Effect of Phloroglucinol (PG) on in vitro growth and multiplication of the date palm cv.Barhee.

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Abstract

This study was carried out to investigate the effects of different concentrations of Phloroglucinol (PG) (0, 25, 50 and 100) $mg.l^{-1}$ on callus growth and shoot regeneration of in vitro cultures of the date palm cv. Barhee. The addition of PG in a concentration of 50 $mg.l^{-1}$ to the culture medium was more effective in increasing the weight of callus, percentage of callus response for shoots regeneration, as well as, substantially increasing the number of shoots per a jar, compared to the other treatments, including the control treatment, during different study periods. In addition, our results showed a decrease in the browning and vitrification percentage of cultured tissues at 50 $mg.l^{-1}$ of PG, compared to other treatments.

Keywords: Callus, Multiple shoots, Micropropagation, Phloroglucinol, Browning. Vitrification

Introduction

Date palm (*Phoenix dactylifera* L.) is a 'tree of life' which belongs to the family Arecaceae; palm fruit is one of the most common fruits in the Middle East, in addition to its value in fruit production , date palm plays a key role in generating employment and creating equal oasis ecosystems. (Al-Bulushi et al., 2017). Plant tissue culture technique is the most promising propagation method for producing high-quality active plant materials (Sane et al 2006; Jasim, et al., 2009). The technique of tissue culture has many advantages as large scale multiplication within a short time, production healthy plantlets (disease and pest-free), production of genetically uniform plants (Cubbin et al., 2004). Organogenesis in date palms has a low efficiency because of the low number of shoots generated in vitro, the long-time required for the initiation stage, as well as the low multiplication rate. Using these techniques, date palm can be micropropagated either through somatic embryogenesis (Al-Mayahi, 2010; Al-Khayri, 2013;Mazri et al., 2017) or by organogenesis (AlKhateeb, 2006; Al-Mayahi, 2014). Tissues and organs are grown in vitro on artificial culture media, which provides most of the needs of cultures for growth. The success of tissue culture technique as a means of plant propagation is largely affected by the nature of the medium used (Al-Mayahi., 2016 a). Phloroglucinol (PG) (1,3,5–trihydroxybenzene) or Phloroglucin (PG) tautomer is a phenolic compound and that has growth regulating properties (Sarkar and Naik, 2006). Also, phloroglucinol (PG) increases the regeneration and multiplication of shoot when added to culture media (jani et al., 2015 ; Londe et al., 2017) noted that the addition of phloroglucinol (PG) improved the multiplication of shoot when added at 200 µM to an MS medium as compared to the control treatment, while higher concentrations decreased the growth and development of shoots in vitro. In field plant tissue culture research, there is an urgent need for continuous research for new substances that can be added to the culture medium that may lead to better multiplication and growth. Therefore, the purpose of the current study was to evaluate the effects of phloroglucinol on the growth and multiplication of date palm cv. Barhee in vitro shoots.

Materials and Methods

The experiments for this study were carried out in The Tissue Culture Laboratory of Date Palm Research Centre, Basrah University, Basrah, Iraq. After the callus propagated, it was separated and cultured on the media composed of Murashige and Skoog(1962)(MS) salt mixture containing the macronutrients and micronutrients. It content with 30 gm l^{-1} sucrose, 7 gm l^{-1} agar, with additional 0.5 mg l^{-1} NAA 0.5 mg l^{-1}BA, 0.5 mg l^{-1} kinetin (K) and 0.5 g l^{-1} activated charcoal 1 mg l^{-1} (Al-Mayahi, 2016b). To study the effects of Phloroglucin (PG) on the growth of callus and formation of buds. Different concentrations of PG were used: (0, 25, 50, and 100) mg l^{-1}. All jars with media were autoclaved at 121°C and 1.04 kg.cm^2 for 20 min. The cultures were maintained under room temperature 27± 1°C, photoperiod day/night 16/8. The results of the experiments, regarding the callus weight were recorded after 4 and 8 weeks of callus culturing on propagation medium, the number of buds and their weights were recorded after 10, 12 and 14 weeks of callus culturing on organogenesis medium, while, the number of shoots and their weights (new shoot number per jar), were recorded after 20 and 24 of callus culturing

Browning percentage (%):

It was estimated by dividing the number of brown cultures by the total number of cultures .

Vitrification percentage (%):

The vitrification percentage (%) was calculated depending on their external appearance. The shoots were classified that is suffering vitrification, had glassy and watery appearance compared to normal shoots :

- No shoot –vitrification., + low shoot - vitrification., ++ Severe shoot – vitrification.

Experimental design and statistical analysis:

A randomized complete block design was used (C.R.D). Statistical variance analysis was performed using Analysis of Variance (ANOVA) table. Using the GenStat softwar. Differences were compared using the least significant difference (L.S.D) at the 5% probability level.

Results and Discussion

Effect of concentrations of PG on callus weight of date palm cv. Barhee

According to the results obtained, the growth of callus was affected significantly by different PG concentrations in vitro after 1 and 2 months of culturing. The highest weight of callus was recorded at 50 mgl-1 PG, while the lowest weight of callus was recorded at 0 mgl-1 PG (control treatment), for both dates above (Fig. 1, picture.1).

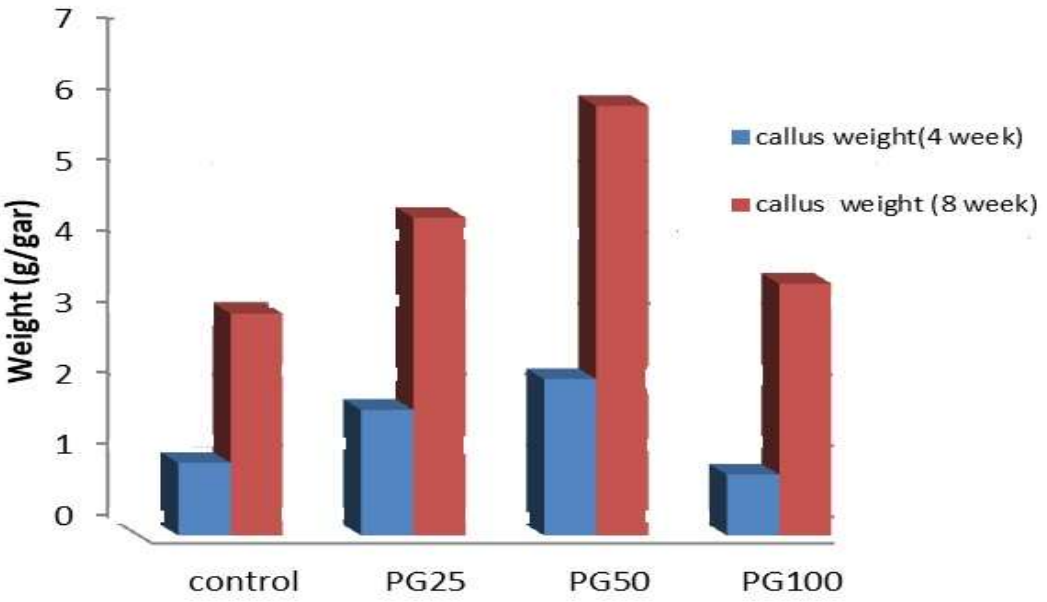
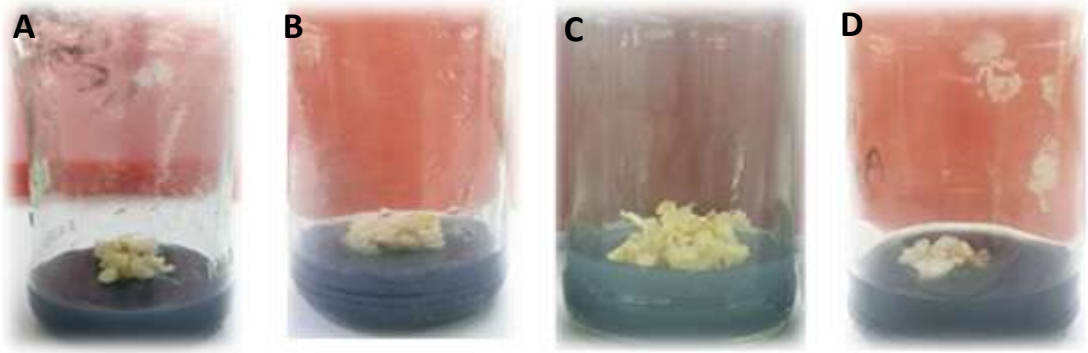


Figure 1. Effect PG concentrations on the weight of callus after 4 and 8 weeks of culturing.



Picture1. Callus growth on MS basal medium containing (A) control treatment "0 PG" (B) 25 mgl-1 PG (C) mgl-1 PG(D) 50 mgl-1PG (E)100mgl-1 PG.

The effect of various PG concentrations on the regeneration and development of date palm buds. cv. Barhee

The results show the development of bud tissues of date palm as changing in bud number and bud weight after three times 10, 12, and 14 weeks of culturing (Figs.2 and 3), it is noted the number of buds was significantly with PG concentration, but there was no significant effect between control treatment and PG100, the PG50 was recorded the highest value, however, the changes in bud number at period of reculture was increased.

The increasing number of buds and buds weight was taken as an indicator for the development of cultures; our results showed that there is an increase in the weight of the buds commensurate with their number, there were increases in weight of bud gradually with increasing concentration PG. The highest increase in bud weight was recorded at the PG50 in all periods of study, but there was no significant difference with PG25 and PG 100, However, the result show in general, positive effects for PG on number and buds weight.

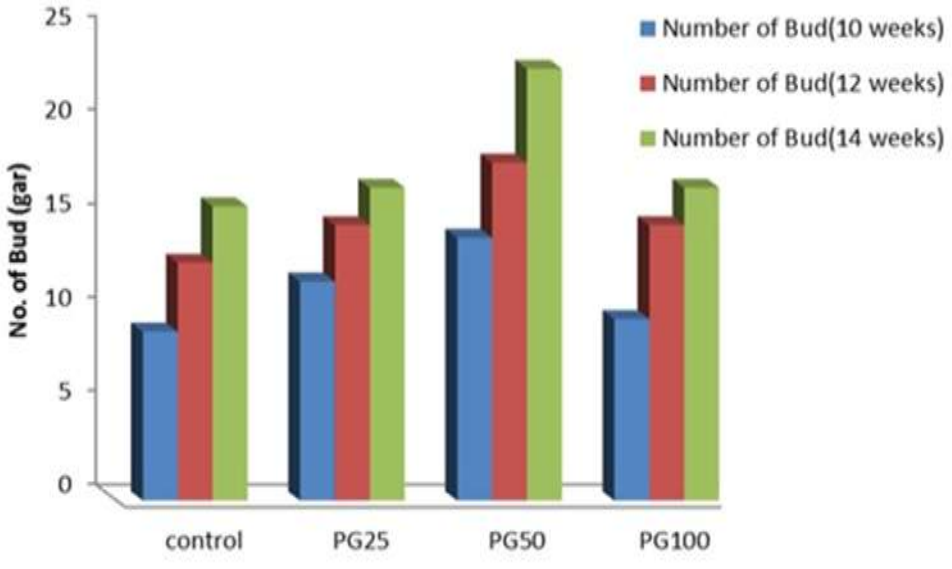


Fig.2 Effect of PG concentrations on the number of buds date palm cv. Barhee in vitro after 10, 12 and 14 weeks of culturing.

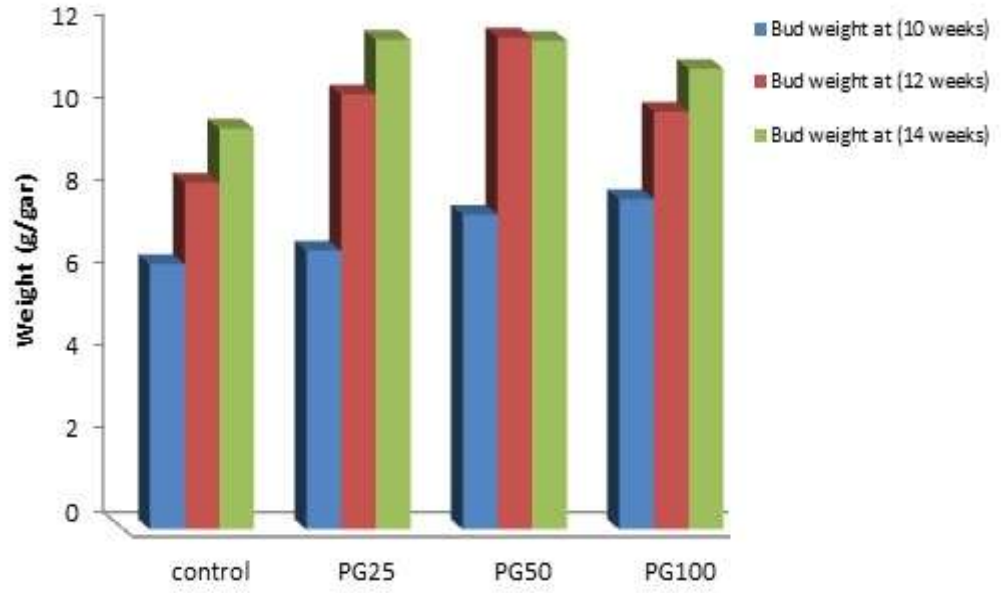


Fig.3. Effect of PG concentrations on the weight of buds date palm cv. Barhee in vitro after 10, 12 and 14 weeks of culturing.

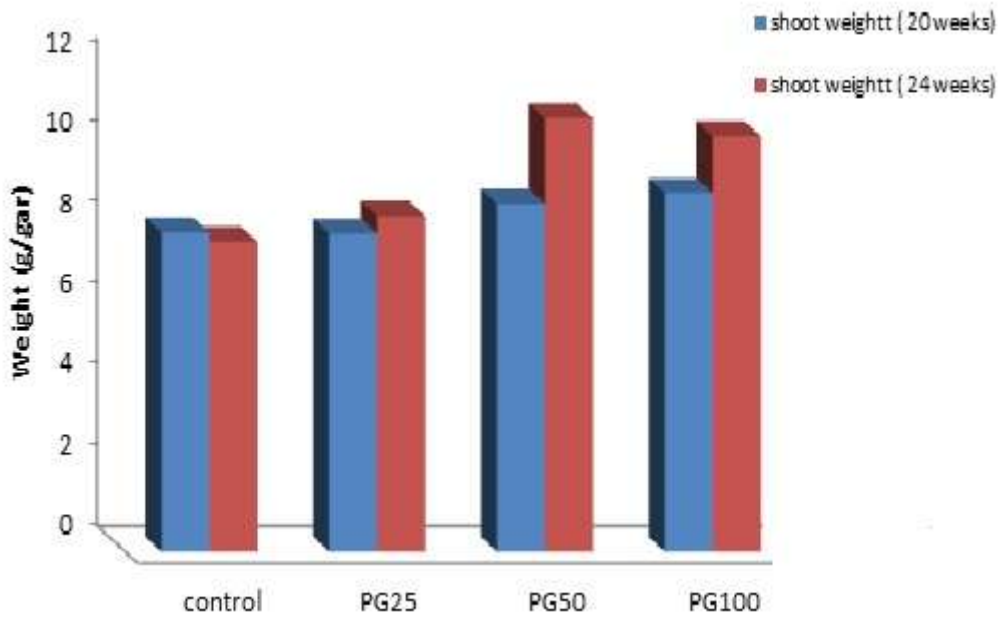


Fig.4 .Effect of Different concentrations of PG on weight of shoot regeneration of date palm cv. Barhee.

Effect of different concentrations of PG on the number and weight of shoots (Figs. 4 and 5). The highest response weight and the number of the shoots were obtained in medium containing 50 mgl-1 compared with the cultures grown in media containing

0 mgl-1 PG (control treatment), However, there were no significant effects between all treatment but there was a significant effect with control treatment specially at 24 week . The weight of shoot increased gradually with PG increased concentration and recorded a height weight in PG50 which was no significant effect with PG100mg.l-1.

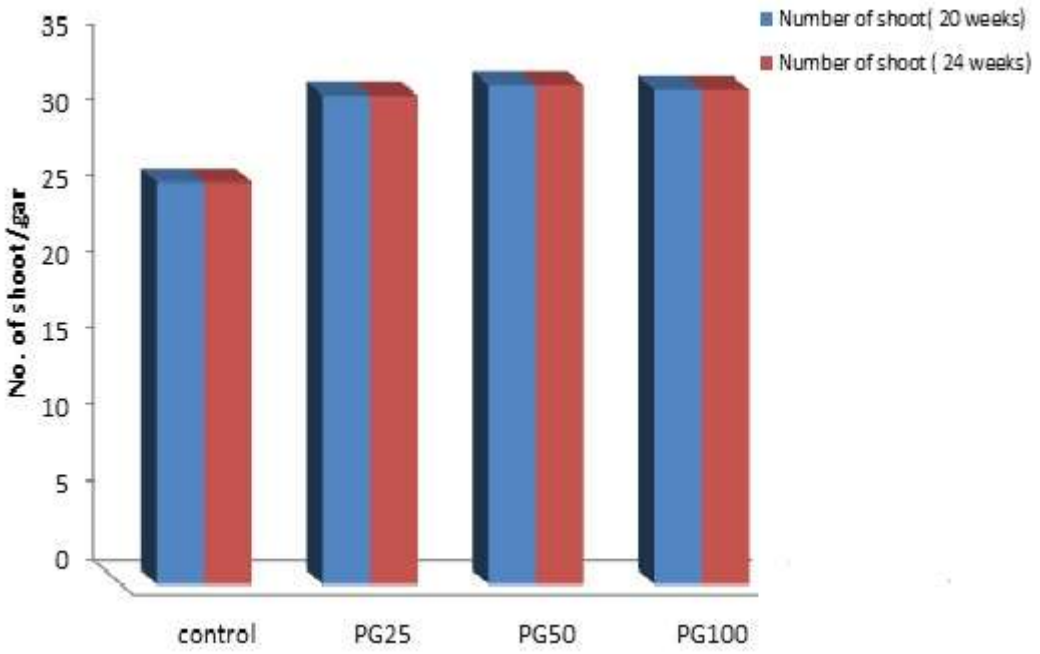


Fig.5 Effect of PG concentrations on the number of shoots date palm cv. Barhee in vitro after 20 and 24 weeks of culturing.

Picture.2. Shoots induction from callus cultured in MS medium supplemented with different concentrations of PG (A) Control treatment"0 PG"(B) 25 mg.l^{-1} PG (C) mg.l^{-1} PG(D) 50 mg.l^{-1} PG (E)100mg.l^{-1} PG.

For date palm, organogenesis appears to be a powerful method for large- scale propagation. However, some major obstacles have been recorded by the organogenesis method that may limit the application of this method such as low multiplication rate and decrease of regeneration capacity (Al-Khatib, 2008). Phloroglucinol stimulates shoot formation in many horticultural plants (Steephen et al., 2010). The exact mechanism of PG activity is not entirely clear, but it most likely may affect the internal synthesis of the ratio between auxins and cytokinin (Siwach and Gill, 2011). PG can stimulate cell growth and shoot multiplication may be due to the physiological role of PG which is considered as cytokinin in its effect in the micropropagation, therefore, they affect the success of multiplication in vitro. Studies have shown the beneficial effects of PG is due to its ability to protect endogenous auxin by keeping the cell in a low state of oxidation (Agud et al.,2010). In general, the regeneration of the organs in the monocotyledons including date palm occurs by the increased concentration of the cytokenines over the auxins Some reports indicated the positive effects of phloglucinol on the promotion of growth and shoot regeneration which have been noted in many of plant woody species (Siwach and Gill, 2011; Jani et al., 2015). This result is in agreement with those reported by Salekjalali. (2012), who found that PG increased the shoot proliferation of Rosa damascena Mill .Effect PG concentrations on the browning percentage. Results of browning percentage revealed that the lowest percentage of browning recorded at the treatment of PG 50 mgl-1 reached 0.0 % , which did not record a significant difference with PG25, while high browning percentage was observed in the tissue cultured in media containing 100 $mg.l^{-1}$ PG (Fig.6)

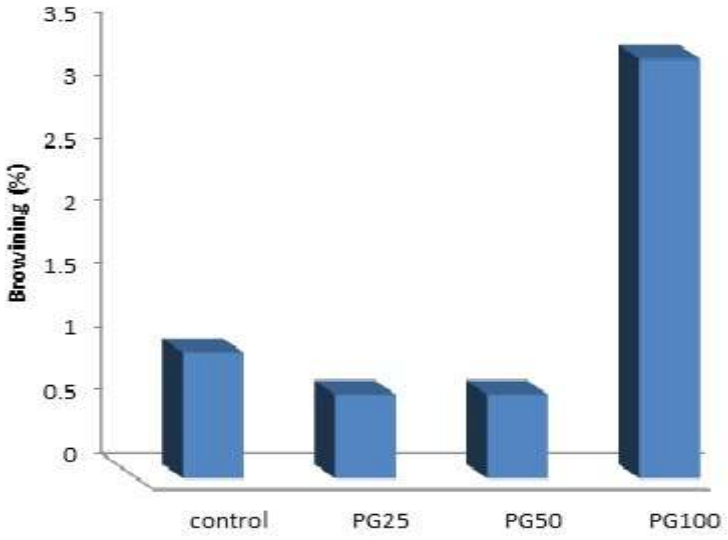


Fig. 6. Effect of PG concentrations on the percentage of browning (%) of date palm tissue cv. Barhee.

Browning of tissues is one of the most important problems frequently observed during the in vitro establishment of the date palm. Browning of cultured explants occurs due to the oxidation of phenolic compounds within the tissues resulting from the composition of quinones which causes toxicity to plant tissue and lead to death (Alkhateeb and Ali-Dinar, 2002)

Effect of PG concentrations on the vitrification percentage (%)

According to the results obtained, the highest percent of vitrification was recorded in the control treatment (without the addition of PG). While all concentrations of PG reduce of the percentage of the buds vitrification (Table 1)

Table.1. Effect of PG concentrations on the vitrification of date palm buds cv. Barhee.

Treatments PG mgl^{-1}	Percent of vitrification (%)
0.0	++
25	-
50	-
100	-

Excessive dehydration is a physiological disorder that refers to an accumulation of water content in the cultured plant tissue in the ‹culture media. This phenomenon is common in both ways of propagation: somatic embryogenesis and organogenesis of date palm (Mazri, 2015). There are many studies that dealt with reducing or preventing vitrification in plant tissue culture, such as cold pretreatment (Almayahi et al., 2018), changing the concentrations of PGRs or ions in the culture medium (Wu et al. 2011; Almayahi , 2019), choice of appropriate gelling agent or changing lighting periods and their intensity (Ascencio-Cabral et al. 2008 Almayahi, 2015). PG could be used to decrease vitrification and enhance proliferation (Debabrata and Prakash, 2000). The production of phenol compounds is directly related to the C / N ratio and phenolics affect lignification, this may explain how the addition of PG to the media can help plants undergoing vitrification to return to their normal state AL-Maarri and Al-Ghamdi (2000). Jaime et al., (2013) reported the PG is preventing vitrification in micropropagation. Phan and Hegedus, (1985) explained that adding PG to the medium for apple and sunflower shoots prevented the emergence of vitrification . The reason for this was attributed to increasing the activity of enzymes involved in lignin synthesis.

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تاثير اضافة Phloroglucinol (PG) على نمو وتضاعف نخيل التمر *Phoenix dactylifera* L. صنف البرحي خارج الجسم الحي

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الخلاصة

اجريت هذه الدراسة لمعرفة تأثير تراكيز مختلفة من Phloroglucinol (PG) (0 و 25 و 50 و 100) ملغم.لتر$^{-1}$ على نمو الكالس والتوالد الخضري لنخيل التمر صنف البرحي المكثر خارج الجسم الحي. اظهرت النتائج ان اضافة PG بتركيز 50 ملغم.لتر$^{-1}$ كان التركيز الاكثر تأثيرا في زيادة وزن الكالس والنسبة المئوية لاستجابة الكالس للتوالد الخضري، فضلاً عن زيادة ملحوظة في عدد النموات الخضرية لكل جارة، مقارنة بالتراكيز الاخرى ومعاملة المقارنة خلال فترات الدراسة المختلفة. كما اظهرت نتائج الدراسة انخفاض نسبة التلون البني والتزجج في الاسنجة المزروعة على اوساط تحتوي على PG بتركيز 50 ملغم.لتر$^{-1}$ مقارنة ببقية المعاملات.