# Role of Picloram, PEG and Sugar Alcohol on Somatic Embryogenesis of Date Palm cv. Barhee Immature Female Inflorescence

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## Abstract

Among the various explants used to propagate date palm, immature inflorescence has been recently used in a wide range. Somatic embryogenesis approach appears to be a reliable option for mass propagation. However, maturation, germination and plant regeneration from somatic embryos are the main challenges of this approach. This research describes in vitro propagation of date palm 'Barhee' through immature inflorescence. Spiklet explants were removed gently from sterilized spaths and cultured on MS medium supplemented with picloram (1.25, 2.5 and 5mg/l). Within 6 subcultures, the direct somatic embryos and embryonic callus were took place in different treatments at various levels. Embryonic callus were shifted onto maturation medium augmented with sorbitol, mannitol and PEG at 5, 10 and 15g/l to record their impact on embryonic callus growth and somatic embryo formation. Subsequently, somatic embryo clusters formed previously were cultured on germination medium with the same concentrations of osmoticum types to form healthy shoots. Paclobutrazol and sucrose at different levels and combinations were added to rooting medium to improve root system. The highest percentages of direct somatic embryos and embryonic callus were achieved on 5mg/l picloram. The addition of PEG to maturation medium at 10 g/l maximized somatic embryo numbers, while mannitol at 15 g/l encouraged significantly the embryonic callus fresh weight. On germination and multiplication stage, mannitol was found to be more effective in the case of shoot formation. PBZ-enriched medium has the positive effect on root formation. Healthy rooted plantlets were successfully transferred to greenhouse conditions with higher survival percentage.

**Keywords:** Embryonic callus, embryo maturation & germination, mannitol, paclobutrazol, sorbitol.

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## Introduction

Date palm (*Phoenix dactylifera* L.), a monocotyledonous, dioecious tree species belonging to Arecaceae family, is one of the oldest fruit crops cultivated in the desert areas of the world, mainly in the Middle East and North Africa countries. Date palms are traditionally propagated by offshoots. However, the use of in vitro propagation protocols is essential for large-scale propagation, and production of true to type plants

(Aleid *et al.*, 2015). Somatic embryogenesis and organogenesis are the two approaches currently used for in vitro propagation of date palm cultivars (Al Kaabi *et al.*, 2007). Numerous researchers have described various techniques to stimulate somatic embryogenesis of date palm different cultivars; some through modifying culture media additives (Hassan *et al.*, 2007; Hassan and Taha, 2012) and technical methods (Othmani *et al*., 2009; Ibrahim *et al*., 2012). Also exposing to stress could be useful to improve growth and development of date palm somatic embryogensis (Taha and Hassan, 2014). For decades, date palm has been micro propagated using shoot tip explants (Abul-Soad *et al*., 2004). However, other meristematic tissues such as axillary buds, immature inflorescences and mature embryos were tested (Khiralla *et al.*, 2017). Recently, the inflorescence explants are successfully used to propagate distinguish and rare cultivars of date palm (AlKhayri, 2003, Hassan *et al*., 2021 and Taha *et al*., 2021) Nutrient medium composition and exogenous application of PGRs have a vital role in in vitro growth, differentiation and plant regeneration from date palm cultured tissue (George *et al*., 2008). Picloram (4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid) has been previously used to induce somatic embryogenesis from date palm different explant; some via immature inflorescence (Hassan *et al*. 2017 and 2021), other from juvenile leaves of date palm cv. Boufeggous (Othmani *et al.* 2009) and from bud and leaf of date palm cv. Bream (Khierallah *et al*., 2015). Previous researches have shown that date palm somatic embryos maturation and germination can be controlled by some factors, including medium physical form (Mazari *et al*., 2019 and Fki *et al*., 2003), auxin concentration and medium type and its different strengths ( Mazari *et al*., 2018, Beauchesne *et al*., 1986 and Hassan *et al.*, 2012), partial desiccation and technical methods (Ibrahim *et al*., 2012 and Othmani *et al*., 2009) .Others tested ABA, PEG and sugar alcohol (Hassan *et al*., 2007 and Mazari *et al*., 2019). The present work aims to develop a new regeneration protocol for date palm cv. Barhee via somatic embryogenesis using immature inflorescence explant. Accordingly, the influence of picloram concentration during induction stage, concentrations of osmoticum various types during maturation & germination stages were studied. In addition, different combinations of sucrose and paclobutrazol were investigated during rooting stage.

## Materials and methods

This study was carried out at the Tissue Culture laboratory of Agricultural Genetic Engineering Research Institute and The Central Laboratory of Date Palm Researches and Development, Agriculture Research Center, Giza, Egypt, during the period of 2021-2023. Barhee cv. immature inflorescences were used, as explant material, for in vitro propagation of date palm in this investigation.

### Plant materials and sterilization

Date palm (*Phoenix dactylifera* L.) cv. Barhee was used throughout this study. Immature female inflorescences (7- 10 cm in length) were taken at late January to

early February from mature palm about 15 years old (Fig. 1a-d). Sterilization of explants was done according to the method described by Hassan *et al*., (2021).

**Initiation stage**

Sterilized spikelet's were divided into small pieces (1-2 cm in length) and cultured on modified MS medium (Murashige and Skoog, 1962) supplemented with 100 mg/l glutamine, 100 mg/l myo inositol, 1mg/l biotin, 5mg/l thiamin HCl, 40 g/l sucrose, 6 g/l agar and 1g/l activated charcoal. Growth regulator "Picloram" was added to initiation medium at 1.25, 2.5 and 5 mg/l to study its impact on somatic embryo formation. The medium pH was adjusted to 5.7-5.8 prior the addition of agar. All culture media were distributed into small jars (200 ml) at rate of 40 ml/jar and covered with polypropylene closures, then sterilized at 121 °C and 15 Ibs\ins$^2$ for 20 min. Explants were re-cultured on fresh medium with the same composition every 6 weeks intervals. After six$^{th}$ subculture, direct embryo formation and embryonic callus were observed on the cultured inflorescence in related to picloram concentration. Browning degree, swelling degree, direct embryo formation percentage and direct embryo numbers per jar, callus formation percentage embryonic callus and degree direct embryo formation were estimated. Browning, swelling and callus formation degrees were determined by degrees according to Pottino (1981) as followed: low (1), below average (2), average (3), good (4) and excellent (5).

**Maturation of callus culture**

To test the impact of osmoticum on embryonic callus proliferation and subsequent somatic embryo appearance, callus from previous stage (0.5 g) was cultured on MS solid medium supplemented with 0.1mg/l NAA, 100mg/l glutamine, 1mg/l biotin, 5mg/l thiamin HCl, modified to contain sugar alcohol (sorbitol and mannitol each at 5, 10, and 15 g/l) and polyethylene glycol (PEG) at 5, 10 and 15 g/l. The cultures were maintained for 12 weeks and subcultured at 4 weeks intervals. After this period, the average of browning, embryonic callus degree and average number of somatic embryos were recorded.

**Germination and multiplication of somatic embryos**

Direct and indirect somatic embryo clusters (2-3 embryos) from initiation stage were cultured on sorbitol, mannitol and PEG previous concentrations to show its effects on somatic embryo growth and development. Different treatments were added to 3/4 MS basal medium containing 0.1mg/l NAA, 0.05 mg/l Benzyl Adenine (BA) and 6 g/l Agar. Average numbers of embryosaverage numbers of germinated embryos (shoots) were counted after 3 subcultures (1 month interval).

**Rooting and acclimatization stage**

Plantlets of Barhee.; 6-8 cm in length were transferred to rooting medium consists of half strength MS solid medium augmented with combination of sucrose at 30, 45 and 60 g/l and paclobutrazol at 0.0, 0.1, 0.2 and 0.3 mg/l in a factorial experiment contained twelve treatments to examine their influence on root formation. The media

were dispensed into 25×250 mm long tubes. Plantlets were moved to fresh rooting medium after 6 weeks. At the end of rooting stage, the average number of roots/shoot and the average root length (cm) were recorded. Growth vigor and root thickness average values were estimated according to Pottino (1981). Healthy rooted plantlets were shifted from rooting medium, rinsed with tab water to remove nutrients residuals and then immersed in fungicide solution at 1g/l for 15 min. After that, plantlets were cultured on torpedo (5 diameters and 18 cm in length) filled with peat: perlite 2:1 v/v and covered with polyethylene bags for 1.5 month with regular checkup to remove any contaminated or wilted plants. Polyethylene covers were removed gradually after 1.5 month. Plantlets were irrigated and fertilized according to Hassan (2012).

**Statistical analysis**

The experimental design was completely randomized with 3 replicates and each replicate contains 3 explants in each treatment. Data were subjected to analysis of variance using the statistical software and means were compared by Duncan (1955) multiple range test at $p \leq 0.05$ level of confidence.

## Results

**Initiation stage**

In the present investigation, date palm cv. Barhee immature inflorescence explants were cultured on MS basal salts supplemented with 100 mg/l glutamine, 100 mg/l myo inositol, 1mg/l biotin, 5mg/l thiamin HCl, 40 g/l sucrose, 6 g/l agar and 1g/l activated charcoal. Picloram various concentrations were added to study their effect on inflorescence response after 6 subcultures. Data from Table (1) revealed that increasing picloram gradually from 1.25, 2.5 to 5 mg/l in initiation medium raised browning degree without significant differences. In respect to swilling degree, data showed that the highest swilling degree (4.33) was found with picloram at 2.5 mg/ l (Fig.1e) followed insignificantly by 5mg/l picloram. While 1.25 mg/l showed the least degree. Data concerning embryonic callus formation % showed that, the highest percentage was achieved with picloram at 5 mg/l (29.63%) followed by 2.5 mg/l which produced (22.22%) while, 1.25 mg/l recoded the lowest percentage. With respect to direct embryo formation% and embryo numbers, data observed that the presence of 5mg/l in initiation media maximized significantly the values of the two parameters as the results were **(**66.66% and13.67 somatic embryos, respectively**)** compared with other treatments (Fig.1f). Concerning embryonic callus degree, raising picloram level to 5mg/l increased significantly the callus degree and embryo differentiation in the cultured spikelet's as shown in (Fig. 1g, h).

**Table (1):** Effect of picloram concentrations on response of immature inflorescence of date palm Barhee cv. during initiation stage

| Picloram con. | Browning degree | Swelling degree | Embryonic callus formation% | Embryonic callus degree | Direct embryo formation% | Direct embryo number |
|---|---|---|---|---|---|---|
| **1.25** | 1.67 A | 2.67 B | 14.81C | 1.33 B | 0.74 C | 1.67 C |
| **2.5** | 2.33 A | 4.33 A | 22.22 B | 2.00 A | 29.63 B | 4.67 B |
| **5.0** | 2.67 A | 3.33 AB | 29.63 A | 2.67 A | 66.66 A | 13.67 A |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level

**Maturation stage**

Embryonic callus from initiation stage weighed (0.5g) was cultured on maturation media supplemented with osmotic different concentrations to notice their impacts on browning degree, callus fresh weight (g) and somatic embryos number/ culture. Results in Table (2) showed that, no significant difference could be noticed in embryonic callus browning degree among osmotic types. However, the lowest average of browning degree was recorded with mannitol. Data concerning concentration revealed that, using lowest concentration (5g/l) decreased callus browning degree in compared with other concentrations. Interaction in this respect pointed that, mannitol at 5g/l recorded the lowest average of browning. On the other hand, PEG at 15 g/l achieved the maximum value.

**Table (2):** Effect of osmoticum concentrations on browning of embryonic callus culture during maturation stage

| Osmoticum | Concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| **Sorbitol** | 2.00 c | 2.67 b | 3.00 ab | 2.56 A |
| **Mannitol** | 1.33 d | 2.00 c | 2.50 b | 1.94 A |
| **PEG** | 2.00 c | 2.67 b | 3.26 a | 2.64 A |
| **Mean** | 1.78 B | 2.44 AB | 2.92 A | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

Data in Table (3) reflected that, osmoticum types and concentrations have an effective role on embryonic callus cultures of Barhee cv. The heaviest embryonic callus cultures were shown with maturation medium enriched with mannitol **(**Fig.1i**)**. On the other hand, fresh weight observed with sorbitol and PEG did not differ significantly. In respect to concentration, data showed that the moderate concentration (10 g/l) was the best to achieve the highest significant fresh weight (5.66 g). Interaction revealed that, mannitol at 15 g/l and PEG at (10g/l) recorded the highest significant fresh weight (6.30 and 6.17 g, respectively).

**Table (3):** Effect of osmoticum types and concentrations on embryonic callus culture fresh weight (g) during maturation stage

| Osmoticum | Concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| **Sorbitol** | 4.50 d | 5.20 c | 4.00 ef | 4.57 B |
| **Mannitol** | 5.20 c | 5.60 b | 6.30 a | 5.70 A |
| **PEG** | 3.75 f | 6.17 a | 4.30 de | 4.74 B |
| **Mean** | 4.48 B | 5.66 A | 4.87 B | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

Data in Table (4) revealed that, maturation medium enriched with PEG was the most significant reliable medium for somatic embryo production in our research. In this medium, many somatic embryos appeared uniformly from the embryonic callus mass either during the first or the second subculture. While sorbitol and mannitol supplemented media induced close values (15.83 and 15.67 embryo/culture, respectively). The addition of osmoticum different types at 5 and 10 g/l to maturation media encouraged the highest significant embryo numbers. However, raising the concentration to 15 g/l reduced significantly the embryos number. Interaction in this respect showed that, maturation medium contained 10 g/l PEG maximized somatic embryos number compared with other treatments in this experiment **(**Fig. 1j**)**.

**Table (4):** Effect of osmoticum concentrations on embryos number from embryonic callus culture during maturation stage

| Osmoticum | Concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| **Sorbitol** | 22.50 b | 12.00 g | 13.00 fg | 15.83 B |
| **Mannitol** | 18.00 c | 14.00 ef | 15.00 de | 15.67 B |
| **PEG** | 19.00 c | 31.00 a | 16.00 d | 22.00 A |
| **Mean** | 19.83 A | 19.00 A | 14.67 B | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

**Multiplication and germination stage**

In this stage, culture media were modified to include 0.1 mg/l NAA mg/l and 0.05 mg/l BA in addition to sorbitol, mannitol and PEG concentrations to determine their effects on somatic embryo growth and development through two subcultures. Results in Table (5) showed that, osmoticum types and concentrations had a pronounced impact on the process of somatic embryogenesis of date palm Barhee cv. Mannitol maximized the number of embryos (18.17/somatic embryo) compared with sorbitol and PEG. Also, data about concentration reflected that, the addition of 5g/l irrespective to osmoticum types was the best; where the number of embryos was (13.67/somatic embryo). Interaction in this respect showed that, the addition of mannitol at 15 g/l surpassed other treatments.

**Table (5):** Effect of osmoticum concentrations on average number of embryo during multiplication and germination stage

| Osmoticum | Concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| **Sorbitol** | 12.00 c | 8.00 de | 6.00 f | 8.67 B |
| **Mannitol** | 20.00 a | 13.50 b | 21.00 a | 18.17 A |
| **PEG** | 9.00 d | 8.50 d | 7.30 e | 8.27 B |
| **Mean** | 13.67 A | 10.00 B | 11.43 B | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

Data in Table (6) revealed that, the presence of osmoticum at different concentrations had a strong effect on somatic embryo germination process. The highest significant number of germinated embryos (shoots) (10.67 shoot/ explant) was noticed with mannitol. This number was lowered significantly by using PEG and sorbitol. Interaction showed that, mannitol and PEG at 10 g/l were the best compared with other treatments. Data about osmoticum concentration revealed that, using medium with 10 and 5g/l raised significantly the number of germinated embryos. However, at concentration higher than the optimal, significant decrease in germinated embryo number was occurred. In regard to interaction data in this Table showed that, certain concentrations of osmoticum type had a stimulatory effect on germination of somatic embryos. The addition of mannitol at (5 and 10g/l) and PEG at 10 g/l enhanced the ability of somatic embryos to convert into shoots (Fig. 1k).

**Table (6):** Effect of osmoticum types and concentrations on average number of germinated embryo during multiplication and germination stage

| Osmoticum | Concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| **Sorbitol** | 11.00b | 8.00 c | 4.00 e | 7.67 C |
| **Mannitol** | 12.00 a | 12.00 a | 8.00 c | 10.67 A |
| **PEG** | 8.00 c | 11.70 a | 7.00 d | 8.90 B |
| **Mean** | 10.33 A | 10.57 A | 6.33 B | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

**Rooting and acclimatization stage**

Paclobutrazol (PBZ) and sucrose concentrations were added to rooting media consists of half strength of MS medium supplemented with 0.5 mg/l from NAA and IBA to record their influence on root formation of Barhee plantlets. Data in Table (7) mentioned that, supplementing the rooting medium with PBZ stimulated root formation compared with control. Among the various concentrations of PBZ tested, the highest number of roots (3.10 roots/ plantlets) was obtained on rooting medium containing 0.3 mg/l followed insignificantly by the medium with 0.2 mg/l (2.83 roots/ plantlet). Data about the effect of sucrose concentrations showed that, the mean number of roots significantly increased to 2.82 when plantlets were cultured on 45g/l

sucrose-containing medium. Increasing the concentration of sucrose to 60 g/l did not improve root formation. The combination of PBZ at 0.3 mg/l and sucrose at 45g/l sucrose induced root numbers to highest significant value (3.8 roots/ plantlet) compared with other combinations.

**Table (7):** Effect of paclobutrazol and sucrose concentrations on average number of root/shoot of Barhee plants during rooting stage

| Paclobutrazol con. (mg/l) | Sucrose concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 30 | 45 | 60 | |
| **0.0** | 1.00 h | 1.97 f | 1.25 g | 1.41 C |
| **0.1** | 2.40 de | 2.60 d | 3.00 bc | 2. 67 B |
| **0.2** | 2.60 d | 2.90 c | 3.00 bc | 2. 83 AB |
| **0.3** | 3.20 b | 3.80 a | 2.30 e | 3.10 A |
| **Mean** | 2.30 B | 2.82 A | 2.39 B | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level

The presence of PBZ in the culture medium increased the growth vigor of Barhee plantlets as shown in Table (8). In PBZ-free medium, the growth vigor was (3.53). The addition of 0.1 mg/ L PBZ to the culture medium resulted in the highest significant value of growth vigor. Increasing this concentration to 0.2 or 0.3mg/L did not improve plant growth vigor. Concerning the effect of sucrose concentrations, data revealed that no significant differences could be observed among tested concentrations. Interaction between PBZ and sucrose reflected the same greatest values of growth vigor with media containing PBZ at 0.1mg/l + 45 or 60 g/l sucrose, PBZ at 0.2 + 45g/l sucrose and PBZ at 0.3mg/l + 30 g/l sucrose (Fig. 1 l).

**Table (8):** Effect of paclobutrazol and sucrose concentrations on growth vigor of Barhee plants during rooting stage

| Paclobutrazol con. (mg/l) | Sucrose concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 30 | 45 | 60 | |
| **0.0** | 3.00 f | 4.00 cd | 3.60 e | 3.53 B |
| **0.1** | 4.22 c | 5.00 a | 5.00 a | 4.74 A |
| **0.2** | 4.80 a | 5.00 a | 4.00 cd | 4.60 A |
| **0.3** | 5.00 a | 4.50 b | 3.80 de | 4.43 A |
| **Mean** | 4.25 A | 4.62 A | 4.10 A | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

**Root length**

In case of root length, the highest root length was detected on medium supplemented with 0.2 mg/l PBZ. It was assumed that, the rise in PBZ concentration to 0.3 mg/L might have an adverse effect on root length. Control treatment gave the lowest significant root length. In respect to sucrose concentration effects as shown in table (9), it is evident that increasing sucrose concentration from 30 to 60 g/l restricted root length significantly. Results about interaction between PBZ and sucrose

concentrations showed that, there were significant differences among different treatments for root length of all plantlets. The greatest significant root length was recorded when plantlets were cultured on medium with 0.2 mg/l PBZ and 30 g/l sucrose while the lowest root length could be obtained on medium with 60 g/l sucrose without PBZ addition.

**Table (9):** Effect of paclobutrazol and sucrose concentrations on of Barhee plant root length (cm) during rooting stage

| Paclobutrazol con. (mg/l) | Sucrose concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 30 | 45 | 60 | |
| **0.0** | 2.00 e | 1.20 h | 1.00 i | 1.40 C |
| **0.1** | 1.80 f | 2.60 b | 2.40 cd | 2.27 B |
| **0.2** | 4.00 a | 2.50 bc | 2.25 d | 2.92 A |
| **0.3** | 1.50 g | 1.75 f | 1.50 g | 1.58 C |
| **Mean** | 2.32 A | 2.01 AB | 1.79 B | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level

**Root thickness**

Data about the effect of PBZ and sucrose concentrations on root thickness of Barhee plantlets are shown in Table (10). With regarding to PBZ concentrations, the highest significant value of root thickness (3) was produced on medium added with 0.1 mg/l PBZ. However, the addition of 0.2 and 0.3 mg/l PBZ to the rooting medium decreased the root thickness. No significant differences could be observed among all tested concentrations of sucrose. Combination between 0.1 mg/l PBZ and 60g/l sucrose recorded the highest statistically value of root thickness (3.5) while rooting medium added with 30g/l sucrose without adding PBZ resulted in the lowest statistically value (1.5). Healthy rooted plantlets transferred successfully to greenhouse with highest percentages (Fig. 1m-p).

**Table (10):** Effect of paclobutrazol and sucrose concentrations on root thickness of Barhee plantlets during rooting stage

| Paclobutrazol con. (mg/l) | Sucrose concentration (g/l) | | | Mean |
|---|---|---|---|---|
| | 30 | 45 | 60 | |
| **0.0** | 1.50 f | 1.90 e | 2.20 d | 1.87 C |
| **0.1** | 2.50 c | 3.00 b | 3.50 a | 3.00 A |
| **0.2** | 3.00 b | 2.50 c | 2.50 c | 2.67 B |
| **0.3** | 3.00 b | 2.50 c | 2.00 e | 2.50 B |
| **Mean** | 2.50 A | 2.47 A | 2.55 A | |

Means followed by the same letter (s) in each column are not significantly different from each other at 5% level.

## Discussion

In past decades, the auxin 2,4-dichlorophenoxy acetic acid (2,4-D) was widely incorporated to culture medium of date palm different explants to stimulate somatic

embryogenesis induction (Eshraghi *et al*., 2005). However, it was found that using higher levels of 2,4-D may cause some abnormalities in regenerated plants (Fki *et al.*, 2011). So, using other types of auxins at low levels to stimulate somatic embryogenesis in date palm cultivars would be benefit. In our experiment, initiation medium supplemented with 5mg/l picloram induced direct embryo and embryonic callus formation percentages from date palm cv. Barhee immature inflorescence explants. Abahmane (2010) reported that 4-amino-3,5,6-trichloropicolinic acid (picloram) and 3,6-dichloro-o-anisic acid (dicamba) could be used as an alternative auxin in *in vitro* culture of some species without somaclonal variation in regenerates. In peach palm picloram-enriched medium stimulated primary and secondary somatic embryos (Steinmacher *et al*., 2007). This auxin was successfully used to induce embryogenic competence in African oil palm (Teixeira *et al*., 1995) and was the favorable auxin source in arecanut palm somatic embryo formation (Karun *et al*., 2004) and macauba palm (Moura *et al*., 2008). In the case of date palm, Mazri *et al*., 2017 found that the addition of 45 µM picloram to culture medium improved somatic embryogenesis in cv. Najda. More recently, Hassan *et al*., 2021 induced somatic embryogenesis from immature inflorescence explants of Medjool cv. using picloram at 2mg/l. On the other hand, Othmani *et al.* (2009) mentioned that picloram at different concentrations failed to stimulate embryonic callus from juvenile leaves of date palm cv. Boufeggous. The somatic embryogenesis process included several phases, started with the induction of embryonic calli, maturation, germination of somatic embryos, and finally plantlet formation (Thuzar *et al*., 2011). However, maturation and germination phases constitute the main challenges in the regeneration process. Abscisic acid (ABA) has been widely added to culture media to stimulate maturation of somatic embryos (Rai *et al.*, 2011). In addition, liquid media (Fki *et al*., 2003) and MS medium strength (Mazari *et al*., 2019) were found to be effective in maturation process of date palm somatic embryos. In the present study, we evaluated the effects of osmotically active solutes; sorbitol, mannitol and PEG on the maturation and germination of date palm somatic embryos. Our results showed that PEG at 10 g/l was more suitable for enhancing somatic embryo number during maturation of embryonic calli, while mannitol at 15 g/l and PEG at 10g/l maximize fresh weight. In fact, PEG (non-plasmolyzing osmoticum) was found to accelerate maturation process by causing a medium statue like to desiccation (Zhang *et al*., 2007). Al-Khateeb *et al*., (2018) found that, PEG and mannitol worked as non-metabolic osmotic agents in callus growth stage and they improved callus fresh and dry weights of the date palm cultivar Khalas. Somatic embryo maturation was also noticed when liquid media combined with PEG or mannitol (Yaseen *et al*., 2013). Al Khayri and Al-Bahrany (2012) mentioned that ABA, PEG-8000, thiamine, biotin and silver nitrate affected significantly date palm somatic embryo growth and development. The non-permeating osmoticum (PEG) surpassed sorbitol and mannitol in the case of somatic embryo maturation in some conifer species (Attree *et al*., 1991). Conversion process of date palm somatic embryos into plantlets was widely studied by several researches (Hassan *et al*., 2012, Taha and Hassan, 2014, Ibrahim *et al*., 2011, Mazri *et al*., 2017

and Al Khayri &Al-Bahrany, 2012). In our investigation, inclusion of mannitol at 10 g/l to germination medium improved somatic embryo germination and shoots formation. Mazri *et al*., (2016) observed the beneficial impacts of mannitol on shoot bud proliferation of date palm cv. Mejhoul but with lower effect than sucrose. Khaun (2021) found that, salysilic acid and mannitol supplemented media promoted shoot bud multiplication and elongation of date palm cv. Owidi. Sugar alcohol mannitol, move easily within plant cells, and involved in several vital processes related to cell division, plant growth and development such as; respiration, energy release, and ATP production. Mannitol also, involved in carbohydrate metabolism (Taiz and Zeiger, 2006; Meena *et al*., 2015). Mannitol, sorbitol and PEG the osmotically active solutes serve as osmotic regulator during somatic embryogenesis (Neto and Otoni, 2003; Shoji *et al.,* 2006). Rooting of plants considered to be an important step in date palm micropropagation approaches and subsequent plant acclimatization. Our results showed that supplementing rooting medium with PBZ at different concentrations enhanced root number, root length, growth vigor and root thickness compared with control medium. On the same line, Abd El-Baky (2006) mentioned that, growth retardants improved the initiation of strong roots and increased the number of roots of date palm. Also, Sidky *et al*. (2009) reported that 4 mg/l of PBZ increased shoot growth vigor, accelerated root formation and stimulated hairy root formation of date palm. In addition, Ibrahim *et al.*, (2012) mentioned that the addition of ABA, ancymidol, cycocel or PBZ at different levels to rooting medium of date palm as a pre-acclimatization treatments enhanced survival percentage and growth vigor of plants in greenhouse. In general, growth retardants reduced cell division and elongation, raised chlorophyll and protein accumulation, increased nutrients absorption, increased osmotic conditions and subsequently reduced water consumption. Aforementioned impacts could enhance plant tolerance to environmental stresses (Grossmann, 1992). In this research, 45g/l sucrose improved growth vigor, root number, root length and root thickness compared with 30 and 60g/l. These results are associated with the results of Ibrahim *et al*., (1999) who reported that raising the concentration of sucrose from 30 g/l to 50 g/l in the rooting medium increased root and shoot growth, but the negative effect was noticed with 60 g/l sucrose. Abu El Soad and Jatoi (2014) enhanced adventitious roots formation by incorporating 40 g/l sucrose to full strength MS rooting media. Ibrahim *et al.,* (2012) and Hassan *et al.,* (2008) improved root system of date palm cultivars by using rooting medium containing 45 g/l sucrose.

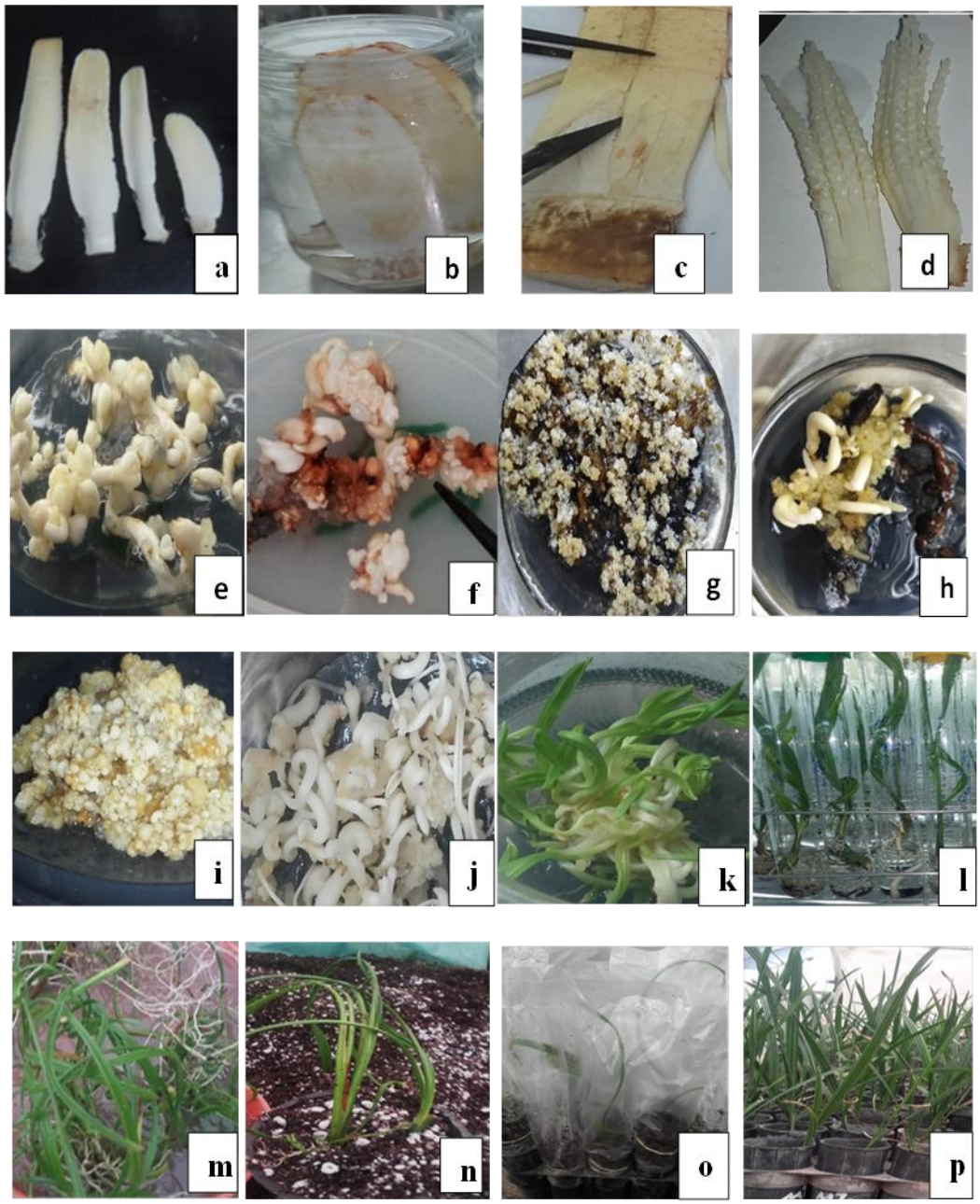


**Fig (1)**: Stages of date palm cv. Barhee immature inflorescence micropropagation. Spaths size **(a),** sterilization of spaths **(b)**, removal of outer protective sheath **(c)**, divided spiklets **(d),** swilling of spiklets on 2.5 mg/l picloram **(e)**, direct embryo formation on 5 mg/l picloram **(f)**, the highest callus degree and embryo differentiation on 5mg/l picloram **(g and h)**, the heaviest callus culture on mannitol during maturation stage **(i)** highest number of somatic embryo on 10g/l PEG during maturation stage(j), highest shoot formation on 15 g/l mannitol during germination stage **(k)**, vigor plantlets on rooting media containing PBZ **(l)** and procedures of plantlets acclimatization on greenhouse **(m-p)**.

## Conclusion

This study successfully demonstrated the in vitro propagation of date palm 'Barhee' using immature inflorescence as the explant source. The use of picloram at 5 mg/l proved optimal for inducing direct somatic embryos and embryonic callus formation. Maturation of embryonic callus was enhanced significantly by the addition of PEG at 10 g/l, which maximized somatic embryo production, while mannitol at 15 g/l effectively increased embryonic callus fresh weight. During the germination and multiplication phases, mannitol also showed superior efficacy in promoting shoot formation. Furthermore, the incorporation of paclobutrazol in the rooting medium facilitated robust root development. The resultant healthy, rooted plantlets exhibited high survival rates upon transfer to greenhouse conditions, indicating the effectiveness of this protocol for mass propagation of date palm. Overall, this research provides a reliable framework for the large-scale propagation of date palm through somatic embryogenesis, contributing to the advancement of date palm cultivation and preservation efforts.

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# دور البيكلورام و والبولي إيثيلين جليكول والسكريات الكحولية في تكوين الأجنة الجسدية من النورة المؤنثة غير الناضجة لصنف البرحي

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## الملخص العربي

تم حديثًا استخدام النورات الزهرية غير الناضجة كأحد الأجزاء النباتية المختلفة المستخدمة في إكثار نخيل البلح على نطاق واسع. وتُعد طريقة الإكثار عن طريق تكوين الأجنة الجسدية بكميات كبيرة خيارًا موثوقًا به للإكثار. وعلى الرغم من ذلك، فإن نضج وإنبات الأجنة وتكوين النبات من الأجنة الجسدية تمثل تحديات رئيسية لهذا الخيار. يصف هذا البحث بروتوكولًا للإكثار المعملي لصنف البرحي من خلال استخدام النورات الزهرية غير الناضجة. تم استخراج الشماريخ الزهرية من الأغاريض المؤنثة المعقمة واستخدامها كأجزاء نباتية، وتم زراعتها على بيئة موراشيج وسكوج (MS) مضافًا إليها البيكلورام بتركيزات (١.٢٥، ٢.٥، و٥ ملجم/لتر). تم تكوين الأجنة الجسدية المباشرة والكالس الجنيني خلال ٦ نقلات في جميع المعاملات بنسب مختلفة. تم نقل الكالس الجنيني إلى بيئة النضج المضاف إليها السوربيتول والمانيتول والبولي إيثلين جليكول (PEG) بتركيزات (٥، ١٠، و١٥ جم/لتر) لتسجيل تأثيرها على نمو الكالس الجنيني وتكوين الأجنة الجسدية. بعد ذلك، تم زراعة مجموعات الأجنة الجسدية التي تكونت سابقًا إلى بيئة الإنبات المضاف إليها التركيزات السابقة من المواد الأسموزية لتكوين أفرع جيدة. تمت إضافة باكلوبيوترازول والسكروز بمستويات مختلفة إلى بيئة التجذير لتحسين نمو الجذور. تم الحصول على أعلى النسب المئوية للأجنة الجسدية المباشرة والكالس الجنيني عند استخدام البيكلورام بتركيز ٥ ملجم/لتر في مرحلة البداية. كما أدت إضافة PEG إلى بيئة النضج عند تركيز ١٠ جم/لتر إلى زيادة أعداد الأجنة الجسدية إلى الحد الأقصى، بينما أدت استخدام المانيتول عند ١٥ جم/لتر إلى زيادة الوزن الطازج للكالس الجنيني. وفي مرحلة الإنبات والتضاعف، وُجد أن مادة المانيتول كانت الأكثر تأثيرًا، حيث أدت إلى زيادة تكوين الأفرع الخضرية. وُجد أيضًا أن إضافة البكلوبوترازول (PBZ) إلى بيئة التجذير له تأثير إيجابي على تكون الجذور. تم نقل النباتات القوية ذات الجذور إلى صوبة الأقلمة بنسب نجاح عالية.

**الكلمات الدالة:** الكالس الجنيني، نضج وإنبات الجنين، مانيتول، باكلوبوترازول وسوربيتول.