IMPROVING DATE PALM (Phoenix dactylifera L. CV. ESTAMARAN) CALOGENESIS BY THE USE OF ZINC OXIDE NANOPARTICLES

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ABSTRACT

To study the effects of zinc nanoparticles on date palm cv. Estamaran (Sayer) callus induction under in vitro condition, a research with two separate experiments was conducted using a completely randomized design. In the first experiment, MS basal salt medium containing 100 mg/l 2,4-D, 3 mg/l 2ip, 3 g/l activated charcoal, 30 g/l sucrose, 40 mg/l adenine sulfate, and 1 mg/l biotin along with different concentrations of zinc sulfate (ZnSO₄), including 0, 2.43, 4.86 mg/l or zinc oxide nanoparticles (ZnO NPs), including 0, 2.43, 4.86, 7.29 mg/l, were used. While in the second experiment, the effect of various medium strengths including full strength MS, ½ MS, and ¼ MS was examined on callus formation. At the end of each experiment, total explants induced callus, embryogenic callus formation percentage, and callus fresh and dry weight were evaluated. Results of the first experiment showed that the best callus induction and callus fresh and dry weight belong to MS medium containing 2.43 mg/l ZnO NPs. Further, higher ZnO NPs concentrations had negative effects on callus formation. However, results of the second experiment indicated that highest callus formation and callus fresh and dry weight are obtained on full strength MS basal salt medium.

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1 Introduction

Date palm (Phoenix dactylifera L.) is a monocot, perennial and dioeciously plant which belongs to the Aricaceae family (Zaid, 1999). Traditional method of date palm propagation is through offshoots. However, various limitation such as very slow growth, less number of offshoots in mother palm trees (Sharma, 1990) and has the risk of contamination of pests and emergence of diseases (Gueye et al., 2009). Therefore, tissue culture technique or in vitro regeneration can be used as an alternative method for date palm mass propagation. Micropropagation of date palm is generally performed by two different methods, somatic embryogenesis and direct organogenesis. In embryogenesis method, vegetative embryos are produced from embryogenic callus. In this technique, date palm meristem is cultured as explants on the medium containing high concentrations of auxin, which consequently results in the callus formation. The second method is more common and practical (Al-Khatteeb, 2008; Othmani et al., 2009; Khan & BiBi 2012). However, direct organogenesis has some limitations such as fewer plantlets regeneration from an explant.

Among indirect embryogenesis processes, callus induction is very difficult because the date palm is a monoeccious, perennial woody plant (Gueye et al., 2009). Therefore, need of a suitable and optimized protocol in order to prepare a nutrient medium for date palm callus induction by the researchers, it would be very helpful for large scale date palm propagation. To date, many researchers have conducted numerous studies on in vitro date palm callus induction. Badawy et al. (2009), used shoot tip of date palm cv. Sakooti for the production of callus on MS medium supplemented with 100 mg/l 2,4-D, 3 mg/l 2ip and 2 g/l activated charcoal for 9 months, and subculture each after 6 weeks intervals. A similar study was reported for induction of embryogenic callus in date palm cv. Karama, using an MS medium containing 100 mg/l 2,4-D and 3 mg/l 2ip with activated charcoal (Gabr & Abd-Alla 2010). Al–Kaabiy et al. (2011), during their study on the effect of water stress on callus induction from shoot tip of date palm cv. Barhee, showed that application of polyethylene glycol to the medium caused significant increase in callus fresh weight.

The use of nanotechnology in different fields of science is increasingly growing in popularity day by day. On the basis of nanotechnology, some metals such as zinc oxide-based nanoparticles increase the permeability and create new holes in the cell wall of bacteria. The application of nanoparticles is an easy way to study plant cells, as they can increase transfer into the plant cell (Sondi & Salopek-Sondi, 2004; Brayner et al., 2006). Stampoulis & Sinha (2009) studied the effects of five nanomaterials (multiwalled carbon nanotubes [MWCNTs], Ag, Cu, ZnO, Si) and their corresponding bulk counterparts on seed germination, root elongation, and biomass of Cucurbita pepo (zucchini) and reported that zinc oxide nanoparticles have no negative effects on the variables under study. However, Lin & Xing (2007) reported that zinc oxide nanoparticles and nano zinc were dissuasive to the germination of rye and corn seeds. Rye grass plants were planted in zinc oxide nanoparticles and zinc ions (Zn²⁺) feeding medium and it was observed that zinc oxide nanoparticles and zinc ions had toxic effects at high concentration level (Lin & Xing 2008). Among the important factors which directly influencing in vitro explants establishment and callus induction, macronutrient concentration and media strength are most important one (Alturki et al., 2013). This study aimed to assess the effects of zinc oxide nanoparticles and MS medium strength (full, 1/2 and 1/4 of macronutrients concentration) on improve callus induction stage of date palm cv. Estamaran (Sayer) under in vitro condition.

2 Material and Methods

This study was carried out at Tissue Culture Laboratory of Department of Horticulture, Shahid Chamran University, Ahwaz.

2.1 Plant material

Explants were collected from 3-4 year old date palm offshoot cv. Estamaran (Sayer) which was obtained from Date Palm and Tropical Fruits Research Institute of Iran, Ahwaz. After selecting the mother palm, their offshoots were carefully removed. Then, the leaves were cut by a knife one after another, from the outermost leaves clinging to the offshoot. Finally, the remained sample with a dimension of 8 × 3 cm containing the shoot tip was rapidly transferred to a cold antioxidant solution containing 100 mg/l ascorbic acid and 150 mg/l citric acid and held there until the sterilization of the surface.

2.2 Surface sterilization the explants

Prior to dividing the shoot tip into smaller pieces and culture on callus induction medium, surface sterilization was carried out to eliminate contamination agents. For sterilization under a sterile hood, the samples were removed from the antioxidant solution, disinfected by a 2.5% calcium hypochlorite solution containing a few drops of Tween 20, and strongly hand shaken for 20 minutes followed by being washed three times with sterile distilled water. After sterilization, the date palm shoot tip was divided into smaller explants. Each 3 explants were cultured in a single petri dish.

2.3 Nano Zinc Oxide treatment

In this experiment, different concentrations of ZnO NPs and ZnSO₄ were added to an MS medium. The ZnO NPs were in form of white yellowish powder with purity of 99.8% and dimension of 6-12 nanometers, which were purchased from Nano Pars Spadana Company. For preparation of homogenous suspension of nanoparticles in water, ultrasonic (100 watt, 40 k hrtz in 35 °C) was used for 40 min.
For preparation of the MS medium with different concentrations of ZnO NPs, the nanoparticles were separately added to an MS basal salt medium supplemented with 3 mg/l 2ip, 100 mg/l 2,4-D, 40 mg/l adenine sulphate, 3 g/l activated charcoal, 30 g/l sucrose, 1 mg/l biotin and gently shaken or stirred. The pH was adjusted on 5.7, before adding 7.5 g agar, and autoclaved in 121°C under 15 psi pressure for 15 min. The treatments included MS medium without zinc (control), MS medium with pure zinc concentrations of 2.43 and 4.86 mg/l from ZnSO₄, and an MS medium with ZnO NPs in different pure zinc concentrations of 2.43, 4.86 and 7.29 mg/l. This study was conducted by using a completely randomized design with 3 replications.

2.4 Media strength treatment

This experiment was conducted to determine the effect of basal salt concentration (ionic strength) of the medium on callus formation of date palm explants under the in vitro condition with three treatments, including full, half and quarter MS medium (MS, ½ MS, and ¼ MS) by using a completely randomized design with three replications. To provide ½ MS and ¼ MS, the macro salts were added to the medium in ½ strength and ¼ strength, respectively.

2.5 Parameters tested

In the both experiments, the measured factors included percentage of explants that produced callus, callus type (friable or compact), and callus fresh and dry weight. After the explants were cultured on callus induction medium and sealed by parafilm, they were transferred to the growth chamber in the darkness at 27 ± 1°C.

2.6 Statistical analysis:

The data were analyzed using SAS software. The means were compared by the Duncan's test and charts were drawn by Excel.

3 Results

3.1 Effect of ZnO NPs on total callus induction

The ZnO NPs and ZnSO₄ concentrations had significant effects on the percentage of total callus induction. Fig. 1(a) showed the comparison of means of zinc compounds at different concentrations on total callus induction of date palm cv. Estamaran (Sayer). According to the obtained results, the highest and lowest total callus was induced by 2.43 mg/l ZnO NPs treatment (80.95%) and 7.29 mg/l ZnO NPs treatment (18.09%), respectively.

3.2 Effect of ZnO NPs on embryogenic callus induction

In this experiment, the ZnO NPs and ZnSO₄ concentrations also significantly affected the percentage of embryogenic callus induction at 0.05 probability level. Fig. 1(b) shows the comparison of means of zinc compounds at different concentrations on embryogenic callus induction of date palm cv. Estamaran (Sayer). The results indicated that the highest amount of embryogenic callus was induced when using 2.43 mg/l ZnO NPs (51.61%), whereas the lowest amount of embryogenesis callus was obtained when using 7.29 mg/l ZnO NPs (14.28%).

3.3 Effect of ZnO NPs on type of callus

No significant effects of the ZnO NPs and ZnSO₄ concentrations was reported on the type of callus (compact vs friable) at 0.05 level (P<0.05).

3.4 Effect of ZnO NPs on callus fresh and dry weight

The ZnO NPs and ZnSO₄ concentrations have also significant effects on callus fresh and dry weight. Both the highest callus fresh weight (1.6615 g) and dry weight (0.1613 g) belonged to the MS medium containing 2.43 mg/l ZnO NPs that has significant differences with other concentrations (Figs. 1(c) and (d)). While both the lowest fresh weight (0.1867 g) and dry weight (0.0312 g) belonged to the MS medium containing 7.29 mg/l ZnO NPs.

3.5 Effect of medium strength on total callus induction

The medium strength (full MS, ½ MS and ¼ MS) has a significant impact on total callus induction of date palm cv. Estamaran (Sayer) explants. Fig. 2(a) showed the means comparison of medium strength effects on total induced callus percentage. According to the results, the highest and lowest callus percentage was obtained in the full strength MS with the mean of 57.14% and in the ¼ MS with the mean of 9.52%, respectively.

3.6 Effect of medium strength on embryogenic callus production

The medium strength significantly influenced the embryogenic callus production. Fig. 2(b) showed the means comparison of medium strength effects on embryogenesis callus production percentage. It was observed that the highest and lowest embryogenesis callus was obtained in the full MS medium with the mean of 38.09% and in the ¼ MS medium with the mean of 0%, respectively.

3.7 Effect of medium strength on compact callus induction

The MS medium strength did not significantly influence the compact callus production.

3.8 Effect of medium strength on callus fresh and dry weight

The results indicate that the callus fresh and dry weight was significantly affected by changes in the medium strength. Figures 2(c) and 2(d) indicate the mean comparison of callus fresh and dry weight in different medium strengths.
Figure 1 Effect of different Zinc type and concentration on date palm total callus induction (a) embryogenic callus (b) callus fresh weight (c) and callus dry weight (d) \textit{in vitro} condition.

Figure 2 Effect of different medium strength on date palm total callus induction (a); embryogenic callus (b); callus fresh weight (c) and callus dry weight (d) \textit{in vitro} condition.
Based on the findings, both the highest callus fresh weight with the mean of 0.7247 g and the highest callus dry weight with the mean of 0.1072 g were found in the MS medium and both the lowest callus fresh weight with the mean of 0.1036 g and the lowest callus dry weight with the mean of 0.0150 g were found in the ¼ MS medium.

4 Discussions

The results demonstrated that ZnO NPs were more effective on date palm callus production under in vitro condition as compared to bulk Zn (ZnSO₄). There are a few reports on the effect of ZnO NPs on the callus production of plants under the in vitro condition. Goue et al. (2013) found that, at certain concentration of ZnO nanoparticle (0.025 ml/l), optimum in vitro growth of Cayratia trifolia callus was observed.

ZnO NPs may have different effects on plant growth. Generally, ZnO NPs in low concentrations have positive effect on growth parameters. However, they may become toxic at high concentrations. The Moringa peregrine plants sprayed with Hogland solution containing ZnO NPs showed an increase in growth parameters either under saline or normal condition (Soliman et al., 2015). Further, Alharby et al. (2016) showed that the ZnO NPs could mitigate salt stress in tomato callus culture in vitro at concentration 15 mg/l. Du et al. (2011) found that ZnO NPs have higher solubility than TiO₂ NPs, dissolved in the soil, and increased the root wheat uptake of excessive zinc.

Zinc as an essential element belongs to micronutrients group (Jammi et al., 2009) and due to having nutritional values, is needed for living organisms' cells. Furthermore, Zn acts as enzymes cofactor and is involved in protein binding, transcriptional and translational regulation, enzyme activity, and signal transduction (Zhang et al., 2005). However, despite its nutritional values, Zn, like other metals, can be toxic depending on its concentration, exposure time, and plant genotype (Chowdhuri et al., 2004). In the in vitro condition, the explant directly contacts the medium containing Zn due to lack of root system; therefore, they may act the same as heavy metal sensitive plants which cannot keep metals out of their roots (Chang et al., 2012). In this situation, the Zn concentration and source type (bulk or nanosized) may play a critical role. The results obtained in this study showed that ZnO NPs were more effective on callus production compared to bulk Zn. Nano sized ZnO particles have large surface areas (Yang & Xie, 2006; Borm et al., 2006) and high reactive activity and electronic density (Pisanic et al., 2006), compared to bulk Zn. Therefore, they may have more ability to interact with solvent molecules. Moreover, ZnO NPs show faster dissolution and higher solubility than bulk Zn (Yang & Xie, 2006; Borm et al., 2006). They dissolve in the extracellular region, and increase the level of intracellular [Zn²⁺] (Pandurangan & Kim, 2015). In this study, ZnO NPs in the medium interacted with explant tissue biomolecules and prevented those with negative effects on callus induction.

The results also showed that incorporation of 2.43 mg/l ZnO NPs in culture medium was the best treatment for callus production, as compared to either lower or higher concentrations. The increase of callus production in 2.43 mg/l ZnO NPs may be due to bioavailability of Zn element at appropriate concentration which was sufficient for nourishing the tissue.

In high concentrations of ZnO NPs, increase of the superoxide radical (O²⁻) formation and ROS accumulation can take place in cells which lead to oxidative stress (De Berardis et al., 2010). High oxidative stress may also lead to the modification of lipids, nucleic acids, and proteins. Binding the proteins in ZnO NPs is a major change which leads to unfolding behavior. Finally, by increasing the ROS production, DNA may be damaged or intracellular Ca²⁺ may be released, each of which causes mitochondrial perturbation and cell death (Xia et al., 2008).

In relation to medium ionic strength, the callus induction and growth were found to be decreased with the reduction in the MS medium salt concentration. Moreover, the mineral element concentration has also been reported to be effective on date palm callus regeneration (Shasany et al., 1998; Bhat et al., 2002). Alturki et al. (2013) studied 3 date palm cultivars (Khalas, Ruziz and Shishi) in 4 mediums (MS, ¾ MS, ½ MS, and ¼ MS) and reported a reduction in explants growth and callus induction with the decrease in medium strength.

This may be due to the deficiency in mineral elements. In fact, with MS medium salt concentration reduction to ¼MS, the medium becomes poor in terms of macronutrient elements and thus, explants lose their growth ability. Kazemiani et al. (2012) reported that a high concentration of minerals is essential for callus induction and growth of potato cv. Agra explant, the results of which correspond to the results obtained in this study. It seems that interaction of minerals nutrients at higher concentration has no negative effects on callus induction and growth. It also seems that, there is a negative correlation between medium strength and secondary metabolites accumulation in date palm tissue. This is may be due to the fact that MS mainly improved primary metabolites and cell growth and thereby, resulted in the callus growth and a reduction in browning (Alturki et al., 2013).

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.
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