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 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 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- furfuryliminopurine. TDZ: Thidiazuron N-phenyl-N'-1,2,3-thiadiazol- 5-ylurea, BAP: Benzyl amino Purine, NOA: Napthoxy acetic acid, TiO: titanium Oxide, PVP: Polyvinylpyrrolidine, 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 Areccacea 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 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.

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.
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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 100 mg/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 charcoal at 1 g/l were used and all types of solid media which used in this study were solidified with purified agar-agar at 7 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 twenty minutes, then the media left 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 100 mg/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 4 mg/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 inhibit polyphenol oxidation which is activated 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 + 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.

2.4.2 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 charcoal 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 charcoal at 1 g/l, supplemented with Picloram 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 (2-3 cm) were cultured on MS medium salts at full strength used supplemented with vitamins, Inositol at 100 mg/l, glutamine at 200 mg/l, adenine at 100 mg/l, citric acid at 150 mg/l, a scorbic acid at 150 mg/l, sucrose at 30 g/l, and PVP at 2 g/l, nanoparticles 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 condition 16 hour for three re-cultures. Numbers of shoots and average shoot length (cm) /culture were listed every six weeks for three sub-cultures. This experiment contained 1 auxin type’s × 5 concentration storage there experiment in 2 cytokinin type × 5 concentrations through three sub-culture. 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 3rd 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/microshoot & 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 different 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.
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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 nanoparticles 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 contamination 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 Khamran Safavi (2012) who indicated that, nano silver and Titanium oxide (TiO2) had a good potential for removing the bacterial contamination in plant tissue culture procedures of potato (Solanum tuberousum 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 Shamsavar (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     | 72.0 A            | 32.0 A          | 28.0 D     |
| chitosan at 70% 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)                        | Fungal %          | Bacterial %      | Total contamination % | Browning degree | Survival % |
|-----------------------------------------|-------------------|------------------|-----------------------|-----------------|------------|
| 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                | 12.0 BC         | 48.0 C     |
| 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                | 40.0 C          | 60.0 B     |
| N.chito. at 3.0+NAA at 50               | 16.0 BCD          | 24.0 B           | 52.0 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            | 12.0 BC               | 8.0 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-D at 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 value 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.

| 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            | 2.6 AB     |
| 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    |
| NAA at 4.0         | 32.0 D             | 1.2 D      |
| NAA at 10.0        | 32.0 D             | 1.6 CD     |
| 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 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-di-chlorophenoxy acetic acid (2,4-D), naphthalene acetic acid (NAA), Picloram, Dicamba, 2,4,5-trichlorophenoxy 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 ‘Za-dai’ & ‘Muzati’ on MS medium add up to 2,4-D at 1.5 mg l. The active concentration, however, varied in ranged 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 6.0 | 6.1 B | 3.2 B | 1.4 BC |
| NAA at 10.0+2ip 6.0+kin 3.0 | 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) |
|------------------|-----------------------|--------------------------|
|                  | 1<sup>st</sup> sub. | 2<sup>nd</sup> sub. | 3<sup>rd</sup> sub. | 1<sup>st</sup> sub. | 2<sup>nd</sup> sub. | 3<sup>rd</sup> 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 nanoparticles 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/l for 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 *Teconomella 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 enhanced the shoot growth and length of *Vigna 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 0.5 mg/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 (4.60 & 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 observed 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 at 4.0 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 gave the highest mean number of shoot per explant and highest mean shoot length of date palm 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 conjuction 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 |
|------------------------|-------------|---------------------------|
|                        | 0.5 mg/l NAA | 1 mg/l NAA | 4 mg/l NAA |
|                        | 2 ip + Kin  | 2 ip + Kin  | 2 ip + Kin  |
|                        | 0           | 0.5         | 1           |
|                        | 1           | 1.44        | 1.96        |
|                        | 2           | 2.64        | 3.40        |
|                        | 4           | 3.96        | 4.04        |
| Mean                   | C           | B           | 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) | 1st subculture | 2nd subculture | 3rd subculture |
|------------------------|----------------|----------------|----------------|
|                        | 2ip + Kin      | 2ip + Kin      | 2ip + Kin      |
|                        | 0 0.5 1 2 4    | 0 0.5 1 2 4    | 0 0.5 1 2 4    |
| 0.0 mg/l NAA           | 2.800 3.600 4.800 5.580 5.940 3.504 | 2.000 4.000 4.800 4.680 2.776 | 2.000 3.600 4.600 4.900 2.880 |
| gh efgh bcd ef abc abc C | fg bcd cd cd abcd C | ij fghj cdefgh bcde abc | D |
| 0.5 mg/l NAA           | 2.800 4.320 4.700 4.800 5.50 3.704 | 2.000 4.000 5.140 3.323 | 2.000 4.000 4.600 4.660 3.320 |
| gh cdefg bcd ef abcd C | efg abc bcd abcd C | ghij cdef cdefg bcde bc | CD |
| 1 mg/l NAA             | 3.300 4.600 5.600 4.800 6.30 4.844 | 2.000 3.560 4.500 4.500 3.560 4.47 | 2.000 3.600 3.360 3.600 3.792 |
| fgh cdef bcd ef abc B | g de abcd abcd a B | hij bcde deghi deghi a | BC |
| 2 mg/l NAA             | 3.860 2.200 4.800 4.900 6.60 5.056 | 3.480 2.600 4.000 4.600 4.600 4.930 | 3.600 1.800 3.600 3.600 6.260 4.312 |
| delgh h bcd ef abcde a B | def efg abcd ab ab B | cdefgh j bcd a | B |
| 4 mg/l NAA             | 4.760 3.800 4.320 5.200 6.620 6.192 | 3.600 3.400 4.400 4.640 4.500 5.216 | 4.000 3.200 3.600 4.400 4.960 5.308 |
| bcd efgh cdefg abcde a A | de def abcd abcd ab abc A | cdefg efghij cdefgh bcde abc | A |
| Mean                   | 4.544 4.424 4.920 4.47 4.94 4.81 | 3.81 3.86 4.01 4.10 4.24 3.580 | 4.012 3.856 4.132 4.032 4.032 |
|                        | A A A A A A | A A A A A A | A A A A A 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 plantlets. Bekheet (2013) he explained that, 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 charchoal (AC), in Pakistani date palm cultivars “Gajar”, “Kashho-wari”, 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 percentage of 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 (12:1:1) and medium mixtures sand: peat: vermiculite: perlite (12:1:1)
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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 1 : 1 : 1       | 80.0 A     |
| Sand : Peat : Vermiculite : perlite 2 : 2 : 1 : 1   | 33.3 B     |
| Sand : Peat : Vermiculite : perlite 2 : 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: 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

باستخدام النورات الزهرية الغير ناضجة

أبو ظبي

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الموجز

في دراسة لتأثير جسيمات الفضة والشيتوزان والكروموميترية في التعقيم وكذلك جزيئات الحديد والزنك النانوميترية على تكوين الكالس النباتي من زراعة الأغراض الزهرية غير الناضجة في مرحلة التأسيس لنخيل البلح صنف البارحي سواء التي تم غمس الأجزاء الزهرية فيها أو التي تم أضافتها إلى البيئة الغذائية كانت كالتالي: سجلت أقل نسبة للتلوث الكلي وأعلى نسبة بقاء للمنفصلات مع بيئة MS مضافا لها جسيمات الفضة النانوميترية بتركيز 20 ملم/لتر ومع أضافتها أيضا جسيمات الحديد النانوميترية بتركيز 27.8 ملم/لتر ومع أضافتها جسيمات الزنك النانوميترية بتركيز 4.3 ملم/لتر. وذلك خلال النقلة الأولى دون أي فرق معنوي بينهم. أما التجربة الثانية فشملت إضافة 2ip بتركيز 4 ملم/لتر مع NAA بتركيز 2 ملم/لتر و Kin بتركيز 2 ملم/لتر و NAA BAP بتركيز 2 ملم/لتر مع NAA بتركيز 2 ملم/لتر. سجلت أعلى نسبة للتلوث الكلي وأعلى نسبة بقاء للمنفصلات مع بيئة MS مضافا لها جسيمات الفضة النانوميترية بتركيز 20 ملم/لتر ومع أضافتها أيضا جسيمات الحديد النانوميترية بتركيز 27.8 ملم/لتر ومع أضافتها جسيمات الزنك النانوميترية بتركيز 4.3 ملم/لتر. وظلت هذه النتائج مرفوعة وتظل مستقية حتى النقلة الثانية.

نسبة تجذير الأوراق: سجلت أعلى نسبة في بيئة MS مضافا لها NAA BAP بتركيز 2 ملم/لتر مع Kin بتركيز 2 ملم/لتر. سجلت نسبة تجذير الأوراق 87.5% ونسبة بقاء الأوراق 95%.

نسبة تجذير الأوراق: سجلت أعلى نسبة في بيئة MS مضافا لها NAA BAP بتركيز 2 ملم/لتر مع Kin BAP بتركيز 2 ملم/لتر. سجلت نسبة تجذير الأوراق 87.5% ونسبة بقاء الأوراق 95%.

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نسبة تجذير الأوراق: سجلت أعلى نسبة في بيئة MS مضافا لها NAA BAP بتركيز 2 ملم/لتر مع Kin BAP بتركيز 2 ملم/لتر. سجلت نسبة تجذير الأوراق 87.5% ونسبة بقاء الأوراق 95%.

نسبة تجذير الأوراق: سجلت أعلى نسبة في بيئة MS مضافا لها NAA BAP بتركيز 2 ملم/لتر مع Kin BAP بتركيز 2 ملم/لتر. سجلت نسبة تجذير الأوراق 87.5% ونسبة بقاء الأوراق 95%.

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