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IMPROVING α-TOCOPHEROL ACCUMULATION IN *ARGANIA SPINOSA* SUSPENSION CULTURES BY PRECURSOR AND NANOPARTICLES FEEDING

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Abstract

Argan (*Argania spinosa*) is an endangered plant and one of the endemic agroforestry species of Morocco, which belongs to family of Sapotaceae. The plant contains α-tocopherol; the major vitamin E compound that is essential for medical purposes and human nutrition. Plant tissue culture has great advantages for potential production of bioactive plant metabolites. The objective of this study was the enhancement of α-tocopherol *in vitro* production in argan callus and suspension cultures using tyrosine as a precursor and titanium dioxide (TiO_2) and silicon dioxide (SiO_2) nanoparticles as elicitors. White friable callus was induced by culturing seedling leaves on full strength Murashige and Skoog (MS) medium supplemented with 4.5 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 5 µM β–naphthalene acetic acid (NAA). In suspension cultures, tyrosine (275.953 µM) led to the increase of α-tocopherol percentage to 0.0602% with about 2.17-fold increase after 10 days of culture, compared to the control treatment. The combination of tyrosine (275.953 µM) with either TiO_2 or SiO_2 nanoparticles (5 ppm) after 10 days of culture gave higher production of α-tocopherol, which reached 0.276 and 0.283% with 4.59- and 4.7-fold increase for TiO_2 and SiO_2 nanoparticles, respectively. This study proves the function of nanoparticles as elicitors for enhancing *in vitro* production of secondary metabolites in plants.

Keywords: Argan, *in vitro* production, secondary metabolites, tyrosine, elicitors

Introduction

Plants are main sources of secondary metabolites of medicinal importance for the production of pharmaceuticals, cosmetics and various valuable products. Biotechnological tools with special emphasis on tissue culture, have a significant role in the production of valuable secondary metabolites from plants. The commercial importance of these active compounds resulted in a great interest in enhancing their production by tissue culture technology (Tiwari and Rana, 2015).

Argania spinosa (L.) Skeels (liquid gold) is an evergreen tree, endemic to southwestern Morocco. The plant can reach up to 10 meters in height and lives approximately 200-250 years. It is a multipurpose oil yielding tree and its oil is now the most expensive edible oil in the world. It is also used in cosmetics industry (Zahidi *et al*., 2014). Argan oil is rich in tocopherols of 620 mg/kg, compared to olive oil that contains only 320 mg tocopherols/kg (Guillaume and Charrouf, 2011).

Tocopherols are natural potential antioxidants and have many applications in food preservation, pharmaceutical and cosmetics industry. They have significant effect in preventing diseases, inhibiting peroxidation of acylglycerol, suppressing liver production of cholesterol, protecting against cancer, enhancing immune system and reducing cell aging (Badrhadad *et al*., 2013). Tocopherols called vitamin E and consist of a family of eight derivatives, which are related in structure, but have different biological activities and α-tocopherol is the most potent. Nowadays, most tocopherols are produced by chemical synthesis, which resulting in compounds with less biological activity compared to the natural ones, which have the highest effectiveness. Therefore, tocopherols naturally synthesized are preferred for medical purposes (Geipel *et al*., 2014).

The biosynthesis of secondary metabolites in plants is affected by environmental stresses, therefore their accumulation can be enhanced by precursors and elicitors (Zhao *et al*., 2010). Precursors are intermediate compounds in the biosynthesis pathways of secondary metabolites, and should be added to the culture media in the right stage and concentration (Gueven and Knorr, 2011). Tocopherol *in vitro* production was effectively stimulated by application of biosynthetic precursors. Tyrosine is an aromatic amino acid, which is essential for the synthesis of protein in all organisms. In plants, tyrosine is a precursor of numerous secondary metabolites of various physiological roles. Tocopherols or vitamin E are tyrosine derived plant natural products (Schenck and Maeda, 2018).

Elicitation is a useful way for the induction of biosynthetic pathways of secondary metabolite. Nanomaterials are one of the most significant inventions, especially for their stable physical properties, depending on their size (1 to 100 nm). Also, the exceptional range of nanoparticle (NP) properties is owing to their large surface area, which can be fitted for different biological functions (Wang *et al*., 2011).

Nanoparticles as elicitors have the efficiency to be applied to *in vitro* cultures for the elicitation of secondary metabolites production (Sharafi *et al*., 2013; Ghorbanpour and Hadian, 2015 and Hatami *et al.*, 2016). Although a diversity of NPs has been utilized in plant biotechnology; metallic oxide NPs are considered the safest for their significant features and stability (Pandurangan and Kim, 2013 and Javed *et al*., 2017). Many NPs were used for elicitation of the secondary products *in vitro*, such as Ag NPs, which were used to enhance artemisinin production in *Artemisia annua* (Zhang *et al*., 2013) and stevioside and rebaudioside A in Stevia (Golkar *et al*., 2019). Pb $(NO_3)_2$, AS_2O_3, $CuSO_4$ and $CdCl_2$ NPs to enhance conessine production in *Holarrhena antidysenterica* (Kumar *et al*., 2018) and CuO NPs for gymnemic acid II and phenolic compounds production in *Gymnema sylvestre* (Chung *et al*., 2019). Both titanium dioxide (TiO_2) and silicon dioxide (SiO_2) NPs gave promising results when used as elicitors for the enhancement of *in vitro* production of secondary

metabolites; such as Aloin content in *Aleo vera* that was elicited with TiO_2 NPs (Raei *et al*., 2014). Also, production of secondary metabolites in cell suspension cultures of flax (*Linum usitatissimum*) was improved using TiO_2 NPs (Karimzadeh *et al*., 2019), while rosmarinic acid was elicited in *Dracocephalum kotschyi* by SiO_2 NPs (Nourozi *et al*., 2019). Therefore, the aim of the present study was to develop a strategy to optimize the callus production from argan seedling leaves and study the effect of tyrosine (the precursor of α-tocopherol), either individually or in combination with TiO_2 and SiO_2 NPs, to increase α-tocopherol production in suspension cultures.

Materials and Methods

Callus induction

Leaves of argan were excised from seedlings germinated from seeds planted in the greenhouse of Tissue Culture Unit, Desert Research Center, Cairo, Egypt. The leaves were washed under running tap water for one hour, then surface sterilized under complete aseptic conditions in the Laminar Air Flow Hood (Holten LaminAir HVR 2448, USA). Leaves were immersed in sterile distilled water with two drops of Dettol (cloroxylenol 4.8%) for 10 min, then rinsed three times with sterile distilled water. Leaves were given a quick rinse (10 seconds) in 95% ethyl alcohol, then rinsed again three times with sterile distilled water. After that, leaves were soaked in 15% commercial bleach (Clorox containing 5.25% sodium hypochlorite) for 15 min, before rinsing for 5-6 times with sterile distilled water to remove all traces of the disinfectant.

The sterilized leaf segments were cultured under complete aseptic conditions on Murashige and Skoog (MS; Caison, USA) medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and 0.01% (w/v) myo-inositol (Fluka AG, Switzerland). Different concentrations of plant growth regulators (PGRs; min. 90%, Sigma-Aldrich, Germany); 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin and β–naphthalene acetic acid (NAA) were added to MS medium for callus induction. MS medium supplemented with 2,4-D at concentrations of 4.5, 9 and 13.5 µM was examined, either individually or in combination with 2.35 µM kinetin or 5 µM NAA or both. MS medium without PGRs served as control.

The pH of the medium was adjusted to 5.7±0.1 before adding the gelling agent (0.3% w/v phytagel; Duchefa, Haarlem, the Netherlands). The culture jars were covered with polypropylene caps and autoclaved (Harvey Sterilemax autoclave, Thermo Scientific, USA) at 121°C and a pressure of 1.1 kg/cm² for 15 min. All cultures were maintained at a temperature of 26±2°C under a photoperiod of 16 hours, with cool-white fluorescent tubes (F 140t9d/38, Toshiba) and under relative humidity of 60-65%. Percentage of callus induction (%), mean fresh weight (g), colour and texture of callus were recorded after eight weeks of culture. Subculturing of callus was carried out every eight weeks for five times.

Establishment of suspension culture and precursor feeding

Suspension cultures were initiated by transferring 5 g of fresh homogenous friable callus into 250 ml Erlenmeyer flasks containing 100 ml liquid MS medium supplemented with 3% (w/v) sucrose, 0.01% (w/v) myo-inositol, 4.5 µM 2,4-D and 5 µM NAA (phytagel free). Different concentrations (275.953, 551.907 and 1103.813 µM) of tyrosine (Alpha Chemika, India) were added to the medium as a precursor of α-tocopherol. The precursor was prepared as a concentrated stock solution and added to the cultures after filter sterilization with Millex syringe driven filter unit (0.22 µm) at the appropriate concentrations. The pH of the media was adjusted to 5.7-5.8 before autoclaving as mentioned previously. The MS medium without tyrosine served as control. Flasks were closed with cotton plugs for gas exchange and were incubated on a rotary shaker (Daihan Scientific, Korea) at 110 rpm and 25±0.5ºC in the dark. The suspension cultures were harvested at different durations (10, 20 and 30 days) and filtered with filter paper and freeze-dried by liquid nitrogen. Samples were stored in a freezer until the extraction and determination of α-tocopherol was carried out.

Quantitative determination of α-tocopherol

The accumulated α-tocopherol in argan cultures was determined by HPLC through the following steps:

α-tocopherol extraction

Extraction of α-tocopherol was carried out by protecting the operation from both light and oxidizing conditions. A weight of 5 g freeze-dried material was converted to powder by grinding, then stirred with 100 ml methanol for three hours at 25°C in the darkness. The mixture was sonicated in ultrasound bath for 40 min. After filtration and centrifugation at 5200 rpm for 20 min, the supernatant was dried using a rotary evaporator, then resuspended in 100 ml n-hexane and sonicated and centrifuged again at the same conditions. The supernatant was filtered through 0.45 µm membrane filter then dried and stored at −20°C until used (Antognoni *et al*., 2009 and Badrhadad *et al*., 2013).

Separation of α-tocopherol by HPLC

Samples of α-tocopherol were injected in High Performance Liquid Chromatography (HPLC, Ultimate 3000) according to the method of Moreno and Salvado (2000).

Separation conditions

Injected	20 µl
Column	Reversed phase C18 column 2.5× 30 cm
Mobile phase	Acetonitrile (solvent A) and methanol (solvent B) = 75: 25 (v/v)
Flow rate	1.0 ml/min
Temperature	25°C
Detection	The UV absorption spectra of the standards and samples were recorded at 230–400 nm.

Calculation of α-tocopherol

The peak area and concentration of α-tocopherol in argan samples were determined by comparing their relative retention time (min) with the standard using DELL-compatible computer supported with Cromelion7

interpretation program. The concentration of α-tocopherol was calculated as mg/100 g fresh weight.

Application of nanoparticles

Once α-tocopherol was determined by HPLC to know the best tyrosine concentration that yielded the highest α-tocopherol content, suspension cultures were taken place by the addition of different concentrations (5, 10 and 20 ppm) of TiO_2 NPs with size of about 25 nm and SiO_2 NPs of about 5-15 nm in size (Sigma-Aldrich, Germany) to MS medium supplemented with 4.5 µM 2,4-D, 5 µM NAA and 275.953 µM tyrosine (the best concentration of the precursor from the previous experiment). Nanoparticles were added after 15 days of incubation. The suspension cultures were harvested at different durations (10, 20 and 30 days), filtered with filter paper and frozen with liquid nitrogen. Samples were stored in a freezer until extraction and determination of α-tocopherol was carried out as mentioned previously.

Experimental design and statistical analysis

All experiments were subjected to completely randomized design. Variance analysis ANOVA was done using Costat software program for statistical analysis. The differences among the means of all treatments were tested for their significance at 5% level by using Duncan (1955) multiple range tests as modified by Snedecor and Cochran (1990).

Results and Discussion

Callus induction

Callus was induced from argan seedling leaf segments as shown in Table 1. Data reveal that the highest growth induction of callus (90%) was achieved on MS medium supplemented with 4.5 or 13.5 µM 2,4-D in combination with 5 µM NAA and 9 µM 2,4-D with 2.35 µM kinetin and 5 µM NAA. However, the highest mean fresh weight of callus (1.65 g) was achieved on MS medium supplemented with 4.5 µM 2,4-D in combination with 5 µM NAA and gave white friable callus (Fig. 1A). This result is supported by Lamaoui *et al*. (2019), who obtained, the highest callus rate when axillary buds of argan were cultured on half-strength MS (½ MS) medium supplemented with 4.5 µM 2,4-D and 5 µM NAA under dark conditions. Also, Koufan *et al.* (2020) found that, ½ MS medium supplemented with 4.5 µM 2,4-D and 5 µM NAA was the most efficient combination for callogenesis of argan after two months of culture, when cotyledons were used as explants. The medium supplemented with 9 µM 2,4-D in combination with 5 µM NAA ranked next concerning main callus fresh weight, that reached 1.41 g and gave also white friable callus. On the other hand, MS medium supplemented with 2,4-D individually resulted in lower callus fresh weight. Also, it is noticed from Table 1 that, the presence of 2,4-D either individually or with NAA was correlated to the white friable callus formation. The greenish compact callus was obtained when kinetin was added to medium (Fig. 1B). Callus morphology (colour and texture) was varied according to PGRs and their concentrations (Kumar and Nandi, 2015).

Table 1: The effect of different concentrations of PGRs on callus induction of argan after eight weeks of culture.

PGRs conc. (µM)			Callus Induction (%)	Fresh weight of callus (g)	Callus colour and texture
2,4-D	Kinetin	NAA			
0.0	0.00	0	0	0.00	-
4.5	0.00	0	20^{cd}	0.30^{cd}	W/F
9.0	0.00	0	40^{bc}	0.40^{cd}	W/F
13.5	0.00	0	50^{abc}	0.50^{bcd}	W/F
4.5	2.35	0	70^{ab}	0.78^{bc}	G/C
9.0	2.35	0	60^{abc}	0.86^{bc}	G/C
13.5	2.35	0	80^{ab}	1.13^{ab}	G/C
4.5	0.00	5	90^{a}	1.65^{a}	W/F
9.0	0.00	5	70^{ab}	1.41^{a}	W/F
13.5	0.00	5	90^{a}	0.60^{bcd}	W/F
4.5	2.35	5	80^{ab}	0.67^{bcd}	G/C
9.0	2.35	5	90^{a}	0.35^{cd}	G/C
13.5	2.35	5	60^{abc}	0.40^{cd}	G/C

Means followed by the same letter within a column are not significantly different at $P \leq 0.05$.
W= white G= green F= friable C= compact

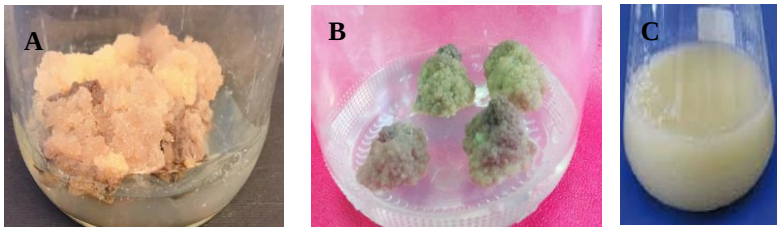


Fig. 1: Callus of argan induced from seedlings leaf explants. **(A)** White friable callus on MS medium supplemented with 4.5 µM 2,4-D and 5 µM NAA, **(B)** Greenish compact callus on MS medium supplemented with 13.5 µM 2,4-D and 2.35 µM kinetin, and **(C)** Suspension culture of callus cells.

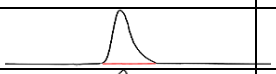
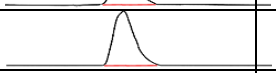
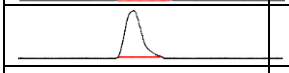
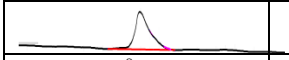
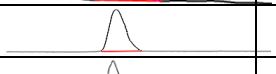
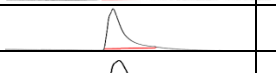
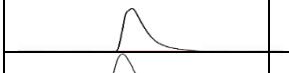
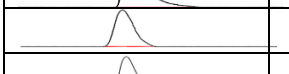
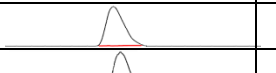
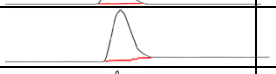
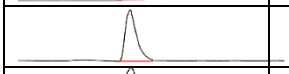
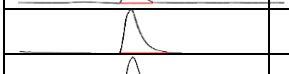
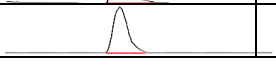
Establishment of suspension cultures

Effect of tyrosine on α-tocopherol accumulation

The PGRs combination that induced the highest callus induction percentage and fresh weight was selected for the suspension culture experiment (Fig. 1C). The effect of adding tyrosine at different concentrations (275.953, 551.907 and 1103.813 µM) in suspension cultures of argan on α-tocopherol accumulation percentage (g/100 g fresh weight of callus) was investigated as represented in Table 2. The increase of α-tocopherol percentage was achieved only at the first 10 days of culture. Tyrosine at the concentration of 275.953 µM gave the highest α-tocopherol accumulation (0.0602%) with about 2.17-fold increase compared to the control treatment (medium without tyrosine at zero time), followed by the concentration of 551.907 µM, which gave about 1.58-fold increase compared to the control medium. However, tyrosine concentration of 1103.814 µM caused a decrease in α-tocopherol with 5.56-fold at the same duration (10 days). The prolonged exposure of culture to the media either with or without tyrosine at all concentrations led to the decrease in α-tocopherol content.

Several studies on cell cultures showed that many criteria; such as specificity and concentration of precursor, time of exposure, culture conditions and cells growth stage influence the elicitation of secondary metabolites production (Wiktorowska *et al*., 2010). The positive effect of tyrosine on the *in vitro* production of secondary metabolites was reported previously by Raghavendra *et al.* (2018), who found that tyrosine at all tested concentrations (50, 100, 150, 200 and 250 mM) had a greater impact on the activity of tyrosine hydroxylase and accumulation of L–Dopa in cell suspension cultures of *Mucuna prurita* from day 3 to day 15 of exposure. Also, Gabotti *et al.* (2019) elicited the production of phenylalanine ammonia-lyase, tyrosine aminotransferase and antioxidant activities from *Cannabis sativa* by the addition of tyrosine in combination with methyl jasmonate, which showed about 51% increase after four days of feeding.

Table 2: Effect of different concentrations of tyrosine on the accumulation of α-tocopherol (g/100 g fresh weight of callus) in suspension culture of argan on MS medium supplemented with 4.5 µM 2,4-D and 5 µM NAA.

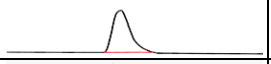
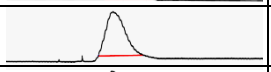
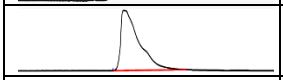
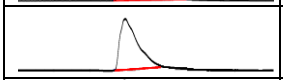
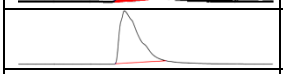
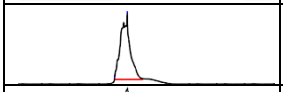
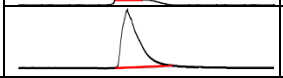
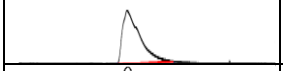
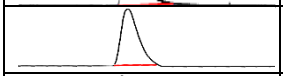
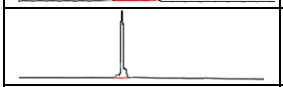
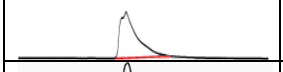
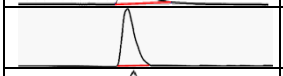
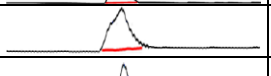
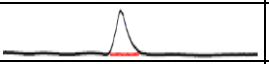
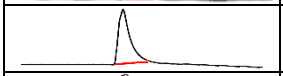
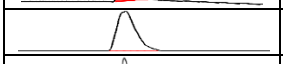
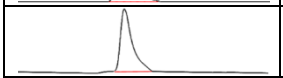
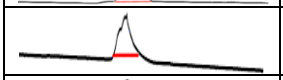
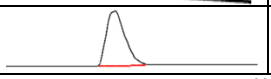
Treatments		α- tocopherol content			
Tyrosine (µM)	Duration (days)	Peaks	Conc. (%)	Increase (fold)	Decrease (fold)
0.000	0		0.0278	-	-
0.000	10		0.0286	1.0288	-
275.953			0.0602	2.1655	-
551.907			0.0440	1.5827	-
1103.814			0.0050	-	5.5600
0.000	20		0.0023	-	12.0870
275.953			0.0043	-	6.4651
551.907			0.0040	-	6.9500
1103.814			0.0037	-	7.5135
0.000	30		0.0003	-	92.6667
275.953			0.0011	-	25.2727
551.907			0.0010	-	27.8000
1103.814			0.0007	-	39.7143

Effect of nanoparticles on α-tocopherol accumulation

Data in Table 3 represent the effect of different concentrations of TiO_2 and SiO_2 NPs, as elicitors, in combination with 275.953 µM tyrosine (in liquid MS medium supplemented with 4.5 µM 2,4-D, 5 µM NAA and 275.953 µM tyrosine) on α-tocopherol accumulation in suspension cultures of argan at different durations. Nanoparticles were added to the liquid medium after 15 days of culture.

After 10 days of TiO_2 NPs application at the concentration of 5 ppm, α-tocopherol reached about 0.276% (g/100 g fresh weight of callus) with about 4.59-fold increase compared to the control (media supplemented with 275.953 µM tyrosine without NPs at zero time of exposure). Increasing TiO_2 NPs concentration decreased accumulation of α-tocopherol at each duration of exposure. Also, using SiO_2 NPs at 5 ppm caused α-tocopherol accumulation to reach about 0.283% with about 4.7-fold increase than the control, which was the best treatment for enhancing tocopherol accumulation. Although, increasing the concentration of SiO_2 NPs decreased α-tocopherol accumulation at each duration of exposure. Also, for both TiO_2 and SiO_2 NPs, increasing the duration of exposure (20 and 30 days) caused a gradual decrease in the accumulation of α-tocopherol. Its content dropped to the minimum of

Table 3: Effect different concentrations of TiO_2 and SiO_2 NPs on the accumulation of α-tocopherol (g/100 g fresh weight of callus) in suspension cultures of argan on MS medium supplemented with 4.5 µM 2,4-D, 5 µM NAA and 275.953 µM tyrosine.

Treatments			α- tocopherol content			
NPs		Duration** (days)	Peaks	Conc. (%)	Increase (fold)	Decrease (fold)
Type	Conc. (ppm)					
Control*		0		0.0602	-	-
TiO_2	5	10		0.2761	4.5864	-
	10			0.1550	2.5748	-
	20			0.0707	1.1744	-
SiO_2	5			0.2832	4.7043	-
	10			0.2550	4.2359	-
	20			0.0829	1.3771	-
TiO_2	5	20		0.0572	-	1.0524
	10			0.0235	-	2.5617
	20			0.0091	-	6.6154
SiO_2	5			0.0620	1.0300	-
	10			0.0456	-	2.1049
	20			0.0286	-	2.1678
TiO_2	5	30		0.0228	-	2.6404
	10			0.0071	-	8.4789
	20			0.0053	-	11.3585
SiO_2	5			0.0278	-	2.1655
	10			0.0201	-	2.9950
	20			0.0064	-	9.4063

*Control = without NPs at zero time of exposure, **Duration of cultures after NPs addition

0.0053 for TiO_2 and 0.0064% for SiO_2 NPs with a decrease of 11.3585 and 9.4063-fold, respectively, at 20 ppm after 30 days of exposure.

These results agree with Nourozi *et al.* (2019), who elicited rosmarinic acid content in the hairy roots of *Dracocephalum kotschyi* by SiO_2 NPs (100 ppm) after 48 h exposure time, which caused a great increment in the production of rosmarinic acid (1016.6 µg/g FW) with 8.26-fold increase, compared to the control. Also, enhancement of spinach (*Spinacia oleracea*) biomass after the treatment with TiO_2 NPs has been observed by Gao *et al.* (2008). However, Al-oubaidi and Kasid (2015) reported that, the concentrations 4.5 and 6 ppm of TiO_2 NPs caused a huge accumulation in most of the secondary metabolites from embryo callus of *Cicer arietinum*.

Navarro *et al.* (2008) reported that the response of plants to NPs may be either positive or negative, depending on the type of plant and NPs characteristics. It was found that TiO_2 NPs inhibit growth by the induction of DNA damage and rising lipid peroxidation in *Allium cepa* and *Nicotiana tabacum* at 4 and 2 mM concentrations, respectively (Ghosh *et al.*, 2010). Many studies showed that, addition of NPs to

the cell suspension cultures reduce cells viability by modifying the expression of nucleic acid; inducing damaging of DNA and cell membrane, increasing ROS production and disturbing chlorophyll synthesis (Jamshidi *et al*., 2016; Kim *et al*., 2017 and Yang *et al*., 2017).

Nanoparticles penetrating plant cells and interact with intracellular organelles and structures. They have the ability to induce stress and produce excessive reactive oxygen species (ROS), which subsequently affect cell components/molecules, DNA, proteins, carbohydrates, lipids and secondary metabolites in plants (Hossain *et al*., 2015). Studies on the mechanism of NPs uptake, binding sites and transportation in plant cells have also led to more understanding of the elicitation evidences of these NPs for improving *in vitro* secondary metabolite production (Sharafi *et al*., 2013).

It is worth to mention that α-tocopherol was elicited in many other plants by *in vitro* suspension culture, but with lower amounts and increase than that obtained in the present study. For instance, Chong *et al.* (2004) found that, α-tocopherol content in *Morinda elliptica* was increased about 40–50 mg/100 g fresh weight in the 7th day of culture and reduced to 5–15 mg/100 g fresh weight on the days 14 and 21. Gala *et al.* (2005) enhanced α-tocopherol accumulation by adding 5 µM jasmonic acid to the culture medium in both *Arabidopsis* and sunflower cell cultures, in which α-tocopherol was increased to 49 and 66%, respectively, after a period of 72 h and decreased with long durations in cell suspension cultures. Moreover, Antognoni *et al.* (2009) increase α-tocopherol to 5.96 mg/100 g in *Amaranthus caudatus* and *Chenopodium quinoa* using methyl jasmonate. Badrhadad *et al.* (2013) elicited α-tocopherol production in *Elaeagnus angustifolia* with jasmonic acid and salicylic acid and reached 0.1964 mg/100 g. Harish *et al.* (2013) mentioned that, homogentisic acid and phytol were used to increase α-tocopherol content (3.64 mg/100 g) through cell suspension culture in tobacco. Also, Cetin *et al.* (2014) elicited α-tocopherol production in cell suspension culture of *Vitis vinifera* using cadmium chloride, where the increase in α-tocopherol production reached 0.126 mg/100 g.

Conclusion

Argan suspension culture represents one of the effective tools for the enhancement of α-tocopherol production *in vitro*. The present study reveals that tyrosine as a precursor of α-tocopherol and SiO_2 and TiO_2 NPs as elicitors found to have significant effects on improving α-tocopherol production in argan suspension cultures. Nanoparticles application in combination with tyrosine showed a significant enhancement in α-tocopherol accumulation compared to using tyrosine individually. The maximum increase in α-tocopherol content was achieved with about 4.7-fold by adding SiO_2 NPs at 5 ppm with 275.953 µM tyrosine after 10 days of exposure. Therefore, NPs application offers a new strategy for the elicitation of secondary metabolites *in vitro* production.

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