FOLIA OECOLOGICA – vol. 48, no. 1 (2021), doi: 10.2478/foecol-2021-0012

Effects of different types of gelling agents on *in vitro* organogenesis and some physicochemical properties of date palm buds, Showathy cv.

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Abstract

Al-Mayahi, A.M.W., Ali, A.H., 2021. Effects of different types of gelling agents on *in vitro* organogenesis and some physicochemical properties of date palm buds, Showathy cv. *Folia Oecologica*, 48 (1): 110–117.

Some obstacles are associated with *in vitro* propagation of date palm, such as explant tissue browning, slow callus growth and development, low organogenesis and multiplication efficiency, and frequent tissue vitrification. This investigation studied the effect of five types of gelling agents (Danish Agar, Cero Agar Type 8952, Chile Agar, Gerlite Food Grade, and Agar-Agar.) on *in vitro* regeneration and bud multiplication of *Phoenix dactylifera* L. cv. Showathy. The results showed that the highest percentages of callus producing buds and average bud formation (77.78%, 11.5 buds, and 72.23%, 10.9 buds) were obtained in response to 7 g l^{-1} Danish Agar and Cero Agar Type 8952, respectively. A decrease in browning percentage was observed in tissues cultured in the medium gelled with Danish Agar. Observations showed that Danish Agar and Cero Agar Type 8952 eliminated also shoot vitrification. Compared with other treatments, the total amount of phenolic compounds was significantly reduced to 0.79 and 0.82 mg GAE/g in buds cultured in the media gelled with Danish Agar and Cero Agar Type 8952, respectively. The macronutrient phosphor, calcium, sodium, and micronutrient boron and copper significantly increased in the *in vitro* shoots regenerated on the media gelled with Danish Agar and Cero Agar Type 8952.

Keywords

browning, multiplication, nutrients, phenol compounds, vitrification

Introduction

Date palm (*Phoenix dactylifera* L.), which belongs to the Arecaceae family, is an important fruit crop grown widely in arid and semi-arid regions because of its multifaceted uses and high economic returns (Abdelaziz et al., 2019; Al-Mayahi et al., 2020a). Date palm is commonly propagated vegetatively by offshoots. However, micropropagation is the preferred means to produce a mass number of healthy plants genetically identical to the mother plant (Al-Khayri and Naik, 2017; Al-Mayahi, 2019). The formation and multiplication of *in vitro* shoots are affected by many factors, including the chemical and physical characters (Al-Mayahi, 2012 and 2014; Ibrahim et al., 2013), such as the type and concentration of the gelling agent (Lebedev et al., 2019). Gelling agents are usually added to the culture medium to make it's viscous enough to support plant tissues floatation

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above the nutrient medium (Raina, 2017). Gelling agents used to solidify plant growth medium can contain many mineral nutrients reported to affect plant growth (Joshi, 2009). Generally, many studies have shown that the type of gelling agent used can influence tissues' growth *in vitro* (Quiala et al., 2014; Ramesh and Ramassamy, 2014, 2015; Al-Mayahi, 2015; Raina, 2017), therefore the study on selecting commercial grades of gelling agents is very important. There are limited studies about gelling agent effect routinely used in micropropagation on palm plant growth and development (Palanyandy et al., 2020; Al-Mayahi, 2015). The study aimed to compare the effects of five gelling agents commonly used to solidify plant tissue culture media (Danish Agar, Cero Agar Type 8952, Chile Agar, Gerlite Food Grade, and Agar-Agar) on the growth and multiplication *in vitro* of date palm buds and analysis of some physicochemical parameters.

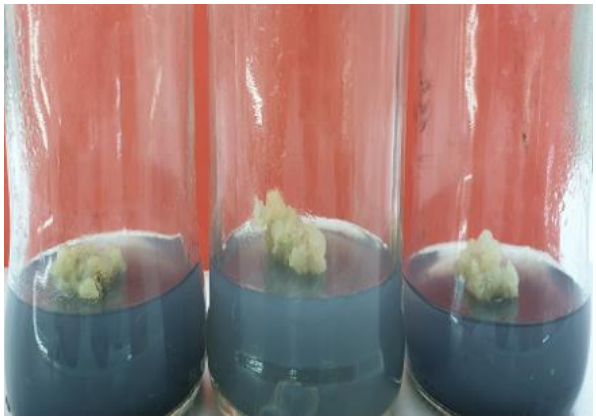

Fig. 1 Callus induced from shoot tip explant of date palm (*P. dactylifera* L. Showathy cv.) is used in this study.

Materials and methods

The present study was performed in The Tissue Culture Laboratory of Date Palm Research Centre, Basra University, Basrah, Iraq, during 2017 and 2018.

The shoot tip terminal, about 1 cm long, was sectioned longitudinally into four sections. In order to induce callus formation, explants were transferred to Murashige and Skoog (1962) (MS) basal medium supplemented with 3 mg l^{-1} 6-(γ,γ-Dimethylallylamino)purine, (synonym: N^6-(2-Isopentenyl)adenine (2iP)), 30 mg l^{-1} NAA, 3 g l^{-1} activated charcoal, 100 mg l^{-1} glutamine, 5 mg l^{-1} thiamine HCl, 1 mg l^{-1} biotin, 30 g l^{-1} sucrose, and solidified with Agar-Agar at 7.0 g l^{-1} (Al-Mayahi, 2014). Cultures were kept under complete darkness at 27 ± 2 °C. The cultures were transferred to fresh media, with the same composition every 6 weeks until the callus began to grow. After 10 weeks, callus at the weight of about 100 mg in average (Fig. 1) was separated and cultured on the medium composed of MS 0.5 mg l^{-1} Naphtalene acetic acid (NAA), 0.5 mg l^{-1} 6-Benzylaminopurine (synonym: N^6-Benzyladenine (BA)), 5 mg l^{-1} Kinetin (K) and 0.5 g l^{-1} activated charcoal according to Al-Mayahi, (2016). The medium pH was adjusted to 5.8 before the adding of gelling agent.The medium was solidified; with five different gelling agents; Danish Agar, Cero Agar Type 8952, Chile Agar, Gerlite Food Grade, and Agar-Agar. The concentrations used in this study were chosen based on our previous researches. Each gelling agent was added to the culture medium at a concentration of 7 g l^{-1}, except for the Gerlite Food Grade, which was added at a concentration of 3 g l^{-1}. Callus tissues (100 mg) were weighed and cultured in 340 ml glass jars containing 50 ml of culture media, closed with a metal cap. The cultures were incubated in a growth chamber under 27 ± 2 °C and 16-h light / 8-h dark cycle with illumination from cool white fluorescence lamps 40 μmol^{-2} m^{-2} s^{-1}. Subcultures were made at 6-week intervals on the same medium with the aim to obtain shoots. Results regarding the percentage of bud regeneration and buds number per 100 mg callus (jar) were recorded after 10 weeks. Treatments were arranged in a completely randomized design with 18 replications.

Morphological parameters

Browning percentage (%)

It was estimated by calculation of the number of brown cultures from the total number of cultures.

Vitrification percentage (%)

The vitrification percentage (%) was calculated depending on their external appearance; the shoots were classified as suffering vitrification and had a glassy and watery appearance compared to normal shoots.

Chemical analysis

Moisture content

Weight losses for fresh buds were obtained until a constant mass was reached. Moisture was determined from shoot weight loss after oven drying at 60 °C until reaching constant weight .

Total phenolic content

Phenolic content was extracted according to the method described by Singleton and Rossi (1965). Gallic acid was used as a reference standard; 0.2 ml of extract solution and 0.2 ml of Folin–Ciocalteu reagent was added, and the contents mixed thoroughly. After 5 min, 1 ml of 15% of sodium bicarbonate Na_2CO_3 was added, and then the mixture was left at room temperature for 2 h. The absorbance was measured at 765 nm. Results were expressed as milligram of Gallic acid equivalent (mg GAE/g).

Mineral analysis of shoots

Content of total sodium (Na), potassium (K), calcium (Ca), and phosphorus (P) in shoots were analyzed according to the method described by Cresser and Parsons (1979). 0.2 g of shoot samples (as dry weight) were taken into a caldal flask with a capacity of 100 cm^3 and digested with a mixture of sulfuric acid (69%) and perchloric acid (62%) under heating for one hour; subsequently, the digested solution was transferred into volumetric flask 50 cm^3, and volumes were completed in size with distilled water. Chemical analyses were performed using the following methods: Phosphorus was measured by a spectrophotometer at 880 nm, according to Murphy and Riley (1962). K, Ca, Mg, and Na were determined by atomic absorption spectrometry, according

to the method described by Black (1968). For B analysis, shoots were dried and digested with 10 ml 0.36 mol l^{-1} H_2SO_4 (Gaines and Mitchell, 1979). B was quantified by the spectrometer. For Cu analysis, each sample was dissolved in 5 ml of a 20% HCl solution. Cu in shoots were determined by atomic absorption spectrophotometry. There were three replicates of each analysis.

Statistical analysis

The experiments were conducted in a completely randomized design (CRD). Data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software version 20. Treatment means were compared using the least significant difference (LSD $P < 0.05$). All treatments were replicated 15 times.

Results

Effect of gelling agents on some morphological parameters: the influence of the gelling agent on the *in vitro* bud induction rates of date palm Showathy cv.

Table 1 showed that different types of a gelling agent had significant effects on buds induction rates. The cultured callus showed significant variations in percentage and the regeneration of buds cultured in the media supplement with different gelling agents (Table 1). The cultures were grown on the medium gelled with Danish Agar, and Cero Agar Type 8952 showed a better result in a percentage of bud formation and bud number compared with the cultures grown in media containing Chile Agar, Gerlite Food Grade, and Agar-Agar (Table 1) (Fig. 2). On media with Danish agar and Cero Agar Type 8952, 77.78 and 72.23% of callus produced bud with average number of buds of 11.5 ± 0.81 and 10.9 ± 0.09 buds/100 mg (jar), respectively.

Browning percentage (%)

The results in Figure 3 showed that the different types of a gelling agent had significant effects on the browning percentage. The cultures grown on the medium gelled with Danish Agar and Cero Agar Type 8952, showed the best results in reducing the browning percentage where the browning reached 12.5%, compared with the cultures grown on the media supplemented with other types of gelling agent, Chile agar, Gerlite food grade and Agar-Agar, where the browning reached 25.0%, 25.0% and 37.5% respectively (Fig. 3).

Effect of gelling agents on vitrification

Visual observation of the effect of gelling agent type on vitrified bud percentage showed that Danish Agar, Cero Agar Type 8952, Chile Agar, and Agar-Agar gelled media reduced the vitrification of shoots to the minimal

Table 1. Effect of various gelling agents on a percentage (%) of callus forming bud and number of buds/100 mg of callus after 10 weeks of culture of date palm Showathy cv.

Solidifying agent	Response of callus for buds regeneration (%)	Mean number of buds/ 100 mg (jar)
(7 g l^{-1}) Danish Agar	77.78 ± 5.77a	11.5 ± 0.17a
(7 g l^{-1}) Cero Agar Type 8952	72.23 ± 3.61a	10.9 ± 0.09a
Chile Agar (7 g l^{-1})	61.12b ± 4.16c	9.2 ± 0.21b
(3 gl^{-1}) Gerlite Food Grade	55.56 ± 9.29c	7.6 ± 0.18c
(7 gl^{-1}) Agar-Agar	44.45 ± 3.06d	6.1 ± 0.39d

± Standard error (n = 18). Values followed by the same letter are not significantly different at $p < 0.05$.

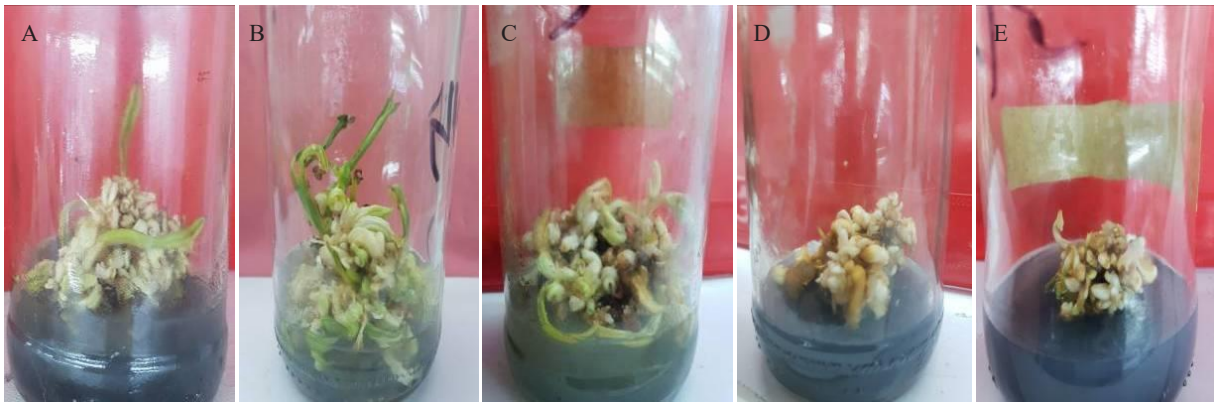

Fig. 2. *In vitro* buds of date palm Showathy cv., developed on media gelled with (A) Danish Agar, (B) Cero Agar Type 8952, (C) Chile Agar, (D) Gerlite Food Grade, and (E) Agar-Agar.

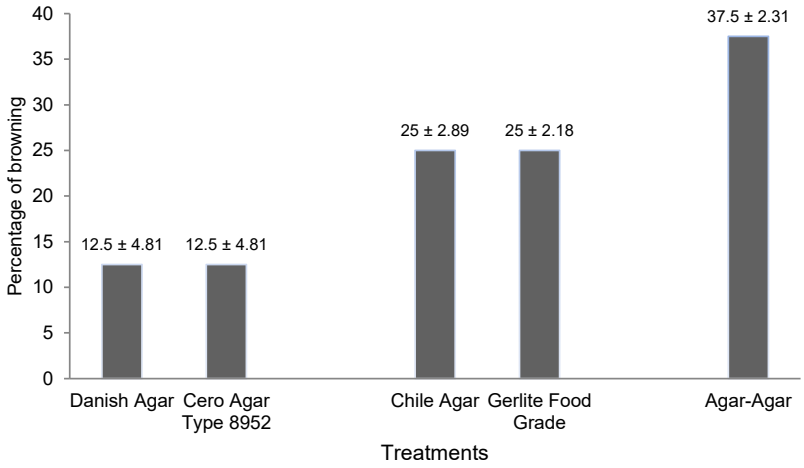

Fig. 3. The effect of various gelling agents on a browning percentage (%) (mg GAE/g) of date palm buds Showathy cv., *in vitro*.

rates (0.0, 0.0, 12.5 and 12.5 % and 12.5% respectively), while Gerlite Food Grade aggravated the occurrence of vitrification to 37.5% (Table 2) (Fig. 4).

Table 2. Effect of various gelling agents on vitrification percentage (%) of date palm buds Showathy cv., *in vitro* (n = 8

Solidifying agent	Percentage of vitrification shoots (%)
(7 g 1^{-1}) Danish Agar	0.0 0.0a
(7 g 1^{-1}) Cero Agar Type 8952	0.0 ± 0.0a
Chile Agar (7 g 1^{-1})	12.5 ± 1.2a
(3 g1^{-1}) Gerlite Food Grade	37.5 ± 2.1b
(7 g 1^{-1}) Agar-Agar	12.5 ± 1.2a

± Standard error (n = 18).Values followed by the same letter are not significantly different at $p < 0.05$.

Fig. 4. Effect of Gerlite Food Grade on vitrification of date palm buds Showathy cv., *in vitro.*

Effect of gelling agents on biochemical parameters

Moisture percentage

The buds cultured on Gerlite Food Grade (3 g l^{-1}) had a higher moisture percentage (90%) than those cultured in media gelled with the other gelling agents. Buds cultured on 7 g l^{-1} Danish Agar had the least moisture percentage, 65% (Fig. 5).

Phenolic compounds contents percentage

The phenol compounds were found highest in buds tissues of Showathy cv., on the medium gelled with Agar-Agar, which was 1.75 mg GAE/g. Simultaneously, this means value decreased significantly and reached the lowest level on the medium solidified with Danish and Cero Agar Type 8952, which were 0.79 and 0.82 mg GAE/g, respectively (Fig. 6).

Mineral content

The medium gelling agent significantly affects the accumulation of elements in shoot tissues of date palm cultured *in vitro*. A significant increase of P, Ca, Na and most microelements studied, such as B and Cu were recorded in the shoots tissues cultured on Danish Agar and Cero Agar Type 8952. Shoots cultured on Gelrite had a higher Mg concentration than media supplemented with other gelling agents. At the same time, statistical analyses did not appear significant differences in the content of K in shoots on all studied media (Table 3).

Discussion

During *in vitro* culture, date palms are exposed to many problems, which directly affect commercial production. Tissue culture media solidified with an appropriate gelling agent that provides a suitable milieu for plant cells and tissue growth are needed. During the present study, we tested the influence of different gelling agents (Danish Agar, Cero Agar Type 8952, Chile Agar, Gerlite Food Grade, and Agar-Agar) on the micropropagation of date palm Showathy cv., to identify the appropriate type that produces a high number of good-quality date palm shoots. Efficient

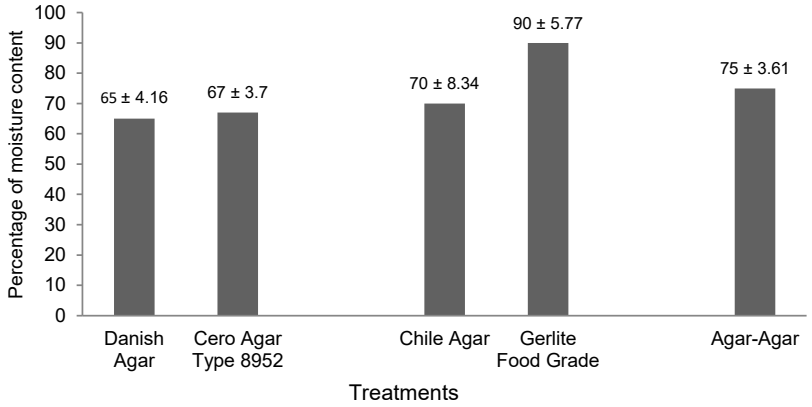

Fig. 5. The effect of various gelling agents on the percentage of moisture content (%) of date palm buds Showathy cv., *in vitro.*

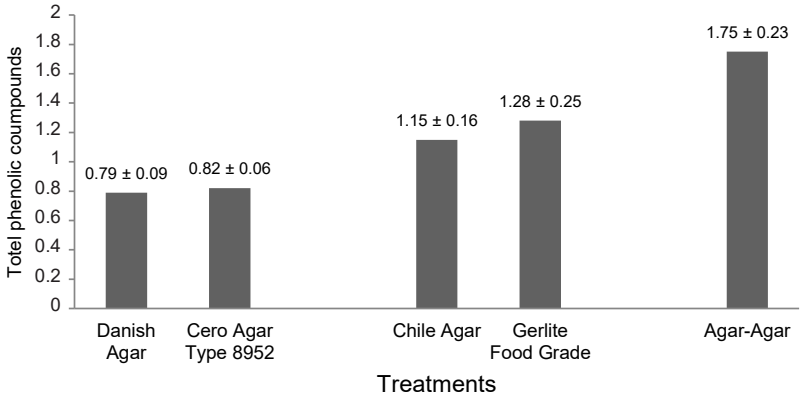

Fig. 6. The effect of various gelling agents on total phenolic compounds (mg GAE/g) of date palm buds Showathy cv., *in vitro*.

bud induction rate is the important economic aspect in the commercial micropropagation of the date palm. The media were optimized when using Danish Agar, Cero Agar Type 8952, and produced a maximum 11.5 and 10.9 number of buds per 100 mg of callus, respectively (Table1). Ensure proper media hardness is of importance in plant tissue cultures. The soft media inclined to liquidity. In that case, the hyperhydricity can be frequent in the *in vitro* cultures while the high solid media are making difficult to absorb the nutrients by the tissue-cultured, thus causing growth inhibition (Gangopadhyay et al., 2009). The choice of gelling agent strongly affects the growth and development of cultivated tissues (Tremblay and Tremblay, 1991; Al-Mayahi, 2015). There are some advantages among gelling agents affecting the *in vitro* growth characters by water retention on gels and the availability of nutrients for cultured tissues (Ramesh and Ramassamy, 2015). Also, the variations appear in responses of plant tissue cultured due to interaction with media components (Romberger and Tabor, 1971), impurities (Nairn et al., 1995) and gelling strength. Ebrahim and Ibrahim (2000) explained that the gelling agents usually is combined with water and absorb variously other compounds. Therefore, it can be concluded that the gelling agents used in this study were different in their effect**.** The phenomenon of vitrification is one of the serious problems that occur during *in vitro* culture of date palm, which directly affects production on a commercial scale (Yadav et al., 2003; Al-Asadi et al., 2020). Our study indicated that the vitrified buds had higher tissue water content than non-vitrified buds (data not shown). The harmful effects of tissue vitrification due to high relative humidity can be reduced using different gelling agents in the medium. Variation in the responses of shoots on different galling agents can be attributed to differences in the water potential of the medium reflected in the leaves what affects plant growth (Buah et al., 1999). Te-Chato et al. (2005) reported that some types gelling agents produce problems related to tissue vitrification. It was observed that low agar concentrations in the medium cause the wet weight gain of *Nicotiana tabacum* L. (Joshi, 2009). There was a decrease in the percentage of vitrification shoots when the gerlite concentration increased from 0.1% to 0.4%, with crabapple (Turner and Singha, 1990). Numerous treatments have been reported to control hyperhydricity "Vitrification", including the use of solid media with a high level of a gelling agent or use a gelling agent with higher gel strength (Debergh,1983). This phenomenon occurs in many plant propagated

Table 3. The effect of various gelling agents on the minerals content in the shoots of date palm buds Showathy cv., *in vitro*

Solidifying agent	P (mg g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Mg (mg g^{-1} DW)	Na (mg g^{-1} DW)	B (mg g^{-1} DW)	Cu (mg g^{-1} DW)
(7 g l^{-1}) Danish Agar	3.358 ± 0.31a	3.160 ± 0.2a	19.088 ± 1.09a	12.780 ± 1.0b	2.926 ± 0.17a	3.690 ± 0.5a	1.37 ± 0.13a
(7 g l^{-1}) Cero Agar Type 8952	3.175 ± 0.28a	3.011 ± 0.08a	18.986 ± 0.88a	12.690 ± 1.5b	2.794 ± 0.25a	3.430 ± 0.4b	1.29 ± 0.08a
Chile Agar (7 gl^{-1})	2.498 ± 0.19b	2.985 ± 0.17a	17.43 ± 0.87b	11.838 ± 0.9c	2.477 ± 0.19b	2.690 ± 0.2c	1.10 ± 0.06b
(3 g l^{-1}) Gerlite Food Grade	1.961 ± 0.06c	3.438 ± 0.7a	16.812 ± 0.52c	13.034 ± 1.2a	2.066 ± 0.08d	1.93 ± 0.3e	0.88 ± 0.04c
(7 g l^{-1}) Agar- Agar	2.028 ± 0.08c	2.808 ± 0.04a	16.986 ± 0.47	11.693 ± 1.0d	2.214 ± 0.18c	2.380 ± 0.2d	1.03 ± 0.05b

± Standard error (n = 5). Values followed by the same letter are not significantly different at $p < 0.05$.

in vitro, including date palms. It reduces their chances of success due to the weakness rate of survived plants obtained. It was proven that agar concentration in the culture medium has an important role in hypersensitivity of *Gypsophila paniculata* L. tissue (Rady, 2006). The water potential of the culture medium is reduced with increasing concentration of gelling agents in harmony with the results of Buah et al. (1999), who reported that high agar concentrations lead to reduced media water potential. Gelrite increases vitrification in date palm; similar results have been found with different species (Ivanova and Van Staden, 2010; Quiala et al., 2014). Thus, the vitrification of cultured tissues was reported in this paper in accordance with many other researchers who reported that raising agar content could reduce the vitrification of produced shoots (Rady, 2006).

The presence of phenol substances in cultured tissue is one of the serious problems that hinder the micropropagation of date palm (Al-Mayahi et al., 2010; Al-Mayahi 2020b). These phenolic compounds are exuded from the cut parts of explants and oxidized due to the enzymes such as peroxidases (POD) and polyphenolase (PPO) (an enzyme involved in tissue browning, is a tetramer that contains four atoms of copper per molecule, and binding sites for two aromatic compounds and oxygen), or when exposed to air (Onuoha et al., 2011), resulting in the culture medium turning brown and death of cultured tissues (Aliyu, 2005). The present study also proved that the Danish Agar, Cero Agar Type 8952, and Chile Agar treatments used in all experimental investigations effectively reduce phenolic compounds (Fig. 3). Choose the proper type of gelling agent is an important factor for plants *in vitro* regeneration (Kacar et al., 2010). The properties of different gelling agents depend on several factors including the degree of clarity or purity, polymerizability and water retention capacity, which in turn affect the availability of nutrients (Beruto et al., 1999). Inorganic compounds in gelling agents and the interaction gelling agent-medium-tissue play an important role during tissue growth in vitro (Scholten and Pierik, 1998). Improvement growth of date palm buds was observed at 7 g l^{-1} Danish Agar, Cero Agar Type 8952. The superiority of the Danish Agar and Cero Agar Type 8952 may be due to their element content, such as P, Ca, Na, B, and Cu in sufficient quantities for the growth and development of date palm tissues (Table 3).

Conclusion

The growth of date palm buds *in vitro* was affected by the type of gelling agent mainly, which causes the difference in the physical and chemical properties of the buds cultured. The MS medium gelled with 7 g l^{-1} of Danish Agar and Cero Agar Type 8952 is most suitable for *in vitro* induction of date palm Showathy cv. buds with the aim to obtain healthy cultures. The chemical analysis of the cultured buds on media supplemented with various gelling agents revealed that Danish Agar and Cero Agar Type 8952 had a high P, Ca, Na, B, and Cu content compared to the other gelling agents. Therefore, we suppose that adding Danish

Agar, or Cero Agar Type 8952 to the culture medium, can promote plant propagation under *in vitro* conditions.

Acknowledgements

The authors thank and appreciate all the staff at the Date Palm Research Center, especially in the Date Palm Propagation Lab.

References

Abdelaziz, A.M., Soliman, S.S.A., Ahmed, T.A.S., Heakal, R.M., Hassanin, A.A., 2019. Micropropagation of Zaglol and Barhy date palm cultivars using immature female inflorescence explants: effect of growth regulatorsbalance. *Zigzag Journal Agriculture Research*, 46 (6A): 2023–2035.

Al-Asadi, A.Z., AL-Mayahi, A.M.W., Abdulwahid, A.H., 2020. Effect of phloroglucinol (PG) on in vitro growth and multiplication of the date palm cv. Barhee. *Basrah Journal of Date Palm Research*, 14 (1): 31–45.

Al-Khayri, J.M., Naik, P.M., 2017. Date palm micropropagation: advances and applications. *Ciência e Agrotecnologia*, 41 (4): 347–358.

Al-Mayahi, A.M.W., Ahmed, A.N., Al-Khalifa, A.A., 2010. Isolation and diagnose of fungi associated with the cultivation of tissues of five cultivars of date palm (Phoenix dactylifera L.) and the effect of fungicide Benlate in control. *Basrah Journal for Date Palm Research*, 9 (2): 79–97.

Al-Mayahi, A.M.W., 2012. Effect of the media on vegetative buds and rooting of vegetative shoot of date palm cv. Khadrawi propagated by in vitro culture. *Journal of Agricultural* and *Veterinary* Sciences, 5 (1): 27–46.

Al-Mayahi, A.M.W., 2014. Effect of copper sulphate and cobalt chloride on growth of the in vitro culture tissues for date palm (Phoenix dactylifera L.) cv. Ashgar. *American Journal of Agricultural and Biological Sciences*, 9 (1): 6–18.

Al-Mayahi, A.M.W., 2015. An efficient protocol for indirect somatic embryogenesis and shoot organogenesis from leaf segments of date palm (Phoenix dactylifera L.) CV. Quntar. *African Journal of Agricultural Research*, 10 (10): 1031–1042.

Al-Mayahi, A.M.W., 2016. Effect of red and blue light emitting diodes ‘‘CRB-LED’’on in vitro organogenesis of date palm (Phoenix dactylifera L.) cv. Alshakr. *World Journal of Microbiology and Biotechnology*, 32: art. no. 160.

Al-Mayahi, A.M.W., 2019. Effect of aluminium on the growth of the in vitro culture tissues of the date palm (Phoenix dactylifera L.) cv. Um-Adelhin. *Folia Oecologica,* 46 (2): 164–169.

Al-Mayahi, A.M.W., Jafar, O.N., Mohsen, K.A., 2020a. Effect of glutathione (GSH) on Date palm (Phoenix dactylifera L.) micropropagation. *Folia Oecologica*, 47 (1): 64–69.

Al-Mayahi, A.M.W., 2020b. Effect of calcium and boron on growth and development of callus and shoot regeneration of date palm cv. Barhee. *Canadian Journal of Plant Science*, 100 (4): 357–364.

Aliyu, O.M., 2005. Application of tissue culture to cashew (Anacardium occidentale L.) breeding: an appraisal. *African Journal of Biotechnology*, 4 (13): 1485–1489.

Beruto, M., Beruto, D., Debergh, P., 1999. Influence of agar on in vitro cultures. II. Biological performance of Ranunculus on media solidified with three different agar brands. *In Vitro Cellular and Developmental Biology – Plant,* 35: 93–101.

Buah, J. N., Kawamitsu, Y., Sato, S., Murayama, S.,1999. Effects of different types and concentrations of gelling agents on the physical and chemical properties of media and the growth of banana (Musa spp.) in vitro. *Plant Production Science*, 2 (2): 138–145.

Cresser, M.S., Parsons, J.W., 1979. Sulphuric perchloric acid digestion of plant material for the determination of nitrogen, phosphours, potassium, calcium and magnesium. *Analytica Chimica Acta*, 109: 43–436.

Debergh, P.C., 1983.Affect of agar brand and concentration on the tissue culture medium. *Physiologia Plantarum*, 59: 270–276.

Ebrahim, M.K.H., Ibrahim, I.A., 2000. Influence of medium solidification and pH value on in vitro propagation of Maranta leuconeura cv. Kerchoviana. *Scientia Horticulturae*, 86: 211–221.

Gaines, T.P., Mitchell, G.A., 1979. Boron determination in plant tissue by azomethine H method. *Communication in Soil Science and Plant Analysis***,** 10:1099–1108.

Gangopadhyay, G., Roy, S.K., Mukherjee, K.K., 2009. Plant response to alternative matrices for in vitro root induction. *African Journal of Biotechnology*, 8 (13): 2923–2928.

Ivanova, M., Van Saden, J., 2010. Influence of gelling agent and cytokinins on the control of hyperhydricity in Aloe polyphylla. *Plant Cell Tissue Organ Culture*, 104: 13–21.

Joshi, N., 2009. In vitro *g*rowth and shoot multiplication in Nicotiana tabacum L. – influence of gelling agent and carbon source. *International Journal* of *Plant Developmental Biology*, 3 (1): 29–33.

Ibrahim, M.A., Al-Mayahi, A.M.W., Al-Taha, H.A.,2013. Plantlet regeneration from root segments of Date palm tree (Phoenix dactylifera L. cv. Barhee) producing by in vitro culture. *Advances in Agriculture & Botanics – International Journal of the Bioflux Society* 5 (1): 45–50.

Kacar, Y.A., Bicen, B., Varol, I., Mendi, Y.Y., Serce, S., Çetiner, S., 2010. Gelling agents and culture vessels affect in vitro multiplication of banana plantlets. *Genetics and. Molecular Research*, 9 (1): 416–424.

Lebedev, V., Arkaev, M., Dremova, M., Pozdniakov, I., Shestibratov, K., 2019. Effects of growth regulators and gelling agents on ex vitro rooting of raspberry. *Plants*, 8 (3): 1–10.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay of tobacco tissue cultures. *Physiologia Plantarum*, 15: 473–497.

Murphy, J., Riley, J. P., 1962. A modified method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27:31-36.

Nairn, B. J., Furneax, R.H., Stevenson, T.T., 1995. Identification of an agar constituent responsible for

hydric control in micropropagation of radiata pine. *Plant Cell Tissue Organ Culture*, 43: 1–11.

Onuoha, C. I., Chinonye, J. E., Unamba, C.I.N., 2011. In vitro prevention of browning in plantain culture. *Online Journal of* Biological Sciences, 11 (1): 13–17.

Palanyandy, S.R., Gantait, S.,Sinniah, U.R., 2020. Effects of some gelling agents and their concentrations on conversion of oil palm polyembryoids into plantlets. *Journal of Genetic Engineering and Biotechnology*, 18: art. no. 5. https://doi.org/10.1186/s43141-019-0018-z

Quiala, E., Jimenez-Tello, M.V., Barbon, R., Chavez, M., De Feria, M., La, O.M. Perez, M., 2014. Influence of 6-Benzyladenine and gelling agent on the reduction of hyperhydricity in Tectona grandis L. *Revista Colombiana de Biotecnología,*16 (1): 129–136.

Rady, M.R., 2006. In vitro culture of Gypsophila paniculata L. and random amplified polymorphic DNA analysis of the propagated plants. *Biologia Plantarum*, 50 (4): 507–513.

Raina, R.J., 2017. Gelling agents for plant tissue culture media: a comparative study. *International Journal Innovative Research in Science and Engineering*, 3 (1): 324–331.

Ramesh, Y., Ramassamy, V., 2014. The effect of gelling agents on in vitro multiplication of Banana var. poovan. *International Journal of Advanced Biological Research*, 4 (3): 308–311.

Ramesh, Y., Ramassamy, V., 2015. Influence of gelling agents in micropropagation of banana var. Grand Naine. *International Journal of Current* Research *in Biosciences and Plant Biology*, 2 (5): 174–178.

Romberger, A. Tabor, C.A., 1971. The Picea abies meristem in culture. 1. Agar and autoclaving effects. *American Journal* of *Botany*, 58: 131–140.

Scholten, H.J., Pierik, R.L.M., 1998. Agar as gelling agent: differential biological effects in vitro. *Scientia Horticulturae*, 77: 109–116.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungsic acid reagents. *American* J*ournal of Enology and Viticulture*, 16 (3): 144–158.

Te-Chato, S., Petsut, P., Nuchum, P., 2005. Effect of gelling agents on shoot growth and multiple shoot formation of mangosteen. *Songklanakarin Journal of Science and Technology*, 27, Suppl. 3: 637–643.

Tremblay, L., Tremblay, F.M., 1991. Effects of gelling agents, ammonium nitrate and light on the development of Picea mariana (Mill.) BSP (black spruce) and Picea rubens Sarag. (red spruce), somatic embryos. *Plant Science* , 77: 233–242.

Turner, S. R., Singha, S., 1990. Vitrification of crabapple, pear, and geum on gellan gum-solidified culture medium. *Horticultural Science*, 5(12): 1648–1650.

Yadav, M.K., Gaur, A.K., Garg, G.K., 2003. Development of suitable protocol to overcome hyperhydricity in carnation during micropropgation*. Plant Cell Tissue Organ Culture*, 72: 153–156.

Received August 19, 2020
Received December 13, 2020

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