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THE EFFECT OF TREATMENT WITH CONCENTRATIONS OF POLLEN EXTRACT AND GIBBERELLIC ACID ON SOME ANATOMICAL CHARACTERISTICS OF THE DATE PALM FRUITS, BARHI CULTIVAR

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Abstract

The study was conducted during the 2024 growing season at the Garmat Ali district, Agricultural Research Station, College of Agriculture, University of Basrah, Iraq. Five 9-year-old female date palm trees cv. 'Barhi' was selected. These trees were pollinated with red Ghanami pollen on March 17, 2024, by hand pollination. The palm bunches were sprayed with two concentrations of pollen extract, 30 and 60 gL^{-1}, and four concentrations of gibberellic acid, 25, 50, 75, and 100 mgL^{-1}, in addition to the control treatment with two sprays, the first one month after fruit set and the second one month after the first spray. The effect of these treatments on some anatomical characteristics of the fruits during the Khalal stage was studied. The 75 mgL^{-1} gibberellic acid treatment recorded the largest thickness of the cuticle and epidermis layers compared to the other applications, reaching 12.92 and 17.27 µm, respectively. The 60 gL^{-1} pollen extract treatment recorded the largest diameter of parenchyma cells and the highest thickness of the outer mesocarp layer, reaching 49.55 and 692.45 µm, respectively. The 30 gL^{-1} pollen extract treatment recorded the largest diameter of tannin cells in the fruit pulp, reaching 64.12 µm. The control treatment recorded the highest diameter of stone cells in the fruit pulp, reaching 28.31 µm.

Keywords: Cuticle, Endocarp layer, Epidermis, Mesocarp layer, Stone cells, Tannin cells,

I. Introduction

The date palm (*Phoenix dactylifera* L.) belongs to the palm family Arecaceae (formerly Palmae), which is considered one of the most important plant families. According to the classification of the Swedish botanist Carolus Linnaeus, the date palm belongs to the order Arecales. Date palms are evergreen, woody, monocotyledonous trees with dioecious flowers (male flowers on one tree and female flowers on another). Pollination in date palms is cross-pollination (Al-Jabouri, 2002; Ibrahim, 2008). Date palms belong to the genus *Phoenix* and to the species *P. dactylifera*, which is distinguished from other genera by its ability to produce offshoots. Date palm trees are found in arid and semi-arid areas and are characterized by their tolerance to high temperatures and salinity (Kreuger, 2001). The Arabian Gulf region, particularly the southern region of Iraq, is considered the original home of the date palm, according to popular

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belief, as various cultivars of date palms originated from this area and later spread to India and North Africa (Wrigley, 1995).

There are numerous date palm cultivars in Iraq, which are classified into three categories based on their importance: commercial, local, and rare. Barhi is an important local Iraqi cultivar, characterized by its fruit having a distinct flavor that sets it apart from other cultivars, which is sweet and of high quality. This cultivar has spread to several countries through tissue culture propagation (Ismail, 2010; Al Nahyan, 2007). Cultivation of this cultivar has spread in many other countries using tissue culture propagation, transitioning from local cultivars to commercial cultivars. What distinguishes Barhi is that it can be eaten at all stages of ripeness (Khalal, Rutab, and Tamar) due to its sweet flavor. The dates of this cultivar are soft (Al-Aqeedi, 2010).

The pollen source used in the pollination process has a significant impact on the physical and chemical properties of the fruit and its ripening time (Ibrahim, 1996). The effects of pollen source on date palm fruit characteristics are known as metaxenia, and the phenomenon continues to attract the attention of researchers and growers specializing in date palm production worldwide (Khalifa *et al*., 1980). The male cultivar "Red Ghanami" is considered one of the most important males in Iraq, where its pollen is used to pollinate female cultivars, including the "Barhi" cultivar (Ibrahim and Khalaf, 2003). Researchers and growers are now interested in using plant extracts in agriculture as alternatives to plant growth regulators and growth promoters because they are natural substances that do not cause any harm to human health. Sayed *et al*. (2018) found that spraying with a pollen extract from the date palm cultivar "Zaghloul" at a concentration of 800 mg L^{-1} led to an improvement in yield and fruit quality.

Date palm fruit is a berry, a simple fruit with a single seed, developed from a single ovary. The date palm fruit consists of three layers. The first, outermost layer is known as the exocarp, a thin, leathery covering surrounded by the cuticle. The second, middle layer is called the mesocarp and comprises the majority of the edible fruit flesh. The third and final layer, called the endocarp, is a thin, membrane-like layer that directly surrounds the seed and separates it from the fruit flesh (Sakr *et al*., 2010). Date palm fruits reach their final size at the end of the Khalal stage, which can take 14–15 weeks after fruit set. The fruit seed begins to harden during this stage and its color changes from white to brown (Ibrahim, 1996). A study conducted on the effect of spraying with gibberellic acid on the anatomical structure of date palm fruits of Hillawi date palm cultivar at concentrations (0, 50, 100, and 150 mg L^{-1}) showed significant differences between treatments in some anatomical traits. The treatment fruits with a concentration of 150 mg. L^{-1} of gibberellic acid was significantly superior to the rest of the treatments in the layer thickness of the Exocarp, Outer Mesocarp, and Inner Mesocarp, and diameter of Tannin and Stone cells, recording 159.9 µm, 1.80 mm, 4.22 mm, 0.859 µm, and 0.123 µm, respectively (Al-Abrisam and Sweid, 2016).

The study was conducted to determine the effect of spraying with different concentrations of red Ghanami pollen extract and gibberellic acid on some anatomical characteristics of date palm fruits of the Barhi cultivar propagated via tissue culture technique.

II. Materials and Methods

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This study was conducted at the Agricultural Research Station and Laboratories of the College of Agriculture, University of Basrah, Iraq. Five Barhi date palm trees propagated by the tissue culture technique were selected. The male inflorescences of the red Ghanami date palm cultivar were selected. Several mature inflorescences of the red Ghanami male cultivar were selected. The inflorescences were taken, separated, and spread out for drying in a shaded room at 25°C. The pollen grains were then stored for pollination. The following treatments were prepared:

1. (C1 and C2) are control treatments (spraying with distilled water).

2. Spraying with pollen extract at a concentration of 30 gL^{-1}

3. Spraying with pollen extract at a concentration of 60 gL^{-1}

4. G1: Spraying with GA_3 at 25 mgL^{-1}

5. G2: Spraying with GA_3 at 50 mgL^{-1}

6. G3: Spraying with GA_3 at 75 mgL^{-1}

7. G4: Spraying with GA_3 at 100 mgL^{-1}

*The pollen extract was prepared by dissolving 30 or 60 g of red Ghanami pollen in a liter of warm distilled water with the addition of 2-3 drops of surfactant (Tween 20). The extract was stored in the dark.

*The concentration was prepared, 25 mgL^{-1} of gibberellic acid was prepared by weighing 25 mg of gibberellic acid using a sensitive balance and placing it in a one-liter flask. 0.1 N NaOH was added to dissolve the acid in 5 ml. After it was completely dissolved in the base, the volume was brought up to 1 liter. The remaining gibberellic acid concentrations were prepared in this way, taking into account the weight of gibberellic acid according to the desired concentration. The first treatment was carried out on the selected female cultivar Barhi bunches on May 20, one month after fruit set, in the early morning. Each treatment was sprayed until bunches were completely wet, separately. This process was repeated one month after the first treatment using the same method.

Fruit samples were collected, and anatomical sections of the fruits were prepared using the paraffin technique according to the method followed by Alnajjar *et al*. (2020), where molten and solid paraffin wax were used, and tissue sections were made according to the following steps:

Fixation

The first step in preparing tissue for histological tests is to preserve the tissue and its contents in the same or close to the state they were in the organism's body. Samples are fixed in F.A.A. (formalin, acetic acid, alcohol) for 24-84 hours in volumetric proportions (90 ml of 70% ethyl alcohol, 5 ml of glacial acetic acid, and 5 ml of formalin).

Washing

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To remove any remaining traces of the fixative from formalin-fixed samples, the sample must be thoroughly washed with running tap water and then with 70% ethyl alcohol for an hour to remove traces of the fixative solution.

Dehydration

This method replaces the water in the tissue with alcohol, and the sample is passed through a series of gradually increasing concentrations of ethyl alcohol.

Clearing

In this process, a dehydrating agent is substituted, allowing the paraffin wax to penetrate the tissue in the next step. This prevents the alcohol used for dehydration from mixing with the paraffin wax. Therefore, a clearing agent is used that is soluble in both alcohol and paraffin wax and also renders the tissue transparent. The samples were then placed in a mixture of absolute ethyl alcohol and xylene in ratios of 1:3, 1:1, and 3:1, and pure xylene for 30 minutes each. They were then transferred to a mixture of xylene and paraffin wax in an oven at 60°C for four hours.

Impregnation or infiltration

The process of completely replacing the immersed material with the filtered material. Paraffin wax is one of the most popular materials used for tissue impregnation, as it quickly penetrates the sample without damaging its tissue structure. It also provides strong support for microtome and helps preserve the sample under normal conditions for long periods without harm. The samples were then transferred to the paraffin wax and placed in an oven at 60°C overnight.

Embedding Process

The purpose of this process is to create a mold of the sample to surround and support the embedding material. The oven temperature is the most important factor in selecting the type of wax, as it is used to create sections and determine the quality of the sample under examination. To create the wax mold, molten wax is filled inside the mold, then the sample is transferred with tongs and positioned in the desired orientation. The mold is then left on an ice surface for a short period until its outer surface cools. Pure paraffin wax, melted in the oven at 60°C, is then poured into metal cubes. The samples are placed inside, marked, and left under running water to cool overnight, ready for cutting.

Trimming Process

After the wax molds were prepared, the sample was trimmed with a sharp blade until it was in a suitable cutting position. The edges were parallel and could fit against the edge of a micrometer knife.

Sectioning Process

When the sample was sectioned, it was placed on a Specimen holder in a microscope equipped with a very sharp blade. The desired section thickness (7-12 micrometers) was determined for the paraffin, and wax molds were cut. After obtaining ribbons or a series of sections, these ribbons were placed on a black plate to make it easier to identify their sections.

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Mounting

The process of placing the tissue section on a glass slide is as follows:

The tissue section is placed in a water bath at 40-45°C and left to float on the surface of the water for one to two minutes until it is completely flat. A drop of Myer's albumin (equal parts egg white and glycerol) is placed under the glass slide, and the section is secured to the center of the slide. This is accomplished by lifting the slide upwards in the direction of the section, preventing any air bubbles from forming. The slide is then left in a slide dryer at 45°C for approximately 24 hours until the water has evaporated, the samples have dried completely, and the slides have adhered to the slides.

Staining Process

Paraffin wax must be completely removed from tissue sections using xylene. This process was repeated three times to ensure complete wax removal from the sections. The glass slides were then placed in a Coplin jar filled with ethyl alcohol in descending concentrations (from absolute alcohol to 50% alcohol, as follows: 50, 70, 80, 90, 95, and 100%) for 15 minutes for each concentration, followed by distilled water. The slides were then transferred to a Coplin jar containing safranin stain (prepared by dissolving 1 g of the stain in 100 ml of 70% ethyl alcohol). The slides were then left in the stain for 30-60 minutes. The slides were then transferred to a Coplin tester containing 50% ethyl alcohol to remove excess staining. They were then dipped in Fast green (prepared by dissolving 1 g of the stain in 100 ml of absolute ethyl alcohol) for 15 seconds. The slides were then washed thoroughly with absolute alcohol and passed through xylene three consecutive times for five minutes each, leaving them to dry for five minutes. After the staining process was completed, the slides were prepared for permanent preservation. A waxy or plastic preservative, such as Canada balsam or D.P.X (Distrene Plasticizer Xylene), was used. A cover slide was then carefully placed at a 45-degree angle to prevent air bubbles from forming. This created a permanent slide. The slides were then left to dry in a slide dryer at 60°C for several hours, after which they were examined under a compound light microscope.

III. Results and Discussion

Data from Table 1 indicate the effect of pollen extract and gibberellic acid treatments on some anatomical traits of Barhi date palm fruits at the Khalal stage, 115 days after fruit set. The 60 g L^{-1} pollen extract treatment recorded the highest cuticle thickness, reaching 14.62 µm, compared to the other treatments (Figure 1, C). This trait is important for maintaining post-harvest fruit quality and extending their storage life (Lara *et al*., 2019; Fernandez-Munoz *et al*., 2022). The results from the same table also show that the cuticle thickness increased with the 75 mg L^{-1} gibberellic acid treatment, compared to the 25 and 50 mg L^{-1} gibberellic acid treatments, which reached 12.92 µm. This is due to the role of gibberellins in increasing the deposition of the cuticle layer in the fruits and increasing its thickness within optimum concentrations (Domínguez *et al*., 2011; Zhang *et al*., 2023). Meanwhile, the 100 mg L^{-1} gibberellic acid treatment recorded the lowest cuticle thickness, compared to the other gibberellic acid concentrations, reaching 9.52 µm. Excess phytohormones beyond the optimum concentrations have opposite physiological effects (Weyers and Paterson, 2001). The control treatment recorded the lowest cuticle thickness compared to all studied treatments, reaching 6.52 µm (Figure 1, B). This result is clear evidence of the role of the optimal concentration of pollen extract and gibberellic acid in increasing the thickness of the cuticle layer in fruits.

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Table 1: Effect of concentrations of pollen extract and gibberellic acid on some anatomical characteristics of date palm fruits cv. Barhi during the Khalal stage

Treatment	Cuticle Layer Thickness (µm)	Epidermis Thickness (µm)	Parenchyma Cells Diameter (µm)
30 gL^{-1} (P1)	12.07±0.02	15.57±0.48	35.95±0.74
60 gL^{-1} (P2)	14.62±0.31	14.72±0.33	49.55±0.49
25 mgL^{-1} (G1)	12.07±0.04	12.17±0.91	39.25±0.15
50 mgL^{-1} (G2)	12.07±0.02	15.57±0.45	47.85±0.65
75 mgL^{-1} (G3)	12.92±0.70	17.27±0.16	41.85±0.43
100 mgL^{-1} (G4)	9.52±0.31	13.02±0.01	41.85±0.66
Control (C)	6.52±0.34	10.47±0.32	29.05±0.03

The results in Table 1 showed that fruits treated with 75 mg L^{-1} gibberellic acid recorded the highest epidermal thickness, reaching 17.27 µm, compared to the other treatments (Figure 1, F). This is due to the role of gibberellins in increasing cell size and expansion, as a result of their direct role in increasing the flexibility of cell walls, which leads to an increase in the thickness of the epidermal layer (Falcioni *et al*., 2018; Ritonga *et al*., 2023). The results also indicate that the thickness of the epidermal layer increased with increasing gibberellic acid concentration. However, the thickness of the epidermal layer decreased at the highest concentration of gibberellic acid (100 mg L^{-1} gibberellic acid), recording 13.02 µm. Fruits treated with 30 and 60 g L^{-1} pollen extract also outperformed the control treatment in epidermal thickness, which reached 15.57 and 14.72 µm, respectively. The control treatment, however, recorded the thinnest epidermal layer, at 10.47 µm (Figure 1, B). The 60 g L^{-1} pollen extract treatment recorded the highest parenchyma cell diameter compared to the other treatments, which reached 49.55 µm (Figure 1, C). This is due to the pollen extract's rich content of hormones, vitamins, and nutrients, which improved fruit growth and physiological maturity (Al-Abbasi *et al*., 2023). It also increased the size of the parenchyma cells, which are responsible for storing food and, consequently, expanding their diameter. Parenchyma cells are the food storage cells and constitute the majority of the edible flesh of the fruit, an important indicator of fruit quality and increased economic value (Abbas *et al*., 2012).

The 50 mg L^{-1} gibberellic acid treatment resulted in parenchyma cells with a larger diameter than the control treatment, which reached 47.85 µm (Figure 1, E). This is because gibberellins affect the softness of the inner walls, leading to the expansion of the parenchyma cells and their widening diameters (Ritonga *et al*., 2023). Meanwhile, the control treatment recorded the lowest parenchyma cell diameter in the fruit pulp, reaching 29.05 µm (Figure 1, B). From Table 2, it is noted that the fruits treated with pollen extract at a concentration of 30 g L^{-1} recorded the highest tannin cell diameter in the fruit flesh compared to the other treatments studied, which reached 64.12 µm (Figure 1, A). This is due to the pollen grains' content of hormones, vitamins, and nutrients that contribute to increasing the diameter of the tannin cells in the date palm fruits (Shahsavar and Shahhosseini, 2021). Meanwhile, the fruits treated with 100 mg L^{-1} gibberellic acid recorded the lowest tannin cell diameter in the fruit flesh, reaching 40.32 µm (Figure 1, G). Tannins are cells found in the outer layer of the fruit's skin (exocarp layer), where astringent substances are stored. Upon ripening, these substances become insoluble and accumulate within these cells, dissolving the astringent taste and making the fruit palatable (Rustioni *et al*., 2014). When astringent tannins accumulate in large quantities, the cells will be large.

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Table 2: Effect of concentrations of pollen extract and gibberellic acid on some anatomical characteristics of date palm fruits cv. Barhi during the Khalal stage

Treatment	Tannin Cell Diameter (µm)	Stone Cell Diameter (µm)	Outer Mesocarp Thickness (µm)
30 gL^{-1} (P1)	64.12±0.06	19.81±0.41	344.11±0.09
60 gL^{-1} (P2)	56.42±0.13	23.21±0.01	692.45±0.41
25 mgL^{-1} (G1)	59.82±0.41	20.66±0.41	500.92±0.59
50 mgL^{-1} (G2)	41.22±0.11	18.11±0.09	534.48±0.33
75 mgL^{-1} (G3)	50.52±0.31	19.81±0.32	380.34±0.21
100 mgL^{-1} (G4)	40.32±0.15	23.21±0.11	382.67±0.45
Control (C)	55.62±0.31	28.31±0.12	604.29±0.21

The control treatment recorded the highest diameter of stone cells in the fruit flesh compared to the other treatments, reaching 28.31 µm (Figure 6). The 30 g L^{-1} pollen extract and 75 mg L^{-1} gibberellic acid treatments recorded the lowest diameter of stone cells in the fruit flesh, both at 19.81 µm (Table 17). Stone cells are hardened due to the accumulation of insoluble organic matter, forming hard, fossilized cells called stony cells (Lin et al., 2023; Peco *et al*., 2023). These cells are concentrated in the outer layer of the fruit (the pericarp).

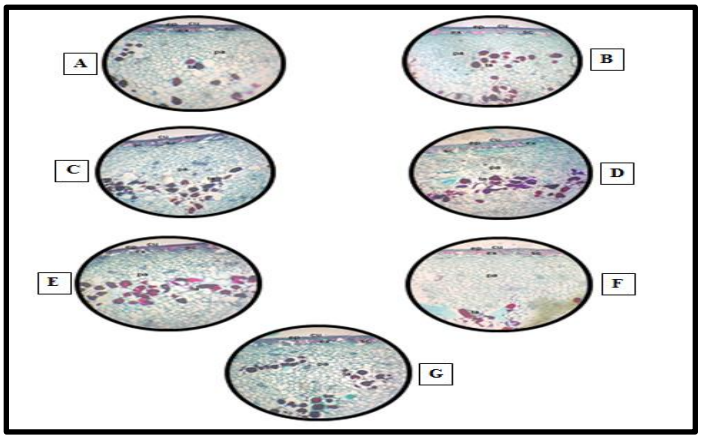

Figure 1: Effect of different concentrations of pollen extract and gibberellic acid on some anatomic characteristics. A: 30gL^{-1} pollen extract treatment; B; Control treatment; C: 60gL^{-1} pollen extract treatment; D: 25mgL^{-1} GA_3 treatment; E: 50mgL^{-1} GA_3 treatment; F: 75mgL^{-1} GA_3 treatment; G: 100mgL^{-1} GA_3 treatment.

The data from Table 2 also indicate that the thickness of the outer fleshy layer in the mesocarp (middle layer) of the fruit flesh varied according to the type of treatment studied. The fruits treated with 60 g L^{-1} pollen extract recorded the highest thickness value, reaching 692.45 µm (Figure 7). Meanwhile, the fruits treated with 30 g L^{-1} of pollen extract recorded the lowest outer mesocarp thickness, reaching 344.11 µm. An increase in the mesocarp layer, which

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represents part of the edible fleshy layer, is an important indicator of fruit quality and increased economic value. The 60 g L^{-1} pollen extract treatment had a superior outer mesocarp thickness, due to the pollen containing hormones, nutrients, vitamins, and active ingredients, which improved fruit growth, which was positively reflected in the fleshy layer of the fruit (Shahsavar and Shahhosseini, 2021).

IV. CONCLUSION

Spraying Barhi date palm fruits with a concentration of 75mgL^{-1} of gibberellic acid improved the thickness of the cuticle and epidermis layers. Spraying Barhi date palm fruits with a pollen extract at a concentration of 60gL^{-1} improved the diameter of the parenchyma cells and the thickness of the outer mesocarp layer.

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