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RESEARCH ARTICLE

ANATOMICAL AND HORMONAL STUDIES OF FLORAL AND FRUITING BEHAVIOR OF *PHOENIX DACTYLIFERA*, CV. BARHEE

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A B S T R A C T

This study was conducted on date palm trees,cv Barhee grown in Basrah region to investigate hormonal levels and anatomical characters of floral and fruiting behavior during the growing season of 2013. Results showed that auxins and gibberellins concentrations in flowers and fruits were higher at early stages of fruit development and then decreased, gradually as the fruit of both normal and abnormal behavior advance toward maturity stage. It was noticed that fruit of normal development had higher concentrations of both auxins and gibberellins than those of abnormal behavior with the exception of abnormal fruits having a slightly higher concentration of auxins at the period of 55 days after pollination.

Anatomical sections of internal structure of normal and abnormal flowers and fruits showed the development of flower in normal behavior, but in abnormal behavior the style is not connected firmly with the ovary causing its death and then failure of pollen grains formation. Also, the style and stigma are separated from the ovary resulting in failure of the process of fertilization, as well as clear disconnection from the ovary. In longitudinal and cross sections of the ovary, it was found that no zygote was detected in the ovary during the developmental stages of abnormal flower. Abnormal male flower had small anther and small pollen sac, with pollen grains being severely reduced in abnormal anther as compared with that of normal anther. Eventually, most of the parthenocarpic fruits remained on bunches until harvest time and have significant influence on the economic value of fruits in this genotype.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most important horticultural crops in Iraq. It is native to the Mediterranean region and originated in the Arabian Gulf and Southern part of Iraq (Al-Bakr, 1983; Wrigley, 1995). Date palm belongs to the order Palmae and the family Arecaceae. The most recent estimate is that it contains 190 genera and 2364 species (Govaerts & Dransfield, 2005). These are widely spread in tropical areas throughout the world, with the greatest density of species existed in America and Southeast Asia. Palms are usually abundant in tropical ecosystems, especially in lowland and mountain moist forests (Henderson, 2006). Dates are nutritious food products with high amounts of sugars and energy, fats, vitamins, proteins, minerals, organic and amino acids, representing an important source of food to local people (El-Sohaimy and Hafez, 2010). Dates can be also preserved, stored and transported to long distances. Shabana *et al.* (2006) reported that growth and development of both seeded and seedless date palm fruits are regulated by endogenous hormones, such as, auxins and gibberellins as well as environmental factors. Mater (1991) reported that pollen grains, un mature seeds and ovary tissues produce endogenous auxins and gibberellins which stimulate cell division and enlargement and induce ovary cells to create more auxins, thereby controlling cell size and number of the developing date fruits. In contrast, the absence of seeds reduces ovary growth due to lacks of endogenous hormones resulting in abnormal formation of small seedless dates with low quality characters, called "Shees". Abd-Alaal *et al.* (1982) found that spraying exogenous auxins and gibberellins on flowers of Khadrawi date cultivar promote natural development of parthonocarp dates.

From the botanical viewpoint, date palm is a dioecious plant with separate male and female trees. Both sexual flowers of date palm are formed in a special type of inflorescence called a spadix. Male date flower has 6 stamens bearing pollen grains in their anther, whereas staminate flower consists of 3 carpels surrounded by a short perianth. Pollination occurs by wind and by artificial transferring of pollen grains collected from male spikelets onto female inflorescence. The fertilized carpel grows and develops faster than the other two carpels, to become a mature fruit, whereas the remaining two carpels degenerate and drop shortly after pollination (Osman *el al.*, 1974). However, unpollinated flowers with all three carpels may develop into seedless, parthenocarpic, fruits of inferior quality (Carmichael *et al.*, 1980).The formation of abnormal multi-carpel flowers and fruits with 6 to 7 carpels were reported by Cohen *et al.* (2004) and Al-Khalifah *et al.*(2007). Cohen, *et al*. (2004) characterize a low fruit setting phenotype which was found widely among tissue culture-produced date palms of Barhee cultivar. Most flowers in such trees turn into parthenocarpic fruitlets having three carpels. Moreover, supernumerary carpels are formed in severe cases, and other flower abnormalities include distortions of carpels and stigmas can be detected also. Many flowers on the abnormal trees have impaired pollen tube elongation, with growth being limited to the stigma or to regions near its point of joining the carpel. As a result, pollen tubes either grow in different directions or stop growing completely. Therefore, the present study focuses on a new phenomenon of abnormal behavior of the developing fruits noticed in Barhee date palm cultivar after pollination, with attention given to the hormonal levels and anatomy of the flowers and fruits.

MATERIALS AND METHODS

Fifteen –years – old trees of Barhee date palm cultivar grown at a private orchard in Abi El- Khasseb District, Basrah Governorate, were used in this study.The orchard soil texture is clay with an organic matter of 2.16 % and a pH at 7.8. Selected date palm trees were subjected to the common cultural practices. Pollination was performed using pollen grains of Khikri date male local cultivar at the commencement of flowering in March, 2013. Hormonal analysis and anatomical sectioning were conducted during the current season growth on normal and abnormal flower and fruit samples. Samples of flowers and developing fruits were collected four times as mentioned in Table (1).

Table 1 Dates of blooming, pollination and fruit growth of Barhee date palm cultivar

Observation Dates	Stages of development
12 th March 2013	Appearance of flower clusters (inflorescence)
27 th March 2013*	Spadix opening
29 th March 2013	Pollination by pollen grains of Khikri male cultivar
14 th May 2013	Start of Kimri stage
23 th May 2013*	Early Kimri stage
27 th June 2013*	Middle Kimri stage
21 st July 2013*	Early Khalal stage

* Spikelet bearing flowers or fruits taken for hormonal analysis

The collected samples were immediately stored at -20 °C until hormonal analysis. Defrosted samples of 1 gram fresh weight were homogenized in 5 ml of 80 % methanol and left overnight at 5 °C. The extracts were then filtered through a Whatman No. 5 filter paper, the supernatants were re-homogenized with the same solution and the extracts were combined. The extracts were evaporated in a rotary evaporator at 40°C to yield a concentrated aqueous residue which was furthered partitioned as described in Goren *et al.* (1971). It was then adjusted to pH 2.5 with 1 N HCl, extracted with ethyl acetate (3 xv) and dried under vacuum at 40 °C. The residue was dissolved in 1 ml methanol and reduced to 100 µl under vacuum, and then line-loaded onto a Whatman No.1 chromatography paper with 30 cm length and 2.5 cm width. Standard IAA and GA_3 were also spot- loaded at both edges of the paper. The paper was allowed to develop for 20 cm in the vertical direction using isopropanol: ammonia: water (10: 1: 1, V / V / V) as the solvent solution. The positions of IAA and GA_3 were detected under UV light and marked. Corresponding spots to the RF values of the standards were scraped off, dissolved in 0.5 ml methanol and hormones were measured using UV–Visible Spectrophotometer (C CHT-USA) at a wave length of 280 nm for IAA (Saimoto *et al*., 1990) and at 205 nm for GA_3 (Mc Cmillan, 1983). The results were expressed as $\mu g.kgm^{-1}$ fresh weight. For anatomical sectioning, fresh materials of flowers and fruits were fixed at least 48 hours in formalin acetic acid alcohol solution (FAA) and preserved in 70% alcohol, then dehydrated in ethyl alcohol series, sectioned on a Rotary Microtome and stained in Safranin and Fast green, and then mounted in Canada balsam (Johansen, 1949). The sections were examined with Olympus CH4 light microscope and photographed with Digital camera type DCE-2. Anatomical terms used are cited from (Esau, 1965; Ditcher, 1974; Radford, 1974).

RESULTS AND DISCUSSION

Endogenous plant hormones

The IAA and GA_3 concentrations were different during flowering and fruiting stages of Barhee date palm cultivar (Table 2). IAA concentration was highest during the flowering stage recording a value of 388.55 $\mu g.kgm^{-1}$ fresh weight, and then decreased gradually to reach the lowest concentration at the fruiting stages of Kimri and Khalal in both normal and abnormal fruits.

Table 2 Endogenous auxins and gibberellins concentrations ($\mu g.kgm^{-1}$ fresh weight) of flowers and fruits of Barhee date palm cultivar.

Sampling time (Days)	Auxins as IAA		Gibberellins as GA_3	
	Normal	Abnormal	Normal	Abnormal
2 days before pollination	388.55*		230.15*	
55 days after pollination	250.40	262.14	211.66	200.11
90 days after pollination	235.11	120.25	197.42	176.36
114 days after pollination	110.45	53.11	162.41	102.44

*Values represent the mean of 3 readings.

Abnormal fruits had higher concentration of IAA (262.14 $\mu g.kgm^{-1}$ fresh weight) than that of normal fruits (250.40 $\mu g.kgm^{-1}$ fresh weight) at early Kimri stage. However, the reverse was true for both normal and abnormal fruits at early Khalal stage recording IAA concentrations of 110.45 $\mu g.kgm^{-1}$ fresh weight and 53.11 $\mu g.kgm^{-1}$ fresh weights respectively. These findings indicate that high levels of IAA during the flowering stage possibly have a positive effect on promoting flower setting into fruits. On the other hand, a higher level of IAA at early stage of Kimri in abnormal fruits may play a role in advancing these fruits to grow toward maturity. However, a lack of IAA source to feed the developing ovaries of abnormal fruits resulted in lower levels of IAA at early stage of Khalal. Al-Bakr (1972) reported similar results in date palm cultivars as a flower set into parthenocarpic fruit forming three sessile seedless fruits which grow slowly to become abnormal small fruits as compared to normal seeded fruits.

In contrast to abnormal fruits, lower levels of IAA at early kimri stage of normal fruits may indicate a high demand for the auxins to stimulate and accelerate the process of active cell division in the growing ovary. However, the increasing percentage of IAA level (51.91%) in normal fruits at early Khalal stage may due to the un mature seed as a source of creating auxins and to the accumulation of auxins in the enlarged cells of the fruit ovary tissues as compared to that of abnormal fruits (Mater, 1991; Shabana *et al*., 2006). GA_3 had the highest concentration during the flowering stage with a value of 230.15 µg.kgm^{-1} fresh weight, but its level was decreased constantly to reach the lowest values at the stages of Kimri and Khalal in both normal and abnormal fruits (Table 2). GA_3 concentration of normal fruits was slightly higher than that of abnormal fruits during early Kimri stage, but the difference in GA_3 concentration of both kinds of fruits at early Khalal stage was high with normal fruits recording an increasing percentage at 36.93% over abnormal fruits. These findings indicate that GA_3 levels during the flowering stage may play a role in flower setting into normal and abnormal fruits. The high level of GA_3 in normal fruits, as compared with that of abnormal fruits, during the developmental stage of Kimri and Khalal may stimulate ovary cells to grow, enlarge and induce auxin production which contribute to normal formation of fruits (Shabana *et al*., 2006), on the other hand, the low level of GA_3 presented in abnormal fruits may be involved in the formation of fruits to become mature " Shees ". Abuo – Aziz *et al*.(1982)found that foliar spray of GA_3 at concentrations of (50 and 100) mg.L^{-1} on inflorescence of Sewy date palm cultivar shortly after opening resulted in the formation of parthenocarpic seedless fruits. Similar result was also achieved by Hugairy (1981) who found that spraying Zahdi cultivar inflorescences with GA_3 at concentrations of (100 and 200) mg.L^{-1} produced seedless fruits.

Floral description of Barhee date cultivar

Inflorescence

The inflorescence is branched to one order, with a strong odor. The staminate flowers closed with the spadix bract. The peduncle is pale orange, and cylindrical in shape. The rachis and rachilla are cream-colored (Fig 1A). It can be seen from that flowers before blooming consist of a long anther and a pistal surrounded by sepals and petals.

Staminate flower

The staminate flowers are elliptical, irregular, a symmetrical in shape, and sessile. The sepals are connate at the base, with three triangular apical lobes, acuminate apex, and glabrous. Petals have three free valvar, lanceolate and a symmetrical.There are six free stamens, with wider filaments at the base. The anthers have a symmetric tip and are dorsifixed, with longitudinal dehiscence, and introrse (Figure 1 B,C, D).

Pistillate flower

The gynoecium has three distinct regions: An apical region, with the stigma, middle region with a short style and a basal region of the ovary. The pistillate flowers are trimerous, a symmetrical pyramidal and cream in color. The sepals and petals are free, pyramidal with petals being longer than sepals. The stigma is tripartite, regular and apical. The ovary is superior, syncarpic, tricarpellary and trilocular, with one ovule to each locule and basal lateral placentation (Figure 1 E, F, G). The pistal consists of circular (ovoid) ovary with three carpels. The style base is attached to the ovary terminal and its end by papilate stigma.

Anatomy of fruit development

Most of the flowers in the examined trees turned into parthenocarpic fruitlets with three carpels (Figure 2). Female flowers of date palm have three carpels surrounded by a short perianth. After fertilization, one of the carpels grows and develops to reach final growth phase. Carpels may continue to grow and develop into seedless fruit even if they are not pollinated. These fruits either drop or remain on bunches until maturity stage. This was due to un successful fertilization of the carpels in which they grow to form fruits. Such fruits are called parthenocarpic or poly carpels fruits (Figure 2 B,C). However, under normal conditions, only one carpel develops into fruit, no clear answer is provided to explain the development of a single carpel at the expense of the two adjacent carpels. Investigators confirmed that two carpels degenerate after pollination. Osman *et al*. (1974) studied the development of date palm fruit at very early stages of development, and found that the fertilized carpel grows and develops faster than the other two carpels, and the carpel started to enlarge in size at the end of the third week of pollination. On the other hand, parthenocarpic fruits were observed on date palm trees propagated by tissue culture almost in all experimental trees (Damankeshan and Panahi,2013).

Anatomical sections of internal structure of normal and abnormal flowers and fruits are shown in Figures (3, 4, 5). Figure (3A) showed the development of flower in normal behavior, the pistil is surrounded by the perianth, and it consists of the ovary, style and stigma, but in abnormal behavior the style is not connected firmly with the ovary (Figure 3 B,C,D) which either caused stigma dryness and then its death resulting in failure of pollen grains growth, or the style and stigma are separated from ovary causing failure of the process of fertilization, and clear disconnection from the ovary (Fig 3D).

As shown in longitudinal and cross sections of Khikri male flower (Figure 4), abnormal growth was observed in the anther (Figure 4 B, C, E). In addition, abnormal flower had small anther and small pollen sac, with pollen grains being severely reduced in abnormal anther as compared with that of normal anther (Figure 4 A, D). Longitudinal section of flowers taken 2 days before pollination (Figure 5) showed flowers with and without zygotes in their ovaries, indicating that abnormal flowers failed to form zygote (Figure 5 A), whereas early development of the zygote was noticed as meristematic cells in the ovary of normal flowers (Figure 5 B). Longitudinal and cross section taken shortly after fertilization (Figure 5 C and D) showed an early development of zygote to become an embryo, there by forming a normal fruit with clear layers of exocarp, mesocarp and endocarp (Figure 5 D), whereas inability of forming zygote was seen in abnormal fruit featuring increases in exocarp and decreases in endocarp layers (Figures 5 C).

Longitudinal and cross sections of normal fruits, 46 days (six weeks) following pollination (Figure 6, A,B), two carpels appeared to form zygotes, grow and develop but the third carpel failed to form a zygote (Figure 6 C,D). Cross sections of abnormal fruits (Figure 6 E and F) revealed the development of one carpel only and a reduction in size of the adjacent two carpels. It can be seen also that the septa located between carpels are formed by the fusion of their margins. However, this fusion is incomplete, forming cavities, one per septum, arranged radially from the base of the ovary to the base of the stigma. Therefore, it can be concluded that the abnormal behavior of the developing fruits may be due to: the absence of the zygote; the damage occurring in the embryo sac and endocarp layer; the reduction in embryo tissue which become the main reason of embryo growth failure.

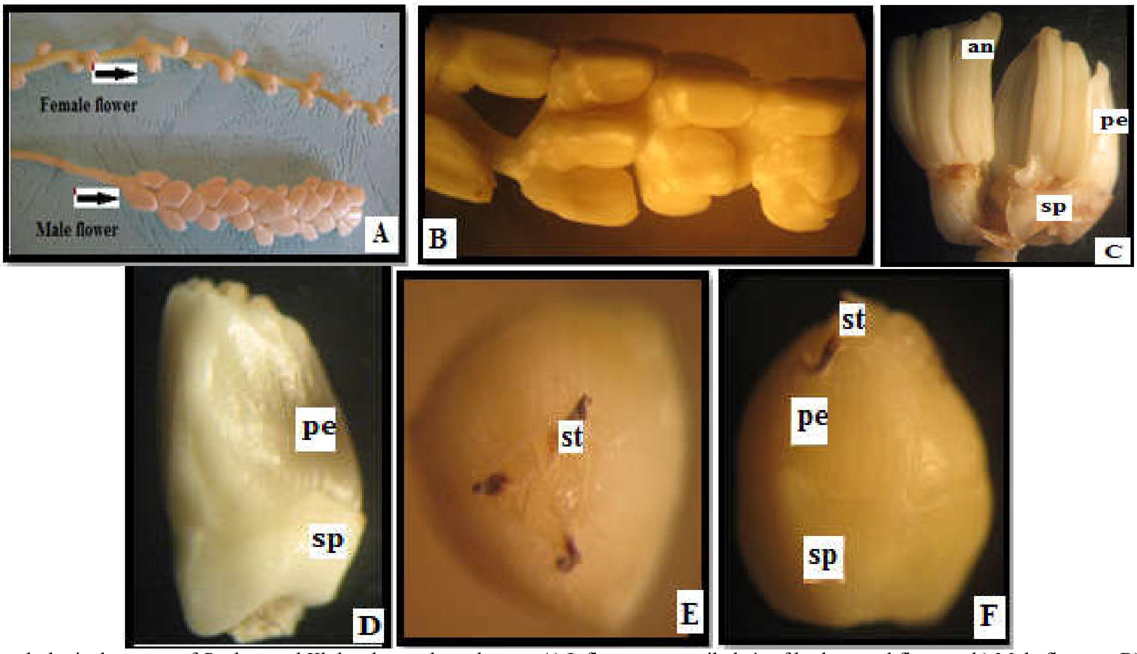

Figure 1 Morphological aspects of *Berhee and Khikri date palm cultivars*. A) Inflorescence spikelet's of both sexual flowers, b) Male flowers; B) Staminate flowers filament (arrow) and Arrangement of the anthers, (arrow). C) Open staminate flowers: D) Anther with sepals and petals: E) Pistillate flower with three stigma: F) Gynoecium, Pistillate flower with sepal and petals ;(pe,: petal, sp: sepal, st: stigma).

Figure 2 Normal and abnormal developing fruit of Barhee date palm cultivar. **A**) Normal development; **B**-C. Abnormal development) Young fruit with three carpels)

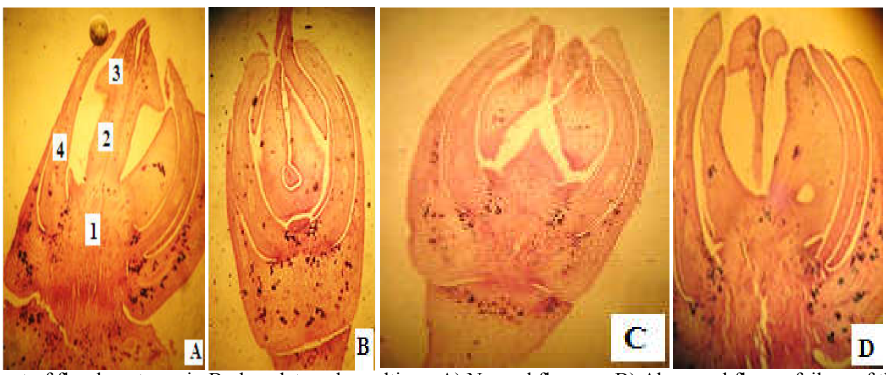

Figure 3 A, B- Development of floral anatomy in Barhee date palm cultivar; A) Normal flower , B) Abnormal flower failure of the process of fertilization , C) The style and stigma are separated from ovary D) The style is not connected firmly with the ovary, causing failure of the process of fertilization. (1- Ovary 2- Style 3- Stigma 4- Perianth).

The developmental stages of date palm carpel to become mature fruit, on the expense of the other two carpels, have been studied in details by Long (1943), Osman *et al.* (1974) and EL-Jarrah, & AL-Ani (1981). The pollinated and fertilized carpel grow and enlarge in size shortly after pollination. Al-Bakr (1972) reported that date palm embryo starts to develop one week following pollination, occupying one third of the ovule cavity and reaching its full length six to seven months later as the fruit matures. The development of a single carpel towards the stage of fruit maturity, may be a result of: un successful pollination and/ or fertilization of the remaining two carpels; domination of the fertilized carpel to become a fruit over the remaining two carpels due to a competion on space and nutrition; embryo abortion of the other two carpels shortly after fertilization.

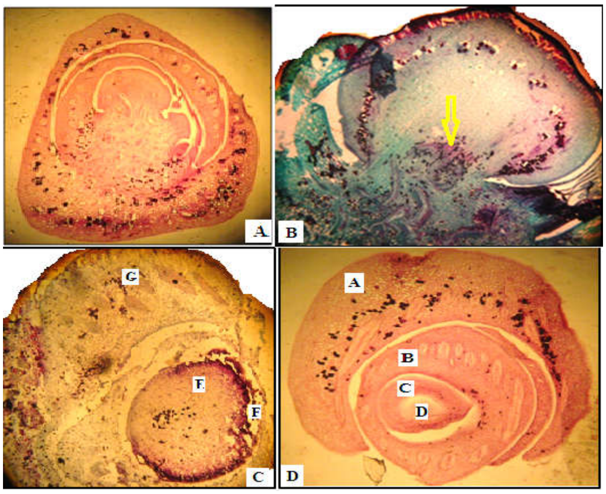

Figure 5 Sections in flower and fruit Barhee cultivar, A) Longitudinal section of abnormal flower; B) Longitudinal section of normal flower; C) Cross section of abnormal fruit; D) Cross section of normal fruit. A-exocarp B- mesocarp containing vascular bundle C- endocarp D- embryo E- Ovary F- micropyl G- Vascular bundle

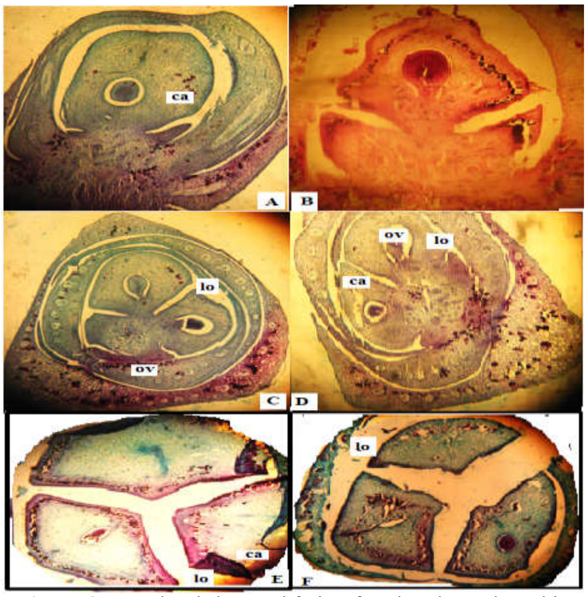

Figure 6 Normal and abnormal fruits of Barhee date palm cultivar. A,B) Longitudinal section in normal fruits, forming zygote; C,D) Cross section in normal flower, development of carpels; E,F) Cross section in abnormal flower, abnormalities in fruit development. (ca- carpel , ov= ovary , lo- locule)

Omar and Arif (1985) showed that the failure of the other two carpels to grow with time could be due to the presence of the perianth around the carpels which affected their progress of growth. Observations on abnormal behavior of tissue culture

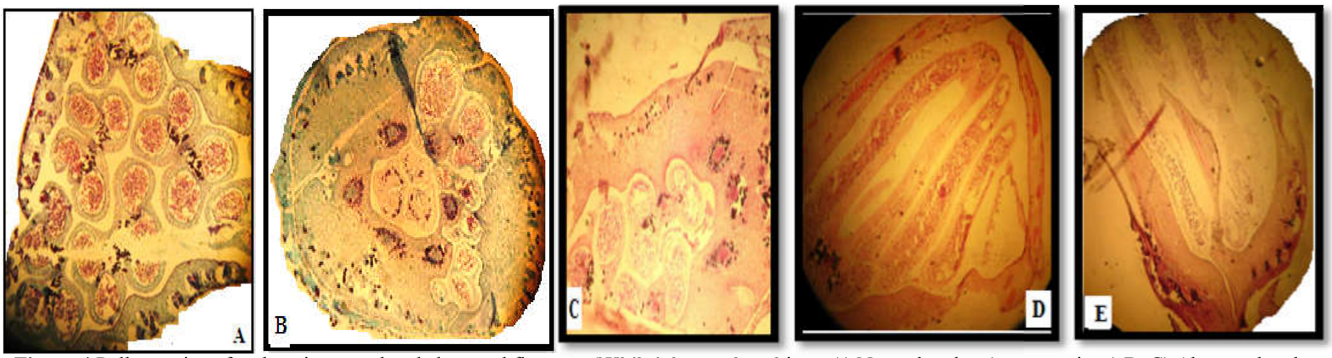

Figure 4 Pollen grains of anthers in normal and abnormal flowers of Khikri date male cultivar. A) Normal anther (cross sections) B, C) Abnormal anther, reduced of pollen grains (cross sections) ; D, E) Longitudinal section D) Normal anther E) Abnormal anther.

derived date palm have been reported by McCubbin *et al.* (2000). Such abnormality include slow growth rate with deformed leaves and wide leaflets, variegated leaves, non-flowering and low fruit setting. Similar findings were also found in tissue culture derived populations of date palms, such as, abnormal multi-carpel flowers and fruits with six to seven carpels (Djerbi, 2000; ;Al-Wasel, 2000 and 2001; Cohen *et al.*, 2004; Al-Kaabi *et al.*, 2007; Al-Khalifah *et al.*, 2007).

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