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# EFFECTS OF PACLOBUTRAZOL AND SUCROSE IN DATE PALM (*PHOENIX DACTYLIFERA* L.) MICROPROPAGATION VIA DIRECT ORGANOGENESIS

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## Abstract

The study was conducted in the Date palm micropropagation unit *in vitro* at College of Agriculture, University of Kufa during the period 2015 to 2016 to show the effect of the growth regulator (*Paclobutrazol*) that was added to the nutrient media at a concentration of (0, 5, 10, 15 mg.$L^{-1}$) and its interaction with sucrose at a concentration of (30, 60, 80 g.$L^{-1}$) in the program of micropropagation of date palms (Barhee cultivar) via direct organogenesis in the multiplication stage after culturing the quarters of the apical meristem. The study results showed that the biomass cultivated in the multiplication media (MS) that provided with 5 mg.$L^{-1}$ Paclobutrazolrecorded the highest average number of buds (33.4 bud) and dry weight (1.050 g). The concentration of 10 mg.$L^{-1}$ recorded an increase in the number of formed shoots (10.89 shoots), It did not have a significant effect on the fresh weight of the biomass. As for the effect of sucrose, the addition of (60 g.$L^{-1}$) led to recording the highest average for the trait of number of formedshoots (17.67 shoot) and fresh and dry weight of biomass (6.56, 1.617g), respectively.While it did not have a significant effect in the number of multiplied buds.

***Key word:*** Date palm, Paclobutrazol, PBZ, Sucrose, Direct organogenesis.

## Introduction

Dates palm (*Phoenix dactylifera* L.) is one of the most important trees belonging to Arecaceae family and to the Palmae order, which is considered one of the most important orders of plants, one of the most important fruit trees as a food and its economy of millions of people in the Middle East and North Africa (Al Baker,1972). The common method in propagation of date palm is by the offsets resulting from the side buds of the mother's palm, But this method does not meet the purpose because the number of offsets that produced from each palm is limited, and because of the decline in the number of palm trees in Iraq during the previous decades as a result of wars in the region and neglect of the date palm, therefore the production of dates palm in relatively large quantities and the production of disease-free cultivars became necessary thing, which led to propagation of date palm modern methods by tissue culture to meet the growing demand for good cultivars and to make a lot of effort to find the right way to explore the best possible pathways of rapid propagation date palm *in vitro* (Zaid, 2002). In the last three decades of the last century, research has focused on the study of the appropriate nutrient media for the propagation of dates palm *in vitro* in terms of the type and balance of growth regulators, carbohydrates and others. In the past few years, researchers have noticed the effective effect of the growth regulators of Paclobutrazol (PBZ) in the field of tissue culture of several plants. Many scientific research and reports indicated its correlation to more than one physiological role, which led to scientific recommendations to be included in the programs and protocols of propagation of many plants *in vitro* (George *et al.*, 2008) For this purpose, the study was conducted.

## Materials and methods

In the study, offsets of the palm (Barhee cultivars) were used that their age ranged between 3-4 years, which

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weighed 8-12 kg. The quarters of the apical meristem were cultured on the propagation media in order to obtain the buds by a direct organogenesis method. Buds biomass was then transferred to the multiplication media. [MS (Murashige and Skoog,1962] with 0.5 mg.$L^{-1}$ Kinetin, 0.5 mg.$L^{-1}$ BAP and 1 mg. $L^{-1}$ NAA). The growth regulator PBZ was added to the medium with concentrations of (0, 5, 10, 15 mg.$L^{-1}$). As for the energy source represented by sucrose, the laboratory sucrose was added with concentrations (0, 30, 60, 90 g.$L^{-1}$). In addition, myo-inositol was added with a concentration of (100 mg.$L^{-1}$), 170 mg.$L^{-1}$ disodium hydrogen phosphate ($NaO_2PO_4.2H_2O$) and the amino acid (Glutamine) with a concentration of (200 mg.$L^{-1}$). Adenine Sulphate was added to the media with concentration of (80 mg.$L^{-1}$), and 100 mg.$L^{-1}$ Inositol. A 1.5 g.$L^{-1}$ activated charcoal was added, and the hardened material (Agar) with concentration of 7 g.$L^{-1}$ was added. The pH of the media was adjusted at 5.7.The nutrient media was distributed with amount of 50 mL in glass jars with capacity of 300 mL and the jars that containing of nutrient media and the laboratory tools used in cultivation were sterilized for 20 min in the Autoclave at 121°C and pressure of (1.05 kg.$cm^2$).Plant parts were cultivated and incubated in the growth chamber for four months with re-culturing every two months at a temperature of $\pm$ 27°C and lighting approximately 1000 lux. The number of buds , the number of formed shoots and the fresh and dry weight of the biomass were recorded.

## RESULTS

### Number of buds

Table 1 shows that the addition of PBZ in the multiplication media and at all its added concentrations has led to a significant increase in the number of formed vegetative shoots compared with the control treatment, which gave the highest when treated with PBZ at a concentration of (5 mg.$L^{-1}$) amounted of 33.4 buds and without significant differences from the other treatments with PBZ, while the lowest number of buds at the control treatment was 20.5 buds. The results showed that there were no significant differences in this trait under the effect of addition of sucrose to the multiplication media, Although it record the highest average at the treatment of (60 g.$L^{-1}$) which amounted of (29.6 bud), As for interaction, the interaction treatment between 5 mg.$L^{-1}$PBZ and 90g.$L^{-1}$ of sucrose recorded the highest average for multiplication of 56.3 buds, while the interaction treatment between 0 mg.$L^{-1}$ of PBZ and 30 g.$L^{-1}$ of the sucrose gave the lowest of bud multiplication amounted of 10.4 bud.

**Table 1:** Effect of Paclobutrazol and sucrose and their Interactions in the Number of Formed Buds in the multiplication stage After Four Months of Culturing in the Multiplication media

| Paclobutrazol (mg.$L^{-1}$) | sucrose (g.$L^{-1}$) | | | Mean |
|---|---|---|---|---|
| | 30 | 60 | 90 | |
| **0** | 10.4 | 32.9 | 18.3 | 20.5 |
| **5** | 27.5 | 16.3 | 56.3 | 33.4 |
| **10** | 41.7 | 34.7 | 16.3 | 30.9 |
| **15** | 30.4 | 34.5 | 18.8 | 27.9 |
| **Mean** | 27.5 | 29.6 | 27.4 | |
| **LSD (0.05)** | Sucrose = **5.06** Paclobutrazol = **5.84** Interaction **10.11** | | | |

### Shoots number

Table 2 shows that the addition of Paclobutrazol in the multiplication media and at all its added concentrations has led to a significant increase in the number of formed shoots, which amounted to (10.89 shoots) at the treatment of 10 mg.$L^{-1}$ and without significant differences from the other treatments with Paclobutrazol compared to the control treatment, which recorded the lowest average of (6.33 shoots).The sucrose had an effect that reached to a significant level in the number of shoots which recorded the highest number when adding sucrose to the growth media at a concentration of 60 g.$L^{-1}$(13.25 shoots) while recorded the lowest average at the highest concentration of 90 g.$L^{-1}$ reached of (3.25 shoots). As for the interaction, 10 mg.$L^{-1}$ Paclobutrazolwith 60 g.$L^{-1}$ sucrose was significantly excelled by recording it the highest average of shoots (17.67 shoots), while the lowest shoots(1 shoot) recorded by the two interaction treatments between (0 mg.$L^{-1}$Paclobutrazol + 90 g.$L^{-1}$ sucrose) And (10 mg.$L^{-1}$Paclobutrazol + 90 g.$L^{-1}$ sucrose).

**Table 2:** Effect of Paclobutrazol and sucrose and their Interactions in the Average number of formed shootsin the multiplication stage After Four Months of Cultivation in the Multiplication media

| Paclobutrazol (mg.$L^{-1}$) | sucrose (g.$L^{-1}$) | | | Mean |
|---|---|---|---|---|
| | 30 | 60 | 90 | |
| **0** | 6.00 | 12.00 | 1.00 | 6.33 |
| **5** | 10.00 | 11.00 | 8.00 | 9.67 |
| **10** | 14.00 | 17.67 | 1.00 | 10.89 |
| **15** | 13.00 | 12.33 | 3.00 | 9.44 |
| **Mean** | 10.75 | 13.25 | 3.25 | |
| **LSD (0.05)** | Sucrose = **1.547** Paclobutrazol = **2.096** Interaction **3.096** | | | |

### Fresh weight for biomass

Table 3 shows no significant differences due to the treatment with PBZ concentrations in the effect on the trait of the fresh weight for the biomass.The results of the same table indicate that there were significant

differences between the treatments under study in the trait of fresh weight as a result of the effect of used sucrose concentrations, where recorded the highest average amounted of 6.56 g at concentration of (60 g.L$^{-1}$) followed by the 30 g.L$^{-1}$ treatment, which also excelled on the treatment at a concentration of 90 g.L$^{-1}$, which also recorded at which the lowest average of 3.55 g. As for the results of the interaction between the experiment factors in the trait of the average of fresh weight, the results in the same table indicate that there are significant differences between the bi-interaction treatments where recorded the highest average of branch when the treated of the multiplication media with 10 mg.L$^{-1}$ PBZ and 60 g.L$^{-1}$ sucrose which amounted of 7.87 g. While interaction treatment between PBZ at a concentration of 15 mg L$^{-1}$ and sucrose at a concentration of 90 g.L$^{-1}$ gave the lowest average amounted of 2.47g.

**Table 3:** Effect of PBZ and sucrose and their Interactions in the average of fresh weight for biomass in the multiplication stage After Four Months of Cultivation in the Multiplication media

| Paclobutrazol (mg.L$^{-1}$) | sucrose (g.L$^{-1}$) | | | Mean |
|---|---|---|---|---|
| | 30 | 60 | 90 | |
| **0** | 4.25 | 5.41 | 3.79 | 4.48 |
| **5** | 4.99 | 5.77 | 4.73 | 5.16 |
| **10** | 7.71 | 7.87 | 3.21 | 6.26 |
| **15** | 6.43 | 7.18 | 2.47 | 5.36 |
| **Mean** | 5.84 | 6.56 | 3.55 | |
| **LSD (0.05)** | Sucrose =**1.802** Paclobutrazol = **2.081** Interaction **3.604** | | | |

### Dry weight for biomass

Table 4 indicates that the response of date palm tissue significantly differed in the extent to which they responded to the treatment with PBZ for the trait of average of dry weight. The treatment with PBZ at concentration of (5 mg.L$^{-1}$) was significantly excelled on the other treatments under study by giving them the highest average of dry weight amounted of 1.050 g. As for the other treatments did not differ significantly between them, it recorded the lowest average amounted of 0.681 g at treatment with the highest concentration of PBZ (15 mg.L$^{-1}$).The results of the same table showed that there were significant differences between the different concentrations of added sucrose to the media in the trait of dry weight, where the results indicated that the treatment with sucrose at a concentration of 60 g.L$^{-1}$ led to recording the highest dry weight (1.141 g), with a significant excelling on the remaining two treatments, which did not differ significantly between them, while recorded the lowest average of 0.635 g in the control treatment (30 g.L$^{-1}$). The interaction between the two study factors was significant according to the presented results in the same table. The interaction treatment between 5 mg.L$^{-1}$ PBZ and 60 g.L$^{-1}$ sucrose, while the interaction treatment between PBZ at the highest concentration (mg.L$^{-1}$) and 30 g.L$^{-1}$ of sucrose gave the lowest average of 0.270 g.

**Table 4:** Effect of PBZ and sucrose and their Interactions in the average of dry weight for biomass in the multiplication stage

| Paclobutrazol (mg.L$^{-1}$) | sucrose (g.L$^{-1}$) | | | Mean |
|---|---|---|---|---|
| | 30 | 60 | 90 | |
| **0** | 0.717 | 0.953 | 0.717 | 0.796 |
| **5** | 0.817 | 1.617 | 0.717 | 1.050 |
| **10** | 0.737 | 0.740 | 0.810 | 0.762 |
| **15** | 0.270 | 1.253 | 0.520 | 0.681 |
| **Mean** | 0.635 | 1.141 | 0.691 | |
| **LSD (0.05)** | Sucrose =**0.1149** Paclobutrazol = **0.1481** Interaction= **0.2297** | | | |

## Discussion

Tables (1, 2, 4) show that PBZ has a significant effect on the number of buds, the number of formed shoots and the dry weight for biomass. The effect of PBZ in increasing the number of growing and multiplying buds may be due to the effect of PBZ in reducing vegetative growth and directing the nutrients to tissues and storing instead of depleting them in vegetative growth, which may lead to an increase in the growth and division of bud cells and shoots [Looney, 1975]. Or its effect may be due to its role in increasing biosynthesis and increasing the effectiveness of natural cytokinines in tissues that treated with PBZ [Zhu *et al.*, 2004]. The reason for the superiority of PBZ concentrations in studied traits may be that it consists of two rings, one containing nitrogen, which has the role of providing energy to increase the number of divisions and protein synthesis, resulting in increased growth [Remotti,1995]. The results of our study agree with the results of other studies in their general framework in terms of positive impact for the presence of PBZ in the nutrient media in the formation of buds and shoots as found in the results on pineapples (Escalon *et al.*, 1999), sweet potato (Meneses *et al.*, 2000) bananas (Albany *et al.*, 2005) apples (*Malus domestica*) (Kepenek and Karoglu, 2011) and *Spathiphyllum floribundum* (Mosonyi *et al.*, 2014). In terms of its positive effect on the increase of fresh and dry weights of tissue cultures our results agree with the result of Nagaraju *et al.*, (2002) in the *Gladiolus*, Kucharska and Orlikowska (2008) in chrysanthemum, Mazher *et al.*, (2014), in *Schefflera arboricola* and Khierallah *et al.*, (2015) in the date palm. As for the effect, it is clear from the results of tables (2, 3, 4) that the treatment of sucrose

has a significant effect on the traits: number of formed shoots, fresh and dry weight of the biomass. The effect of Sucrose may be due to the emergence and multiplication to the importance of the addition of Sucrose to the growth media related to the emergence of the buds directly and multiply, as well as increase the number of formed buds by increasing the added concentration reaching the optimal concentration of (60 $g.L^{-1}$), Which is higher than the standard sucrose concentration of MS media, It is well known that the addition of sucrose to the nutritional media of plant tissue culture aims at processing the plant part planted with the energy needed for its growth and development in the desired direction and lead to Inhibition the formation of chlorophyll [George and Sherrington,1993]. The culture *in vitro* lose their ability to build photosynthesis significantly, so It cannot build glucose, which is the source of energy-provided carbon. Sucrose, added to the nutrient media, is partially decomposed into its essential constituents glucose and fructose, when it is sterilized with steam in autoclave and then completely decompose into tissues and plant cells via the invertase of the cell wall, or through the reverse reaction of the sucrose synthase in the cytoplasm (Dennis *et al.*, 1997), the cells first absorb glucose from the nutrient media and then fructose (Ramawat, 2004). Thorpe (1978) has indicated that the metabolic processes of glucose increased during the formation of primary meristems, and sucrose is the carbon-energy source during morphogenesis processes in implanted cells *in vitro*. The positive effect of sucrose in organic organogenesis is due to its association with Auxin in vascular differentiation of cultured Xylem and bark tissues. It was found to be a prerequisite for the differentiation of Trachieds and also determines the ratio between the Xylem vessels and the differentiated Sieve elements (Aloni, 1980). The results showed that increasing the concentration of sucrose in the nutrient media from the standard limit of MS (30 $g.L^{-1}$) has increased the rate of formation of the Adventitious buds from the cultivated tissue. This may be due to an increase in energy processing rates for implanted cells and tissues indirectly. So it works to increase sucrose concentrations in the nutrient media increase the activity of $NO_3$ and ammonium nitrate $NH_4^+$ (Gamborg *et al.*, 1974). As well as increase the effectiveness of cytokinein in the stimulation of cell division, and the decrease in the formation of Adventitious buds with increase in concentrations of sucrose more than (60 $g.L^{-1}$) is due to increase Osmotic pressure for nutrient media, where sucrose plays an important role in maintaining the Osmotic pressure of the nutrient media and increasing its concentration will increase the Osmotic pressure. Thus, the tissues and cells are exposed to water stress and their growth rates and development accordingly(George andSherrington, 1993). These results are consistent with what was found by Drira and Benbadis (1985), Hameed (2001) and Alkhateeb (2006) in date palms who found that the addition of sucrose in the nutrient media (45-60 $g.L^{-1}$) had a significant effect in stimulating the formation of Adventitious buds on the apical meristem of the date palm.

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