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Impact of Nanoparticles of *In Vitro* Propagation of Date Palm *cv.* Barhee by Immature Inflorescences

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Abstract

The impact of nano silver & nano chitosan particles on sterilization, nano Fe and Zn on callus formation of immature inflorescence of date palm cv. Barhee during the establishment stage was 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 at200 mg/l, and nano chitosan at 150, and 200 mg/l. The lowest contamination %recorded in medium culture containing silver nanoparticles at 4mg/l with NAA at 100mg/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 6mg/l & Kin at 6 mg/l during callus proliferation. 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 , MS medium culture supplemented 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, 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. Interaction between cytokinins and auxin concentration, indicated, 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 1st, 2nd & 3rd subcultures, respectively. The highest rooting percentage and number of roots/ microshoots were obtained 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, Immature Inflorescence, *In Vitro*, Propagation, Nanoparticles, Acclimatization

Abbreviation: 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, NS: nano silver particles, N chito: nano chitosan particles.

1 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 Egypt harvested area of date palm about 122371.59 fedan and produce about 1590414 tonnes (FAO 2019).

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.

Inflorescence explants had proved useful in avoiding many obstacles that face shoot-tip explants, such as high percentage of contamination, browning, and long initiation stage (Mohan et al 2011). Inflorescence-based micropropagation 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 (Mohan et al 2011).

The term nanosilver 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 sa ionic silver, nanosilver particles are very potent killer of bacteria, fungi, algae, and some viruses, including HIV (Becker et al 2000). Newly, nanosilver have been showed at concentrations as low as 0.14 µg/ml to be toxic to several species of nitrifying bacteria (Reidy et al 2013). In 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 (Sidky 2014).

2 Materials and Methods

This study was achieved through 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:

2.1 Plant materials and explant types

The inflorescences are collected from 10-year-old trees planted in the Giza area during the flowering season, from February to March from the mother tree. The spathe dimensions are variable, measuring 15-25 cm.

2.2 Sterilization procedure experiment

All of plant material disinfection were done in several steps; first, the 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 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 Spikelet species with (2-3) florets were immersed for 7 min in nano particles materials solutions as follows:

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

The nano particles materials were obtained from a private company that was equipped for this study. Spikelet fragments with at least 2 or 3 florets were cultured on MS medium full strength to indice 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.

In this investigation we study the activity antimicrobial of nano silver and nano chitosan. The ability of nano silver and nano chitosan after confirm to reduce the microorganism, we decide to using and adding NS and chitosan to tissue culture media can reduce and remove microorganisms in MS media and then the explants can growth very well and we try effort to establish an *in vitro* propagation protocol of date palm cv. Barhee by immature inflorescences.

2.3 Culture media and incubation condition

MS media (Murashige and Skoog 1962) salts at full strength were supplemented with vitamins, Inositol 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. 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 7g/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 twenty minutes, then the media lefted to cool and harden for 24 hours before being used.

2.4 Establishment stage

2.4.1 Effect of auxin type, concentration, silver, and chitosan nanoparticles concentration added to MS medium on contamination %, browning degree and survival percentage of date palm cv. Barhee immature inflorescences

MS medium containing auxin 2,4-D at (5,10,50 and 100mg/l), NAA at (5,10,50 and 100 mg/l, silver nanoparticles at (1, 2, 3 and 4 mg/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 at 25 ± 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 auxin types × 4 concentration of auxin each with of one + 2 nanoparticles material × 4 concentration+ 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 Pottino (1981). Negative browning =1 Small browning = 2 Medium browning =3 Large browning =4.

2.4.2 Effect of auxin type, concentration added to MS medium on callus formation and callus size of date palm cv. Barhee 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. 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 Pottino (1981).

This experiment contained 2 auxin types × 4 concentration+ other one type(NAA) × 3 concentration supplemented 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.

2.4.3 Effect of auxin type and concentration on callus formation percentage, callus size and browning degree of date palm cv. Barhee 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 types × 3 concentration+ 2 cytokinin type × 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 Pottino (1981). Small callus= 1 Medium callus = 2 Large callus =3 Extra-large callus = 4.

2.5 Multiplication stage

2.5.1 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 cv. Barhee callus culture during shoot formation stage

Microshoots about (2-3cm) were cultured on MS medium salts at full strength used 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 concentration + 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.

2.5.2 Effect of auxin and cytokinins concentration on number of shoot / culture and average shoot length (cm) of date palm Barhee 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 and day-light condition16 hour for three re-cultures. Numbers of shoots and average shoot length (cm) /culture were listed every six weeks for three subcultures. This experiment contained 1 auxin type's ×5 concentration storage there experiment in 2 cytokinin type × 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.

2.6 Rooting Stage

2.6.1 Effect of auxin type & concentration on rooting percentage, number of roots and root length (cm) of date palm cv. Barhee microshoots during rooting stage

Microshoots of 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 concentration = 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.

2.7 Acclimatization stage

2.7.1 Effect of medium mixtures on survival % of date palm cv. Barhee plantlet during acclimatization stage

Plantlets of date palm cv. Barhee 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.

2.8 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 Snedecor and Cochran (1982).

3 Results and Discussions

3.1 Establishment stage

3.1.1 Sterilization procedure experiment

3.1.1.1 Effect of different silver and chitosan nanoparticles concentration on contamination percentages, browning degree & survival percentage of date palm cv. Barhee immature inflorescences

Data offered in **Table 1** exhibit, the effect of different silver & chitosan nanoparticles concentration on total contamination percentages, browning degree and survival percentage of date palm cv. Barhee immature inflorescences. Results showed that the lowest significant total contamination percentage with nano silver particles at 100,200 mg/l, and nano chitosan at 150, 200 mg/l without significant differences among them. On the other hand, the highest values were occurred with commercial bleach clorox at 30% and ethanol 70% nano silver particles at 50 mg/l, chitosan at 50 mg/l, clorox & ethanol with MC at 200mg/l. As for the effect of silver and chitosan nano particles on browning degree, results indicated that the lowest degrees of browning were noticed with nano silver at 50 mg/l. The highest survival percentage was noticed with silver nano particles at 200 mg/l and nano chitosan particles at 100, 150& 200 mg/l, without significant differences.

Data presented in **Table 2** showed that, the effect of auxin type, concentration, silver and chitosan nanoparticles added to MS medium on contamination percentage, browning degree and survival percentage, results refers that the lowest value of contamination % occurs with added silver nanoparticles at 4.0mg/l + NAA at 100.0 mg/l. moreover, the lowest value of browning degree with treatment 1 mg/l silver nanoparticles with 2,4-D 5.0 mg/l and 3.0 mg/l silver nanoparticles with 2,4-D 50.0mg/l and 1 mg/l nano chitosan with NAA at 5 mg/l. On the contrary, the highest total ontamination was achieved with MS free hormones without nanoparticles.

The highest survival percentage was occurred with 4 mg/l N.S. + NAA 100.0mg/l and 4 mg/l N.chito + 2,4-D 100.0 mg/l, without any significant differences among them.

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 Jo et al (2009).

The results gained from this study are consistent with Kamaran Safavi (2012) 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. Antibiotics have been extensively tested for their ability to inhibit or prevent the growth of bacteria in plant *in vitro* cultures. However, using of antibiotics has confirmed limitations. For example, antibiotics are expensive; their range of activity against kinds of bacteria is often narrow, usually are heat-labile, phytotoxic and only effective against bacteria and not fungi or do it another way, capable of altering the behavior of cultured plant tissues by default and inhibition of plant growth.

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 (Richards et al 1984) and interaction with hydrogen bonding processes had been demonstrated to take place (Russell & Hugo 1994). As specific surface area of nanoparticles is increased, their biological effectiveness can be increased due to the increase in surface energy (Willems 2005). Also, Rostami and Shahsavar (2009) recommended adding low concentration of nano silver particles to *in vitro* media culture of woody plant such as Olive cv. Mission.

Table 1. Effect of different silver and chitosan nanoparticles concentration on contamination percentages, browning degree & Survival percentage of date palm cv. Barhee immature inflorescences

Treatments (mg/l)	contamination %	Browning degree	Survival %
Nano silver at 50.0	60.0 AB	8.0 C	40.0 C
Nano silver at 100.0	32.0 C	16.0 B	68.0 B
Nano silver at 200.0	24.0 C	24.0 AB	76.0 A
Nano chitosan at 50.0	72.0 A	20.0 B	28.0 D
Nano chitosan at 100.0	48.0 B	28.0 A	52.0 C
Nano chitosan at 150.0	29.3 C	28.0 A	70.7 AB
Nano chitosan at 200.0	20.0 C	28.0 A	80.0 A
Commercial bleach clorox at 30% and ethanol 70%	72.0 A	32.0 A	28.0 D
commercial bleach clorox and ethanol with MC at 200.0	64.0 AB	32.0 A	36.0 CD

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

Table 2. Effect of auxin type, concentration, silver, and chitosan nanoparticles concentration added to MS medium on contamination percentage, browning degree & survival percentage of date palm cv. Barhee immature inflorescences

Treatments (mg/l)	Contamination %			Browning degree	Survival %
	Fungal %	Bacterial %	Total contamination %		
N.S at 1.0 + 2,4-D at 5	24.0 BCD	24.0 B	48.0 C	8.0 C	52.0 C
N.S. at 2.0 + 2,4-D at 10	12.0 CD	12.0 CD	24.0 D	16.0 B	76.0 A
N.S. at 3.0 + 2,4-D at 50	12.0 CD	12.0 CD	24.0 D	8.0 C	76.0 A
N.S. at 4.0 + 2,4-D at 100	12.0 CD	12.0 CD	24.0 D	16.0 B	76.0A
N.chito. at 1.0 +NAA at 5.0	36.0 AB	28.0 B	64.0 B	8.0 C	36.0 D
N.chito at 2.0+NAA at 10	24.0 BCD	28.0 B	52.0 C	12.0 BC	48.0 C
N.chito. at3.0+NAA at 50	16.0 BCD	24.0 B	40.0 C	24.0 A	60.0 B
N.chito.at 4.0+NAA at 100	12.0 CD	16.0 C	28.0 D	28.0 A	72.0 A
N.S. at 1.0 + NAA at 5	20.0BCD	20.0 BC	40.0 C	12.0 BC	60.0 B
N.S. at 2.0 + NAA at 10	12.0CD	16.0 C	28.0 D	12.0 BC	72.0 A
N.S. at 3.0 + NAA at 50	12.0 CD	12.0 CD	24.0 D	24.0 A	75.0 A
N.S. at 4.0 + NAA 100	8.0 D	8.0 D	16.0 E	28.0 A	84.0 A
N.chito. at 1.0 +2,4-D at 5	32.0 BC	20.0 C	52.0 C	12.0 BC	48.0 C
N.chito. at 2.0 +2,4-D at 10	12.0 CD	16.0 C	28.0 D	12.0 BC	72.0 A
N.chito.at 3.0 .+2,4-D at 50	12.0 CD	12.0 CD	24.0 D	24.0 A	75.0 A
N.chito. at 4.0 +2,4-Dat 100	12.0 CD	8.0 D	20.0 D	28.0 A	80.0 A
MS free hormones without nano particles	44.0 A	52.00 A	96.0 A	16.0 B	4.0 E

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

The results in this study referred that using 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 Kamaran Safavi et al (2011) 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.

3.1.1.2 Effect of auxin type & concentration on callus formation percentage and callus size of date palm cv. Barhee immature inflorescences during establishment stage

Data presented in **Table 3** showed, impact of auxin type & concentration on callus formation percentage and callus size of date palm cv. Barhee immature inflorescences during initiation stage. The highest percentages of callus formation were registered (80.0 & 72.0) with treatments of picloram at 8 and 6mg/l, respectively. The highest value of callus size (2.8 & 2.6) was noticed with picloram at 8 &6 mg/l, respectively. The lowest values of callus formation percentage were recorded with picloram at 2.0 mg/l (36.0), 2,4-D at 4.0 mg/l (24.0) & with NAA at 4.0, 10.0 mg/l (32.0) without significant differences among them.

Table 3. Effect of auxin type & concentration on callus formation percentage and callus size of date palm cv. Barhee immature inflorescences during initiation stage

Treatments (mg/l)	Callus formation %	Callus size
Picloram at 2.0	36.0 CD	1.8 BCD
Picloram at 4.0	56.0 B	1.6 CD
Picloram at 6.0	72.0 AB	2.6AB
Picloram at 8.0	80.00 A	2.8 A
2,4-D at 4.0	24.0 D	1.2 D
2,4-D at 10.0	40.0 C	2.2 ABC
2,4-D at 15.0	44.0 C	2.4 ABC
2,4-D at 25.0	44.0 C	2.6 AB
NAA at 4.0	32.0 D	1.2 D
NAA at 10.0	32.0 D	1.6CD
NAA at 20.0	56.0 B	2.4 ABC

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

3.1.1.3 Effect of auxin and cytokinins concentration on callus weight, callus size& browning degree of date palm cv. Barhee immature inflorescences during callus proliferation stage

Data in **Table 4** pointed out; the highest value of callus weight (9.2) and highest value of callus size (4.0) were occurred with NAA at 10.0 mg/l +2ip at 6 mg/l + kin at 6 mg/l. On a contrary, the lowest values of callus weight and callus size were observed with NAA at 5.0mg/l + 2ip at 6.0mg/l + kin at 3.0mg/l. The lowest browning degree (1.0) occurred with NAA at 5.0mg/l+2ip at 3.0 mg/l+ kin at 3.0mg/l, NAA at 5.0mg/l+2ip at 6.0 mg/l+ kin at 0.0 mg/l and NAA at 5.0mg/l+2ip at 6.0 mg/l+ kin at 6.0 mg/l without significant differences among them.

Many reports showed that, the combination of auxin like NAA and cytokinins has a significantily 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 & environmental signal play a key role in somatic embryogenesis in different plant species (Rao 1996; Dodeman et al 1997; Feher 2006). Kurup (2014). 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. Junaid et al (2009) 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 (Mazri et al 2017).

When Malht et al (2019) 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.

3.2 Multiplication Stage

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

In **Table 5** data indicated, the highest number of shoot / culture (7.4, 7.2 & 6.4) was occurred with MS medium supplemented by Fe nano particles at 20.85 mg/l, MS medium supplemented by Fe nano particles 27.8mg/l, and MS supplemented Zn nano particles at 4.3mg/l in the first subculture without any significant differences among them. 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.

Table 4. Effect of auxin and cytokinins concentration on callus weight, callus size& browning degree of date palm cv. Barhee 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	3.9 C	1.6 DE	1.0 C
NAA at 5.0+2ip 6.0+kin 0.0	4.0 C	2.2 CD	1.0 C
NAA at 5.0+2ip 6.0 +kin 6.0	6.2 B	2.8 BC	1.0 C
NAA at 5.0+2ip 6.0+kin 3.0	3.1 C	1.4 E	2.0 AB
NAA at 10.0+2ip 3.0+kin 3.0	4.1 C	1.8 DE	2.2 A
NAA at 10.0+2ip 6.0+kin 0.0	4.4 C	2.0 DE	1.6 ABC
NAA at 10.0+2ip 6.0 +kin 3.0	6.1 B	3.2 B	1.4 BC
NAA at 10.0+2ip 6.0+kin 6.0	9.2 A	4.0 A	1.6 ABC
NAA at 20.0+2ip 3.0+kin 3.0	6.3 B	3.2 B	1.6 ABC

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

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 cv. Barhee callus culture during shoot formation stage

Treatments (mg/l)	N. of shoots /culture			Average shoot length (cm)		
	1st sub.	2nd sub.	3rd sub.	1st sub.	2nd sub.	3rd sub.
MS medium	3.4 D	3.4 C	2.6 D	3.56 CD	3.00 D	3.50 BC
MS + Fe N.Ps at 6.95	3.6 D	4.0 BC	3.2 CD	3.70 CD	3.80 CD	3.40 BC
MS+ Fe N.Psat13.9	5.2 BC	5.0 B	4.2 BC	4.60 BC	4.84 ABC	4.30 ABC
MS+ Fe N.Ps at 20.85	7.4 A	6.8 A	4.8 AB	6.26 A	5.42 AB	4.66 ABC
MS+ Fe N.ps at 27.8	7.2 A	7.8 A	5.8 A	6.28 A	6.10 A	5.80 A
MS+ Zn N.Ps at 2.15	3.8 CD	4.2 BC	3.0 CD	3.80 CD	4.20 BCD	3.20 C
MS+Zn N.Ps at 4.3	6.4 AB	6.8 A	5.0 AB	5.88 AB	6.06 A	4.80 AB
MS+Zn N.Ps at 6.45	3.0 D	4.2 BC	3.2 CD	3.00 D	4.20 BCD	3.40 BC
MS+ Zn N.ps at 8.6,	3.4 D	4.2 BC	3.2 CD	3.86 CD	4.20 BCD	3.80 BC

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

Data in **Table 5** illustrated that, the highest average shoot length (cm) (6.28, 6.26 & 5.88) was obtained with MS medium supplemented by Fe nano particles at 27.85 mg/l, MS medium supplemented by Fe nano particles at 20.8 mg/l, and MS supplemented Zn nano particles at 4.3 mg/lin the first subculture without any significant differences among them. 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 & 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 (Helaly et al 2014). Silver nanoparticles, BAP at 40 mg/l and IAA at 20 mg/l gave the highest % of explants per shoots and highest mean number & length of shoots per explants of *Tecomella undulate* (Roxb) Aghdaei et al (2012).

Zaho et al *(*2014) 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.

3.2.2 Effect of auxin & cytokinins concentration on number of shoot per culture of date palm Barhee callus culture during shoot multiplication stage

Effect of auxin concentration, the highest significant number of shoots/culture (4.24, 4.08 and 3.44) were achieved by NAA at 4.0 mg/l in the 1st, 2nd and 3rd 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 4.0 mg/l. On a contrary, control treatment (0.0 mg/l) gave the lowest values during the three subcultures. The interaction between cytokinin & auxin concentration, the highest number of shoots / culture (6.60 & 5.60) (6.40, 5.20) and (5.20, 4.80) 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 (1.0 j) was occurred with NAA at 0.0mg/l, with 2ip & kin at 0.5mg/l of first subculture.

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

Effect of auxin concentration, data in **Table 7**. Pointed that, the highest average shoot length (cm) (6.1, 5.2 and 5.3) was occurred with auxin treatment by NAA at4.0 mg/l in the 1st, 2nd & 3rd subcultures. The lowest average shoot length (cm) (3.50, 2.7, 2.8) was recorded with NAA at 0.0 mg/l respectively, without any significant differences among them in the 1st, 2nd and 3rd subcultures.

3.2.4 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) (6.2 and 6.6) 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) (2.2, 2.0 and 1.8) 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 (Jain et al 2011). The significantly effective of cytokinins on *in vitro* mature inflorescences was well-celebrated and-comprehended in the literature (Wang et al 2001). 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 (Hee et al 2007).

The previous results pointed that, the use of 2iP at 1.5 mg/l, BAP at 1 mg/l and NAA at 1 mg/l give the highest mean number of shoot per explant and highest mean shoot length of date plam cv. Barhee (Jazinizadeh et al 2015). Similarly, Masmoudi-Allouche et al (2010) 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 (Al-khateeb 2006) 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 Zaid et al (2006) and Aaouine (2000). In a similar way, Khan and Tabassum (2012) 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

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

Cytokinins conc.(mg/l) / Auxin conc.	Number of shoots/culture																				
	1St subculture						2nd subculture						3rd subculture								
	2ip + Kin						2ip + Kin						2ip + Kin								
	0	0.5	1	2	4	Mean	0	0.5	1	2	4	Mean	0	0.5	1	2	4	Mean
0.0mg/l NAA	1.20	1.00	1.60	1.40	2.00	**1.56**	1.40	1.60	1.20	1.60	2.40	**1.84**	1.20	1.00	1.40	1.40	2.00	**1.40**
	ij	j	hij	ij	ghij	**C**	gh	gh	h	gh	efgh	**C**	g	g	fg	fg	efg	**C**
0.5mg/l NAA	1.20	1.80	2.00	1.60	2.40	**1.88**	1.40	1.40	2.40	2.00	2.60	**2.08**	1.00	1.60	2.00	2.00	2.00	**1.56**
	ij	hij	ghij	hij	ghi	**C**	gh	gh	efgh	fgh	defg	**C**	g	fg	efg	efg	efg	**C**
1 mg/l NAA	1.40	1.60	2.80	2.80	4.60	**2.68**	1.60	1.40	2.600	2.600	3.800	**2.68**	1.40	1.20	2.60	2.20	3.20	**2.36**
	ij	hij	fgh	fgh	bcd	**B**	gh	gh	defg	defg	cd	**B**	fg	g	def	efg	cde	**B**
2 mg/l NAA	1.80	2.20	3.20	4.20	**6.60**	**3.08**	2.40	2.60	3.20	3.80	**6.40**	**3.04**	1.60	1.80	2.20	4.60	**5.20**	**2.92**
	hij	ghij	efg	cde	**a**	**B**	efgh	defg	cdef	cd	**a**	**B**	fg	fg	efg	ab	**a**	**A**
4 mg/l NAA	2.20	2.80	3.80	5.40	5.60	**4.24**	2.40	3.40	4.00	5.20	5.20	**4.08**	1.80	2.20	3.60	4.40	4.80	**3.44**
	ghij	fgh	def	abc	ab	**A**	efgh	cde	bc	ab	ab	**A**	fg	efg	bcd	abc	ab	**A**
Mean	**1.44**	**1.80**	**2.64**	**3.60**	**3.96**		**1.64**	**1.96**	**2.40**	**3.68**	**4.04**		**1.40**	**1.72**	**2.12**	**3.08**	**3.36**	
	C	**C**	**B**	**A**	**A**		**C**	**BC**	**B**	**A**	**A**		**C**	**BC**	**B**	**A**	**A**	

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

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

Cytokinins conc.(mg/l) / Auxin conc.	Average shoots length (cm)																			
	1St.subculture						2nd subculture						3rd subculture							
	2ip + Kin						2ip + Kin						2ip + Kin							
	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	2.800 gh	3.600 efgh	4.800 bcdef	5.580 abc	5.940 abc	**3.504 C**	2.200 fg	4.200 bcd	4.000 cd	4.000 cd	4.680 abcd	**2.776 C**	2.000 ij	2.800 fghij	3.60 cdefgh	4.600 bcde	4.900 abc	**2.880 D**
0.5 mg/l NAA	2.800 gh	4.320 cdefg	4.700 bcdef	4.800 bcdef	5.50 abcd	**3.704 C**	2.600 efg	2.400 efg	4.980 abc	4.200 bcd	5.140 abc	**3.232 C**	2.600 ghij	4.200 cdef	4.000 cdefg	4.600 bcde	4.660 bcd	**3.320 CD**
1 mg/l NAA	3.300 fgh	4.600 cdef	5.600 abc	4.800 bcdef	6.30 ab	**4.844 B**	2.000 g	3.560 de	4.420 abcd	4.500 abcd	5.600 a	**4.528 B**	2.200 hij	4.600 bcde	3.360 defghi	3.360 defghi	5.760 ab	**3.792 BC**
2 mg/l NAA	3.860 defgh	2.200 h	4.800 bcdef	4.900 bcdef	6.60 a	**5.056 B**	3.480 def	2.600 efg	4.600 abcd	4.400 abcd	5.460 ab	**4.300 B**	3.600 cdefgh	1.800 j	4.400 bcde	4.600 bcde	6.260 a	**4.312 B**
4 mg/l NAA	4.760 bcdef	3.800 efgh	4.320 cdefg	5.200 abcde	6.620 a	**6.192 A**	3.600 de	3.400 def	4.640 abcd	4.400 abcd	5.200 abc	**5.216 A**	4.000 cdefg	3.200 efghij	3.600 cdefgh	4.400 bcde	4.960 abc	**5.308 A**
Mean	**4.544 A**	**4.424 A**	**4.920 A**	**4.47 A**	**4.94 A**		**3.81 A**	**3.86 A**	**4.01 A**	**4.10 A**	**4.24 A**		**3.580 A**	**4.012 A**	**3.856 A**	**4.132 A**	**4.032 A**	

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

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).

Similarly results by Malht et al (2019) 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.

3.3 Rooting Stage

3.3.1 Effect of auxin type & concentration on rooting percentage, number of roots & root length (cm) of date palm cv. Barhee microshoots during rooting stage

In **Table 8** data illustrated, the highest rooting percentage (83.3) was recorded with MS medium supplemented by NAA at 0.5 mg/l. On the contrary, the lowest rooting percentage (33.3) was showed with MS medium with NAA at 0.2 mg/l and IBA at 1.0 mg/l. Moreover, the highest number of roots/ microshoots (4.7& 4.2) was occurred with MS medium with NAA 0.5 & 1.0mg/l, respectively. The highest values of root length (cm) (6.4& 5.9) were recorded with MS medium with IBA at 3.0 & 2.0 mg/l.

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 (Shaheen, 1990). 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 Bekheet (2013) 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 (Asemota et al 2007). On the other hand, Tissert (1984) 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. Helaly et al (2014) 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 (Jazinizadeh et al (2015). Mushtaque et al (2015) 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”. Elghayaty et al (2016) 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 Malht et al (2019) showed that, NAA 1.0 mg/L induced the highest rooting percentage & microshoots length of date palm cv. Sewi microshoots.

Table 8. Effect of auxin type & concentration on rooting %, number of roots and root length (cm) of date palm cv. Barhee 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	4.3 AB
NAA at 1.0	66.6 B	4.2 AB	4.0 AB
IBA at 1.0	33.3 D	2.2 C	4.3 AB
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.

3.4 Acclimatization stage

3.4.1 Effect of medium mixtures on survival percentageof date palm cv. Barhee plantlet during acclimatization stage

In **Table 9** data indicated that, the highest survival percentage (83 & 80) were occurred with medium mixtures sand: peat: vermiculite: perlite (1:2: 1:1) and medium mixtures sand: peat: vermiculite:

perlite (2: 1: 1: 1) respectively, without any significant differences between them of date palm cv. Barhee plantlet. On the contrary, the lowest survival percentage (21.33) was recorded with medium mixtures sand: peat: vermiculite: perlite (1: 1: 1:1) of date palm cv. Barhee plantlet.

Table 9. Effect of medium mixtures on survival % of date palm cv. Barhee plantlet during acclimatization stage

Medium mixture	Survival %
Sand : Peat : Vermiculite : perlite 1 : 1 : 1 : 1	21.33 C
Sand : Peat : Vermiculite : perlite 2 : 1 : 1: 1	80.0 A
Sand : Peat : Vermiculite : perlite 2 : 2 : 1: 1	33.3 B
Sand : Peat : Vermiculite : perlite 1: 2 : 1: 1	83.0 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 (Abul-Soad et al 1999; Hegazy and Abo shama 2010; Taha et al 2007).

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. Tissert (1984) 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. El-Sharabasy et al (2001) 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 (Mushtaque et al (2015) and Gabr and Abd-Alla (2010) 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. Al-Khayri (2010) 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 (Mazri et al 2016).

Also, Malht et al (2019). 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.

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تأثير جسيمات النانو على الأكثار المعملي لنخيل البلح صنف البارحي بواسطة النورات الزهرية الغير ناضجة

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الموجـــــــــز

فى دراسة لتأثير جسيمات الفضة والشيتوزان النانوميترية فى التعقيم وكذلك جزيئات الحديد والزنك النانوميترية على تكوين الكالس الناتج من زراعة الأغاريض الزهرية غير الناضجة فى مرحلة التأسيس لنخيل البلح صنف البارحي سواء التي تم غمس الأجزاء الزهرية فيها أو التي تم أضافتها الى البيئة الغذائية كانت كالتالي: سجلت أقل نسبة للتلوث الكلي وأعلى نسبة بقاء للمنفصلات مع المعاملة جسيمات الفضة النانوميترية بتركيز 200 ملجم/لتر، والمعاملة بجسيمات الشيتوزان النانوميترية بتركيزي 150 و 200 ملجم/لتر. كذلك سجلت أقل نسبة تلوث فى البيئة الغذائية عندما أضيف لها جسيمات الفضة النانوميترية بتركيز 4 ملجم/لترمع أضافة NAA بتركيز 100 ملجم/لتر وكذلك مع جسيمات الشيتوزان النانو ميترية بتركيز 4 ملجم/لتر وأضافة 2,4–D بتركيز 100 ملجم /لتر. سجلت أفضل نسبة لتكوين الكالس وحجم الكالس الناتج مع بيئة MS مضافا لها البكلورام بتركيز 8 ملجم/لتر. كما كان أفضل وزن وحجم للكالس الناتج مع أضافة NAA بتركيز 10 ملجم/لتر و Kin, 2ip بتركيزي 6 ملجم/لتر. فى مرحلة التضاعف: سجلت أعلى عدد للأفرع الناتجة / منفصل مع بيئة MS مضافا لها جسيمات الحديد النانوميترية بتركيز 20.8 ملجم/لتر ومع أضافة أيضا جسيمات الحديد النانوميترية بتركيز 27.8 ملجم/لتر ومع أضافة جسيمات الزنك النانوميترية بتركيز 4.3 ملجم/لتر الى بيئة MS. وذلك أثناء النقلة الأولي بدون أية فروق معنوية بينهم. بينما سجل أعلى متوسط لطول الأفرع مع بيئة MS مضافا لها جسيمات الحديد النانوميترية بتركيزي، 28.8، 20.8 ملجم/لتر وبيئة MS مضافا لها جسيمات الزنك النانوميترية بتركيز 4.3 ملجم/لتر. وذلك خلال النقلة الأولى بدون أية فروق معنوية بينهم. وكان التفاعل بين تركيزي السيتوكينينات والأوكسين حيث سجلت أعلى عدد للأفرع الناتجة/منفصل مع المعاملة NAA بتركيز 2 ملجم/لتر مع 2ip بتركيز 4 ملجم/لتر و Kin بتركيز 4 ملجم/لتر و 1 ذلك خلال النقلة الأولى والثانية خلال مرحلة التضاعف بدون أية فروق معنوية بينهما. سجلت أفضل نسبة تجذير وأفضل عدد جذور/نبات مع بيئة MS مضافا لها NAA بتركيز 0.5 ملجم/لتر بينما سجلت أعلى نسبة بقاء للنباتات خلال مرحلة الأقلمة مع مخلوط البيئة المكون من رمل: بيتموس: فيرموكليت: بيرليت (1:2:1:1) و (1: 1: 1: 2) على التوالي.