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Araştırma / Research

Developmental Features of Reproductive Organs in *Viburnum tinus* L.

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Abstract

Viburnum tinus, which belongs to Adoxaceae family, is a plant that commonly used in pharmacy and landscape architecture. Flowers located in corymb-type of the inflorescence are white, fragrant, hermaphrodite. The development of the flower buds begins with the differentiation of the apical meristem as a small bulge. Afterwards, the apical apex is expanded, flattened and transforms into a floral meristem. Floral meristem cells have large volume and abundant cytoplasm. Concomitant with the development, firstly five stamen primordia and then three carpel primordia differentiate from the floral meristem. Anthers are tetrasporangiate. Anther wall is formed by epidermis, endothecium with fibrous thickening, ephemeral middle layer and, plasmodial tapetum. Tapetal cells degenerate at the young pollen stage. Pollen grains are discharged by the opening of stomium. Carpel primordial cells lengthen upwards, merge and form a style above the ovary. An ovule differentiates into each ovarian loculi. But the development continues only in one them and transforms into the mature embryo sac. Ovules are unitegmic and tenuinucellate. The style has a transmitting channel. Stigma is three-lobed and wet typed. The transmitting tissue and stigmatic papillae start to degenerate after pollination.

Keywords: developmental biology, flower ontogeny, reproductive biology, sexual reproduction, *Viburnum tinus*

Viburnum tinus L.' nin Üreme Organlarının Gelişimsel Özellikleri

Öz

Adoxaceae familyasına ait olan *Viburnum tinus* eczacılık ve peyzaj mimarisinde sıklıkla kullanılan bir bitkidir. Korimbus tip çiçek durumunda bulunan beyaz ve hoş kokulu çiçekler hermafrodittir. Çiçek tomurcuklarında gelişim apikal meristemin küçük bir çıkıntı olarak farklılaşması ile başlar. İlerleyen evrelerde apikal tepe genişleyip düzleşerek floral meristeme dönüşür. Floral meristem hücreleri büyük hacimli ve bol sitoplazmalıdır. Gelişimin ilerlemesi ile beraber floral meristemden ilk önce beş adet stamen taslağı, ardından 3 adet karpel taslağı farklılaşır. Anterler tetrasporangiyattır. Anter çeperi epidermis, fibroz kalınlaşma gösteren endotesyum, kısa ömürlü ara tabaka ve plazmodyal tapetumdan oluşur. Tapetum hücreleri genç polen evresinde körelir. Polen taneleri stomiumların açılması ile doğaya salınır. Karpel taslakları yukarı doğru uzayıp, birleşerek ovaryumun üzerinde stilusu oluşturur. Her ovaryum

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lokusunda bir tohum taslağı farklılaşır. Ancak tohum taslaklarından sadece bir tanesi olgun embriyo kesesine dönüşür. Tohum taslakları tek integümentli ve tenuinusellat tiptedir. Stilus nakil doku içerir. Stigma üç loblu ve ıslak tiptedir. Stilusun nakil dokusu ve stigma papilleri tozlaşmadan sonra körelmeye başlar.

Anahtar Kelimeler: çiçek ontogenisi, eşeyli üreme, gelişim biyolojisi, üreme biyolojisi, *Viburnum tinus*

1.Introduction

Viburnum tinus L. belongs to the Adoxaceae family comprises more than 200 species of shrubs or small trees. It is mainly used as a medicinal plant and, is preferred in landscape architecture (Konarska, 2017). *Viburnum tinus* is characterized by a corymb-like inflorescence containing 15-50 flowers. Flowers are white, lightly scented and hermaphrodite with five stamens and one pistil (Jin et al, 2010). Fruits are dark, blue-black and have ornamental importance (Darras et al., 2010).

Sexual development of a flower includes two main stages; floral initiation and floral organ development (Sandoval-Oliveros et al., 2017). Floral initiation starts by the transformation of the apical meristem into the floral meristem which forms flower organ primordia in forthcoming stages (Çetinbaş-Genç and Ünal, 2017). Flower organ development continues with the maturation of these organ primordia and proceeds with the embryological process (Bernier et al., 1993). Reproductive features seen in these durations are very considerable since the reproductive biology of plants supply beneficial information for the working area of programmed cell death of cell biology, systematic and seed production (Kinney et al., 2008).

Despite extensive morphological and pharmacological studies in *Viburnum tinus* L., minor information is known about reproductive biology. The main goal of the paper is to analyze the developmental features of male and female reproductive organs during flower development. Information on the developmental features of reproductive organs will help advance our comprehension of reproductive behaviour.

2.Material-Methods

Materials were collected from Marmara University Campus (Istanbul/Turkey). Flower buds were fixed by ethyl alcohol-glacial acetic acid solution, grounded in paraffin and sliced by rotation microtome. Slides were stained by Hematoxylin for general structure, by Periodic acid-Schiff (PAS) for insoluble polysaccharide (Feder and O'Brian, 1968), by Coomassie Brilliant Blue (CBB) for protein (Fisher, 1968), by Auramine O for cuticle (Heslop-Harrison and Shivanna, 1977). Sections were stained by 4,6-Diamidino-2-phenylindole (DAPI) solution to detect the nuclear disorders (Schweizer, 1976). Preparations were investigated by an Olympus BX-51 light and fluorescence microscope (Auramine O and DAPI).

3.Results and Discussion

V. tinus have corymb-like inflorescences contains only fertile hermaphrodite flowers as in *V. lantana* and *V. dilatatum*. However, some inflorescence of *Viburnum* species such as *V. macrocephalum*, *V. sympodiale*, and *V. sargentii* have both fertile and sterile flowers. Also some species such as *V. macrocephalum*, inflorescence includes only sterile flowers (Donoghue et al., 2003; Jin et al., 2007).

Flower development starts with the differentiation of apical meristem consisting of consecutive layers of the cell (Figure 3.1a). Afterward, the apical meristem becomes flattened and transforms into the floral meristem. Teeri et al. (2006) indicated that enlargement of floral meristem appears as a result of the enhancement in division ratio of the floral meristem cells. Floral meristem cells have a bigger volume and dense cytoplasm (Figure 3.1b). In the following stages, floral meristem forms floral organ primordia. Firstly, five stamen primordia differentiate as a roundish bulge from the floral meristem (Figure 3.1c). Shortly after the stamen primordia induction, floral meristem cells differentiate into the three carpel primordia (Figure 3.1d). The similar organ primordia differentiation model is seen in *Adoxa moschatellina* belongs to the Adoxaceae family (Roels and Smets, 1994).

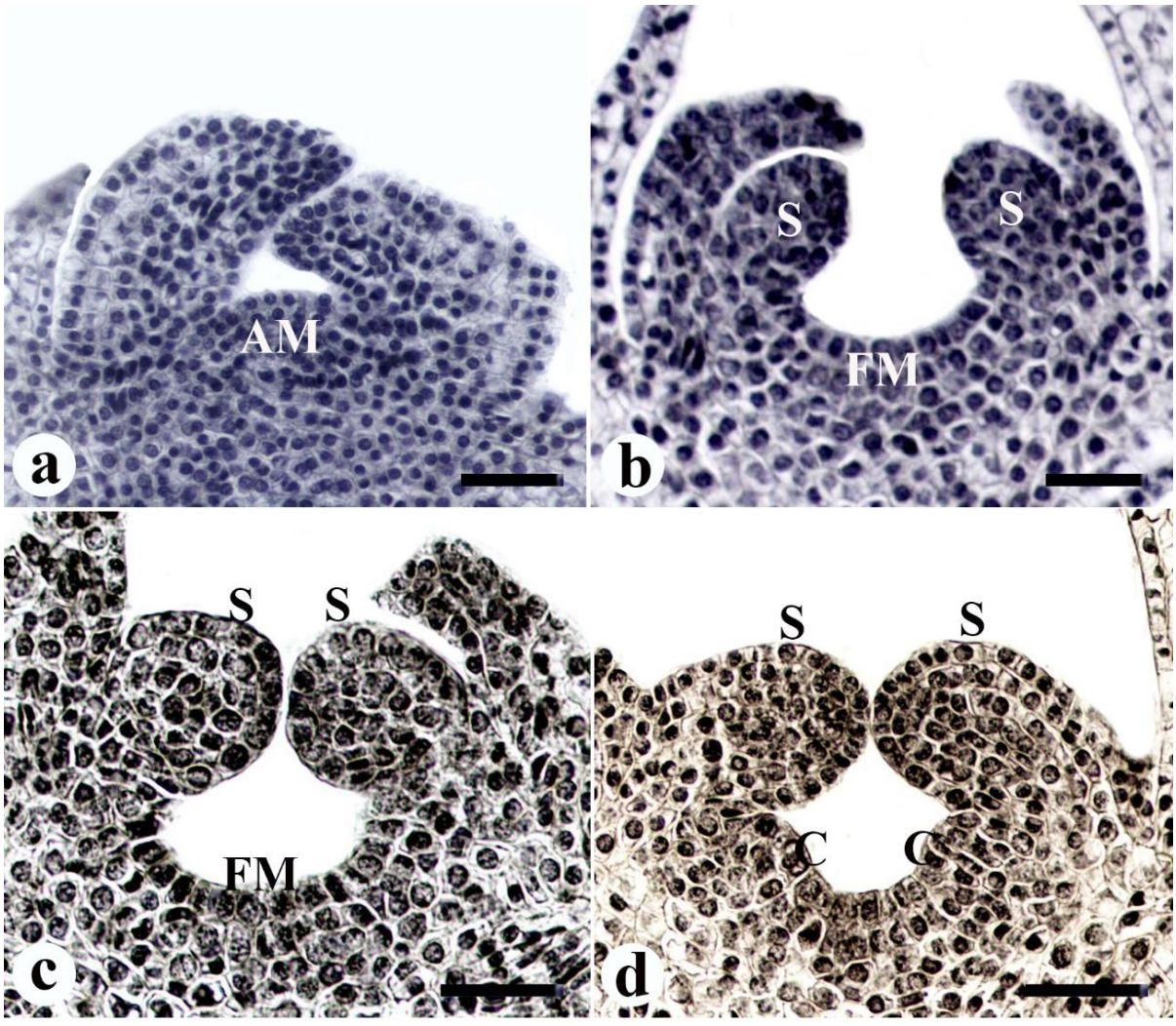

Figure 3.1. Flower ontogeny in *V. tinus*. a. Apical meristem, b. Floral meristem, c. Stamen primordia initiation, d. Carpel primordia initiation. AM: Apical meristem, FM: Floral meristem, S: Stamen primordia, C: Carpel primordia. Bar: 50 µm.

While stamen primordia start to lengthen, firstly filament and then anther differentiates (Figure 3.2a). This is the most outstanding condition in angiosperms and, many species have been observed such as *Helianthus annuus* (Çetinbaş and Ünal, 2012), *Crataegus tanacetifolia* (Çetinbaş and Ünal, 2015) and, *Salvia viridis* (Çetinbaş-Genç and Ünal, 2017). The anther enlarges during the development and becomes tetrasporangiate (Figure 3.2b). Tetrasporangiate anther is a prevalent situation in Adoxaceae family (Backlund and Bittrich, 2016). Anther wall is composed of epidermis, endothecium, middle layer and, tapetum. These

anther layers locate as proper cell lines at the beginning of the development (Figure 3.2b). Epidermis and endothecium with fibrous thickening stay unspoiled until anther dehiscence; however, middle layer and tapetum dissolve during development (Figure 3.2c). Fibrous endothecium is very common in the Adoxaceae family (Ghimire at al., 2018). Mature pollen grains are discharged by the opening of stomium (Figure 3.2d). Ghimire et al., (2018) also have reported anther dehiscence by the longitudinal slit in some species of Adoxaceae family. Tapetum is plasmodial typed which tapetal cells break down during microspore development. Benko-Iseppon and Morawetz (2000) indicated that *Viburnum* species can also have glandular tapetum. Disorders in tapetal nuclei start to be visible from the microspore tetrad stage (Figure 3.2e, h). Disorganization and nuclear fragmentations are more prominent at the young pollen stage (Figure 3.2f, i) and finally, tapetum cannot be observed at the mature pollen stage (Figure 3.2g, j). Similar tapetal nuclei disorders have been shown in many species such as *Lathyrus undulatus* L. (Vardar and Ünal, 2012) and *Crataegus tanacetifolia* (Çetinbaş and Ünal, 2015).

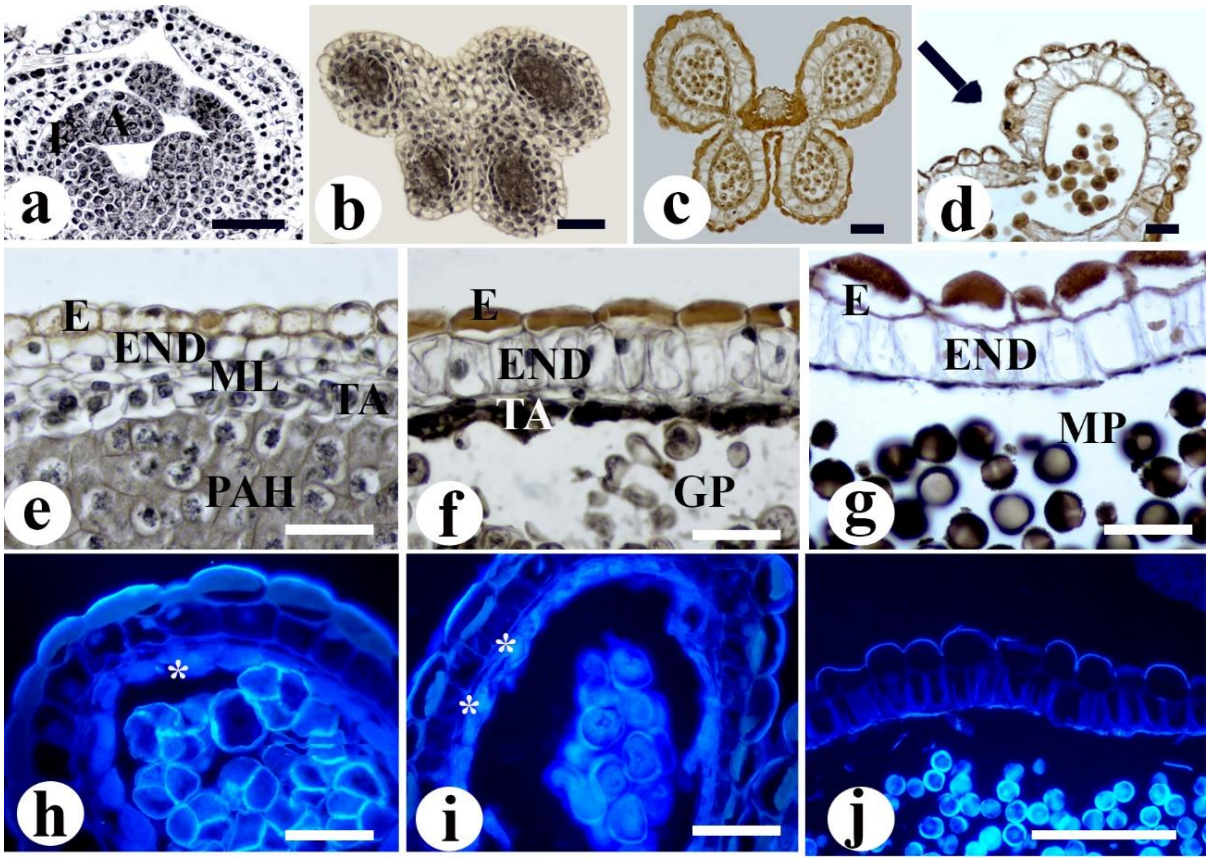

Figure 3.2. Anther development in *V. tinus*. a. Differantiation of anther and filament, b. Young tetrasporangiate anther, c. Mature tetrasporangiate anther, d. Anther dehiscence by stomium (arrow), e. Ordered wall layer at young anther, f. Deteriorated tapetum layer at young pollen stage, g. Anther wall layers at mature pollen stage, h. Tapetal nuclei at tetrad stage (asteriks), i. Tapetal cells with degenerated nuclei at young pollen stage (asteriks), j. Mature anther. A: Anther, F: Filament, E: Epidermis, END: Endothecium, ML: Middle layer, TA: Tapetum, PMC: Pollen mother cells, YP: Young pollen, MP: Mature pollen. Bar: 50 µm.

Floral meristem cells differentiate into the three carpel primordia shortly after the stamen initiation (Figure 3.3a) as in *A. moschatellina* (Roels and Smets, 1994). Gasser and Beers (1993) stated that carpel primordium initiates in the core of the floral meristem. Three carpel primordia start developing and forming three ovarian loculi between them (Figure 3.3b). In

the subsequent stages, carpel primordial cells divide by mitosis and they lengthen upwards (Figure 3.3c, d). They merge and form a style above the ovary (Figure 3.3e). The ovary has three loculi in *V. tinus*. Ovules initiate as a little mass on the placenta in each loculus (Figure 3.3f, g, h). Ovules are unitegmic, so they have one integument. Also, ovules are tenuinucellate, megaspore mother cell (MMC) differentiates just below the nucellar epidermis (Figure 3.3g). An ovule differentiates into each loculus. However the development continues only in one of them and transforms into the mature embryo sac (Figure 3.3i, j). In others, the development ends at generally megaspore mother cell stage. These ovules are called abortive ovules (Figure 3.3k). Wilkinson (1948) reported the abortion of two of the three ovules in some Adoxaceae members. The style has a transmitting channel (Figure 3.3l, m). Before the pollination, the nuclei of the transmitting channel are spherical and regular (Figure 3.3n). However, disorders and nuclear fragmentations of nuclei are more obvious after the pollination (Figure 3.3o). Stigma is three-lobed and contains papillae as in *V. opulus* and *V. lanatana* (Konarska, 2017).

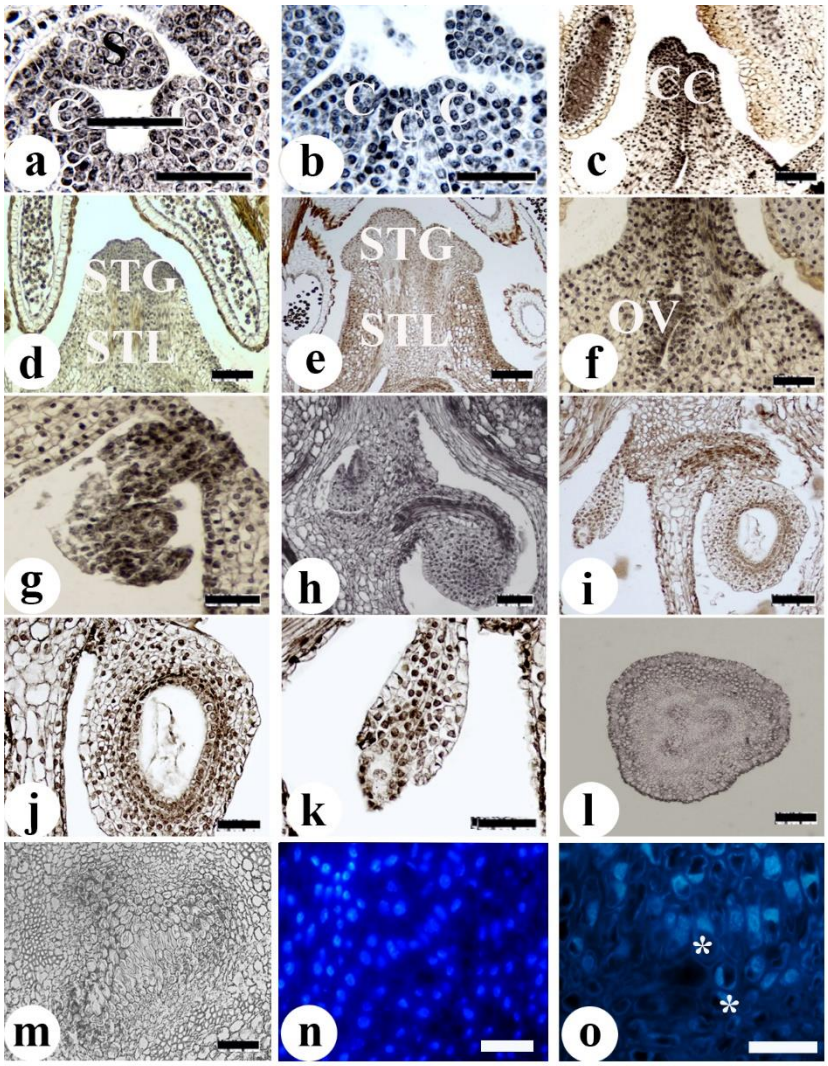

Figure 3.3. Pistil development in *V. tinus*. a. Carpel primordia initiation, b. Developing carpel primordia, c. Formation of ovarian loculi, d. Young pistil, e. Mature pistil, f. Ovule differantiation, g. Integument and megaspore mother cell, h. Early stage of ovule development, i. Advanced stage of ovule development, j. Mature ovule with mature embryo sac, k. Abortive ovule with megaspore mother cell, l. Cross section of a style, m. Transmitting channel of the style, n. The cells of transmitting channel before the pollination,

o. The cells of transmitting channel after the pollination. C: Carpel primordia, S: Stamen primordia, STG: Stigma, STL: Style, OV: Ovule. Bar: 50 µm (b, f, g, j, k, l, m, n, o) and 100 µm (a, c, d, e, h, i).

The papillae cells have a large amount of organic matter (Figure 3.4a, b). Stigma is wet typed and there is no pellicle layer stained by Coomassie Brilliant Blue (Figure 3.4b). There is a cuticle layer stained by Auramine O which is an indicator of lipoidal substance (Figure 3.4c). Similarly, *V. opulus* and *V. lantana* have wet type stigma and stigmatic exudate are rich in organic matter content (Konarska, 2017). Although there are no nuclear disorders before the pollination in papillae cells (Figure 3.4d, e), nuclear fragmentations can be seen after pollination (Figure 3.4f). Similar nuclear disorders in papillae after pollination have been shown in *Olea europaea* (Irene et al., 2010).

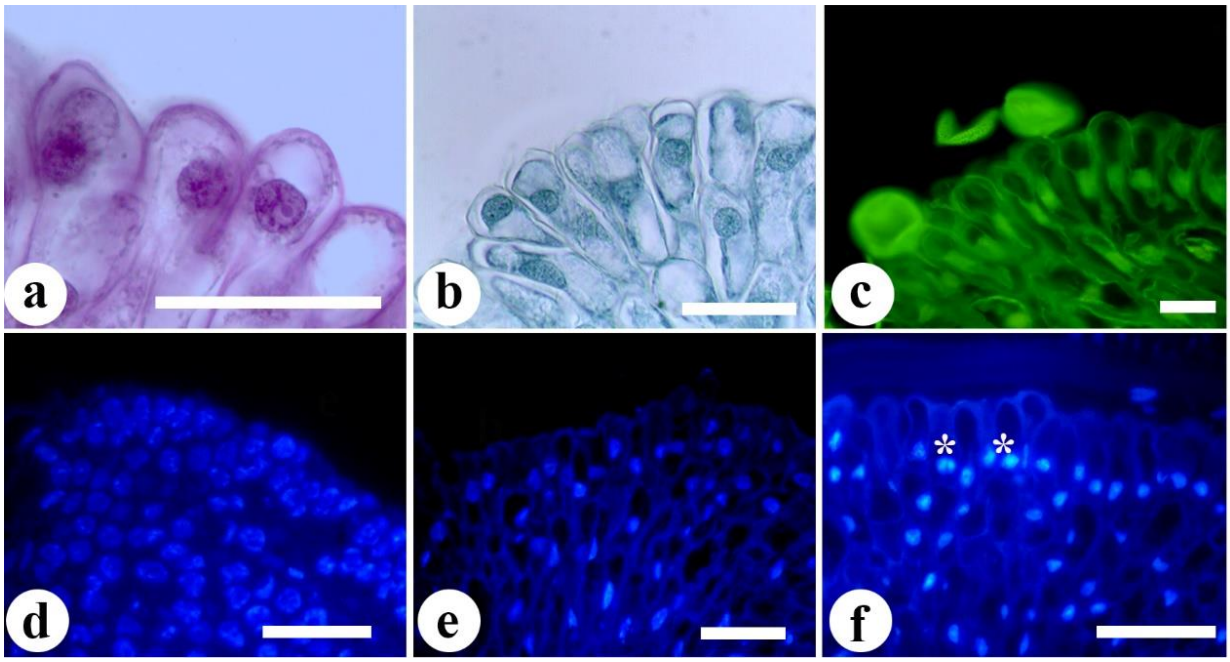

Figure 3.4. Stigmatic papillae of *V. tinus*. a. PAS stained papillae, b. Coomassie Brillat Blue stained papillae, c. Auromine O stained papillae, d. Stigma papillae with shperical nuclei at young stigma, e. Stigma papillae with shperical nuclei before pollination, f. Degenerated nuclei of stigmatic papillae after pollination. Bar: 20 µm (c), 50 µm (a, b, d, e, f). Bar: 50 µm.

4.Conclusion

Some developmental and embryological features of *V. tinus* flower were defined in this study. The findings present valuable information that can be used in systematic, cell biology and, plant reproduction. Moreover, the findings emphasize the importance of biochemical processes and programmed cell death in plant sexual reproduction.

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