ORIGINAL ARTICLE

Ultrastructure of microsporogenesis and microgametogenesis in *Campsis radicans* (L.) Seem. (Bignoniaceae)

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Abstract In the present study, microsporogenesis, microgametogenesis and pollen wall ontogeny in *Campsis radicans* (L.) Seem. were studied from sporogenous cell stage to mature pollen using transmission electron microscopy. To observe the ultrastructural changes that occur in sporogenous cells, microspores and pollen through progressive developmental stages, anthers at different stages of development were fixed and embedded in Araldite. Microspore and pollen development in *C. radicans* follows the basic scheme in angiosperms. Microsporocytes secrete callose wall before meiotic division. Meiocytes undergo meiosis and simultaneous cytokinesis which result in the formation of tetrads mostly with a tetrahedral arrangement. After the development of free and vacuolated microspores, respectively, first mitotic division occurs and two-celled pollen grain is produced. Pollen grains are shed from the anther at two-celled stage. Pollen wall formation in *C. radicans* starts at tetrad stage by the formation of exine template called primexine. By the accumulation of electron dense material, produced by microspore, in the special places of the primexine, first of all protectum then columellae of exine elements are formed on the reticulate-patterned plasma membrane. After free microspore stage, exine development is completed by the addition of sporopollenin from tapetum. Formation of intine layer of pollen wall starts at the late vacuolated stage of pollen development and continue through the bicellular pollen stage.

Introduction

Male gametophyte is produced in the pollen sacs of the anther by two successive developmental phases, microsporogenesis and microgametogenesis. Microsporogenesis includes the series of progressive developmental stages from sporogenous cells to haploid unicellular microspores. During microsporogenesis, the diploid sporogenous cells differentiate as microspore mother cells (meiocytes) which undergo meiotic division to form four haploid microspores (tetrad) enclosed by the callose envelope. By the release of microspores from the callose envelope, microsporogenesis is completed and microgametogenesis, which gives rise to mature microgametophytes from one celled microspores starts.

Studies on pollen ontogeny make significant contributions to the taxonomic and phylogenetic studies and understanding of the reproductive biology of the seed plants. For this reason, numerous cytochemical and ultrastructural studies on pollen development (microsporogenesis and microgametogenesis) have been carried out in angiosperms (Polowick and Sawhney 1992; Hess 1993; Owen and Makaroff 1995; Rosenfeldt and Galati 2005; Liu et al. 2012; Galati et al. 2012; Gotelli et al. 2012). However, in Bignoniaceae these studies are limited (Mehra and Kulkarni 1985; Galati and Strittmatter 1999; Bittencourt and Mariath 2002) and so far, no ultrastructural studies on the pollen ontogeny of genus *Campsis* have been published. Therefore, in the present study series of ultrastructural changes, which lead to the development of bicellular

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pollen grain from sporogenous cells in the anther tissue of *Campsis radicans*, were investigated. Since pollen wall architecture varies among different species of plants and it has taxonomic value, microsporogenesis and microgametogenesis in *Campsis radicans* were examined with a special attention to the sporoderm (pollen wall) development.

Although ultrastructure of microsporogenesis and microgametogenesis in *C. radicans* is being reported for the first time in the present study, a few morphological and ontogenic studies have been carried out on its pollen grains and anther formerly. Previous morphological and ontogenetic studies made on *C. radicans* anther have revealed that (1) it has spheroidal 3-colpate pollen grains with reticulate ornamentation and semi-tectate tectum (Halbritter 2000; Tütüncü et al. 2007), (2) microspore cytoplasm contains variable amounts of proteins, lipids, and insoluble carbohydrates at different stages of pollen development (Tütüncü Konyar and Dane 2013a), (3) its anthers are tetrasporangiate. The wall of the young anther consists of epidermis, endothecium, middle layer and the secretory type tapetum with Ubisch bodies. (Tütüncü Konyar and Dane 2013b), (4) distribution of carbohydrates, lipids, and proteins in the anther tissue changes regularly depending on the physiological activities such as differentiation, cell division, callose breakdown, and material synthesis that takes place during pollen development (Tütüncü Konyar et al. 2013).

Materials and methods

Fresh flower buds of different sizes and at different stages of development were collected from *C. radicans* plants found as ornamental plants in parks and gardens of Edirne in European Turkey.

Following the dissection from the buds, anthers belonging to successive developmental stages (from 1 up to 7 mm in length) were measured and classified into groups according to their length related to the pollen development stage (Table 1). Their stage of development had been determined before by examining paraffin-embedded sections stained with Alcian blue and Nuclear fast red under a light microscope (LM) (Tütüncü Konyar and Dane 2013b).

For transmission electron microscopy (TEM) studies, the anthers were prefixed immediately in 3 % glutaraldehyde in Sorensen's buffer (pH 7.4) for 4 h at 4 °C and rinsed in Sorensen's buffer three times. After postfixation with 1 % osmium tetroxide in the same buffer (pH 7.4) for 2 h (at room temperature), the anthers were dehydrated in a series of increasing concentrations of ethanol in water and were embedded in Araldite according to the usual method of electron microscopy (Glauert and Glauert 1958).

Table 1 Correlation between anther size and developmental stage of microspores of *Campsis radicans* (Tütüncü Konyar and Dane 2013b)

Anther length (mm)	Pollen developmental stage
1	Undifferentiated anther
1.5	Sporogenous cell stage
2–3	Microspore mother cell stage
3	Meiotic divisions of microspore mother cells
3.5–4	Tetrad stage
4.5–5	Free microspore stage
5.5–6	Vacuolated microspore stage
7	Two-celled pollen (mature pollen)

Ultrathin sections (50–120 nm) were cut from the Araldite embedded material with a Leica Em UC6 ultramicrotome and stained with uranyl acetate and lead citrate. Electron micrographs were taken with a FEI Tecnai G2 (120 kv) transmission electron microscope.

Terminology

The terminology of the different exine layer of *C. radicans* was defined according to Hesse et al. (2009).

Results

Although microsporogenesis and microgametogenesis are a continuous process, to describe the events taking place during the pollen ontogeny in *C. radicans* clearly, the process was classified into six developmental stages as sporogenous cell, pollen mother cell, tetrad, free microspore, vacuolated microspore, and bicellular pollen stages. The developmental stages are synchronized in the microsporangia of the same anther.

Sporogenous cell stage

Sporogenous tissue stage has been determined in anthers 1–1.5 mm in length. Mostly, two longitudinal rows of sporogenous cells that completely fill the locular space were observed in the crescent-shaped anther locule. Sporogenous tissue had slightly polygonal cells with large centrally located nuclei and prominent nucleoli (Figs. 1a, c, 2a). Sporogenous cells were surrounded by thin cell walls having plasmodesmata. As reported earlier (Tütüncü Konyar et al. 2013), cytoplasm of the sporogenous cells were poor in insoluble polysaccharides, protein, and lipid bodies. Furthermore, numerous mitochondria and a few vacuoles were observed in the cytoplasm (Figs. 1a–c, 2). Sporogenous cells increased in number by mitotic divisions.

Fig. 1 TEM micrographs of section through a single anther locule showing sporogenous tissue and initially differentiated tapetal cell in 1-mm length anther. **a–c** Ultra structure of sporogenous cells. *Arrow* shows the plasmodesma between adjacent sporogenous cells. **d** Ultra structure of inner tapetal cell. *Arrows* show the plasmodesmata between the tapetal cell and sporogenous cell. (*CW* cell wall, *iTa* inner tapetum, *N* nucleus, *Nu* nucleolus, *Spc* sporogenous cell, *V* vacuole)
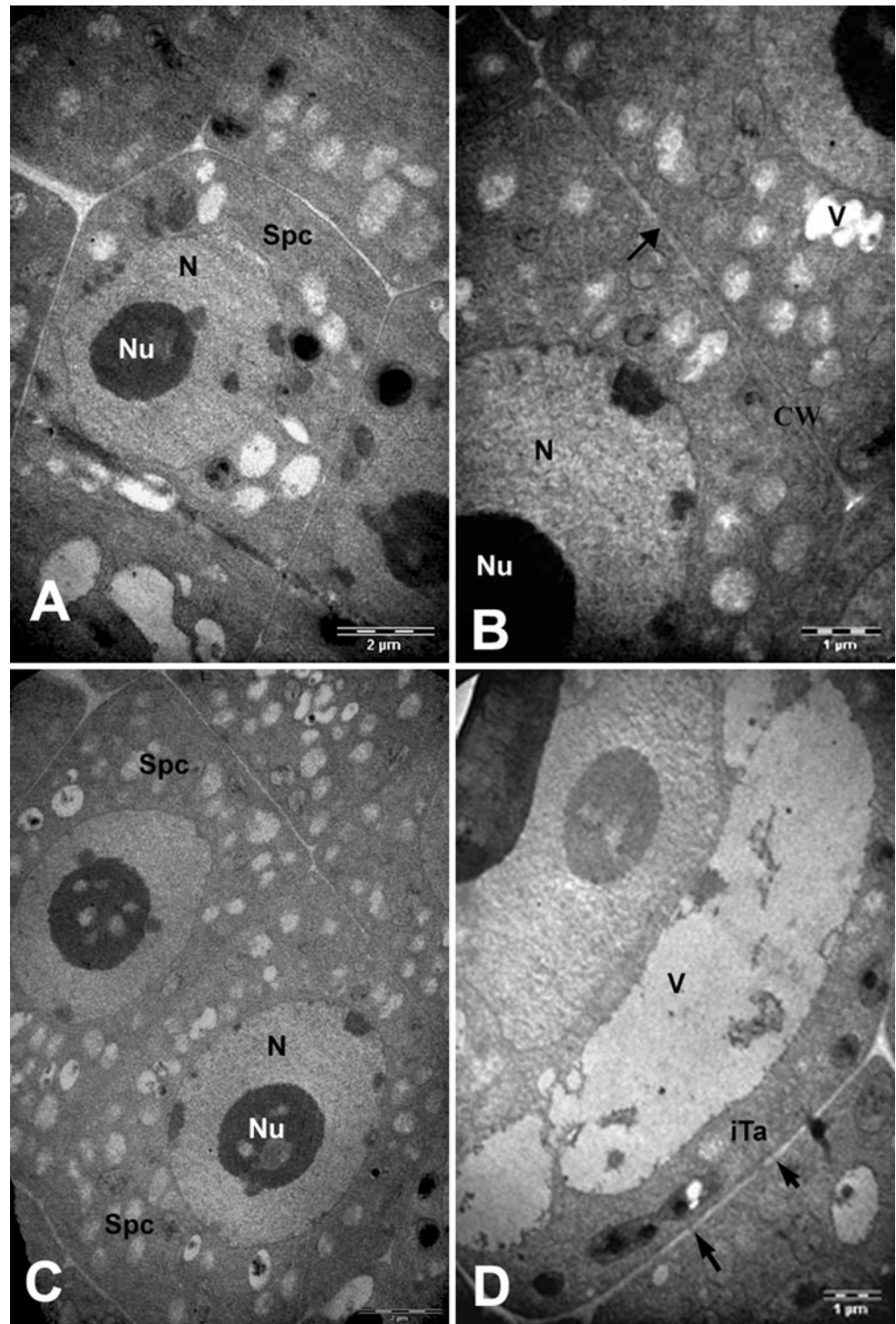
During the early sporogenous cell stage, anther wall was undifferentiated. However, as development proceeds, it completed its differentiation and outer tapetum having large vacuole and one or two nucleus were observed (Fig. 2b). Tapetal cells were also connected to each other and to the microsporocytes by plasmodesmata.

Microspore mother cell stage

Anthers in the microspore mother cell (MMC) stage range in length from 2 to 3 mm. At early premeiotic stage (2.5 mm anther), MMCs still had a centrally located large nucleus and were connected to each other and to the tapetal
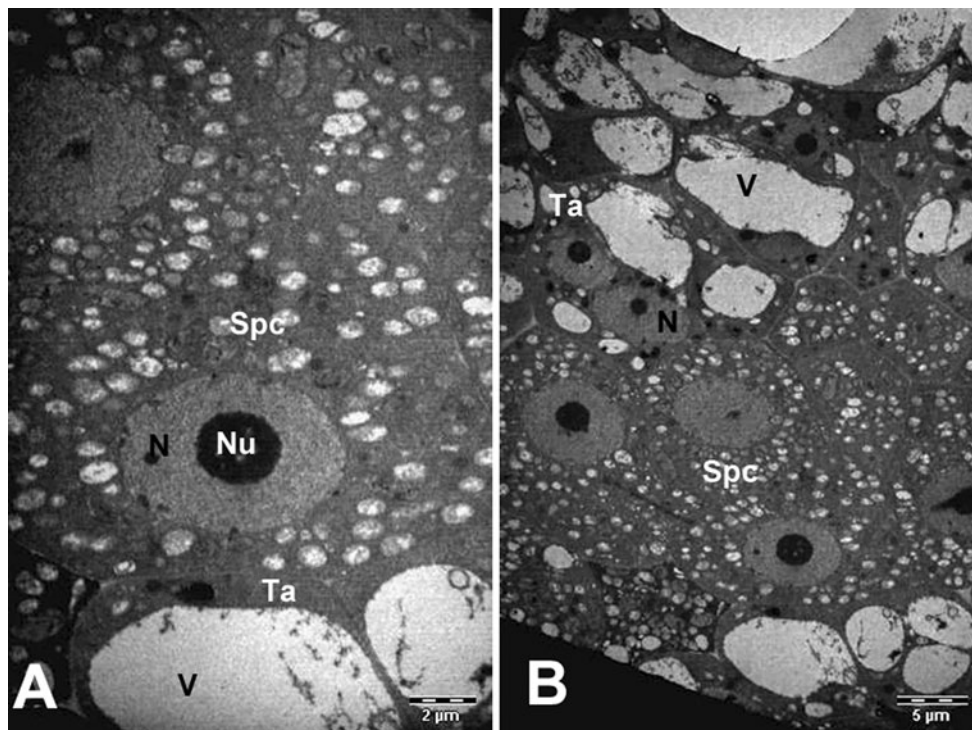
Fig. 2 TEM micrographs of transverse section through a single anther locule showing sporogenous tissue and initially differentiated tapetal cell in 1.5-mm length anther. **a**, **b** Ultrastructure of sporogenous tissue and tapetal cells. (*N* nucleus, *Nu* nucleolus, *Spc* sporogenous cell, *Ta* tapetal cell, *V* vacuole)

cells by plasmodesmata (Fig. 3a–c). Subsequent to accumulation of callose around the MMCs, plasmodesmata were replaced by the large cytoplasmic channels, whereas tapetal cells were connected to each other by their original plasmodesmata. At premeiotic stage, numerous plastids (containing starch), mitochondria, and small vacuoles were observed throughout the microsporocyte cytoplasm (Fig. 4). The amount of reserve materials (polysaccharides, lipids and proteins) in microsporocyte cytoplasm had increased compared with the previous stage (observed in semi-thin sections; data is not shown). At meiotic stage, the meiocytes increased in volume and rounded. They lost their polygonal shape (Fig. 5), and were surrounded by thick callose wall.

Tetrad stage

At the end of meiotic division, numerous tetrads with tetrahedral arrangement (Fig. 6a) and a few tetrads with isobilateral arrangement were formed by simultaneous cytokinesis. Each tetrad had four haploid microspores and was surrounded by a thick asymmetrical callose wall. Numerous mitochondria, plastids with internal membranes, lipid globules, Golgi vesicles small vacuoles, and endoplasmic reticulum of the rough type were observed in the dense cytoplasm of microspores (Fig. 6a–d). Vacuoles were observed usually near the plasmalemma (Fig. 7b, d). Fibrillar matrix called primexine started to develop between the plasmalemma and callose wall surrounding the each tetrad (Figs. 6, 7).

Exine formation

Pollen wall formation in *C. radicans* started at tetrad stage by the formation of exine template called primexine. At early tetrad stage the plasmalemma, which surrounded the each microspore in a tetrad, was flat and was in contact with the wall enclosing the microspore. However, later it drew away from the callose wall, undulated and its surface became irregular. Undulations were observed at the whole region of the microspores except aperture regions (Fig. 6b, c). Subsequently, by the accumulation of substances between plasmalemma and callose wall surrounding the tetrad, fibrillar matrix called primexine was formed (Fig. 7a). Primexine served as the exine template during the pollen wall development. Deposition of electron dense material at the special sites within the reticulate pattern of the exine template caused the formation of protectum, on the protruding site of the invaginated plasmalemma. Subsequently, procolumellae, in the form of colons extending from the fibrillar material embedded in the lower part of
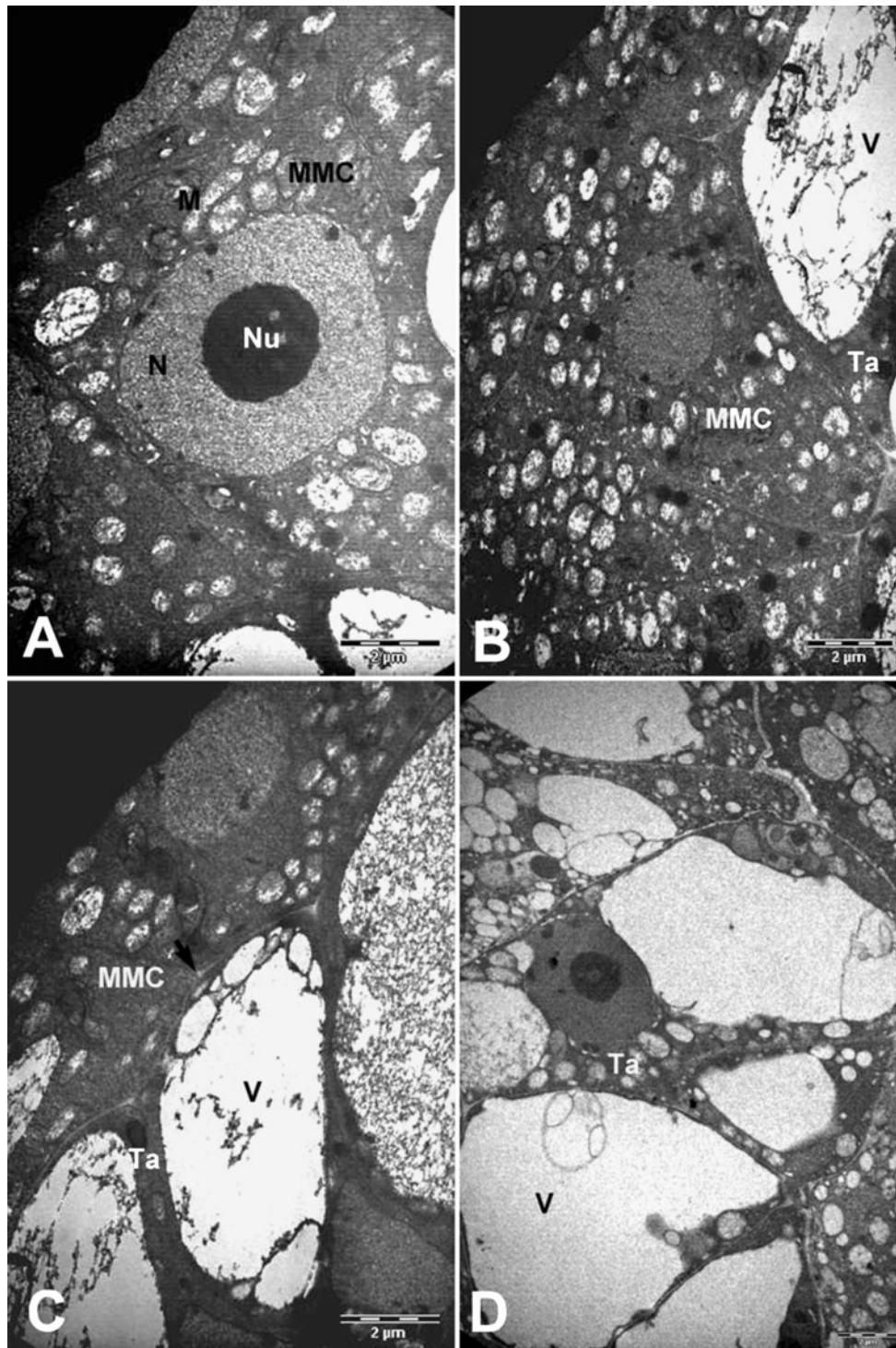
Fig. 3 TEM micrographs of transverse section through anther showing microspore mother cells and tapetal cells at early microspore mother cell stage of microsporogenesis (in 2-mm length anther). **a, b** Ultrastructure of microspore mother cells. **c** Plasmodesmata (*arrow*) between tapetal cell and microspore mother cell. **d** Ultrastructure of outer tapetal cell. (*M* Mitochondrion, *MMC* microspore mother cell, *N* nucleus, *Nu* nucleolus, *Ta* tapetal cell, *V* vacuole)

the plasmalemma to the protectum, were observed (Figs. 6, 7). During the building of exine, many small vesicles and vacuoles were seen near the plasmalemma. Some vesicles were in close contact with the plasmalemma (Fig. 7b, d).

Tapetum

Tapetal cytoplasm was dense and rich in plastids, mitochondria, lipid globules, and dictyosomes (Fig. 8a, b).
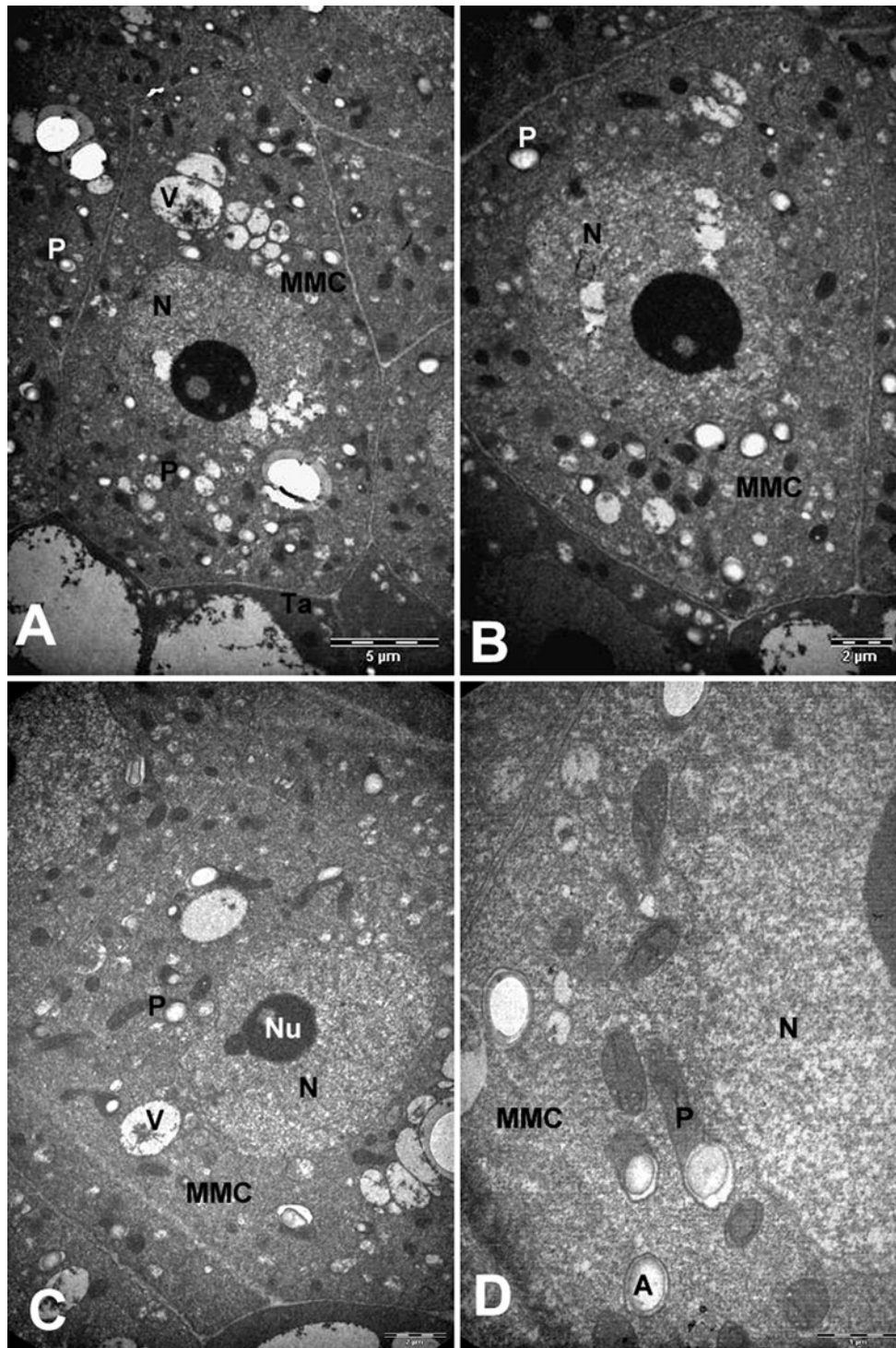
Fig. 4 TEM micrographs of microspore mother cells at premeiotic stage of microsporogenesis. **a**, **b** General view of microspore mother cells. **c**, **d** Details of the cytoplasm of microspore mother cells. Note the numerous plastids and mitochondria in the cytoplasm. (*A* amyloplast, *M* Mitochondrion, *MMC* microspore mother cell, *N* nucleus, *Nu* nucleolus, *P* plastid, *Ta* tapetum, *V* vacuole)

Compared with the previous stage, rough endoplasmic reticulum was more numerous and prominent. The endoplasmic reticulum cisternae were distributed through the cytoplasm of tapetal cells in the form of dense, thick rows (Fig. 8c). Tapetal cells had one or two polarized nuclei (Fig. 8a). Most of the tapetal cells had one centrally located large vacuole, while others had a few small vacuoles. As reported earlier (Tütüncü Konyar and Dane 2013b), proorbicules (Pro-Ubisch bodies) were observed on the inner tangential walls of tapetal cells.
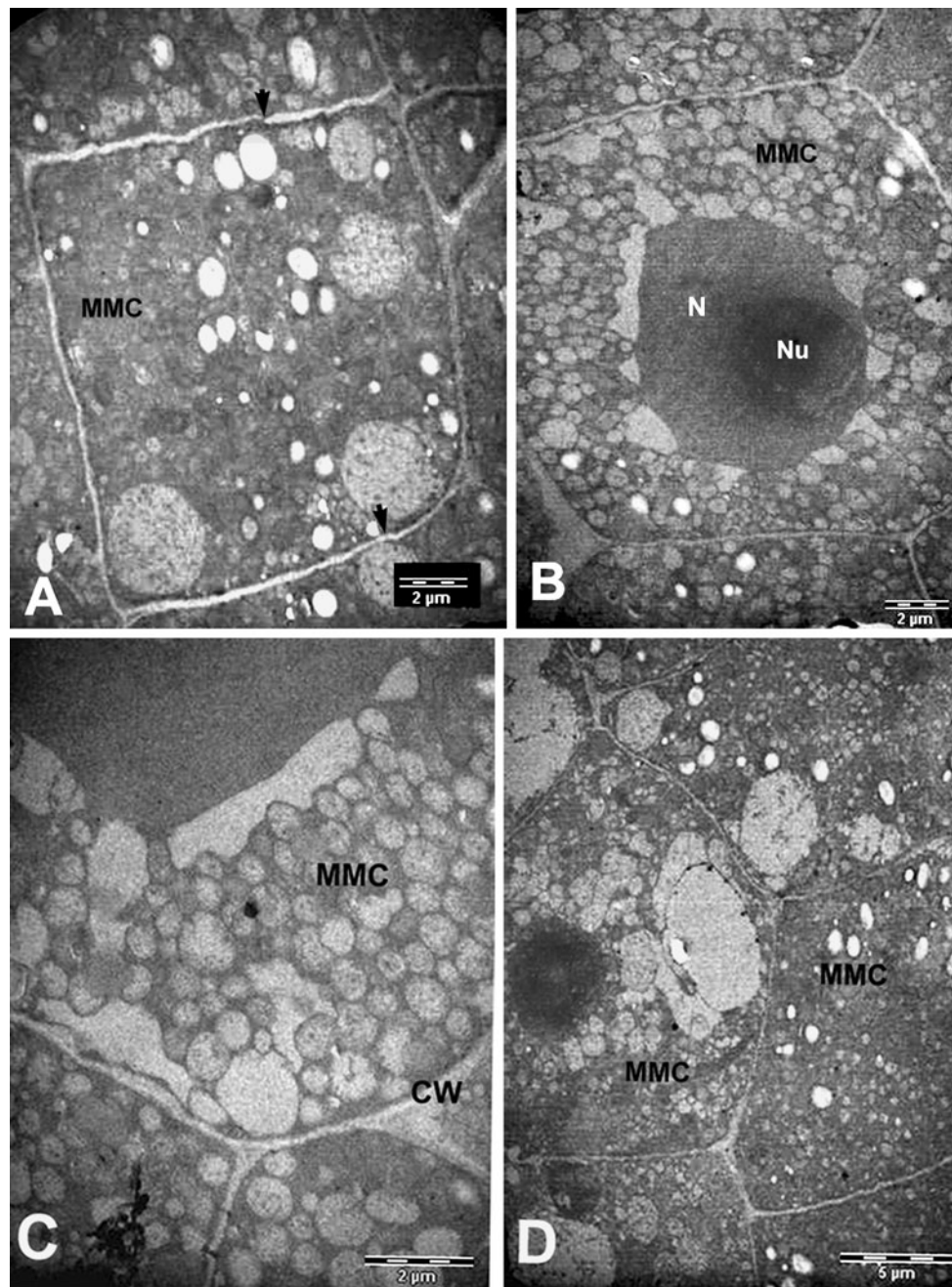
Fig. 5 Microspore mother cells at meiotic stage of microsporogenesis. Note that the cytoplasm of the microspore mother cells is very dense and there are cytoplasmic connections (*arrows*) between them. (*CW* cell wall, *MMC* microspore mother cell, *N* nucleus, *Nu* nucleolus)
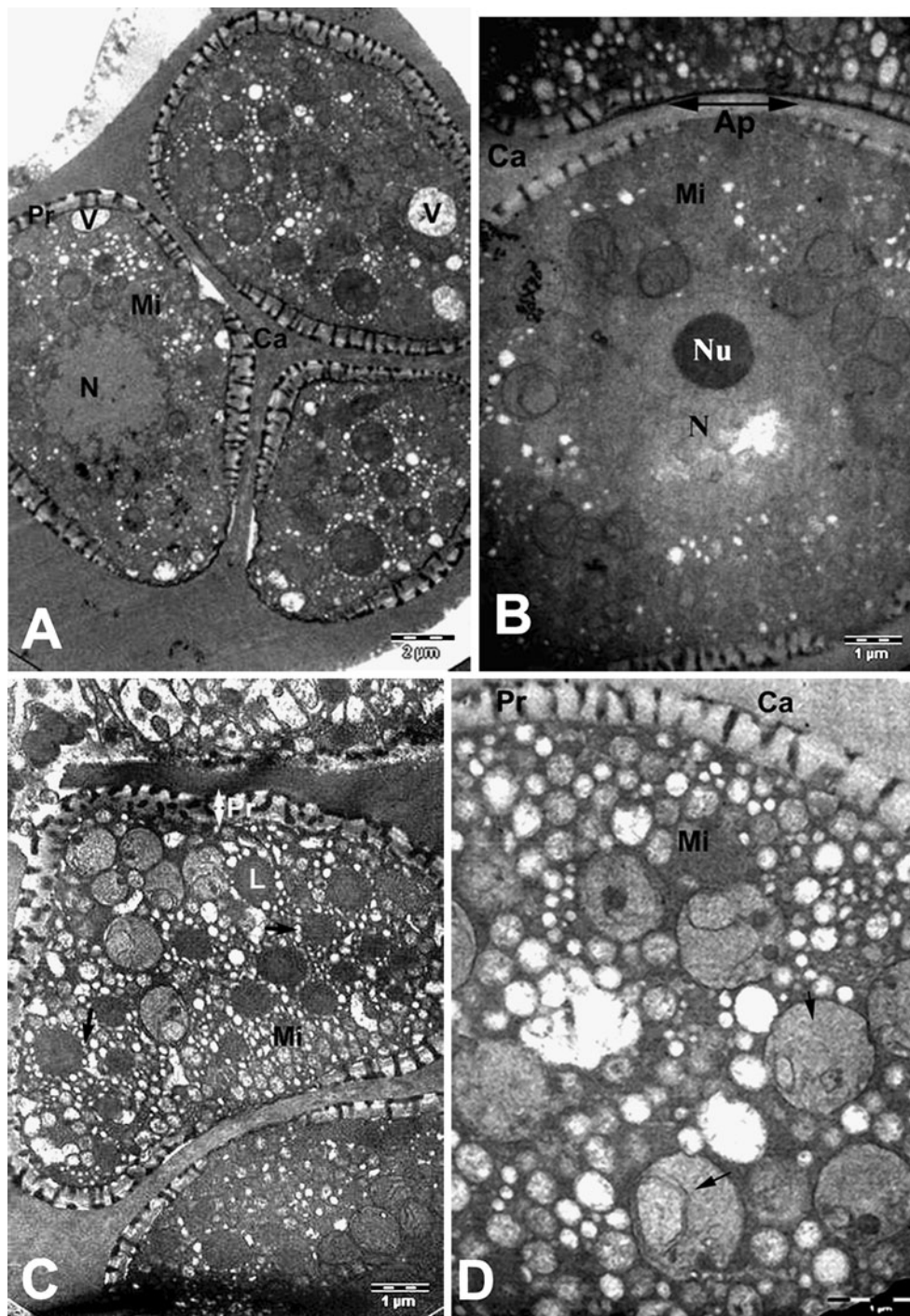
Free microspore stage

Microspore

Following the dissolution of the callose envelope that holds the four microspores together at tetrad stage, microspores became free in the anther locule. The amount of fibrillar material present throughout the locules had increased at this stage (Fig. 9a); at early free microspore stage, microspores were small, wrinkled and somewhat angular in shape, but later they swell and became more rounded. Most of the microspores had a conspicuous nucleus that was located at the center or near the plasmalemma (Fig. 9e, f). Numerous mitochondria, plastids (with or without starch), Golgi vesicles, large osmiophilic globules, were observed in the microspore cytoplasm (Figs. 9a–f, 10a, b). However, reserve substances were scarce in microscopes at this stage. Ubisch bodies were observed on the inner tangential walls of tapetal cells (Fig. 10b–d).

Fig. 6 TEM micrographs of microspores at tetrad stage of microsporogenesis. **a** General view of the tetrad in tetrahedral arrangement. **b** Ultrastructure of single microspore in a tetrad. **c** Ultrastructure of cytoplasm of microspore. There are numerous Golgi vesicles (*arrows*) in the cytoplasm. **d** Details of microspore cytoplasm and primexine. Note that the cytoplasm is very dense and there are many plastids with internal membranes (*arrows*). (*Ap* aperture region, *Ca* callose, *L* lipid body, *Mi* microspore, *N* nucleus, *Nu* nucleolus, *Pr* primexine, *V* vacuole)

Sporoderm

Exine construction, which began at the tetrad stage, progressed through the free microspore stage by addition of sporopollenin from tapetum to primexine. Both the ektexine and endexine were formed at this stage of development. Although the ektexine could be distinguished easily from the endexine layer in PAS staining (see Tütüncü Konyar and Dane 2013a) they were hardly distinguishable in TEM micrographs (Fig. 9).

Vacuolated microspore

Microspore and sporoderm

Many mitochondria were observed in the microspore cytoplasm (Fig. 11a–d). Big vacuole, which was formed by the joining of small vacuoles pushed the nucleus and a part of the cytoplasm towards the one side of the cell. Furthermore, intine layer started to build between endexine and plasma
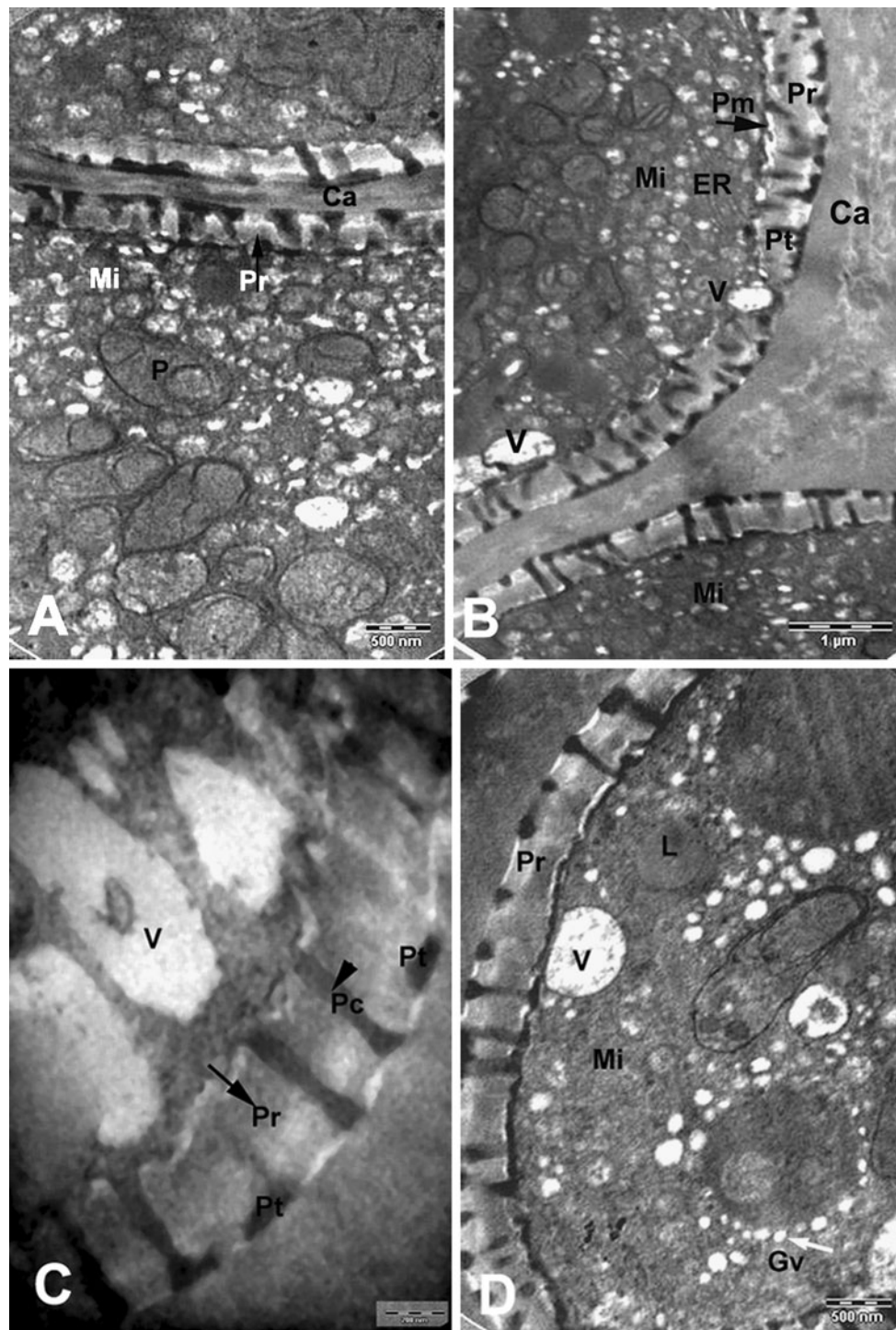
Fig. 7 Ultrastructure of microspore cytoplasm and sporoderm construction in tetrad stage of microsporogenesis. **a** Primexine deposition against the callosic envelope. **b–d** Formation of protectum and procolumellae. (*Ca* callose, *ER* endoplasmic reticulum of rough type, *Gv* golgi vesicles, *L* lipid, *Mi* microspore, *P* plastid, *Pc* procolumellae, *Pm* plasma membrane, *Pr* primexine, *Pt* protectum, *V* vacuole)

membrane (Fig. 11c, d). Exine layer of microspore had the same electron density with Ubisch bodies.

At late vacuolated microspore stage, big vacuoles were divided by a pinching-in of the tonoplast (Fig. 11a).

Fragmentation of large vacuoles into smaller vacuoles was followed by the mitotic division and asymmetrical cytokinesis which lead to the formation of binucleated pollen grains (Fig. 12a).
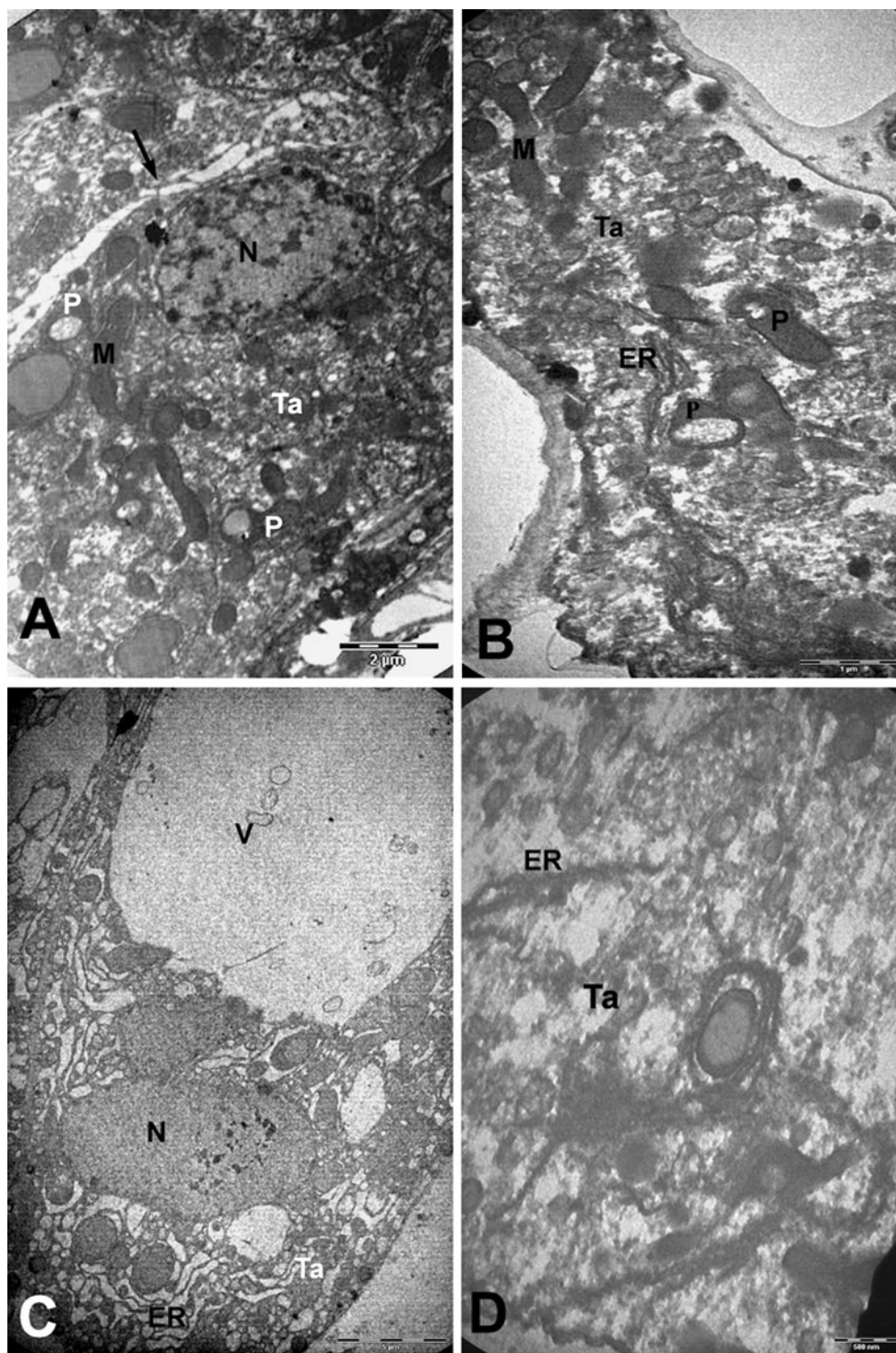
Fig. 8 a–d Ultrastructure of tapetal cells at tetrad stage of micro-sporogenesis. **a**, **b** Cytoplasm of the tapetal cells is very dense and contains numerous plastids and mitochondria. Note the plasmodesma (*arrow*) between adjacent tapetal cells. **c** Note the endoplasmic reticulum of rough type extending through the tapetal cytoplasm. (*ER* endoplasmic reticulum of rough type, *M* mitochondrion, *N* nucleus, *P* plastid, *Ta* tapetal cell)

Tapetum

Since autolysis had started in the tapetum, only the remnants of the tapetum could be seen and nucleoli were observed only in some tapetal cells. In addition to these, many vesicles with osmiophilic contents were observed in tapetal cells (Fig. 12c, d). As reported earlier (Tütüncü Konyar and Dane 2013b), during the maturation of
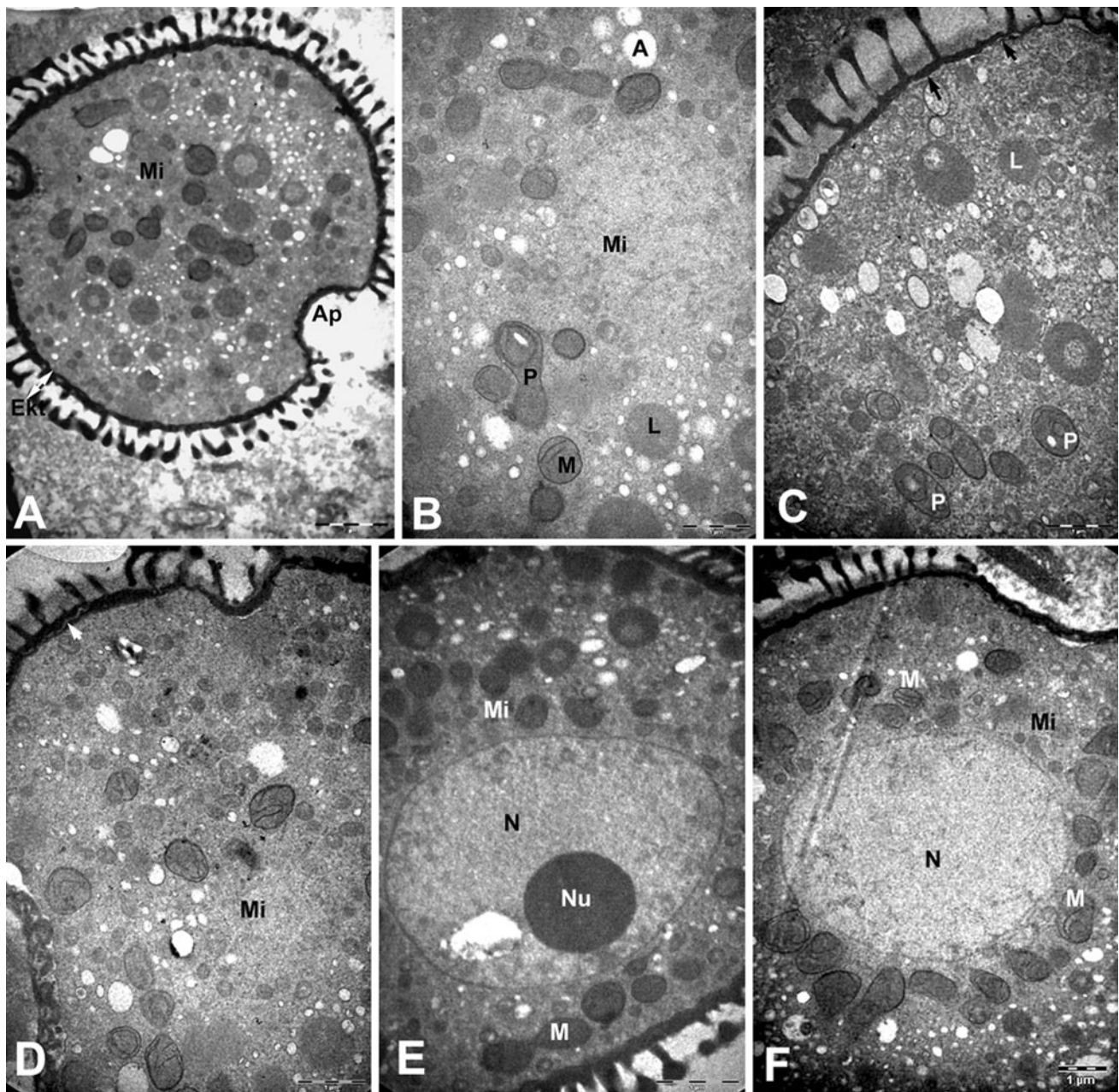
Fig. 9 Ultrastructure of microspores at free microspore stage of development. **a** Polar view of a microspore. **b** Details of the microspore cytoplasm. Note the numerous plastids and mitochondria in the cytoplasm. **c**, **d** Details of pollen wall development and microspore cytoplasm. Note the *single white line* (arrows) below orbicules (Ubisch bodies), pro-orbicules found in them disappeared leaving small cavities with irregular shapes (Fig. 12c, d). Some materials were secreted from tapetal cells into anther locule. However, amount of fibrillar material in the anther locule was less than the previous stage. which the endexine develops. **e**, **f** Polar view of microspores showing the large nucleus in their cytoplasm. Note the numerous mitochondria surrounding the nucleus in **f**. (*A* amyloplast, *Ap* aperture region, *Ekt* ektexine, *L* lipid body, *M* mitochondrion, *Mi* microspore, *N* nucleus, *Nu* nucleolus, *P* plastid)

Bicellular pollen stage

Cytoplasm of bicellular pollen

In the two-celled pollen grain, which was produced by the asymmetric mitotic division, a lens-shaped generative cell
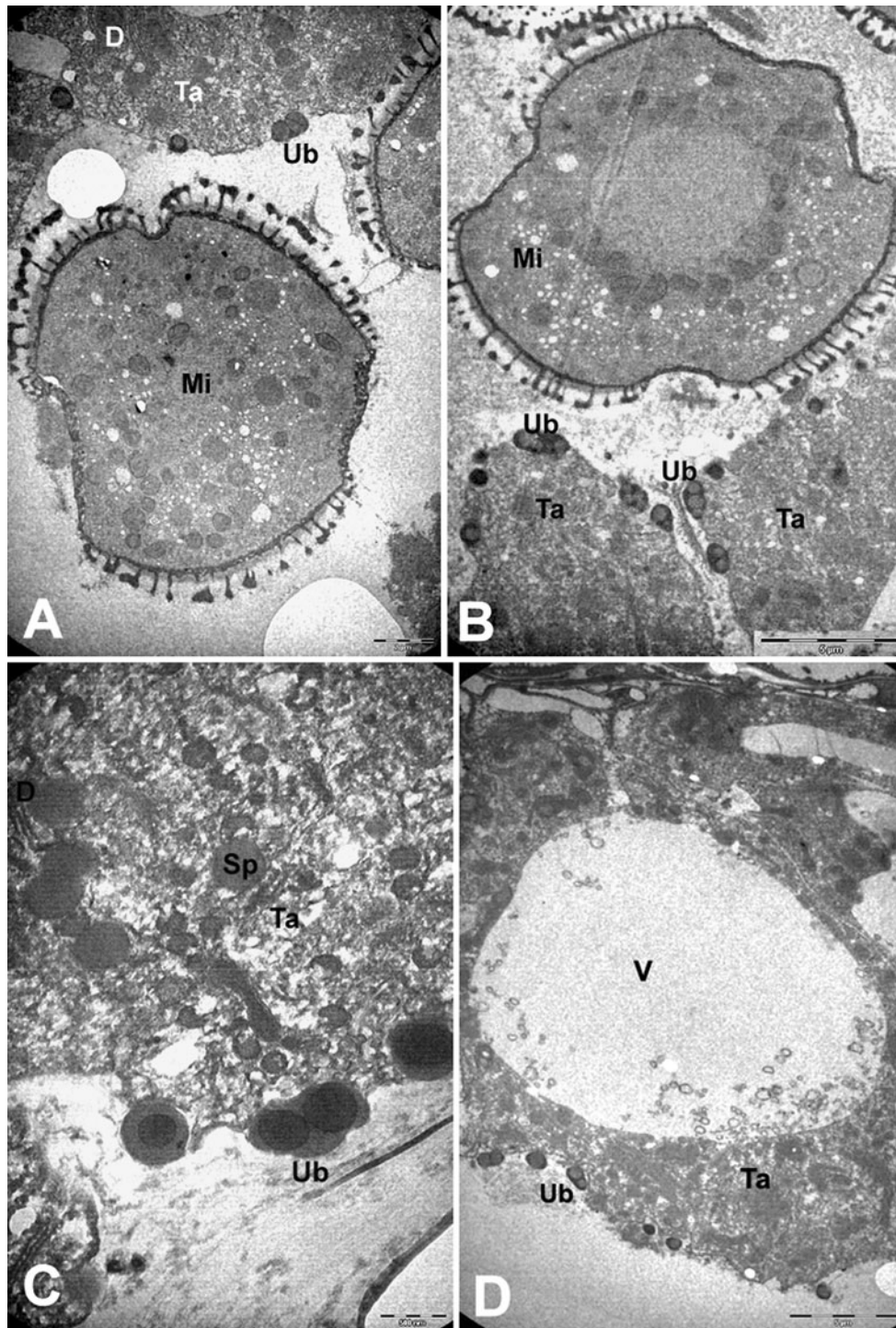
Fig. 10 TEM micrographs of transverse sections through anthers of *C. radicans* at free microspore stage. **a**, **b** Free microspore and parts of the tapetal cells. Note the Ubisch bodies on the inner tangential wall of the tapetal cells. **c** Details of the cytoplasm of the tapetal cell. **d** General view of the tapetal cell. (*Di* dictyosome, *Mi* microspore, *Sp* spherosome, *Ta* tapetal cell, *Ub* ubisch body, *V* vacuole)

was located near the side of the pollen grain adjacent to the vegetative cell. At the early bicellular pollen stage, the wall that surrounds the generative cell was attached to the intine layer (Fig. 12a), but later it detached from the intine and moved in the vegetative cell cytoplasm towards the generative nucleus (Fig. 12b). Cytoplasm of vegetative cell was very dense. Numerous starch granules, small vacuoles, lipid bodies were observed in the cytoplasm (Fig. 13a–c).

Tapetal cells had degenerated completely, and sporopollenin-like tapetal membrane was formed and
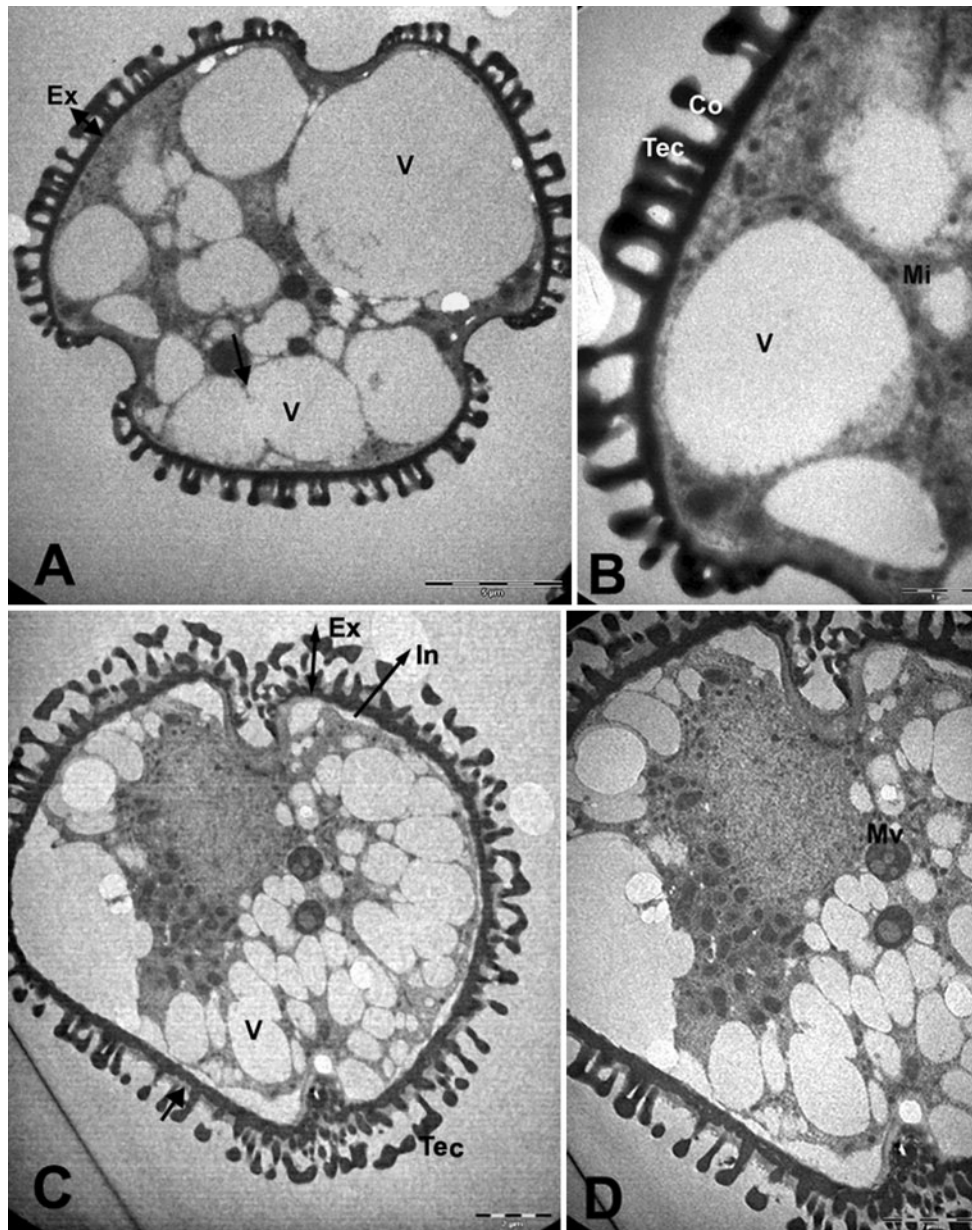
Fig. 11 TEM micrographs of vacuolated microspores. **a** Polar view of microspore. Note that vacuole is dividing by a pinching-in of the tonoplast (*arrow*). **b** Magnified view of a part of the **a**. **c, d** Ultra structure of the microspore cytoplasm and sporoderm at late vacuolated microspore stage. Note the remnants of primexine between columellae (*arrow*). (*Co* columellae, *Ex* exine, *In* intine, *Mi* microspore, *Mv* multivesicular body, *Tec* tectum, *V* vacuole)

surrounded the anther locule thoroughly. Ubisch bodies were observed on tapetal membrane next to the tangential wall of the endothecial cells.

Sporoderm

At the bicellular pollen stage, exine building was completed (Fig. 13a–d). However, intine construction continued by the addition of materials from the vesicles which were produced by the microspore (Fig. 13c). It was observed that, at aperture region, intine protruded towards

the cytoplasm and its thickness was twice as much of the non-aperture region (Fig. 13e, f).

Discussion

A study of the microsporogenesis and microgametogenesis in *C. radicans* showed that microspore and pollen development in *C. radicans* can substantially be considered as normal. Sporogenous cells are surrounded by thin cell walls having plasmodesmata, and prior to the
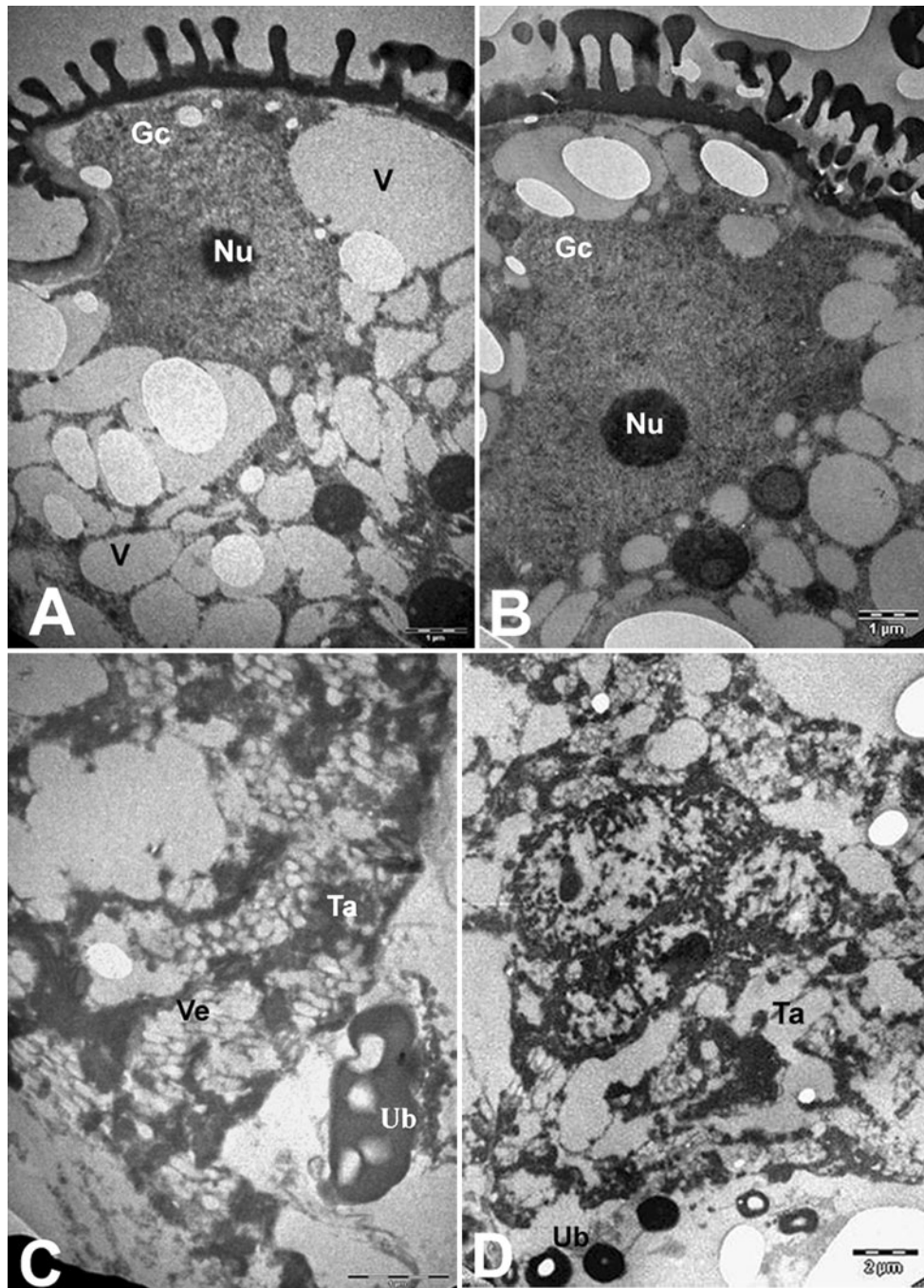
Fig. 12 TEM micrographs of transverse sections through anther of *C. radicans* at late vacuolated microspore stage and early bicellular pollen stage. **a** A portion of the vacuolated microspore just after the first mitosis showing newly formed generative cell attached to intine layer. **b** A portion of the microspore showing generative cell moving towards the center of the vegetative cell. **c**, **d** Degenerating tapetal cells. (*Gc* generative cell, *Gn* generative nucleus, *Mi* microspore, *N* nucleus, *Nu* nucleolus, *Ta* tapetum, *V* vacuole, *Ve* vesicle, *Ub* ubisch body)

differentiation into microspore mother cells, they increase their number by mitotic divisions.

Microsporocytes secrete callose wall which causes the sealing of plasmodesmata. Now microsporocytes are connected to each other by large cytoplasmic channels. Following meiotic division, tetrahedral and rarely isobilateral tetrads are formed by simultaneous cytokinesis. After released from the tetrad, free microspores starts to develop, and subsequent to first mitotic division mature bicellular pollen grains are produced. As in most angiosperms and other members of the Bignoniaceae such as *Tabebuia Pulcherrima* (Bittencourt and Mariath 2002) and *Jacaranda*
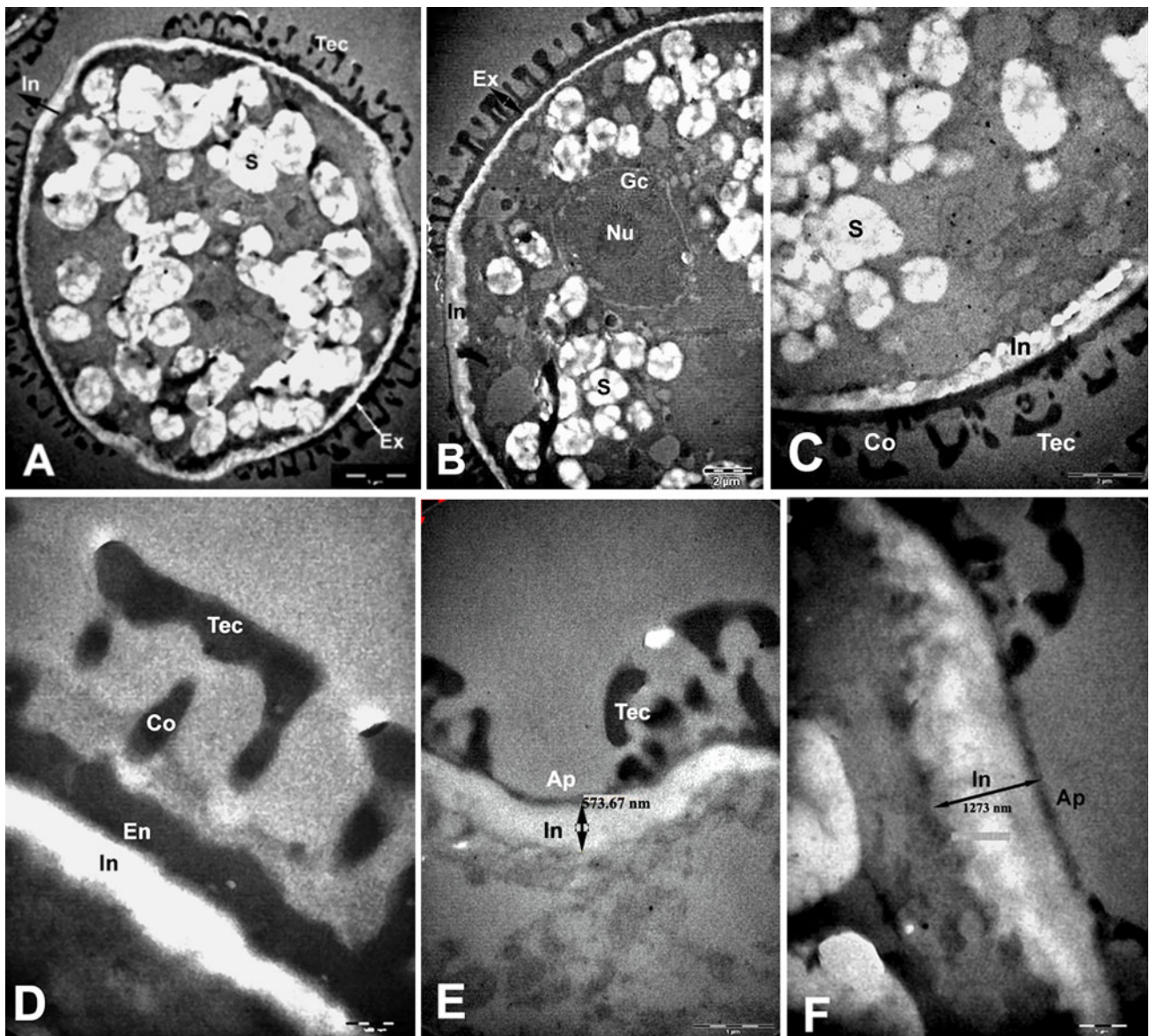
Fig. 13 TEM micrographs of the two-celled pollen grain of *C. radicans*. **a** Polar view of two-celled pollen. **b, c** Details of cytoplasm and pollen wall. Note that intine synthesis is still proceeding in **c**. **d** Details of sporoderm (pollen wall). **e, f** Details of the intine layer. Note that thickness of the intine in aperture region is twice of the non-aperture region. (*Ap* aperture region, *Co* columellae, *En* endexine, *Ex* exine, *In* intine, *S* starch, *Tec* tectum)

Mimosifolia (Galati and Strittmatter 1999), in *C. radicans* pollen grains are shed from anther at two-celled stage.

According to Rowley (1993), tapetal cells undergo cycles of hyperactivity during their development that can be detected from the changes taking place in their structure and secretory activity. In the tapetal cells of *C. radicans* anther, two hyperactive periods, tetrad and vacuolated microspore stages, have also been determined that coincide with the building and maturation of the exine. During these periods, fibrillar material in the anther locule increases indicating the transfer of fibrillar materials, vesicles, and lipids from the tapetal cells to the developing pollen.

Ontogeny of pollen wall (Sporoderm)

Until today, various models about the origin of exine template have been suggested by many authors including Heslop-Harrison (1963), Hideux (1981), Sheldon and Dickinson (1983, 1986), Dunbar and Rowley (1984), Dickinson and Sheldon (1986), Takahashi (1986, 1989, 1994), and Takahashi and Kouchi (1988). These studies have confirmed the idea that pollen wall template is determined during meiosis or just after the meiosis; however, agreement has not been reached on the initial process of exine template formation. From the developmental

perspective, three main views on the exine pattern formation have been proposed. One suggests that exine pattern is determined by the primexine, which is deposited within the invaginated plasma membrane and callose wall. According to primexine model (Heslop-Harrison 1963), exine formation starts with the appearance of probacules in the primexine matrix. The other view asserts that plasma membrane—associated compound, glycocalyx, determines the initial exine pattern (Rowley and Skvarla 1975; Rowley and Dahl 1977; Rowley 1990). Another view with different perspective proposes that the exine pattern is determined by invagination of the microspore plasma membrane at the early tetrad stage (Takahashi 1989, 1993, 1994; Takahashi and Skvarla 1991a, b, and protectum is formed directly on the plasma membrane before the primexine matrix and probacule formation (Takahashi and Kouchi 1988; Takahashi 1989). Therefore, plasma membrane rather than the primexine is responsible from the determination of exine pattern.

Although many authors have agreed upon the formation of primexine, there are different ideas about the sequence of events which leads to the conversion of primexine into exine through sporopollenin accumulation in different taxa. In other words, there is confliction about the order in which the earliest components of exine occur. Hideux (1981) has suggested three distinct sequences in which components of exine are formed before the degradation of the callose wall. (1) Bacules are formed first with varying amounts of tectum, and the foot layer; (2) tectum and some parts of the bacules are formed; and (3) only tectum is formed.

Whilst Heslop-Harrison (1963), Fernandez and Rodriguez-Garcia (1988) and El-Ghazaly et al.(2001) have suggested that exine formation starts with the appearance of probacules in the primexine matrix, Dunbar and Rowley (1984) have reported in *Betula* that only the protectum component of exine is present until after the tetrad period. Results that are similar to exine development in *Betula* (Dunbar and Rowley1984) have been reported by Liu and Huang (2003) for exine initiation in *Dumasia miaoliensis*. Liu and Huang (2003) have pointed out that, the protectum is formed on the protruding site of the invaginated plasma membrane and after that the probacules are formed under the protectum. In addition, Romero et al. (2003) have declared in *Hypecoum imberbe* that protectum is the first exine element appeared in the primexine matrix.

Recent studies on exine development (Gabarayeva and Grigorjeva 2010; Blackmore et al. 2010; Gabarayeva et al. 2011) have been made to confirm the earlier hypothesis (Gabarayeva and Hemsley 2006; Hemsley and Gabarayeva 2007), which have put forward the idea that there is a considerable input of micelle self-assembly processes in sporoderm development of any species.

In the present study, the initial process of exine pattern formation in *C. radicans* starts with the undulation of microspore plasma membrane which is followed by the primexine formation, and protectum is the first exine component deposited in the fibrillar primexine matrix. Small vesicles and vacuoles that are in close contact with the plasmalemma (Fig. 7b, d) at the tetrad stage indicates the fact that materials deposited in the special sites of primexine are produced by microspore. Furthermore, amount of fibrillar material which secreted from tapetal cells was high in anther locule at free microspore stage, but when the exine formation was completed fibrillar material disappeared in the anther locule. This situation denotes that fibrillar material could be used during exine construction.

Foot layer and endexine

The thickness of the foot layer is approximately half of the endexine. Since the foot layer has the same electron density with the endexine, in TEM micrographs it can only be distinguished when single, indistinct, and discontinuous white line indicating the initial deposition of endexine forms below it (Fig. 9c, d). However, in PAS staining foot layer could easily be distinguished from the endexine layer (Observed in semi-thin sections. Data is not shown here).

The difficulty in distinguishing between the foot layer and the endexine using conventional TEM techniques is not exclusive to *C. radicans*; it has also been reported in other angiosperms such as *Nelumbo* (Kreunen and Osborn 1999), *Rondeletia odorata* (El-Ghazaly et al. 2001) and *Hypecoum imberbe* (Romero et al. 2003).

In most angiosperms including *Olea europaea* (Fernandez and Rodriguez-Garcia 1988), *Lycopersicon esculentum* (Fernandez et al. 1992), *Epilobium* and *Poinciana* (Rowley 1995), *Nelumbo* (Kreunen and Osborn 1999), *Artemisia vulgaris* (Rowley et al. 1999) and *Rondeletia odorata* (El-Ghazaly et al. 2001) lamellated endexine is reported during its development. However in *C. radicans* endexine is not lamellated and it breaks into separate units as reported in *Hypecoum imberbe* (Romero et al. 2003). According to some authors (Blackmore and Barnes 1986; Suarez-Cervera and Seoane-Camba 1986; Fernandez et al. 1992; Suarez-Cervera et al. (2001); Romero et al. 2003), this discontinuous structure of endexine facilitates its role in harmomegathy.

Intine

Intine development shows variations among different species, especially in the number of strata it contains. In some species such as *Anigozanthus viridi* (Rowley and Rowley 1996), *Nelumbo lutea* (Kreunen and Osborn 1999),

Hypecoum imberbe (Romero et al. 2003), and *Tarenna gracilipes* (Vinckier and Smets 2005), two strata have been reported. On the other hand, three strata have been stated in Poaceae (Marquez et al. 1997a, b) and Euphorbiaceae (Suarez-Cervera et al. 2001). Furthermore, a single homogeneous stratum has been described in *Olea europaea* (Fernandez and Rodriguez-Garcia 1988) and *Lycopersicon esculentum* (Fernandez et al. 1992). In the present study, TEM observations of mature pollen wall of *C. radicans* did not reveal the presence and absence of some strata in the intine layer; however, in semi-thin sections it seems to have two strata (data is not shown here).

Although it has reported in most taxa that the initiation of the intine occurs after microspore mitosis (Blackmore and Barnes 1990), in *C. radicans* it has occurred at the late vacuolated stage just before the microspore mitosis.

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