiation of callus and induction of somatic embryogenesis. Different parameter recorded after 8 weeks including both percentage and type and number of somatic embryogenesis induced from immature embryo.

Experiment 3. Induction of somatic embryogenesis from callus of immature embryo

According to the results of experiment (2) callus obtained with 1mg/l 2,4-D was as a source for differentiation experiment "as explants". Each explant contends all callus produced from one immature embryo. That callus formed from the embryos of immature embryo of *Mangifera indica* L. cv Zebda, Sedeek and Hindi was cultured for induction of embryos differentiation on B5 medium with the same various concentrations of 2,4-D used at experiment 2 (0.0, 0.5, 1.0, 1.5and 2.0mg l^{-1}). Number and percentage of somatic embryogenesis were recorded after 8 weeks.

The experimental design was completely randomized with 10 replications, each one of a jar (350 mm containing 50 ml medium) with one embryo, callus per immature embryo or somatic embryogenesis per callus. The pH of all media was adjusted to 5.8 before autoclaving. All media were sterilized by autoclaving for 20 min at 121ºC and 15 lbs. pressure. All cultures were maintained in complete darkness at 25 ± 2° C.

Experiment 4. Effect of some factors affecting development of somatic embryogenesis into cotyledonary stage

According to the results of experiment (3), only somatic embryos of the cultivars (Zebda and Sedeek) were used as explants for the development into cotyledonary stage. The explants were cultured on different media {full MS (Murashige and Skoog, 1962), full B5 and half MS + half B5}. These media were further supplemented with different sucrose concentrations (30, 50 and 70 g l^{-1}) 1 mg l^{-1} BA, 400 mg l^{-1} L-glutamine and 0.8% agar (w/v). Different parameter was recorded after 8 weeks including

number and percentage of somatic embryogenesis which developed into cotyledonary stage. The experimental design was completely randomized with 5 replicates in treatment each replicate contained 3 jars (350 mm containing 50 ml medium) which each had 20 embryos (explants). Cultures were incubated in complete darkness at 25 ± 2° C.

Experiment 5. Germination of somatic embryos

For embryo germination (embryos with developed root and visible plumule), only embryos developed into cotyledon stages were these embryo of both cultivars Zebda and Sedeek. Embryos were cultured on the same various types of media (full MS, full B5 and half MS + half B5) supplemented with different concentrations sucrose (30, 50 and 70 g l^{-1}) 1 mg l^{-1} BA, 1.0 mg l^{-1} GA3, 400 mg l^{-1} L-glutamine and 0.8% agar (w/v). Different parameters were recorded after 8 weeks as number and percentage of germinated embryos. The experimental design was completely randomized with 5 replicates in treatment each replicate contained one jars(350 mm containing 50 ml medium) which each had 20 embryos (explants). Culture was incubated of a 16 h photoperiod provided by cool white fluorescent light (1500 lux) at 25 °C.

Layout of the experiments

All experiments were designed in factorial completely design and data were compared according to method described by (Snedecor & Cochran, 1989).

3. Results and Discussion

Experiment 1. Effect of developmental stages of embryo on its responses *in vitro*

Different embryo ages (15, 30, 45, 60, 75, 90, 105 and 120 –day-old after pollination) were used as explants to study their responses. The results obtained from this experiment are presented in Table (1) and Fig (1). Results show that, all 15 of day-old immature embryos of cultivars Zebda, Sedeek and Hindi were died of after two months.All other examined embryo ages 30, 45, 60, 75, 90, 105 and 120 –day-old of cultivars (Zebda, Sedeek and Hindi) showed a swelling after two months of cultivars. Concerning callus formation, results indicated that the formation of callus was affected by the cultivar and age of each. As the callus were obtained on the embryo of Zebda cultivar with ages 45, 60 and 75 day-old. The same response was obtained with Sedeek embryo cultivar of ages 45, 60, 75, 90 and 105 day – old and Hindi cultivar of age 45 and 60-day – old.

As for direct somatic embryogenesis, *in vitro* observation in Table (1) show that the direct somatic embryos was induced on embryos of age 45 and 60 –day-old of Zebda and 45, 60 and 75 day –old of Sedeek. However, Hindi cultivar did not induce somatic embryos at all examined embryo ages. It was clear that the ages 120-day–old of all examined cultivars (Zebda, Sedeek and Hindi) showed germination of the cultured embryos (explants) without callus formation or somatic embryos. Some other studies showed that different immature embryo ages of this affected study, callus and somatic embryogenesis induction. Thirty to sixty-day-old fruits, harvested after pollination are suitable for induction of somatic embryogenic culture from the nucellus (Litz et al., 1982; Dewald et al., 1989; Pliego-Alfaro et al., 1996; Ara et al., 1999; Singh et al., 2002; Sulekha and Rajmohan, 2004).The percentage of explants showing embryogenesis was 10- 20% less in case of nucellus of older fruits as compared to that of younger fruits. Furthermore, the average number of developed embryos formed in younger explants was more, i.e., 20.75 than in case of nucellus of older fruits where it was only 12.5 (Chaturvedi et al., 2004). The embryogenic response is strongly cultivar dependent. On the basis of their embryogenic response, Litz et al., (1998) classified some varieties as highly embryogenic (polyembryonic Hindi and Parris), moderately embryogenic (monoembryonic Lippens and Tommy Atkins) and difficult-to-regenerate (polyembryonic Nam Doc Mai).

Experiment 2. Induction of callus and somatic embryogenesis from immature embryos

Embryos of 45 –day-old of the three examined cultivars were used in this explants, immature embryo cultivars (Zebda, Sedeek and Hindi) of age 45 -day-old were cultured B5 medium contained 2,4-D for callus induction in Table (2) and Fig. (4). Induction callus varied according to the different cultivars and the different 2,4-D concentrations. Data of the main effect of 2,4-D concentration in the Table (2) indicate that,the highest response of callus formation in immature embryo (31.67 %) was observed with B5 medium contained 1mg l^{-1} 2,4-D. Data of main effect of cultivars show that,Sedeek cultivars showed the highest response of callus formation (31.00%). Concerning the interaction, an excellent callus induction of immature embryo cv Sedeek was obtained on B5 medium supplemented with 1 mg l^{-1} 2, 4-D (60%) compared with Zebda and Hindi (25 and 10% respectively). B5 medium supplemented with 1 mg l^{-1} 2,4-D produced light yellowish /dark brown compact callus having good growth with cv Zebda, white yellow/ dark brown friable callus having good growth of cv Sedeek and initials of greenish white/ dark brown compact callus which

turned brown later with no further growth on the case of cv Hindi (Fig. 2).

The importance of 2,4-D, in callus induction of immature embryo mango, was also reported by Ara et al. (2000) who showed that callusing of the nucellus Amrapali and Chausa cultivars occurred in 3–5 weeks only on the medium supplemented with 0.1 to 2.5 mg l^{-1} 2,4-D. Higher concentrations (5.0 and 10.0 mg l^{-1}) of 2,4-D caused total inhibition. The initial callus was dark-brown or black and moist. From these calli pale-yellow or cream-colored, shiny and translucent pro-embryogenic calli (PEC) initiated in the next 2–3 weeks on fresh medium of the same composition. Laxmi et.al., (1999) mentioned that the callus induction medium consisted of half-strength MS supplemented with 4.5 mM 2,4-D, 20 mM BAP and 6% sucrose, gelled with 0.8% agar-agar. Data presented in Table (2) and Fig. (3) show the effect of 2, 4-D concentrations on the induction of direct somatic embryo from the embryos of different *mangifera indica* cultivars (Zebda, Sedeek and Hindi). In Table (2) data on the main effect of the 2, 4-D concentrations indicate that the cultured explants of *Mangifera indica* on B5 nutrient medium without 2,4-D (control) recorded the best result for number of direct somatic embryogenesis induction (13.33/explant) compared to other concentrations of 2,4-D. The lowest response was significantly observed with the levels 2.0mg/l 2,4-D. Data on the main effect of immature embryo cultivars Zebda and Sedeek significantly showed higher values of number of somatic embryogenesis (8.20 and 9.40/explant respectively) compared to cv Hindi which had in that concern no response. As for the interaction, the highest number of direct somatic embryos (40) was produced from immature embryo of cv Sedeek cultured on B5 medium free 2,4-D compared to all other cultivars and concentrations of 2,4-D (Fig. 3).

Experiment 3. Induction of somatic embryogenesis from callus of immature embryo

Well-proliferated calli derived from the immature embryo after four months cultures were used for regeneration studies. Calli derived from immature embryo different cultivars (Zebda, Sedeek and Hindi), were placed on the B5 medium containing the same different concentrations of 2,4-D. Regeneration of number and percentage of somatic embryogenesis occurred in these calli after two months (Table 3). In Table (3) data on the main effect of the 2,4-D concentrations show that the addition of the medium with 2.0mg l^{-1} 2,4-D showed lowest effect on number and percentage of somatic embryogenesis (6.67 and 3.33%/callus from immature embryo) compared to control (71.67 and 79.33 % /callus from immature embryo). Data on the main effect of cv Sedeek significantly presented similar higher values of somatic embryogenesis number and percentage (55 and 33.67% /callus from immature embryo). Lower responses were significantly observed at number and percentage of somatic embryogenesis of cv Hindi (7 and 18%/callus from immature embryo). The original data (interaction between different 2,4-D and cultivars of immature embryo mango) show that the addition of 2,4-D to the medium was effective in enhancement the somatic embryos formation as the media contained 1.0,1.5 and 2mg l^{-1} 2,4-D of cv Zebda and Sedeek observed without of somatic embryos formation of cv Hindi. Moreover, the highest somatic embryogenesis number and percentage of cv Zebda (85 and 95%/ callus from immature embryo) and Sedeek (100 and 73% / callus from immature embryo) was obtained on the medium without 2,4-D compared to interaction between cv Hindi and 2,4-D concentrations (Fig.4).

In that concern, Litz et al. (1984) showed reported somatic embryogenesis in mango cultivars 'Tommy Atkins', 'Ruby' and 'Irwin' on half-strength MS medium fortified with 2,4-D but embryos did not develop beyond globular stage. Ara et al. (1999) mentioned that from the embryogenic callus produced globular-stage somatic embryos within 4 weeks on 2,4-D-free medium in darkness. Laxmi et al. (1999) as well observed maximum embryo production on half strength MS medium supplemented with 20.0 µM BAP devoid of 2,4-D.ified with 2,4-D but embryos did not develop beyond globular stage. Ara et al. (2000) showed that the proembryogenic calli produced up to 130 somatic embryos when transferred to 2,4-D-free medium. The presence of 2,4-D in the medium inhibited progression of development of somatic embryos. Litz and Gomez-Lim (2005) observed that after subculture on medium without 2,4-D, the advanced development stages of somatic embryos were observed. Krishna and Singh, (2007) mentioned that the inductive phase mediated by 2,4-D is necessary for establishment of a embryogenic culture in monoembryonic mango genotypes. Though, persisted presence of 2,4-D in maintenance medium exerts a considerable negative influence on the somatic embryos production by hindering its development beyond globular stage.

Experiment 4. Some factors affecting development of somatic embryogenesis to cotyledonary stage

Data presented in Table (4) clearly show the effect of media type (full MS, full B5 and half MS + half B5) and sucrose concentrations (30, 50 and 70gl^{-1}) on somatic embryos formation (number and

percentage of somatic embryos) during cotyledonary stage of mango cultivars (Zebda and Sedeek).

The data in Table (4) and Fig.(5) show that the main effect of mango cultivars, significantly presented similar higher number and percentage of cotyledonary stage from somatic embryogenesis of cv Sedeek (24.71 and 41.18%) compared to cv Zebda (18.47and 30.78%). The number and percentage of somatic embryos development to cotyledonary stage were increased by increasing the concentration of sucrose from 30 to 50 g l^{-1}. Further, increase of the sucrose concentration from 50 to 70 g l^{-1} decreasing number and percentage of cotyledonary stage from somatic embryogenesis. The main effect of different media, the highest significantly number and percentage of somatic embryogenesis development to cotyledonary stage were achieved in half-strength MS + half- strength B5 medium containing 50g l^{-1} sucrose (45.50 and 75.84%), followed by full MS and B5 medium containing 30 or 70g l^{-1} sucrose. As for the interaction, the highest number and percentage development of somatic embryogenesis to cotyledonary stage obtained from cv Zebda (40 and 66.67%) and Sedeek (51and 85%) were cultured on half-strength MS + half- strength B5 medium containing 50g l^{-1} sucrose compared to other treatment (Fig.5). Lower rates of embryo to cotyledonary stage in some media may be attributed to embryo abnormalities. This result agreement with, Ara et al., (2000) showed that the best medium for the production, development and maturation of somatic embryos was the modified M4E medium which contained full-strength B5 macrosalts, MS microsalts, MS iron-EDTA and MS organics along with 400 mgl^{-1} L-glutamine, 6% (w/v) sucrose and 0.8% (w/v) agar. The mature somatic embryos gave rise to plantlets in liquid medium containing half-strength B5 macrosalts and 1.0 mgl^{-1} GA3. Laxmi et al. (1999) mentioned that the conversion of globular embryos to torpedo stage was higher on half-strength MS medium supplemented with 20 µM BAP than on B5, MS or half-strength B5 medium. Lower rates of conversion of embryos to torpedo stage in other media may be attributed to embryo abnormalities. However, the further conversion of torpedo stage embryos to cotyledonary stage was maximum on B5 medium. Litz et al. (1984) showed reported somatic embryogenesis in mango cultivars 'Tommy Atkins', 'Ruby' and 'Irwin' on half-strength MS medium fortified with 2,4-D but embryos did not develop beyond globular stage. Aiqing et al. (2011) indicated that the effect of improved B5 was better than MS or WPM for the study of SE of mango. When applied on improved B5 media, it would induce normal cotyledons, and most of the somatic embryos developed normally at the early stage of heart-shape embryo.

Experiment 5. Germination of somatic embryos

Embryos germination (embryos with developed root and visible plumule) of cultivar (Zebda and Sedeek) were cultured on the different media (full strength MS, full strength B5 and half strength MS+ half strength B5) supplemented with different concentrations of sucrose (30, 50 and 70gl^{-1}). Data of the main effect of different media, the minimum number of embryos germination and percentage % was recorded on full strength MS medium supplemented with 50g/l sucrose. The maximum was recorded on half strength B5 + half strength MS medium with 30gl^{-1}sucrose (Table 5). Increase of sucrose concentration resulted in lower germination of embryos were cultured on all media. Data of the main effect of mango cultivars, the maximum germination number and percentage (%) of embryos derived from cotyledonary stage were recorded of Sedeek. Concerning the interaction, an excellent germination of embryos Sedeek were cultured on half strength B5 medium + half strength MS medium supplemented with (30 and 50g/l sucrose) or with 30gl^{-1} sucrose of Zebda (Fig 6). On the other hand, increase sucrose concentration to (70gl^{-1}) with full strength MS resulted in dead all embryos of Zebda and Sedeek. The importance of sucrose and salts strength, in developing embryos of mango, was also reported by Laxmi et al. (1999) showed that lowering of sucrose concentration and addition of GA3 and N6-benzylamino purine for improved somatic embryo germination. Germination was achieved on a medium with B5 major salts, MS minor salts and organics. Litz (2003) and Thomas (1999) indicated that the germination medium contains no filter sterilized coconut water and has reduced sugar concentration, i.e.2.0%. Litz and Gomez-Lim (2005) also advocated reduction in concentration of sucrose in maturation medium from 6 to 4%. The sucrose concentration is gradually reduced to 1.0% during sequential subculture to fresh medium owing to different requirements of developing embryos from heart stage to complete maturity stage. Furthermore, cessation of mango somatic embryo elongation at maturity has also been observed in the presence of high sugar concentration.

Table 1. Effect of time period (day) after pollination on immature embryo responses of *Mangifera indica* cultured on B5 medium after two months *in vitro*.

Time after pollination and fertilization in days	Responses of embryo		
	Zebdia	**Sedeek**	**Hindi**
15	-	-	-
30	+	+	+
45	+++	+++	++
60	+++	+++	++
75	++	+++.	+
90	+	++	+
105	+	++ Germination	+ Germination
120	+ Germination	+ Germination	+ Germination
-	**Dead embryos**		
+	**Swelling**		
++	**Swelling + Callus**		
+++	**Swelling + Callus + pre-embryo formation**		

Table 2. Effect of different concentrations of 2, 4- D on callus and somatic embryogenesis induction from immature embryo *Mangifera indica* after two months *in vitro*.

2,4-D conc. (mg l^{-1})	Responses of immature embryo							
	Percentage of callus formation				Number of somatic embryos			
	Zebda	**Sedeek**	**Hindi**	**Mean (A)**	**Zebda**	**Sedeek**	**Hindi**	**Mean (A)**
0.0	0.0 f	0.0 f	0.0 f	**0.0 d**	0.0 d	40.0a	0.0d	**13.33 a**
0.5	10.0 de	30.0 c	5.3 ef	**15.11 bc**	20.0 b	7.0 d	0.0d	**9.00 b**
1.0	25.0 c	60.0 a	10.0 de	**31.67 a**	10.0 c	0.0d	0.0d	**3.33 c**
1.5	15.0 d	40.0 b	4.0 ef	**19.67 b**	6.0 cd	0.0d	0.0d	**2.00 c**
2.0	5.0 ef	25.0 c	0.0 f	**10.00 c**	5.0cd	0.0d	0.0d	**1.40 c**
Mean (B)	11.00 b	31.00 a	3.86 c	**Mean (A)**	8.20 a	9.40 a	0.0 b	
Different letters within a column indicate significant differences at P = 0.05 by Duncan's multiple range.								

Table 3. Effect of different concentrations of 2, 4-D on number and percentage of somatic embryogenesis from callus of immature embryo (*Mangifera indica*) after two months.

2,4-D conc. (mgl^{-1})	Somatic embryogenesis from callus/embryo							
	Number				Percentage			
	Zebda	**Sedeek**	**Hindi**	**Mean (A)**	**Zebda**	**Sedeek**	**Hindi**	**Mean (A)**
0.0	85ab	100 a	30 de	**71.67 a**	95 a	73 b	70 b	**79.33 a**
0.5	70 bc	75 b	5 f	**50.00 b**	50 c	50 c	20 d	**40.00 b**
1.0	30 de	50 cd	0 f	**26.33 c**	20 d	20 d	0 d	**13.33 c**
1.5	30 de	30 de	0 f	**20.00 c**	5 d	15 d	0 d	**6.67 c**
2.0	0 f	20 ef	0 f	**6.67 d**	0 d	10 d	0 d	**3.33 c**
Mean (B)	**43.00 b**	**55.00 a**	**7.00 c**		**34.00 a**	**33.67 a**	**18.00 b**	
Different letters within a column indicate significant differences at P = 0.05 by Duncan's multiple range.								

Table 4. Effect of media type and sucrose concentrations on development of somatic embryos to cotyledonary stage of mango cultivars (Zebda and Sedeek) after two months *in vitro*.

Media type	Sucrose conc.(gl^{-1})	Development of somatic embryos to cotyledonary stage					
		Number			Percentage		
		Zebda	Sedeek	Mean (A)	Zebda	Sedeek	Mean (A)
Full MS	30	0.00 i	0.00 i	**0.0 h**	0.00	0.00	**0.00**
	50	5.33 h	6.67 h	**6.00 g**	8.88	11.12	**10.00**
	70	0.00 i	4.33 h	**2.16 h**	0.00	7.22	**3.61**
Full B5	30	12.33 g	15.67 fg	**14.00 f**	20.55	26.12	**23.33**
	50	17.67 f	41.00 b	**29.33 d**	29.45	68.33	**48.89**
	70	30.67 d	35.00 c	**32.83 c**	51.12	58.33	**54.73**
Half B5 + Half MS	30	22.00 e	27.33 d	**24.67 e**	36.67	45.55	**41.11**
	50	40.00 b	51.00 a	**45.50 a**	66.67	85.00	**75.84**
	70	38.00 bc	42.33 b	**39.67 b**	63.33	70.55	**66.94**
Mean (B)		**18.47 b**	**24.71 a**		**30.78**	**41.18**	

Different letters within a column indicate significant differences at P = 0.05 by Duncan's multiple range.

Table 5. Effect of media type and sucrose concentrations on germination of somatic embryos of Mango cultivars (Zebda and Sedeek) after two months *in vitro*.

Media type	Sucrose conc. (gl^{-1})	Germination of somatic embryo					
		Number			Percentage		
		Zebda	Sedeek	Mean (A)	Zebda	Sedeek	Mean (A)
Full MS	30	2.00 ij	4.33 h	**3.17 f**	10	21.56	**15.78**
	50	1.33 jk	3.33 hi	**2.33 f**	6.65	16.65	**11.65**
	70	0.0 k	0.0 k	**0.00 g**	0.00	0.00	**0.00**
Full B5	30	8.67 ef	9.33 e	**9.00 d**	43.35	46.65	**45.00**
	50	10.00 de	13.00 c	**11.50 c**	50.00	65.00	**57.50**
	70	4.67 h	6.67 g	**5.67 e**	23.35	33.35	**28.35**
Half B5 + Half MS	30	15.67 b	17.67 a	**16.67 a**	78.35	88.35	**83.35**
	50	14.33 bc	14.67 b	**14.50 b**	71.65	73.35	**72.50**
	70	7.67 fg	11.00 d	**9.33 d**	38.35	55.00	**46.68**
Mean (B)		**7.16 b**	**8.90 a**		**35.80**	**44.50**	

Different letters within a column indicate significant differences at P = 0.05 by Duncan's multiple range.

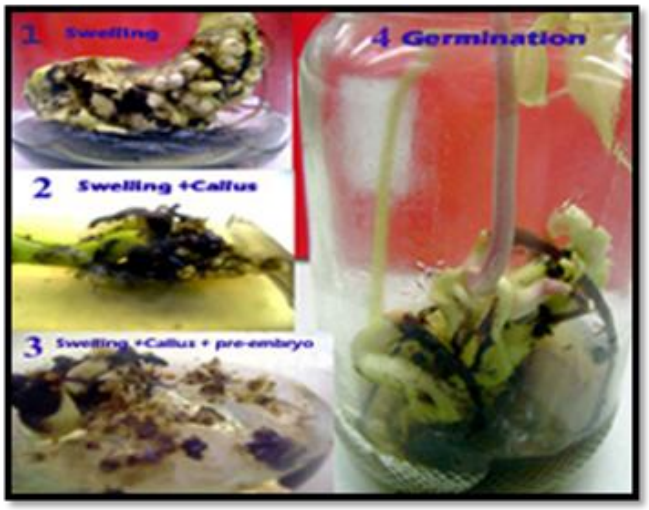

Figure 1. I*n vitro* response of *Mangifera indica* embryos cultured on B5 medium after two months.

Figure 2. The differences in callus type according to the different cultivars mango cultured on B5 medium supplemented with 1 mg/l 2, 4-D after two months *in vitro*.

Figure 3. Direct somatic embryogenesis from immature embryos of Zebda and Sedeek cultivars after two months.

Figure 4. Indirect somatic embryogenesis from callus on the medium without 2, 4-D per immature embryo of *Mangifera indica* after two months.

Figure 5. Development of somatic embryo to cotyledonary stage of mango cultivars (Zebda and Sedeek).

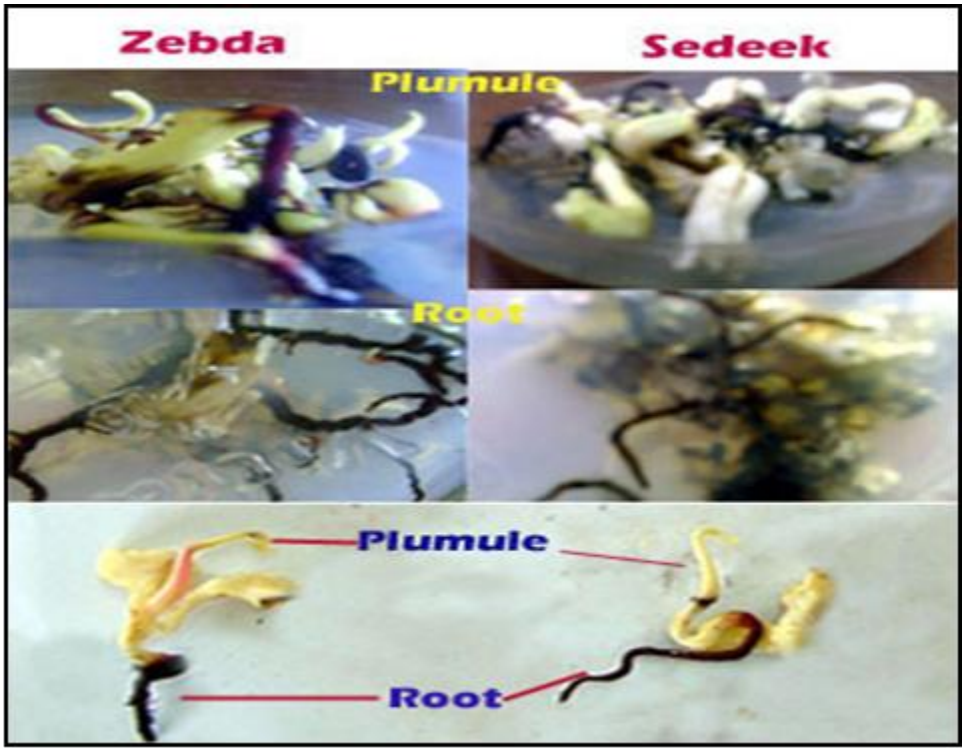

Figure 6. Somatic embryos of Mango cultivars (Zebda and Sedeek) in germination and subsequently to *in vitro* mango plantlets.

4. Conclusion

A protocol for somatic embryogenesis which were found to be reproducible from nucellus of some highly prized polyembryonic of *Mangifera indica*, L. cultivars (Zebda, Sedeek and Heendy) in Egypt.

Callus was obtained from 45- day-old (after pollination) embryos of Zebda, Sedeek and Hindi were cultured on B5 medium supplemented with 1 mgl^{-1} 2,4-D. Induction of direct somatic embryos from immature embryos of cultivars Zebda and Sedeek cultured on B5 medium without 2, 4-D after two months. The cultivar Hindi showed no somatic embryogenesis.

Indirect somatic embryos were obtained from mango callus which obtained from mango embryos (45- day-old) and cultured on B5 medium supplemented with 1 mg l^{-1} 2,4-D while direct somatic embryos were induced from immature embryo cultivar Sedeek on free 2,4-D after two months. Regeneration from callus of mango cultivars using B5 medium contend different 2,4-D concentrations has been examined, as it was possible to produce somatic embryos from callus cultured on B5 medium lack 2,4-D after two months.

The embryos were developed of somatic embryos during cotyledonary stage when somatic embryos of Zebda and Sedeek cultivars on half-strength MS + half- strength B5 medium with 50g l^{-1} sucrose. At cotyledonary stage, embryos cultivars of Zebda, Sedeek were germinated (embryos with developed root and visible plumule) on half-strength MS + half- strength B5 medium with 30 gl^{-1} sucrose and mango plantlets were produced.

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