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Anther Ontogeny and Microsporogenesis in *Helianthus annuus* L. (Compositae)

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Abstract

In this study, anther ontogeny and microsporogenesis were analysed in *Helianthus annuus* L. The undifferentiated anther is ovoid-shaped and the differentiation starts with the appearance of archesporial cells. Mature anthers are tetrasporangiate. The anther wall is composed of epidermis, endothecium, middle layer and plasmodial tapetum. Endothecial cells show no fibrous thickening. Tapetum is amoeboid type with binucleate cells. Epidermal layer remains intact until anther dehiscence; however, middle layer, endothecium and tapetum disappear during development. At the end of regular meiotic division tetrahedral microspore tetrads are formed. Pollen grains are triporate, suboblate and angulaperturate.

Keywords: anther ontogeny, anther wall, Asteraceae, microsporogenesis

Introduction

Helianthus annuus L. is a member of Asteraceae (Compositae), which is the largest family of flowering plants, with approximately 1,620 genera and more than 23,600 species (Anderberg *et al*., 2007). Therefore, Asteraceae family needs more data about the additional taxonomic characters such as anther and pollen development. The number of anther wall layers, tapetum type and number of tapetal nuclei are taxonomically important characters.

The anther wall is formed by a specific number of cell layers, those originate in the earliest developmental stages (Garcia, 2002). Four types of anther wall development were described by Davis (1966) based on the secondary parietal layers: basic type, dicotyledonous type, monocotyledonous type and reduced type. Anther wall formation of *H. annuus* L. is dicotyledonous type (Davis, 1966).

In most angiosperms, the anther wall consists of four layers from outer to inner: an epidermis, an endothecium, one or two rows of middle layer(s) and tapetum. The outermost cell layer of the anther is epidermis, which in most plants is slightly modified during development (Rezanejad, 2008). This layer is a multifunctional tissue, playing important roles in water relations, defence and pollinator attraction (Rezanejad, 2008). The endothecium with cell walls of uneven thickness due to irregular lignification is responsible for the tensions that lead to splitting of the anther (Wilson *et al*., 2011). In angiosperms, middle layer have one or two rows, and the number of rows may have taxonomic value.

Two types of tapetum are recognized in plants. In the secretory type, the tapetal cells remain associated with the anther wall until their degeneration and secrete substances to the anther locule. In the plasmodial type, the tapetal cell walls are broken down and the protoplasts protrude into the locule fusing to form a coenocytic periplasmodium around the microspore mother cells or developing microspores. The tapetum plays an important role in the nutrition and development of microspores (Chapman, 1987; Pacini, 1990; Polowick and Sawhney, 1992; Shivanna and Johri, 1985) and it also involves directly in the breakdown of the callose wall around the microspore tetrads (Shivanna and Johri, 1985). The type of tapetum may have taxonomic value in the sense that members of most angiosperm families have the same type (Davis, 1966; Johri *et al*., 1992). Although the member of Asteraceae family have either a secretory or amoeboid tapetum (Pacini *et al*., 1985), *H. annuus* L. have amoeboid tapetum (Pacini, 1996).

In the present study, the events taking place during the anther ontogeny in *H. annuus* L. were documented with a special attention to the structure and the development of the anther wall using light microscope and scanning electron microscope (SEM).

Material and methods

The capitulum of *H. annuus* L. was collected from Tekirdağ (Turkey) and hermaphrodite flowers (florets) of capitulum were morphologically analysed and picked up under stereomicroscope. Stamens were carefully excised and fixed in acetic alcohol (1:3, v/v) and embedded in paraffin. Then sliced at 8-10 μm by a rotation microtome. For developmental studies, sections were stained with Delafield's hematoksilin. For cytochemical analysis, sections were stained with periodic acid-Schiff (PAS) for insoluble polysaacharides

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(O'Brien and McCully, 1981), with Coomassie brilliant blue for proteins (Fisher, 1968). The preparations were photographed with an Evolution LC color camera and an Olympus BH-2 microscope, and the images were analyzed with Image-Pro Express Version 6.0 scientific image processing and analysis software.

For investigtion of anther and pollen morphology by SEM analysis, the material was fixed in 2.5% glutaraldehyde in 50 mM cacodylate buffer, pH 7.0 (Platt *et al*., 1983) and then dehydrated with an increasing ethanol gradient: from 70% up to 100%. Then, the material for drying was kept in various percentages of ethanol-HMDS solution at room temperature (Topçuoğlu *et al*., 2009) and coated with 11 nm of gold by using an automated sputter coater and then examined with a SEM (JEOL JMS-59 10LV).

Results

The developmental proceses in the anthers of *H. annuus* L. are classified into four stages: sporogen tissue, pollen mother cell, microspore tetrad and mature pollen stage. Anther wall development and microsporogenesis were investigated according to this classification. The correlation between anther lenght and developmental stages is presented at Table 1.

Table 1. Correlation between anther lenght and developmental stage in *H. annuus* L.

Anther lenght (mm)	Developmental stage
≤ ~ 0.5	Undifferantiated anther
~ 0.5-1.5	Sporogenous tissue
~ 1.5-2.2	Pollen mother cell
~ 2.2-4	Microspore tetrad
~ 4-10	Mature pollen stage

Structure of the androecium

The inflorescence of *H. annuus* L. has two types of flowers (or florets) on a single capitulum, central hermaphrodite disc florets and peripheral pistillate ray. Androecium of *H. annuus* L. in hermaphrodite florets consists of five stamens, with free filaments and fused anthers that produce a tube surrounding the style. Anthers are yellow, bazifiks and tetrasporangiate. Mature stamens are 10-12 mm in length (Fig. 1A) and anthers are longer than the filaments (Fig. 1B). Filaments are around 2 mm in length at maturity. Anthers have sharp tops and trichomes (Fig. 1C).

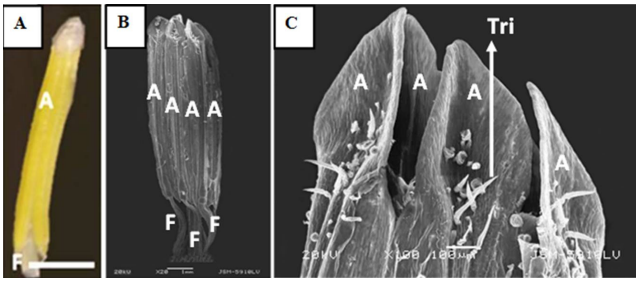

Fig. 1. Male reproductive organ in the hermaphrodite florets of *H. annuus* L. A- Mature anther viewed by stereomicroscope; B- Long anthers and anther tube viewed by SEM; C- Anthers with sharp tops and trichomes viewed by SEM; inside images: A- Anther, F- Filament, Tri- Trichome; Bar: 1 mm (A, B), 100 μm (C)

Ontogeny

In transverse sections, the undifferentiated anther of *H. annuus* L. was monitored as a homogeneous mass of cells surrounded by a single layer of epidermal cells (Fig. 2A). Then differentiation started in each sporangium by the development of the archesporial cells. They have prominent nuclei and dense cytoplasm, which make them distinguishable from the rest of cells (Fig 2B). Archesporial cells undergo periclinal divisions forming the primary parietal layer towards the epidermis and the sporogenous layer to the connective site. Then, the cells of parietal layer result in the formation of an epidermis, endothecium, a uniseriate middle layer and tapetum, by successive periclinal and anticlinal divisions. The sporogenous cells undergo mitotic divisions to give pollen mother cells. The anthers enlarge during the meiotic division and they become tetrasporangiate (Figs. 2C-F).

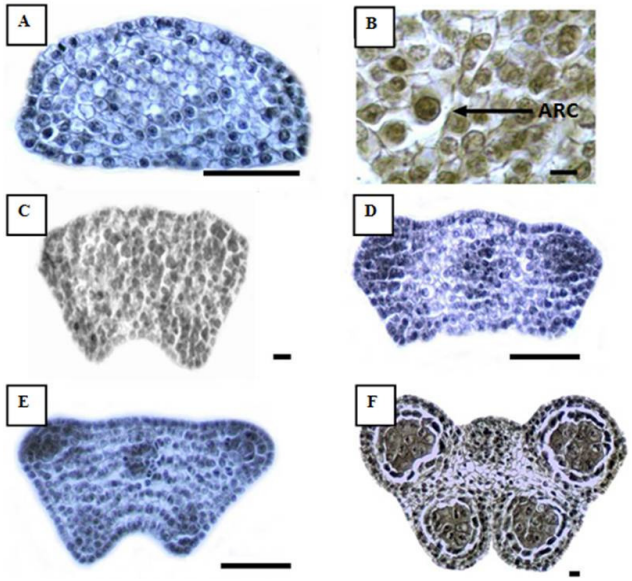

Fig. 2. Ontogeny of an anther in transverse sections of *H. annuus* L. A- Undifferentiated anther; B- Archesporial cell with prominent nucleus and dense cytoplasm; C- The anther become slightly lobed; D- Sporogenous tissue started to become visible; E- General view of four-lobed anther; F- Mature, tetrasporangiate anther with four pollen sacs; ARC: Archesporial cell. Bar: 10 μm (B, C, F) and 100 μm (A, D, E)

Structure and development of the anther wall

The anther wall of *H. annuus* L. consists of four layers from outer to inner: an epidermis, endothecium, one row of middle layer and a layer of tapetum (Fig. 3).

In sporogen tissue stage, four anther layers locate as proper cell lines. Epidermis is a single row and consists of swollen and flattened cells (Fig. 3). The epidermal cells tangentially elongate in the course of maturation and preserve their vitality until the period of anther dehisce (Fig. 4A-D).

The single layer of endothecium consist of longitudinal elongated cells (Fig. 3). Endothecial layer develops until the period of anther dehiscence (Figs. 4A-C) and they show no wall thicknening in the course of development. At the end of the development, the connection of endothecium with internal tissues is cut and the volume of cells start to shrink. The endothecial layer becomes more thin.

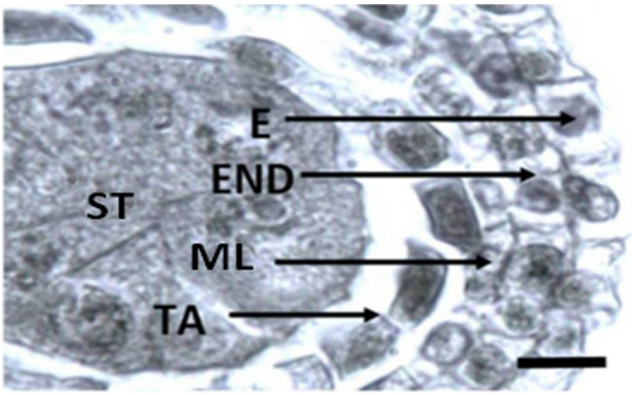

Fig. 3. Anther wall layers and sporogenous tissue of *H. annuus* L.; E: Epidermis, END: Endothecium layer, ML: Middle layer, TA: Tapetum, ST: Sporogenous tissue. Bar: 10 µm

The middle layer is a single row and ephemeral. It consists of flattened cells (Fig. 3), which disappears when the pollen mother cells are at tetrad stage (Fig. 4B).

Tapetum is composed of a single layer of cells. They have bigger volume, dense cytoplasm and larger spherical nuclei than the rest of cells of anther wall (Fig. 3). Tapetum is of periplasmodial type in *H. annuus* L. As a result of the mitosis, the tapetal cells become binucleate (Fig. 4A) and the dissolution of the cell walls starts. Inner transverse and longitudinal wall of tapetal cells are broken down at microspore tetrad stage (Fig. 4B). After dissolution of walls, the protoplasts move into the anther loculi (Fig. 4C). Afterwards, the protoplasmic mass merges and generates tapetal periplasmodium. When tapetal cells are binucleated, middle layer starts to disappear (Fig. 4A). Meanwhile, elongation of epidermis and endothecial cells continue.

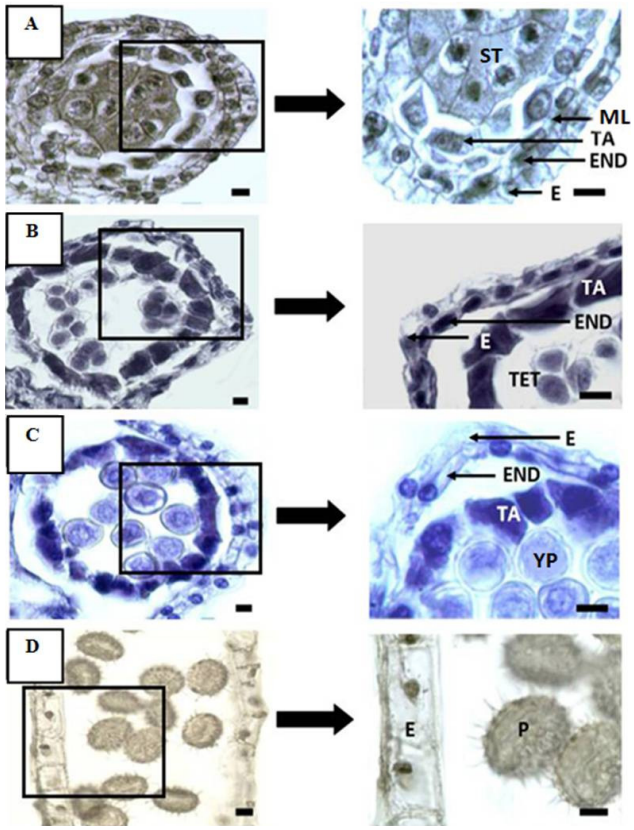

Fig. 4. Anther wall layers at different developmental stages; A- At sporogenous tissue stage; B- At microspore tetrad stage; C- At young pollen stage; D- At mature pollen stage; ST: Sporogenous tissue, E: Epidermis, END: Endothecium, ML: Middle layer, TA: Tapetum, TET: Tetrad, YP: Young pollen, P: Pollen. Bar: 10 µm

In advanced stages, the tapetal periplasmodium is fully inserted into the anther loculi and surrounds microspores firmly (Fig. 4C). While epidermal layer continues to exist, endothecium crushes by the pressure of the surrounding tissues. At mature pollen stage, tapetum is consumed and endothecium completely dissappears (Fig. 4D). In this stage, only epidermis is observed as an anther layer (Fig. 4D).

In order to detect the presence of proteins and insoluble polysaccharides in tapetum, the tissue was stained with Comassie brillant blue and PAS. It stained intensely with Comassie brilant blue, revealing its rich protein content (Fig. 5A), but it gave weak reaction with PAS, suggesting its poor content of insoluble polysaccharides (Fig. 5B).

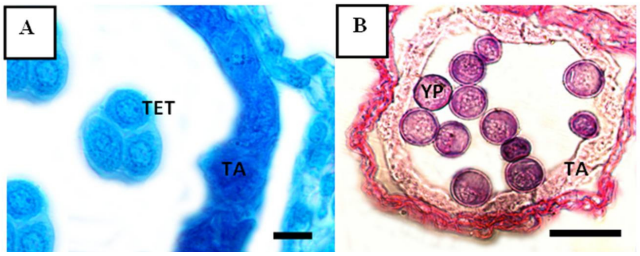

Fig. 5. *H. annuus* L.' s anthers stained with Coomassie brillant blue (A) and with PAS (B); TET: Tetrad, TA: Tapetum, YP: Young pollen. Bar: 10 µm (A), 50 µm (B)

Meiosis at pollen mother cell

Pollen mother cells are isodiametric cells, which have generous cytoplasm. They undergo regular meiosis (Figs. 6A-C), forming tetrahedral tetrads surrounding by callose wall. At tetrad stage, spores are independent cells and there is no celluler connection between them (Fig. 5A). Young microspores are small-volumed cells and they have dense cytoplasm, without vacuoles and centrally located nuclei (Fig. 6D). When pollen grains start to increase in volume, numerous small vacuoles appear. In the course of development, small vacuoles fuse to generate a big one. The nucleus is pushed towards one side of the pollen grain. At the

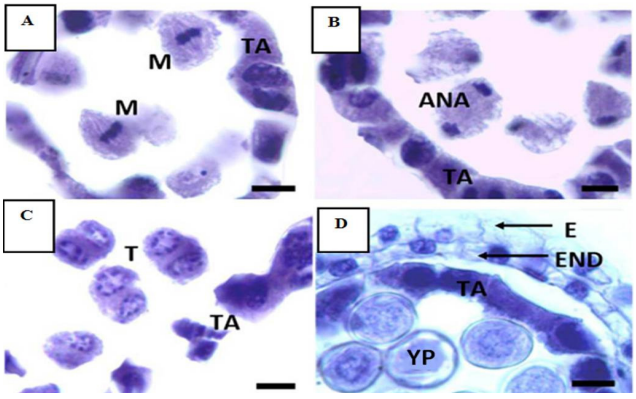

Fig. 6. Some stages of meiosis in the pollen mother cells of *H. annuus* L; A- Metaphase I; B- Late anaphase I; C- Late telophase I; D- Young pollen; TA: Tapetum, E: Epidermis, END: Endothecium layer, YP: Young Pollen, M: Metaphase, ANA: Anaphase, T: Telophase. Bar: 10 µm

end of mitosis, two-celled pollen grains are formed: the larger vegetative cell and smaller generative cell.

Pollen morphology

Pollen grain of *H. annuus* L. is triporate, suboblate and angulaperturate (Horner, 1977). The pollen wall is composed of intine, at innermost layer and exine, at outermost layer (Fig. 7A). Intin gave positive reaction with PAS, indicating that it is made up of insoluble polysaccharides (Fig. 7C). Exine of mature pollen grain is quite thick and it has thin, short and spike like projections. Cytoplasm of mature pollen grains was filled with proteins (Fig. 8B) and insoluble polysaccharides (Fig. 7C). The ornate structure of exine was analysed by SEM (Fig. 8).

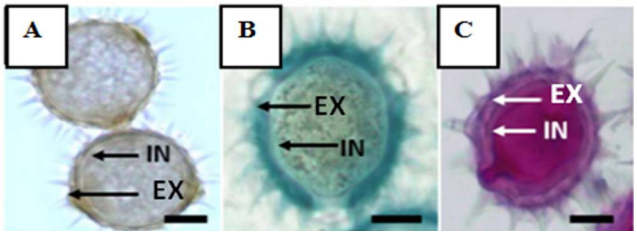

Fig. 7. Pollen grains stained with hematoksilin (A); Coomassie brilliant blue (B); PAS (C); IN: Intine, EX: Exine. Bar: 10 μm

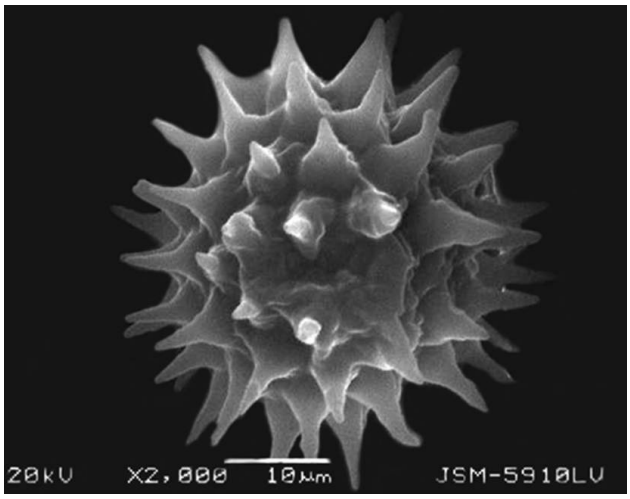

Fig. 8. Scanning electron micrographs of pollen grain to show exine pattern in *H. annuus* L. Bar: 10 μm

Discussions

In Asteraceae family, anther wall ontogeny has been studied in only a few genera (Li *et al*., 2009). This is the first report on anther wall ontogeny in *H. annuus* L.

Although, Venkateswarlu and Devi (1955) were reported that the members of Asteraceae family has bisporangiate or tetrasporangiate anther, the anthers in *H. annuus* L. are tetrasporangiate.

The pattern of anther development in *H. annuus* L. is similar to other flowering plants in general, as well as other members of the Asteraceae, such as *Chrysanthemum morifolium* (Li *et al*., 2009). The anther wall development in the *H. annuus* L. is of the dicotyledonous type, as specified by Davis (1966).

H. annuus L. shares a number of embryological features with other Compositae species as compiled by Venkateswarlu and Devi (1955). Young anthers consist of epidermis, endothecium, middle layer and tapetum, as specified by Gotelli *et al*. (2008). Epidermis is permanent (Venkateswarlu and Devi, 1955). In most of angiosperms, fibrous thickenings develop from the inner tangential and radial walls of the endothecial cells during microsporogenesis, but they show no thickenings in the walls of *H. annuus* L. After the first meiotic division, the middle layer cells start a slow degeneration process (Gotelli *et al*., 2008).

All members in two families, such as in the Gramineae and Leguminosae, share the same type of tapetum; other 12 families of angiosperms have two types of tapetum (Davis, 1966; Johri *et al*., 1992). In the Asteraceae, however, there are many types of tapetum. Pacini (1996) defined four types of tapetum, such as the *Sonchus oleraceus* type, the *Cosmos bipinnatus* type, the *Helianthus annuus* type and the *Cichorium intybus* type. The tapetum of the *Helianthus annuus* type belongs to the periplasmodial type. Similar phenomenon was also reported by Laveau *et al*. (1989) and Lersten and Curtis (1990) in *H. annuus* L. and *Ambrosia trifida*, respectively.

In *H. annuus* L. pollen mother cells undergo meiotic division simultaneously and produce tetrahedral microspore tetrads, as in *Vernonia divergens* and *Adenostema lavenia*, while in *Elephantopus scaber* (Vernonieae) and *Adenostema rugosum* (Euparieae), pollen tetrads have an isobilateral arrangement (Pullaiah, 1979). Mature pollen grains are suboblate, triporate and angulaperturate with spherical amb as specified by Gotelli *et al*. (2008).

Conclusions

The present study reveals that the anther wall ontogeny and microsporogenesis of *H. annuus* L. Anther wall formation of *H. annuus* L. is dicotyledonous type. Mature anthers are tetrasporangiate, composed of epidermal layer, endothecium layer, middle layers and amoeboid tapetum layer. Only epidermis layer preserves their vitality until the period of anther dehisce. Pollen mother cells undergo regular meiosis. Microspore tetrads are tetrahedral. Pollen grains are triporate, suboblate, big size and angulaperturate.

Acknowledgments

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