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The effect of nanoparticles of in vitro propagation of seedling male date palm by immature inflorescences

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Abstract

The effect of nano silver and nano chitosan particles on sterilization, Fe & Zn nanoparticles on callus formation of immature inflorescence of date palm seedling male during the establishment stage were investigated with immersion and adding to MS culture medium. The lowest total contamination percentage and the highest survival percentage were achieved with nano silver particles at 200 mg/l, and nano chitosan at 150, and 200 mg/l. The lowest contamination % recorded in medium culture containing silver nanoparticles at 4 mg/l with NAA at 100 mg/l and chitosan nanoparticles at 4 mg/l with 2,4-D at 100 mg/l. The optimum callus formation percentage and callus size were obtained on MS medium supplemented with picloram at 8 mg/l. The highest callus weight and size were showed with NAA at 10 mg/l, 2ip at 6 mg/l & Kin at 6 mg/l during callus proliferation. Seedling male in multiplication stage, the highest number of shoot / culture were occurred on MS medium culture supplemented with Fe nano particles at 20.8 mg/l and with other treatment by MS medium culture by Fe nano particles at 27.8 mg/l and Zn nano particles at 4.3 mg/l in the first subculture without any significant differences among them. The highest average shoot length (cm) was obtained with MS medium containing Fe nano particles at 27.8 mg/l and the treatment with MS medium supplemented by Fe nano particles at 20.8 mg/l and Zn nano particles at 4.3 mg/l in the first subculture without any significantly differences among them. Impact of interaction among cytokinins and auxin concentration, revealed that, the highest number of shoots / culture were achieved with NAA at 2.0 mg/l, 2ip at 4 mg/l, kin at 4mg/l during the 1 st, 2 nd & 3 rd subcultures, respectively. In rooting stage, the highest rooting percentage and number of roots/ microshoots were recorded with MS containing NAA at 0.5 mg/l. The highest survival percentages in acclimatization stage were occurred with medium mixtures of sand: peat: vermiculite: perlite at (1:2: 1:1) and (2: 1: 1:1), respectively.

Keywords: Date palm seedling male, Immature Inflorescence, In Vitro, Propagation, Nanoparticles, Multiplication, Rooting, Acclimatization.

1. Abbreviation

MS: Murashige and skoog medium1962 NAA: α-Naphthalene Acetic Acid IBA: Indole-3-butyric Acid 2ip: N6-(2-isopentyl) Adenine Kin: 6-furfurylaminopurine TDZ: Thidiazuron N-phenyl-N'- 1,2,3-thiadiazol- 5-ylurea BAP: Benzyl Amino Purine NOA: Napthoxy acetic acid TiO: Titanium Oxide PVP: Polyvinylpyrrolidone. N.S: Nano Silver Particles N. chito: Nano Chitosan Particles.

2. Introduction

Date palm (Phoenix dactylifera L.) is one the most important fruit crops of the world in arid region, a diploid with 2n = 36, is a member of the monocotyledon's family Arecacea classified as a dioecious tall evergreen. In Arab Republic of Egypt harvested area of date palm about 122371.59 fedan and produce about 1590414 tonnes [12]. The fruit of date palm is a berry consisting of skin, pulp, inner layer and seeds. There are over 600 varieties of dates around the world that differ in shape, size and

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properties of the fruit pulp. In addition, dates vary in color, shape and texture, depending on the ripening phase in which they are harvested: Hababouk, Kimri, Khalal, Rutab and Tamr.

Micropropagation has great potential for the multiplication of female and male date palms of commercially grown cultivars by using inflorescences. This approach is simple, convenient, and much faster than the conventional method of using shoot-tip explants. The potential of inflorescence explants have been verified to develop direct and indirect of somatic embryos formation and organogenesis [15].

The inflorescence of male date palm explants had proved useful in avoiding many problems that face shoot-tip tiusse culture explants, such as high percentage of contamination, browning, and long initiation stage [30].

The immature inflorescence-based micro-propagation gave great potential for the propagation of individual recalcitrant female and male date palms and cultivars of commercial interest and is particularly useful when offshoot availability is limited. This type of propagation can be skillful in a short time with minimal effort compared with the traditional practice of using shoot-tip explants [30].

The nanoparticles materials indicate nanoparticles of silver ranging in size between 1 nm & 100 nm. Thus a single silver atom (Ag) or silver ion (Ag+) is not nanomaterial. A particle of nanosilver may or may not be charged on its surface or generate silver ions. Such as ionic silver, nanosilver particles are very potent killer of bacteria, fungi, algae, and some viruses, including HIV [7]. resently, particles of nanosilver have been showed at concentrations as low as 0.14 µg/ml to be toxic to several species of nitrifying bacteria [35]. In tissue culture of date palm, The optimal concentrations for successful inflorescence growth was 5 or 10 mg/ l Picloram and through studying the residuals effect of Picloram on inflorescences proliferation in the presence of three concentrations of TDZ, it found, TDZ at 0.5 mg /l combined with NAA at 0.1 mg/l was more effective to induce direct somatic embryos and gave the highest inflorescence proliferation percentage, while the high level of Picloram induced callus [40].

The application of small iron oxide particles in in vitro diagnostics has been practised for nearly 40 years. In the last decade, increased investigations with several types of iron oxides have been carried out in the field of nanosized magnetic particles (mostly maghemite, g-Fe2O3, or magnetite, Fe3O4, single domains of about 5–20 nm in diameter),among which magnetite is a very promising candidate since its biocompatibility has already proven [49]. magnetic iron oxide NPs became the strong candidates, and the application of small iron oxide NPs in in vitro diagnostics has been practiced for nearly half a century [50]. Magnetic iron oxide NPs have a large surface-to volume ratio and therefore possess high surface energies. Consequently, they tend to aggregate so as to minimize the surface energies. Moreover, the naked iron oxide NPs have high chemical activity, and are easily oxidized in air (especially magnetite), generally resulting in loss of magnetism and dispersibility. Therefore, providing proper surface coating and developing some effective protection strategies to keep the stability of magnetic iron oxide NPs is very important [50].

The investigation was aimed to studding who to controlling of the activity antimicrobial of nano silver and nano chitosan. The ability of nano silver and nano chitosan after confirm to decrise the microorganism, we decide to using to adding NS and chitosan in tissue culture media can decrise and remove contamination in MS media and then the explants can growth very well and we try effort to establish an in vitro propagation protocol of seedling male date palm by immature inflorescences. Also we using Fe and Zn nanoparticles to improve shoot multiplication rate in multiplication stage of seedling male date palm.

3. MATERIALS AND METHODS

This study was carried out during three successive years of 2015 to 2018 in the Tissue Culture Technique Laboratory, Central Laboratories Network- National Research Center - Dokki - Egypt. This investigation was performed throughout four stages:

Plant materials and explant condition types:

The male inflorescences are collected from 10-year-old trees planted in the Giza area during the flowering season, used this variety of pollination of date palm tree in Giza area from February to March from the mother tree. The spathes dimensions are variable measuring 15-25 cm.

Sterilization procedure experiment:

All of plant material disinfection were done in several steps; first, the male spathe was immersed for 10 minutes in a solution fungicide containing 3 g/l of Topsin, then, soaked in clorox solutions at 30% v/v commercial bleach (sodium hypochlorite percent at 5.25%) containing two drops of Tween 20 per 100 ml solution for 25 minutes and then soaked for 5 minutes

in mercuric chloride at 200 mg/l (as a control treatment), then soaked for 1 minut in ethyl alcohol solution at 70%. The male spathes were opened under aseptic conditions and the spikelets were washed three times carefully with sterile distilled water and cut into a small pieces (1-2 cm) and kept into an antioxidant solution (ascorbic acid 100 mg/l , citric acid 150 mg/l) to protect the plant material from browning.

Some the male spikelet spieces with (2-3) florets were immersed for 7 min in nano particles materials solutions as follows:

1- Nano silver particles solutions at 50 mg/l.
2- Nano silver particles solutions at 100 mg/l.
3- Nano silver particles solutions at 200 mg/l
4- Nano chitosan particles solutions at 50 mg/l.
5- Nano chitosan particles solutions at 100 mg/l.
6- Nano chitosan particles solutions at 150 mg/l.
7- Nano chitosan particles solutions at 200 mg/l.
8- Commercial bleach clorox at 30% with ethanol at 70%.
9- Commercial bleach with mercuric chloride (Hg Cl2) at 200 mg/l.

The nanoparticles materials were obtained from a private company that was equipped for this study.

Male spikelet fragments with at least 2 or 3 florets were cultured on MS medium full strength to induce callus formation.

Experiments were designed in a completely randomized design. Nine treatments×three replicates ×3 jars. After one month, contamination percentage, browning degree and survival percentage were recorded.

Culture media and incubation condition:

MS media [31] salts at full strength were supplemented with vitamins, myo Ino-sitol at 100 mg/l, glutamine at 200 mg/l, adenine at 100mg/l, citric acid at 150 mg/l, ascorbic acid at 150 mg/l during establishment stage, callus formation and shoot multiplication stage s. Sucrose at 30 g/l, and activated charcol at 1 g/l were used and all types of solid media which used in this study were solidified with purified agar-agar at 8 g/L. The pH was adjusted to 5.7 ± 0.02 by NaOH and HCl. The media were autoclaved at 100 K. pa (15 P.S.I) and 121° C for 25 minutes, then the media lefted to cool and harden for 24 hours before being used.

1- Establishment stage:

Effect of auxin type, concentration, nanoparticles of silver, and chitosan concentration added to MS medium on contamination %, browning degree and survival % of seedling male immature inflorescences explant.

MS medium containing auxin 2,4-D at (5,10,50 and 100 mg/l), NAA at (5,10,50 and 100 mg/l, silver nanoparticles at (1,2,3 and 4mg/l) and chitosan nanoparticles at (1,2,3 and 4mg/l) were added to MS culture medium supplemented with cytokinins (2ip at 3 mg/l& kin at 3 mg/l) during establishment stage. Cultures were incubated in darkness and room temperature was maintained at25± 2°C during establishment stage. During the first three months of incubation, cultures were incubated under complete darkness to inhibt polyphenol oxidation which is activited under light conditions. Total contamination (fungal % and bacterial %), browning degree and survival percentage were recorded after 6 weeks under dark incubation.

This experiment contained 2 types of auxin × 4 concentrations of each one + 2 nanoparticles materials × 4 concentrations of each one + MS free hormones without nanoparticles= 17 treatments. Experiment was designed in a completely randomized design. The degree of browning was evaluated visually as scores (index values), using the method described by [33]. Negative browning =1 Small browning= 2 Medium browning =3 Large browning =4.

Effect of auxin concentration s and types added to MS medium on callus formation and callus size of date palm seedling male immature inflorescences during initiation stage.

Callus about 3 g were transferred to MS medium salts at full strength contained sucrose at 30 g/l, and activated charcol at 1 g/l, supplemented with Picloram at (2,4,6,and 8 mg/l) , 2,4-D at (4,10,15 and 25 mg/l) and NAA at (4,10 and 20 mg/l) with cytokinins (2ip at 6 mg/l + Kin at 6 mg/l) to improve callus formation and callus size. Cultures were incubated in complete darkness and room temperature was maintained at 25± 2 °C to improve callus formation and avoid polyphenol oxidation which is catalyzed under light conditions.Callus formation percentage and callus size were recorded after 12 weeks of cultivation. The degree of callus formation and callus size were evaluated visually as scores (index values), using the method described by [33].

This experiment contained 2 auxin types × 4 concentration s + other one type(NAA) × 3 concentration s supplemented with cytokinin type with 1 concentration= 11 treatments. Experiment was coordinated in a completely randomized design. Each

treatment contained 3 replicates and each replicate contained 3 jars, each jars include one cluster.

Effect of auxin concentrations on callus formation %, callus size and browning degree of date palm seedling male immature inflorescences during callus proliferation stage.

Callus about 3 g were transferred to MS medium salts at full strength contained sucrose at 30 g/l, and activated charcol at 1 g/l, NAA at (5,10 and 20 mg/l), 2ip at (3 and 6 mg/l) & Kin at (0, 3 & 6 mg/l). Callus weight, callus size and browning degree were recorded during callus proliferation after three months. Callus subculture was carried out every six weeks.

This experiment contained 1 auxin type × 3 concentration+ 2 cytokinin types × 3 concentration= 9 treatments. Experiment was harmonious in a completely randomized design. Every treatment contained 3 replicates and each replicate contained 3 jars, each jars include 3g callus.The degree of callus formation and browning degree were rated visually as scores, using the method qualified by [33]. Small callus =1 Medium callus = 2 Large callus =3 Extra-large callus = 4

2-Multiplication stage

2.1. Effect nanoparticles of Fe and Zn concentration added to MS culture medium on number of shoots/culture and average shoot length (cm) of date palm seedling male callus culture during shoot formation stage.

Microshoots of seedling male about (2-3cm) were cultured on MS medium salts at full strength supplemented with vitamins, Ino-sitol at 100mg/l, glutamine at 200 mg/l, adenine at 100mg/l, citric acid at 150 mg/l, a scorbic acid at 150 mg/l, sucrose at 30 g/l, and PVPat 2 g/l, nano particles were tested for culture media in the stage of shoot multiplication with MS medium, Fe nanoparticles (1x=27.8,¾= 20.85, ½=13.9, & ¼= 6.95 mg/l) and Zn nanoparticles(1x=8.6,¾= 6.45, ½= 4.3& ¼= 2.15 mg/l).

This experiment contained 2 nanoparticles types × 4 concentrations + MS medium macro and micro elements= 9 treatments. Experiment was coordinated in a completely randomized design. Every one treatment contained 3 replicates and each replicate contained 3 jars, every one jar include one shoot.

2.2. Effect of cytokinin and auxin concentrations on number of shoot per culture and average shoot length (cm) of date palm seedling male callus culture during shoot multiplication stage.

NAA (0, 0.5, 1, 2 & 4 mg/l), 2ip and kin at (0, 0.5, 1,2 &4 mg/l) also were tested.MS medium containing Fe SO4.7H2O at 27.8 mg/l and Zn SO4 at 8.6 mg/l were used a control treatment. Shoot cultures were incubated under culture room 26± 2 °C Cand day-light condition16 hour for three recultures. Numbers of shoots and average shoot length (cm) /culture were listed every six weeks for three subcultures.

This experiment contained 1 auxin type ×5 concentrations Stoge ther experiment in 2 cytokinin types × 5 concentrations through three subculture. Experiment was harmonious in a factorial completely randomized design. Every one treatment contained 3 replicates and each replicate contained 3 jars, each jars include one shoots.

3-Rooting Stage

Effect of auxin concentrations and types on rooting %, number of roots and root length (cm) of date palm seedling male microshoots during rooting stage.

Microshoots of seedling male date palm about 5-7 cm length produced after the 3th subculture were transferred to MS rooting medium at ½ strength supplemented with NAA at 0.2, 0.5 & 1.0 mg /l or IBA at 1.0, 2.0, & 3.0 mg /l. Rooting percentage, number of roots /microshoots & average root length (cm) were on record after six weeks on rooting medium.

This experiment contained 2 auxin types × 3 concentrations = 6 treatments. Experiment was coordinated in a completely randomized design. Every one treatment contained 3 replicates and each replicate contained 3 jars, each jar include one microshoot.

4-acclimatization stage.

Effect of mixtures of medium on survival percentage of seedling male date palm plantlet during acclimatization stage.

Plantlets of date palm seedling male about 10 -12 cm in length and have a more developed root system were rinsed carefully with water distilled and sterile to remove adhering medium and transplanted into torpedo plastic pots 30 cm containing a mixture of sand: peat: vermiculite: perlite with difference ratio (by volume) (1:1:1:1), (2:1:1:1), (2:2:1:1) and (1:2:1:1), Plantlets were grown in greenhouse condition and covered with clear polyethylene bag for four weeks, the polyethylene bags were progressively removed after two weeks. The plantlets were sprayed with MS medium salts solutions at half strength weekly. Survival percentages were recorded after nine

weeks from transplanting.This experiment contained 4 medium mixtures as 4 treatments. Experiment was harmonious in a completely randomized design. Every treatment include 3 replicates and each replicate contained one torpedo pot, each torpedo pot contained one plantlet.

Data taken and statistical analysis:

Each treatment contains three replicates, each one replicate represented by three explants or jars. Recorded data were analyzed by Analysis of Variance (ANOVA) using MSTAT method. Duncan's multiple rang test was employed for mean comparisons accord to [41].

RESULTS AND DISCUSSIONS

1. Establishment stage.

1.1. Sterilization procedure experiment:

1.2. Effect of different silver and chitosan nanoparticles concentration on contamination percentages, browning degree & survival percentage of date palm seedling male immature inflorescences.

Data presented in Table (1) showed the effect of different silver and chitosan nanoparticles concentration on contamination percentage and browning degree of date palm seedling male immature inflorescences. Results clearly indicated that, the lowest total contamination percentage was noticed with silver nanoparticles at 200 mg/l and chitosan nanoparticles at 150 & 200 mg/l without significant differences among them. On the other hand, the highest contamination percentage and was occurred with control Clorox at 30% and ethanol at 70%.

Concerning, the effect of silver and chitosan nano particles on browning degree. Results indicated that the lowest browning degree with nano silver at 50, 100 mg/l and nano chitosan 50,100 mg/l. No significant differences between other treatments of nanoparticles silver and chitosan in contamination percentage.

The highest survival percentage obtained with silver nanoparticles at 200mg/l, chitosan nanoparticles at 150& 200 mg/l, respectively without significant differences among them.

Generally, results clearly indicated that the silver nanoparticles at 100,200 mg/l, and chitosan nanoparticles at 150,200 mg/l recorded the lowest contamination percentage and the highest survival percentage.

Data in Table (2) illustrated that, the lowest fungal percentage was occurred with 4mg/l silver nanoparticles with 2, 4-D at 100mg/l , 4mg/l silver nanoparticles with NAA at 100mg/l and4mg/l chitosan nanoparticles with 2,4-D at 100mg/l. The lowest bacterial percentage was recorded with 2, 3 and 4 mg/l silver nanoparticles and 4mg/l chitosan nanoparticles.

The lowest browning degree was achieved with addition 1mg/l chitosan nanoparticles with 2, 4-D and NAA at 5mg/l and 1mg/l silver nanoparticles with 2,4-D at 5mg/l. On the other hand, the highest contamination percentage was recorded with MS free hormones and free nanoparticles. The highest browning degree occurred with chitosan nano particles at 4mg/l with 2, 4-D at 100 mg/l and nano silver particles at 4mg/l with NAA at 100mg/l.

The lowest values of total contamination were occurred with treatments of silver nanoparticles at 4 mg/l + 2,4-D at 100 mg/l and chitosan nanoparticles at 4mg/l with NAA at 100 mg/l, respectively, without any significant differences between them. On the contrary, the highest total contaminations were achieved with MS free hormones without nanoparticles and silver nanoparticles and chitosan nanoparticles at 1 mg/l with 2,4-D and NAA at 5.0 mg/l, respectively, without any significant differences between them.

Table (1): Effect of different silver and chitosan nanoparticles concentration on contamination percentage and browning degree and survival percentage of date palm seedling male immature inflorescences.

Treatment (mg/l)	Contamination %	Browning degree	Survival %
Nano silver at 50.0	56 C	12.00 B	44 C
Nano silver at 100.0	36 D	16.00 B	64 B
Nano silver at 200.0	24 E	28.00 A	76 A
Nano chitosan at 50.0	60 C	12.00 B	40 C
Nano chitosan at 100.0	48 C	12.00 B	52 B
Nano chitosan at 150.0	28 E	28.00 A	72 A
Nano chitosan at 200.0	24 E	28.00 A	76 A
Clorox at 30% and ethanol 70%	85 A	28.00 A	15 D
Clorox and ethanol with MC at 200.0	64 B	28.00 A	36 CD

Means in each column with similar letter (s) are not significantly different at 5% level.

Data in Table (2) indicated that, the highest callus formation percentage of date palm cv. male immature inflorescences respectively, with added by silver nanoparticles at 3 mg/l with 2, 4-D at 50 mg/l. the lowest callus formation percentage of date palm cv. male immature inflorescences respectively, recorded with MS free hormones without nanoparticles.

Results indicated that, use of nano particles of silver and chitosan as immersion and adding to culture media controlled of internal and external contamination fungal and bacterial in explants. The current study of date palm indicated that nanoparticles silver and chitosan solution as immersion and adding to culture media significantly reduces contamination internal and external of date palm explant compared to colorx , mercuric chloride and they are not effect of viability of explant and callus culture compare with Clorox and mercuric chloride. Since the activity of silver is greatly influenced by timing of application, preventative applications of silver nanoparticles and ions work better before spores penetrate and colonize within the tissue of plant. Role of the activity of silver on different species of pathogens like soil borne sterile fungi that rarely produce spores [20]. The results gained from this study are consistent with [23] who indicated that, nano silver and Titanum oxide (TiO2) had a good potential for removing the bacterial contamination in plant tissue culture procedures of potato (Solanum tuberosum L.). He referred that combine nano silver (50 mg/l) to media and evaluate at second week was fully effective to control the microorganism infection. This research shows that NS had a good potential for removing of the bacterial contaminants in tissue culture plant procedures.

The results showed that NS nano silver particles can reduce and remove microorganisms in MS media and then the explants can growth very well. Cell division inhibition and damage to bacterial cell wrapper are also recorded by [36] and interaction with hydrogen bonding processes had been demonstrated to take place[38]. As specific surface area of nanoparticles is increased, their biological effectiveness can be increased due to the increase in surface energy[45]. Also, [37] recommended adding low concentration of nano silver particles to in vitro media culture of woody plant such as Olive cv. Mission.

The results in this study referred that using explant in culture medium after surface sterilization by sodium hypochlorite compared with immersion explants in alcohol following submerge in nano silver particles "NS" solution was more effective to reduce both of fungal and bacterial contaminations as well as had less adverse effects on viability and regeneration of explants. our results agreed with those obtained by [22] who reported that using nano silver in culture medium after surface sterilization displayed a more noticeable effect on removing contaminations fungal and bacterial in Tobacco plants tissue culture.

Our resultus are agreeing with in silver and chitosan nanoparticles greatly prevented contamination in explant of date palm cv. Barhee. [48].

Effect of auxin type with concentration on callus formation percentage and callus size of date palm seedling male immature inflorescences during establishment stage.

Data presented in Table (3) showed, impact of auxin type with concentration on callus formation percentage and callus size of date palm seedling male immature inflorescences during Callus proliferation stage. The highest percentages of callus formation were registered with treatments of picloram at 6 and 8 mg/l, respectively. The highest value of callus size was noticed with picloram at 6 & 8 mg/l, respectively.

Meanwhile, the lowest of callus formation percentage was noticed with at 2.0 2,4-D at 4.0 mg/l and with NAA at 4.0 mg/l.

Effect of auxin and cytokinins concentration on callus weight, callus size and browning degree of date palm seedling male immature inflorescences during callus proliferation stage.

Data in Table (4) showed that, the highest value of callus weight and highest value of callus size were occurred with NAA at 10.0 mg/l +2ip at 6 mg/l + kin at 6 mg/l. On the contrary, the lowest values of callus weight and callus size were observed with NAA at 5.0mg/l + 2ip at 6.0 mg/l + kin at 3.0 mg/l. The lowest browning degree was recorded with NAA at 5.0 mg/l+2ip at 3.0 mg/l+kin at 3.0mg/l, NAA at 5.0 mg/l+2ip at 6.0 mg/l+ kin at 0.0 mg/l and NAAat 5.0mg/l+2ip at 6.0mg/l+ kin at 6.0mg/l without significant differences among them.

Table (2): Effect of auxin type, concentration, silver, and chitosan nanoparticles concentration added to MS medium on contamination percentage, browning degree, survival percentage and callus formation percentage of date palm seedling male immature inflorescences.

Treatments (mg/l)	Total contamination	Browning degree	Survival %	Callus formation %
1.0 N.S. + 2,4-D 5.0	72.0 AB	12.0 C	28.0 E	36.0 BC
2.0 N.S. + 2,4-D 10.0	36.0 CD	24.0 BC	64.0 B	32.0 CD
3.0 N.S. + 2,4-D 50.0	32.0 D	24.0 BC	68.0 AB	60.0 A
4.0 N.S. + 2,4-D 100.0	24.0 E	28.0 B	76.0 A	52.0 B
1.0 N.chito. +NAA 5.0	72.0 AB	12.0 C	28.0 E	28.0 D
2.0 N.chito.+NAA10.0	60.0 B	28.0 B	40.0 D	32.0 CD
3.0 N.chito.+NAA 50.0	44.0 C	32.0 AB	68.0 AB	48.0 BC
4.0 N.chito.+NAA 100.0	28.0 E	32.0 AB	68.0 AB	40.0 BC
1.0 N.S. + NAA 5.0	72.0 AB	24.0 BC	64.0 B	40.0 BC
2.0 N.S. + NAA10.0	60.0 B	16.0 BC	40.0 D	48.0 BC
3.0 N.S. + NAA 50.0	44.0 C	32.0 AB	56.0 C	60.0 A
4.0 N.S. + NAA 100.0	32.0 D	36.0 A	68.0 AB	56.0 B
1.0 N.chito. +2,4-D 5.0	56.0 C	12.0 C	44.0 D	28.0 D
2.0 N.chito.+2,4-D 10.0	48.0 C	20.0 BC	52.0 C	36.0 BC
3.0 N.chito.+2,4-D 50.0	32.0 D	24.0 BC	68.0 AB	40.0 BC
4.0 N.chito.+2,4-D 100.0	20.0 E	36.0 A	80.0 A	44.0 BC
MS free hormones without nano particles	92.0 A	20.0 BC	8.0 E	24.0 E

Means in each column with similar letter (s) are not significantly different at 5% level.

Table (3): Effect of auxin type and concentration on callus formation percentage and callus size of date palm seedling male immature inflorescences during callus proliferation stage.

Treatments (mg/l)	Callus Formation %	Callus Size
picloram at 2.0	28.0 CD	1.6 AB
picloram at 4.0	52.0 ABC	1.6 AB
picloram at 6.0	76.0 A	2.6 A
picloram at 8.0	76.0 A	2.6 A
2,4-D at 4.0	24.0 D	1.8 AB
2,4-D at 10.0	44.0 BCD	2.0 AB
2,4-D at 15.0	28.0 CD	2.2 AB
2,4-D at 25.0	44.0BCD	2.4AB
NAA at 4.0	28.0CD	1.40B
NAA at 10.0	40.0 BCD	1.80 AB
NAA at 20.0	56.0 AB	2.40 AB

Means in every column with similar letter (s) are not significantly different at 5% level.

Many reports showed that, the combination of auxin like NAA and cytokinins has a significantly effective on regeneration of plant. The cytokinins which encourage cell division in plant and have active role on maturation of callus and embryos. Some of researchers believed that auxins such as 2,4-dichlorophenoxy acetic acid (2,4-D), naphthalene acetic acid (NAA), Picloram, Dicamba, 2,4,5-tricholorophenoxy acetic acid) 2,4,5 T) and endogenous hormone metabolism which are influenced by genetic, physiological with environmental signal play a key role in somatic embryogenesis in different plant species [34]; [9]; [13] [15] and [25].

Table (4): Effect of auxin and cytokinins concentration on callus weight, callus size and browning degree of date palm seedling male immature inflorescences during callus proliferation stage.

Treatments (mg/l)	Callus weight	Callus Size	Browning degree
NAA at 5.0 +2ip 3.0+kin 3.0	3.1 C	1.4 E	1.4 AB
NAA at 5.0 +2ip 6.0 +kin 0.0	4.1 BC	2.2 CD	1.6 AB
NAA at 5.0 +2ip 6.0 +kin 6.0	3.3 C	2.0 D	1.0 B
NAA at 5.0 +2ip 6.0 +kin 3.0	3.9 C	2.0 D	1.8 AB
NAA at 10.0 +2ip 3.0 +kin 3.0	6.2 AB	2.8 ABC	1.6 AB
NAA at 10.0 +2ip 6.0 +kin 0.0	3.4 C	2.4 BCD	2.2 A
NAA at 10.0 +2ip 6.0 +kin 3.0	6.3 A	3.0 AB	2.2 A
NAA at 10.0 +2ip 6.0 +kin 6.0	7.7 A	3.4 A	2.0 A
NAA at 20.0 +2ip 3.0 +kin 3.0	6.2 AB	3.2 A	1.8 AB

Means in every column with similar letter (s) are not significantly different at 5% level.

Who reported that, the combination of BAP with NAA is thinked to be the potential factor to devise a rapid response of callus induction of date palm cv. Khenizi. [21] reported that, the maximum callus induction was observed in date palm cv. 'Khalasah' follow up by 'Zadai' & 'Muzati' on MS medium add up to 2,4-D at 1.5 mg /l. The active concentration, however, varied in rranged from 0.5 to 1.5mg/l; but, the higher concentration inhibit callus induction and growth.

The highest % (90.0%) of bud explants producing callus of date palm cv. Najda was observed on MS medium supplemented with 45 µM 2,4-D and 4.5 µM 2iP. Explants from bud-derived were displayed a high embryogenic potential when cultured on MS medium supplemented with 2,4-D or picloram [27].

When [29] indicated, the higher significant callus formation percentage of date palm cv. Sewi were obtained with 2,4-D and picloram at 4mg/l, the higher embryo formation of date palm cv.Sewi with MS medium supplemented with Picloram at 4 mg/l.

2. Multiplication Stage

2.1.Effect of Fe and Zn nanoparticles concentration added to MS culture medium on number of shoots/culture and average shoot length (cm) of date palm seedling male callus culture during shoot formation stage.

In Table (5) data indicated, the highest number of shoot / culture was occurred with MS medium supplemented by Fe nano particles at 27.8 mg/l.

The results took the same trend in the second and third subculture. On the other hand, control MS without either Fe or Zn nanoparticles gave the lowest values in the three subcultures.

Data in Table (5) illustrated that, the highest average shoot length (cm) was obtained with MS medium supplemented by Fe nano particles at 27.85mg/l.

The results took the same trend in the second and third subculture.

Results cleared that nanoparticles of Fe and Zn added to culture media are significantly increase the number of shoots per culture and average shoot length (cm) of date palm cv. Barhee callus culture compared with MS medium free nanoparticles. Good shooting, rooting & regenerated plantlets of banana sp were spotted also in MS+Zinc nanoparticles and ZnO at 100 mg/l. The nanoparticles led to accumulation of both proline and chlorophyll and the activity of antioxidant enzymes and developed more dry weight accumulation than the control[17]. Silver nanoparticles , BAP at 40 mg/l and IAA at 20 mg/l gave the highest % of explants per shoots and highest mean number and length of shoots per explants of Tecomella undulate (Roxb) [3].

[46] reported that using Zn, Fe and Cu oxide NPs at 50 ppm as foliar spray enhancement the shoot growth and length of Vigina radiate . Zinc oxide NPs at 400 mg/Kg enhancing the uptake of micronutrients of Cu, Mn and Zn of Cucumis sativus fruits.

[48] Indicated that, when they used Fe and ZincO nanoparticles inhanced the number of shoots per culture and average shoot length (cm) in date palm cv. Barhee.

Table (5): Effect of Fe and Zn nanoparticles concentration adding to MS culture medium on number of shoots/culture and average shoot length (cm) of date palm seedling male callus culture during shoot formation stage.

Treatments (mg/l)	No. of shoots /culture			Average shoot length (cm)		
	1st sub.	2ndsub.	3rdsub.	1st sub.	2nd sub.	3rd sub.
MS medium	3.6 CDE	4.2 B	2.8 C	3.60 CD	3.80 C	2.60 C
MS + Fe N.Ps at 6.95	3.0 DE	4.0 B	3.6 BC	3.20 CD	3.80 C	3.78 BC
MS+ Fe N.Psat13.9	4.2 BC	5.0 AB	3.6 BC	4.40 BC	4.78 AB	3.32 BC
MS+ Fe N.Ps at 20.85	4.8 AB	4.6 B	3.8 BC	4.40 BC	4.20 B	3.80 BC
MS+ Fe N.ps at 27.8	5.4 A	6.0 A	5.2 A	5.40 A	6.00 A	5.34 A
MS+ Zn N.Ps at 2.15	5.0 AB	4.6 B	3.4 BC	4.74 B	4.40 AB	4.32 AB
MS+Zn N.Ps at 4.3	4.8 AB	5.2 AB	4.6 AB	4.90 A	4.60 AB	4.08 B
MS+Zn N.Ps at 6.45	2.8 E	4.4 B	2.8 C	2.80 D	4.40 B	4.40 AB
MS+ Zn N.ps at 8.6,	4.0 BCD	4.0 B	3.0 C	4.20 C	4.10 B	3.58 BC

Means in every column with similar letter (s) are not significantly different at 5% level.

Effect of auxin and cytokinins concentration on number of shoot per culture of date palm seedling male callus culture during shoot multiplication stage.

Data in Table (6) revelead that, effect of auxin concentration, the highest significant number of shoots/culturej were achieved by NAA at 4.0 mg/l in the 1st, 2 nd and 3 rd subculture, respectively. Meanwhile, the lowest values were noticed with NAA at 0.0 or 0.5mg/l.

The effects of cytokinins: the highest number of shoots/culture were recorded by 2ip +kin at 2.0 or 4.0mg/l. On a contrary, control treatment (0.0 mg/l) gave the lowest values during the three subcultures.

Data in Table (6) indicated that, the interaction between cytokinin and auxin concentration, the highest number of shoots / culture were achieved with NAA at 2.0 mg/l, 2ip at 4mg/l, kin at 4mg/l and NAA at 4.0 mg/l, 2ip at 4.0 mg/l, kin at 4.0 mg/l respectively, without any significant differences among them in first, second and third subcultures. Otherwise, the lowest number of shoots / culture in first, second and third subculture was occurred with NAA at 0.0 mg/l, with 2ip & kin at 0.5 mg/l of first subculture.

Effect of auxin and cytokinins concentration on average shoots length (cm) of date palm seedling male callus culture during shoot multiplication stage.

Effect of auxin concentration,data in Table (7) . Pointed that, the highest average shoot length (cm) was occurred with auxin treatment by NAA at4.0 mg/l in the 1st, 2nd & 3 rd subcultures. The lowest average shoot length (cm) was recorded with NAA at 0.0 mg/l respectively, without any significant differences among them in the 1st,2nd and 3rd subcultures.

Effect of cytokinin concentration, there are insignificant differences among them all the cytokinins treatments in the 1st, 2nd and 3rd subcultures of average shoot length (cm).

The interaction between cytokinin and auxin concentration, the highest average shoot length (cm) were achieved with NAA at 4.0 and 2.0 mg/l with 2ip at 4.0 mg/l, kin at 4.0 mg/l, respectively , without any significant differences among them in 1st and 3rd subcultures. On a contrary, the lowest average shoot length (cm) was occurred with NAA at 0.0, 1.0 & 2.0mg/l, with 2ip & kin at 0.5 mg/l in first, second and third subcultures.

Pervious studies In vitro in date palm immature inflorescences effected by multifaceted factors like light, photoperiod, pH of the medium, and nutrients. Many studies have also converge on the effect of plant growth regulators on in vitro flowering process in other species[18]. The significantly effective of cytokinins on in vitro mature inflorescences was well-celebrated and comprehended in the literature[44]. The action of BA (6-benzyladenine) or combined impact of BA with phytohormones on early in vitro has inflorescences also been reported for different plant species [17].

The previous results pointed that, the use of 2iP at1.5 mg/l, BAP at 1 mg/l and NAA at 1mg/l give the highest mean number of shoot per explant and highest mean shoot length of date plam cv. Barhee [19]. Similarly, [26] resulted, an in vitro flower induction experiment of one year old date palm cv. Barhee plantlets which was hold on basal MS medium, with sucrose (50 g/l) & phytohormones (NAA: 2.68 µM , BAP: 4.44 µM, Kin: 4.64 µM & IPA: 5.28 µM). Studing on in vitro propagation of date palm cv. Sukry by [4] submitted, the highest propagation was occured in the MS medium, with 0.05 mg/l Kin 0.025 mg/l 2ip, BAP, IAA, NOA & NAA. The same results were also obtained by [47] and [1]. In a similar way [24] gave this conclusion that after using 3 mg/l 2iP & BAP at initiation stage, the quantity of cytokinins decreased to 0.5 mg/l Kin & BAP respectively. As well, they are revealed that utilizing a conjunction of two cytokinins (BAP & Kinetin) and one auxin (NAA) in multiplication stage demonstrated more hopeful for making cultures with sufficient mean number of shoots with best shoot lengths. In present study, shoots good developed adequate (number of shoots per culture & average shoot length) just after 3 subcultures without increasing, as we used combination of cytokinins (2iP & Kin) with auxin (NAA) like [28].

Similarly results by [29] indicated, Kin at 0.25 mg/l significant increasing average number of adventitious shoot per culture of date palm cv. Sewi and refereed, Kin and 2ip had gave the highest significant number of shoots per culture.

Similarly results by [48] revelaed that NAA,2ip and Kin were recorded the highest number per culture and the highest average shoot length in the three subculture during multiplication stage of date palm cv. Barhee.

Table (6).Effect of cytokinins and auxin concentration on number of shoot / culture of date palm male callus culture during shoot multiplication Stage.

Cytokinin conc. / Auxin conc.	Number of shoots/culture																	
	1st.subculture						2nd subculture						3rd subculture					
	2ip + Kin(mg/l)						2ip + Kin(mg/l)						2ip + Kin(mg/l)					
	0	0.5	1	2	4	Mean	0	0.5	1	2	4	Mean	0	0.5	1	2	4	Mean
0.0 mg/l NAA	1.2 hi	1.0 i	1.4 ghi	1.4 ghi	2.0 efghi	**1.64 C**	1.4 jk	1.6 ijk	1.6 ijk	1.6 ijk	2.6 fghi	**1.88C**	1.4 fghi	1.4 fghi	1.4 fghi	1.4 fghi	1.8 efghi	**1.36 C**
0.5 mg/l NAA	1.4 ghi	1.2 hi	1.8 fghi	1.4 ghi	2.2 defgh	**1.72 C**	1.6 ijk	1.0 k	2.4 ghij	1.2 k	2.4 ghij	**2.00C**	1.0 hi	0.80 i	2.2 cdefg	1.2 ghi	2.2 cdefg	**1.64 C**
1 mg/l NAA	1.4 ghi	1.6 fghi	2.4 cdefg	2.2 defgh	3.4 bc	**2.24 B**	1.6 ijk	1.8 hijk	3.0 efg	2.8 efgh	3.8 cde	**2.76 B**	1.4 fghi	1.8 efghi	2.4 cdef	2.0 defgh	3.2 bc	**2.28 B**
2 mg/l NAA	2.0 efghi	2.4 cdefg	2.6 cdef	3.2 cd	4.8 a	**2.52 B**	2.4 ghij	2.8 efgh	3.2 efg	4.6 bcd	4.8 abc	**3.04 B**	1.4 fghi	1.8 efghi	2.4 cdef	2.8 bcde	3.8 b	**2.24 B**
4 mg/l NAA	2.2 defgh	2.4 cdefg	3.0 cde	4.4 ab	4.8 a	**3.40 A**	2.4 ghij	2.8 efgh	3.6 def	5.0 ab	5.8 a	**3.88 A**	1.6 fghi	2.4 cdef	3.0 bcd	3.8 b	5.2 a	**3.24 A**
Mean	**1.40 C**	**1.60 C**	**2.20 B**	**3.00 A**	**3.32 A**		**1.76 C**	**1.72 C**	**2.60 B**	**3.56 A**	**3.92 A**		**1.48 C**	**1.48 C**	**2.16 B**	**2.44 B**	**3.20 A**	

Table (7): Effect of cytokinins and auxin concentration on average shoots length (cm) of date palm seedling male callus culture during shoot multiplication stage.

Cytokinin conc. / Auxin conc.	**Average shoots length (cm)**																	
	1St.subculture						**2nd subculture**						**3rd subculture**					
	2ip + Kin(mg/l)						**2ip + Kin(mg/l)**						**2ip + Kin(mg/l)**					
	0	**0.5**	**1**	**2**	**4**	**Mean**	**0**	**0.5**	**1**	**2**	**4**	**Mean**	**0**	**0.5**	**1**	**2**	**4**	**Mean**
0.0 mg/l NAA	3.20 efg	2.80 fg	3.14 efg	4.20 abcdef	4.64 abcde	3.28 D	2.40 fg	4.40 abcd	3.86 cdef	4.30 abcde	5.20 abc	2.98 C	1.80 fg	4.00 bcd	3.80 bcd	3.60 bcd	4.40 abc	2.51 C
0.5 mg/l NAA	2.80 fg	4.80 abcd	3.90 abcdefg	4.460 abcde	5.00 abc	4.14 BC	2.60 efg	3.400 defg	4.20 abcde	4.00 bcdef	5.60 ab	3.66 C	1.60 g	3.60 bcd	4.20 abcd	3.00 def	4.80 ab	3.92 B
1 mg/l NAA	2.40 g	3.52 cdefg	3.20 efg	4.60 abcde	5.40 a	3.59 CD	2.00 g	3.300 defg	3.40 defg	4.60 abcd	5.16 ab	3.65 C	2.20 efg	4.240 abcd	3.200 cde	3.600 bcd	5.480 a	3.68 B
2 mg/l NAA	3.40 defg	5.00 abc	3.80 bcdefg	4.20 abcdef	4.60 abcde	4.50 AB	3.70 cdefg	3.800 cdef	3.20 defg	4.60 abcd	5.90 a	4.55 B	3.14 cdef	3.80 bcd	4.20 abcd	4.20 abcd	4.20 abcd	3.72 B
4 mg/l NAA	4.60 abcde	4.60 abcde	3.92 abcdef	5.08 ab	5.26 ab	4.98 A	4.20 abcde	3.40 defg	3.60 cdefg	5.26 abc	4.80 abcd	5.33 A	3.84 bcd	4.00 bcd	3.00 def	4.20 abcd	4.34 abcd	4.64 A
Mean	**3.74 B**	**4.12 AB**	**3.66 B**	**4.28 AB**	**4.69 A**		**4.03 A**	**3.96 A**	**3.69 A**	**4.24 A**	**4.25 A**		**3.52 A**	**3.44 A**	**3.74 A**	**3.90 A**	**3.87 A**	

3-Rooting Stage:

Effect of auxin type and concentrations on rooting percentage, number of roots and root length (cm) of date palm seedling male microshoots during rooting stage.

Data in Table (8) . Showed that, the highest rooting percentage was recorded with MS medium supplemented by NAA at 1.0 mg/l. On the contrary, the lowest rooting percentage was showed with MS medium containing IBA at 0.2 and 1.0 mg/l, respectively.

However, the highest number of roots/ microshoots was occurred with MS medium supplemented with NAA at 1.0 mg/l and IBA at 3 or 2 mg/l, respectively. On the contrary, The lowest number of roots / microshoots were recorded with IBA at 1.0 mg/l and NAA at 0.2 mg/l. The highest value of root length (cm) was recorded with MS medium with IBA at 3.0 mg/l.

Table (8): Effect of auxin type and concentrations on rooting percentage, number of roots and root length (cm) of date palm seedling male microshoots during rooting stage.

Auxin treatments (mg /L)	Rooting %	Number of roots/microshoots	Root length (cm)
NAA at 0.2	33.3 D	2.2 C	2.9 B
NAA at 0.5	83.3 A	4.7 A	4.3AB
NAA at 1.0	66.6 B	4.2 AB	4.0AB
IBA at 1.0	33.3 D	2.2 C	4.3AB
IBA at 2.0	50.0 C	3.2 BC	5.9 A
IBA at 3.0	66.6 B	3.0 BC	6.4 A

Means in each column with similar letter (s) are not significantly different at 5% level.

Thereafter, in order to do the root formation, good developed and normal morphologically regenerated shoots were recultured to MS medium, supplemented with different levels of NAA. Root formation is a essential stage in micropropagation of date palm, as it allow the subsequent success of production of food date palm plantlets [39]. Our results revealed, the highest rooting percentage & number of root per microshoots were obtained with NAA at 0.5 mg/l and IBA at 3.0 mg/l, respectively. Similar of our results with in vitro rooting of date palm was explained by [8] he showed that NAA (1 mg/L) was the best for in vitro root formation in compar with IAA or IBA with same concentrations. The stimulative effects of NAA treatment on rooting also has been obtained in other explants. Roots efficiently developed when cultured leaf explants of date palm on the Eeuwen's induction medium with 5 & 15 mg/L NAA [6]. On the other hand, [43] revealed that unusual rooting of date palm plantlets were gained on medium with 0.1 mg/L NAA. He was showing that, high levels of NAA in a negative way affected to root length of date palm cv. Barhee plantlets. [17] showed that, NAA at 1.0 mg/L dosage was preferable because it gaving the best effective on encourage the regeneration of plantlets of banana with well-formed root systems.

The highest percentage of rooting (90.9%) recorded with NAA 0.5 mg/l was employed. The highest root number per microshoots were observed in the existence of 1.0 mg/l NAA and they decreased as NAA level was decreased (at 0.5 and 0.2 mg/l) or increased (2.0 mg/l) of date palm cv. Barhee[19]. [32] indicated that, best rooting in 1/4 MS medium with NAA 0.1 mg/l in absence of activated charchol (AC), in Pakistani date palm cultivars “Gajar”, “Kashoowari”, and “Dedhi”. [11] studied the rooting in “Hayani” after 8 weeks, noticed that a combination of 1.0 mg/l each of IBA & NAA in MS medium significantly increase the number of root formations & root length when one shoot was cultured / test tube.

Similarly results reported, by [29] showed that, NAA 1.0 mg/L induced the highest rooting percentage & microshoots length of date palm cv. Sewi microshoots.

Similarly results reported, by [48] using NAA and IBA gives the best rooting percentage, root length and number of root per microshoots of date palm cv. Barhee.

4. Acclimatization stage.

Effect of medium mixtures on survival percentage of date palm seedling male plantlet during acclimatization stage.

The highest survival percentage (80.0) were found with medium mixtures sand: peat: vermiculite: perlite (2: 2: 1:1) of date palm male plantlet.

On the other hand, the lowest survival percentage (20.0 C) was recorded with substrate mixtures of sand: peat: vermiculite: perlite (1 : 1 : 1:1) of date palm male plantlet.

Table (9). Effect of medium mixtures on survival % of date palm seedling male plantlet during acclimatization stage.

Medium mixture	Survival %
Sand : Peat : Vermiculite : perlite 1 : 1 : 1 : 1	20.0 C
Sand : Peat : Vermiculite : perlite 2 : 1 : 1: 1	46.6 B
Sand : Peat : Vermiculite : perlite 2 : 2 : 1: 1	33.3 B
Sand : Peat : Vermiculite : perlite 1: 2 : 1: 1	80.0 A

Means in each column with similar letter (s) are not significantly different at 5% level.

This study and based on the utilization of rooting stage formation of adventitious roots and accurate handling for the plant material, the survival percentage extended to more than 80 - 83 %. Using of medium mixture contained sand: Peatmoss: perlite: vermiculite at ratio (1: 2 : 1: 1) and (2:2: 1:1 v/v) gave the best survival percentage in acclimatization stage. Several soil mixtures have been used to transfer plantlets ex vitro.

Rooting superiority of the ex vitro plantlets of date palm was the dynamic factor increased the survival percentage in the greenhouse. Most of the studies registered low survival percentage 25-35% during acclimatization stage rather than it used to be a big problem in complete micropropagation protocol [2]; [16] &[42].

The major mixture characteristic that effectiveness plant growth is moisture which should not be excessive to escaping fungi attacks roots and not too low to avoid plantlet dryness. [43] showed the best survival rate was recorded for 10–12 cm date palm plantlets transferred to peat moss: vermiculite mixture (1:1 v/v) and covered with transparent plastic. [10] reported that the best results were occurred with a planting medium containing equivalent parts of peat, sand and vermiculite. Survival percentage was reached to 80% after eighteen months. The survival percentage of some Pakistani date palm cultivars reached more than 95%. The used soil bed was a simple mixture of washed sand and peatmoss (1:1 volume / volume) with few amount of perlite. The acclimatized plants with at least one compound leaf were shifted to the field conditions [32], [14] indicated that pre-acclimatization is a very useful & important step to full micropropagation process. Plantlets grown in lab under optimum conditions (moisture, salts, sucrose and water), slim cuticle layer in leaves with high transpiration rate.Water supply must be keep an eye on carefully during the 1st month of acclimatization process. If the moisture are too much can lead to plantlet root and too little moisture in the substrate can lowering the relative humidity around the plants and cause their rapid wilt. [5] spotted a survival range of 72–84% in date palm cvs. Khasab and NaboutSaif. In date palm cv. Najda organogenesis, recorded, the survival rate depends upon the elongation-rooting medium; and a high survival rate of 100% was recorded in plantlets that have been cultured on plant growth regulators free in solid medium before acclimatization.

Highest survival % (88–92.5%) were also obtained in date palm cv. Mejhoul propagated by through organogenesis [27] and [28] .

Also, [29] Indicated, the higher significant survival percentages (83%) during acclimatization stage of date palm cv. Sewi were observed with plantlets produced from Indole-3-butyric acid (IBA) at 0.5 mg/l during rooting stage.

[48] indicated that, the best survival percentage was occurred with media mixture at sand : petmoss: vermcolut: perlit (2:2:1:1) and (1:2:1:1), respectively of date palm cv. Barhee.

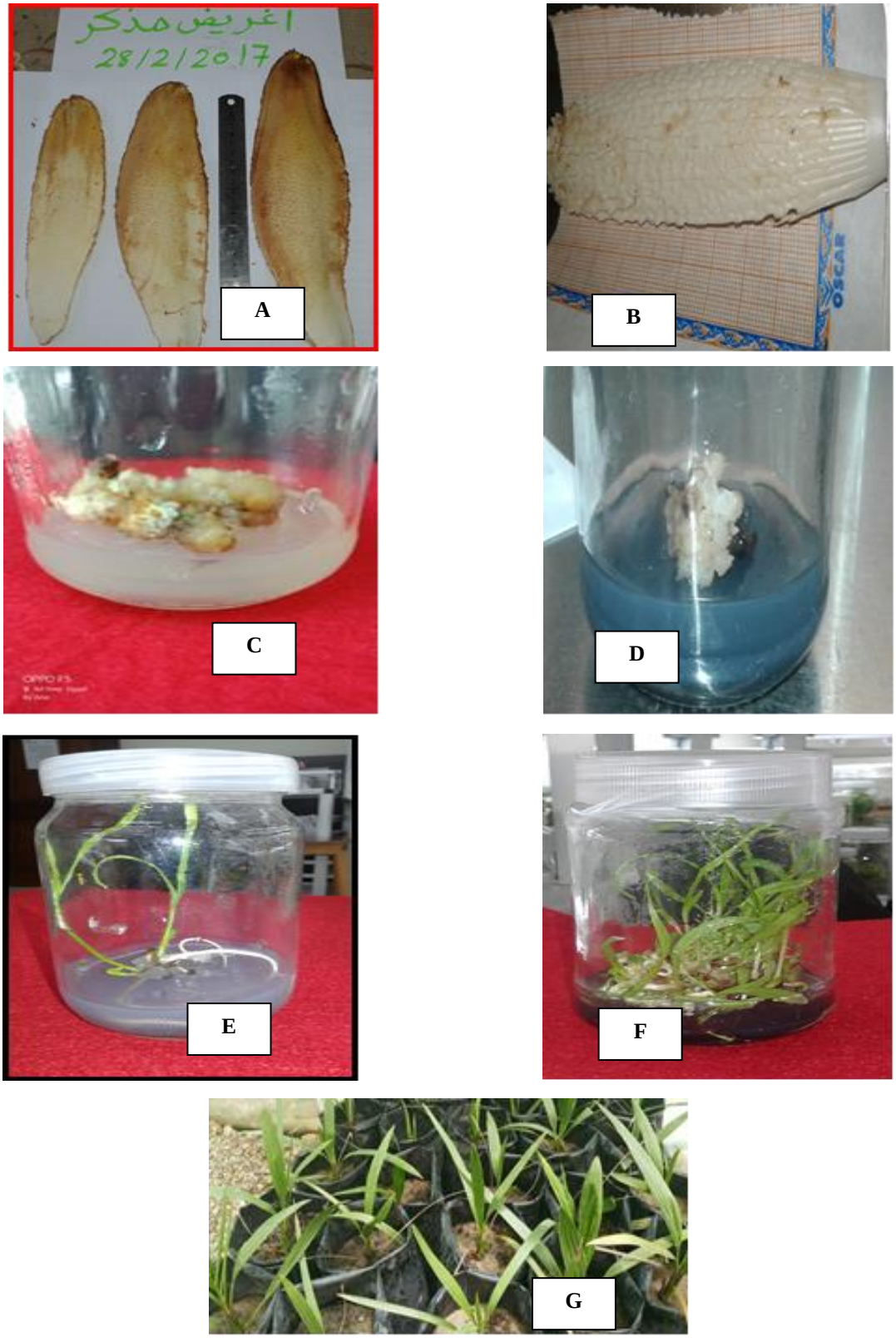

Fig. (1) (a). Immature spathes of date palm male seedling, (b). Open immature spikelets of date palm male seedling, (c and D): Callus formation produced on MS medium containing nano silver particles at 4mg/l + NAA at 100 mg/l, (f): Proliferated shoots on MS medium containing NAA 4.0mg/l +2ip 2mg/l + Kin 2 mg/l, (e): Microshoots rooted on MS including NAA at 1.0 mg/l, (g): Plantlets acclimatized on medium mixtures Sand: Peat: Vermiculite: perlite at (2: 2 : 1: 1) after 3 months of transplantation.

References

[1] Aaouine, M. (2000). Production of date palm in vitro plants: the Moroccan experience. Proceedings of the Date Palm International Symposium, Windhoek, Namibia.

[2] Abul-Soad, A.A., Ibrahim I.A., El-Sherbeny N.R. and Baker S.I. (1999). In vitro and ex vitro optimization for rooting and acclimatization of date palm. Proc. first Inter. Conf. in Egypt on plant tissue culture and its Application, 12-14 September, Egypt, pp. 227-241.

[3] Aghdaei M. ;Salehi H. and Sarmast M.K. (2012).Effects of silver nanoparticles on Tecomellaundulata (Roxb.)SeemMicropropagation. Adv. Hort. Sci., 26(1): 21-24.

[4] Al-khateeb, A.A. (2006). Role of cytokinin and auxin on the multiplication stage of date palm (Phoenix dactylifera L.) cv. Sukry. Biotechnology 5 (3): 349-352.

[5] Al-Khayri J. M. (2010). Somatic embryogenesis of date palm (Phoenix dactylifera L.) improved by coconut water. Biotechnology. 9:477–484. doi: 10.3923/biotech. 477.484.

[6] Asemota, O., Eke, C.R. and Odewale, J.O.(2007). Date palm (Phoenix dactylifera L.) in vitro morphogenesis in response to growth regulators, sucrose and nitrogen. African J.biotechnol. 6 (20): 2353-2357.

[7] Becker D. K, Dugdale B, Smith M. K, Harding R. M and Dale J. L (2000). Genetic transformation of Cavandish banana (Musa spp. AAA group) cv 'Grand Nain' via microprojectile bombardment. Plant Cell Rep 19:229–234.

[8] Bekheet, S.(2013).Direct organogenesis of date palm (Phoenix dactylifera L.) for propagation of true-to- type plants.SciAgri 4(3):85-92.

[9] Dodeman V. L., Ducreux G. and Kreis M (1997). Zygotic embryogenesis versus somatic embryogenesis. J Exp Bot 48(313):1493-1509.

[10] El Sharabasy, S. F. ;Bosila, H. A and Ibrahim, I. A. (2001). Micropropagation studies on Zaghlool and Sewicvs of date palm (Phoenix dactylifera L.): III. Plantlet acclimatization. Proceedings second international conference on date palm, Al Ain, UAE, pp 523–530.

[11] Elghayaty, S.H.; Edriss, M.H.; Abdrabboh, G.A.; Elsharabasy, S.F. and Abd-El-kariem G. E. (2016). An optimized protocol for direct shoot regeneration from shoot tips cultures of date palm (Phoenix dactylifera L.) cv. Hayani. World Rural Observations ;8.(2).

[12] Fao (2019). FAOSTAT. Food and Agricultural Organization of the United Nations. Available in: www.fao.org/faostat/en/; access in: June 20.

[13] Feher A (2006). Why somatic plant cells start to form embryos? In: Plant cell monographs. Mujib A., Samaj J. (eds), 2:85-101, Springer-Verlag Berlin Heidelberg.

[14] Gabr, M.F. and Abd-Alla M.M. (2010). Micropropagation of Phoenix dactylifera L. var. karama. New York Science Journal, 3(12): 64-69.

[15] Hee, K. H., Loh, C. S. and Yeoh, H. H. (2007). Early in vitro flowering and seed production in culture in Dendrobium Chao Praya Smile (Orchidaceae)."Plant cell reports 26 (12): 2055-2062.

[16] Hegazy, A.E. and Aboshama, H.M (2010). An efficient novel pathway discovered in date palm micropropagation. Acta Hort. 882: 167-176.

[17] Helaly, M. N.; El-Metwally, M. A,; El-Hoseiny, H; Abdelaziz, S. O. and El-Sheery, N. I (2014).Effect of nanoparticles on biological contamination of 'in vitro' cultures and organogenic regeneration of banana .Australian journal of crop science, V.8,(4).

[18] Jain S. M ;Al-Khayri J.M and Johnson D.V (eds) (2011). Date palm biotechnology. Springer, The Netherlands, pp 47–68.

[19] Jazinizadeh E, Zarghami R, Majd A, Iranbakhsh A and Tajaddod G (2015).In vitro product of date palm (Phoenix dactylifera L.) cv. 'Barhee' plantlets through direct organogenesis. Biol Forum 7(2):566–572.

[20] Jo Y. K., B. H. Kim and Jung G. (2009) . Antifungal Activity of Silver Ions and Nanoparticles on Phytopathogenic Fungi. Plant Dis. 93:1037-1043.

[21] Junaidaslam and Khan S.A(2009). In vitro micropropagation of 'khalas' date palm (Phoenix dactylifera l.), an important fruit plant.journal of fruit and ornamental plant research vol. 17(1): 15-27.

[22] Kamran, S. (2011). In vitro antibacterial activity of nanomaterial for using in tobacco plants tissue culture. World academy of science, engineering and technology, volume: 55. p372-375.

[23] Kamran S. (2012).Evaluation of Using Nanomaterial in Tissue Culture Media and Biological activity. 2nd International Conference on Ecological, Environmental and Biological

Sciences (EEBS'2012) Oct. 13-14, Bali. Indonesia.

[24] Khan S and Tabassum B.B. (2012). Direct shoot regeneration system for date palm (Phoenix dactylifera L.) cv. Dhakki as a means of micropropagation. Pak. J. Bot. 44(6): 1965-1971.

[25] Kurup, S. S. (2014). Rapid in vitro regeneration of date palm (Phoenix dactylifera L.) cv. Kheneizi using tender leaf explant. Emirates Journal of Food and Agriculture, 26(6):539-544.

[26] Masmoudi-Allouche F, Meziou B, Kriaaˆ W, Gargouri-Bouzid R, Drira N (2010). In vitro flowering induction in date palm (Phoenix dactylifera L.). J Plant Growth Regul 29:35–43.

[27] Mazri MA, Meziani R, El Fadile J, Ezzinbi A.(2016). Optimization of medium composition for in vitro shoot proliferation and growth of date palm cv. Mejhoul. 3 Biotech.;6:111. doi: 10.1007/s13205-016-0430-x.

[28] Mazri M. A., Ilham B., Meziani R., Mokhless B. and Souad N. (2017). Somatic embryogenesis from bud and leaf explants of date palm (Phoenix dactylifera L.) cv. Najda. 3 Biotech.May; 7(1): 58. Morocco.

[29] Malhat M. M. H, El-Wakeel H., Abd El-Hamid A., Khalil S. M. and Mona M. Hassan. (2019).Direct embryogenesis and indirect organogenesis of date palm (Phoenix dactylifera L.) cv. Sewi using immature inflorescences. AUJAS, Ain Shams Univ., Cairo, Egypt, special Issue, 27(1).

[30] Mohan Jain S. ; Al-Khayri J. M. and Johnson D. V (2011).Date Palm Biotechnology.Book.Springer.

[31] Murashige, T., and Skoog, F.A. (1962). Revised medium for rapid growth and bioassays with tobacco tissue cultures.Physiol Plant 15: 473-97.

[32] Mushtaque, A. J. ;Abul-soad,A. A.;Solangi , N. and Markhand. G. S (2015). Establishment of an Efficient Protocol for Micropropagation of Some Pakistani Cultivars of Date Palm (Phoenix dactyliferaL) using novel inflorescence explants. Pak. J. Bot., 47(5): 1921-1927, 2015.

[33] Pottino, B.G. (1981).Methods in plant tissue culture.Dep.of Hort. Agric. College, Maryland Univ. College Park, Maryland, U.S.A., PP. 8-29.

[34] Rao K. S(1996). Embryogenesis in flowering plants: recent approaches and prospects. Biosci 21(6):827-841.

[35] Reidy Bo. ; Haase, A ;Luch, A. ; Dawson, K. A. and Lynch, I. (2013). Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials (Basel). Jun; 6(6): pp2295–2350.

[36] Richards, R. M. E., Odelola H. A. and Anderson B. (1984).Effect of silver on whole cells and spheroplasts of a silver resistant Pseudomonas aeruginosa.Microbios. 39: 151-157.

[37] Rostami A.Aand shahsavar, A (2009).Nano silver particles eliminate contamination of olive :Mission explants. Asian Journal of plant science. ISSN 1682- 3974.

[38] Russell, A.D and Hugo, W.B. (1994(. Antimicrobial activity and action of silver.Prog. Med. Chem., 31: 351 - 370.

[39] Shaheen, M.A (1990). Propagation of date palm through tissue culture: A review and an interpretation. Ann Agric Sci, Ain-Shams Univ., Cairo, Egypt. 35: 895-909.

[40] Sidky R. A (2014). The Effect of Picloram and Thidiazuron Concentrations on ProliferationSomatic Embryos from Immature Inflorescence of Date palm. Assiut J. Agric. Sci., (45) No. (1) 58-67.

[41] Snedecor, G.W. and Cochran, W.G. (1982). Statistical Methods, 8th Ed. Iowa State Univ. Press, Ames, Iowa, USA.

[42] Taha, H.S., Hassan M.M. and El-Bahr M.K. (2007). Micropropagation of some Egyptian date palm dry cultivars, 1- Maturation of somatic embryos. Arab J. Biotech., 10(2):333-340.

[43] Tissert, B.(1984).Propagation of date palm by shoot tip cultures.Hort Science., 19: 230-231.

[44] Wang, S., Tang, L. and Chen, F. (2001)."In vitro flowering of bitter melon." Plant cell reports, 20(5): 393-397.

[45] Williams R. O. , Yang W J and Peters I. (2005). Inhaled nanoparticles- A current review. International Journal of Pharmaceutics. V 356, Issues 1–2, , Pp 239-247.

[46] Zaho L., Peralta-Videa J. R and Cyren Rico M. (2014). CeO2 and ZnO nanoparticles change the nutritional qualities of cucumber (Cucumis Sativus L.) supporting information. Univ. of Texas. USA.

[47] Zaid, A., Al Kaabi H.H. and El-Korchi, B. (2006). Impact of lower concentration of growth regulators on the multiplication stage of date palm organogenesis.3rd Intl. date palm Conf. Abu Dhabi, U.A.E.

[48] Rohim F. M., El-Wakeel H., Abd El-Hamid A. A. and Eman A. A. (2020). Impact of Nanoparticles of In Vitro Propagation of Date Palm cv. Barhee by Immature Inflorescences. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt. V. 28(4), pp. 1187-1202.

[49] Schwertmann U., Cornell R.M. (1991). Iron oxides in the laboratory: preparation and characterization. Weinheim, Cambridge: VCH.

[50] Wu W., Quanguo H., Changzhong J. (2008). Magnetic Iron Oxide Nanoparticles: Synthesis and Surface Functionalization Strategies. Nanoscale Res Lett 3:397–415.