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EFFECT OF SILVER NANOPARTICLES ON MICROPROPAGATION OF DATE PALM (*PHOENIX DACTYLIFERA* L, *CV.* SEWI AND MEDJOOL)

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Abstract

The current research was carried out to assess the potential effects of silver nanoparticles on contamination and *in vitro* development of date palm, immature inflorescences explants of Sewi and Medjool date palm were cultured on MS medium supplemented with 1mg L^{-1} 2,4-D, 1mg L^{-1} 2iP and 1g L^{-1} activated charcoal. Silver nanoparticles treatments (0, 125, 250 and 500µg L^{-1}) were applied during establishment, callus differentiation and multiplication stage. Contamination%, direct somatic embryos%, callus%, callus growth degree, average number of embryos per cluster and number of shoots per cluster were recorded. The obtained results showed that silver nanoparticles has a good potential for removing contaminants in date palm tissue culture, 500µg L^{-1} of silver nanoparticles considered as the most successful disinfection treatment, which decreased contamination to 2.77% compared with 19.44% in control treatment during establishment stage and recorded zero during multiplication & germination stage. Regarding number of embryos and shoot number, the lower concentrations of AgNPs gave better values compared with higher concentration while, the highest callus formation, callus growth and globularizaton were recorded at the higher concentrations of silver nanoparticles.

Key words: Date palm, Nanotechnology, Silver nanoparticles, Micropropagation

Introduction

Date palm (*Phoenix dactylifera* L.) cultivation considered as one of the most economically important activities in the arid zones of the Middle East and North Africa (El Hadrami and Al-Khayri, 2012). Traditionally, date palm propagated by off-shoots, this method produces limited number of palm trees depending on the palm genotype and age (Jain, 2012 and Al-Khalifah *et al.,* 2013). Tissue culture provides an effective method for mass propagation, production of pathogen free and true to type palms (Smith and Aynsley, 1995; Khosroushahi *et al.,* 2006 and Kriaa *et al.,* 2012). Tissue culture was used for propagation of date palm using both of organogenesis and somatic embryogenesis (Sharma *et al.,* 1986; Bouguedoura *et al.,* 1990; El Hadrami *et al.,* 1995; George *et al.,* 2008 and Fki *et al.,* 2011), different types of palm explants can be used; including lateral buds (Abul-Soad and Mahdi, 2010 and Abahmane, 2011), root segments (Ibrahim *et al.,* 2013), female inflorescences (Sidky and Eldawyati, 2012) and lateral leaves of shoot tip (Fki *et al.,* 2003 and Abd El Bar and El Dawayati, 2014). Contamination of plant tissues is a prevailing problem in plant micropropagation (Cassells, 1991). Date palm explants are exposed to microbial contamination at all stages of tissue culture, contamination coming with the explants, or occurring during the propagation procedures (Al-Mussawi, 2010), contamination in date palm tissue culture laboratories is not easy to eliminate by surface sterilization (Abass, 2013).

Nanoparticles have a wide range of applications in the agricultural field due to their unique properties, including high penetration ability, larger surface area and high chemical activity (Agrahari and Dubey, 2020). Metal particles in the nanometer size exhibit physical properties that are different from both of ion the bulk material (Singh *et al.,* 2008). Among the different types of nanomaterials, silver nanoparticles (AgNPs) are the most popular, especially in the agricultural sector (Yan & Chen 2019). The antimicrobial effects of silver against a broad range of bacteria, fungi and other micro-organisms have been reported (Gong *et al.,* 2007 and Jo *et al.,* 2009 and Sarmast and Salehi 2016). According to El-Sharabasy and Zayed, (2018) silver nanoparticles are able to reduce bacterial and fungal contamination of date palm explants

during establishment stage. Moreover, several reports have proven the positive effects of NPs on callus induction, number of somatic embryos, shoot multiplication and plant growth under *in vitro* conditions (Roustan, *et al.,* 1990; Al-Khayri and Al-Bahrany, 2001; Aghdaei *et al.,* 2012 and Sarmast and Salehi 2016). Medium concentrations of silver NPs improved amount of embryogenic callus and produce the highest number of embryos (Roshanfekrrad *et al.,* 2017). According to Al-Khayri and Al-Bahrany (2004) Ag had a stimulatory effect on callus growth in date palm, the degree of stimulation differed among genotypes and concentration. The use of nanoparticles in plant tissue culture still a new approach, which needs further study and research for proper understanding and implementation, the aim of the current study was to evaluate the effect of silver nanoparticles on date palm micropropagation.

Materials and Methods

The current investigation was carried out at the Central Laboratory for Date Palm Researches and Development, Agric. Res. Center, Giza, Egypt, throughout successive period 2017-2018 to study the effect of applying silver nanoparticles as an optimization tool of date palm micropropagation protocol.

Sterilization of plant material

Female inflorescence (15-20 cm) were collected at late January to early February from mature healthy trees of Medjool and Sewi date palm growing at experimental farm of the Central Laboratory for Date Palm Researches and Development. The spaths were washed with tap water and liquid soup for half an hour. Surface sterilization in laminar air flow hood was performed using 50% commercial bleach (5.25% sodium hypochlorite) containing 2 drops of tween-20 for 20 minutes. Sterilized spaths were opened and the isolated spiklets immersed in 0.2% mercuric chloride solution for 10 minutes, then rinsed with sterilized distilled water three times.

Characterization of silver nanoparticles (Ag NPs)

AgNPs were synthesized at the Department of Molecular Genetics, Faculty of Agriculture, Ain Shams University. Particle size measurements of AgNPs were carried out at Nanotechnology and Advanced Material Central Lab (NAMCL), Agriculture Research Center with a Zetasizer (Nano-ZS90, Malvern, UK), the size of the silver particles were found to be <70 nm.

Establishment stage

Explants of sterilized spiklets were cultured on MS media supplemented with 1mg L^{-1} 2,4-D, 1mg L^{-1} 2iP, 6g L^{-1} agar, 40g L^{-1} sucrose and 1g L^{-1} activated charcoal. The silver nanoparticles applied at four concentrations (0, 125, 250 and 500 µg L^{-1} in the culture media). After that, jars were incubated at dark conditions with regular transferring to fresh media with the same components every two months. After eight months percentage of contamination, percentage of explants produced direct somatic embryos or callus were recorded according to the following equations:

Callus induction % =

$$\frac{Number\ of\ florets\ for \min g\ callus}{Total\ number\ of\ florets} \times 100$$

Direct embryos % =

$$\frac{Number\ of\ florets\ for \min g\ direct\ embryo}{Total\ number\ of\ florets} \times 100$$

Callus differentiation stage

0.5g callus mass were cultured on MS medium supplemented with 0.5mg L^{-1} 2,4-D, 1mg L^{-1} 2iP, 40g L^{-1} sucrose, 6g L^{-1} agar and 1g L^{-1} activated charcoal, in addition to aforementioned silver nanoparticles treatments; then, it was incubated at dark conditions, after three months, the globularizaton and callus growth degree was recorded (Pottino 1981).

Multiplication stage

Clusters of somatic embryos (3-4 embryos) cultured on MS medium supplemented with 0.1mg L^{-1} NAA, 0.05mg L^{-1} BA, 0.25mg L^{-1} ABA, 6g L^{-1}, 40g L^{-1} sucrose agar and 0.5g L^{-1} activated charcoal in addition to aforementioned silver nanoparticles treatments. Then, it

Table 1: Effect of silver nanoparticles on contamination percentage of Sewi and Medjool date palm *cvs.*

AgNPs concentrations (µg L^{-1})	Contamination percentage		
	Establish-ment stage	Callus stage	Multiplication & germination stage
Sewi *cv.*			
0.0	19.44	11.11	08.33
125	11.11	08.33	05.55
250	05.55	05.55	02.77
500	02.77	02.77	00.00
Mean	09.71	06.94	4.16
LSD at $_{0.05}$	12.96	08.26	5.57
Medjool *cv.*			
0.0	19.44	11.11	08.33
125	11.11	08.33	05.55
250	05.55	05.55	02.77
500	02.77	00.00	00.00
Mean	09.71	06.24	04.16
LSD at $_{0.05}$	12.96	07.05	05.57

was incubated at 16 hours light using cold white fluorescent lamps giving intensity of 2000 lux. After three months, the average number of embryos/cluster and number of shoots/cluster were recorded.

Statistical analysis

All experiments were run in a randomized complete design and statistically analyzed with three replicates according to Snedecor & Cochran (1989). The mean values were compared using LSD method at 0.05% level. The percentages were transformed to the arcsine to find the binomial percentages according to Steel & Torrie (1980).

Table 2: Effect of silver nanoparticles on Sewi and Medjool date palm cvs. during callus stages.

Ag NPs concentrations (µg L^{-1})	Callus formation%	Callus growth	Globular embryos formation
Sewi cv.			
0.0	46.66	01.66	09.00
125	48.33	02.33	17.33
250	65.00	03.33	14.00
500	73.33	03.66	09.00
Mean	58.33	02.75	12.33
LSD at $_{0.05}$	12.07	00.99	01.79
Medjool cv.			
0.0	43.33	02.33	08.00
125	50.00	02.66	16.00
250	56.00	03.66	13.00
500	76.66	04.00	08.33
Mean	56.49	03.16	11.33
LSD at $_{0.05}$	13.39	00.99	02.48

Results and Discussions

Data in table 1 showed that contamination percentage was affected by increasing Ag NPs concentrations in culture media. Data clearly show that medium supplemented with 500µg L^{-1} Ag NPs recorded the lowest contamination percentage during the different micropropagation stages of both cultivars, followed by 250µg L^{-1} AgNPs as compared with other concentrations, the lowest contamination percentage was recorded for 500 µg L^{-1} AgNPs in Sewi *cv.* during multiplication & germination stage and in Medjool *cv.* during callus and multiplication & germination stages. Based on the fact that contamination is a serious problem in tissue culture, silver NPs were previously used at different concentrations to reduce microbial contamination (Spinoso-Castillo *et al.,* 2017). AgNPs have broad spectrum of antimicrobial activity and reduce various contamination types caused by fungal and bacterial pathogens in plant tissue culture (Abdi *et al.,* 2008; Jo and Kim 2009; Ismail *et al.,* 2017 and Hwan *et al.,* 2017). Previous studies have shown that AgNPs can remove microbial contamination in potato tissue culture (Safavi and Mortezaeinezhad 2012). Silver attacks a wide range of biological processes of microorganisms, penetrating bacterial cell wall and changing the structure and functions of the cell membrane (Singh *et al.,* 2008).

Results concerning the effect of Ag NPs concentration on micrpropagation of date palm, data in

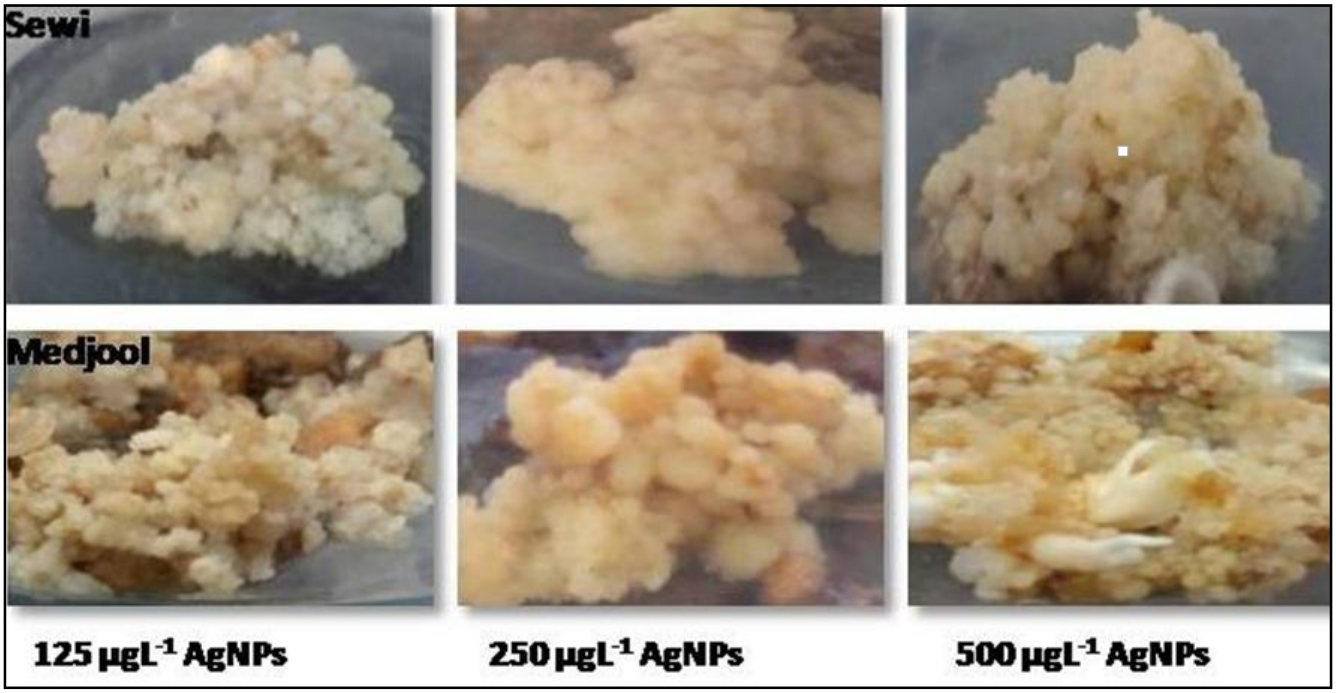

Fig. 1: Effect of AgNPs on callus induction, growth and somatic embryogenesis of Sewi and Medjool date palm *cvs.*

table 2 and Fig. 1 showed the positive effects of AgNPs on callus induction, callus growth and differentiation; 500µg L^{-1} significantly induced the highest callus formation percentage in both cultivars (73.33 and 76.66 in Sewi and Medjool respectively) compared with other concentrations, with respect to the callus growth and differentiation, results reveal that the highest degree of callus growth was recorded with 500µg L^{-1} AgNPs in both cultivars, followed by 250µg L^{-1} without any significant difference, while the lowest degree of callus growth was recorded for the control treatments. The formation of globular embryos was significantly affected by AgNPs treatments 125µg L^{-1} AgNPs recorded the highest value compared with the other concentrations, it's obvious that the high AgNPs had a negative effect on globular embryos formation.

Table 3: Effect of silver nanoparticles on Sewi and Medjool date palm cvs. during embryo stages.

Ag NPs concentrations (µg L^{-1})	Direct embryo formation %	Embryo multiplication	Embryo germination
Sewi cv.			
0.0	28.33	00.22	00.33
125	68.33	01.00	00.92
250	41.66	00.77	00.81
500	35.00	00.55	00.73
Mean	43.33	00.63	00.69
LSD at $_{0.05}$	14.75	00.28	00.11
Medjool cv.			
0.0	31.66	00.33	00.33
125	68.33	01.00	00.81
250	53.33	00.88	00.73
500	33.33	00.66	00.69
Mean	46.66	00.71	00.64
LSD at $_{0.05}$	12.44	00.17	00.11

Data in table 3 and Fig. 2 reveal that, direct somatic embryo formation was affected by different AgNPs concentrations. Data clearly show that medium supplemented with 125µg L^{-1} AgNPs recorded the highest percentage of direct somatic embryo formation (68.33% in both cultivars) followed by 250µg L^{-1} (41.66 and 53.33 % in Sewi and Medjool respectively) compared with the other concentrations. Somatic embryo multiplication & germination was significantly affected by different AgNPs concentrations, the lower concentrations produced the highest embryo multiplication and germination value in both cultivars.

Previous studies showed that, addition of silver NPs to MS medium, increased callus fresh weight and callus formation degree (Ewais *et al.,* 2015). Also, addition of silver NPs in MS medium increased the number of shoots per explant of *Tecomella undulata* (Roxb.) (Aghdaei *et al.,* 2012) and increased shoot regeneration of *Brassica* sp. (Wang *et al.,* 2017). Shoot number and shoot length of *T. undulata* were significantly enhanced on medium supplemented with Ag NPs (Sarmast *et al.,* 2015). Spinoso-Castillo *et al.,* (2017) investigated the effects of Ag NPs on shoot regeneration of *Vanilla planifolia*, the highest number of shoots was obtained on medium

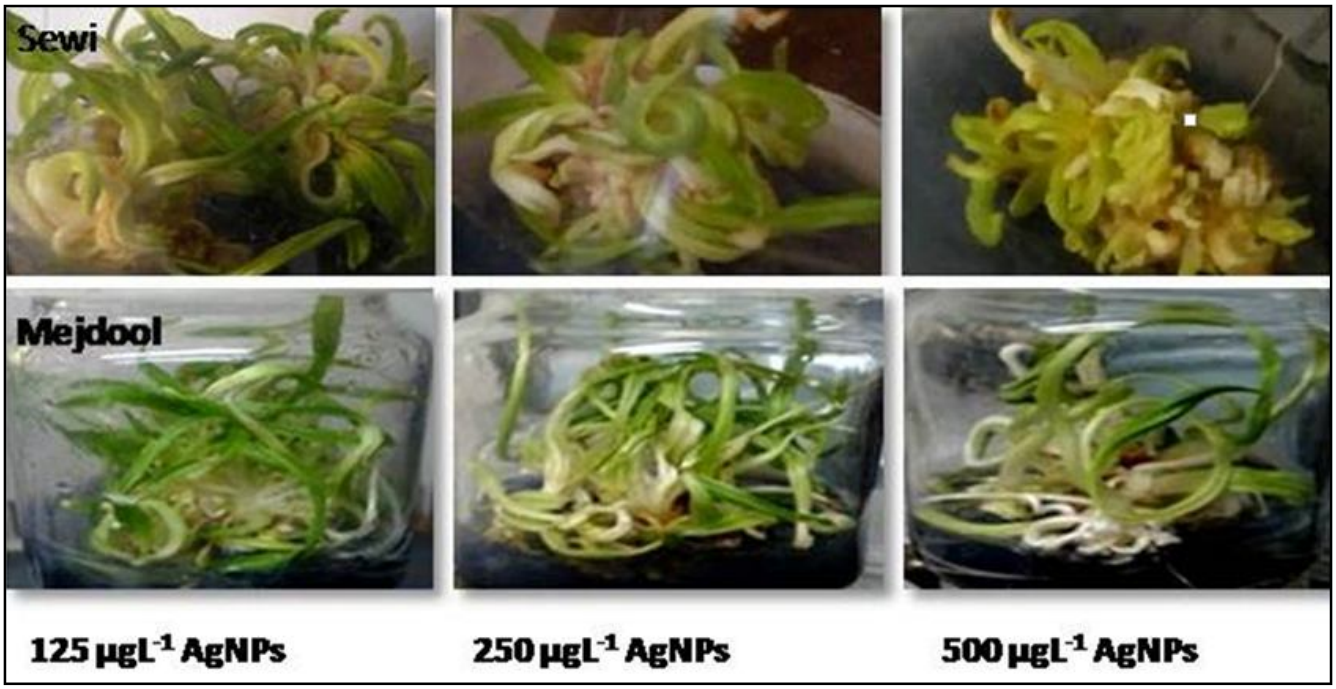

Fig. 2: Effect of AgNPs on embryo formation, multiplication and somatic embryogenesis germination of Sewi and Medjool date palm *cvs*.

supplemented Ag NPs, whereas the lowest number of shoots were observed on higher Ag NPs concentrations. These results may be due to Ag NPs effect on plants physiological status. Zuverza-Mena *et al.,* (2016) demonstrated that Ag NP decrease radish (*Raphanus sativus*) water content in a dose-dependent manner; the nutrient content was also significantly reduced, suggesting that AgNPs may affect plant growth by changing water and nutrient content.

Mena *et al.,* (2016) reported that AgNPs also affect plant hormones; silver nanoparticles caused a significant increase in total cytokinins in the leaves of *Capsicum annuum*., Sun *et al.,* (2017) found that AgNPs reduced auxin accumulation, while gene expression analysis suggested that auxin receptor-related genes were down regulated upon AgNP exposure. Vinkovic *et al.,* (2017) found that AgNP accumulation in pepper tissue resulted in a significant increase in total cytokinin levels, suggesting the importance of cytokinin in the plant's response to AgNPs. Recently Aghdaei *et al.,* (2012) demonstrated that ethylene accumulation during micropropagation of *Tecomella undulata* decrease chlorophyll content and would result in the demise of explants and the positive effects of AgNPs on organogenesis may be due to inhibition of ethylene production; Ag NP treatment delayed explants senescence and increased survival by down regulation of the TuACS gene which involved in biosynthesis of ethylene.

Conclusion

The obtained results showed that silver nanoparticles has a good potential for removing contaminants during date palm micropropagation, the lower concentrations of silver nanoparticles has a positive effect on number of embryos, secondary embryo number and shoot number, while higher concentrations improved callus formation, callus growth and globularizaton.

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