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

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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 *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 ($\mu M.l^{-1}$)	2-iP ($\mu M.l^{-1}$)	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 (H_2SO_4) 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).

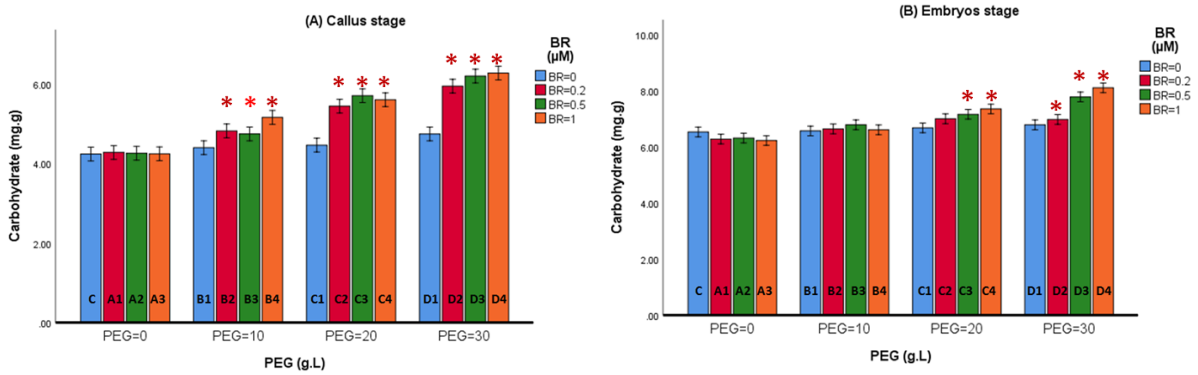

Fig. (1): The interaction effect between BR and PEG on the tissue total carbohydrates (mg g^{-1}) at the (A) Callus and (B) Embryos stages.

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).

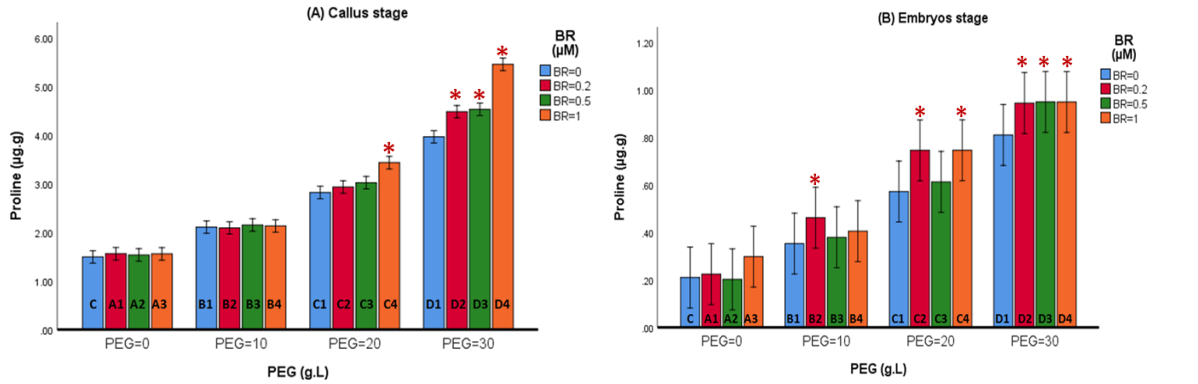

Fig. (2): The interaction effect between BR and PEG on the tissue proline (µg g^{-1}) at the (A) Callus and (B) Embryos stages.

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).

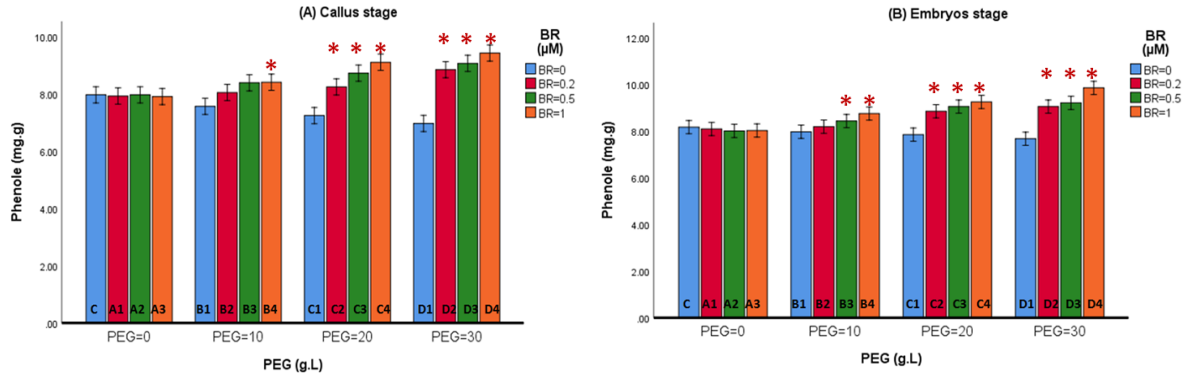

Fig. (3): The interaction effect between BR and PEG on the tissue total phenols (mg g) at the (A) Callus and (B) Embryos stages.

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).

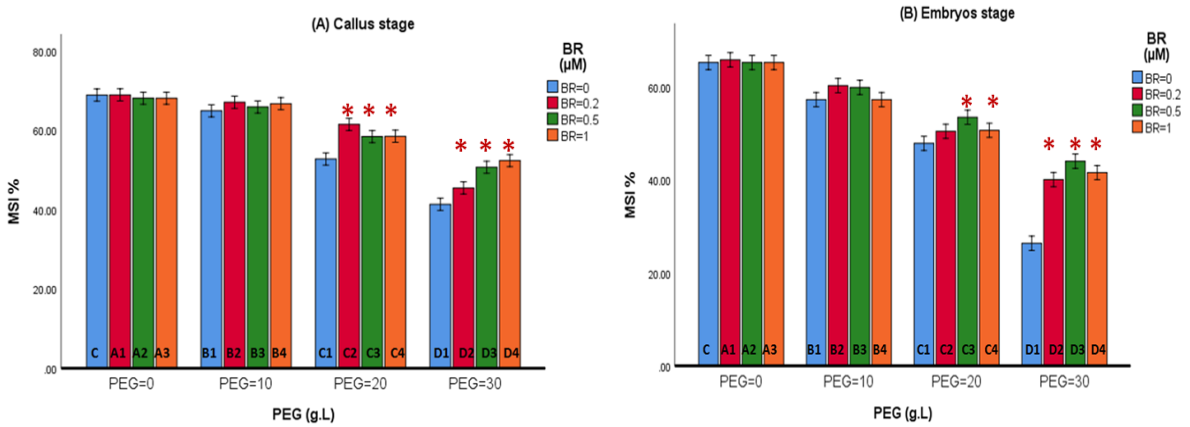

Fig. (4): The interaction effect between BR and PEG on the tissue MSI% at the (A) Callus and (B) Embryos stages.

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.

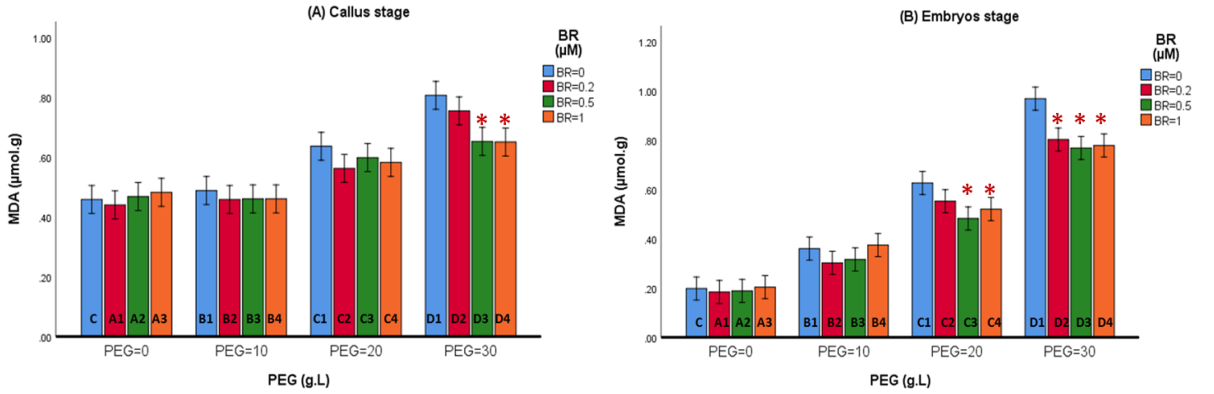

Fig. (5): The interaction effect between BR and PEG on the tissue MDA% at the (A) Callus and (B) Embryos stages.

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 e*t al*., 2017; Hasanzadeh-Naemi *et al*., 2021; Pereira e*t 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).

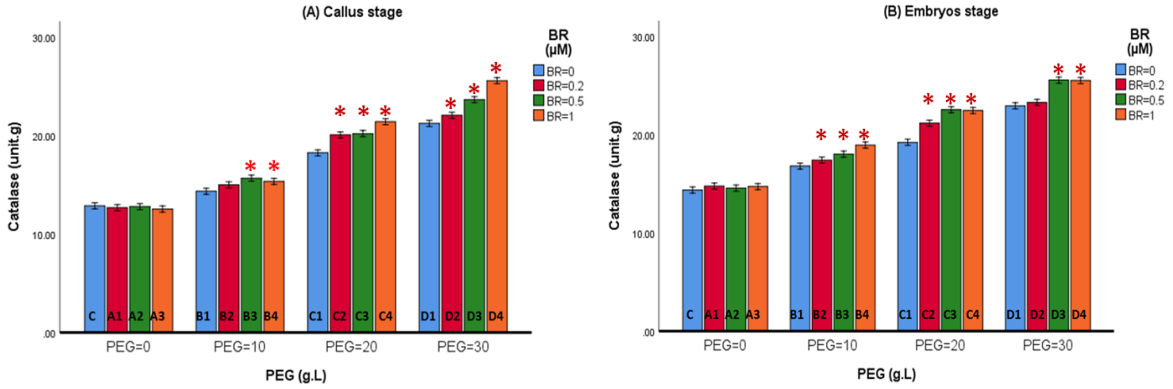

Fig. (6): The interaction effect between BR and PEG on the tissue CAT (unit g) at the (A) Callus and (B) Embryos stages

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 & Savouré, 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 Abscisic 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.

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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.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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

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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، و30 غم لتر$^{-1}$) مع أربع تركيزات من الهرمون (BR) (0، 0.2، 0.5، و1 ميكرومول لتر$^{-1}$) في الوسط الغذائي لأنسجة نخيل التمر في مرحلتي 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، انخفاض 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 نباتًا مقاومًا للإجهادات البيئية.

الكلمات المفتاحية: انزيم الكاتاليز، التخلق الجنيني، مالونديالدهيد، بولي إيثيلين جليكول، البرولين.