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Research Article
Impact of Antioxidants on *in vitro* Rooting and Acclimatization of Two Egyptian Dry Date Palm Cultivars

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Abstract

Background and Objectives: Date palm (*Phoenix dactylifera* L.) is propagated vegetatively via offshoots, which is limited by either the offshoots numbers produced from a superior selected plant, or the occurrence of these offshoots only during the juvenile phase of the date palm life cycle. As a result, the *in vitro* propagation could be considered an alternative technique for large scale propagation of date palm. Obtaining well-developed root system is considered the most important step in establishment of reliable protocol for successful production of date palm and subsequently successful acclimatization of the *in vitro* derived plants. The aim of this study is to obtain a well-developed root system through using different antioxidants, with detecting the similarity between the *in vitro* derived plants and the mother plants using RAPD, long RAPD and ISSR techniques. **Materials and Methods:** Individual plantlets obtained from maturation of somatic embryos *in vitro* of about 5-7 cm in length with 2-3 leaves were used as plant material. Plantlets were cultured on half strength MS liquid medium supplemented with 0.5 mg L^{-1} thiamine-HCl+2.0 mg L^{-1} glycine+0.1 mg L^{-1} biotin+40 g L^{-1} sucrose+ 0.1mg L^{-1} NAA with different concentrations from either $AgNO_3$ or citric or ascorbic acids (0.0, 0.5, 1, 2 mg L^{-1}). Growth development, root number and root length (cm) were evaluated at the end of the second subculture (12 weeks). Data were reported as Mean±Standard deviation (SD). Data were subjected to one way-analysis of variance ($p<0.05$). Results were processed by Excel (2010). **Results:** Among the different antioxidants with different concentrations used, generally it was found that 2 mg L^{-1} of each agent gave the highest values of growth development, roots number and roots length. However, using 2 mg L^{-1} $AgNO_3$ gave the best results with all parameters. Regarding the response of date palm cultivar, it was remarkable that Bartamoda showed relatively better results than Sakkoty cultivar. According to PCR reactions, the results of RAPD, long RAPD and ISSR profile of tissue culture-derived plantlets grown on a medium supplemented with 2 mg L^{-1} $AgNO_3$ obviously revealed high similarity to mother plants. **Conclusion:** It could be concluded that using 2 mg L^{-1} $AgNO_3$ gave the best results for growth development, root numbers and length of the two cultivars but Bartamoda showed relatively better results than Sakkoty cultivar. The tissue culture-derived plantlets on this medium (2 mg L^{-1} $AgNO_3$) revealed high similarity to mother plant as a result to RAPD, long RAPD and ISSR profiles.

Key words: Egyptian date palm, silver nitrate, ascorbic and citric acids, *in vitro* rooting, acclimatization, RAPD, long RAPD and ISSR

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), is an economically important commodity, it is an monocotyledonous tree widely in arid regions of the Middle east, North Africa and is the major plantation crop in some Arabic countries[1]. Date palm is a fruit tree that can afford diverse climatic conditions especially in arid regions of the Middle East and North Africa. The date fruit have high nutritional value and possess also different medicinal values[2]. It has different beneficial effects on human health, the phenolic compounds in it act as anti-cancer and protecting from cardiovascular diseases[3]. The annual international market including import and export of the date palm reached about 1.9 billion USD according to FAO[4], from which it is obvious that it may be a cornerstone to support and strengthen the economy of any country. Date palms are propagated vegetatively via offshoots, which is limited by either the numbers of offshoots produced from a superior selected plant, or the occurrence of these offshoots only during the juvenile phase of the life cycle of date palm[5]. As a result, the *in vitro* propagation is increasingly becoming an attractive alternative technique for large scale propagation of date palm.

Development of well root system *in vitro* is considered the most important step in establishment of reliable protocol for successful production of date palm through tissue culture technique. In this context[6-7] reported that development of root system and vascular elements, as revealed by anatomical investigation, may significantly affect *in vitro* rooting and *ex vitro* acclimatization of tissue culture derived date palm plantlets. They suggested that successful acclimatization of date palm *in vitro* derived plants starts from succeeding in establishment of well-developed root system *in vitro* as adaptation or pre acclimatization starts *in vitro*[8].

To improve the low survival percentage of *in vitro* derived date palm plantlets during acclimatization stage, several trials was examined. It is well known that ethylene accumulate during plant incubation in tissue culture technique, which decrease plant growth[9]. Silver ion (Ag^+), which is added to the *in vitro* medium either in the form of silver nitrate ($AgNO_3$) or as silver thiosulfate (STS), can act as an inhibitor for ethylene action[10]. In addition it was found that Ag^+ promotes IAA which can influence *in vitro* root emergence and can improve rooting efficiency[11]. In this respect, addition of $AgNO_3$ to the culture media greatly improved the regeneration of both dicot and monocot plant tissue cultures through its positive effect on shoot multiplication, root formation and flowering *in vitro*[12-17].

Auxin appears to be the primary factor controlling growth and morphology of roots. Some scientists suggest that rooting of micropropagated plants can be improved by treatment with antioxidants *in vitro*[18]. Antioxidants can potentially protect the natural plant rooting hormones from oxidation, enhancing rooting and increasing the tolerance of plants to greenhouse conditions[19]. The biosynthesis of L-ascorbic acid (Vitamin C) ASC and its roles as cellular antioxidant, stress response factor and enzyme co-factor have been the subject of many reviews over the past years[20-24].

As mentioned before, the date palm is propagated vegetatively through off-shoots which is limited with some obstacles so tissue culture may offer a solution, one of the most important step in the establishment of reliable tissue culture protocol for date palm is the rooting stage.

In this study the *in vitro* derived shoots of 2 Egyptian date palm cultivars (Bartamoda and Sakkoty) were sub cultured on modified MS-medium containing different concentrations of three antioxidants i.e., silver nitrate ($AgNO_3$), Ascorbic and citric acids aiming to obtain well developed root system *in vitro* and subsequently successful acclimatization of *in vitro* derived plants. RAPD, long RAPD and ISSR profile of tissue culture-derived plantlets were performed and compare it with the mother plant.

MATERIALS AND METHODS

This study was carried out in Plant Biotechnology Department, National Research Centre, Giza, Egypt. During the period from January, 2016 till December, 2018.

Plant material: Slices of sterilized shoot-tips were initially cultured on a medium consisting of MS[25] medium supplemented with 40 mg L^{-1} adenine sulfate+3.0 mg L^{-1} 2-isopentenyl adenine (2iP)+10.0 mg L^{-1} 2,4-dichlorophenoxy acetic acid (2,4-D)+30 g L^{-1} sucrose+170 mg L^{-1} sodium di-hydrogen orthophosphate (NaH_2PO_4)+2.0 g L^{-1} activated charcoal+8.0 g L^{-1} agar. Cultures were incubated at 26±2°C under complete darkness for 8 month with re-culturing on MS medium supplemented with 200 mg L^{-1} glutamine+ 70 mg L^{-1} NaH_2PO_4 without growth regulators to obtain mature embryos according to Sharma *et al.*[26]. Mature embryos were sub-cultured onto the same previous medium in addition to 0.1mg L^{-1} NAA to obtain germinated embryos according to Mater[27] for at least four subcultures. Individual plantlets of about 5-7 cm in length with 2-3 leaves were used as plant material.

Growth development

Effect of different antioxidants on growth development of the 2 dry date palm cultivars (Bartamoda and Sakkoty): Plantlets were cultured on half strength MS liquid medium supplemented with 0.5 mg L^{-1} thiamine-HCl+2.0 mg L^{-1} glycine+0.1 mg L^{-1} biotin+40 g L^{-1} sucrose+0.1 mg L^{-1} NAA with different concentrations from either $AgNO_3$, citric or ascorbic acids (0.0, 0.5, 1 and 2 mg L^{-1}).

The pH was adjusted to 5.7-5.8 with adding few drops of either 0.1 NaOH or 0.1 HCl. The media were dispensed into culture tubes (28×250 mL). The culture vessels were immediately capped with polypropylene closure then were autoclaved at 1.1 kg cm^{-2} at 121°C for 20 min. Plantlets were cultured on culture tubes and incubated at 26±2°C for 12 weeks (two subcultures) in growth room under 16 h illumination of 3000 lux white fluorescent lamps. Each treatment consists of three replicates. After 12 weeks of culturing, the following data were recorded

Growth development: Growth development was evaluated at the end of the second subculture (12 weeks) through determining fresh weight of the whole plant in grams.

Rooting stage

Effect of different antioxidants on rooting of the 2 dry date palm cultivars (Bartamoda and Sakkoty): Plantlets cultured on half strength MS liquid medium supplemented with 0.5 mg L^{-1} thiamine-HCl+2.0 mg L^{-1} glycine+0.1 mg L^{-1} biotin+40 g L^{-1} sucrose+0.1 mg L^{-1} NAA with different concentrations from either $AgNO_3$, citric or ascorbic acids (0.0, 0.5, 1 and 2 mg L^{-1}) for 2 sub cultures (12 weeks) and then the following data were recorded:

- Root number
- Root length (cm)

Adaptation stage: For adaptation of the 2 dry date palm cvs. Bartamoda and Sakkoty, 20 plantlets derived from the previous rooting stage about (12-14 cm in length) were rinsed thoroughly with tap water to remove the agar from roots and then immersed in 0.5% (w/v) benlate. Plantlets were planted in plastic pots (torpedo) (5×18 cm) containing mixture of peat moss, vermiculite and washed sand at equal volume (1:1:1 v/v/v).

Molecular analysis

DNA extraction: CTAB extraction buffer [CTAB (2%), EDTA (10 mM), Tris-HCl (50 mM), pH 8.0 and poly vinyl pyrrolidone (PVP, 1%), LiCl (4 M)] as described by Doyle and Doyle[28].

Polymerase chain reaction (PCR)

Random amplified polymorphic DNA (RAPD): RAPD amplification was carried out according to the procedure described by Hussein *et al.*[29]. PCR amplification was performed in 0.1 mL reaction mixture containing 20 ng template DNA, 0.5 unit Taq polymerase (Promega. USA), 200 lM each of dATP, dCTP, dGTP, dTTP. 10 pmol random primers as shown:

Primer	M09	D05
Primer sequence	GTCTTGCGGA	TGAGCGGACA

The PCR products were separated on100 mL agarose gel solution 1.5% in 1×TBE buffer containing 5 µg of red safe for about 2 h at 80 V. Gel was photographed under UVlight with Tracktel GDS-2 gel documentation system.

Long rapid amplified polymorphic DNA (LRAPD): The main difference between RAPD and LRAPD is the primer length. PCR amplification was performed in 0.1 mL reaction mixture containing 20 ng template DNA, 0.5 unit Taq polymerase (Promega. USA), 200 lM each of dATP, dCTP, dGTP, dTTP. 10 pmol random primers as shown:

Primer	AM05	AM09
Primer sequence	CACACACACACACAG	ATGTGTTGTCTGGCTTGGTA

The PCR products were separated on100 mL agarose gel solution 1.5% in 1×TBE buffer containing 5 µg of red safe for about 2 h at 80 V. Gel was photographed under UVlight with Tracktel GDS-2 gel documentation system.

ISSR (inter simple sequence repeat) analysis: The PCR reactions were prepared by using 50 ng of genomic DNA, 1×PCR buffer, 200 µM dNTP, 2 mM $MgCl_2$ and 2 units of ampliTaq polymerase (RTS-Taq DNA polymerase) and 15 ng ISSR primers. The following temperature profile was used for amplification: 94°C for 5 min followed by 45PCR cycles of 94°C for 1 min, 49°C for 45 sec and 72°C for 2 min. A final extension step of 7 min at 72°C was also carried out according to Lei *et al.*[30]. ISSRs primers were designed and screened for PCR amplification as follow:

Primer	HB10	HB08
Primer sequence	GAGAGAGAGAGACC	GAGAGAGAGAGAGG

The PCR products were separated on100 mL agarose gel solution 1.5% in 1×TBE buffer containing 5 µg of red safe for about 2 h at 80 V. Gel was photographed under UV light with Tracktel GDS-2 gel documentation system.

Statistical analysis: Each treatment consists of three replicates. Data were reported as Mean±standard deviation (SD). Data were subjected to one way-analysis of variance ($p<0.05$). Results were processed by Excel (2010).

RESULTS

The present study showed that the different concentrations of $AgNO_3$, citric and ascorbic acids (0.5, 1, 2 and 3 mg L^{-1}) affected growth development, root number and length of the two dry Egyptian date palm cv. Bartamoda and Sakkoty as shown in Table 1-3 and Fig. 1.

Effect of different antioxidants on growth development of the 2 dry date palm cultivars (Bartamoda and Sakkoty): Data presented in Table 1 showed the effect of different antioxidants on growth development, data declared that there is a statistically significant difference among the two dry cultivars used especially with using $AgNO_3$ since Bartamoda gave higher results than Sakkoty. Plantlet cultured on medium supplemented with $AgNO_3$ generally showed a higher values of growth development compared with those cultured on citric or ascorbic acids.

However, adding 2.0 mg L^{-1} $AgNO_3$ to the medium produced the highest value of growth development (4.33 and 3.33 g) in the two dry cultivars (Bartamoda and Sakkoty) respectively compared with the other treatments. On the other hand, ascorbic acid showed better effect on growth development than citric acid. Also data showed that there were no significant differences between the two dry cultivars regarding ascorbic and citric acids treatments.

Rooting stage

Effect of different antioxidants on roots number of the 2 dry date palm cultivars (Bartamoda and Sakkoty): Data in Table 2 showed the effect of different antioxidants on roots number/plantlet of grown date palm plantlets. Data declared that using $AgNO_3$ at 1.0 or 2.0 mg L^{-1} increased significantly roots number/plantlet as results (4.33, 5.0, 4.0 and 5.0 roots/plantlet) in the 2 dry cultivars Bartamoda and Sakkoty respectively compared with other treatments. Also data showed that there were no significant differences between citric and ascorbic acids treatments (Fig. 1).

Using 1.0 mg L^{-1} was the best concentration compared with the other concentrations of citric and ascorbic in case of Bartamoda cultivar. While, using 0.5 mg L^{-1} gave the higher roots number/plantlet in case of Sakkoty cultivar.

Table 1: Effect of silver nitrate, ascorbic or citric acids added to the rooting medium on growth development (fresh weight in (g)) of plantlet of the 2 cultivars (Bartamoda and Sakkoty)

	Growth development (fresh weight (g))	
Treatments	Bartamoda	Sakkoty
Silver nitrate (mg L^{-1})		
0.0	1.00±0.2	1.00±0.26
0.5	3.67±0.75	2.67±0.15
1.0	4.33±0.50	2.33±0.15
2.0	4.33±0.51	3.33±0.56
Ascorbic acid (mg L^{-1})		
0.0	1.00±0.26	1.00±0.26
0.5	2.67±0.35	2.67±0.25
1.0	3.00±0.20	2.33±0.40
2.0	2.00±0.26	2.33±0.25
Citric acid (mg L^{-1})		
0.0	1.00±0.36	1.33±0.05
0.5	1.33±0.15	1.33±0.20
1.0	1.33±0.32	2.33±0.50
2.0	2.00±0.10	2.00±0.30

Data represent Mean±SD

Table 2: Effect of silver nitrate, ascorbic or citric acids added to the rooting medium on roots number/plantlet of the 2 cultivars (Bartamoda and Sakkoty)

	Roots number/plantlet	
Treatments	Bartamoda	Sakkoty
Silver nitrate (mg L^{-1})		
0.0	1.00±0.0	2.00±1.0
0.5	3.66±0.57	3.30±1.52
1.0	4.33±0.57	4.00±1.0
2.0	5.00±1.0	5.00±1.0
Ascorbic acid (mg L^{-1})		
0.0	1.00±0.0	2.00±1.0
0.5	3.33±0.57	3.66±0.57
1.0	3.66±0.57	3.00±1.0
2.0	2.66±0.57	2.33±0.57
Citric acid (mg L^{-1})		
0.0	1.00±0.0	2.00±1.0
0.5	3.33±0.57	3.66±1.15
1.0	3.66±0.57	3.00±1.0
2.0	2.66±0.57	2.33±0.57

Data represent Mean±SD

Effect of different antioxidants on root length (cm) of the 2 dry date palm cultivars (Bartamoda and Sakkoty): Data presented in Table 3, showed the effect of different antioxidants on root length. Data obviously declared that adding $AgNO_3$ to the rooting medium increased significantly root length of date palm plantlets. Among the different concentrations of $AgNO_3$ it was found that using 2.0 mg L^{-1} increased significantly root length which recorded (6.44 and 6.67 cm) in Bartamoda and Sakkoty respectively, compared with other treatments. Also data showed that there were no significant differences reported between citric and ascorbic acids treatments.

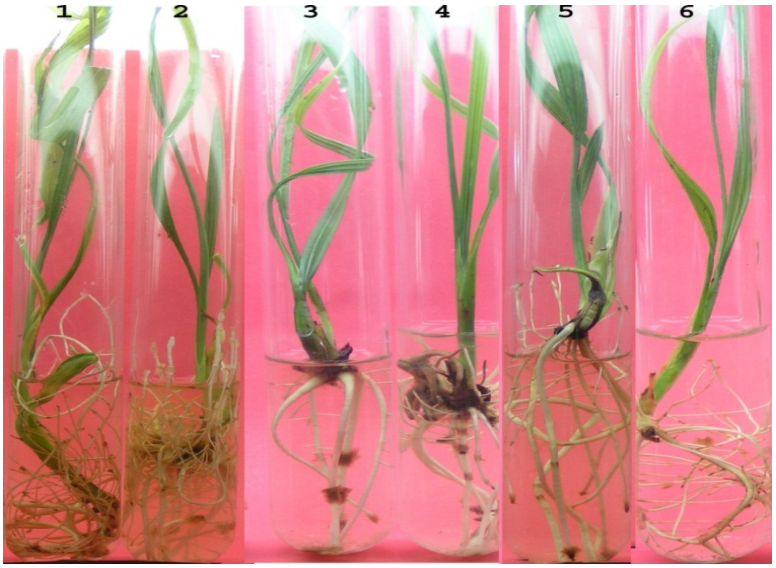

Fig. 1: Effect of 2.0 mg L^{-1} $AgNO_3$, citric and ascorbic acids added in the liquid media of the 2 dry date palm cultivars (Bartamoda and Sakkoty)

1: Bartamoda cultured on 2.0 mg L^{-1} $AgNO_3$, 2: Sakkoty cultured on 2.0 mg L^{-1} $AgNO_3$, 3: Bartamoda cultured on 2.0 mg L^{-1} ascorbic acid, 4: Sakkoty cultured on 2.0 mg L^{-1} ascorbic acid, 5: Bartamoda cultured on 2.0 mg L^{-1} citric acid, 6: Sakkoty cultured on 2.0 mg L^{-1} citric acid

Table 3: Effect of silver nitrate, ascorbic or citric acids added to the rooting medium on roots on root length (cm) of the 2 cultivars (Bartamoda and Sakkoty)

	Root length (cm)	
Treatments	Bartamoda	Sakkoty
Silver nitrate (mg L^{-1})		
0.0	3.00±0.50	3.02±0.30
0.5	5.43±0.58	5.47±0.63
1.0	4.33±0.22	4.25±0.25
2.0	6.43±0.58	6.67±0.29
Ascorbic acid (mg L^{-1})		
0.0	3.00±0.1	3.02±0.01
0.5	5.10±0.85	3.17±0.20
1.0	2.25±0.25	2.50±0.50
2.0	4.00±1	4.27±0.23
Citric acid (mg L^{-1})		
0.0	3.00±0.70	3.02±0.51
0.5	4.10±0.52	3.17±0.58
1.0	3.25±0.25	3.50±0.50
2.0	4.00±0.50	4.27±0.23

Data represent Mean±SD

Adaptation stage: Concerning the effect of acclimatization period (6 month), the highest significant value of survival percentage (90%) was achieved after 2 month then the survival percentage was reduced significantly to reach 80 and 65% after 4 and 6 month, respectively.

Regarding the interaction between the acclimatization period and the residual effect of different treatments, it was obvious that the highest significant values of survival percentage was achieved when plantlets received from different concentrations of $AgNO_3$ especially with 0.5, 1.0 and 2.0 mg L^{-1} after 2 months as it recorded 85, 90 and 90%, respectively.

The plantlet succeeded in acclimatization continued their growth and development in green house with higher survival percentage as shown in Fig. 2.

Molecular analysis

RAPD technique: RAPD analysis was performed on the DNA of tissue culture-derived plantlets of the 2 cultivars. Among the 2 primers tested only one primer (M09) gave sufficient and reproducible amplification products Fig. 3. The results of the RAPD profile of tissue culture-derived plantlets especially that from medium supplemented with 2 mg L^{-1} $AgNO_3$ obviously revealed high similarity to mother trees for the 2 cultivars with this primer.

Long RAPD technique: Using long RAPD, to investigate the genetic stability and somaclonal variation of the tissue culture-derived plantlets. There are two primers used, only one of them (AM08), Fig. 3 gave high similarity between the tissue culture-derived plantlets from medium supplemented with 2 mg L^{-1} $AgNO_3$ and mother trees for the 2 cultivars.

ISSR technique: To investigate the genetic stability and somaclonal variation of the tissue culture-derived plantlets, ISSR analysis was performed on the DNA of tissue

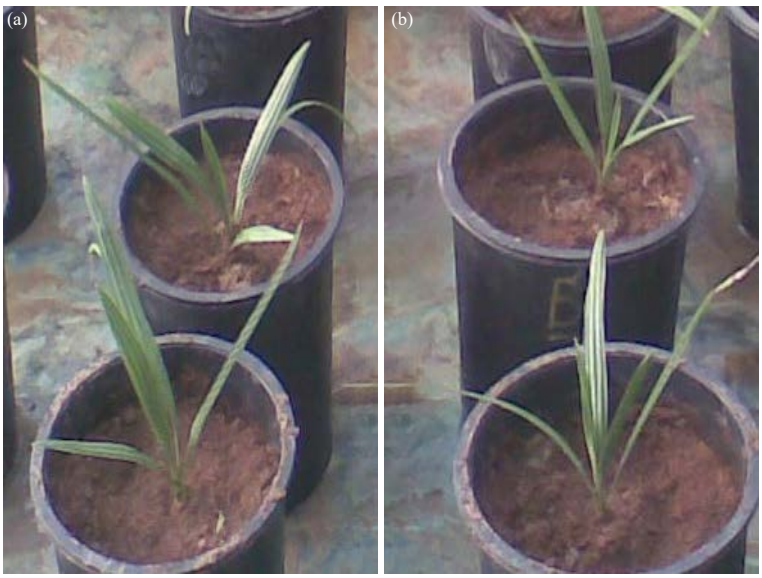

Fig. 2(a-b): Date palm dry cultivars (a) Sakkoty and (b) Bartamoda plantlets after 6 month from adaptation

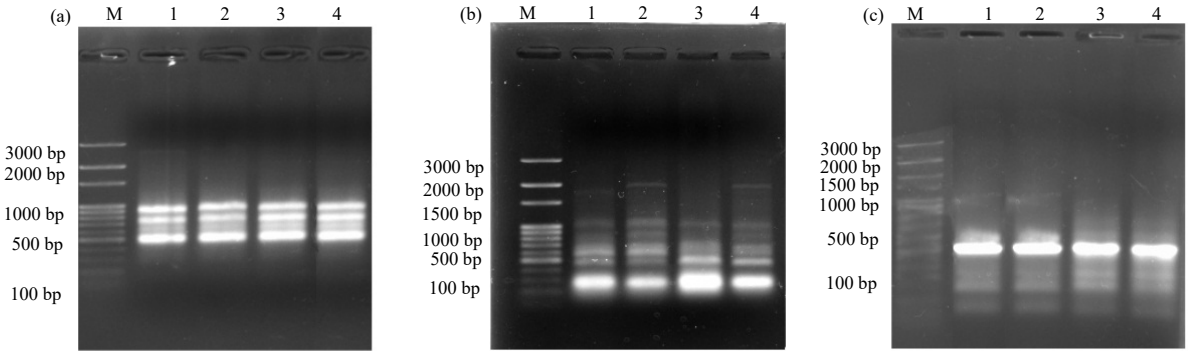

Fig. 3(a-c): Agarose gel electrophoresis of (a) Randomly RAPD with M09 primer, (b) Long RAPD with AM08 primer and (c) ISSR with HB10 primer

M: DNA marker (bp), Lane 1: Bartamoda mother plant, Lane 2: Tissue culture-derived Bartamoda plantlets from medium supplemented with 2 mg L^{-1} $AgNO_3$, Lane 3: Sakkoty mother plant, Lane 4: Tissue culture-derived Sakkoty plantlets from medium supplemented with 2 mg L^{-1} $AgNO_3$

culture-derived plantlets of the two cultivars. Only one primer (HB10) among the two primers used in ISSR analysis, that revealed an obviously high similarity between the tissue culture-derived plantlets from medium supplemented with 2 mg L^{-1} $AgNO_3$ and mother trees for both cultivars (Fig. 3).

DISCUSSION

Effect of different antioxidants on growth development of the 2 dry date palm cultivars (Bartamoda and Sakkoty): This work was carried out mainly to overcome the problem of transferring the plantlets (shoots with primary roots) from *in vitro* to *ex vitro* (green house), i.e., to obtain well developed root system on the *in vitro* derived shoots. Results under discussion showed that the addition of $AgNO_3$ at 2.0 mg L^{-1} produced the highest value of growth development and gave the higher values for roots number and length. Firstly, it is could be clarified that factors affecting endogenous auxin levels within the cultured tissues are important in controlling growth and morphogenesis of plant tissue culture. Modification of the rate of oxidation by enzymes within the group of peroxidase isozymes seems to be important way by which internal IAA (auxin) levels are regulated[31]. Several research workers have suggested that di-and tri-hydroxy phenols and their polymers protect the auxin by keeping the cell at a lower redox potential[32-33] thus depressing the rate of IAA degradation[34]. It is highly probable that the beneficial effect of $AgNO_3$, citric and ascorbic acids on

growth development and rooting of date palm dry cultivars Bartamoda and Sakkoty plantlet as presented in this study may be due to its ability to depress the peroxidase activity, thereby protecting the auxin from peroxidase-catalyzed oxidation.

Rooting stage: As mentioned before, results declared that the addition of $AgNO_3$ at 2.0 mg L^{-1} produced the highest value of growth vigor and the highest root number and length. In this regard, Bidding ton[35] demonstrated that the use of ethylene inhibitors such as $AgNO_3$ might promote root formation in shoot culture of apple. Shiblirida *et al.*[36] found that $AgNO_3$ was effective in inhibiting ethylene synthesis and promoted growth in *Vaccinium pahalae*. Also, Naik and Chand[37] recorded similar results with *Punica granatum*. In the same way, Hwida[38] reported that the highest percent of rooting and the greatest number of root formed per shootlet was recorded by using 7.0 mg L^{-1} $AgNO_3$+3 g L^{-1} activated charcoal on *Sequoia sempervirens* (D.Don) Endl. On the same respect, El-Ashry *et al.*[39] on their study on *Gardenia jasminoides* reported that, the addition of silver nitrate at 3 mg L^{-1} to the rooting medium supplemented with 2 mg L^{-1} from both NAA and IBA gave the highest mean length of roots. Also, Oumar *et al.*[40] declared that, longest roots and the highest number of roots were obtained on MS medium supplemented with 1 mg L^{-1} 2-iP+0.5 mg L^{-1} NAA+10 or 15 mg L^{-1} $AgNO_3$ after 3 weeks of culturing regenerated shoots of banana varieties (FHIA-21 and PITA-3).

While, Kaviani[41] reported that supplementation of ascorbic acid to the medium increased length of cells in cell elongation zone of root and also increased distance between root hairs zone and root tip.

As for date palm, Alromaihi and Elmeer[42] declared that the combined role of carbohydrate and auxin is effective in root formation of date palm somatic embryos, they mentioned that the best combination resulted in a mean of 2.5 roots per embryo and reached up to 10 healthy roots when the rooting medium was supplemented with 60 g L^{-1} sucrose and 1 mg L^{-1} NAA. Successful plant acclimatization of date palm should be started at the rooting stage. In this regard, Abd-Alhady and Abo El-Fadl[43] reported that using different concentrations of polyethylene glycol (PEG) increased root number and decreased root length of date palm seedling strain (Shaba) by increasing PEG concentration. They reported that, addition of 4 g L^{-1} PEG to MS medium resulted in the highest number of roots.

Adaptation stage: Results declared that, the highest significant value of survival percentage (90%) was achieved after 2 month then the survival percentage was reduced significantly to reach 80 and 65% after 4 and 6 month, respectively on an acclimatization mixture of peat moss, vermiculite and washed sand at equal volume (1:1:1 v/v/v). In this respect, Hassan *et al.*[44] reported that the highest survival percentage was achieved in an acclimatization medium consist of peatmoss: Perlite (2:1) and when plantlets were cultured greenhouse conditions in autumn or spring.

Molecular analysis: Since morphological identification of off-types is unreliable at the juvenile stage of the date palm, molecular marker is required at this plant development stage for the possible detection of plant off-types. The introduced DNA-based techniques have the potential to identify polymorphisms represented by differences in DNA sequences. The obtained results are accordance with those reported by Ahmed *et al.*[45]. They mentioned that clonal plants of date palm were regenerated from juvenile leaves. Success depends on the concentration of 2,4-D tested. RAPD patterns of the plantlets were identical with the original plant mother, indicating that 2,4-D did not induce somaclonal variation that can be detected by RAPD technique. In this respect, Moghaieb *et al.*[46] used RAPD technique to study the genetic stability in date palm tissue-cultured, Ferhi cultivar. Among the 20 random primers used, 10 have shown polymorphism between accessions.

For future recommendation, successful commercial production of date palm *in vitro* derived plants starts from succeeding in establishment of well-developed root system *in vitro* as adaptation or pre acclimatization starts *in vitro*.

CONCLUSION

Finally as a conclusion, it was found that using antioxidants especially $AgNO_3$ at 2 mg L^{-1} $AgNo_3$ gave the best results for growth development, root numbers and length of the two cultivars. Bartamoda showed relatively better results than Sakkoty cultivar. The tissue culture-derived plantlets on the medium supplemented with 2 mg L^{-1} $AgNO_3$ revealed high similarity to mother plant as a result to RAPD, long RAPD and ISSR profiles.

SIGNIFICANCE STATEMENT

This study open the door for the commercial production of date palm *in vitro* derived plants, since it succeeded in establishment of a well-developed root system which enables it to successfully pass the adaptation stage and this is the main obstacle that face the *in vitro* production of date palm.

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