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Research article

STUDY OF ANATOMICAL STRUCTURE OF VEGETATIVE ORGANS, FLORAL MERISTEM AND POLLEN DEVELOPMENT IN SESAME

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ABSTRACT: Sesame (*sesamum indicum* L.) is recognized as the oldest oilseed and belongs to the Pedaliaceae family. It is cultivated in tropical and temperate regions. In order to study anatomical and developmental process, the samples were collected from Babol in summer of 2014. The vegetative organs were fixed in alcohol-glycerin. Likewise, collected flowers and meristems were fixed in F.A.A and then the slides were prepared for staining and microscopic studies. The vegetative organs present dicotyledonous-type structure. The microsporogenesis in sesame presents perfect flowers, tetrasporangiate anthers, tetrahedral tetrads arrangement and secretory tapetum. This report is a contribution to consider sesame pollen ontogeny in Pedaliacea family.

Key words: Microsporogenesis, secretory tapetum, tetrahedral tetrads

INTRODUCTION

People's lives from the past up to now have been impressed by plants. In 19th century, the developmental studies could complete the anatomical perusals. Plant development studies guide us to a better understanding of the functioning of cells. Studies of growth, differentiation and organogenesis are the developmental investigation aims [21]. Due to the diversity of plant species, plant anatomical and developmental information can be very valuable for the classification of herbs. A cellular and tissue structure researches is very important even for physiological and molecular investigations. Therefore, the anatomical and developmental studies have major roles to a better perception in plant genotypic and phenotypic researches [16].

Sesame (*sesamumindicum*L.) belongs to the Pedaliaceae family and it has a somatic number 2n=26. There are 17 generaand 60 species in this family. The Pedaliaceae family has herbaceous, rarely shrubs genera. Although the number of sesame species is unclear, 40 species have been introduced for it. But the most famous species is *s. indicum*.In some cases, the sesame origin has been described in Africa. Except one species, other species were found in Africa [14]. On the other hand, according to kumar (2008), the origin of sesame was in India [13]. All these statements caused debates on the exact origin place of this valuable herb. Sesame is an annual herb and its height can reach more than one meter. Its stem is usually square and green. The upper part of the stem is hairy. The leaves are stipulate and they are arranged opposite and alternate [14]. The inflorescence type is spike and the flowers are zygomorphic, and they are in leaf axile. Flowers have five petals with white and pink color. The lower petal is longer and the lip is folded over the top. The stamens are didynamous and the ovary is hypogynous, tetracarpellate and each carpel has two locules. Its fruit is capsule and consists of seeds. Most of the capsules are dehiscent [13].

The study of floral development is important in helping to understand phylogenetic relationships between plants [4]. Male organ developmental process in plants was started with the differentiation of anther primordia. Pollen ontogeny studies are effective for understanding the evolutionary and reproduction investigation in seed plants. Microsporogenesis is a process that pollen microspore mother cells are generated by meiosis in the male organs [23]. Suarez-Cervera *et al*. (1992) studied the pollen morphology in the Pedaliaceae family [25]. Osman and Yermanos (1982) observed genetic male sterile line in sesame, and have found a gene responsible for the male sterility [18]. Abnormal tapetum played an important role in this process [10]. In recent years, we can see many studies on the materials that are neededfor the plant growth, but not in the anatomical studies. Although there is some information about sesame pollen morphology, but there is no information about sesame flower development.

To the better understanding of the physiological processes, structural studies of cells and tissues of the plants can be worthy. In this present paper we report the results of the anatomical structure of the vegetative organs, floral meristem development and pollen ontogeny in sesame.

MATERIALSANDMETHODS

In order to carry out the histological analysis, vegetative organs (stem, leaf and root) and the flowers of *sesamum indicum* L. were collected in all different sizes and times of its development from Babol, Mazandaran, Iran in summer of 2014. Then, vegetative organs have been fixed in alcohol- glycerin (1:1, v/v). The sections were cut manually and were double stained by methylene blue- Congo red. Flowers were fixed in alcohol-formalin-acetic acid (17:2:1, v/v/v) about 12 hours. The samples were dehydrated in an alcohol series, cleared in toluene and embedded in paraffin. 8-12 µm thick sections were obtained by using a rotary microtome. Slides were cleared in toluene, rehydrated and stained with hematoxylin and eosin. Sections were analyzed with light microscope (Olympus BX41). For scanning electron, the microscopy (SEM) was followed by Irish and Sussex (1990) protocol [11]. Images were taken by canon camera, and then we used Adobe Photoshop for other processes.

RESULTS

Anatomical analysis

The cross section of the root (Figure 1) shows that the exodermise layer is lost. The periderm layers are the outer layers of the root that include rectangular arrangement and compact of cells. The parenchyma cells are thin-walled and have irregular arrangements. Central cylinder was represented by vascular bundles. Xylem tissue has been developed to thecentral part of the root that is separatedby rays (Figure1-B).

The epidermis in stem cross section (Figure 2) consists of 1-2 layers of cells. The cortex is compact, thin-walled and consists of two layers of lamellar collenchyma. The endodermis layer is the innermost layer of cortex. Phloem tissue is on the top of xylem tissue. The vascular bundles are collateral. The xylem has thickened walls (Figure2-B).

The cross section through the main vein in leaf (Figure 3-A) shows that there are angular collenchyma layers (Figure 3-C). Vascular bundles can be seen. The xylem has thickened walls (Figure 3-B). The lamellar collenchyma can be seen in the cross section of the petiole (Figure 3-D), which is limited to the epidermis. The upper epidermis layer is compact and has anyzocitic stomata in lamina cross section (Figure 4-B). The lower epidermis has a similar structure as upper one. The mesophyll is heterogeneous (Figure 4-A).

In longitudinal section of the shoot apex, two layers of tunica are the outermost layers. The tissue internal to the tunica is the corpus. Below the corpus layer, the rib meristem in the central zone is shown. The peripheral zone is the site of formation of new leaf primordium. It is found out that the leaves are arranged opposite (Figure 5-A). In longitudinal section of root apical meristem (RAM), the root cap is outer layers of the RAM. The root cap arises from the calyptrogen, and the quiescent center is above this zone (Figure 5-B).

Floral meristem development

Formation of the leaf primordium has been gradually stopped by reducing initial ring activity. These changes which caused formation of primary floral meristem and maximum cell divisions have occurred in this phase. The cell differentiation was resulted to a prominent dome in the central zone of the floral meristem. The formation of the first and second whorl, in other words, sepals and petals formation can be seen at the early stages of floral development (Figure 6-A). After that stamen primordium was noticed. At the beginning of this stage, the stamen primordium was shown on the receptacle and in an epipetalous form (Figure 6-B). Stamens genesis have occurred before the carpel formation and the anthers wall layers were observed at that time (Figure 6-C, D). After the anthers development was completed, carpel primordium started to generate gynoecium. Ovary, style and stigma were observed in this stage (Figure 6-E).

Pollen development

In the cross section of young anthers, the tetrasporangiate anther connects with the interface tissue. Each pollen sac includes a group of archesporial cells in the early stage of the anther development. The archesporial cell divisions and differentiation generate wall layers. These layers consist of epidermis, endothecium, middle layers and tapetum (Figure 7-A). Pollen mother cells (PMC), formed as a large group of cells, are located in the middle of pollen sacs, and they are surrounded by a tapetum layer. PMCs have dense cytoplasm and conspicuous nuclei (Figure 7-B). First, the meiosis occurred in Microsporocytes, and forming dyads that are separated by thin callose wall (Figure 7-C). In the end of meiosis II, tetrads were observed with a tetrahedral arrangement. The tapetum layer is diminished during the tetrads formation. The tetrad microspores are separated. The tapetum persists to the end of the stage of tetrads, so it appears the secretory type (Figure 7-D).

The young microspore was observed. The epidermis and endothelium layer were seen in this stage (Figure 8-A). Each microspore nucleus undergoes mitotic division and generates a vegetative cell and a generative cell (Figure 8-B).After the pollen maturity, the epidermis and endothecium layers were still observed. But, the middle layer and tapetum layer were diminished. The surface structure of the pollen grains was observed by SEM. The exine surface was covered with gemate sculpture appearance. The size of the mature pollen grain is 54.77 µm.The microspores have 13 furrows and two pores (Figure 8-C, D). There is a process that causes loss of cell layers of the connective tissue. Septum degeneration creates a bilocular anther and pollen grain pressure leads the anther to complete longitudinal dehiscence (Figure 9-A, B).

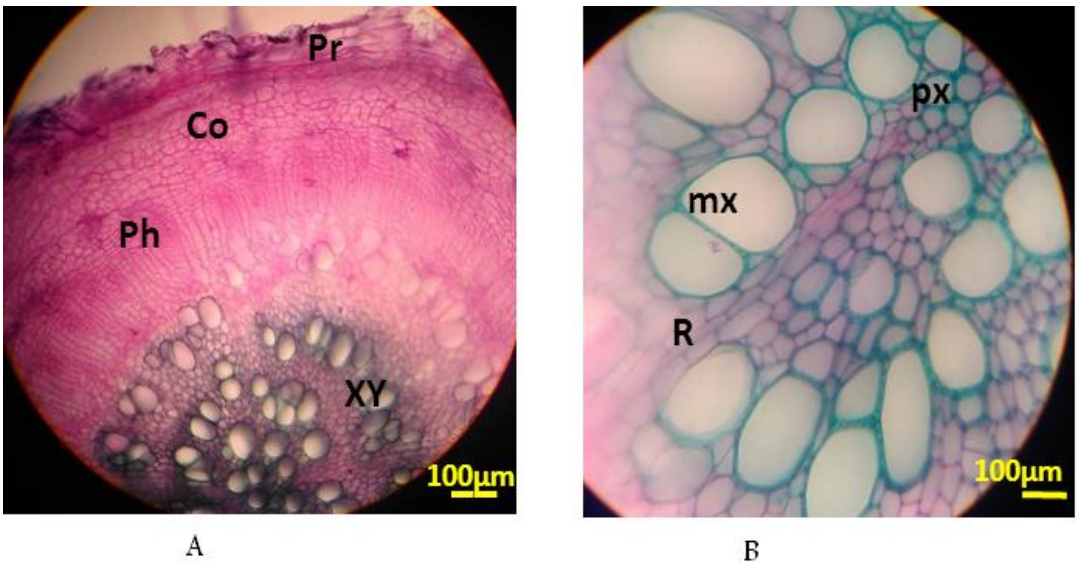

Figure 1. Sesame root section. (A) The secondary sturucture is shown compact cells in periderm layers and a collatelar vascular bundle. (B) A thick-walled xlyem that is seperated by ray. Periderm (Pr), Cortex (Co), Phloem (Ph), Xylem (Xy), Metaxylem (Mx), Protoxylem (Px), Ray (R).

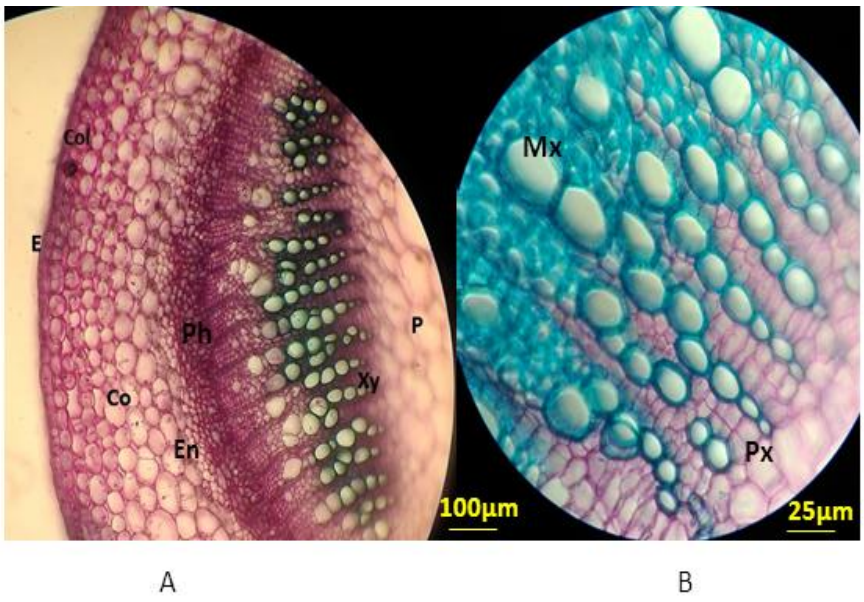

Figure 2. Cross section of the young stem of sesame. (A) It isillustrating the collateral vascularbundles of which the primaryvascular system is comprised. (B) the xylem has thickend wall.Epiderm (E), Collenchyma (Col), Cortex (Co), Endodermis (En), Phloem (Ph), Xylem (Xy), Pith (P), Metaxylem (Mx), Protoxylem (Px).

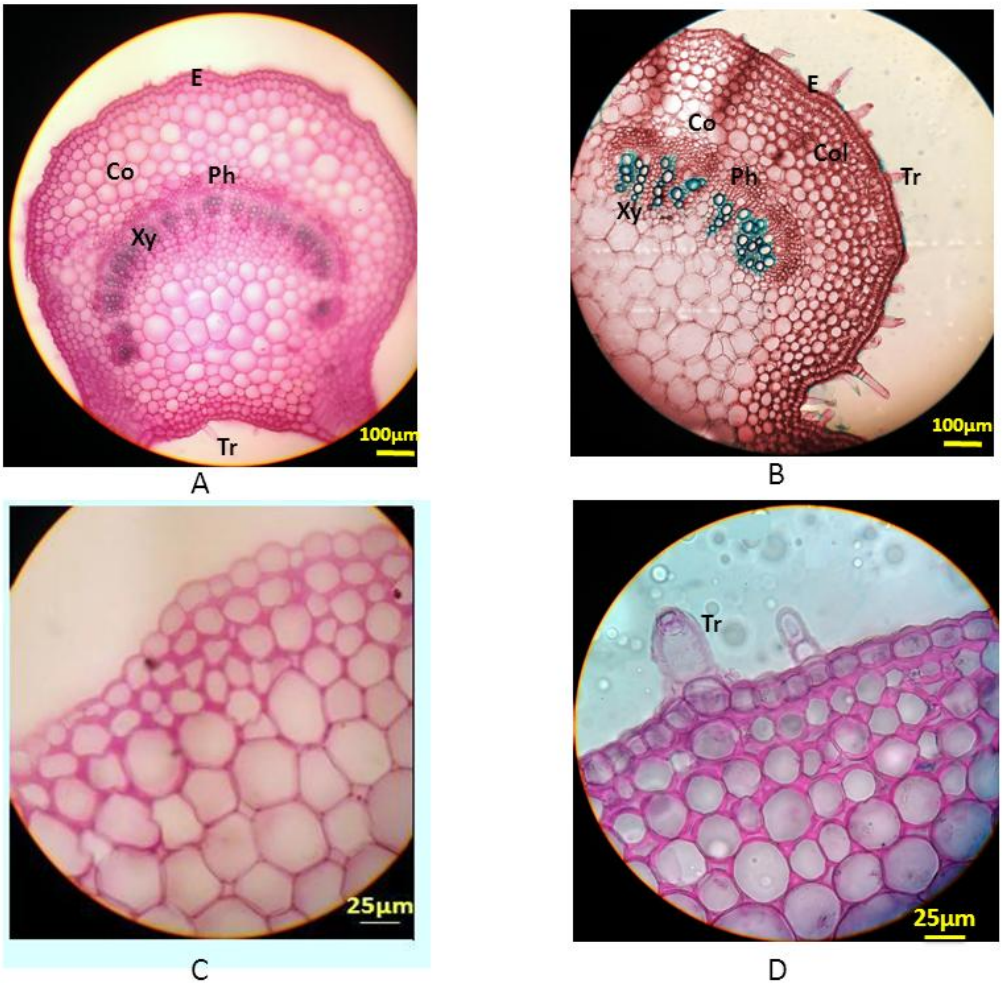

Figure 3. The cross section of the main vein (A) and the petiole (B) of sesame. (A)large epidermal cells and collateral vascular bundlesare noticed. (B) Trichomes are observed on the epidermal surface of petiole.vascular bundles arranged in collateral form. (C) and (D) lamellar collenchyma in the main vein (C) and the petiole (D). Epiderm (E), Cortex (Co), Collenchyma (Col), Phloem (Ph), Xylem (Xy),Trichomes (Tr).

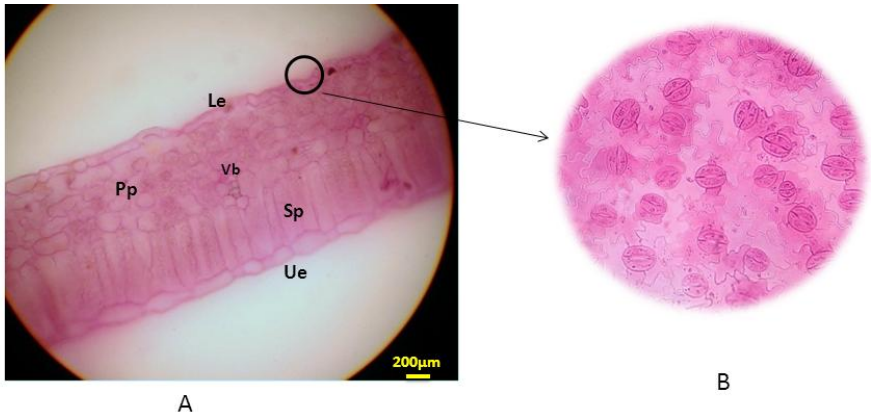

Figure 4. Cross section in lamina of sesame. (A) The heterogeneousmesophyllconsists of large parenchymatous cells. (B) Anyzocitic stomata. Lowe epidermis (Le), Palisade parenchyma (Pp), Vascular bundle (Vb), Spongy parenchyma (Sp), Upper epidermis (Up).

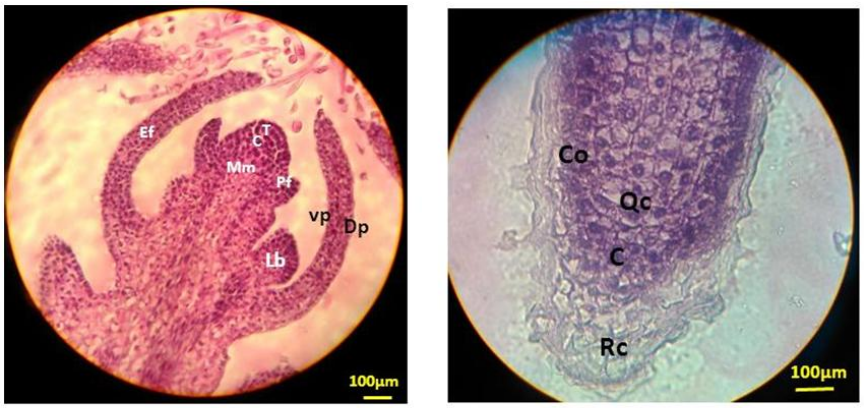

Figure 5. A longitudinal section of the shootapex (A) and the root apical meristem (B) of sesame. (A) The leaf primordia and young leaves (ebouche foliaire) are produced and arranged in opposite.the increase in length of theapical meristem followingproduction of a leaf primordium. It is illustrating lateral buds in top of each leaf that is showing spike inflorescence. (B) longitudinal view of root apic showing the calyptrogen that generates root cap. Tunica (T), Corpus (C), Mother meristem zone (Mm), Primordium foliaire (Pf), Ebouche foliaire (Ef), Ventral parenchyma (Vp), Dorsal parenchyma (Dp), lateral bud (Lb), Cortex (Co), Quiescent center (Qc), Calyptrogen (C), Root cap (Rc).

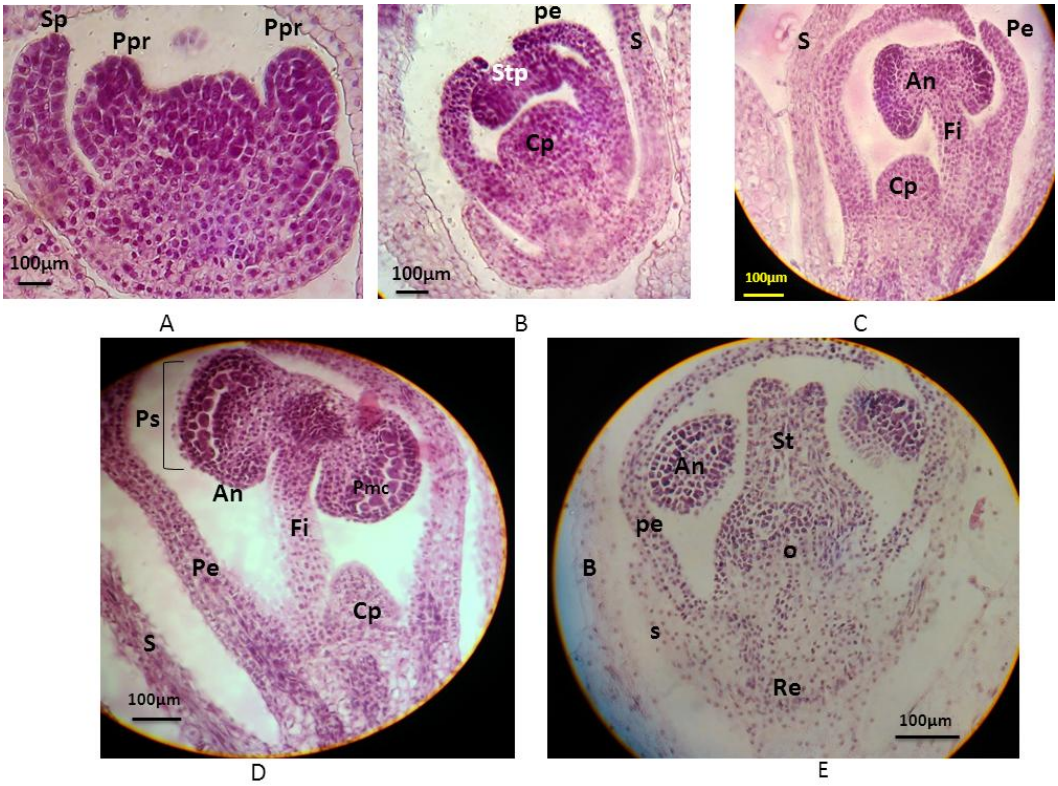

Figure 6. Longitudinal sections of floral meristems during floral development. (A) Sepal and petal primordia start to form. (B)Stamen primordia and carpel primordia eventually form in sesame. (C, D) anther wall layers formation. (E) carpel starts elongating and generating gynoecium. Sepal primordium (Sp), Petal primordium (Ppr), Sepal (S), Petal (Pe), Stamen primordium (Stp), Carpel primordium (Cp), Anther (An), Filament (Fi), Pollen sac (Ps), Pollen mother cell (Pmc), Style (St), Ovary (o), Receptacle (Re).

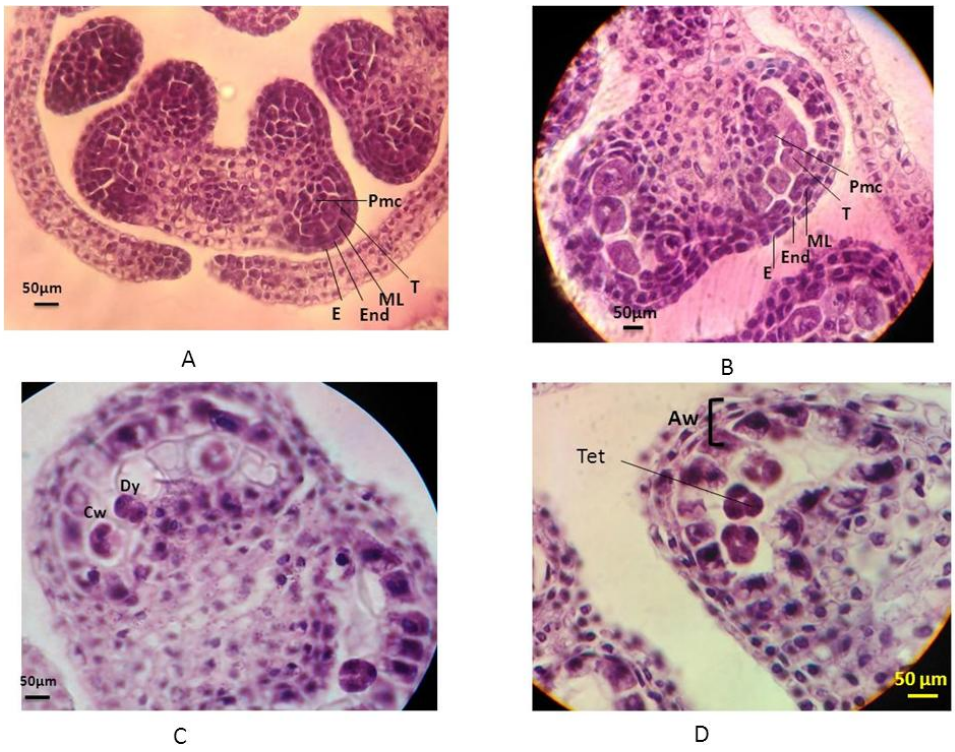

Figure 7. Microsporangium tissues in different stages of development in *sesamum indicum* L. (A) anther wall containing of epidermis, endothecium, middle layers and tapetum. (B) Tapetum layer degeneration. (C)dyad surrounding callose wall. (D) Tetrads with tetrahedral configuration. Ollen mother cell (Pmc), Epidermis (E), Endothecium (End), Middle layer (Ml), Tapetum (T), Callose wall (Cw), Dyad (DY), Anther wall (Aw), Tetrad (Tet).

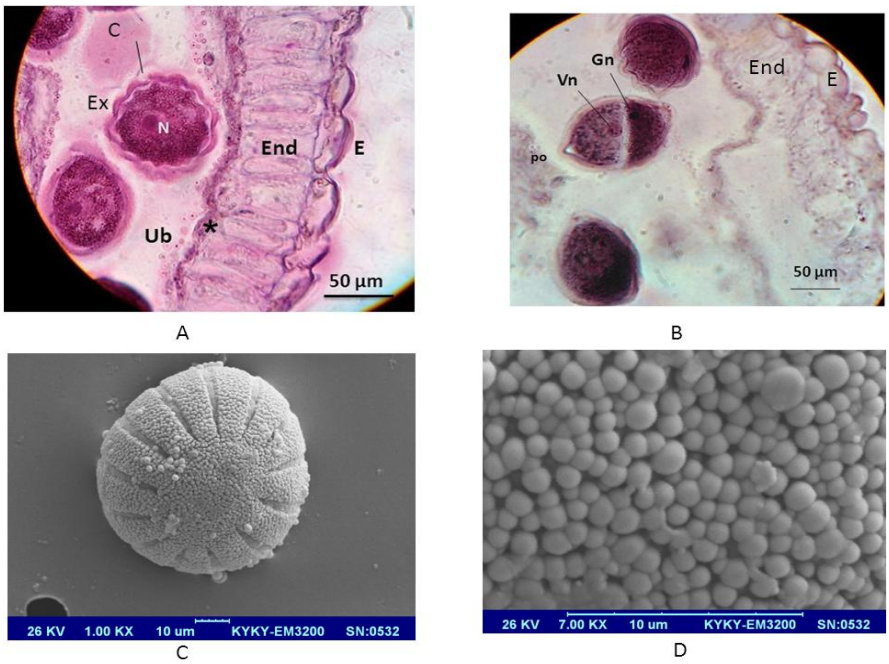

Figure 8. Cross section of anthers in later developmental stages of sesame flower. (A) Young microspores, showing epidermis, endothecium and degenerated tapetum (*). (B) Mature pollen grain with 2 pores. (C, D) The electron microscopy (SEM) scanning of pollen grain with 13 furrows and gemate sculpture appearance in exine surface can be seen. Epidermis (E), Endothecium (End), Ubish body (Ub), Exine (Ex), Nucleus (N), Colpus (C), Pore (po), Vegetative nucleus (Vn), Generative (Gn).

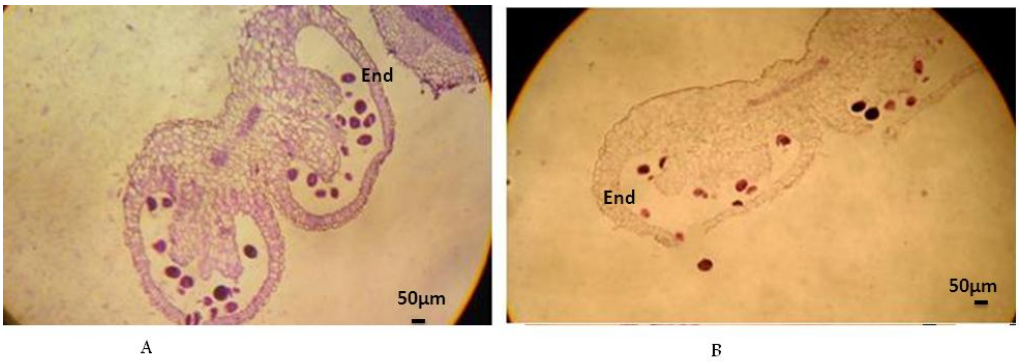

Figure 9. Cross section of mature anthers. (A) lysis of the stomium combined with the pressure from the expansion of the pollen grains. (B) The broken septum and releasing pollen grains. Endothecium (End).

DISSCUTION

Anatomical analysis

According to Mirela *et al*. (2009), the root's secondary tissues are produced by the vascular cambium and the phellogen. The phellogen produces phellem and phelloderm which comprise the periderm. During the periods of growth the vascular cambium divides and produces secondary phloem and secondary xylem. Stems are encased by a transparent epidermis, which is usually about one cell thick. Phloem is formed before the xylem and it differentiates on the outside of the bundle [17]. Xylem is formed on the inside of the bundle. Cortical cells are photosynthetic and often store starch. In dicotyledons, the ground tissue with the parenchyma cells in the center of the stem is specialized for the storage. The sesame root and stem structures are compatible with Wenliang *et al*. (2013) reports [27]. Fleming (2002) reported that in dicotyledons the leaf mesophyll is parenchymatous and collenchyma provides support the leaf margins. The leaf epidermis is a compact parenchyma tissue. In upper epidermis, the palisade mesophyll, consists of compact cells and in the lower epidermis, the spongy mesophyll, is consists of irregularly shaped cells. During leaf development, the rate of cell division within different regions may be related to its mature shape [8].

According to Clark (2001), Anticlinal divisions in the tunica are caused by increasing the surface of the apical dome. The corpus periclinal divisions are caused by enhancing the volume of apical dome [6]. Cells of the tunica and corpus are connected by plasmodesmata. The central mother cell zone is the source of the cells of the rib meristem. The peripheral zone is meristematic and is the site of new leaf primordium. As a leaf primordium develops, the meristem diminishes in size [2]. As the development proceeds, the strands of provascular tissue differentiate within the residual meristem [1]. In roots of dicotyledons, the various regions can be traced to distinct meristematic tiers. The root apex consists of three tier, root cap, ground meristem and provascular. The quiescent center, the low frequency of cell division has been observed in the upper region of calyptrogen. Ponce *et al*. (2000) described that the quiescent center has a significant role in development and also found the communication between the quiescent center and the associated apical initials [19].

Floral meristem and pollen development

Brand *et al*. (2000) mentioned that the first step in floral development is the transformation of the apical meristem into a floral meristem; and finally the growth of the flower's organs. An external stimulus is required to trigger the differentiation of the meristem into a flower. This stimulus will activate cell division in the meristem, especially on its sides where new primordia are formed. Some genes will regulate the maintenance of the stem cell's characteristics [3]. Flowers of dicotyledons usually are organized into four whorls of organswhich the first whorl is sepals. Petals are the second whorl that arisesat the next step in the floral meristem. First sepal primordium is initiated abaxially and then petal primordia are apparent on its inner surface. The third whorl contains stamens. The stamen primordia differentiate and give rise to the filament and the anther [31]. Jack (2004) described that the identity of floral organ primordia is controlled by three gene classes termed A, B, and C.A dome-like carpel primordium is initiated after stamens formation [12].

Pollen development includes cell division and differentiation. The archesporial cells have undergone a periclinal cell division, forming the primary sporogenous and primary parietal cells [24]. The latter undergoes periclinal divisions to form secondary sporogenous cells, which finally differentiate into pollen mother cells. The PMCs are surrounded by the tapetum layer. Primary parietal cells undergo periclinal divisions, resulting in two layers of secondary parietal cells [30].

After these two layer division, the anther wall layers will be formed. At this stage the PMCs have a dense cytoplasm, with numerous mitochondria, endoplasmic reticulum of rough type, plastids and free ribosomes [32]. The tapetal cells have a dense cytoplasm with abundant mitochondria, plastids and free ribosomes. The cytoplasm of the tetrad microspore has a high metabolic activity; it is dense, with numerous ERr, dictyosomes with vesicles. In most of the angiosperms, the tapetal cells play an important nutritive role in the formation of the pollen grains [7].The tapetal cells reach their maximum development at the tetrad stage [20]. Galati (2003) mentioned that one of the main features of the secretory tapetum is the production of orbicules at the tetrad stage. Orbicules have a thick wall of sporopollenin [9].

In free microspores stage the exine wall is formed by a basal layer, bacula and tectum. The cytoplasm of microspores contains abundant vacuoles, mitochondria, ERr and plastids. In mature pollen grain stage, tapetal cells are degenerated [7]. According to Chen *et al*. (2012) in the cytoplasm of the generative cell small vesicles and some mitochondria are observed. The vegetative cell possesses numerous mitochondria and abundant vesicles [5]. The sesame pollen morphology is compatible with Haaster (1990) and Yang et al. (2008) reports [26, 29]. The pollen grains cause outward pressure exerted from the inside of the locule on the anther. Enzymatic lysis of the stomium combined with the pressure from the expansion of the pollen and causes the septum to break to form a single locule. At this time, the anther walls dehydration causes an increased tension on the stomium region to release pollen grains [28].

CONCLUTION

To the better understanding of plants organ's function, the anatomical and developmental analysis can be useful. In this survey, we analyzed anatomical structure of vegetative organs and pollen ontogeny in sesame. The vegetative organs present dicotyledonous-type structure. Tetrasporangiate anthers, tetrahedral tetrads arrangement and secretory tapetum were observed in the anthers cross sections during the microsporogenesis. Therefore, this information can be important for distinguishing the differences between other species in this family.

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