The effect of polyamines and silver thiosulphate on micropropagation of date palm followed by genetic stability assessment

Ahmed Madi Waheed Al-Mayahi

Received: 26 December 2021 / Accepted: 9 May 2022 / Published online: 1 June 2022

© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
There are some limitations in date palm micropropagation. These include low multiplication efficiency, low rooting rate, and high mortality experienced by in vitro raised plantlets during laboratory to soil transfer. The objective of the study was to determine the effect of the polyamines and Silver Thiosulphate (STS) on the enhancement of shoot multiplication and genetic stability of in vitro cultures of date palm cultivar Quntar. Media supplemented with 75 mg L\(^{-1}\) SPD in combination with 10 mg L\(^{-1}\) 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 on root formation and the number of roots per shoot, where the best result, 91.67% and 6.37 roots per shoot, respectively, were obtained using 75 mg L\(^{-1}\) PUT and 10 mg L\(^{-1}\) STS, resulting in fast-growing plantlets during acclimatization phase, reaching 80% 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 was beneficial to improving shoot organogenesis, rooting, and production of genetically stable date palm plants.

Keywords Shoot regeneration · Multiplication · Rooting · Acclimatization · RAPD

Abbreviations
NAA Naphthaleneacetic acid;  
IAA Indoleacetic acid  
2iP N6-(2-Isopentenyl) adenine  
PUT Putrescine  
SPD Spermidine  
STS Silver thiosulfate

Introduction
The date palm (Phoenix dactylifera L.), a tree of the palm family (Arecales), is cultivated for its sweet, edible fruits. The date palm has been prized from the ancient times and may have originated in ancient Mesopotamia, now known as Iraq (Wrigley 1995). The date palm can be propagated either from seeds or from offshoots. When plants are grown from seeds, about half of the palms turn to be males, which can be identified only upon 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 few 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 et al. 2018; Al-Mayahi and Ali 2021). The success of plant tissue culture largely depends on selecting the right culture medium. Nutritional requirements for optimal tissue growth in vitro may vary with the species. Even tissues from different parts of a plant may have different requirements for satisfactory growth; it is essential to work out a medium that will fulfill the specific requirements of that tissue (Al-Mayahi 2019, 2020). Organogenesis is one of the growth pathways through which somatic embryos 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 (Dey et al. 2019; Muhusen et al. 2020; Rakesh et al. 2021). 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. Kielkowska and Adamus (2021) reported that putrescine "PUT" and spermidine "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 inhibit ethylene production in the culture medium (Panizzaa et al. 1993; Laukkainen and Sarjala 1997). Thus it is likely to affect growth and development in such systems. The ethylene effects on in vitro morphogenesis are not fully understood, but the role of this hormone in senescence has been widely reported (Kumar et al. 1998; Wang et al. 2002). Senescence is a natural phenomenon related to ethylene and oxidative stress (Wang et al. 2002). The reactive oxygen species (ROS) are toxic molecules naturally produced as a result of aerobic metabolism, and therefore they should be rapidly and efficiently scavenged by antioxidant systems (Mittler 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 Silver Thiosulphate (STS) was more effective than AgNO₃ on shoot regeneration from cotyledon and hypocotyl explants of Brassica napus. The acclimatization process is crucial in the 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 in general and PUT in particular are nitrogen sources that have an anti-stress influence on stressed plants (Chen et al. 2019). 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). Maintaining the true-to-type nature of in vitro propagated plants in commercial and marketing processes is crucial for upholding certain agronomic and horticultural traits when using elite genotypes (Alizadeh et al. 2015). Nevertheless, plant micropropagation technology has a phenotypic and genetic variation of propagated plants known as somaclonal variation (Larkin and Scowcroft 1981). 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). Srivastav et al. (2010) suggested that RAPD markers are more efficient than ISSR for assessing genetic variation in date palms. Several researchers used RAPD technique to examine genetic variability. It is an efficient and reliable technique for screening true to types of nature of tissue culture-derived plants (Moghaieb et al. 2011; Devi et al. 2013; Chaudhary et al. 2015). This study aimed to explore the effects of polyamines (putrescine "PUT" and spermidine "SPD") and Silver Thiosulphate (STS) supplementation on the performance of the growth date palm ‘Quntar’ and to determine the impact of these compounds on genetic stability for tissue cultured in vitro.

**Materials and methods**

The experiments of this study were carried out in the date palm micropropagation laboratory for the Date Palm Research Center at Basrah University, Basrah, Iraq. Young offshoots (2–3 years old) of date palm cv. Quntar were detached from the mother palm (Fig. 1a). Outer leaves and fibrous tissues at their bases were removed gradually until the shoot tip zone was exposed (Fig. 1b). Sheathing leaf base enclosing the very young leaves of the heart of the offshoot was left in place to protect it from disinfection solutions. The explants were taken and kept in antioxidant solution (100 mg L⁻¹ ascorbic acid 150 mg L⁻¹ citric acid). Sterilization of explants was performed using 70% ethanol for 1 min and 2.5% sodium hypochlorite for 20 min. Explants were then rinsed three times with sterile distilled water. 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). Culture 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 supplemented with 3 mg L⁻¹ 6-(dimethylallyl) amino purine (2iP), 30 mg L⁻¹, naphthalene acetic acid (NAA), 1.5 g L⁻¹ activated charcoal, 30 g L⁻¹ sucrose, 2.0 g L⁻¹ activated charcoal, and solidified with agar–agar at 6.0 g L⁻¹ were used. The pH of the medium was adjusted to 5.7 with 0.1N NaOH or HCl, before the addition of agar. Cultures were kept under complete darkness at 27 ± 2 °C. (Fig. 1c). The cultures were transferred to fresh media, with the same composition every 6 weeks until callus induction (Fig. 1d). For multiplication, the callus was transferred to MS media.
supplemented with 0.5 g L⁻¹ activated charcoal, 6.0 mg L⁻¹ NAA and 2.0 mg L⁻¹ 2iP. To study the effects of polyamine type on the multiplication of buds, the callus was divided and subcultured on shoot induction media supplemented as mentioned above, except for the growth regulators concentrations i.e., 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). Media were dispensed into culture jars and sterilized by autoclaving at 121 °C temperature and 1.04 kg cm⁻² pressure for 20 min. All the cultures were incubated at 27 ± 2 °C and irradiated for 16 h with a diffuse light of daylight fluorescent lamps. Based on the result of our previous experiment in this study, the appropriate type of polyamine was selected in combination with Silver Thiosulphate (STS). To study the effects of these two compounds on enhancing organogenesis of shoots from callus, and shoot multiplication. MS medium was modified with different concentrations of PUT (0.0, 25, 75, and 150 mg L⁻¹) in combination with STS (0, 5, 10, and 15 mg L⁻¹). Each treatment included 12 replicated jars, incubated at room temperature 25 ± 2 °C, with a 16 h white fluorescent light photoperiod. The percentage of root induction and root number per shoot were evaluated six weeks after the inoculation of shoots on the media. There were ten replicates of each treatment, as shown in Table 2.

**Table 1** Treatments applied in the organogenesis stage

| No | Treatments (mg L⁻¹) | No | Treatments (mg L⁻¹) |
|----|---------------------|----|---------------------|
| 1  | 0.0 SPD + 5 STS     | 7  | 75 SPD + 10 STS     |
| 2  | 25 SPD + 5 STS      | 8  | 150 SPD + 10 STS    |
| 3  | 75 SPD + 5 STS      | 9  | 0.0 SPD + 15 STS    |
| 4  | 150 SPD + 5 STS     | 10 | 25 SPD + 15 STS     |
| 5  | 0.0 SPD + 10 STS    | 11 | 75 SPD + 15 STS     |
| 6  | 25 SPD + 10 STS     | 12 | 150 SPD + 15 STS    |

**Optimization of rooting and plant acclimatization**

Shoot clusters with no visible signs of root formation were collected in the elongation stage; the shoots were separated individually and cultured on rooting medium consisted of MS medium combined with Gamborg’s vitamins and supplemented with 30 mg L⁻¹ sucrose, 7.0 mg L⁻¹ agar, 0.5 mg L⁻¹ NAA and 0.5 g L⁻¹ activated charcoal. The media were supplemented with two polyamines, PUT and SPD, in concentrations (0.0, 25, 75 and 150 mg L⁻¹). The cultures were maintained at 16 h photoperiod, 25 ± 1 °C temperature and at16 h photoperiod at 25 ± 1 °C and irradiance of 13.5 µmol m⁻² s⁻¹ provided by cool white fluorescent tubes. Each treatment included 12 replicated jars. 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 in this study, the appropriate type of polyamine was selected in combination with Silver Thiosulphate (STS) to study their effects on the rooting percentage (%) and root number. The rooting percentage was calculated as the number of shoots forming roots out of the total number of shoots cultured. Culture media were supplemented with different concentrations of PUT (0.0, 25, 75, and 150 mg L⁻¹) in combination with Silver Thiosulphate (STS) at four concentrations (0, 5, 10, and 15 mg L⁻¹). Each treatment included 12 replicated jars, incubated at room temperature 25 ± 2 °C, with a 16 h white fluorescent light photoperiod. The percentage of root induction and root number per shoot were evaluated six weeks after the inoculation of shoots on the media. There were ten replicates of each treatment, as shown in Table 2.

**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 (Benlate 500 mg L⁻¹) for 20 min and...
transferred to plastic pots containing autoclaved mixture of peat moss and perlite (2:1). Cover the plants with glass bottles to maintain humidity. After 6 weeks, glass bottles were removed, and plants were gradually irrigated with 1/2 strength MS salts. After eight weeks the percentage for plantlets acclimated were calculated as follows:

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

### Genetic stability of regenerated plants

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 3.

#### The PCR mixture

The reaction mixture (20 μl) contained 10 ng DNA, 200 μM deoxynucleotide riphosphates (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 (35 cycles) 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 a 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

Callus tissues showed significant variations in their response percentage, and Callus tissues in medium containing SPD at 75 mg L⁻¹ showed a better response rate (73.34%) of callus producing shoots and average shoots number (9.18 shoots/jar) (Table 4). 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 75 mg L⁻¹ to the culture medium, the highest response percentage of callus producing buds with the highest number of shoots was recorded (73.34% and 9.18, respectively).

From the results of our current study (Table 5), 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 mg L⁻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 to 10 mg L⁻¹ proportional to the concentration in the medium, but after that 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 4, 5). The highest response percentage and numbers of shoots (83.3% and 16.3) were obtained on the

### Table 2

| No | Treatments (mg L⁻¹) | No | Treatments (mg L⁻¹) |
|----|---------------------|----|---------------------|
| 1  | 0.0 PUT + 5 STS     | 7  | 75 PUT + 10 STS     |
| 2  | 25 PUT + 5 STS      | 8  | 150 PUT + 10 STS    |
| 3  | 75 PUT + 5 STS      | 9  | 0.0 PUT + 15 STS    |
| 4  | 150 PUT + 5 STS     | 10 | 25 PUT + 15 STS     |
| 5  | 0.0 PUT + 10 STS    | 11 | 75 PUT + 15 STS     |
| 6  | 25 PUT + 10 STS     | 12 | 150 PUT + 15 STS    |

### Table 3

| Primers | Sequences |
|---------|-----------|
| OPA02   | TGC CGA GCTG3 |
| OPC 04  | CCGCATCTAC  |
| OPD 07  | TTGGCACGGG   |
| OPE-15  | ACGCACAACC  |

---

Springer
media supplemented with 75 mg L⁻¹ SPD and 10 mg L⁻¹ STS, respectively (Fig. 2g).

**Rooting and plantlets induction**

From the data presented in Table (6), adding two types of polyamines separately at the studied concentrations to the nutrient medium 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 mg L⁻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 PUT positively affects root length compared to 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 a single additive alone (Tables 6, 7). 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 PUT with all concentrations of STS positively affects root length compared to other treatments.

**Acclimatization**

The obtained data (Fig. 4) showed that adding polyamines PUT to silver thiosulfate (STS) improved the survival percentage. Maximum survivability was noticed for plants cultured in media containing both 75 PUT + 10 STS (Fig. 5), followed by plants cultured in media containing 75 PUT + 5 STS. The media did not contain STS, and the addition of PUT or STS at high concentrations was not suitable for date palm acclimation, where the 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. 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. dactylifera* 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, achieving shoot, 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, 2020, 2021). This study evaluated the effect of medium composition on shoot organogenesis. Our experiment indicates that using polyamines (PAs) with STS plays a synergistic role in promoting 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. The best type and

| 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 ± 0.70      |
| 75 PUT                          | 80                                       | 5.75 ± 0.10     | 4.9 ± 0.20      |
| 150 PUT                         | 30                                       | 2.66 ± 0.32     | 5.2 ± 0.60      |
| 25 SPD                          | 30                                       | 3.34 ± 0.20     | 3.0 ± 0.17      |
| 75 SPD                          | 60                                       | 4.66 ± 0.20     | 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)
concentration of polyamines used was SPD at 75 mg L\(^{-1}\). 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,

**Table 7** 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 (mg L\(^{-1}\)) | Frequency (%) | Root number | Root length (cm) | Treatments (mg L\(^{-1}\)) | Frequency (%) | Root number | Root length (cm) |
|-----------------------------|--------------|-------------|------------------|-----------------------------|--------------|-------------|------------------|
| 0.0 PUT + 5 STS             | 16.67 ± 1.53 * | 2.50 ± 0.25 | 2.30 ± 0.42      | 75 PUT + 10 STS            | 91.67 ± 6.40 | 6.37 ± 0.40 | 5.9 ± 0.80       |
| 25 PUT + 5 STS              | 41.67 ± 3.89  | 3.80 ± 0.50 | 3.50 ± 0.50      | 150 PUT + 10 STS           | 33.34 ± 2.06 | 3.25 ± 0.40 | 6.1 ± 0.40       |
| 75 PUT + 5 STS              | 75.00 ± 3.37  | 5.56 ± 0.20 | 4.80 ± 0.20      | 0.0 PUT + 15 STS           | 8.34 ± 0.81  | 2.00 ± 0.30 | 2.1 ± 0.09       |
| 150 PUT + 5 STS             | 25.00 ± 3.89  | 3.00 ± 0.20 | 5.11 ± 0.60      | 25 PUT + 15 STS            | 33.34 ± 2.06 | 3.50 ± 0.40 | 2.9 ± 0.20       |
| 0.0 PUT + 10 STS            | 25.00 ± 3.89  | 2.66 ± 0.40 | 2.50 ± 0.25      | 75 PUT + 15 STS            | 58.34 ± 4.81 | 4.70 ± 0.42 | 3.2 ± 0.10       |
| 25 PUT + 10 STS             | 50.00 ± 2.75  | 4.16 ± 0.37 | 4.10 ± 0.50      | 150 PUT + 15 STS           | 16.67 ± 1.53 | 2.50 ± 0.25 | 4.3 ± 0.42       |

LSD < 0.05 13.90 0.70 1.20

* ± Standard error (n = 12)

**Fig. 3** Rooting in in vitro raised date palm 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.

**Fig. 4** Effect of putrescine (PUT) and silver thiosulfate (STS) on acclimatization of date palm cv. Quntar after eight weeks from transition to plastic pots.
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 (Muhsen 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 mg L⁻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 (Table 1, 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 calyx growth and plant regeneration in vitro (Saiprasad and Raghuvee 2007; Sarropoulou et al. 2016). Sridhar et al. (2011) reported that STS significantly increased the shoot regeneration response and average buds in Solanum nigrum. Similar to our results, this finding is in a harmony with the results of Thiruvengadam and Chung (2015) who reported a positive correlation between SPD and shoot regeneration in gherkin (Cucumis anguria L.). However, the addition of SPD (75 mg L⁻¹) in MS containing STS (10 mg L⁻¹) produced a higher percentage of response as well as the number of shoots/jar when compared to STS alone (Table 5). 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. 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; Arun et al. 2014; Roh et al. 2012; Muhsen et al. 2020).

The number, length, and development of roots are essential in the in-vitro development with micropropagation. PAs play a vital role in rooting. 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 essential in differentiation (Tiburcio et al. 2014). PAs play a major role in cell division and different morphogenetic processes, including rooting (Kielkowska and Adamus 2021). 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 a marker of root induction in vitro. Its catabolism could be the basis for root growth by providing H₂O₂ (Neves et al. 2002). It has previously been shown that PUT to MS media increases endogenous PUT 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 adding ethylene inhibitor to the culture medium along with an auxin significantly augmented the induction of roots.

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 amplified bands’ quantity, quality, and reproducibility. 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). Similar results were obtained by some other authors (Abdol vand et al. 2018; Al-Mayahi 2021c), who used molecular markers to confirm the genetic stability of micropropagated date palm plantlets.

The banding pattern analysis confirmed no somaclonal variation and, therefore, the reliability of the micropropagation protocol for producing true-to-type plantlets of date palm cv. Quntar on a mass scale.

Conclusion

This study provides an efficient in vitro propagation protocol for producing genetically uniform date palm plants. Our study indicated that SPD alone or in combination with STS plays a synergistic role in improving shoot regeneration from callus tissues of date palm cv. Quntar in vitro. Also, particular emphasis should be given on PUT, which in combination with STS, in the rooting medium, was essential in stimulating a high rooting percentage with high quality of roots; thereby, 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 could be used for the large-scale production of genetically stable date palm cv. Quntar.

Acknowledgements The author wishes to thank all the Date Palm Research Center staff, especially in the Date Palm propagation Lab.

Author contributions AMWA 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.

Funding The authors have not disclosed any funding.

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

References

Abdol VB, Zarghami R, Ari AA (2018) The effects of Agno3 and 2iP on a different stage of somatic embryogenic in date palm cv Medjool. Pak J Bot 50(2):495–502
Adawy SS, Hussein Ebtissam HA, Saker MM, El-Iriby Hanaiya A (2004) Intra- and Inter-varietal variation of Upper Egypt date palm cultivars (Phoenix dactylifera L.): I As revealed by RAPD and inter simple sequence repeat markers. Proc Int Conf Genet Eng Appl Sharm El-Sheikh South Sinai 2004:165–179
Alizadeh M, Krishna H, Eltekhari M, Modareskia M, Modareskia M (2015) Assessment of clonal fidelity in micropropagated horticultural plants. J Chem Pharm Res 7(12):511–514
Al-Mayahi AMW (2010) The effect of amino acids and silver nitrate in the growth and organogenesis of adventitious buds for date palm (Phoenix dactylifera L.) cv. Showaithy by in vitro. Damas Univ J Agric Sci. 26(2):95–110
Al-Mayahi AMW (2016) Effect of red and blue light emitting diodes “CRB-LED” on in vitro organogenesis of date palm (Phoenix dactylifera L.) cv. Alshakt. World J Micro Biotechnol 32:160
Al-Mayahi AMW (2019) Effect of aluminium on the growth of the in vitro culture tissues of the date palm (Phoenix dactylifera L.) cv. Um-Adelhin. Folia Oecol 46(2):164–169
Al-Mayahi AMW (2020) Effect of calcium and boron on growth and development of callus and shoot regeneration of date palm cv. Barhee. Can J Plant Sci 100(4):357–364
Al-Mayahi AMW (2021a) The effect of humic acid (HA) and zinc oxide nanoparticles (ZnO-NPS) on in vitro regeneration of date palm (Phoenix dactylifera L.) cv. Quntar. Plant Cell Tiss Organ Cult 145:445–456
Al-Mayahi AMW (2021b) In vitro plant regeneration system for date palm (Phoenix dactylifera L.): effect of chelated iron sources. J Genet Eng Biotechnol 19:83
Al-Mayahi AMW (2021c) The effect of phenyl acetic acid (PPA) on micropropagation of date palm followed by genetic stability assessment. J Plant Grow Regul. https://doi.org/10.1007/s00344-021-10500-5
Al-Mayahi AMW, Ali AH (2021) Effects of different types of gel agents on in vitro organogenesis and some physicochemical properties of date palm buds, showathy cv. Folia Oecol 48(1):110–117
Al-Mayahi AMW, Ali AH, Shareef HJ (2018) Influence of cold pretreatment on shoot regeneration from callus in date palm (Phoenix dactylifera L.) cv. ‘Barhee.’ J Gen Eng Biotechnol 16:607–612
Al-Mayahi AMW, Jafar ON, Mohsen KA (2020) Effect of glutathione (GSH) on Date palm (Phoenix dactylifera L.) micropropagation. Folia Oecol 47(1):64–69
Arun M, Subramanyam K, Theboral J, Ganapathi A, Manickavasagam M (2014) Optimized shoot regeneration for Indian soybean: the influence of exogenous polyamines. Plant Cell Tiss Organ Cult 117(305):309
Bader SM, Khierallah HS (2009) The role of silver thiosulphate and glutamine on direct organogenesis of two date palm (Phoenix dactylifera L.) cultivars. J Biotechnol Res Cen 3(1):37–45
Bais HP, Ravishankar GA (2002) Role of polyamines in the ontogeny of plants and their biotechnological applications. Plant Cell Tissue Org Cult 69:1–34
Baron K, Stasolla C (2008) The role of polyamines during in vivo and in vitro development. In Vitro Cell Dev Plant 44:384–395
Chae SC (2016) Shoot organogenesis of Echinacea angustifolia DC as influenced by polyamines. Life Sci J 13(1):16–19
Chaudhary DS, Kajla A, Poonia B, Brar S, Duhan JS (2015) Molecular assessment of genetic stability using ISSR and RAPD markers in commercial banana cultivar cv. Robusta India. J Biotechnol 14(3):420–424
Chen D, Shao Q, Yin L, Younis A, Zheng B (2019) Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. Front Plant Sci 9:1945
Couée I, Hummel I, Salmon C, Gouesbet G, Amrani AE (2004) Involvement of polyamines in root development. Plant Cell Tissue Organ Cult 76:1–10
Denaxa NK, Roussos PA, Vemmos SN (2014) The possible role of polyamines to the recalcitrance of “Kalama” olive leafy cuttings to root. J Plant Growth Regul 33:579–589

Devi SP, Kumaria S, Rao SR, Tandon P (2013) In vitro propagation and assessment of clonal fidelity of Nepenthes khasiana Hook. f.: a medicinal insectivorous plant of India. Acta Physiol Plant 35:2813–2820

Dey A, Hazra AK, Nongdam P, Nandy S, Tikendra L, Mukherjee A, Banerjee S, Mukherjee S, Pandey DK (2019) Enhanced bacoside content in polyamine treated in-vitro raised Baccaea monnieri (L.) Wettst. S Afr J Bot 123:259–265

El-Bahr MK, El-Ashry AAE, Gabr AMM (2019) Impact of antioxidants on in vitro rooting and acclimatization of two Egyptian dry date palm cultivars. Pak J Biol Sci 22:435–443

Harathi K, Naidu CV (2016) Influence of ethylene inhibitor silver nitrate on direct shoot regeneration from in vitro raised shoot tip explants of Sphaeranthus indicus Linn. an important antiaulcer medicinal plant. Am J Plant Sci 7:525–532

Hausman JF, Kevers C, Gaspar T (1995) Auxin-polyamine interaction in the control of the rooting inductive phase of poplar shoots in vitro. Plant Sci 110:63–71

Ibrahim MA, Waheed AM, Al-Taha H, Al-Taha H (2013) Plantlet regeneration from root segments of Date palm tree (Phoenix dactylifera L. cv. Barhee) producing in vitro culture. AAB BIOFLUXAAB Bioflux 5(1):45–50

Ilevinsh G, Kruzmane D, Rusite E, Arente G, Gertnere D (2000) Modulation of Solanum tuberosum L. morphogenesis and antioxidative status in a stem explant culture by limitation of gas exchange: putative effects of ethylene. J Plant Phys 156(5–6):717–723

Kielkowska A, Adamus A (2021) Exogenously applied polyamines reduce reactive oxygen species, enhancing cell division and the shoot regeneration from Brassica oleracea L. var capitata polyplasts. Agronomy 11:735

Kumar P, Lakshmanan P, Thorpeee TA (1998) Review: regulation of cytokinin side content in polyamine treated in-vitro raised S. indicus. Br J Biol Sci 22:435–443

Kumar P, Lakshmanan P, Thorpeee TA (2007) History of plant tissue culture. Mol Biotechnol 318:9–32

Larkin PJ, Scowcroft WR (1981) Somaclonal variation: a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60(4):197–214

Laukkanen H, Sarjala T (1997) Effect of exogenous polyamines on Scots pine callus in vitro. J Plant Physiol 150:167–172

Mattoo AK, Minoscha SC, Minoscha R, Handa A (2010) Polyamines and cellular metabolism in plants: transgenic approaches reveal different responses to diamine putrescine versus higher polyamines spermidine and spermine. Amino Acids 38:405–413

Mittler R (2002) Oxidative stress antioxidants and stress tolerance. Trends Plant Sci 7(9):405–410

Moghaieb REA, Abdel-Hadi AA, Ahmed MRA (2011) Genetic stability among date palm plantlets regenerated from petiole explants. Afr J Biotechnol 10(65):14311–14318

Muhusen KA, Hantosh EA, Darweesh MA (2020) The effect of putrescine and sal salic acid and their interaction on the multiplication of vegetative bud and their characteristics for date palm cultivar Al-Barhi in vitro. Plant Cell Biotechnol Mol Biol 21(19&20):112–125

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497

Ney M (2017) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Neves C, Santos H, Vilas-Boas L, Amânisco S (2002) Involvement of free and conjugated polyamines and free amino acids in the adventitious rooting of micropropagated cork oak and grapevine shoots. Plant Physiol Biochem 40:1071–1080

Panizza M, Mennuzzi-aodi A, Togni F (1993) Role of ethylene in axillary shoot proliferation of lavandin - interaction with benzyladenine and polyamines. J Exp Bot 44(259):387–394

Park E, Bae H, Park WT, Kim YB, Chae SC, Park SU (2012) Improved shoot organogenesis of gloxinia (Sinningia Speciosa) using silver nitrate and putrescine treatment. Plant Omics J 5:6–9

Rakesh B, Sudheer WN, Nagella P (2021) Role of polyamines in plant tissue culture: an overview. Plant Cell Tissue Organ Cult 145:487

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol 5:69–76

Roh KH, Kwak BK, Kim JB, Lee KR, Kim HU, Kim SH (2012) The influence of silver thiosulfate and thidiazuron on shoot regeneration from cotyledon explants of Brassica napus. J Plant Biotechnol 39:133–139

Saiprasad GVS, Raghuvpeer P (2007) Influence of ethylene inhibitors and ethrel on production of protocorm like bodies in orchid - Dendrobium ‘Sonica.’ J Hortic Sci 2(1):13–18

Saker MM, Bekheet SA, Taha HS, Fahmy AS, Moursy HA (2000) Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. Biol Plant 43:347–351

Sarropoulou V, Dimassi-theriooou K, Therios I (2016) Effect of the ethylene inhibitors silver nitrate, silver sulfate, and cobalt chloride on micropropagation and biochemical parameters in the cherry rootstocks CAB-6P and Gisela 6. Turk J Biol 40:670–683

Sharaf MM, Khamis MA, El-Bana A, Abd El-Galeil LM, Zaid ZE (2012) Improvement of date palm plantlets during rooting stage by silver ion. In: Third international conference on radiation sciences and applications, pp 709–719

Shenoy VB, Vasil IK (1992) Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napiergrass (Pennisetum purpureum K. Schum). Theor Appl Genet 83:947–955

Sivanandhan G, Salammal T (2011) The effect of polyamines on the efficiency of multiplication and rooting of Withania somnifera (L.) Dunal and content of some withanolides in obtained plants. Acta Physiol Plant 33:2279–2288

Sridhar TM, Preethi D, Naidu CV (2011) Effect of silver thiosulphate on in vitro plant regeneration of Solanum nigrum (Linn): an important antihyperglycaemic medicinal plant. Curr Bot 2(7):14–16

Srivashtav VS, Kapadia CV, Mahatma MK, Jha SK, Jha S, Ahmad T (2013) Genetic diversity analysis of date palm (Phoenix dactylifera L.) in the Kutch region of India using RAPD and ISSR markers. EMI J Food Agric 25(11):907–915

Takahashi T, Kakehi J (2010) Polyamines: ubiquitous polycations with unique roles in growth and stress responses. Ann Bot 105(1):1–6

Tang W, Newton R (2005) Polyamines promote root elongation and growth by increasing root cell division in regenerated virginia pine (Pinus virginiana Mill.) plantlets. Plant Cell Rep 24(10):581–589

Thiruvengadam M, Chung M (2015) Phenolic compound production and biological activities from in vitro regenerated plants of Withania somnifera (L.) using silver ion. J Med Plants Res 6(19):3579–3585

Thorpe TA (2007) History of plant tissue culture. Mol Biotechnol 318:9–32

Tiburcio AF, Gendy CA, Van TT (1989) Morphogenesis in tobacco smoke exposure. J Biotechnol 18:295–301

Tiberio SB, Pinto M, Ford K, Houghton CA, Varma NR, Varma V, Yotsuyanagi H, Bungard J, Atkinson G, Violante F, Attfield P, Tieman DM, Marson D, Denaxa NK, El-Behery A, Alumkal J, Bi C, Pham D, McVittie KJ, Voorrips RE, Prinsen CA, Weijers D (2018) Genetic diversity analysis of date palm (Phoenix dactylifera) using RAPD and ISSR markers. EMI J Food Agric 25(11):907–915

Vasudevan A, Selvaraj N, Ganapathi A, Kasthurirengan S, Ramesh-Anbazhagan V, Manickavasagam M, Choi C (2008) Leucine and spermidine enhance shoot differentiation in cucumber (*Cucumis sativus* L.). In Vitro Cell Dev Biol Plant 44:300–306

Wang KL, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14:131–151

Wrigley G (1995) Date palm. In: Smart J, Simonds NW (eds) Evolution of crop plants, 2nd edn. Longman, London, pp 399–403

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.