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EFFECT OF PHLOROGLUCINOL(PG) ON PROTEIN PATTERN CHANGES IN OF DATE PALM MICROSHOOTS (PHOENIX DACTYLIFERA L.) CV. BARHEE

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Abstract:

The current study was conducted in Basra Governorate - Iraq for the season 2019-2020 to study the effect of phloroglucinol (PG) on the protein pattern and phenotypic characteristics of the vegetative buds of date palm cultivar Al-Barhee cultivar tissue cultured. Between(2-8bands) and by 4 bundles for each treatment, while the two treatments 75 and 100 micromoles recorded 8 molecular bands with a molecular weight that ranged between (68.8-10.44 kDa). The results of the phenotypic study also showed that the 25 micromoles treatment was significantly superior to most vegetative traits, while increasing the compound concentration led to a negative impact on the significance of the results, so that the 100 micromoles treatment recorded the least significant differences.

Keywords: Organ, phenotypic study

INTRODUCTION

The decline in date palm *Phoenix dactylifera* L. in Iraq in general and Basrah Governorate in particular is due to many reasons, and the resulting neglect of these vital and influential aspects in the country's economy (Ali & Hama, 2016). Practical applications in the field of biotechnology, in particular plant tissue culture

There are two main approaches to palm propagation histologically, the first is through organogenesis, that is, the organs are formed directly from the plant tissue without going through the callus stage, so they are identical to the mother plant (Kushairi et al., 2010) and the second is through the formation of somatic embryogenesis directly from the plant tissue or through The path of passing through the stage of the callus, from which the embryos are formed, by cultivating the plant part in sterile industrial food media (Mazri et al., 2017)

The researchers were interested in using some chemical compounds that have an effect in stimulating organ formation and stimulating rooting, and these compounds used are Phloroglucinol (PG, 1,3,5-trihydroxybenzene). Organ growth and development and has been used in tissue culture with some plants, yet the roles of phloroglucinol(PG) are still not understood (Petti, 2020)

Chemical compounds are added to the growth media in tissue culture in order to obtain good results, and the excessive addition causes chemical stress in high concentrations that affect the molecular level of the plant leading to the synthesis of proteins of different molecular weights Abbas *et al*,. (2015), so this study was conducted to find out the effect of phloroglucinol(PG) compound in the stability of the pattern

MATERIAL AND METHOD

Experimental Materials

Adventitious buds of date palm (*Phoenix dactylifera* L.) cultivar 'Barhee' was obtained from Fadak for Agricultural Plant and Animal Production Private Company, Basrah Governorate, Basrah, Iraq during the period 2019-2021. Buds were multiply continuously on Bud growth and proliferation medium (BGPM) containing Murashige and Skoog inorganic salts (Murashige and Skoog, 1962), 50.000 mg.L^{-1} sucrose, 100 mg.L^{-1} myo-inositol, 187.5 mg. L^{-1} Sodium dihydrogen phosphate dehydrate (NaH2Po4.2H2O), 200 mg.L^{-1} glutamine, 1500 mg.L^{-1} Calcium nitrate tetrahydrate Ca(No3)2.4H2O, 80 mg.L-1 Adenine sulfate, 500 mg.L^{-1} Polyvinyl pyrrlidone, 0.1 mg.L^{-1} kinetin(KN), 0.1mg.L^{-1} benzylaminopurine (BAP) and 0.1 mg.L^{-1} 6-(γ,γ-Dimethylallylamino)purine (2iP) (Almusawi, *et al*., 2017).

Adventitious buds obtained from the last step of multiplication were subjected to various concentrations of PG(0, 25, 50, and 75 µmol) to study theon bud growth, proliferation rate and development in vitro. To achieve this step a clump of vegetative buds (8- 10 buds) were inoculated to a (BUGM) supplemented with different concentrations of phloroglucinol

Incubation conditions.

The cultures were incubated in growth chamber at 27±2ºC. A16/8 h (light/dark) photoperiod provided by 100 w. LED light fixed 40 cm above the rack.

Preparation of Extraction Solution Sample extraction solution was prepared according to the method of Bavei et al., (2011), where Tris-HCl buffer 6.8 was prepared by taking 1.5 g of Tris-Hcl and it was dissolved in a volume of distilled water 25 ml, then 0.0175 g of Phenyl Methane Sulfonyl Fluoride (PMSF) was taken. Which was dissolved in a small amount of distilled water and added to the first solution after adjusting its pH value to 6.8 using HCL and NaoH.Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Preparation of sample of each 0.3 g sample was taken from the crushed samples and crushed in a ceramic mortar with 1 ml of the prepared solution Tris-Hcl buffer at a temperature of 4 °C, then the samples were centrifuged at a speed of 15,000 revolutions / min for 30 minutes at a cooling temperature of 4 °C, then the filtrate was taken and the precipitate was left after which it was saved Samples extracted in a refrigerated reefer container until deportation.

(SDS–PAGE) was performed on date palm buds on a 12% polyacrylamide gel (BioRad, Hemel Hempstead, UK). A sample of DSPC and soy protein isolate (SPI) was run on the gel. A protein molecular weight ladder (BioRad, Hemel Hempstead, UK) was also run on the gel, to allow molecular weight determination. Gels were stained overnight with colloidal Coomasie blue and destained(10% [v/v] ethanol and 2% [v/v] orthophosphoric acid) until the background become clear, and protein bands were visible. Gels were scanned using a BIO-RAD Molecular imager (ChemiDocTM XRS+), to estimate the molecular weight of protein bands(Skriver et al,.1990).

Phenotypic: Estimation of morphological parameters

Bud numbers, length and diameter

Bud numbers, length and diameter were calculated after 8 weeks of bud inoculation on (BUGM) supplemented with different concentrations of PG.

Fresh weight

The fresh weight (average value) of vegetative buds was assessed directly for three replicates of each treatment.

RESULTS AND DISCUSSION

The results of electrophoresis of proteins extracted from vegetative buds formed and developing in MS-media growth media and treated with fluoroglycinol at concentrations (25, 50, 75 and 100 µmol) and as shown in (Fig. 1)large sizes with a molecular weight of 68.8 kDa and four small and low density bundles with a molecular weight ranging between 15-22 kDa, while the two treatments 25.50 micromol of fluoroglycinol did not notice any differences in the resulting patterns compared to the neutral treatment "untreated" Changes in protein composition may be due to changes in mRNA translation efficiency or regulation, RNA transcription, transport and stability at high concentrations of the chemical compound. Expression of A-proteins correlates with adaptation to stresses to plants, one of which is those caused by chemical stress. Previous studies indicated that one of the precise and important mechanisms involved in protecting the cell and genetic material from stresses is the induction of ribosomes to synthesize a group of new proteins (Bassiouny et al. (2008) (Sudha et al,.2019).

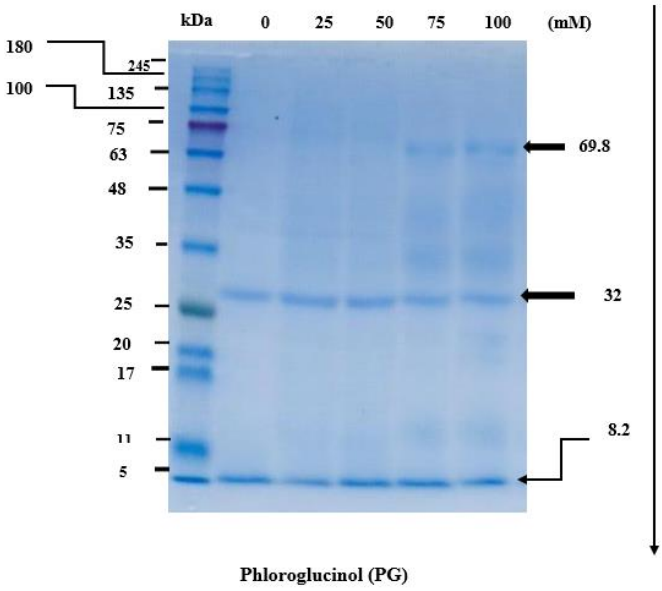

Figure (1): Electrophoresis using SDS-PGAE of date palm bud proteins of Barhee cultivar growing in media containing phloroglucinol(PG) using Coomassie Blue for staining.

Estimation of morphological parameters

The results of (Table 2-fig-2) indicate the moral effect of the compound PG when added to the culture medium and at low concentrations, as the 25 micromol treatment recorded the highest rate of fresh weight with a significant difference of (20.79 and 24.30 g) after 8 and 12 weeks, respectively, compared to the standard treatment, which recorded an average weight of (19.73 and 23.66 g). Whereas, the increase in the concentration of the PG compound had a negative impact on the growth and development of the buds by affecting the fresh weight, as it was observed that the weight decreased with the increase in concentrations, where the treatment of 100 µmol recorded the lowest average weight after 8 and 12 weeks, which amounted to (9.19 and 11.87 g), respectively. From planting with an increase in brown discoloration with increasing concentration, it was observed that new live masses formed from the masses of shoots affected by brown discoloration

The data (Table - 2) indicates the effect of adding (PG) to growth media in the indicator of the number of buds produced in the doubling stage to the significant decrease in the average number of buds between the standard treatment and the study coefficients, as the comparison treatment recorded the highest rate of the number of buds, which amounted to (29.17), while The treatments of the PG compound with a concentration of 100 micromoles recorded the lowest average number of buds, which amounted to (7.50) buds. It is noted that the treatment of 25 micromols was significantly superior compared to the rest of the study treatments, which recorded an average number of buds of (25.47) buds. It is clear from the results of the current study that fluoroglycinol It has significantly affected the development and growth of buds and caused their delay in addition to noting the blackening and death of the buds by increasing the concentration of the PG compound.

The current study found that the PG25 micromol treatment was significantly superior in the average length of buds compared to the rest of the other treatments, while the results did not record a significant difference between the PG 25 treatment and the standard treatment, which recorded an average length of 12.13, while the results were negative with an increase in the concentration of fluoroglycinol, as the treatment 100 micromoles recorded less The average length of the bud was 2.33 cm. But it was found through the study that the average diameter of the buds increased significantly with the increase in the concentration of PG. The treatment of 50 micromole recorded the highest average diameter, which was 4.00 mm compared to the comparison treatment, which recorded an average diameter of 1.80 mm (Table-2).

The results of the current study agree with Raghu et al., (2006), as their results indicated that the addition of fluoroglycinol affected the characteristics of vegetative growth, and when the concentrations of it increased, it led to a higher percentage of plant blackening, while its addition led to an improvement in the rooting of plants.

Sharifian et al., (2009) attributed the reason for the significant increase in vegetative growth indicators in low concentrations to the role of fluoroglycinol in acting as a synergist with auxin in stimulating bud development, while the excessive increase in concentrations led to exposing the formed buds to chemical stresses that negatively affected growth rates. An increase in concentration above the normal limit results in an accumulation of phenolic substances and thus leads to an adverse effect on bud growth

Table (1): Effect of different concentrations of phloroglucinol on the vegetative characteristics of growing palm buds on the multiplication medium

Measurements						
Bud diameter (mm)	**Bud length (cm)**	**Bud numbers**	**Fresh weight (g) 12 weeks**	**Fresh weight (g) 8 weeks**	**Concentrations (mm)**	Treatments
1.80±0.52	12.90±0.53	29.17±0.76	23.66±0.59	19.73±1.05	0	Control
3.67±0.76	12.13±0.81	25.47±0.50	24.30±0.36	20.79±0.87	25	
4.00±1.00	11.00±1.00	18.00±1.73	19.40±0.92	15.78±1.75	50	treatments
2.33±0.29	8.70±0.61	11.77±0.67	16.20±0.79	12.27±0.31	75	PG
1.50±0.50	2.33±1.15	7.50±0.50	11.87±1.63	9.19±1.15	100	
0.994	1.253	1.408	1.313	1.546		L.S.D

± The averages represent the average of three replicates followed by the standard deviation values. The averages followed by the same letter are not significantly different from each other, and their difference indicates a significant difference at the probability level. %5

Figure (2): Buds formed in the multiplication medium containing phloroglucinol (0,25,50,75,100 (µmol).

REFERENCES

1. Abbas, M. F., Jasim, A. M., & Al-zubaidy, B. H. (2015). Effect of NaCl stress on protein pattern changes in embryogenic callus of the date palm (Phoenix dactylifera L.) cv. Ashkar. *AAB BIOFLUX Advances in Agriculture & Botanics- International Journal of the Bioflux Society*, *7*(1), 7–11.
2. Ali, A. S. A., & Hama, N. N. (2016). Integrated management for major date palm pests in Iraq. *Emirates Journal of Food and Agriculture*, *28*(1), 24–33. https://doi.org/10.9755/ejfa.2016-01-032
3. Almusawi, Abdulminam H.A. Abdullah J. Sayegh, Ansam M.S. Alshanaw, and John L. Griffis Jr.(2017). Plantform Bioreactor for Mass Micropropagation
4. Bassiouny, E., Mostafa, H. M., Khawas, H. A. El, Hassanein, S. A., & Monem, S. I. A. El. (2008). Physiological Responses of Wheat Plant to Foliar Treatments with Arginine or Putrescine. Australian Journal of Basic and Applied Sciences, 2(4), 1390–1403.
5. Bavei, V., Shiran, B., Khodambashi, M., & Ranjbar, A. (2011). Protein electrophoretic profiles and physicochemical indicators of salinity tolerance in sorghum (Sorghum bicolor l.). *African Journal of Biotechnology*, *10*(14), 2683–2697. https://doi.org/10.5897/ajb09.754
6. Kushairi, a, Tarmizi, a H., Zamzuri, I., R, S. K., Ooi, S. E., Palm, M., Board, O., Institusi, N. P., & Bangi, B. B. (2010). Production , Performance and Advances in Oil Palm Tissue Culture 1. *International Seminar on Advances in Oil Palm Tissue Culture*, *6*, 1–23.
7. Mazri, M. A., Belkoura, I., Meziani, R., Mokhless, B., & Nour, S. (2017). Somatic embryogenesis from bud and leaf explants of date palm (Phoenix dactylifera L.) cv. Najda. *3 Biotech*, *7*(1). https://doi.org/10.1007/s13205-017-0676-y
8. Petti, C. (2020). Phloroglucinol Mediated Plant Regeneration of Ornithogalum dubium as the Sole " Hormone-Like. Plants, 9(929), 1–15.
9. Raghu, A. V., Geetha, S. P., Martin, G., Balachandran, I., & Ravindran, P. N. (2006). In vitro clonal propagation through mature nodes of Tinospora cordifolia (Willd.) Hook. F. & Thoms.: An important ayurvedic medicinal plant. In Vitro Cellular and Developmental Biology - Plant, 42(6), 584–588. https://doi.org/10.1079/IVP2006824
10. Sharifian, S., Vahdati, K., Mirmasoumi, M., & Ghaem Maghami, S. A. (2009). Assessment of phloroglucinol effect on rooting of tissue cultured Persian walnut. Acta Horticulturae, 812(February), 189–196. https://doi.org/10.17660/ActaHortic.2009.812.22
11. Skriver, K., & Mundy, J. (1990). Gene expression in response to abscisic acid and osmotic stress. Plant Cell, 2(6), 503–512. https://doi.org/10.1105/tpc.2.6.503
12. Sudha, G. S., Ramesh, P., Sekhar, A. C., Krishna, T. S., Bramhachari, P. V., & Riazunnisa, K. (2019). Genetic diversity analysis of selected Onion (Allium cepa L.) germplasm using specific RAPD and ISSR polymorphism markers. Biocatalysis and Agricultural Biotechnology, 17, 110–118. https://doi.org/10.1016/j.bcab.2018.11.007