

Interaction Effect of PEG6000 and Brassinosteroid on Biochemical Characteristics of *in Vitro* Date Palm (Barhi cultivar)

Khudhair M. Al-Kanany^{1*}, Aqeel H. Abdulwahid² & Majid A. Ibrahim³

¹ Department of Date Palm, Basrah Agriculture Directorate, Basrah, Iraq.

² Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Iraq.

³ Department of Biology, College of Science, University of Basrah, Iraq.

*Corresponding author email: K.M.A.: kalkanany@yahoo.com, A.H.A.: Aqeel.hadey@uobasrah.edu.iq,
M.A.I.: majid.abdulhameedl@uobasrah.edu.iq

Received 22nd October 2024; Accepted 22nd February 2025; Available online 24th August 2025

Abstract: Somatic embryogenesis is an essential method of date palm micropropagation that produces large biomass of plants. The callus is the primary plant tissue that goes through the indirect propagation way, which is the mainstay in plant formation that resists environmental stresses. Polyethylene Glycol (PEG) is used in *in vitro* experiments to induce osmotic stress, while Brassinosteroid is a promising hormone used to overcome or reduce water stress in a nutrition medium. The experiment aims to study the effect of PEG and BR on the biochemical characteristics of date palm tissues. Four concentrations of PEG (0, 10, 20, and 30g.L⁻¹) were combined with four concentrations of the hormone (0, 0.2, 0.5, and 1μmol.L⁻¹) in the nutritional media of date palm tissues at the callus and embryo stages. The results showed significant increases in proline, catalase enzyme, and membrane stability index tissue content, which are stress resistance indicators of plant tissues. Conversely, there is a decrease in the MDA content, conceded as an indicator of plant degradation by stress. These are valuable results because they prove the essential role of BR in the stress resistance of plant tissues, and by increasing this resistance; a strong plant can be produced that is resistant to environmental stresses.

Keywords: Catalesa, Embryogenesis, Malondialdehyde, Polyethylene glycol, Proline.

Introduction

The best Date palm (*Phoenix dactylifera*) micropropagation by somatic embryogenesis through an indirect callus induction pathway is the best biomass production method (Fki *et al.*, 2011). This pathway can produce two types of callus. The embryonic calli, which can develop into embryos, and the non-embryonic calli. Embryonic calli and embryos are the first

stages of embryogenesis way which are highly sensitive and affected by abiotic stresses such as water stress (Helaly *et al.*, 2017). Embryo maturation is a fully an understood and complex process that is influenced by a range of chemical and physical factors. These factors include using nutrients, growth regulators, and polyethylene glycol (PEG) (Stasolla *et al.*, 2002).

PEG is a chemical compound used in laboratory experiments to induce the osmotic potential in the nutrient medium and hold the water available to the plant, thus increasing the water stress (Alkhateeb, 2006). PEG is an osmotic, non-toxic compound with a high molecular weight. This compound reduces water availability to plants in the nutrient media and stimulates water stress without harmful or toxic effects on the plant. It can reduce RWC by lowering the water potential of the medium. PEG has negative effect on plant. The bad effect can reduce by adding some chemical compounds and plant hormones to treat drought stress. PEG affects the branch length, plant mass, root mass, and chlorophyll content (AL-Mayahi, 2010; Al-Mayahi, 2016). Enhancing plants' internal defense mechanisms to survive.

Harmful environmental cues are one of the core areas of biotechnological advances in plant improvement. In this context, the external application of plant protection such as brassinosteroids (BRs) is a promising option (Sharma & Banerjee, 2021).

Brassinosteroids (BRs) are polyhydric steroid compounds that represent a group of plant hormones and have a wide range of physiological responses. BRs promote various physiological processes, such as cell differentiation, male fertility, timing of senescence, and tissue development (Clouse, 2011; Al-Kanany *et al.*, 2022). BRs can enhance the plants ability to deal with various stresses. BRs significantly availability water stress, salt stress, low and high-temperature stress, and heavy metal stress (Ali *et al.*, 2008; Bajguz & Hayat, 2009; Hayat *et al.*, 2010).

This research aims to study the interaction effects of BR application and PEG-induced osmotic stress on some biochemical characteristics. The characteristics studies include; proline, catalase enzyme, and MSI as drought-resistant indicators, while MDA as a drought-degradation indicator.

Materials & Methods

The experiment was conducted in the Tissue Culture Laboratory of the Basra Agriculture Directorate in 2022. The experiment aimed to study the physiological characteristics of date palm tissues' and select drought-tolerant tissues using PEG and BR through the tissue culture technique. The experiment was conducted in two stages of date palm propagation using tissue culture technique (callus and embryos). Callus was obtained from pruning a 4-year-old Barhi date palm offshoot by removing the outer leaves up to the apical bud. The apical bud was superficially sterilized by washing it well with running water several times and then sterilized using sodium hypochlorite (NaClO) 5% for 15 min. The explant was cultivated in the previously prepared nutrient medium (Table 1) (Al-Kanany *et al.*, 2023) to induce callus. The explant was incubated in the dark with replanting on the same nutrient medium every four weeks until callus was obtained and doubled. Then a part of the callus was subcultured in the embryo media (Table 1) and incubated in the long-day growth room (16 hours of light and 8 hours of darkness) with 5000 lux lighting with replanting every four weeks.

Table (1): Growth Medium

Media Components	MS (g.l ⁻¹)	Sucrose (g.l ⁻¹)	Agar (g l ⁻¹)	NAA (μM.l ⁻¹)	2-iP (μM.l ⁻¹)	pH
Initiation (culls stage)	4.3	40	7	6	3	7
Embryo formation	4.3	30	7	3	1	7

The laboratory work was conducted using the factorial experiment design with two factors; the first factor is PEG6000 at 0, 10, 20, and 30gm.L⁻¹ interacts with the second factor, which is the BR at 0, 0.2, 0.5, and 1μmol.L⁻¹. The completely randomized design (CRD) was applied, with five replicates. The results were analyzed using analysis of variance and

differences between the means (ANOVA) and the least significant difference (LSD) and the probability level of 1% using Statistical Product and Service Solution (SPSS) Version 25 was used as the statistical program (IBM-Corp, 2017). The treatments are designed as below (table 2). The BR-free treatment with the PEG-free treatment considered a control treatment.

Table (2): Laboratory Work treatments design

Treatment	(C)	A1	A2	A3	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
PEG (gm L ⁻¹)	0	0	0	0	10	10	10	10	20	20	20	20	30	30	30	30
BR (μmol L ⁻¹)	0	0.2	0.5	1	0	0.2	0.5	1	0	0.2	0.5	1	0	0.2	0.5	1

Total carbohydrate was estimated in tissues using sulfuric acid (H₂SO₄) and phenol method, as described by Dubois (1956) and Dubois *et al.*, (1956). Proline content was determined according to Troll (1955) and Troll & Lindsley (1955). Total phenol content was estimated using Folin-Ciocalteu according to Singleton and Rossi (1965) Singleton & Rossi (1965). The MDA content in tissues was estimated according to Heath and Packer (1968) method and Heath and Packers (1968). MSI was calculated according to Lutts *et al.*, (1996). Catalase extraction was carried out from plant tissues according to the Luhova *et al.*, (2003) and Al-Alwani (2006), and the enzyme activity was estimated according to the Goth (1991) method.

Results & Discussion

Total Carbohydrates

Figure (1A) shows significant there were increases in total carbohydrates in tissues at the callus stage for the C2, C3, and C4 treatments compared to the control and C1 treatments. Also, significant increases in the D2, D3, and D4 treatments compared to the control and D1 treatments. There were also substantial increases in the B2 and B4 treatments compared to the control and B1 treatments. In addition, there was a significant increase in the B3 treatment compared to the control treatment, but this increase is insignificant compared to the B1 treatment. Figure (1B) shows substantial increases in total carbohydrates in tissues at the embryonic stage for the C2, C3, and C4 treatments compared to the control and C1 treatments. Also, in the D3 and D4 significantly increase significantly treatments compared to the control and D1

treatments. PEG significantly increased the total carbohydrates in the plant tissues. These results agree with what Shareef & Al-khayri (2021) and Chaves Filho and Stacciarini-Seraphin (2001) found. PEG (alternative to water stress) changes the solubility of

carbohydrates in the tissues. Soluble carbohydrates have a significant role in osmotic modification as they protect the structure of membranes and giant molecules under stress conditions (Hellal *et al.*, 2018).

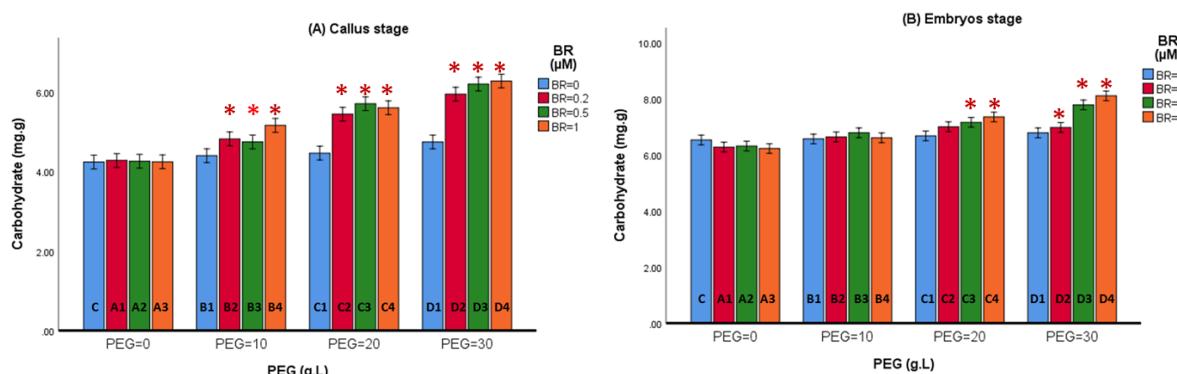


Fig. (1): The interaction effect between BR and PEG on the tissue total carbohydrates (mg g⁻¹) at the (A) Callus and (B) Embryos stages.

BR increased the total soluble carbohydrates in the plant tissues compared to the control treatments under drought stress, because BR works to regulate the sugar metabolism in the plant and arrange the regulation process of the Calvin cycle (Jiang *et al.*, 2012).

Proline Content

The proline content significantly increased in the tissues at the callus stage for D2, D3, and D4 treatments compared to the control and D1 treatments. Also, there was a significant

increase in the C4 treatment compared to the control and C1 treatments (Figure 2A). The proline content significantly increased in the tissues at the embryonic stage in the D2, D3, and D4 treatments compared to the control and D1 treatments. There were also significant increases in the C2 and C4 treatments compared to the control and C1 treatments. Also, there was substantial increase in the B2 treatment compared to the control and B1 treatments (Figure 2B).

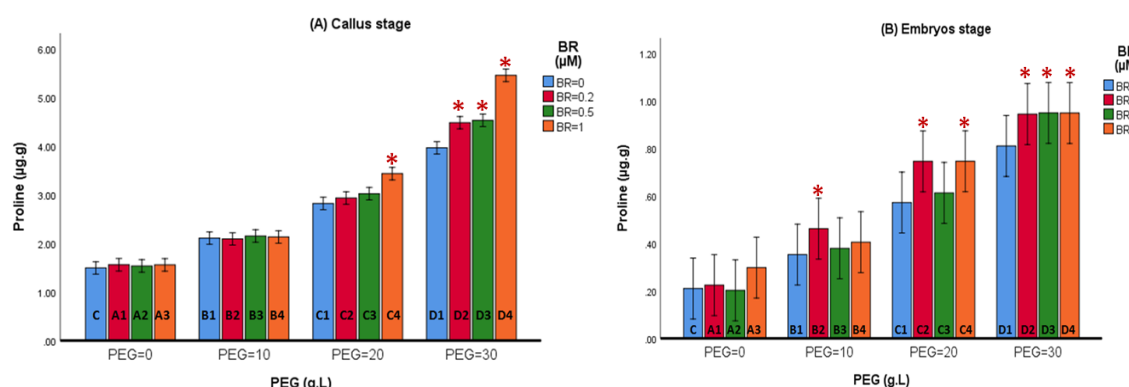


Fig. (2): The interaction effect between BR and PEG on the tissue proline (µg g⁻¹) at the (A) Callus and (B) Embryos stages.

Proline is considered an important indicator of plant protection from abiotic stresses such as water stress, as the proline content in the plant will increase with the increase in water stress (Saleh *et al.*, 2023; Harkousse *et al.*, 2021; Qadir, 2018). Proline is one of the first lines of defense for plant protection from exposure to water stress; as it also works to enhance the tissues' growth and antioxidant activity (Dhawi & Al-Khayri, 2008). BR acts as a key factor that protects the plant by increasing the proline content in the plant under stress conditions (Naveen *et al.*, 2021). Özdemir *et al.*, (2004) indicated that BR induces the gene expression of proline biosynthetic.

Total Phenols Content

The total phenols of tissues at the callus stage significantly increased for the C2, C3, and C4 treatments compared to the control and C1 treatments. Also, there were significant increases for the D2, D3, and D4 treatments compared to the control and D1 treatments. There was also a significant increase in the B4 treatments compared to the control and B1 treatments (Figure 3A). The total phenols of tissues at the embryonic stage significantly increased in the C2, C3, and C4-treated treatments compared to the control and C1 treatments. Also, there were significant increases in the D2, D3, and D4 treatments compared to the control and D1 treatments. Also, there were significant increases in the B3 and B4 treatments compared to the control and B1 treatments (Figure 3B).

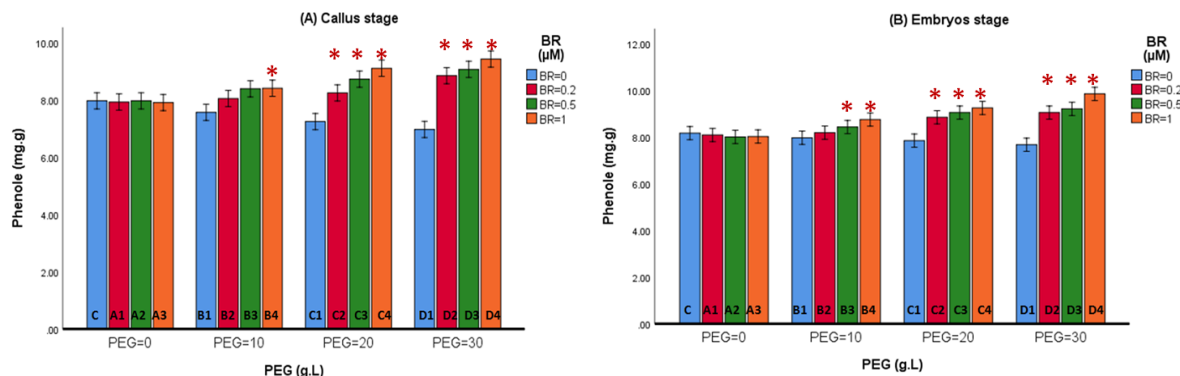


Fig. (3): The interaction effect between BR and PEG on the tissue total phenols (mg g) at the (A) Callus and (B) Embryos stages.

The results showed no clear effect of drought on total phenols in date palm tissues. Researchers have reported differing effects of drought on the phenol compounds, as some studies reported that drought stress significantly increases the tissue content of phenol compounds (Du *et al.*, 2021). On the other hand, others argued that drought decreased the phenol compounds in the tissues.

Also, drought affects phenols according to the plant seasons and stages (Sakran *et al.*, 2018). BRs significantly increased the total phenol content in plant tissues under drought stress conditions (Farooq *et al.*, 2009a; Farooq *et al.*, 2009b). BR enhances the biosynthesis process of soluble phenol compounds, which are drought-tolerant in plants (Anuradha & Rao, 2007).

MDA Content and MSI

Significant increases in the MSI% were observed at the callus stage in the C2, C3, and C4 treatments compared to the C1 treatment. Also, substantial increases in the D2, D3, and D4 treatments compared to the D1 treatment

(Figure 4A). Significant increases were obtained in the MSI% at the embryonic stage in the D2, D3, and D4 treatments compared to the D1 treatment. Also, there were significant increases in the C3 and C4 treatments compared to the C1 treatment (Figure 4B).

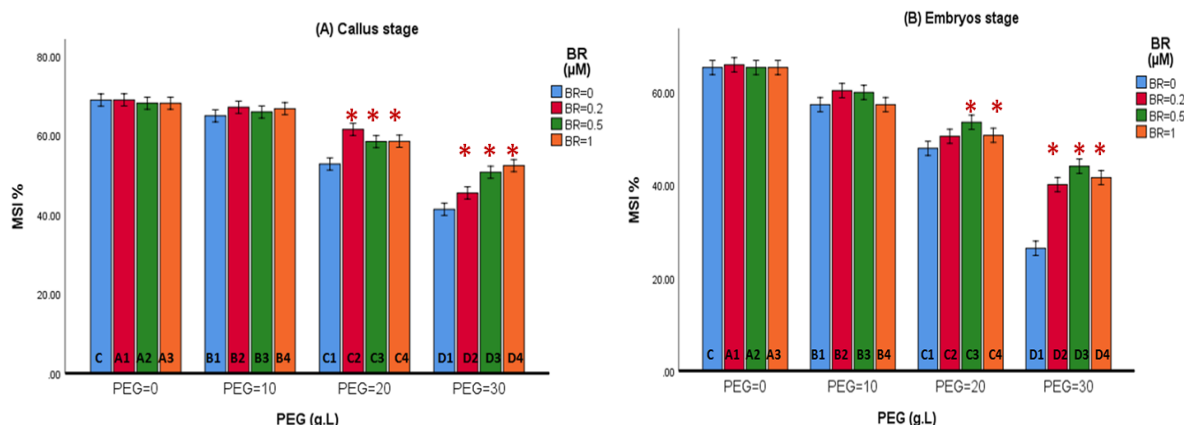


Fig. (4): The interaction effect between BR and PEG on the tissue MSI% at the (A) Callus and (B) Embryos stages.

Figure (5A) shows significant decreases in the MDA content of tissues at the callus stage in the D3 and D4 treatments compared to the D1 treatment. Figure (5B) shows significant decreases in the MDA of tissues at the

embryonic stage in the D2, D3, and D4 treatments compared to the D1 treatment. Also, there were significant decreases in the C3 and C4 treatments compared to the C1 treatment.

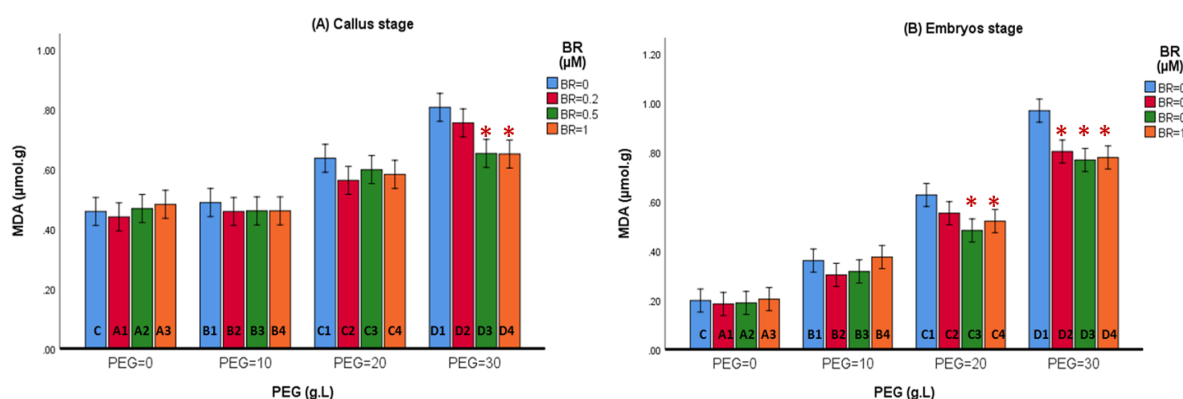


Fig. (5): The interaction effect between BR and PEG on the tissue MDA% at the (A) Callus and (B) Embryos stages.

MDA content is an indicator of plant degradation due to drought stress. However, MSI is an indicator of several stresses and is

often used to measure the tolerance of different plants to water stress. Increasing PEG concentrations increases the MDA content and

decreases MSI in the plant tissues (ElBasyoni *et al.*, 2017; Li-Ping *et al.*, 2006). BR can encourage the tolerance-drought trait in plants by MDA decreasing and MSI increasing in the BR-treated tissues compared to the control treatment (Gill *et al.*, 2017; Hasanzadeh-Naemi *et al.*, 2021; Pereira *et al.*, 2019). The increases in the MSI and the decreases in MDA content in plant tissues are due to the major role of BR in encouraging the activity of the antioxidant enzymes by scavenging the reactive oxygen species (ROS) (Behnamnia *et al.*, 2009; Gill & Tuteja, 2010).

The content of catalase enzyme at the callus stage is characterized by significant increases

in the C2, C3, and C4 treatments compared to the control and C1 treatments. Also, significant increases in D2, D3, and D4 treatments compared to the control and D1 treatments. In addition, significant increases in the B3 and B4 treatments compared to the control and B1 treatments (Fig. 6A). It observed significant increases in the catalase content of tissues at the embryonic stage in the B2, B3, and B4 treatments compared to the control and B1 treatments. Also, significant increases in C2, C3, and C4 treatments compared to the control and C1 treatments. In addition, there were substantial increases in the D3 and D4 treatments compared to the control and D1 treatments (Fig. 6B).

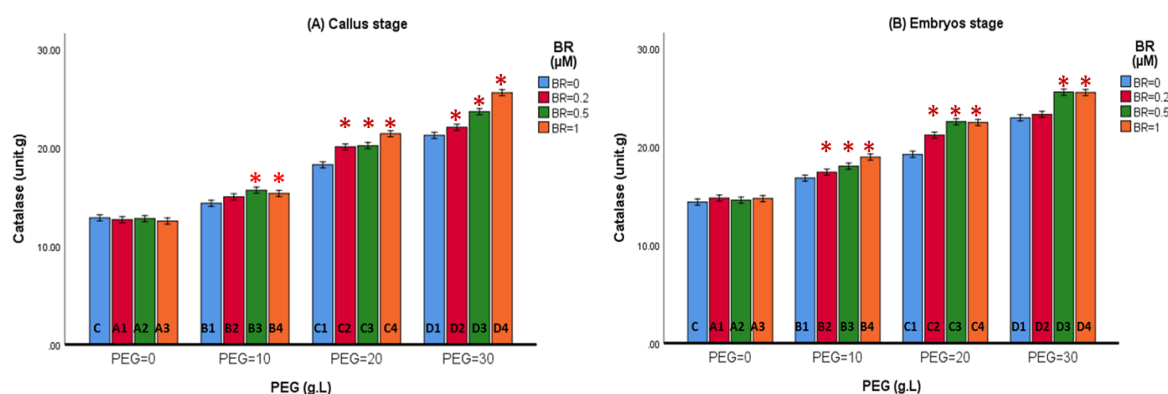


Fig. (6): The interaction effect between BR and PEG on the tissue CAT (unit g) at the (A) Callus and (B) Embryos stages

Catalase Enzyme

Catalase is an essential oxidative enzyme that breaks down nitrogen hydroxide (H₂O₂) into oxygen and water that scavenges ROS. Nitrogen hydroxide destroys and damages lipids, nucleic acids, and proteins, which can ruin the metabolism process in plant cells (Mittler, 2002; Szabados & Savaure, 2010). Increasing PEG leads to increased Catalase content in tissues, which is results from Harkousse *et al.*, (2021) and Abdulwahid (2012). BR applications increase the catalase content in tissues under water stress caused by PEG. BR's role in drought tolerance comes through its work as a mediator factor to stimulate the gene expression of the antioxidant enzymes, especially the catalase

enzyme. Also, BR can stimulate the biosynthesis of endogenous Absciscic acid, which, in turn, encourages the biosynthesis of the enzymatic antioxidant system (Naveen *et al.*, 2021; Xia *et al.*, 2009; Yuan *et al.*, 2010).

Conclusion

Plant tissue culture is considered one of the best techniques for date palm propagation, and it is easy to apply laboratory experiments to know the critical effects of environmental stresses and ways to overcome them using hopeful hormones such as BR. PEG is a stimulator of osmotic stress in the nutrient media, and the interaction between the BR application and PEG led to a reduction in the water stress effect on plant tissues. The research results

highlight BRS key role in reducing the damage caused by water stress. These results showed increasing the proline content and catalase enzyme. Also, BR application has significantly increased the MIS%, while the MDA content in plant tissues has decreased.

Acknowledgements

The authors would like to thank staff of Plant Tissue Culture Division, Date Palm Division, Basra Agriculture Directorate for laboratory work and support provided for the current study.

Contributions of Authors

K.M.A: Collection of specimens, Laboratory techniques, wrote and revised the manuscript.

A.H.A: Suggestion the proposal of the article, revised the manuscript.

M.A.I: Suggestion the proposal of the article, revised the manuscript.

ORCID

A.H.Abdulwahid: <https://orcid.org/0000-0001-7921-8560>

M.A.Ibrahim: <https://orcid.org/0000-0002-8416-7742>

Conflicts of interest

The authors declare that they have no conflict of interests.

References

- Abdulwahid, A. H. (2012). Investigation of the effect of salt stress on the antioxidant enzyme activities on leaves of date palm (*Phoenix dactylifera*) seedling. *Advances in Agriculture & Botany* 4, 94-102.
- Al-Alwani (2006). The reasons for the phenomenon of longevity in terms of the hypothesis of oxidation through the formation of adventitious roots in the cuttings of the Mung bean (*Phaseolus aureus* Roxb). PhD thesis - *College of Science, University of Babylon*.
- Al-Kanany, K. M., Abdulwahid, A. H., & Ibrahim, M. A. (2023). Physical Characteristics of in vitro Date Palm culture under the influence PEG including water stress and Brassinosteroid treatment. *DYSONA Applied Science* 4 (2023), 21-27. DOI: 10.30493/DAS.2022.368280.1067
- Al-Kanany, K. M., Abdulwahid, A. H., & Ibrahim, M. A. (2022). Effect of brassinosteroid on some of the Physico- Chemical Characteristics of Date Palm Fruits (*Phoenix dactylifera* L.) cv. Barhi under Drought Stress. 18, 1309-1317. <https://connectjournals.com/03899.2022.18.1309>
- AL-Mayahi, A. M. (2010). Effect of NAA, 2iP inorganic sulphates, PEG and adenine sulphate on maturation of the somatic embryos in date palm *Phoenix dactylifera* L. cv. Quntar propagated by in vitro culture. *Basrah Journal For Date Palm Research* 9. <https://www.iraqoj.net/iasj/article/49234>
- Al-Mayahi, A. M. W. (2016). Effect of silicon (Si) application on *Phoenix dactylifera* L. growth under drought stress induced by polyethylene glycol (PEG) in vitro. *American Journal of Plant Sciences* 7, 1711-1728. DOI: 10.4236/ajps.2016.713161
- Ali, B., Hasan, S., Hayat, S., Hayat, Q., Yadav, S., Fariduddin, Q., & Ahmad, A. (2008). A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). *Environmental and Experimental Botany* 62, 153-159. <https://doi.org/10.1016/j.envexpbot.2007.07.014>
- Alkhateeb, A. (2006). Somatic embryogenesis in date palm (*Phoenix dactylifera* L.) cv. Sukary in response to sucrose and polyethylene glycol. *Biotechnology* 5, 466-470. DOI: 10.3923/biotech.2006.466.470
- Anuradha, S., & Rao, S. (2007). The effect of brassinosteroids on radish (*Raphanus sativus* L.) seedlings growing under cadmium stress. *Plant Soil and Environment* 53, 465. DOI: 10.17221/2307-PSE
- Bajguz, A., & Hayat, S. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. *Plant physiology and biochemistry* 47, 1-8. <https://doi.org/10.1016/j.plaphy.2008.10.002>
- Behnamnia, M., Kalantari, K. M., & Ziaie, J. (2009). The effects of brassinosteroid on the induction of

- biochemical changes in *Lycopersicon esculentum* under drought stress. *Turkish Journal of Botany* 33, 417-428. DOI: 10.3906/bot-0806-12
- Chaves Filho, J. T., & Stacciarini-Seraphin, E. (2001). Change in osmotic potential and soluble carbohydrates levels in *Solanum lycocarpum* St.-Hil. in response to water stress. *Brazilian Journal of Botany* 24, 199-204. <https://doi.org/10.1590/S0100-84042001000200010>
- Clouse, S. D. (2011). Brassinosteroids. *The arabidopsis book* 9, e0151-e0151.
- Dhawi, F., & Al-Khayri, J. M. (2008). Proline Accumulation in Response to Magnetic Fields in Date Palm (L.). *The Open Agriculture Journal* 2. DOI: 10.2174/1874331500802010080
- Du, B., Kruse, J., Winkler, J. B., Alfarraj, S., Albasher, G., Schnitzler, J.-P., Ache, P., Hedrich, R., & Rennenberg, H. (2021). Metabolic responses of date palm (*Phoenix dactylifera* L.) leaves to drought differ in summer and winter climate. *Tree Physiology* 41, 1685-1700. <https://doi.org/10.1093/treephys/tpab027>
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. t., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry* 28, 350-356. <https://doi.org/10.1021/ac60111a017>
- ElBasyoni, I., Saadalla, M., Baenziger, S., Bockelman, H., & Morsy, S. (2017). Cell membrane stability and association mapping for drought and heat tolerance in a worldwide wheat collection. *Sustainability* 9, 1606. <https://doi.org/10.3390/su9091606>
- Farooq, M., Wahid, A., & Basra, S. (2009a). Improving water relations and gas exchange with brassinosteroids in rice under drought stress. *Journal of Agronomy and Crop Science* 195, 262-269. <https://doi.org/10.1111/j.1439-037X.2009.00368.x>
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. (2009b). Plant drought stress: effects, mechanisms and management. In *"Sustainable agriculture"*, pp. 153-188. Springer. DOI: 10.1007/978-90-481-2666-8_12
- Fki, L., Masmoudi, R., Kriaâ, W., Mahjoub, A., Sghaier, B., Mzid, R., Mliki, A., Rival, A., & Drira, N. (2011). Date Palm Micropropagation via Somatic Embryogenesis. In *"Date Palm Biotechnology"* (S. M. Jain, J. M. Al-Khayri and D. V. Johnson, eds.), pp. 47-68. Springer Netherlands, Dordrecht. DOI:10.1007/978-94-007-1318-5_4
- Gill, M. B., Cai, K., Zhang, G., & Zeng, F. (2017). Brassinolide alleviates the drought-induced adverse effects in barley by modulation of enzymatic antioxidants and ultrastructure. *Plant Growth Regulation* 82, 447-455. DOI:10.1007/s10725-017-0271-6
- Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry* 48, 909-930. <https://doi.org/10.1016/j.plaphy.2010.08.016>
- Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica chimica acta* 196, 143-151. doi: 10.1016/0009-8981(91)90067-m.
- Harkousse, O., Slimani, A., Jadrane, I., Aitboulahsen, M., Mazri, M. A., Zouahri, A., Ouahmane, L., Koussa, T., & Al Feddy, M. N. (2021). Role of local biofertilizer in enhancing the oxidative stress defence systems of date palm seedling (*Phoenix dactylifera*) against abiotic stress. *Applied and Environmental Soil Science* 2021. <https://doi.org/10.1155/2021/6628544>
- Hasanzadeh-Naemi, M., Jari, S. K., Zarrinnia, V., & Fatehi, F. (2021). The effect of exogenous methyl jasmonate and brassinosteroid on physicochemical traits, vase life, and gray mold disease of cut Rose (*Rosa hybrida* L.) flowers. *Journal of the Saudi Society of Agricultural Sciences* 20, 467-475. DOI:10.1016/J.JSSAS.2021.05.007
- Hayat, S., Hasan, S. A., Hayat, Q., & Ahmad, A. (2010). Brassinosteroids protect *Lycopersicon esculentum* from cadmium toxicity applied as shotgun approach. *Protoplasma* 239, 3-14. doi: 10.1007/s00709-009-0075-2.
- Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics* 125, 189-198. doi: 10.1016/0003-9861(68)90654-1.

- Helaly, M. N., El-Hosieny, H. A. R., El-Sarkassy, N. M., & Fuller, M. P. (2017). Growth, lipid peroxidation, organic solutes, and anti-oxidative enzyme content in drought-stressed date palm embryogenic callus suspension induced by polyethylene glycol. *In Vitro Cellular & Developmental Biology - Plant* 53, 133-141. DOI:10.1007/s11627-017-9815-8
- Hellal, F., El-Shabrawi, H., Abd El-Hady, M., Khatib, I., El-Sayed, S., & Abdelly, C. (2018). Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. *Journal of Genetic Engineering and Biotechnology* 16, 203-212. <https://doi.org/10.1016/j.jgeb.2017.10.009>
- IBM-Corp (2017). IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp. Available at: <https://www.ibm.com/products/spss-statistics>
- Jiang, Y.-p., Cheng, F., Zhou, Y.-h., Xia, X.-j., Mao, W.-h., Shi, K., Chen, Z.-x., & Yu, J.-q. (2012). Hydrogen peroxide functions as a secondary messenger for brassinosteroids-induced CO₂ assimilation and carbohydrate metabolism in *Cucumis sativus*. *Journal of Zhejiang University SCIENCE B* 13, 811-823. doi:10.1631/jzus.B1200130.
- Li-Ping, B., Fang-Gong, S., Ti-Da, G., Zhao-Hui, S., Yin-Yan, L., & Guang-Sheng, Z. (2006). Effect of soil drought stress on leaf water status, membrane permeability and enzymatic antioxidant system of maize. *Pedosphere* 16, 326-332. [https://doi.org/10.1016/S1002-0160\(06\)60059-3](https://doi.org/10.1016/S1002-0160(06)60059-3)
- Luhova, L., Lebeda, A., Hedererová, D., & Pec, P. (2003). Activities of amine oxidase, peroxidase and catalase in seedlings of *Pisum sativum* L. under different light conditions. *Plant soil and environment* 49, 151-157. DOI: 10.17221/4106-PSE
- Lutts, S., Kinet, J., & Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of botany* 78, 389-398. <https://doi.org/10.1006/anbo.1996.0134>
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science* 7, 405-410. DOI:10.1016/s1360-1385(02)02312-9
- Naveen, N., Kumari, N., Avtar, R., Jattan, M., Ahlawat, S., Rani, B., Malik, K., Sharma, A., & Singh, M. (2021). Evaluation of Effect of Brassinolide in Brassica juncea Leaves under Drought Stress in Field Conditions. *Horticulturae* 7, 514. <https://doi.org/10.3390/horticulturae7110514>
- Özdemir, F., Bor, M., Demiral, T., & Türkan, İ. (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. *Plant growth regulation* 42, 203-211. DOI:10.1023/B:GROW.0000026509.25995.13
- Pereira, Y., Rodrigues, W., Lima, E., Santos, L., Silva, M. & Lobato, A. (2019). Brassinosteroids increase electron transport and photosynthesis in soybean plants under water deficit. *Photosynthetica* 57, 181-191. DOI: 10.32615/ps.2019.029
- Qadir, S. A. (2018). Wheat Grains Germination and Seedling Growth Performance under Drought Condition. *Basrah Journal of Agricultural Sciences* 31, 44-52. <https://doi.org/10.37077/25200860.2018.99>
- Sakran, M. I., El Rabey, H. A., Almulaiky, Y. Q., Al-Duais, M. A., Elbakry, M., & Faridi, U. (2018). The antioxidant enzymatic activity of date palm seedlings under abiotic drought stress. *Indian Journal of Pharmaceutical Education and Research* 52, 442-448. <https://doi.org/10.5530/ijper.52.3.51>
- Shareef, H. J., & Al-khayri, J. M. (2021). Salt and drought stress exhibits oxidative stress and modulated protein patterns in roots and leaves of date palm (*Phoenix dactylifera* L.). *Acta agriculturae Slovenica* 117, 1-10. <https://doi.org/10.14720/aas.2021.117.1.1829>
- Sharma, S., and Banerjee, M. (2021). Role of Brassinosteroids in Root Growth and Development. In "Rhizobiology: Molecular Physiology of Plant Roots", pp. 239-252. Springer. https://doi.org/10.1007/978-3-030-84985-6_14
- Saleh, A. M., Al-Najjar, M. A. H., & Alpresem, W. F. F. (2023). Effect of polyamines and zeolites on the protein profile of leaves of the date palm cuttings *Phoenix dactylifera* L. grown under heavy metal stress conditions. *Journal of Global Innovations in Agricultural Sciences* 11, 391-6. <https://doi.org/10.22194/JGIAS/23.1104>

- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture* 16, 144-158. DOI: 10.5344/ajev.1965.16.3.144
- Stasolla, C., Kong, L., Yeung, E. C., & Thorpe, T. A. (2002). Maturation of somatic embryos in conifers: morphogenesis, physiology, biochemistry, and molecular biology. *In Vitro Cellular & Developmental Biology-Plant* 38, 93-105. DOI:10.1079/IVP2001262
- Szabados, L., & Saviouré, A. (2010). Proline: a multifunctional amino acid. *Trends in plant science* 15, 89-97. <https://doi.org/10.1016/j.tplants.2009.11.009>
- Troll, W., & Lindsley, J. (1955). A photometric method for the determination of proline. *Journal of biological chemistry* 215, 655-660. [https://doi.org/10.1016/S0021-9258\(18\)65988-5](https://doi.org/10.1016/S0021-9258(18)65988-5)
- Xia, X.-J., Huang, L.-F., Zhou, Y.-H., Mao, W.-H., Shi, K., Wu, J.-X., Asami, T., Chen, Z., and Yu, J.-Q. (2009). Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. *Planta* 230, 1185-1196. doi: 10.1007/s00425-009-1016-1
- Yuan, G.-F., Jia, C.-G., Li, Z., Sun, B., Zhang, L.-P., Liu, N., & Wang, Q.-M. (2010). Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. *Scientia Horticulturae* 126, 103-108. <https://doi.org/10.1016/j.scienta.2010.06.014>

تأثير التداخل بين PEG6000 و Brassinosteroid على الخصائص الكيموحيوية لنخيل التمر النسيجي (صنف برحي)

خضير مطشر الكنانى¹ وعقيل هادي عبدالواحد² وماجد عبد الحميد ابراهيم³

¹قسم النخيل، مديرية زراعة البصرة، البصرة، العراق

²قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة البصرة، العراق

³قسم الأحياء، كلية العلوم، جامعة البصرة، العراق.

المستخلص: توالد الاجنة الجسدية هو طريقة أساسية لإكثار نخيل التمر عن طريق الاكثار الدقيق والتي تنتج كتلة حيوية كبيرة من النباتات. الكالس هو النسيج النباتي الأساسي الذي ينتج عبر طريقة الإكثار غير المباشر (بتقنية زراعة الانسجة)، وهو الدعامة الأساسية في تكوين النبات الذي يقاوم الضغوط البيئية. يستخدم البولي إيثيلين جليكول (PEG) في التجارب المعملية لتحفيز الإجهاد الأسموزي، في حين يعد هرمون براسينوستيرويد هرموناً واعداً يستخدم للتغلب على الإجهاد المائي أو تقليله في وسط غذائي. تهدف التجربة إلى دراسة تأثير البولي إيثيلين جليكول وبراسينوستيرويد على الخصائص البيوكيميائية لأنسجة نخيل التمر (الكالس والاجنة). تم استخدام أربع تركيزات من براسينوستيرويد (0، 10، 20، و 30 غم لتر⁻¹) مع أربع تركيزات من الهرمون (BR) (0، 0.2، 0.5، و 1 ميكرومول لتر⁻¹) في الوسط الغذائي لأنسجة نخيل التمر في مرحلتى الكالس والاجنة. أظهرت النتائج زيادة كبيرة في محتوى الأنسجة من البرولين وإنزيم الكاتالاز ومؤشر استقرار الغشاء، وهي مؤشرات مقاومة الإجهاد لأنسجة النبات. وعلى العكس من ذلك، انخفض في محتوى MDA، والذي يعتبر كمؤشر على تدهور النبات بسبب الإجهاد. هذه نتائج قيمة لأنها تثبت الدور الأساسي للبراسينوستيرويد في مقاومة الإجهاد لأنسجة النبات، وبزيادة هذه المقاومة؛ يمكن للنبات القوي أن ينتج نباتاً مقاوماً للإجهادات البيئية.

الكلمات المفتاحية: إنزيم الكاتالاز، التخلق الجنيني، مالونديالدهيد، بولي إيثيلين جليكول، البرولين.