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# Pollen and Anther Development in *Ziziphus jujuba* L. (Rhamnaceae)

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**ABSTRACT**

The *Ziziphus jujuba* belongs to the Rhamnaceae family. This tree is commonly found in the Mediterranean region. The current study uses *Z.jujuba* lam.var spinosus Bge as an example. The tree has small green-yellow flowers with short peduncles (1 to 5 mm) and cymes, which are located beside the leaves. These flowers contain 5 sepals which are triangular and flat, 5 tiny, separated petals, and 5 stamens. Young buds and flowers of *Ziziphus jujuba* L. were removed at different stages (tiny buds to mature flowers, in 4 sizes) and fixed in FAA, embedded in paraffin and sliced at 7 – 10 µm. Staining was carried out with PAS and contrasted with Hematoxylin. The generative meristems, buds, anther and pollen development were studied after staining by light microscope. Result showed that the slices of the generative meristems have Tunica and Corpus; they are very active, their cells are divided and because of that, meristems are massive and protruding. The surface layers of generative meristem, is called sporangiare meristem and the lower parts of them are less staining which is receptacle meristem, with division of generative meristem, floral structural components, such as bracts, sepal and stamen primordia are determined. the next step, after the stamen are formed, then due to the activity of peripheral sporangiare meristem the petals are also formed opposite the stamen and the final stage, ovary primordium is formed, in more advanced stages respectively, from outside to inside the sepals. Petal primordia, stamen primordia and ovary primordium are detectable. Result showed that un-differentiated anther is ovoid and tetrasporangiated. The anther wall development follows the dicotyledonous type, which is composed of epidermal layer, endothelial layer, one middle layer and then a plasmodial type tapetum layer. The tapetal cells are uniseriate uni or bi-nucleate and the microspore tetrads are tetragonal. Pollen grains are spherical, tricolporate and are shed at a bicellular stage. The nucleus is then divided by the mitosis into two nuclei, small generative and large vegetative nuclei that's why they are called bi-nucleated pollen grain which at last will be two-cell pollen grains are spherical, tricolporate and shed at a bicellular stage.

***Key words:*** *Ziziphus jujuba,* Generative meristem, Vegetative meristem, Pollen grain.

## Introduction

The *Ziziphus jujuba* belongs to the Rhamnaceae family. This tree is commonly found in the Mediterranean region (i.e. Northern Africa, Southern Europe, and Western Asia), and is often found growing as a wild type in areas near the Caspian Sea, and as a semi-wild type in Rasht and Booshehr [1]. This tree can grow as tall as 2-8 meters, and some specimens have been known to reach 12 meters. Its resistance to dryness has manifested in some trees having spines, and some having no spines. The current study uses *Z.jujuba* lam.var spinosus Bge as an example. Its leaves are small, glabrous, and shining, with three veins. There are outgrowths on both sides of the petioles, which grow into firm and sharp spines, then become woody and remain permanently on the shoots. The tree has small green-yellow flowers with short peduncles (1 to 5 mm) and cymes, which are located beside the leaves. These flowers contain 5 sepals which are triangular and flat, 5 tiny, separated petals, and 5 stamens. Its ovary is hypogynous with two chambers, stigma and style. Although style are divided into two parts, on rare occasions three parts can be seen [1]. Jujuba`s fruit can be found in two shapes: an elliptical or spherical shape, or a drupe shape. These fruits are red or brown, shiny, and tend to look like olives. They are edible, with a succulent, white; epicarp.The high concentration of nutrients in Jujuba's fruit can be used in fresh or dry forms to treat a cough or stomach pain, and to aid in digestion. It is also used as a tranquilizer, a lenitive, and fever reducer. The bark of the tree can be used to stop diarrhea, while the fresh leaves can be used as soap. The present paper is the first report and is an attempt to understand the generative meristem and the male gametophyte development in *Z.jujuba*. The development of the

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male gametophyte involves a series of events culminating in the production and release of mature pollen grains from anther [16]. Little is known about *Z.jujuba* microsporogenesis.There are two major types of tapetum, the more primitive secretory type, considered to be the prevalent type in the majority of plants, and amoeboid type that extends to microspores in the anther locule [9].

Four types of anther wall development were described by Davis [7] based on the secondary parietal layers. Basic type (typeI), dicotyledonous type (type II), monocotyledonous type (typeIII) and reduced type were found in each (type IV). In general, one specific type of anther wall development is family. However, some families posses two types of anther wall development, such as the Commelinaceae having type I and II [13] and the family Solanaceae having type I ,II [2].

## Material and methods

Generative meristems and young floral buds were collected from Behshahr. The flower and buds in different stages of development (in 4 sizes) were removed at 15/5/2011, fixed in FAA 70% (Formalin and glacial acetic acid, 70% Ethanol 5:5:90 v/v), stored in 70% Ethanol, embedded in paraffin, and sectioned at 7-10 µm with a microtome. Staining was carried out with PAS (Periodic Acid Schiff) according to protocol suggested by Yeung [17] and contrasted Meyer`s Hematoxylin. Several sections for each generative meristems and anther developmental stage were studied with a Zeiss Axiostar plus light microscope. Many samples were studied before each stage and photomicrographs were made from the best ones.

*Results:*

Generative meristems, Anthers and pollen development were studied by light microscope. The slices of the generative meristems showed that in this meristem, Tunica and Corpus are very active, their cells are divided and because of that, meristems are massive and protruding. The slices of generative meristem (fig 1A) show that it is massive and has raised the intensity of the activity area in Tunica and Corpus. The surface layers of generative meristem, are called sporangiare meristem (Sp.m) and the lower regions, which are less staining are called receptacle meristem (Re.m), with division of generative meristem, flora structural components, such as bracts (br), sepal (se) and stamen primordia (st.p) are detected (fig 1 B). After the stamen are formed, due to the activity of peripheral sporangiare meristem, the petals are also formed opposite the stamen and the final stage, ovary primordium (ov.p) is formed (fig 1 C), in more advanced stages (fig 1 D), respectively, from outside to inside the sepals (Se). Petal primordia (pe.p), stamen primordia (st.p) and ovary primordium (ov.p) are detectable. With proliferation and change of ovary primordium, ovarian cavity and style (sty) are formed. Development of stamen primordia (st.p) is faster than ovary primordium (ov.p).in early stages, a longitudinal slot, the middle region of ovary primordium which is on the top is formed and ultimately leads to the formation of the stigma and style plug. After these change and gradual development of floral organs, sepals (se), petals (pe), and stamen are detected, ovary primordium (o.p) are formed (fig 1 G). Ovule primordia develop to ovule. The fully developed microsporangium consists of a mass of haploid pollen grains enclosed within the sporangial wall. On maturation of the anther, the sporangial wall consists of the epidermis and the endothecium, whereas the middle layers and tapetum disintegrate. The partition wall between the two anther lobes of on each side disintegrates and the two pollen sacs appear as one (fig 1 I). The mass pollen grains exert pressure on the anther wall, which bursts open (↑) liberating the pollen grains (fig 1 H).

*Male gametophyte developmentn:*

In very young buds, the head of stamen primordia protrudes slightly and microspore mother cells are massive diploid and detectable (fig 2A). Each microspore mother cell undergoes meiosis; during which M.M.C undergo successive type. In this type of cytokinesis, each nuclear division is successively followed by cell wall formation. The first nuclear division of meiosis (meiosis I) is accompanied by cell wall formation (↑) resulting in the formation of two haploid cells i.e. a dyad (fig 2 B). The dyad undergoes the second meiotic division (meiosis II), which is followed by wall formation and results in the formation of tetragonal tetrads that is surrounded by special wall (fig 2 C). Next, haploid cells (meiospores) are separated after the degeneration of the special wall, and each of the meiospores is turned into young meiospores with big and central vacuoles (V) and small and peripheral nucleus (N). Exine (Ex) and Intine (In) are formed in this stage (fig 2 E) and tapetum in this plant is plasmodial type. These are tapetal cells moved toward the microspores and extensions of them are visible in the anther locule at the microspores releasing stage (fig 2 E-F). The tapetal cells were degenerated at the stage of binuclear pollen grains and only relics of the tapetum are visible in this stage (fig 2 H). In this species, middle layer is not developed. The pollen grains are tricolporate (fig 2 H). As a result, the mitotic division of the microspore is unequal. Therefore, a darker generative nucleus and a lighter vegetative nucleus appear. So pollen grains are two-celled at the time of shedding (fig 2 G). The mature pollen grain have pores (P) (fig 2 H) and as a result, the pollen tube grows in the anther (fig 2 I).

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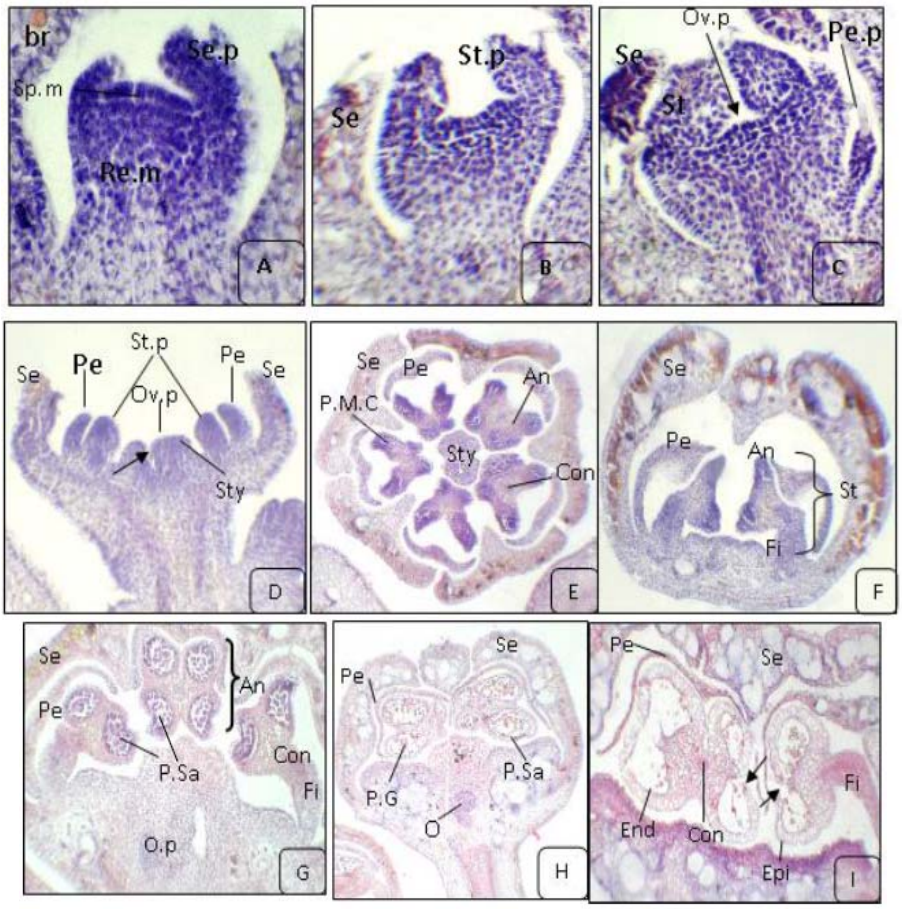


**Fig. 1:** (A): Longitudinal section of Ziziphus jujuba generative meristem showing the formation of sepal primordia, sporangiare meristem (Sp.m) receptacle meristem (Re.m) and bracts (br), (B): Longitudinal section showing sepal (se), stamen primordia (St.p), (C): Longitudinal section showing sepals (se), petal primordia (Pe.p) and ovary primordium (Ov.p), (D): Longitudinal section of bud showing sepals (se), petals (pe), stamen primordia (St.p) and ovary primordium (Ov.p) and longitudinal slot in style (↑). (E): diagram of flower, style (Sty). (F): Longitudinal section of bud size1, filament (Fi), anther (An), stamen (St). (G): bud size2, pollen sac (P.Sa),connective (con) ,ovule primordium(O.P) .(H): bud size 3, ovule (O),pollen grain(P.G). (I): bud size 4, epidermis (Epi), endothecium (End), mass pollen grains exert pressure on the anther wall which bursts open (↑) liberating pollen grain.

*Discussion:*

Results of this research show that showing, Tunica and Corpus are very active, their cells were divided continuously, and meristem become massive and protruding it`s called generative meristem. Surface layer of generative meristem is called sporangiare meristem and lower parts are called receptacle meristem. Petals, stamens and pistil from sporangiare meristem, and sepals, bracts and receptacle from receptacle meristem are formed, which is in accordance with findings of Majd and Jafari [11] and Jafari and Sharee [12]. In the young buds, stamen peimordia developed earlier than ovary primordium. The current study showed that development of three layered anther wall occurred as the dicotyledonous-type in *Z.jujuba* [7]. A sharp correlation was observed between division in microspore mother cells (M.M.Cs) and development of anther`s tapetum. That was reported for other dicotyledonous plant like Asteraceae members [10,4,5,6].

Tapetum cells have a high level of polyploidy which indicates their high metabolic activity [14]. Two basic types of tapetum have amoeboidal (periplasmoidal) type so that its periplasmoidal extensions are observed toward the anther locule. The microspores at releasing time are vacuolated and they have peripheral nucleus. The nucleus is then divided during mitosis into two nuclei, a small generative nucleus, and large vegetative nucleus, hence the name bi-nucleated pollen grain, which is in accordance with findings of Chehregani [4,5,6].

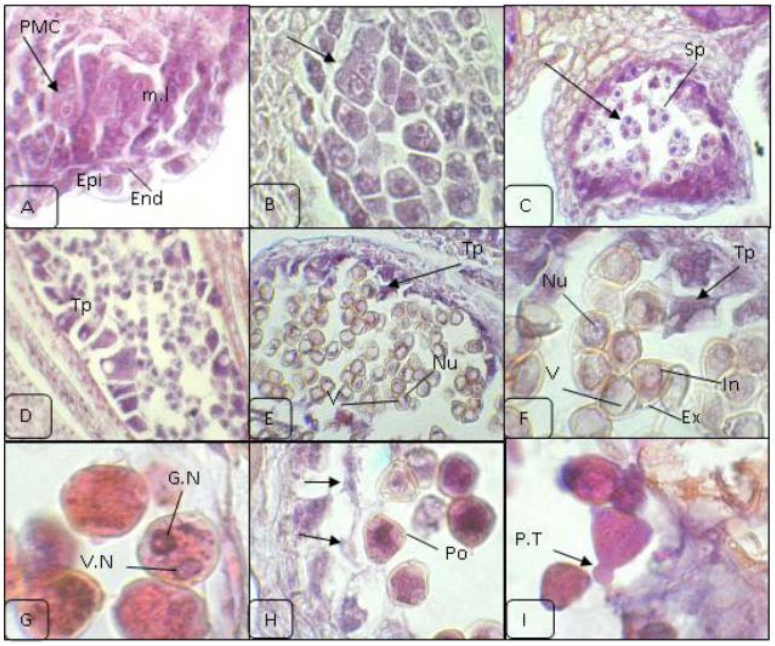


**Fig. 2:** (A): Section of single locule showing early microspore mother cell (MMC). The locule is lined by epidermis (Epi), Endothecium (End), middle layer (m.l.) and tapetum (Tap). (B): Pollen grain in dyad stage (arrow show cell wall). (C): Section through tetragonal tetrad, callosic wall are visible that surrounding early tetrads. (D): Anther at the later tetrad stage. Not those extensions of tapetal cells are visible (Tp). (E), (F): cross section of anther showing young misrospores with Exine (Ex) and Intine (In), they vacuolated considerably and nucleus is peripheral. (G): young pollen grain have generative and vegetative nucleus. Note that generative nucleus is darker than vegetative nucleus. (H): mature tricolpate pollen grain with pores (Po). Relics of tapetum are visible (arrows). (I): mature pollen grain; note that pollen tube grows into the anther (P.T).

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