Effects of Polyamines And Silver Thiosulphate On *Phoenix Dactylifera* L. Cv. Quntar Micropropagation And Molecular Analysis.

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Research Article

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Abstract

There are some limitations in the practical applications of *in vitro* date palm tissue culture, such as low multiplication efficiency, low rooting rate, and high mortality experienced by *in vitro* raised plantlets during laboratory to soil transfer. The objective of the present study is to determine the effect of polyamines (putrescine "PUT" and spermidine" SPD") and silver thiosulfate (STS) on enhancing propagation of date palm cv Quntar *in vitro*. Media supplemented with 75 mg L^{-1} SPD in combination with 10 mgL^{-1} STS gave the highest percentage of callus producing buds (83.34%) and average bud formation (16.3) per jar. The addition of PUT and STS to the medium was most effective in root regeneration and the number of roots per shoot, where the best result 91.67% and 6.37 roots per shoot, respectively, were obtained using 75 mgL^{-1} PUT and 10 mgL^{-1} STS, resulting in fast-growing plantlets during acclimatization phase, reaching 90% of plant survival. The genetic fidelity assessment of plants derived from micropropagation was confirmed by RAPD analysis. Four operon primers were used, and all of them showed amplified unambiguous (OPA02, OPC-04, OPD-07, and OPE-15). All generated bands were monomorphic and had no variation among the tissue culture-derived plants tested. Accordingly, these results indicate that adding polyamines and silver thiosulfate to the nutrient medium of date palm cv. Quntar is beneficial in improving shoot organogenesis, rooting, and production of genetically stable date palm plants.

Key Message

This paper is the first to illustrate the effect of polyamines (putrescine and spermidine) and silver thiosulfate (STS) together in date palm. It provides experimental evidence for these compounds' remarkable role in growth pathways, date palm genetically stability under in vitro conditions, and *ex vitro* acclimatization.

Introduction

The date palm (*Phoenix dactylifera* L.), a tree of the palm family (Arecaceae) cultivated for its sweet, edible fruits. The date palm has been prized from the earliest times and may have originated in ancient Mesopotamia, now known as Iraq. The date palm can be propagated either from seeds or from offshoots. When plants are grown from seeds, about half of the palms turn out to be males, which can be identified only when flowering. Moreover, the plants obtained through seeds are genetically heterogeneous. Consequently, for uniformity of the orchards, date palms are propagated through offshoots only. Due to the little offshoots that trees produce, the date palm is one case that requires serious attention for rapid vegetative propagation. Micropropagation technology has overcome these problems and provides uniform and good quality planting material to establish large-scale plantations (Ibrahim et al., 2013; Al-Mayahi and Ali., 2021). Micropropagation contributes to plant growth and development by modifying biochemical and physical media (Al-Mayahi, 2019; 2020). Organogenesis is one of the growth pathways through which buds can be stimulated to differentiation. Studies have shown that successful organogenesis can be achieved through the appropriate establishment of culture medium components, appropriate explant selection, and control of the physical environment (Thorpe,

2007). It is clear from the literature that polyamines have an important role in plant tissue culture, as their use has expanded in recent years, especially in stimulating and multiplying shoots. Polyamines are low-molecular-weight organic cations essential for tissue growth and development due to their role in cell proliferation, signal transduction, and protein synthesis (Rakesh et al. 2021). Studies have shown that adding polyamines to culture media can enhance shoot growth, callus induction, and root regeneration (Tang and Newton, 2005; Thiruvengadam et al., 2012). Chae (2016) reported enhanced plant regeneration of Echinacea angustifolia DC plants *in vitro* in the presence of polyamines. Kiełkowska and Adamus, (2021) reported that PUT and SPD had a beneficial effect on the mitotic activity of cultured cells, which further affected the plant regeneration process. *In vitro* propagation has limitations, especially when the accumulation of ethylene in culture containers is severe and the genotypes exhibit sensibility to this phytohormone (levinsh et al., 2000). Studies have shown that externally applied polyamines can promote ethylene production in the culture medium. Thus it is likely to affect growth and development in such systems. Ethylene accumulation in culture vessels under in vitro propagation systems appears to be a major problem in date palm regeneration studies. Ethylene inhibitors, including silver thiosulfate (STS) affect ethylene activity, preventing or reducing sensitivity and negative effects on plant tissues (Wang et al., 2002). Meanwhile, ethylene inhibitors can be used as a stimulator to promote callus induction and shoot regeneration (Al-Mayahi., 2010). Roh et al. (2012) also reported that STS was more effective than $AgNO_3$ on shoot regeneration from cotyledon and hypocotyl explants of B. napus. Somatic clonal variations may occur mostly as a result of stresses to the plantlets under laboratory conditions. Suggesting visual phenotypic evaluation may not be sufficient for characterizing the in vitro plants. Hence, an astringent quality test in terms of genetic similarity of the tissue culture-raised plants becomes necessary. Out of various molecular markers used to evaluate in vitro regenerated plants' genetic fidelity, RAPD is one of the most simple, quick and cost-effective methods and require only small amounts of DNA (Chaudhary et al., 2015). Srivashtav et al.,(2013) suggested that RAPD markers are more efficient than ISSR for assessing genetic variation in date palms. Despite the advantages of date palm propagation *in vitro*, low multiplication and rooting rates, and high mortality during acclimatization. Despite the benefits of date palm propagation *in vitro*, multiplication and rooting rates are low, and high mortality during acclimatization. Therefore, this research aimed to evaluate the effects of two types of polyamines (putrescine "PUT" and spermidine" SPD") in combination with silver thiosulfate (STS) on multiplication shoots, rooting, acclimatization, and genetic stability, RAPD indicators were used to determine genetic stability of *in vitro* multiplied materials to determine the protocol effectiveness.

Materials And Methods

The experiments of this study were carried out in the date palm micropropagation laboratory for Date Palm Research Center at Basra University, Basra, Iraq.

Offshoots of Quntar cv.(2–3 years old) were chosen and detached from the mother palm (Fig. 1a). Offshoots were dissected acropetally until the apical buds appeared (Fig. 1b). The apical buds were sectioned longitudinally into four parts. In order to induce callus induction, explants were cultured on the

MS basal medium (Murashige and Skoog,1962). Media was prepared from MS medium salts (4.43 g L^{-1}) mixture containing the macronutrients and micronutrients. It was combined with Gamborg's vitamins and equipped with 3 mg L^{-1} 6- (dimethylallyl amino) purine (2iP), 30 mgL^{-1}, naphthalene acetic acid (NAA), 1.5 g L^{-1} activated charcoal, and solidified with Agar-Agar at 7.0 g L^{-1}., supplemented (Fig. 1c). Cultures were incubated under complete darkness at 27 ± 2°C. Cultures were transferred to new media, with the same composition after every 6 weeks interval until the callus had an induction (Fig. 1d). For callus multiplication, it was transferred to jars containing 25 ml of the MS medium, with Gamborg's vitamins and supplemented with 0.5 g L^{-1} activated charcoal, with the addition of NAA at 6 mg L^{-1} and 2iP at 2 mg L^{-1}. To study the effects of polyamine type on the multiplication of buds, the callus was divided and subcultured on organogenesis media equipped as mentioned above, except for the growth regulators concentrations 1 mg L^{-1} (NAA) and 3.0 mg L^{-1}(2iP). It was also supplemented with Putrescine (PUT) and spermidine (SPD) in concentrations (0.0, 25, 75, and 150 mg L^{-1}). The pH of the culture media was adjusted to 5.7 with 0.1 mol L^{-1} NaOH or HCl before the addition of agar. Media were dispensed into culture jars. Sterilization of the culture's jars with media was performed by autoclaving at 121°C and pressure of 1.04 kg cm^{-2} for 20 min. All the cultures were incubated at 27 ± 2°C and irradiated for 16 h with a diffuse light provided by daylight fluorescent lamps. Based on the result of our previous experiment, the appropriate type of polyamine was selected in combination with silver thiosulphate (STS). To study their effects on buds regeneration and changes in phytochemicals. MS medium was modified at four concentrations of SPD (0.0, 25, 75, and 150 mg L^{-1}) in combination with silver thiosulfate (STS) at four concentrations (0, 5, 10, and 15 mg L^{-1}). Data on the percentage of bud induction and bud number per jar were taken after 12 weeks of culturing callus on the organogenesis media. Treatments were consisted of 12 media, as shown in Table I

Table I. Treatments applied in the organogenesis stage

No.	Treatments (mgL^{-1})	No.	Treatments (mgL^{-1})
1	+5 STS SPD0.0	7	+10 STSSPD75
2	+ 5 STS SPD25	8	+10 STS SPD150
3	+5 STSSPD75	9	+ 15 STS SPD 0.0
4	+5 STS SPD150	10	+15 STS SPD25
5	+10 STS SPD0.0	11	+15 STSSPD75
6	+10 STSSPD25	12	+15 STSSPD150

Optimization of rooting and plant acclimatization Clusters with no visible signs of roots formation in vitro shoots of date palm cv.Quntar were collected in the elongation stage; typical shoots were separated individually and cultured on a rooting MS medium. It was combined with Gamborg's vitamins and supplemented with 30 mg L^{-1} sucrose, 7 mg L^{-1} agar 0.5 mg L^{-1} NAA, and 0.5 g L^{-1} activated. The

media were supplemented with two types of polyamines PUT and SPD in concentrations (0.0, 25, 75 and 150 mg L^{-1}). Each treatment included 12 replicated jars, incubated at room temperature 25 ± 2°C, with a 16 h white florescent light photoperiod. The percentage of root induction and root number per shoot were evaluated 6 weeks after the inoculation of shoots on the media. Based on the result of our previous experiment, the appropriate type of polyamine was selected in combination with STS. To study their effects on the percentage of root induction and root number per shoot were evaluated 6 weeks after the inoculation of shoots on the media. MS medium was modified at four concentrations of PUT (0.0, 25, 75, and 150 mg L^{-1}) in combination with silver thiosulfate (STS) at four concentrations (0.0, 5, 10, and 15 mg L^{-1}). The percentage of root induction and root number per shoot were evaluated 6 weeks after the inoculation of shoots on the media. Treatments were consisted of 12 media, as shown in Table II.

Table II. Treatments applied in the rooting stage

No.	Treatments (mgL^{-1})	No.	Treatments (mgL^{-1})
1	+5 STS PUT0.0	7	+10 STSPUT75
2	+ 5 STSPUT25	8	+10 STS PUT150
3	+5 STSPUT75	9	+ 15 STS PUT0.0
4	+5 STS PUT150	10	+20 STSPUT25
5	+10 STS PUT0.0	11	+15 STSPUT75
6	+10 STSPUT25	12	+15 STS PUT150

Acclimatization Stage. For acclimatization, well-developed plantlets were gently washed with tap water to remove the remnants of agar. Then, the plantlets were washed with distilled water and treated with fungicide (Benlet 500 mg L^{-1}) for 20 min and transferred to plastic pots containing autoclaved a mixture of peat moss and perlite (2:1). Maintaining the plantlets at high humidity for the first few days following transplanting is critical for survival by covered the plants with glass bottles to maintain humidity. After six weeks, glass bottles were removed plants gradually were irrigated with $^1/_2$ strength MS salts.

After eight weeks calculated the percentage for plantlets acclimated as follows:

$$\text{Percentage of plantlets acclimated} = \frac{\text{Number of plantlets acclimated}}{\text{The total number of plantlets}} \times 100$$

Genetic stability among regenerated date palm plantlets

In order to study the genetic similarities, several regenerated plantlets were analyzed at the molecular levels using RAPD analysis

RAPD analysis

Total genomic deoxyribonucleic acid (DNA) was isolated from regenerated date palm plantlets using the CTAB method described in Rogers and Bendich (1985). Polymerase chain reaction (PCR) reactions were conducted using a set of four arbitrary 4-mer primers (Operon Technology, Inc., Alameda, CA, USA). These primers and their sequences are presented in Table III

Table III. The RAPD primers and their sequences used for the genetic fidelity evaluation

Primers	Sequences
OPA02	TGCCGAGCTG3
OPC 04	CCGCATCTAC
OPD 07	TTGGCACGGG
OPE-15	ACGCACAACC

The PCR mixture

The reaction mixture (20 µl) contained 10 ng DNA, 200 µM deoxynucleotide triphosphates (dNTPs), 1 µM primer, 0.5 units of Red Hot Taq polymerase (AB-gene Housse, UK) and 10-X Taq polymerase buffer (AB-gene Housse, UK). For DNA amplification, a Perkin Elmer thermal cycler (2720) programmed as follow: Denaturing: 95°C for 5 min 94°C for 0.45 min. Then annealing (35cycles) 35°C for 1 min. This is followed by 72°C for 1 min and 30 s and finally Extension: at 72°C for 7 min (Adawy et al., 2004). The amplification products were separated in 1% (w/v) agarose gel in 1X Tris/Borate/Ethylenediaminetetraacetic_acid (TBE) buffer and visualized by staining with ethidium bromide. The reproducibility of DNA profiles was determined by replicating all RAPD reactions at least three times using DNA markers. The primers were evaluated from wise pair comparison for the proportion of shared bands amplified (Nei, 1978). The similarity coefficient was calculated by using the statistical software package STATISTICA-SPSS (Stat Soft Inc).

Experimental design and statistical analysis

The experiments were carried out using a completely randomized design(CRD). Data were analyzed using variance (ANOVA) analysis using Statistical Package for Social Sciences (SPSS) software version 20. Treatment means were compared using the least significant difference (LSD) at the $P < 0.05$ level.

Results

Shoot induction and multiplication

From the results of our current study, it was found that the two types of polyamines (PUT and SPD) worked well for the regeneration of date palm cv Quntar (Table 1). The highest response percentage of

callus tissue cultures producing buds with the highest number of shoots was obtained at (75 mgL^{-1}) for each studied polyamines compound. Without polyamines, the control medium recorded the lowest response with the lowest number of buds (26.67 %; 2.75), respectively. Results showed that when SPD was added to the culture medium, the highest response percentage of callus tissue cultures producing buds with the highest number of shoots was recorded at (75 mgL^{-1}) reached (73.34 and 9.18), respectively.

Table 1. Effect of polyamines on the response percentage of callus for bud formation and a number of shoots after 12 wk of culturing for date palm, cv Quntar.

Polyamines treatments (mg L^{-1})	Response of callus for shoot formation	Number of shoots
Control	26.67±3.89	2.75± 0.22
25 PUT	33.34± 3.05	3.50± 0.4
75 PUT	60.00± 4.70	7.66± 0.58
150 PUT	40.00± 3.06	4.33±0.42
25 SPD	40.00±3.06	3.83± 0.50
75 SPD	73.34±4.60	9.18±0.21
150 SPD	46.67±3.89	4.71±0.6
LSD<0.05	13.33	1.1

Standard error (n15).

From the results of our current study (Table 2), it was evidenced that the response percentage of callus tissue cultures producing buds with the number of buds increased with increasing concentration of polyamines used up to 75 mgL^{-1} and then decreased. The percentage of callus producing shoots and the number of shoots per jar also increased with the increase STS concentrations from 0 mg L^{-1} to 10 mgL^{-1} proportional to the concentration in the medium, but thereafter decreased with increasing STS concentrations. The combination between SPD and STS application had the highest response percentage and number of shoots, compared with treatments with no additives or one additive alone (Tables 1and 2). The highest response percentage and numbers of shoots (83.3% and 16.3) were obtained on the media supplemented with 75 mg L^{-1} SPD and 10 mg L^{-1} STS, respectively (Fig. 2g).

Table 2. Effect of spermidine (SPD) and silver thiosulfate (STS) on a response percentage (%) of callus for bud formation, and a number of buds/100 mg callus for date palm, cv. Quntar.

Treatments (mgL^{-1})	frequency [%]	Shoot number	Treatments (mgL^{-1})	frequency [%]	Shoot number
0.0 SPD +5 STS	16.67±1.53	0.503.0±	**+10 STSSPD75**	4.8183.3±	1.9016.3±
25 SPD + 5 STS	33.34±2.06	0.403.75±	**+10 SPD150 STS**	±2.6050.0	0.46.16±
75 SPD +5 STS	75.00± 5.77	13.2±0.80	**0.0 SPD + 15 STS**	16.67±1.53	3.0±0.19
150 SPD +5 STS	41.67±3.89	0.185.6±	**+15SPD25 STS**	4.8125.0±	0.6±3.66
0.0 SPD+10 STS	25.0±4.81	0.503.6±	**+15SPD75 STS**	4.70±66.67	0.6±7.8
25 SPD+10 STS	41.67±3.89	0.185.2±	**+15 SPD150 STS**	2.0633.34±	0.504.0±
LSD<0.05	13.9	0.7	LSD<0.05	13.9	0.7

± Standard error (n112).

Rooting and plantlets induction

From the data presented in Table (3), adding two types of polyamines separately at the studied concentrations to the nutrient medium for MS improved the percentage of rooting and the number of roots cultured for date palm cv Quntar. The highest significant value of percentage of rooting and the number of roots/shoot was obtained at (75 mgL^{-1}) for each polyamine compound studied. The data showed that when PUT was added to the culture medium, the highest significant value was recorded, increasing the percentage of rooting and the number of roots/ shoot (80% and 5.75), respectively. A high concentration of 150 mg L^{-1} PAs positively affects the length of roots as compared with the other treatments

The combination between PUT and STS application had the highest percentage of rooting and the number of roots/shoot, compared with treatments with no additives or one additive alone (Tables 3 and 4). The highest percentage of rooting and the number of roots/shoot (91,67 and 6.37) were obtained on the media supplemented with 75 mg L^{-1} PUT and 10 mg L^{-1} STS, respectively (Fig. 3). While the high concentration of 150 mg L^{-1} of PAs with all concentrations of STS positively affects root length compared to other treatments.

Table 3
Effect of polyamines on rooting of shoots date palm cv Quntar after six weeks of culture

Polyamines treatments (mg L^{-1})	Response of shoot for root Formation (%)	Number of roots	Lengths of roots
Control	20	2.0 ± 0.30	2.3 ± 0.42
25 PUT	40	4.25 ± 0.42	3.6 ± 0.7
75 PUT	80	5.75 ± 0.1	4.9 ± 0.2
150 PUT	30	2.66 ± 0.32	5.2 ± 0.6
25 SPD	30	3.34 ± 0.2	3.0 ± 0.17
75 SPD	60	4.66 ± 0.2	4.1 ± 0.42
150 SPD	20	2.50 ± 0.42	4.4 ± 0.37
LSD < 0.05	19.9	0.35	0.6
*± Standard error (n = 10)			

Table 4
Effect of putrescine "PUT" and silver thiosulfate (STS) on a response percentage (%) of shoots for root formation and a number of roots/shoot for date palm, cv. Quntar.

Treatments (mgL^{-1})	Frequency [%]	Root number	Root leanth (cm)	Treatments (mgL^{-1})	Frequency [%]	Root number	Root leanth (cm)
0.0 PUT +5 STS	16.67 ± 1.53	2.5 ± 0.25	2.3 ± 0.42	**75 PUT + 10 STS**	91,67 ± 6.40	6.37 ± 0.4	5.9 ± 0.80
25 PUT + 5 STS	41.67 ± 3.89	3.8 ± 0.5	3.5 ± 0.5	**150 PUT +10 STS**	33.34 ± 2.06	3.25 ± 0.4	6.1 ± 0.4
75 PUT + 5 STS	75.00 ± 3.37	5.56 ± 0.2	4.8 ± 0.2	**0.0 PUT + 15 STS**	8.34 ± 0.81	2.0 ± 0.3	2.1 ± 0.09
150 PUT + 5 STS	25.00 ± 3.89	3.00 ± 0.2	5.11 ± 0.6	**25 PUT + 15 STS**	33.34 ± 2.06	3.5 ± 0.4	2.9 ± 0.2
0.0 PUT + 10 STS	25.00 ± 3.89	2.66 ± 0.4	2.5 ± 0.25	**75 PUT + 15 STS**	58.34 ± 4.81	4.7 ± 0.42	3.2 ± 0.1
25 PUT + 10 STS	50.00 ± 2.75	4.16 ± 0.37	4.1 ± 0.5	**150 PUT +15 STS**	16.67 ± 1.53	2.5 ± 0.25	4.3 ± 0.42
LSD < 0.05	13.9	0.7	1.2	**LSD < 0.05**	13.9	0.7	1.2
* ± Standard error (n = 12)							

Acclimatization

From the obtained data (Fig. 4) the addition of polyamines PUT to silver thiosulfate (STS) improved the survival percentage. Maximum survivability was noticed for plants cultured in media that containing both 75 PUT + 10 STS (Fig. 5), followed by plants cultured in media that containing 75 PUT + 5 STS. We found media did no contain STS and the addition of PUT or STS at high concentrations were not suitable for date palm hrdening where was survival percentage was low.

RAPD analysis

In this study, we regenerated plants from callus tissues with polyamine and silver thiosulfate STS; hence it becomes necessary to check the genetic stability of the regenerated plant. Random amplified polymorphic DNA markers (RAPD) were used in the present study under the influence of different treatments (Fig. 6). The results showed the genetic stability of in vitro propagated plants, and the PCR amplification results showed a monomeric band in both the *in vitro* derived date palm plants and the mother plants of all primer pairs tested. RAPD analysis micropropagated plant (*P. dactyliferaL* cv. Quntar) indicated a profile similar to that of the control group that clearly showed the genetic stability of those plants (Fig. 6) and the fidelity of the in vitro propagation protocol to produce true–to–type date palm plants, indicating that the use of polyamine and STS during micropropagation phases caused no variation in the plants of this date palm cv. Quntar

Discussion

Although shoot induction occurs, shoot number, elongation, and rooting are challenging in date palm (Al-Mayahi, 2021a). Shoot organogenesis depends on many factors, such as culture medium composition and culture conditions (Al-Mayahi,2016; Al-Mayahi et al.,2020; Al-Mayahi, 2021b). In this study, the effect of medium composition on shoot organogenesis was evaluated. Our experiment indicates that the use of PAs in combination with STS plays a synergistic role in promoting multiple shoot formation from callus tissues of date palm in vitro. SPD with STS increased the regeneration frequency of date palm shoots *in vitro.*

The addition of polyamines to the culture medium was influential in the regeneration of shoots (Table 1). The best type and concentration of polyamines used was SPD at **75** mgL^{-1}. PAs play a major role in cell division, plant growth, and development (Mattoo et al., 2010). It has been shown that PAs interact with plant hormones, act as PGR substances or secondary hormonal messengers, and as carbon and nitrogen storage in culture tissues (Couée et al, 2004). Furthermore, PAs carry amino groups capable of interacting with macromolecules, such as nucleic acids, proteins, phospholipids, and cell wall components, and may have different effects on the culture medium (Takahashi and Kakei, 2010; Tiburcio et al., 2014). Shoots multiplication helps increase the number of plantlets achieved through hormonal combination and PAs (Dey et al. 2019). The positive effect of polyamines on shoot regeneration can be attributed to their stimulatory effect on cell division (Bais and Ravishankar, 2002). It has been suggested that regeneration and differentiation can be significantly improved by applying putrescine in date palm (Muhusen et al., 2020). It has been advocated that shoot regeneration and differentiation can be significantly improved by

using PAs with ethylene inhibitors (Park et al.,2012). In the present study, the influence of ethylene inhibitor STS on *in vitro* culture of date palm was investigated. According to the results obtained, using STS in culture media can enhance the ability of date palm callus tissues to give the highest response percentage of shoots and shoot numbers. Silver thiosulphate is a suitable candidate for use in Quntar cultivar for the regeneration and multiplication of shoots and the rooting of plants. Although this response depends on the concentration of STS used. The highest percentage of callus producing buds and shoot number was achieved on media supplemented with 10 mgl^{-1} STS. High concentrations of STS do not have an important positive role in bud production. However, a medium without PAs and STS is the least effective for organogenesis (Tables 1 and 2). Ethylene produced by plant tissues grown *in vitro* may accumulate in large quantities in culture vessels, thus potentially affecting growth and development. This may be due to the role of silver ions in overcoming the action and metabolism of ethylene. Several studies support that ethylene affects callus growth and plant regeneration *in vitro* (Saiprasad and Raghuveer, 2007; Sarropoulou et al, 2016). Sridhar et al. (2011) reported that STS significantly increased the shoot regeneration response and average number of buds in Solanum nigrum. Similar to our results, the effect of SPD and induced a maximum shoot regeneration in gherkin (Thiruvengadam and Chung, 2015). However, the addition of SPD (75 mgL^{-1}) in MS containing STS (10 mgL^{-1}) produced a higher percentage of response as well as the number of shoots/ jar when compared to STS alone (Table 1). In Cucumis sativus (Vasudevan et al. 2008) and Withania somnifera (Sivanandhan and Salammal, 2011), SPD supplementation of the culture medium improved shoot regeneration compared to putrescine as observed in the current study. Vasudevan et al., (2008) reported that SPD was the most effective for shoots regeneration in Cucumis sativus L. SPD is a nitrogen source that promotes plant differentiation in *Glycine max* L. (Arun et al., 2014). Our results are in agreement with previously reported results showing the stimulative role of PAs or STS in organogenesis in many plants (Bader and Khierallah, 2009; Park et al., 2012; Roh et al.,2012 Tamimi, 2015; Muhusen et al.,2020).

The number, length, and development of roots is an essential factor in the *in-vitro* development of plants. PAs play a vital role in rooting. The effect of polyamines on the number, length and development of roots was studied, and it was found that these factors depend on the type and concentration of polyamines and their combinations with STS. The highest response percentage and numbers of roots were obtained on the media supplemented with 75 mg L^{-1} PUT and 10 mg L^{-1} STS. Although a high concentration of 150 mg L^{-1} PAs positively affects the length of roots compared to the other treatments, it has no significant effect on rooting percentage and the number of the roots. PAs are involved in various cellular and physiological pathways and cycles that promote root growth, proving an essential role in differentiation. PAs play a major role in cell division and different morphogenetic processes, including rooting (Cou´ee et al., 2004). Our results indicate that PUT improved rooting efficiency, whereas spermidine showed less response to root induction. Denaxa et al. (2014) reported that PUT improved the rooting response of difficult-to-root ‘Kalamata’ olive cultivar, compared with SPD, which failed to promote rooting. Endogenous PUT is considered as a marker of root induction *in vitro*, and its catabolism could be the basis for root growth by providing H_2O_2 (Neves et al. 2002). It has previously been shown that PUT to MS media increases endogenous putrescine accumulation to promote root induction and growth

(Hausman et al. 1995). PUT also acts as a second messenger, correlating with the peak of root mitotic activity (Tiburcio et al. 1989). Similarly, PUT induced root induction in Pinus virginiana (Tang and Newton, 2005). The encouragement of palm plants during the rooting stage by STS may be due to the unique function of silver, which appears to be unique among the heavy metals that play an inhibitory role in ethylene biosynthesis. This result is in harmony with Sharaf et al. (2012), who reported a positive correlation between response to rooting and STS. Roh et al., (2012) reported that the medium supplemented with STS compound encouraged roots cultures to elongate and proliferate. Similar results were obtained by Harathi and Naidu, (2016) who suggested that the addition of ethylene inhibitor to the culture medium along with an auxin significantly augmented the induction of roots.

The acclimatization process is a very important step in micropropagation process of date palm plantlets. One of the main obstacles to applying micropropagation technology is the high mortality rate during transfer to the soil. PAs protect against biotic or abiotic stresses by increasing various antioxidant enzymes and regulating osmolysis; they also prevent chlorophyll degradation and protect the cell membrane from oxidation (Romero et al., 2018). Moreover, PAs in general and PUT in particular are nitrogen sources that have an anti-stress influence on stressed plants (Chen et al., 2018). Ethylene produced by plant tissues grown *in vitro* may accumulate in large quantities in culture vessels, thus potentially affecting growth and development. The beneficial effect of STS depends on the function of silver ion acting as an ethylene antagonist (playing as an inhibitor of ethylene biosynthesis) (Sharaf et al., 2012)

Micropropagation cannot be considered completely successful unless complete genetic fidelity is maintained. The regenerated plants from tissue culture were checked for their genetic stability using RAPD primers. RAPD has been extensively used in genetic variation experiments in date palm plants derived from tissue culture (Saker et al. 2000; Moghaieb et al., 2011). Four primers were selected based on the quantity, quality, and reproducibility of the amplified bands. All bands matched perfectly with the DNA of the field donor plant. The detected bands were 100 % monomorphic, indicating that the use of PAs and the STS during micropropagation phases caused no variation in the tissue culture-derived plants of this date palm genotype. (The resultant clones are true-to type of the selected genotype). PAs carry positive charges on nitrogen atoms; this helps electrostatic attraction between DNA, RNA, proteins, and phospholipids. Hence, PAs play a role in membrane fluidity, signal transduction, elicitation, RNA processing, chromatin remodelling, etc. (Baron and Stasolla 2008). Shenoy and Vasil (1992) reported that micropropagation through explants containing organized meristem is generally associated with a low risk of genetic instability. The culture conditions used to achieve plant regeneration from tissue where meristems are already present are less aggressive than those usually needed to induce shoots from differentiation. This result is in agreement with that previously reported by El-Bahr et al.,(2019). Our findings are in agreement with the study of Abdol vand et al., (2018), who reported that the molecular results demonstrated the genetic stability of in vitro derived date palm plants. The banding pattern analysis confirmed no somaclonal variation and, therefore, the reliability of the micropropagation protocol for producing authentic plantlets of date palm cv. Quntar on a mass scale.

Conclusion

This study provides an efficient in vitro propagation method by providing a protocol for producing genetically uniform plants. Our study indicated that SPD use in combination with STS plays a synergistic role in increasing multiple shoot regeneration from callus tissues of date palm cv. Quntar *in vitro*. Also, particular emphasis should be done on PUT, which in combination with STS, in the rooting medium, was essential in stimulating a high rooting percentage with high quality of roots, resulting in fast-growing plantlets during acclimatization phase, reaching 90% of plant survival. On the other hand, no genetic variation was observed by the four RAPD primers tested. The *in vitro* micropropagation protocol developed in this research can be used for the large-scale production of genetically stable date palm.cv. Quntar

Declarations

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Author contributions Al-Mayahi preparing the culture media and the conduct of plant tissue culture of the date palm, and the follow of the growth and development of cultures. The author also analyzed the physiological characteristics of the tissues and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest benefit.

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Figures

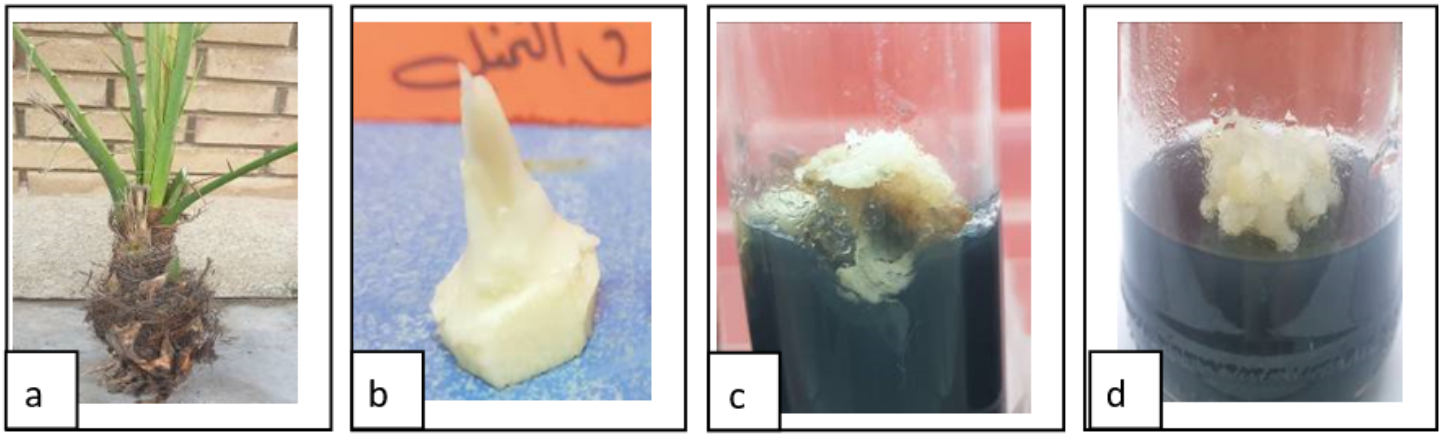

Figure 1

Induction of callus of date palm (P. dactylifera L. cv. Quntar) from apical buds of offshoot: a) Offshoot; b) Apical bud, c) Apical development; d) Callus formation.

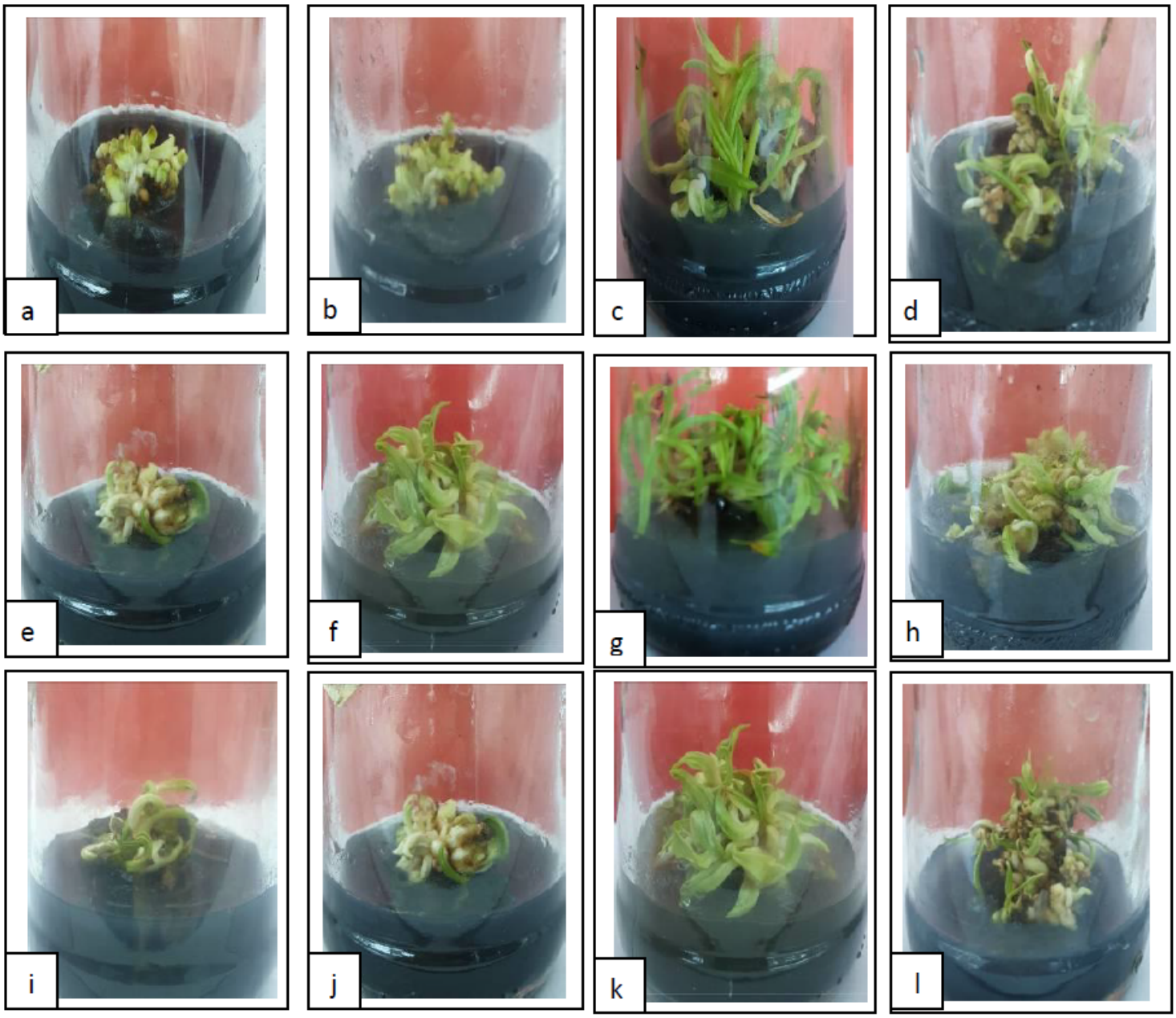

Figure 2

Bud induction on MS media supplemented with polyamines (PAS) and silver thiosulfate (STS) (mg.L-1) of date palm cv Quntar: a control, b 0.0 SPD +5 STS, c 25 SPD + 5 STS, d 75 SPD +5 STS, e 150 SPD +5 STS, f 0.0 SPD+10 STS , g 25 SPD+10 STS, h 75 SPD+10 STS. i 150 SPD +10 STS + 0.0 SPD + 15 STS, j 25 SPD +15 STS, k 75 SPD+15 STS, l 150 SPD+ 15 STS.

Figure 3

Rooting of date palm shoots cv. Quntar on MS medium supplemented with 75 mg L−1 PUT in combination with 10 mg L−1 STS, after 45 days from shoots culture on the rooting medium.

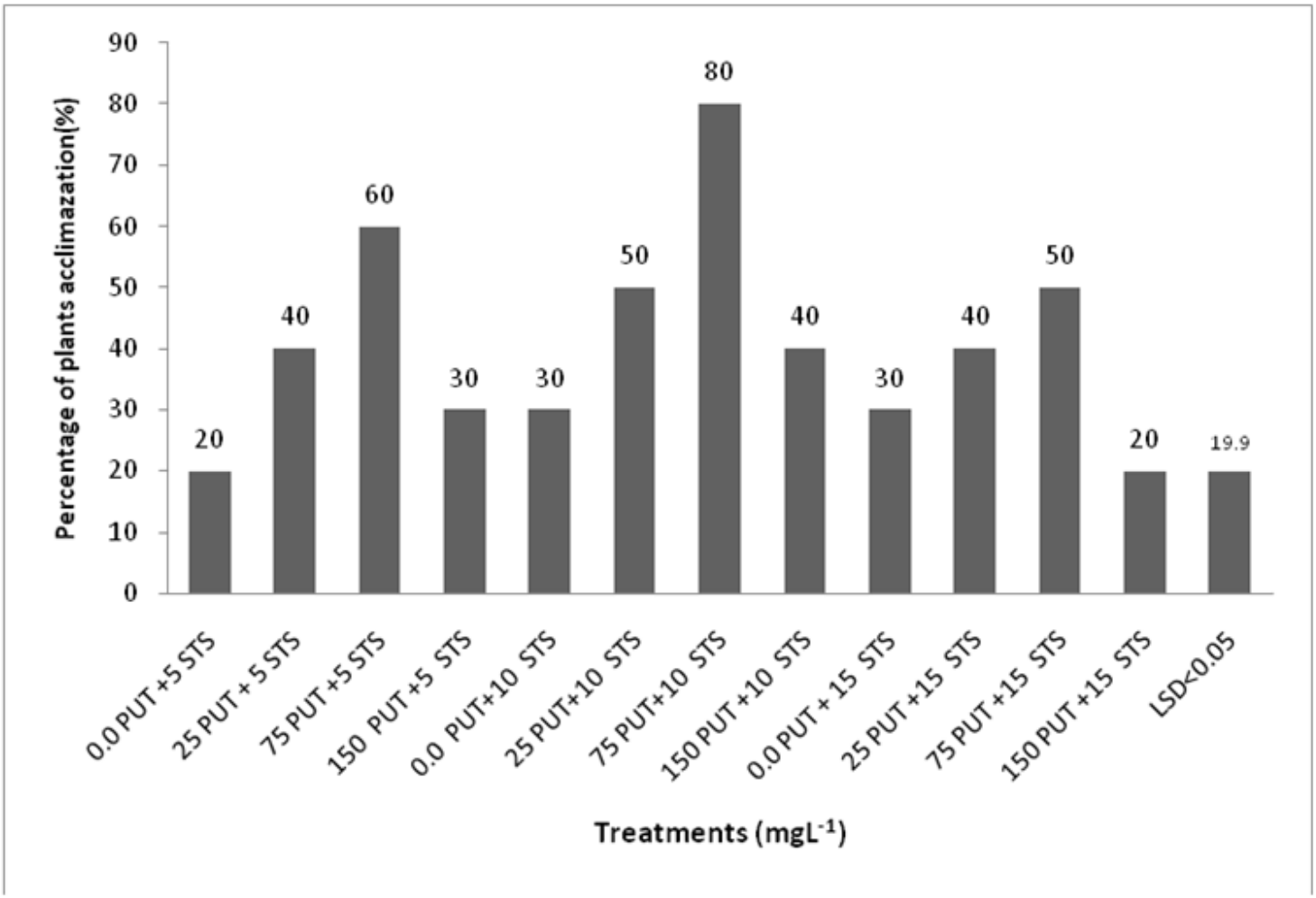

Figure 4

Effect of PUT and STS on acclimatization of date palm cv. Quntar after eight weeks of culture in plastic pots.

Figure 5

Date palm plants eight weeks after transfer from media containing 75 mg L-1 PUT with 10 mg L-1 STS to plastic pots.

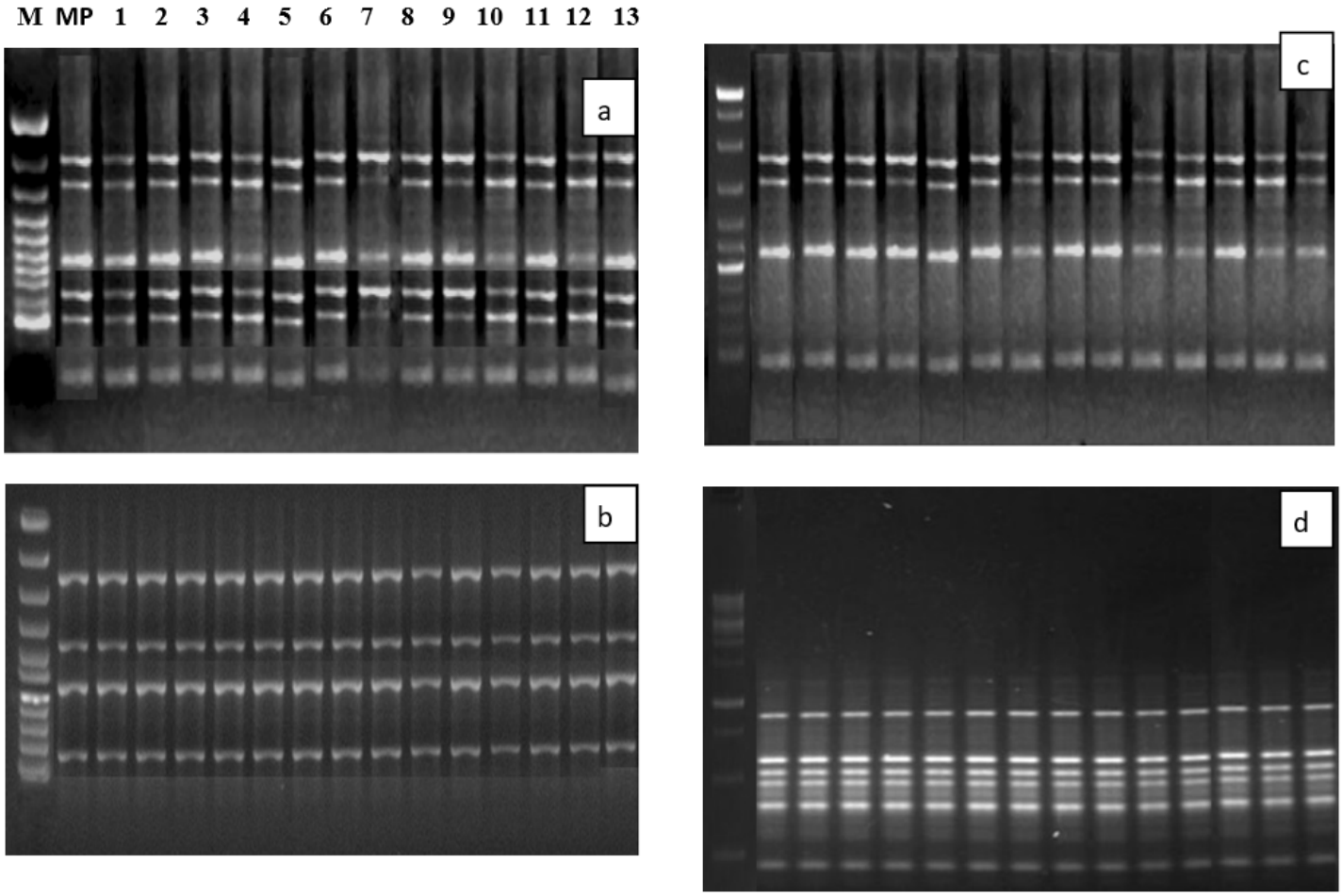

Figure 6

(a- OPA02, b- OPC-04, c- OPD-07 and d- OPE-15). RAPD pattern of regenerated plants of Phoenix dactylifera L. cv Quntar on MS medium supplemented with putrescine (PUT) in combination with silver thiosulfate (STS) (mgL-1): (M) Size marker (1) MP: Mother Plant, (2) 0.0 PUT+5 STS, (3) 25 PUT+ 5 STS, (4) 75 PUT+5 STS, (5) 150 PUT+5 STS, (6) 0.0 PUT+10 STS , (7) 25 PUT+10 STS; (8) 75 PUT+10 STS. (9) 150 PUT+10 STS (10) 0.0 PUT+15 STS, (11) 25 PUT+15 STS, (12) 75 PUT+15 STS (13) 150 PUT+15 STS.