Examination Of Changes In The Anatomical Characteristics Of The Fruits Of Two Cultivars Of Date Palm Fruits (*Pheonix Dactylifera* L.) When Inoculated With Pollen Of Different Males

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Abstract

This study was conducted at one of the private orchards in Al-Rumaitha area of Al-Muthanna Governorate during the two growing seasons 2019. Ten males were selected from date palms of seed origin whose pollen was used in pollination of female pollen of the two date palm cultivars Zuhdi (V1) and Sayer (V2), by three palm trees of the same age. The results of the study showed the superiority of the female Al-Sayer cultivar on the thickness of the cuticle layer and the thickness of the epidermal layer 50.17 and 72.83 µm. The M10 male excelled in the two mentioned characteristics 49.37 and 82.17 micrometers. Also, the cultivar Zuhdi was significantly superior on the thickness of the stone cells, the thickness of the outer mesocosm and the thickness of the subepidermal layer 415.99, 715.60 and 238.19 µm, it was also noted that the thickness of the tannin layer of the female Al-Sayer variety is 196.37 µm. The second interaction recorded a clear significant effect for all the studied traits.

Keywords: anatomical changes, Zuhdi, Sayer, date palm fruits (*Pheonix dactylifera* L.), pollen.

Introduction

The date palm, *Phoenix dactylifera* L. belongs to the palm family (Arecaceae), which includes 200 genus, the most important of them in economic terms and their relationship to human life is the sex Phoenix

To which the date palm belongs and the fruits of the date palm have nutritional, medicinal, preventive and curative value, it was one of the richest fruits in its sugar content, which was easily digested and absorbed by the body (Ibrahim, 2008).

The date palm has an important place in the oases and desert regions, its distinguished morphology has enabled it to adapt in these areas. Botanically, the fruit of the date palm is a simple, single-seeded Berry grape, consists of the wall of the fruit, the

pericap, and it is sometimes called the fruit cover, which is the edible part of the fruit, as well as the seed or kernel (Al-Shurafa, 2014).

Pollination is directly related to the production process through its effect on the rate of fruit set and drop, pollen has a definite effect on the fruits of different date palm cultivars, the first to study this effect was the researcher Swingl in 1926, who mentioned that pollen has a clear effect on the characteristics of fruits and seeds, the effect of the pollen variety on the physical and chemical characteristics of the fruits, the percentage of their setting and the date of maturity, it was called Metaxenia to distinguish it from Xenia, it was the effect that is related to the seed and uses the characteristics of the fruit represented by the nature of the fruit, its shape and the type of its coverings or walls and seeds, as important taxonomic traits in isolating different taxon orders. (Fernando *et al.*, 2013).

The study of the anatomical characteristics of fruits and leaves of fruit trees is important from a taxonomic point of view, the reason for the changes in it is attributed to the nature of the genetic characteristics of the variety and plant type, when studying eight date palm cultivars, it was found (Sakr *et al.* 2010). There are significant differences in the studied anatomical samples of fruits between these varieties, which included the thickness of the epidermal layer, the cuticle and the layer of rocky cells

As well as the thickness of the outer shell (Exocarp), the thickness of the endocarp, the area of tannin cells between them, and the number and density of tannin layers in the fruit (Al-Rubaie, 1998).

Khalaf (2017) noted that when studying the anatomical changes of the fruit during different stages of growth and differentiation, the histological disclosure begins after the pollination process until the end of the interstitial stage, as the tannin cells are in small numbers in the first days of the growth of the fruit and after a while it becomes more clear, it represents specific sites in the fruit, which is a layer under the epidermis, and it also forms a ring that separates the outer mesosphere from the inner.

The anatomical studies conducted on the date palm fruits, it was found that the thickness of the layers and the number of rows of cells or their dimensions, it was affected positively or negatively by several factors, the most important of which is the female type, it was one of the most important factors affecting the anatomical structure of fruits (Sakr et al. 2010). The source or class of pollen This effect is known as Metaxenia as well as the amount of pollen/particulate. Omer (2011) mentioned the effect of pollen grains on the anatomical characteristics of Zaghloul cultivar, the amount of pollen plays a role in influencing the quality of the fruits through some changes in the thickness of the cells associated with the anatomy of the fruit.

The study aims to know the effect of the pollen source on some anatomical characteristics of the fruits and their quality related to the fruits of two date palm cultivars, Zuhdi (V1) and Sayer (V2).

Materials and Methods:

This study was conducted in one of the private orchards in the Al-Rumaitha area of Al-Muthanna Governorate during the two growing seasons 2020 AD, ten date-palm

males of seed origin were selected whose pollen was used in pollinating female pollen of the two date palm cultivars Zuhdi (V1) and Saer (V2). Three palm trees per variety of the same age (15-18) years, growth strength, size, and tree service operations
Nine shoots were left on each palm tree, with three seedlings per seedling, pollinated when they opened. Fruit samples were taken at the beginning of the khalal stage when the color began to change to the color of the distinctive variety.

Anatomical characteristics of fruits.

The method mentioned in Khafaji (2001) was followed in preparing the anatomical sections and taken from (Johouson, 1968).

The Paraffin Technique:

In it, molten and solid paraffin wax is used. The following are the steps used to make textile sections, and they are summarized as follows:

1. Obtaining the specimen.

The fruit samples were collected in the khalal stage at any color change.

2. Installation Fixation

The samples were fixed for (24-48) hours in F.A.A (Formalin, Acetic acid, Alcohol) fixative in volume proportions (90 ml of 70% ethyl alcohol, 5 ml of glacial acetic acid and 5 ml of formalin).

3. Washing process

The samples were washed with running tap water, then with 70% ethyl alcohol, twice, one for an hour and the other for 18 hours, in order to get rid of the effects of the fixative solution.

4. Dehydration process

The cut sections were passed with a mounting series of (95-90-80-70)% ethyl alcohol for an hour in each and then placed in absolute ethyl alcohol for an entire night.

5. Clearing process

The samples were placed in a mixture of absolute ethyl alcohol and xylene in proportions 1:3, 1:1, 3:1 and pure xylene for 30 minutes each, then transferred to a mixture (volume to volume) of xylene and paraffin wax in an oven at 60°C. for 4 hours.

6. The process of impregnation or impregnation

The models were transferred to paraffin wax and placed in an oven at 60°C for a whole night.

7. Embedding process

Pour pure paraffin wax that has been melted in the oven at a temperature of 60°C into metal cubes and put the samples in them, marked and left to cool under running water for a whole night until they are ready for cutting.

8. Trimming process

After preparing the wax molds, they were trimmed with a sharp blade until the sample was in a suitable position for cutting so that its edges become parallel and can fit on the edge of the microtome knife.

9. Sample cutting

The sample was fixed on the specimen holder in the microtom equipped with a very sharp blade. The thickness of the desired sector was determined (7-12) microns for paraffin. Wax molds were cut and after obtaining Ribbons tapes or a series of sectors, these tapes were placed on a black plate so that the sectors could be easily distinguished.

10. Mounting sectors

The sector was placed in a water bath at a temperature of (40-45) degrees Celsius and left the sector to float on the surface of the water for 1-2 minutes until it was completely isolated, passed the glass slide after placing a drop of Myer's albumin (two equal volumes of egg albumin and glycerin with sodium chain) under this strip and snapped so that it adhered to the center of the slide, by lifting the slide towards the sector upwards, not allowing any air bubbles to form, the slide was left to dry on a slide dryer (45) °C for about 24 hours, until the water evaporated completely and the samples adhered to the slides.

11. Staining

The glass slides were placed in a Koblin lab containing ethyl alcohol with decreasing concentrations from absolute alcohol to 50% alcohol as follows: (50, 70, 80, 90, 95, 100 %) for 15 minutes for each concentration. Then the slides were transferred to a Koblin lab containing safranin dye (prepared by dissolving 1 g of dye in 100 ml of 70% ethyl alcohol). The slides were left in the dye for 60-30 minutes. In order to remove the excess dye, the slides were transferred to a Koplin lab containing 50% ethyl alcohol. Then it was placed in Fast green dye (prepared by dissolving one gram of dye in 100 ml of absolute ethyl alcohol) for 15 seconds, then washed thoroughly with absolute alcohol, then passed the xylene three times in a row for 5 minutes each time, then left for 5 minutes to dry.

After the dyeing process was completed, the process of preparing the microscopic slide for permanent preservation began, where used a waxy substance or a plastic

preservative such as Canada Balsam or D.P.X , then the cover slip was placed at an acute angle of 45 degrees and with great care so as not to form air bubbles, and thus what is known as a permanent slide was made, then it was left to dry on the slide dryer at 60°C for several hours and then examined under a microscope.

Statistical analysis

The results were analyzed using analysis of variance for all studied anatomical traits using the SPSS statistical program to ensure that there are significant differences between the studied traits. The averages were also analyzed and significance was tested according to the least significant difference test, RLSD rate and under the probability level of 0.05 (Basheer, 2003).

Results and discussion:

Table (1) shows that the M10 male was significantly superior to the rest of the males included in the study, and it was recorded at 49.37 µm in the thickness of the cuticle layer. The M2 male recorded the lowest values of 27.42 µm with a clear significant difference. The female cultivar Al-Sayer (V2) also recorded the highest values for the studied trait, which amounted to 50.17 µm, with a significant difference from the Zuhdi cultivar (V1), which gave the lowest value of 28.58 µm. It was also noted that there is a difference in the thickness of the cuticle layer to overlap between the males and the female species. The M9 and M10 males with Al-Sayer cultivar recorded the highest readings with a significant difference compared to the rest of the treatments and the values were 60.34 and 60.33 µm, respectively, the lowest values in the treatment were for M2 and M3 males with the ascetic variety and were 19.17 and 18.87 µm, respectively.

It was also noted from the same table that the M10 male was significantly superior in skin thickness characteristic of 82.17 µm, and the lowest values were for the M1 male, which amounted to 58.17 µm. The female cultivar Al-Sayer recorded the highest value significantly compared to the ascetic cultivar for the same trait 72.83 µm, the female cultivars Sayer and Zuhdi were significantly superior to the M10 male compared to the other treatments with respect to the two-way interaction, as it scored (82.00 and 82.33 µm), respectively.

Table (1) Effect of pollen source on cuticle and epidermal thickness (µm) for two date palm cultivars, the female Sayer and Zuhdi.

Male	Cuticle layer thickness		Mean	Epidermal thickness		Mean
	Zuhdi	Sayer		Zuhdi	Sayer	
M1	20.50	37.67	29.08	59.68	56.65	58.17
M2	19.17	35.66	27.42	57.67	74.00	65.83
M3	18.87	34.87	26.87	54.00	72.69	63.34
M4	26.90	47.23	37.07	71.00	70.33	70.67
M5	29.45	55.65	42.55	73.35	73.31	73.33
M6	29.89	55.66	42.78	74.34	74.66	74.50
M7	33.40	56.67	45.03	75.35	67.31	75.83

M8	34.62	57.65	46.63	77.34	76.35	76.84
M9	34.66	60.34	47.50	81.00	81.00	81.00
M10	38.40	60.33	49.37	82.33	82.00	82.17
Mean	28.58	50.17		70.60	72.83	
$L.S.D_{0.05}$	Male	Female	Interaction	Male	Female	Interaction
	2.268	0.926	3.207	1.511	0.615	2.137

Table (2) shows the significant superiority of the Al-Sayer variety compared to the ascetic variety in the thickness of the tannin layer 199.50 µm, the male date palm M8 significantly outperformed the other males, and the M1 male recorded the lowest values in this trait at 155.50 µm, the interaction had a significant significant effect by registering Sayer cultivar with M8 cultivar with the highest values of 238.70 µm compared to the rest of the study coefficients.

From the same table, we notice the superiority of the female cultivar Pink in the characteristic of subcutaneous layer thickness of 238.19 µm, and the M10 male significantly outperformed the rest of the studied males by recording the highest values of 280.83 µm, the table also shows that the interaction between the male and the female cultivars was significant, as the female cultivar Zuhdi and the M10 male were significantly superior compared to the rest of the study coefficients, recording 8958.3 µm.

Table (2) Effect of pollen source on the thickness of the tannin and sub-epidermal layer (µm) for two date palm cultivars, the female Sayer and Zuhdi.

Male	Tannin thickness		Mean	Sub-epidermal thickness		Mean
	Zuhdi	Sayer		Zuhdi	Sayer	
M1	160.34	150.66	155.50	204.33	195.00	199.67
M2	170.65	160.69	165.67	196.00	184.00	190.00
M3	179.35	164.65	172.00	184.33	182.33	183.33
M4	181.32	217.34	199.33	237.67	234.33	236.00
M5	138.00	205.00	171.50	246.00	237.67	241.83
M6	161.65	189.64	175.64	245.00	247.00	246.00
M7	151.00	210.00	180.50	247.33	249.00	248.17
M8	160.30	238.70	199.50	265.00	258.00	261.67
M9	158.33	222.67	190.50	261.33	262.67	262.00
M10	139.34	204.36	171.85	295.00	266.67	280.83
Mean	160.02	196.37		238.19	231.66	
$L.S.D_{0.05}$	Male	Female	Interaction	Male	Female	Interaction
	3.612	1.475	5.108	5.916	2.415	8.366

Table 3 shows a clear significant superiority of the M10 male in the characteristic of stone cell thickness, recorded 478.67 µm, while the male M2 recorded 312.67 µm, which was the lowest reading among the study parameters. The female cultivar Zuhdi was significantly superior in the same trait, which was 415.99 µm, while Sayer cultivar recorded the lowest value of 412.17 µm. The table also shows the difference of study coefficients with respect to the interaction between male and female palms. The variety Zuhdi with the M10 male recorded the highest values with a significant

difference, as its value was 415.03 µm, and the lowest value with a significant difference was when the treatment was the brand Sayer and the M2 male, and its value was 312.67 µm. From the results of the same table, we notice that the M10 male was significantly superior to the rest of the males by recording the highest values of 868.34 µm, and the lowest values were for the M3 male, which recorded 473.17 µm for the outer middle shell trait. The cultivar Zuhdi was significantly superior to this trait by 715.60 µm, and the female cultivar Sayer recorded the lowest value compared to the cultivar Zuhdi 693.96 µm. From the results of the analysis of variance, we find that the two-way interaction has a significant significant effect on the thickness of the outer middle shell, as the Al-Sayer variety with the M10 male scored the highest values by 868.68 µm, which did not differ significantly with the treatment of the variety Zuhdi and the M10 male, which was 686.00 µm, as the Al-Sayer variety with the M3 male recorded The lowest values are 419.00 µm.

Table (3) Effect of pollen source on the thickness of the stone cells and the outer mesosphere (µm) for two cultivars of date palms, the female Sayer and Zuhdi.

Male	Stone cells thickness		Mean	outer mesosphere thickness		Mean
	Zuhdi	Sayer		Zuhdi	Sayer	
M1	337.31	338.34	337.82	595.67	505.00	550.33
M2	332.00	312.67	322.33	572.00	465.00	518.50
M3	314.00	316.33	315.17	527.33	419.00	473.17
M4	422.67	425.00	423.83	697.35	685.65	691.50
M5	432.00	439.00	435.50	657.36	740.64	699.00
M6	444.33	442.05	443.19	775.00	767.67	771.33
M7	449.00	449.00	449.00	749.64	778.36	764.00
M8	467.00	458.31	462.65	851.65	851.00	851.32
M9	478.65	462.35	470.50	862.00	858.67	860.33
M10	483.03	478.67	480.85	868.00	868.68	868.34
Mean	415.99	412.17		715.60	693.96	
$L.S.D_{0.05}$	Male	Female	Interaction	Male	Female	Interaction
	7.654	3.125	10.827	4.044	1.651	5.720

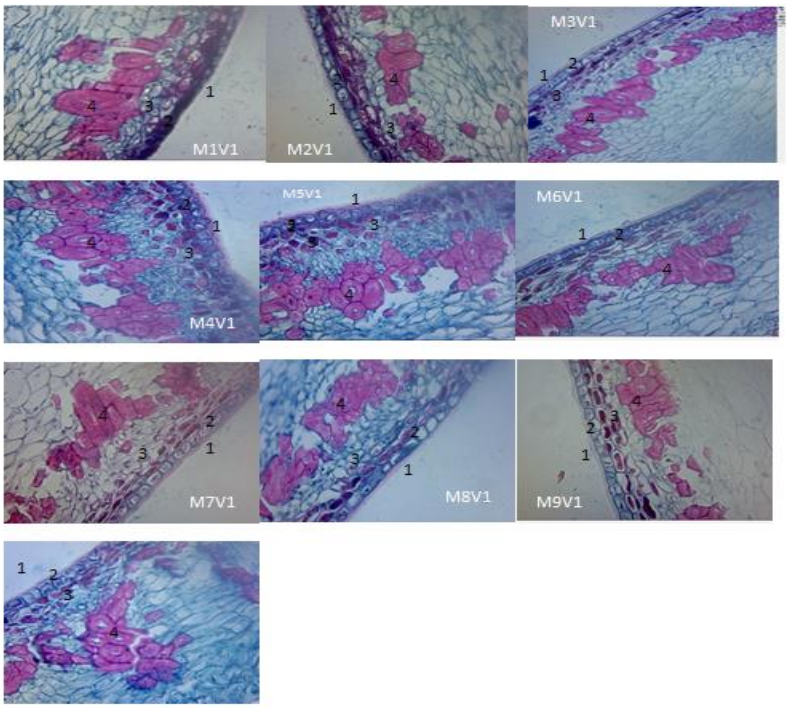

Figure 1. Shows 1- Cuticle 2- Epidermis 3- Sub-epidermal 4- Stone Cells.

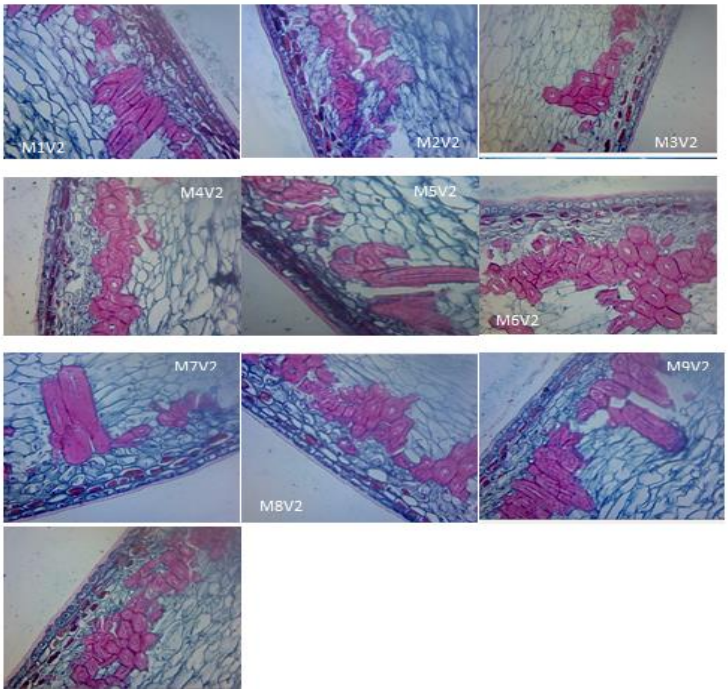

Figure (2) shows the stone cells, the epidermis, and the sub-epidermal cells.

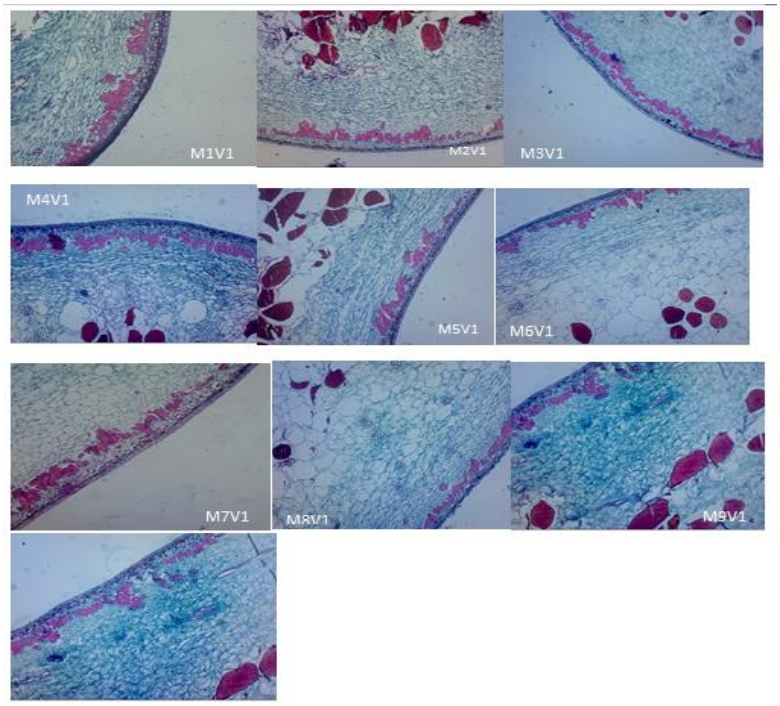

Figure (3) shows the tannin cells.

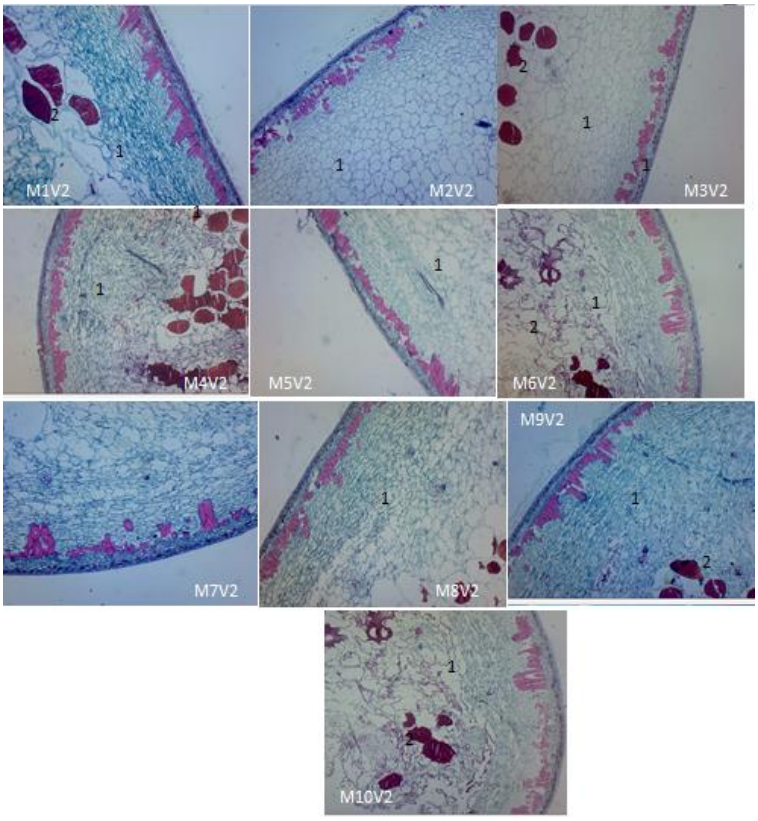

Figure (4) shows 1- the outer mesosphere 2- the tannin cells

Table (1) and Figures (1 to 3) show that the thickness of the cuticle layer ranged from 28.58 to 50.17 µm for the female cultivars Zuhdi and Sayer, respectively, and between 27.42 to 49.37 for the M2 and M10 males, respectively. The thickness of the

epidermal ranged from 58.17 to 72.83 µm for the female cultivars Zuhdi and Sayer, respectively, and between 58.17 to 82.17 for the 1M and M10 µm males, respectively. The increase may be due to an increase in the diameter of the cells. Tannin accumulates in most cells and sclerosis is completed in the cells of the inner mesosphere. The sclerotic cells are arranged so that their longitudinal axis is often oriented in the diagonal direction. The tannin cells that separate the inner and outer mesosphere are fully formed, forming a ring of 5-7 rows of cells, this stage also marks the beginning of the differentiation of vascular bundles.

Regarding the subcutaneous layer whose thickness at this stage ranges from (4-6) cells (Fig. 1). The study recorded that the thickness of this layer ranged between (238.19 to 231.66) micrometers for the female cultivars Zuhdi and Sayer, respectively, the highest values were obtained for the variety Zuhdi (415.99) µm for the thickness of the layer of stone cells, it was also noted that the vascular bundles spread, but their spread decreases towards the inner covering (Fig. 3).

The khalal stage lasts from three to four weeks and begins when the color of the fruit changes from green to a distinctive color, at this stage, there was a continuous increase in the thickness of the outer and inner layers of the mesocarp, this increase continues to the middle of the interstitial stage, the thickness of these two layers is mainly due to the increase in the diameters of the cells that make up their thickness and not to the increase in their number due to the cessation of cell division. These results are consistent with the findings of the current study. The thickness of the outer shell reached 5-6 rows of cells (Fig. 2). The stone cells began to disintegrate and the decay of tannin from the cells of some of the strains under study is also observed (Fig. 4). This stage of growth represents the last stage in which the differentiation of vascular tissues is completed, as a number of them were observed during the anatomical sections.

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