International Journal of Environment, Agriculture and Biotechnology
Vol-6, Issue-6; Nov-Dec, 2021

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Impact of silver nanoparticles on enhancing*in vitro* proliferation of embryogenic callus and somatic embryos regeneration of Date palm cv. Hayani

Sayed, A. A. Elsayh

Department of Breeding, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt

Received: 25 Sep 2021; Received in revised form: 30 Oct 2021; Accepted: 07 Nov 2021; Available online: 12 Nov 2021

Abstract**—***The growth and development of in vitro plants are aided by silver nanoparticles. The Phoenix dactylifera is one in all the economically important fruit crops in many Arab countries. During this study, the various concentrations of Ag NPs (0, 0.1, 0.5, 1.0, 2.0, and 4.0 ml/l) were added to MS basal medium to judge their effects on the embryogenic callus proliferation, differentiation, and development, regeneration of somatic embryos of feather palm Hayani cv. to check the consequences of Ag NPs, research with two separate experiments was conducted. Within the first experiment, MS basal salt medium containing 3.0 mg/l 2,4-D, 1.0 mg/l 2ip (mg/l), and different concentrations of Ag NPs were wont to determinate the embryogenic callus proliferation under in vitro conditions. While within the second experiment, the effect of MS basal medium supplemented with 0.05 NAA, 0.1 BA (mg/l), and different concentrations of Ag NPs were examined on regeneration of somatic embryos. Results of the primary experiment indicated that various concentrations of Ag NPs had a significantly affected on the embryogenic callus proliferation and substantially increased somatic embryos formation on the callus when added Ag NPs at 1.0 ml/l in MS basal medium. Within the second experiment, the appropriate medium for regeneration of somatic embryos was added 1.0 ml/l Ag NPs intothe medium which had a positive effect on the number of somatic embryos and registered to the utmost number of somatic embryos 35.30 embryo/jar with the best length of embryos 1.80 cm, the best number of leaves 43.72 leaf/jar and also the highest length 3.87 cm. During this treatment, the full chlorophyll content was 2.584 mg/g. Further, higher Ag NPs concentrations had negative effects. There was genetic stability between shoots sample which exposure with 1.0 Ag NPs and therefore the mother plant.*

Keywords**— Date palm, Ag NPs, In vitro, Embryogenic callus, Somatic embryos, Chlorophyll content and RAPD (PCR) Molecular Marker.**

I. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) could be a monocotyledonous and dioecious species that belongs to the Arecaceae family and is recognized because the most vital fruit tree in many Arab countries; like Saudi Arabia and Iraq.

A feather palm tree is one among the traditional fruit crops cultivated in North Africa and therefore the Middle East **(Masmoudi-Allouche*et al.*, 2011)**. In addition, it's a multi-purpose tree that's utilized in ornamental and landscape designs; Also, different parts of the tree such as; seeds, leaves, and trunk even have other uses that bring extra profit to this point palm growers **(Al-Khalifah and Shanavaskhan, 2012)**. The date palm tree has a necessary role within the improvement of sustainable agriculture in several drought and salinity damaged regions**(Khierallah and Hussein, 2013)**.

Commonly, a feather palm tree is propagated via seeds or offshoots, but plantlets produced from seeds aren't identical and have less quality than the mother tree. While propagation using offshoots is that the best method, the number of offshoots produced by the mother tree is

proscribed especially in rare cultivars. The survival rate of offshoots is low and a high chance of infection attributed to the abundance of pests happens. Thus to beat these problems and produce a high number of plants freed from disease, it's necessary to develop another method of propagation, like the employment of plant structure culture technique**(Eshraghi*et al*., 2005)**.

One of these options is micro-propagation, which **is that the** true-to-type propagation of a genotype using *in vitro* culture procedures **(Al-Khalifah and Shanavaskhan, 2012)**. The tissue culture technique has numerous advantages, and plants have proven to be popular among farmers. These benefits include the ability to supply disease and pest-free cultivars of plants with desired qualities, large-scale multiplication of an outsized number of plantlets at any time of year, the ability to propagate elite cultivars that lack offshoots and produce seed only derived plants. Additionally to, avoiding the extent of plant quarantine regulations by facilitation of the exchange of plant materials between laboratories for research purposes without the chance of disease or pest transmission**(Johnson, 2011)**.*In vitro* date palm plantlets are produced by either somatic embryogenesis or organogenesis **(Mazri and Meziani, 2015)**. The *in vitro* pathway of somatic embryogenesis involved the induction of embryogenic callus which differentiation to somatic embryogenesis **(Al-Samir, 2015)**. Somatic embryogenesis is that the best date palm micro-propagation regeneration process. **(Fki*et al*., 2003)**. It's noted to be a swift and economical way for extensive propagation of *Phoenix dactylifera* and will even be extremely suitable for breeding programs **(El-Hadrami*et al*., 1998)**. Lots of studies are conducted to enhance the somatic embryogenesis of date palm by altering substance components and physical conditions **(Al-Khairy and Al-Bahrany, 2012; Baharan*et al*., 2015)**. Several previous studies of *Phoenix dactylifera* micro-propagation using the callus and somatic embryo pathways are published. **(McCubbin*et al*., 2000; Fki*et al*., 2011)**. On the opposite hand, Apical meristem tissues from axillary and lateral offshoots are now used because the widely accepted source of explants for date palm tissue culture after showing promising results **(Al-Khalifah*et al*., 2013; Hoffmann *et al*., 2013)**. In general, the development of cultured cells *in vitro* is especially dependent on the nutritional components and plant growth regulators **(Lima *et al.*, 2012)**.

Nanotechnology may be a technology applied at the nano-scale, involved the study of too small materials and its application among many other science fields, like chemistry, physics, biology, engineering, and agriculture. The worth of agricultural products is increased through the utilization of Nanotechnology and helps reduce environmental problems. Nanoparticles and powders have big reactivity because of extended specific surface area; these characteristics make the absorption of fertilizers that produced in nano-scale easier**(Mousavi and Rezaei, 2011)**.Present day, nanotechnology has introduced new valuable components and substances that help progress in ecological and environmental researches **(Miller and Senjan, 2006)**.Tiny metal-based nanoparticles (NPs) of 1–100 nm in size and is extremely low quantity has been tested as substitute plant mineral nutrients and stimulants due to the fast development of nanotechnology **(Saxena*et al*., 2016)**.

Several experiments have examined the influence of different nanoparticles on a number of commercially valuable plant species **(Monica and Cremonini, 2009; Krishnaraj*et al*., 2012)**.The main advantage of using nanoparticles in the field of biotechnology is focused on antimicrobial and nutrient properties and improvement of plant growth with different pathways. As date palm is an important traditional crop, many efforts have been made to provide higher yields and quality. In-plant tissue culture, there are promising indications of the utility of nano-materials to improve plant nutrition, plant growth, and development, seed germination, enhance plant growth and yield; enable plant genetic modification, improve bioactive compound production and achieve plant protection and tolerance to diseases**(Wang *et al*., 2016)**.

Silver Nanoparticles (Ag NPs) are a non-toxic substance with strong antimicrobial properties against fungi, bacteria, and viruses **(Abdi *et al*., 2008)**. The use of Ag NPs has grown in recent years as nanotechnology has advanced **(Luoma, 2008)**. *Brassica juncea*, common bean and corn plants increased their growth processes (shoot length, root length and leaf area) as well as biochemical parameters (chlorophyll, starch, protein content and antioxidant enzymes) **(Salama, 2012; Sharma *et al*., 2012)**. Moreover, the inclusion of Ag NPs in the plant tissue culture medium for the treatment of microbial contamination did not adversely affect shoot multiplication and subsequent rooting **(Gouran*et al.* 2014)**, on the contrary it proved its efficacy in different plant species **(Kumar *et al.*, 2007)**.

The chlorophyll content may be a physiological characteristic that may affect plantlet quality, survival, growth, and development after transplantation. Chlorophyll could be a photoreceptor, an indicator of the photosynthetic potential of plants, and a catalyst for the conversion of sunlight into energy. It plays a significant role within the photochemical synthesis of carbohydrates **(Oliveira *et al*., 2016)**.

The aim of this study was to see how Ag NPs affected the growth and proliferation of embryogenic callus. Also,study the added impact of Ag NPs to medium on improvement and development of somatic embryos of date palm.

II. MATERIALS AND METHODS

This study was carried out in the Department of Breeding, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt during 2018-2020.

2.1Plant material

The healthy offshoots of 5–7 kg in weight and 50–70 cm in length from date palm cv. Hayani were selected and detached from healthy, disease-free mother plants. The offshoots were carefully removed; the leaves were cut using a sharp knife until the shoot tip zone was exposed (apical meristems with leaf primordia). To avoid browning, the explants were washed in flowing tap water for 1 hour before being soaked in an antioxidant solution containing 150 mg/l ascorbic acid and 100 mg/l citric acid for 30 minutes.

2.2 Surface sterilization the explants

Surface sterilization was carried out to eliminate contamination agents. For sterilization under a sterile hood, the explants were removed from the antioxidant solution, then dipped in 70% ethanol for 1 minute and then surface sterilized with 0.1 mg/l $HgCl_2$ containing few drops of Tween-20 with continuous stirring for 75 minutes followed by washing with sterile distilled water 3 times and remove 1–2 leaves. After sterilization, the date palm shoot tips were divided longitudinally into eight segments and cultured on callus induction medium.

2.3 Culture medium

The culture medium used for *in vitro* cultures was the basal salts MS **(Murashige and Skooge, 1962)** supplemented with $Na_2H_2PO_4$ (170.0 mg/l), myo-inositol (100.0 mg/l), glutamine (200.0 mg/l), nicotinic acid (0.5 mg/l), pyridoxine-HCl (0.5 mg/l), thiamine (1.0 mg/l), 40 mg/l adenine sulphate, sucrose (40 g/l), agar (6 g/l), 0.5 g/l of activated charcoal. The pH of the medium was adjusted to 5.7, after that the media was dispensed into small jars (150 ml) in aliquots of 40 ml per jar and capped with polypropylene closures. Subsequently the media were autoclaved at 121 °C and 1.04 kg/cm^2 for 15 min.

2.4 Callus initiation

For callus culture initiation, the pieces of shoot tips were cultured into callus induction medium composed of basal salts MS with additional 10.0 mg/l Di-chlorophenoxy acetic acid (2,4-D) and 3.0 mg/l Isopentenyl adenine (2ip) **(Bekheet*et al.*, 2007)**. All cultures were incubated in a culture room under darkness at 27 ± 2 °C until initiation of white soft callus. The shoot tip explants were subcultures on the same medium and growth conditions every 4 weeks for 4 subcultures.

The primary callus was transferred on the same basal medium with added 5.0 mg/l 2,4-D and 2.0 mg/l 2ip, more callus growth occurs that can be easily separated from the original explants, after three subcultures with four weeks interval induced the embryogenic callus.

Isolate callus growth from original explants and transfer to callus multiplication medium which containing 3.0 mg/l 2,4-D and 1.0 mg/l 2ip and maintain in darkness until desired amount of callus.

2.5 Explant material and treatments used in this experiment:

2.5-1 Ag NPs characterization

Spherical silver nano-powder was purchased from Sigma-Aldrich Corporation. The particle size of Ag NPs was 10 nm, 0.02 mg/ml in aqueous buffer, and sodium citrate was used as a stabilizer, according to the manufacturer. Molecular weight at 107.87 and the pack size at 25 ml **(Fig. 1)**.

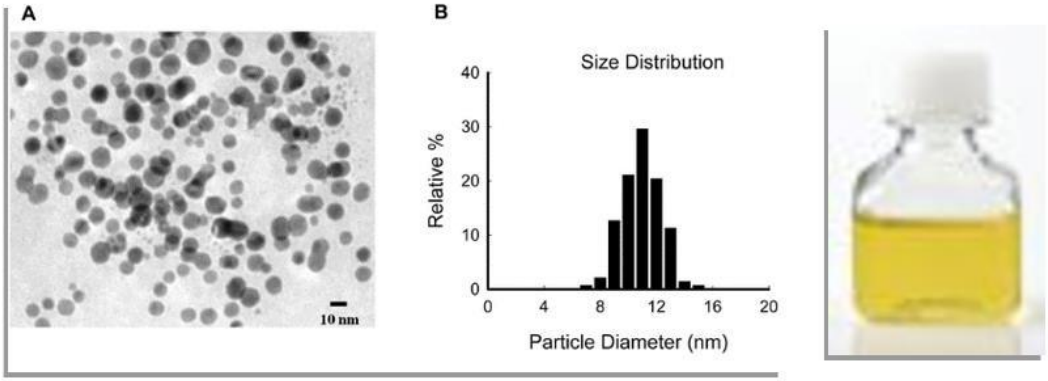

Fig. 1. Characterization of silver nanoparticles. Particles are mostly circular in shape with the average size of 10 nm by using scanning electron microscope

2.5-2 Effect of Ag NPs concentrations embryogenic callus growth and proliferation

The embryogenic callus at 0.5–1.0 g were used as the explant during this experiment to study the effect of Ag NPson the differentiation of embryogenic callus and improvement the growth of somatic embryos during two subcultures and each culture interval 4 weeks. Six treatments were used, five treatments from different concentrations of the Ag NPs at (0.1, 0.5, 1.0, 2.0 and 4.0 ml/l) were addition to MS basal medium supplemented with 2,4-D at 3.0 mg/l + 2ip at 1.0 mg/l and the treatment without Ag NPs was control.The each treatment contained 6 replicates.After the second subculture, the fresh weight of embryogenic callus was measured and also the number of somatic embryos induction on callus was counted.

2.5-3Effect of Ag NPs concentrations on growth and regeneration of somatic embryos

Transfer the small cluster consist of 6-10 somatic embryos of date palm cv. Hayani to differentiation medium which consist of MS basal medium supplemented with 0.05 mg/l Benzyl adenine (BA) + 0.1 mg/l Naphthalen acetic acid (NAA) **(Omar, 1988)**and added the different concentrations of Ag NPs, the cultures were incubated under light condition with 1500 lux for 16 hrs and 8 hrs dark at 27 ±2 °C. The somatic embryos were re-cultured on the fresh medium during two subcultures, each culture interval 4 weeks. At the end of experiment, the number of embryos/jar, length of embryos (cm), number of leaves/jar and length of leaves (cm) were measured.

2.6 Biochemical analyses

The chlorophyll content were determination leaves,Chlorophyll A, B and Carotenoids content (mg/g) as described by **(Lichtenthaler and Buschmann, 2001)** using Thermo Scientific, Orion Aqua Mate 8000, UV-Visible Spectrophotometer at wave lengths 660, 640 and 440 nm.

2.7 Detection of genetic stability at DNA Level Using RAPD (PCR) Molecular Marker

2.7-1 DNA extraction

Total DNA was extracted from mother plant of date palm Hayani cv. and*in vitro* shoots were exposure to 1.0 ml/l of Ag NPs. This work was carried out in the Horticulture Research Institute, Agricultural Research Center. DNA extraction was performed according to a specific procedure detailed by **Marzachì*et al.*** **(1999)**.Green fresh tissues were collected from healthy shoots *in vitro*. The genomic DNA was re-suspended in sterile distilled water in a volume of 100 liters.

2.7-2 PCR amplification

In the PCR reactions, the isolated DNA was used. Amplification was conducted using twelve primers PCRamplification, as shown in **Table (1).** was performed by adding 10 mg of template DNA to a solution consisting of 10 µM Tris-HCl, pH 9.0, 50 µM KCl, 0.1% Triton X-100, 1.5 µM $MgCl_2$, 200 µM DNTPs each, 0.4 µM primers and 2 units of Taq polymerase. The reaction volume was 25 µl. Thermal cycling parameters consisted of 45 cycles and annealing temperatures of 36 °C), 1 min denaturation at 95 °C (except for the first cycle: 5 min), 1 min annealing and 2 min extension at 72 °C (except for the last cycle: 5 min).

2.7-3 Gel Analysis

Amplified products were analyzed by electrophoresis in 1% agarose gel, stained with ethidium bromide and visualized with a UV transilluminator.

Table 1. The sequencing of Random Amplified Polymorphic DNA (RAPD) marker of mother plant of date palm Hayani cv. and sample of shoots exposure to 1.0 ml/l of Ag NPs

Numbe	Primer	Sequencing
1	OPA-1	CAGGCCCTTC
2	OPA-2	TGCCGAGCTG
3	OPA-3	CTCAGTCGCA
4	OPA-4	GTGAGGCGTC
5	OPA-5	GGACCCAACC
6	OPA-6	AGGGGTCTTG
7	OPA-7	TTGGCACGGG
8	OPA-8	GTGACGTAGG
9	OPA-9	GTGATCGCAG
10	OPA-10	AGTCAGCCAC
11	OPA-11	GAAACGGGTG
12	OPA-12	GGACCCAACC

2.8 Statistical analysis

With six replicates in each treatment, the experimental design was completely randomized. The best three results from each treatment were statistically analyzed using MSTAT Computer Program. To verify differences among means of various treatments, means were compared using Ducan's Multiple Range Test as described by **Ducan (1955).**

III. RESULTS AND DISCUSSION

3.1 Effect of Ag NPs concentrations embryogenic callus growth and proliferation

Many physiological and developmental changes during plant cellular growth are caused by the scale, concentration, and association of nano-materials with plant cells **(Khodakovskaya*et al.*, 2012)**.The results as demonstrated in **Table (2) and Fig. (2)** showed that the applications of Ag NPs significantly affected on callus growth and proliferation. The different doses of Ag NPs were employed *in vitro* on MS basal medium supplemented with 3.0 mg/l 2,4-D and 1.0 mg/l 2ip. Results indicated that a significant increase occurred in the fresh weight of embryogenic callus at 1.0 ml/l Ag NPs which recorded the maximum value 4.60 g/jar compared with the control treatment 2.52 g/jar, while a significant decrease in the fresh weight of callus obtained in the treatments 2.0 and 4.0 ml/l Ag NPs which resulted the lowest value 2.15 and 1.26 g/jar, respectively.

Added Ag NPs to medium were resulted increase in callus differentiation and produced somatic embryos, these results dependent on a dose, the concentration of Ag NPs at 0.5 ml/l was an average response which recorded 13.60 embryo/jar. The greatest results were found with MS medium supplemented with 3.0 mg/l 2,4-D, 1.0 mg/l 2ip and 1.0 ml/l Ag NPs, where the number of somatic embryos initiated on callus increased to 19.39 embryo/jar compared with control MS medium which recorded 6.13 embryo/jar. However, further increases in Ag NP concentration decreased the somatic embryos initiated. Addition the concentrations of Ag NPs at 2.0 and 4.0 ml/l, the induction response decreased further to 7.20 and 2.33 embryo/jar, respectively.

The auxin to cytokinin ratio has been shown to assess calli regeneration, and increasing the augmented auxin to cytokinin ratio suppresses calli plant regeneration frequency **(Din *et al.*, 2016)**. The cell culture which exposure to metallic NPs has been promote positive effects on callus induction, shoot regeneration and *in vitro* growth **(Kim*et al.*, 2017)**. Ag NPs can enhance the plant cell's nutrient and water uptake from the culture media by mutilating the cell wall, according to the mechanism of action of Ag NPs in plant cell development **(Ali *et al.*, 2018)**.

Table 2. Effect of Ag NPs concentrations on growth and proliferation of embryogenic callus of in vitro date palm after second subculture

Concentration of Ag NPs (ml/l)	F.W. of embryogenic callus (g)	Number of somatic embryos (embryo/jar)
0.0	2.52 ^{d}	6.13 ^{e}
0.1	3.24 ^{bc}	8.54 ^{c}
0.5	3.89 ^{b}	13.60 ^{b}
1.0	4.60 ^{a}	19.39 ^{a}
2.0	2.15 ^{e}	7.20 ^{cd}
4.0	1.26 ^{f}	2.33 ^{f}

Mean values followed by the same letter(s) within a column are not significantly different (P <0.05)

Low concentrations of NPs had an inductive effect on callus development and subsequent shoot regeneration in the current sample. Authors,it's conceivable that tiny NPs can join explants and affect certain genetic reprogramming features **(Shirazi and Ramezanpour, 2016)**. At a concentration of 20 mg/l, Ag NP induced a significant rise in ethylene and ABA levels, which was detrimental to callus regeneration. Increased ABA levels in plants are a symptom of stress and are dangerous to their growth and production **(Rai*et al.*, 2011)**.

The addition of Ag NPs to the culture media had an important effect on narrow-leaved lavender growth and production *in vitro*. The results differed depending on the metal concentration and the form of NP used. The lavender plants that were exposed to the lowest levels of NPs (1–5 mg dm3 Ag NPs) developed shoots **(Jadczak*et al.*, 2019)**. Determining the optimum concentration of any nano particles at which a plant cell can attain full and stable growth is very important for preventing toxicity concerns related to nanomaterial use on plants. When bean plant explants were treated with various amounts of Ag NPs, the most growth parameters during callus growth, like callus induction percent and biomass, were observed at the optimum concentration (50 mg/ml)**(Mustafa *et al.*, 2017)**. The effects of Ag NPs on the growth and anatomy of *solanumnigrum* callus *in vitro* culture were investigated, furthermore as changes in *S. nigrum*callus morphology, anatomy, including biomass (weight), and deforming cell shape and color, similarly as genetic instability in callus exposed to Ag NPs. They stated that exposure to Ag NPs increased callus fresh weight**(Emad*et al.*, 2015)**.

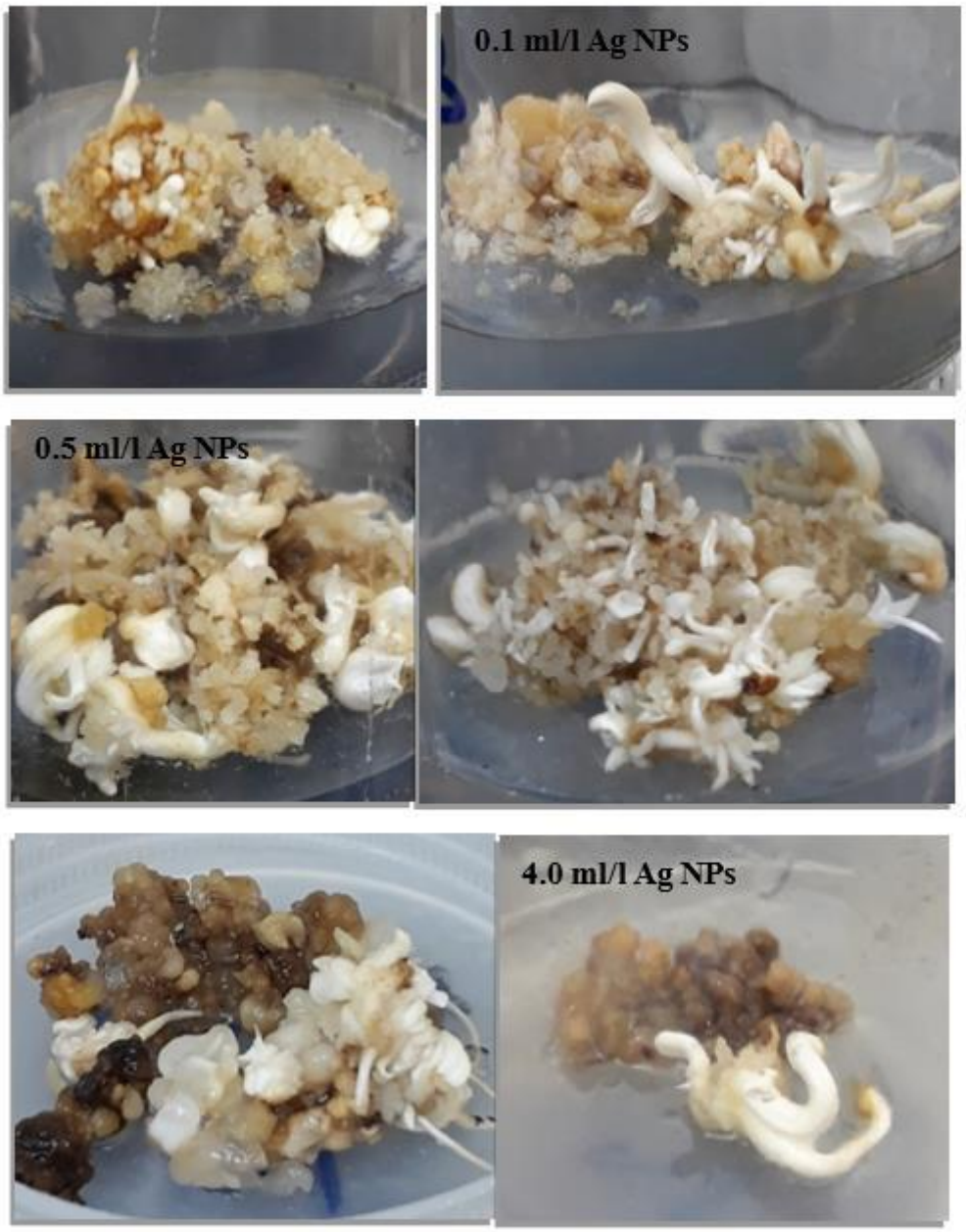

Fig. 2. Effect of different concentrations of Ag NPs on development and proliferation of callus embryogenic date palm Hayani cv. after the second subculture

The results of 8.0 mg/l Ag NPs together with 5.0 mg/l BA and 3.0 mg/l NAA on the event of callus in MS culture media were detected. There is the positive role of Ag NPs on callus induction and growth in the cultures of *Lycopersicumesculentum* supplemented with Ag NPs**(Alia *et al.*, 2019)**. When compared to the control medium, which had a callus induction frequency of 62%, the callus induction response was highest with Ag NPs concentrations of 10 mg/l, with callus induction frequency of 82%, followed by 69%% with 5 mg/l Ag NPs, 37% with 15 mg/l Ag NPs and 16% with 20 mg/l Ag NPs. The calli had a friable texture and a creamy appearance at lower concentrations of Ag NPs, but because the concentrations of Ag NPs rose, the calli became brown. The results of Ag NPs varied after the mediated calli were transferred to regeneration medium. Greening and organogenesis, on the opposite hand, decreased significantly because the concentration of NPs within the culture medium increased **(Manickavasagam *et al.*, 2019)**.

3.2 Effect of Ag NPs concentrations on growth and regeneration of somatic embryos

Results revealed that when adding Ag NPs into the cultivation medium, the response positively resulted on the number of somatic embryos initiated on callus, length of embryos, differentiation of embryos to leaves and length of leaves which supports the findings of **Salama (2012)** and **Sharma *et al.* (2012)** who found that Ag NPs increased root, shoot and leaf development as well as biochemical parameters.

The results as demonstrated in **Table (3) and Fig. (3)** showed that added Ag NPs into the culture medium had a positive effect on the number of somatic embryos. The different concentrations of Ag NPs in proliferation medium were caused produce the highest number of somatic embryos. The treatment with Ag NPs at 0.5 ml/l was resulted in high number of embryos 26.12 embryo/jar, by increasing the concentration of Ag NPs to 1.0 ml/l recorded an increase the number of somatic embryos and registered the maximum value as 35.30 embryo/jar.The other treatments of Ag NPs at 2.0 ml/l and 4.0 ml/l were recorded the lowest number of somatic embryos 17.83 and 10.66 embryo/jar, respectively compared with the control treatment 15.0 embryo/jar. These results were in good agreement with **Mahendran*et al.* (2018)** who reported the highest percentage of somatic embryo production of *Gloriosasuperba*was achieved on the MS medium containing 0.4 mg/l Ag NPs.

Moreover, there was a significant difference in the length of embryos with different concentrations of Ag NPs as shown in **Table (3) and Fig. (3)**. The highest length of embryos 1.80 cm was observed with the treatment 1.0 ml/l of Ag NPs. The other concentrations of Ag NPs (0.1, 0.5, 2.0 and 4.0 ml/l) were recorded a decrease in the embryos length as 0.50, 1.22, 0.65 and 0.33 cm, respectively.

There was a significant difference in the number of leaves by using the different concentrations of Ag NPs. There was an increasing gradually with different treatments of Ag NPs, the treatment 0.1 ml/l Ag NPs was resulted the number of leaves 25.60 leaf/jar which increased to 31.00 leaf/jar with increasing the concentration of Ag NPs to 0.5 ml/l. The highest number of leaves 43.72 leaf/jar were recorded with a treatment 1.0 ml/l Ag NPs. However, increasing Ag NPs to 2.0 ml/l caused the decrease in the number of leaves to 20.14 leaf/jar and also a treatment of Ag NPs at 4.0 ml/l recorded 10.35 leaf/jar which was lower than that in the control treatment 18.32 leaf/jar.

On the other hand, there was a significant difference in the length of leaves with the highest length 3.87 cm recorded with the treatment 1.0 ml/l of Ag NPs. The length of leaves decreased to 1.20 cm by increasing Ag NPs concentration to 2.0 ml/l. The lowest (0.1 ml/l) and highest (4.0 ml/l) Ag NPs concentrations recorded the least leaf lengths 0.58 and 0.65 cm respectively, compared with the control treatment 0.76 cm.

Table 3. Effect of different concentrations of Ag NPs on the development of somatic embryogenesis and shoots proliferation of in vitro date palm after the second subculture

Concentration of Ag NPs (ml/l)	No. of embryos (embryo/jar)	Length of embryos (cm)	No. of leaves (leaf/jar)	Length of leaves (cm)
0.0	15.00 ^{e}	0.76^{c}	18.32 ^{d}	0.76 ^{d}
0.1	19.58 ^{c}	0.50 ^{d}	25.60 ^{c}	0.58^{e}
0.5	26.12 ^{b}	1.22 ^{b}	31.00 ^{b}	1.92 ^{b}
1.0	35.30 ^{a}	1.80 ^{a}	43.72 ^{a}	3.87 ^{a}
2.0	17.83 ^{cd}	0.65 ^{cd}	20.14 ^{d}	1.20 ^{c}
4.0	10.66 ^{f}	0.33 ^{e}	10.35 ^{e}	0.65^{de}

Mean values followed by the same letter(s) within a column are not significantly different (P <0.05)

In brief and after two subcultures, the results showed that Ag NPs with concentration 1.0 ml/l have positive response on the number of embryos and leaves. This result is in agreement with the result achieved by **Do *et al.* (2018)**who reported that adding 1.0 ppm Ag NPs to the banana shoot multiplication medium was found to be the optimum concentration to induce maximum shoot growth, the maximum number of leaves, and maximum total chlorophyll content.

Increasing the concentration of Ag NPs to higher than 1.0 ml/l decreased the number of embryos and length which was lower than the control treatment, which means that low Ag NPs concentrations stimulates proliferation and development while higher concentrations have an

inhibitory effect similar to that reported by **Salama (2012)** for the increase in shoot and root lengths as well as leaf surface area and chlorophyll content of the two tested crop plants and on the contrary with the findings of **Caroline de Oliveira Timoteo*et al.* (2019)**who found a 90% reduction within the number of recent shoots of *C. rufa* nodal segments with 15.4 mg/l Ag NPs. However, the amount and length of leaves decreased by increasing Ag NPs concentration to 0.5 ml/l but re-increased to achieve its maximum with increasing the concentration to 1.0 ml/l.

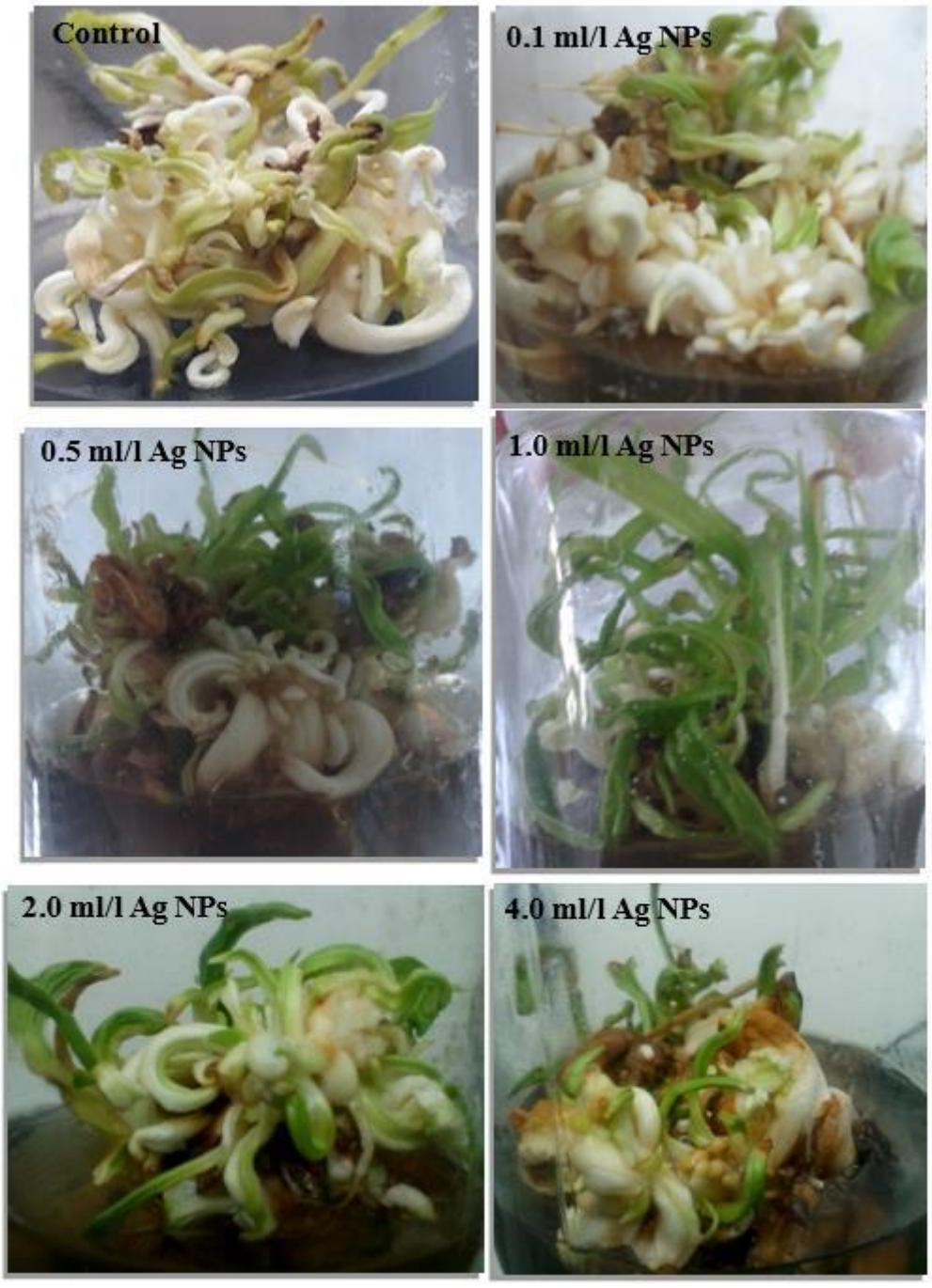

Fig. 3. Effect of different concentrations of Ag NPs on growth and regeneration of somatic embryos date palm Hayani cv. after the second subculture

These results in agreement with, Ag NPs have a large surface area and interact with other particles in the medium to increase efficiency **(Ingle *et al.*, 2008)**.The Plant organisms, age, tissue types, and physiological status are among the parameters influenced by NPs **(Vanninie*t al.*, 2013)**. More recently, silver nanoparticles have also been

used due to their physical and chemical properties and their easy uptake and mobility into plant cells **(Sarmast and Salehi, 2016)**.

Different concentrations of Ag NPs were found to have a substantial positive effect on the growth of shoot and root in Zea mays in another study **(Salama, 2012)**. Shoot multiplication and length were greatly influenced when Ag NPs were added to the culture medium. These results were obtained by **Razzaq*et al.* (2016)** in seedlings of wheat (*Triticumaestuvum*) grown *in vitro* in MS medium with different concentrations of Ag NPs, finding better development in 25 mg/l of Ag NPs. **Spinoso-Castillo *et al.* (2017)** reported that in vanilla (*Vanilla planifolia*), using Ag NPs observed increased shoot production and length at a concentration of 25 mg/l of Ag NPs, whereas the lowest number of shoots were observed on MS medium supplemented with 200 mg/l.

3.3 Determination of chlorophyll content

The chlorophyll contents as represented in **Table (4)** decreased with the lowest Ag NPs concentration (0.1 ml/l) compared to the control then it gradually increased with increasing the Ag NPs concentration from 0.5 to 1.0 ml/l then decreased again with increasing Ag NPs to 2.0 and 4.0 ml/l.

Table 4. Effect of different concentrations of Ag NPs on the chlorophyll contents after the second subculture

Concentration of Ag NPs (ml/l)	Chlorophyll (mg/g)		
	A (660 nm)	B (640 nm)	C (440 nm)
0.0	1.125 [d]	0.583 [c]	1.425 [d]
0.1	1.106 [d]	0.471 [d]	1.151 [e]
0.5	1.461 [b]	0.743 [b]	1.937 [b]
1.0	1.925 [a]	0.815 [a]	2.584 [a]
2.0	1.233 [c]	0.420 [d]	1.683 [c]
4.0	0.519 [e]	0.251 [e]	0.572 [f]

Mean values followed by the same letter(s) within a column are not significantly different (P <0.05)

Chlorophyll results conform to **Castro *et al.* (2019)** who reported that chlorophyll A content was greater in Ag NPs than the control treatment. *In vitro* grown wheat, gross chlorophyll increased significantly at concentrations of25,50, and 100 mg/l of Ag NPs, according to**Razzaq*et al.* (2016)**.**Salama (2012)**, on the opposite hand, found that applying 60 mg/l of Ag NPs to beans and corn increased growth and chlorophyll content. An increase in chlorophyll content was found in the bean at concentrations of 50 mg/l of Ag NPs**(Saeideh and Rashid, 2014)**. The exposure of rice to 0.5 mg/l of Ag NPs has increased the content of chlorophyll and carotenoids **(Nair and Chung, 2014)**. Increased content of photosynthetic pigments in vanilla and sugarcane shoots treated with Ag NPs; this result was possibly caused by increased N, Mg and Fe concentrations in plant tissues exposed to Ag NPs, as these elements are linked to chlorophyll biosynthesis**(Spinoso-Castillo *et al.*, 2017; Bello-Bello *et al.*, 2017)**. The content of chlorophyll a, chlorophyll b, and overall chlorophyll in *A. thaliana* was decreased at 0.2, 0.5 and 3 mg dm^3 of Ag NPs **(Qian *et al.*, 2013)**.

3.4 Detection of genetic stability at DNA Level Using RAPD (PCR) Molecular Marker

The results in the present study revealed that the average polymorphism detected by the RAPD assay, indicating that there were no differences in the amplified DNA fragments among *in vitro* shoots sample of Hayani cv. which treated with 1.0 ml/l of Ag NPs and mother plant (control).Two date palm samples, as shown in **Fig. 4.**were fingerprinted using twelve random primers (RAPD) to detect the DNA polymorphism. Only two primers, namely OPA-5 and OPA-7 showed a difference of 5%, while other primers named (OPA-1, OPA-2, OPA-3, OPA-4, OPA-6, OPA-8, OPA-9, OPA-10, OPA-11 and OPA-12) showed that there were no differences of genetic variation (95%) between the sample treatment and the mother plant.This proves that using 1.0 ml/l of Ag NPs helps to produce true to type shoots for the mother plant.

Molecular diversity in date palm by using molecular marker tool was also documented by many workers **(Mohktar*et al.*, 1999)**. In this analysis, RAPD primers were used to amplify date palm sample and mother plant shoots in a PCR master cycler. RAPD markers produced a banding pattern that specifically identified clusters with genetic similarities.

In present study, RAPD markers have been successfully amplified for cultivar identification and genetic diversity analysis in the date palm Hayani cv. and the RAPD marker has given a good polymorphic data and hence they can be used for genetic diversity analysis. These finding were found similar as given by **(Ravi *et al.*, 2003; Kibria*et al.*, 2009; Hossain *et al.*, 2012)**.

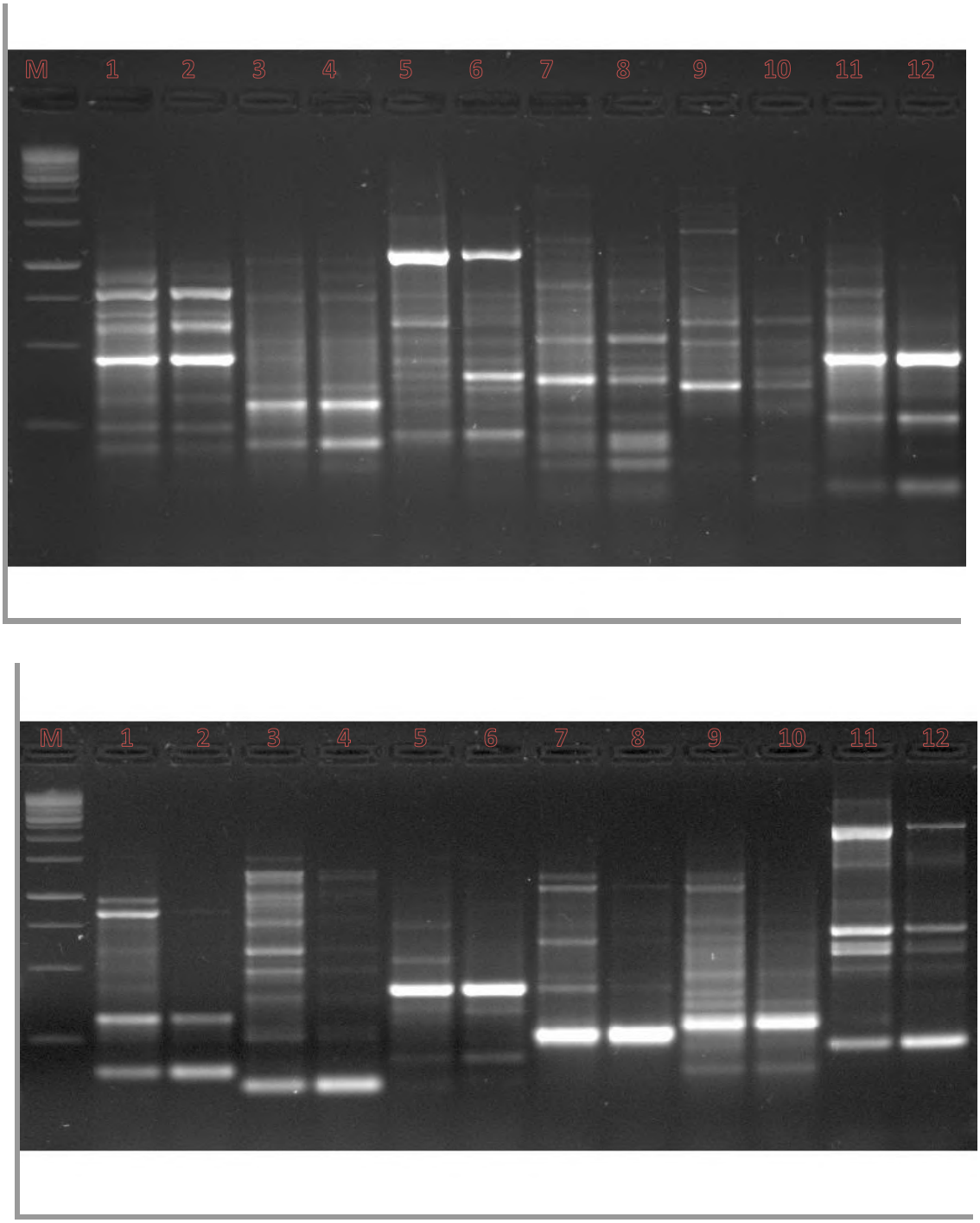

Fig. 4. RAPD's product of primers OPA-1, OPA-2, OPA-3, OPA-4, OPA-6, OPA-8, OPA-9, OPA-10, OPA-11 and OPA-12 showed similarity between the shoots sample which treated with 1.0 ml/l of Ag NPs and mother plant, while primer OPA-5 and OPA-7 showed the differences 5% between sample and mother plant

IV. CONCLUSION

Nano-materials are considered to be one of the most important inventions of modern science **(Wang *et al.*, 2011)**. Date palm organogenesis has gained much interest because of its high multiplication potential and production of true to type plantlets. It can be concluded that the right amount of Ag NPs can help with embryogenic callus growth and proliferation, as well as the development and regeneration of somatic embryos in date palm cv. Hayani*in vitro*.

Culture medium supplemented with 1.0 ml/l of Ag NPs was the best one for embryogenic callus differentiation and produced the highest number of somatic embryos. The suitable medium for regeneration of somatic embryos was added 1.0 ml/l Ag NPs. In this treatment, the number of somatic embryos, length of embryo, number of leaves, length of leaves and total chlorophyll content were 35.30 embryo/jar, 1.80 cm, 43.72 leaf/jar, 3.87 cm per explant and 2.584 mg/g, respectively. It was clear that there was significant effects in embryogenic callus and somatic embryos since exposure to low concentrations of AgNPs which stimulated growth, while increasing the concentration of AgNPs inhibits development.

The result inthis study indicated that the efficiency and ease of using RAPD markers for investigating genetic relationship and identification of varieties is good tool. Only two primers, namely OPA-5 and OPA-7 showed a difference of 5%, while other primers showed there were no differences of genetic variation (95%) between the samples were treated with 1.0 ml/l Ag NPs and the mother plant.

REFERENCES

[1] **Abdi, G.; Salehi, H. and Khosh-khuri, M. (2008).**Nano silver: A novel nanomaterial for removal of Bacterial contamination in Valerian (*V. officinalis*) tissue culture. Acta Physiol. Plant, 30: 709-714.

[2] **Alia, A. B.; Mohammadb, S.; Khanc, M. A.; Rajaa, N. I.; Arifc, M.; Kamilc, A. and Mashwani, Z. R. (2019).** Silver nanoparticles elicited *in vitro* callus cultures for accumulation of biomass and secondary metabolites in *Carallumatuberculata*. Artificial Cells, Nanomedicine, and Biotechnology, 47 (1): 715–724.

[3] **Ali, H.; Khan, M. A.; Ullah, N. and Khan, R. S. (2018).**Impacts of hormonal elicitors and photoperiod regimes on elicitation of bioactive secondary volatiles in cell cultures of*Ajugabracteosa*.Journal of Photochemistry and Photobiology B: Biology, 183: 242–250.

[4] **Al-Khairy, J. and Al-Bahrany, A. M. (2012).**Effect of abscisic acid and polyethylene glycol on the synchronization of somatic embryo development in date palm (*Phoenix dactylifera* L). Biotechnology, 11: 318-325.

[5] **Al-Khalifah, N. S. and Shanavaskhan, A. E. (2012).**Micro-propagation of Date Palms.Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB) and Association of Agricultural Research Institutions in the Near East and North Africa (AARINENA). Riyadh, Saudi Arabia.

[6] **Al-Khalifah, N. S.; Askari, E. and Shanavaskhan, A. E. (2013).**Date Palm Tissue Culture and Genetical Identification of Cultivars Grown in Saudi Arabia.KACST Press. Riyadh, Saudi Arabia.

[7] **Al-Samir, E.; Al-Utbi, S. and Abass, M. (2015).**Phytotoxic effect of 2,4-D and dicamba on date palm (*Phoenix dactylifera* L.) tissue cultures at initiation stage. Advances in Agriculture and Botanics, 7: 96-108.

[8] **Baharan, E.; Pour, P.; Mohammadi, E.; Shahbaziand, S. and Hosseini, Z. (2015).**Effects of some plant growth regulators and light on callus induction and explants browning in date palm (*Phoenix dactylifera* L.) *in-vitro* leaves culture. Iranian Journal of Plant Physiology, 5: 1473-1481.

[9] **Bekheet, S. A.; Taha, H. S.; Saker, M. M. and Solliman, M. E. (2007).**Application of Cryopreservation Technique for *In vitro* Grown Date Palm (*Phoenix dactylifera* L.)Cultures. Journal of Applied Sciences Research, 3 (9): 859-866.

[10] **Bello-Bello, J. J.; Chavez-Santoscoy, R. A.; Lecona-Guzmán, C. A.; Bogdanchikova, N.; Salinas-Ruíz, J.; Gómez-Merino, F. C. and Pestryakov, A. (2017).**Hormetic Response by Silver Nanoparticles on *In Vitro* Multiplication of Sugarcane (*Saccharum spp.* Cv. Mex 69-290) Using a Temporary Immersion System. Dose-Response, 15 (4): 1559325817744945.

[11] **Din, A. R. J. M.; Ahmad, F. I.; Wagiran, A.; Samad, A. A.; Rahmat, Z. and Sarmidi, M. R. (2016).** Improvement of efficient *in vitro* regeneration potential of mature callus induced from Malaysian upland rice seed (*Oryza sativa* cv. Panderas). Saudi Journal of Biological Sciences, 23: S69–S77.

[12] **Do, D. G.; Dang, T. K. T.; Nguyen, T. H. T.; Nguyen, T. D.; Tran, T. T. and Hieu, D.D. (2018).** Effects of nano silver on the growth of banana (*Musa spp*.) cultured *in vitro*. Journal of Vietnamese Environment, 10 (2): 92-98.

[13] **Ducan, D. B. (1955)**. Multiple range and multiple F test. Journal of Biometrics, 11: 1-42.

[14] **El-Hadrami, I.; El-Bellaj, M.; El-Idrissi, A.; J'Aiti, F.; El-Jaafari, S. and Daaf, F. (1998).**Plant biotechnology and improvement of the date palm (*Phoenix dactylifera* L.) Pivot of Moroccan oasis agriculture.Cah. Agric., 7: 463-468.

[15] **Emad, A. E.; Said, A. D. and Ezzat, H. E. (2015)**.Evaluation of Callus Responses of *Solanumnigrum* L. Exposed to Biologically Synthesized Silver Nanoparticles.Nanoscience and Nanotechnology, 5 (3): 45-56.

[16] **Eshraghi, P.; Zarghami, R. and Mirabdulbghi, M. (2005).**Somatic embryogenesis in two Iranian palm cultivars. Afr. J. Biotechnol., 4: 1309–1312.

[17] **Fki, L.; Masmoudi, R.; Drira, N. and Rival, A. (2003).**An optimized protocol for plant regeneration from embryogenic

suspension cultures of date palm, *Phoenix dactylifera* L., cv. DegletNour. Plant Cell Reports, 21 (6): 517–524.

[18] **Fki, L.; Masmoudi, R.; Kriaa, W.; Mahjoub, A.; Sghaier, A. B.; Mzid R.; Mliki, A.; Rival, A. and Drira, N. (2011).**Date palm micro-propagation via somatic embryogenesis. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.). Date Palm Biotechnology.Springer. The Netherlands, pp. 47–68.

[19] **Gouran, A.; Jirani, M.; Mozafari, A. A.; Saba, M. K.; Ghaderi, N. and Zaheri**, **S. (2014).** Effect of silver nanoparticles on grapevine leaf explants sterilization at *in vitro* conditions, 2nd National Conference on Nanotechnology from Theory to Application, Isfahan, Iran, pp. 1–6.

[20] **Hoffmann, F.; Fortier, T. and Hoffmann-Tsay, S. S. (2013).**Micro-propagation of Date Palm (*Phoenix dactylifera*).Phoenix Institute of Desert Agriculture, San Diego, California.

[21] **Hossain, M. M.; Islam, M. M.; Hossain, H.; Ali, M. S. and da Silva, T. (2012).**Genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.) by microsatellite markers. Genes, Genomes and Genomics, 6 (SI1): 42-47.

[22] **Ingle, A.; Gade, A.; Pierrat, S.; Sonnichsen.C and Rai, M. (2008).**Mycosynthesis of silver nanoparticles using the fungus *Fusariumacuminatum*and its activity against Some Human Pathogenic Bacteria.Current Nanoscience,4 (2):141 – 144.

[23] **Jadczak, P.; Kulpa, D.; Bihun, M. and Przewodowski, W. (2019).**Positive effect of Ag NPs and Au NPs in *in vitro* cultures of *Lavandulaangustifolia* Mill. Plant Cell, Tissue and Organ Culture, 139: 191–197.

[24] **Johnson, D. V. (2011).**Date palm biotechnology from theory to practice. In: Jain, S.M., Al-Khayri, J.M., Johnson, D.V. (Eds.), Date Palm Biotechnology. Springer Netherlands. The Netherlands, pp. 1–11.

[25] **Khierallah, H. S. M. and Hussein, N. H. (2013).**The Role of coconut water and casein hydrolysate in somatic embryogenesis of Date Palm and genetic stability detection using RAPD markers.Date Palm Research Unit, College of Agriculture, University of Baghdad, Iraq.

[26] **Khodakovskaya, M. V.; De Silva, K.; Biris, A. S.; Dervishi, E. and Villagarcia, H. (2012).** Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano, 6 (3): 2128–2135.

[27] **Kibria, K.; Nur, F.; Begum, S. N.; Islam, M. M.; Paul, S. K.; Rahman, K. S. and Azam, S. M. M. (2009).** Molecular Marker based Genetic Diversity Analysis in Aromatic Rice Genotypes Using SSR and RAPD Markers. Int. J. Sustain. Crop Prod. 4 (1): 23-34.

[28] **Kim, D. H.; Gopal, J. and Sivanesan, I. (2017).**Nanomaterials in plant tissue culture: the disclosed and undisclosed. The Royal Society of Chemistry Advances, 7: 36492–36505.

[29] **Krishnaraj, C.; Jagan, E. G.; Ramachandran, R.; Abirami, S. M.; Mohan, N. and Kalaichelvan, P. T. (2012).**Effect of biologically synthesized silver nanoparticles on *Bacopamonnieri* (Linn.)Wettst.Plant growth metabolism. Process Biochem., 47 (4): 51–658.

[30] **Kumar, V.; Ramakrishna, A. and Ravishankar, G. A. (2007)**. Influence of different ethylene inhibitors on somatic embryogenesis and secondary embryogenesis from *Coffeacanephora* P ex Fr. *In vitro* Cellular & Developmental Biology-Plant, 43: 602-607.

[31] **Lichtenthaler, H. K. and Buschmann, C. (2001).**Chlorophylls and carotenoids: Measurement and Characterization by UV-VIS. Current Protocols in Food Analytical Chemistry (CPFA), (John Wiley and Sons, M. New York): pp. F4.3.1-F4.3.8. [Nr. 107].

[32] **Lima, G. P.; Campos, R. S.; Willadino, L. G.; Terezinha, J. R. and Vianello, F. (2012).**Polyamines, gelling agents in tissue culture, micro-propagation of medicinal plants and bioreactors. In: Leva A, Rinaldi L., Eds. Recent advances in plant *in-vitro* culture. In Tech, pp. 165-185.

[33] **Luoma, N. S. (2008).** Silver nanotechnologies and the environment: Old problems or new challenges? The Project on Emerging Nanotechnologies.Project on Emerging Nanotechnologies of the Woodrow Wilson International Center for Scholars, 15: 66 pages.

[34] **Manickavasagam, M.; Pavan, G. and Vasudevan, V. (2019).**A comprehensive study of the hormetic influence of biosynthesized Ag NPs on regenerating rice calli of indica cv. IR64. Scientific Reports, 9 (8821): 1-11.

[35] **Marzachì, C.; Veratti, F.; D'Aquilio, M.; Vischi, A.; Conti, M. and Boccardo, G. (1999).**Molecular Hybridization and PCR Amplification of Non-Ribosomal DNA to Detect and Differentiate StolburPhytoplasma Isolates from Italy.Journal of Plant Pathology, 82 (3): 201-212.

[36] **Masmoudi-Allouche, F.; Gargouri-Bouzid, R.; Kriaâ1, W.; Saïdi, M. N.; Jamoussi, H.; Abdelkefi, Y. and Drira (2011)**. Investigation of genetic variability related to the *in vitro* floral hermaphrodism induction in Date palm (*Phoenix dactylifera* L.). Afr. J. Biotechnol., 10: 10567–10574.

[37] **McCubbin, M. J.; Van Staden, J. and Zaid, A. (2000).** A southern African survey conducted for off-types on date palm production using somatic embryogenesis. In: Proceedings of the date palm international symposium. Windhoek, Namibia, pp. 68–72.

[38] **Miller, G. and Senjan, R. (2006).**The disruptive social impacts of nanotechnology.

[39] **Mohktar, T.; Abdelmajid, R.; Ali, O.; Hela, S. and Mohamed, M. (1999).**Differention of Tunisian date-palm varieties by RAPDs technology. The International Conference of Date Palm, Nov. 9-11.

[40] **Monica, R. C. and Cremonini, R. (2009).**Nanoparticles and higher plants.Caryologia., 62 (2): 161–165.

[41] **Mousavi, S. R. and Rezaei, M. (2011).**Nanotechnology in agriculture and food production. Journal of Applied Environmental and Biological Sciences, 1: 414-419.

[42] **Murashige, T. and Skoog, F. (1962).** A revised medium for rapid growth and bio assays with Tobacco tissue cultures. PhysiologiaPlantarum, 15: 473-480.

[43] **Mustafa, H. S.; Oraibi, A. G.; Ibrahim, K. M. and Ibrahim, N. K. (2017).** Influence of silver and copper

nanoparticles on physiological characteristics of *Phaseolus vulgaris* L. *in vitro* and *in vivo*. International Journal of Current Microbiology and Applied Sciences, 6 (1): 834-843.

[44] **Nair, P. M. G. and Chung, I. M. (2014).**Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa* L.) seedlings. Chemosphere, 112: 105–113.

[45] **Oliveira, R. S.; Ma, Y.; Rocha, I.; Carvalho, M. F.; Vosátka, M. and Freitas, H. (2016).***Arbuscularmycorrhizal*fungi are an alternative to the application of chemical fertilizer in the production of the medicinal and aromatic plant *Coriandrumsativum* L. Journal of Toxicology and Environmental Health A, 79 (7): 320-328.

[46] **Omar, M. S. (1988).***In vitro* response of various date palm explants. Date Palm J., 6 (2): 371-388.

[47] **Qian, H.; Peng, X.; Han, X.; Ren, J.; Sun, L. and Fu, Z. (2013).**Comparison of the toxicity of silver nanoparticles and silver ions on growth of terrestrial plant model Arabidopsis thaliana. J. Environ. Sci., 25 (9): 1947–1956.

[48] **Rai, M. K.; Shekhawat, N. S.; Harish; Gupta, A. K.; Phulwaria, M.; Ram, K. and Jaiswal, U. (2011)**.The role of abscisic acid in plant tissue culture—a review of recent progress. Plant Cell Tissue and Organ Culture, 106: 179–190.

[49] **Ravi, M.; Geethanjali, S.; Sameeyafarheen, F. and Maheswaran, M. (2003).** Molecular Marker based Genetic Diversity Analysis in Rice (*Oryza sativa* L.) using RAPD. Euphytica, 133: 243–252.

[50] **Razzaq, A.; Ammara, R.; Jhanzab, H. M.; Mahmood, T.; Hafeez, A. and Hussain, S. (2016).**A novel nanomaterial to enhance growth and yield of wheat. Journal of Nano-science and Technology, 2 (1): 55–58.

[51] **Saeideh, N. and Rashid, J.(2014).**Effect of silver nanoparticles and Pb $(NO_3)_2$ on the yield and chemical composition of mung bean (*Vignaradiata*). Journal of Stress Physiology & Biochemistry, 10 (1): 316-325.

[52] **Salama, H. M. H. (2012)**. Effects of silver nanoparticles in some crop plants, Common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). International Research Journal of Biotechnology, 3 (10): 190-197.

[53] **Sarmast, M. K. and Salehi, H. (2016).** Silver nanoparticles: an influential element in plant nano-biotechnology. Mol. Biotechnol., 58: 441–449.

[54] **Saxena, R.; Tomar, R. S. and Kumar, M. (2016).** "Exploring nano-biotechnology to mitigate abiotic stress in crop plants," Journal of Pharmaceutical Sciences and Research, 8 (9): pp. 974–980.

[55] **Sharma, P.; Bhatt, D.; Zaidi, M. G.; Saradhi, P. P.; Khanna, P. K. and Arora, S. (2012).** Silver nanoparticle mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl. Biochem. Biotechnol., 167: 2225–2233.

[56] **Shirazi, A. M. S. and Ramezanpour, Z. (2016).** "Toxicity effects of SiO_2 nanoparticles on green micro-algae *Dunaliella Salina*", International Journal ofNano-science andNanotechnology, 12 (4): 269-275.

[57] **Smith, F.; Gilles, M. A.; Hamilton, J. K. and Godess, P. A. (1956).**Colorimetric method for determination of sugar and related substances.Anal. Chem., 28: 350-358.

[58] **Spinoso-Castillo, J. L.; Chavez-Santoscoy, R. A.; Bogdanchikova, N.; Pérez-Sato, J. A.; Morales-Ramos, V. and Bello-Bello, J. J. (2017).** Antimicrobial and hormetic effects of silver nanoparticles on *in vitro* regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. Plant Cell, Tissue and Organ Culture, 129: 195–207.

[59] **Timoteo, C. O.; Paiva, R.; Reis, M. V.; Claro, P. I. C.; Silva, D. P. C.; Marconcini, J. M. and Oliveira, J. E. (2019).**Silver nanoparticles in the micro-propagation of *Campomanesiarufa* (*O. Berg*) Nied. Plant Cell, Tissue and Organ Culture,137: 359–368.

[60] **Vannini, C.; Domingo, G.; Onelli, E.; Prinsi, B.; Marsoni, M.; Espen, L. and Bracale, M. (2013).** Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. PLOS One, 8 (7): e68752.

[61] **Wang, J.; Asbach, C.; Fissan, H.; Hulser, T.; Kuhlbusch, T. A. J.; Thompson, D. and Pui, D. Y. H. (2011).** How can nano-biotechnology oversight advance science and industry: Examples from environmental, health and safety studies of nanoparticles (nano-EHS). J.Nanopart. Res., 13: 1373–1387.

[62] **Wang, P.; Lombi, E.; Zjao, F. J. and Kopittke, P. M. (2016).**Nanotechnology: A New Opportunity in Plant Sciences. Trends Plant Sci., 21 (8): 699-712.