Pak. J. Bot., 50(2): 495-502, 2018.

THE EFFECTS OF $AGNO_3$ AND 2iP (N^6-(2-ISOPENTENYL) ADENINE) ON DIFFERENT STAGES OF SOMATIC EMBRYOGENESIS IN DATE PALM (*PHOENIX DACTYLIFERA* L.) (CV. MEDJOOL)

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Abstract

This study is aimed at investigating the effects of silver nitrate ($AgNO_3$) during various stages of somatic embryogenesis in Medjool palm. Silver nitrate is a strong inhibitor of ethylene, which by reducing the negative effects of ethylene, confers growth and development on tissue culture plants in laboratory. In the present study, the effects of different concentrations of AgNO3 and 2iP on embryogenic callus production, the number of embryos, the length of embryos and the germination of somatic embryos were scrutinized. Moreover, the length of the plantlet, rooting percentage, length of root, and the quality of plantlets were examined under effect of different concentrations of AgNO3. The media supplemented with 4 mg/l of $AgNO_3$ induced the highest number of embryogenic callus and the greatest number of embryos. While, the media contained 0.5 mg/l 2iP resulted in the highest length of embryos. Furthermore, a media including 8 mg/l of silver nitrate has a very positive effect on the germination of somatic embryos. In order to prolonging the plantlets and root length, $AgNO_3$ is an invigorating treatment to be utilized, but it did not have a positive effect on rooting of the plantlets. The genetic stability of tissue culture plants derived from somatic embryogenesis, examined and screened by 16 primer pairs of SSR marker. As a result, it had been revealed that somatic embryogenesis method and the protocol used in this study were the suitable and safe method for proliferation of Medjool cultivar in date palm, as no differences were observed among *In vitro* grown plants and the mother plant.

Key words: Date palm; Silver nitrate; Somatic embryogenesis; Tissue culture; 2iP; Microsatellite marker (SSR).

Introduction

Date palm (*Phoenix dactylifera* L.) belonging to the family Arecaceae is considered as one of the most important plants with a great economic value, particularly, in dry and hot areas like Middle East and southern Mediterranean countries. It is a dioecious, cross-pollinated and evergreen species. Palm fruits generally are high in sugars, minerals, and vitamins. Without irrigation, this plant is able to withstand long periods of drought and it can adapt itself well to the saline conditions. More importantly, it can also be a significant contributor to socio-economic and cultural aspects in rural areas (Safwat, 2007). It has been estimated that there are about 150 million date palm trees all over the world (Al-Khayri *et al*., 2015).

Date palm is propagated sexually by seed and vegetatively by offshoots (Bonga, 1982). However, due to various constraints in planting material production in large scale, tissue culture has been widely used in date palm propagation. *In vitro* proliferation is considered as an effective and efficient alternative for the conventional vegetative propagation as it guarantees the rapid proliferation and maintenance of genetic stability of superior cultivars (Al-Khayri, 2005 &2007).

Somatic embryogenesis is one of the asexual *In vitro* reproduction methods for propagating date palm, which is based on meristematic calli (Al-Khayri, 2005). Indeed, this is considered as one of the most successful techniques for mass propagation of date palm (Kunert *et al*., 2003). Moreover, somatic embryogenesis is a suitable method for *In vitro* plant breeding purposes, employed in many laboratories worldwide. Apart from this, it has other capabilities such as preserving plant materials, reaping secondary metabolites and somatic seed production etc.

Sometimes, in the production of plantlets using tissue culture, accumulation of gasses such as ethylene in culture environment inhibits the growth of seedlings or creates a variety of disorders in them (Mele *et al.*, 1982). As a growth regulator, ethylene is produced by plant tissues, callus and plantlets in culture and exerts negative impacts on plant morphology (Brar *et al*., 1999). *In vitro* studies have shown that emission of ethylene as a gaseous plant hormone to the environment influences the growth of calli, shoot regeneration and somatic embryogenesis by aging and slowing down the growth. In order to minimize the adverse effects of ethylene, silver nitrate has been widely used as a precious deterrent in reducing the effect of this hormone and to improve growth and development of a number of tissue culture plants (Cristea *et al*., 2012; Steinitz *et al.*, 2010). Addition of silver nitrate into culture media resulted in increasing regeneration and somatic embryogenesis in tobacco (Purnhauser *et al.*, 1987), rubber (Auboiron *et al.*, 1990) and several monocots such as pearl millet, wheat (Purnhauser *et al.*, 1987) and maize (Songstad *et al.*, 1988). Furthermore, there are a number of reports on the promising effects of silver nitrate on improving the regeneration in somatic embryogenesis in a variety of crop species (Zhang *et al.*, 2001; Vikrant & Rashid, 2002; Parimalan *et al.*, 2010; Cogbill *et al.*, 2010). Beside this, $AgNO_3$ is also known as ethylene inhibitor and once added to the medium it enhances growth of the shoots (Biddington, 1992). Kumar *et al.* (2007) confirmed the efficacy of silver nitrate in different

plants species. $AgNO_3$ has also been used in somatic embryogenesis in date palm, but its effect depends on genotype and cultivar and its concentrations (Al-Khayri & Al-Bahrany, 2001 & 2004). Callus formation occurs in a medium containing 2,4-D (100 mg/l) and 2iP (3 mg/l) with 3 g/l activated charcoal (Shaheen, 1990; Aslam & Khan, 2009). In media containing NAA (0.1 mg/l) and 2iP (0.2 mg/l) a great number of embryo production has been reported (Gaber *et al*., 2010). Maturation of somatic embryos developed in half strength MS medium enriched with 60 g/l sucrose and 2 mg/l ABA, caused thicker and longer embryos with high protein content (Fki, 2005). Taha *et al.* (2007) suggested that the optimum hormone combination for embryo maturation and proliferation of the plantlets is ¾ MS supplemented with 10 mg/l of 2,4-D, 3 mg/l 2iP, 10 mg/l thiamine hydrochloride and 1 mg/l biotin.

The length of the *In vitro* culture procedure, more number of sub-culturing passages and hormonal effect, make micropropagated plants more vulnerable to somaclonal variation which alters the genetic fidelity (Matthes *et al*., 2001). Therefore, monitoring the effect of *In vitro* treatments on genetic variation of derived plants has to be done. Many studies report the use of molecular markers to evaluate the genetic diversity in date palm, including randomly amplified polymorphic DNAs (RAPDs) (Sedra *et al*., 1998; Adawy *et al*., 2005), Amplified fragment length polymorphic (AFLP) (Cao & Chao, 2002; Soumaya *et al.*, 2011), and simple sequence repeat (SSR) (Zehdi *et al*., 2004; Ahmed & Al-Qaradawi, 2009). The sensitivity, reproducibility, co-dominance and strong discriminatory power make microsatellite SSR markers a reliable tool for investigating genetic diversity in date palm (Billotte *et al*., 2004).

The present study is aimed at evaluating the effect of silver nitrate and 2iP on producing embryogenic callus, formation and maturation of embryos, and the effect of silver nitrate on rooting and plantlet formation.

Materials and Methods

In this experiment, the Medjool cultivar, which is non-native Iranian palm with a North-African origin, was used. Its superiority over Iranian counterparts is the production of large fruits. The experiment was conducted in Agriculture Biotechnology Research Institute of Iran, during early 2015 till end of 2016. For sampling, 3-4-year old offshoots of palm trees were collected in winter and their leaves were completely removed leaving only the apical meristems with primary leaves. After disinfection, the off shoots were cut into pieces and the resulted explants (including meristem and primary leaves) were transferred into establishment media.

Establishment medium: In this study, at all stages a basal MS medium was utilized, which was supplemented by (170 mg/l) N_2PO_4, Mayo-Inositol (125 mg/l), glutamine (200 mg/l), thiamine-HCl (1 mg/l), nicotinic acid (1 mg/l), Pyridoxine-hydrochloride (1 mg/l), sucrose (30 g/l), agar (7 g/l) and activated charcoal (1.5 mg/l), which has been changed according to the circumstances and the needs of each stage. Before adding agar, the pH was set at 5.6 to 5.7. Thereafter, the medium has been autoclaved for 20 minutes at 121°C.

The sterilized shoot tips were cultured on an establishment medium containing 2, 4 – D (100 mg /l), 2iP (3 mg /l) and activated charcoal (3 g / l). They were maintained for 12 months in dark at 24±3°C, and sub-cultured in a 3-week interval during above period. Afterwards, it was aimed at producing more callus, the initial callus was kept for another 3 months in the same medium with three weeks intervals subculture.

Embryogenic callus formation: Developed callus were transferred into media containing different treatments to form embryogenic callus (Table 1). Here onwards, the induced calluses were kept for 12 weeks during which they were transferred at a 3 week interval, and evaluated at the end of each 3 weeks. Explants were maintained in light at 24±3°C in phytotron for further embryogenic callus weight assessment. This study was carried out in completely randomized design with 5 replicates (3 Petri dishes in each replica).

Table 1. Six different treatments.

Treatment number	AgNO3 (mg/l)	2iP (mg/l)
1	-	-
2	4	-
3	8	-
4	-	0.5
5	4	0.5
6	8	0.5

Somatic embryo formation: At this stage, in order to develop somatic embryos and its maturation, embryogenic callus maintained for 9 weeks in a medium with different treatments during which they were transferred at a 3 week interval, and evaluated at the end of each 3 weeks (Table 1). This experiment was performed in a completely randomized design with 5 replications (each replication includes 3 Petri dishes). At the end of 9 weeks, the number and the length of embryos that produced in 10 g of embryogenic callus and the percentage of abnormal embryos were assessed.

Rooting and plantlet production: In this research the effect of 3 silver nitrate concentrations (0, 4 and 8 mg) on rooting percentage of germinated embryos of date palm were examined. This experiment was conducted in a completely randomized design with 5 replicates (each replicate contained 3 tubes). Germinated embryos were transferred into test tubes for 14 weeks, during which they were transferred at a 7 week interval to produce roots and proper shoots. At this stage, the treatments were evaluated for rooting percentage, plantlet length, root length, and quality of the plantlet. Length of shoots and roots were measured using a ruler. In order to assess the quality of the plantlets, they were scored from 0 to 5 (e.g. 0 for the plantlets being very low quality and 5 for the plantlets being best quality). Regarding the storing, the tubes containing date palm plantlets were incubated in a phytotron, under illuminated conditions at 24±3°C.

DNA Extraction: Total DNA was extracted from both tissue cultured plants (1 g of leaf tissue) and the originated mother plant (control), following manual instructions of genomic DNA purification kit (Promega, UCSD Core Bio). The quality of obtained DNA was quantified using Nanodrop Spectrophotometer.2 µl DNA was loaded on 1% agarose gel at 80 V for 45 min for further quantity estimation.

PCR Conditions for Microsatellites (SSR) amplification: A set of 16 date palm SSR primer pairs developed by Billote *et al*. (2004) (Table 2), were used in the present study. Total reaction mixture of 25µl were used to perform PCR reaction, containing; 1µl genomic DNA (20ng), 12.5µl of Red Load Taq Master® Mastermix (Takapouzist, Tehran, Iran), 1µl (10 pmol) of primers each and 9.5 nuclease free water.The initial 5 min denaturation step (94°C) was followed by 35 cycle of 1 min denaturation (94°C), 1 min annealing step in specific temperature (Table 2) and the extension phase for 1 min at 72°C followed by final extension in 72°C (1 min) was used to carry out PCR amplification which performed in the thermocycler (Bio-Rad, USA). Electrophoresis was performed in Meta Phoragarose gel (3%) to achieve higher resolution using 1X TBE buffer and stained with ethidiumbromid. Gel visualization performed using a gel documentation system (Bio-Rad, USA) and 50 bp DNA ladder (Takapouzist, Tehran, Iran) helped to comparing the size of amplicons.

Data analysis: Data analysis was performed using SAS software and mean separation was done using Duncan's multiple range test ($p<0.01$).

Table 2. List of SSR primers used in the detection of genetic stability in micropropagated date palm plants.

No.	Primer name	Annealing temperature (°C)	Primer sequence (5'-3')	
			Forward	Reverse
1.	mPdCIR010	58	ACCCCGGACGTGAGGTG	CGTCGATCTCCTCCTTTGTCTC
2.	mPdCIR015	58	AGCTGGCTCCTCCCTTCTTA	GCTCGGTTGGACTTGTTCT
3.	mPdCIR016	52	AGCGGGAAATGAAAAGGTAT	ATGAAAACGTGCCAAATGTC
4.	mPdCIR025	42	GCACGAGAAGGCTTATAGT	CCCCTCATTAGGATTCTAC
5.	mPdCIR032	44	CAAATCTTTGCCGTGAG	GGTGTGGAGTAATCATGTAGTAG
6.	mPdCIR035	56	ACAAACGGCGATGGGATTAC	CCGCAGCTCACCTCTTCTAT
7.	mPdCIR044	56	ATGCGGACTACACTATTCTAC	GGTGATTGACTTTCTTTGAC
8.	mPdCIR048	56	CGAGACCTACCTTCAACAAA	CCACCAACCAAATCAAACAC
9.	mPdCIR050	56	CTGCCATTTCTTCTGAC	CACCATGCACAAAAATG
10.	mPdCIR057	56	AAGCAGCAGCCCTTCCGTAG	GTTCTCACTCGCCCAAAAATAC
11.	mPdCIR063	44	CTTTTATGTGGTCTGAGAGA	TCTCTGATCTTGGGTTCTGT
12.	mPdCIR070	46	CAAGACCCAAGGCTAAC	GGAGGTGGCTTTGTAGTAT
13.	mPdCIR078	46	TGGATTTCCATTGTGAG	CCCGAAGAGACGCTATT
14.	mPdCIR085	46	GAGAGAGGGTGGGTGTTATT	TTCATCCAGAACCACAGTA
15.	mPdCIR090	46	GCAGTCAGTCCCTCATA	TGCTTGTAGCCCTTCAG
16.	mPdCIR093	46	CCATTTATCATTCCCTCTCTTG	CTTGGTAGCTGCGTTTCTTG

Table 3. Variance analysis of mean square for the embryogenic callus weight, number of embryos, length of embryos and the number of germinated embryos in date palm.

Source variation	df	Square mean			
		Embryogenesis callus weight	Embryo number	Embryo length	Number of germinated embryo
Treatments	5	18.767**	2476380.86**	8.818**	480.975**
Error	24	0.278	62.90	0.593	1.323
c.v %		8.87	0.49	8.21	5.14

*,** Were significant at 5% and 1% respectively

Results and Discussion

Embryogenic Callus Formation: Variance analysis of embryogenic callus weight showed significant difference at 1% level among the treatments (Table 3).

According to the results (Fig. 1), treatment containing 4 mg/l silver nitrate with no additional 2iP (7.96 g) followed by the media with 2iP and lacking of silver nitrate (7.61 g) produced the greatest amount of embryogenic callus. On the other hand, the media contained the highest concentrations of silver nitrate (with or without 2iP) had the lowest embryogenic callus production. The results showed that silver nitrate triggered a stimulatory effect on embryogenic callus growth in Medjool date palm, albeit, this amount was dependent on $AgNO_3$ concentration. Accordingly, a medium containing a moderate concentration of $AgNO_3$ is likely to be a more suitable medium for embryogenic callus production. These results are in accordance to those reported by Al-Khairy & Al-Bahrany, (2004) who suggested the contributions of $AgNO_3$ in increasing the growth of embryogenic callus, though high concentrations had no effect on increasing the effect. It is apparent that high concentrations of silver nitrate play no positive role in the production of embryogenic callus. Devoid of 2iP, applying 4 mg/l silver nitrate alone is by far convincing, while doubling this concentration has a decreasing effect on production. In the presence of 2iP when the concentration of $AgNO_3$ was increased it lead to decrease in the production of embryogenic callus. Al-Khayri & Al-Bahrany, (2004) reported that embryogenic callus weight is significantly correspondent to the interaction between $AgNO_3$ and 2iP so

that in the presence of $AgNO_3$ and devoid of 2iP, it was resulted in the highest weights of embryogenic callus. Therefore, silver nitrate increases the embryogenic callus propagation and hence the results of the present study are in accordance with those reported by previous authors.

The formation of somatic embryos: Variance analysis of the number and the length of somatic embryos (Table 3) showed, there were significant differences among the treatments at 1% level.

The interactive effect of $AgNO_3$ and 2iP (Fig. 2) on somatic embryogenic showed that the media containing 4 mg/l silver nitrate devoid of 2iP, yielded 2758.2 embryos per 10g of embryogenic callus, which was the highest number of production. Nevertheless, a medium containing no growth regulators and $AgNO_3$, produced 1809.1 embryos which was the second highest number. According to the results; silver nitrate at low concentrations has a stimulatory effect on the production of somatic embryos (somatic embryo's size in date palm is enough to count by bare eye).

Similar results were obtained by Al-Khayri & Al-Bahrany, (2004) who suggested that the optimum concentration of silver nitrate for cultivars like Barhee, Naboot Saif, and Ruzaiz was different depending on the cultivars used. Especially silver nitrate at low concentrations resulted in a significant increase in the number of embryos, but this effect diminishes at high concentrations. Giridhar *et al*. (2004) reported that applying silver nitrate at different stages of somatic embryogenesis in *Coffea arabica* L. resulted in an increasing effect of somatic embryogenesis. Renukdas *et al*. (2010) claimed that the addition of silver nitrate into culture media increased the maturation of somatic embryos.

The result of this experiment showed that the use of silver nitrate alone produced more embryos, which support the finding results of those by Al-Khayri & Al-Bhrany, (2001) which reported that the number of somatic embryos development was significantly reduced by a combination of silver nitrate and 2iP and increased while the silver nitrate only added in media.

The effect of silver nitrate and 2iP on embryo's length, showed the medium containing 0.5mg/l 2iP, produced the longest embryos (11.9 mm) followed by medium containing the combination of 8 mg/l silver nitrate and 0.5 mg/l 2iP (Fig. 3). Therefore, 2iP has a positive effect on embryo's length. As a result, if silver nitrate was used with 2iP the production of longer embryos would be resulted. A medium lacking both silver nitrate and 2iP is capable of producing the embryos with the minimum lengths. In contrast, the silver nitrate was found to be effective for the elongation of somatic embryos and its increasing effect was enhanced when it was accompanied with 2iP. Just apposing these results, it can be inferred that 2iP has an enlarging effect on the length of embryo. The present results conform to the results of Al-Khayri & Al-Bhrany, (2001) who has explained that silver nitrate has a positive influence on increasing the length of somatic embryos in date palm.

In this experiment, also, the number of abnormal embryos was counted (somatic embryo's size in date palm is enough to count by bare eye) so that the medium containing 0.5mg/l 2iP and devoid of silver nitrate had the lowest number (2%) of abnormal embryos. On the other hand, the medium supplemented with 4 mg/l silver nitrate resulted in the production of greatest higher number (4%) of abnormal embryos. In other treatments, the total number of abnormal embryos was about 3%. Therefore, addition of silver nitrate resulted in increasing number of abnormal embryos, but this number is virtually negligible.

Apart from production and maturation of embryos, the resulted embryos also germinated in same media combination, therefore, 18-week (9 weeks for each stage) culture reduced to 9 weeks which the embryo formation and maturation, and subsequent germination occurred. The results presented below occurred during the 9 week of embryo production, and in this way the number of germinated embryos out of 100 embryos compared. Variance analysis of the number of germinated embryos (Table 3) showed that difference among the treatments were significant at level of 1%.

The effects of different treatments on somatic embryo's germination were studied (Fig. 4). The media containing 2iP are more promising to induce germination and silver nitrate also invigorates the media to prompt germination. Based on these results, it is evident that the medium containing a high concentration of silver nitrate (8 mg/l), with or without 2iP, resulted in a higher number of germination. Nonetheless, the medium without both silver nitrate and growth regulator is least effective for germination. Taking all these results into consideration, it can be clearly seen the positive effect of silver nitrate in the germination so that the medium with silver nitrate had 29.9 germinated embryos, while the medium devoid of growth hormones resulted in only 5.6 embryos which could germinate. Al-Khayri & Al-Bahrany, (2004) stated that although silver nitrate played an important role in the production of somatic embryos, it had no influence on their germination. This was not in agreement with the present results as it was obvious that silver nitrate was effective in promoting germination.

In this experiment, the important role of silver nitrate in embryo production has been revealed. Besides this, it was found that this substance was quite stimulating in embryo germination. Therefore, it could be concluded that in addition to saving time (reducing from 18 weeks to 9 weeks), silver nitrate has an extraordinary effect on the number of germination.

Ethylene is a gaseous phytohormone in the culture dishes and it is well-known as an inhibitor, though it sometimes stimulates *In vitro* growth (Kumar *et al*., 1998). Meanwhile, ethylene inhibitors such as silver nitrate can be used as a stimulus to enhance callus reproduction, somatic embryogenesis, and shoot' regeneration.

Depending on the genotype, the effect of silver nitrate concentrations is varied. Songstad *et al*. (1991) showed that the response to ethylene antagonism of each species was dependent on the needs of each species. This discrepancy of responses to silver nitrate could even occur between genotypes of the same species.

Considering the results of the present study, silver nitrate is a suitable candidate to be used in Medjool cultivar for producing embryogenic callus, embryos and their maturation, and also germination of somatic embryos.

Rooting and plantlets induction: Variance analysis of mean squares of treatments plantlet length, rooting percentage, root length, and quality of plantlets in Medjool palm (Table 4) after 14 weeks (2 subcultures) showed that the different treatments were significant at 1% level.

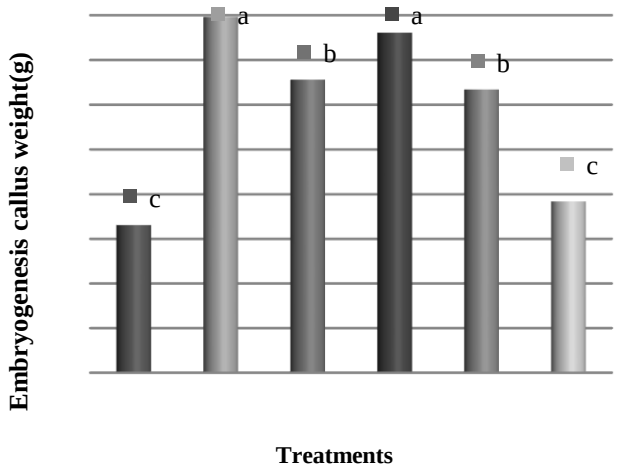

Fig. 1. The effects of different treatments on embryogenic callus weight (g).

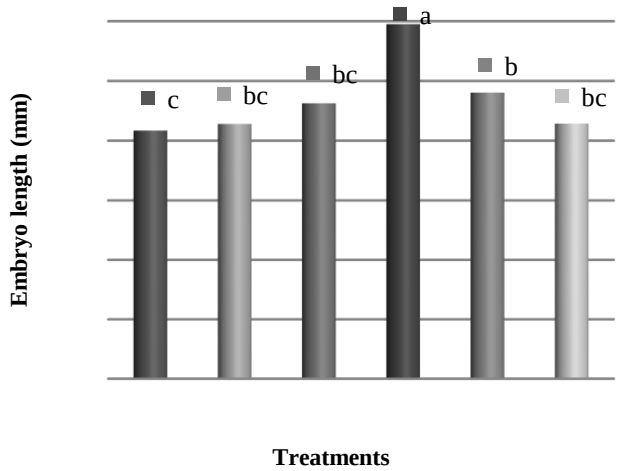

Fig. 3. The effect of different treatments on somatic embryo's length (mm).

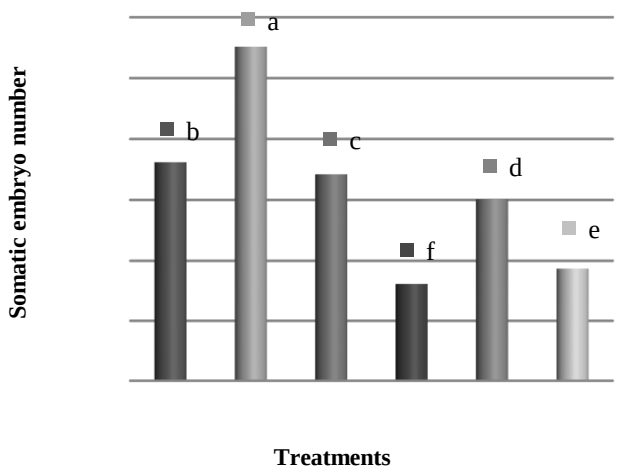

Fig. 2.The effect of different treatments on somatic embryo's number.

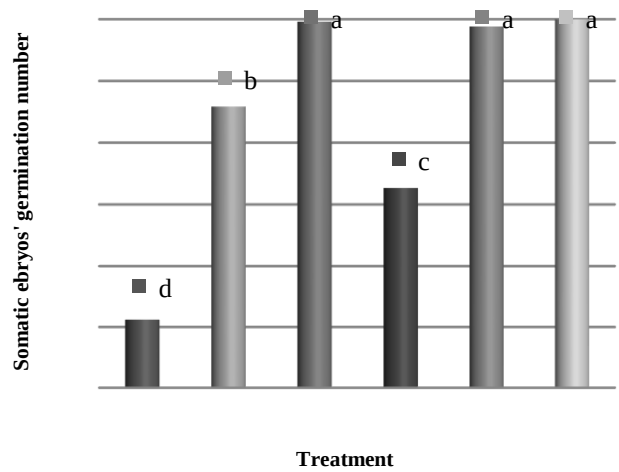

Fig. 4. The effect of different treatments on somatic embryo's germination.

Table 4. Variance analysis of mean square for percentage of rooting, root length, length of plantlets and the quality of plantlets in date palm.

Source variation	df	Square mean			
		Rooting percentage	Root length	Length of plantlet	Plantlet quality
Treatments	2	2372.600**	605.416**	152.016**	0.804**
Error	12	69.22	14.75	4.058	0.289
c.v %		8.87	4.85	7.42	14.88

*,** Were significant at 5% and 1% respectively

The effects of 3 different concentrations of silver nitrate on plantlet length showed in Figure 5. The media containing a high concentration of silver nitrate (8 mg) produced the longest plantlets, with an average of 33.5 mm in length. It has been depicted in the graph, the medium with no silver nitrate has the same effect as the medium with 4 mg/l silver nitrate on increasing the length of the plantlets. The results of the present study clearly demonstrate that silver nitrate can lead to an increase in the length of the plantlets in Medjool dates and this effect has a positive correlation with its concentration.

The effects of three different concentrations of silver nitrate on somatic embryo's rooting percentage in Medjool cultivar is shown in Figure 6. With an average of 80% of rooting, the silver-nitrate-free media accounted for the highest percentage of rooting. The second medium with the best capability of rooting is the one which is supplemented by 4 mg/l silver nitrate. The results demonstrated that silver nitrate had no significant effect on rooting and its negative effect increase by higher concentrations. Therefore, a medium devoid of silver nitrate is more suitable for root induction.

The effect of 3 different concentrations of silver nitrate on the root length is shown in Figure 7. It was elucidated that a medium containing 8 mg/l silver nitrate, produced the maximum length of root with an average of 90 mm in length followed by a medium containing 4 mg/l silver nitrate which recorded elongated roots with an average of 79.5 mm in length.

The effects of 3 different treatments of silver nitrate on the quality of plantlets showed an $AgNO_3$-free medium produced plantlets of high quality, and hence, it can be concluded that the presence of silver nitrate in the medium has no effect on the quality of plantlets (Fig. 8).

$AgNO_3$ is a competent substance for increasing the length of the plantlets in Medjool palm, while it has no positive effect on rooting and the quality of the plantlets.

Park *et al*. (2012) reported that as an ethylene inhibitor in *Sinningia speciosa*, silver nitrate aggrandized the number and the length of the seedlings. Moreover, Sirisom & Te-chato, (2012) suggested that addition of 3-5 mg/l of $AgNO_3$ to basal culture of *Hevea brasiliensis* induced the plantlet. In addition, Petrova *et al*. (2011) reported that a medium supplemented with $AgNO_3$ invigorated rooting of *Gentiana lutea*.

Cristea *et al*. (2012) showed that silver nitrate is a potent inhibitor of ethylene action. Embryogenesis, seedling production, and rooting of seedlings are vital processes of *In vitro* culture in which silver ions in the form of nitric acid play a critical role in the process of somatic embryogenesis. According to the results, the positive role of silver nitrate in different stages of somatic embryogenesis in Medjool palm is by far evident.

Genetic stability analysis: The genetic fidelity of micropropagated plants versus mother date palm plants were tested through molecular technique using microsatellites (SSR) markers. 16 microsatellite primer pairs were employed to find possible genetic changes that had occurred (Billote *et al*., 2004). The PCR amplification results demonstrated monomorphic band in both micropropagated plant and mother plant for all 16 primer pairs tested. The results clearly demonstrated the genetic stability of *In vitro* derived plants (Fig. 9) and the fidelity of the micropropagation protocol to produce true –to- type date palm plants. Our findings are in agreement with others who have reported similar results in many studies (Andrea *et al*., 2004; Lopes *et al*., 2006; Kumar *et al*., 2010). The banding pattern in microsatellite analysis in this study confirmed no somaclonal variation and therefore the reliability of micropropagation protocol for producing authentic plantlets of date palm in mass scale.

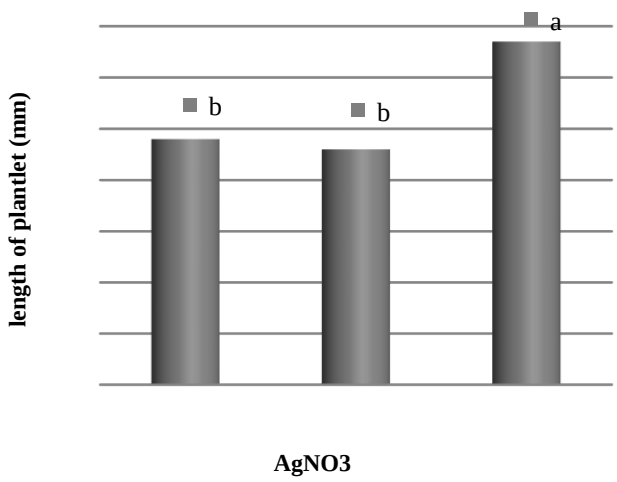

Fig. 5. The effect of different concentrations of silver nitrate on plantlets length.

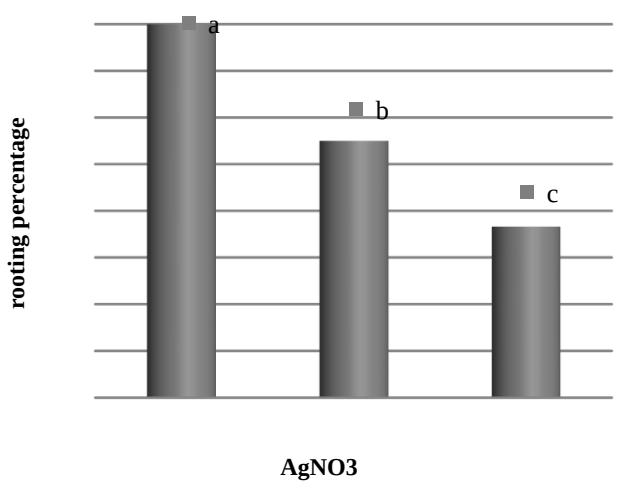

Fig. 6. The effect of different concentrations of silver nitrate on somatic embryo's rooting percentage.

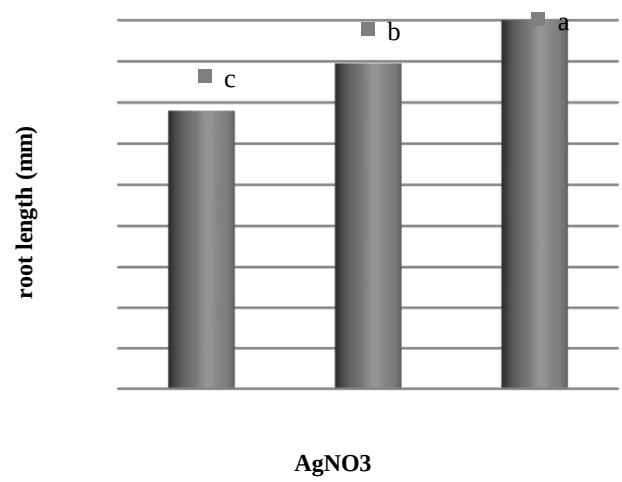

Fig. 7. The effect of different concentrations of silver nitrate on root length.

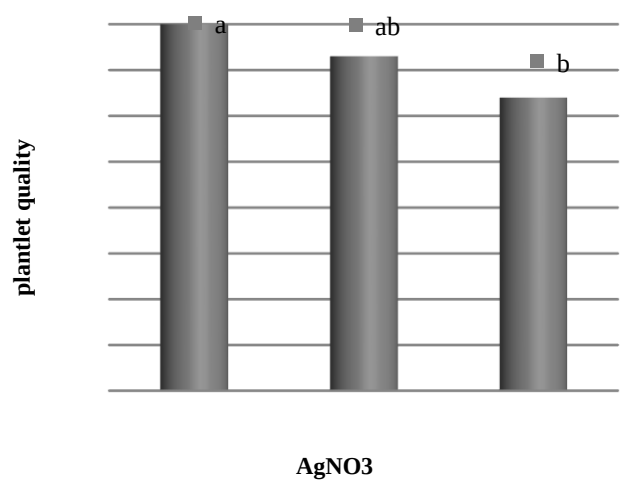

Fig. 8. The effect of different concentrations of silver nitrate on the quality of plantlets.

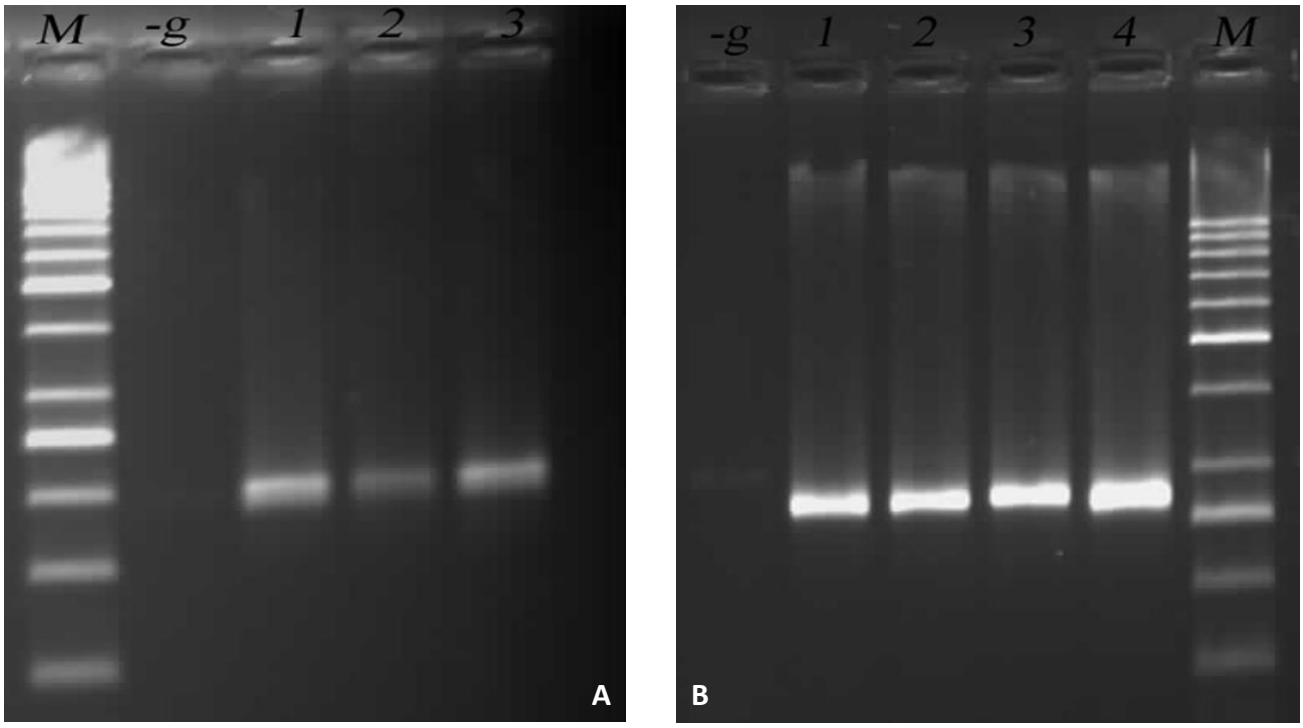

Fig. 9. SSR amplification. (A) Primer No. 6 and (B) Primer No. 5. (M) 50bp ladder. (-g) negative; no DNA (1) Mother plant (2, 3 & 4) Micropropagated plants.

Conclusions

Silver nitrate has a significant influence on increasing somatic embryogenesis in date palm. In the present study, the effects of $AgNO_3$ and 2iP on embryogenic callus induction, embryo production, maturation and germination were investigated. Moreover, the contribution of $AgNO_3$ to rooting and plantlet production in date palm (cv. Medjool) was also scrutinized. According to the results, a medium containing 4 mg/l silver nitrate is optimal for producing embryogenic callus and somatic embryos. Nevertheless, the best medium for germination of somatic embryos is the one containing 8 mg/l of $AgNO_3$. Although high concentration of silver nitrate (8 mg/l) has a positive effect on the length of roots and plantlets, it has no effect on rooting percentage and the quality of the plantlets. The molecular results clearly demonstrated the genetic stability of *In vitro* derived plants and the fidelity of the micropropagation protocol of date palm.

Authors' contributions: B. Abdolvand participated in the experiments and data analysis and also contributed to the writing of the manuscript. R. Zarghami, the corresponding author, designed the research, organized the study, coordinated the data analysis. A. Salari contributed in conducting and analyzing molecular work related to the present study and writing of the manuscript.

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(Received for publication 12 April 2017)