*ACTA BIOLOGICA CRACOVIENSIA Series Botanica* 54/2: 69–78, 2012
DOI: 10.2478/v10182-012-0027-6

# EMBRYOLOGY OF *WITHANIA SOMNIFERA* (L.) DUNAL (SOLANACEAE)

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Received June 9, 2012; revision accepted October 30, 2012

We studied the embryology of *Withania somnifera* (L.) Dunal by light microscopy in order to reveal specific embryological features of the genus, and compared the results with embryological data on other members of the family Solanaceae. The key embryological characters of *W. somnifera* include dicotyledonous-type anther wall formation, simultaneous cytokinesis in pollen mother cells, binucleate tapetal cells, 2-celled mature pollen, anatropous, tenuinucellate and unitegmic ovules, polygonum-type embryo sac formation, the presence of an endothelium, and cellular endosperm formation. We give the first report of the dicotyledonous mode of anther wall formation (previously described as basic type) for the species. Comparative study suggests that anther wall formation, number of nuclei in tapetal cells, number of cells in mature pollen, mode of embryo sac formation and endosperm development are the most variable embryological features in Solanaceae. Some of these embryological features of *W. somnifera* should be of value for comparative study of related species and their phylogenetic relationships within the family.

**Key words:** Embryological features, anther wall formation, embryo sac, endosperm.

## INTRODUCTION

The conventional classification of Solanaceae into two subfamilies, Cestroideae and Solanoideae (D'Arcy, 1979, 1991; Hunziker, 1979, 2001; Olmstead and Palmer, 1992), was revised by Olmstead et al. (2008), who recognized three subfamilies, with Nicotianoideae between Cestroideae and Solanoideae. Solanoideae, with curved embryos in a flattened discoid seed and typically berry-like fruits (D'Arcy, 1979; Hunziker, 1979), was considered the most primitive; Cestroideae, with straight or somewhat bent embryos in small, angular to subglobose seeds and typically capsular fruits, was suggested to be the evolved one. *Withania* was placed in Solanoideae, closely related to *Mellissia*, *Aureliana* and *Athenaea* based on chloroplast DNA, and these were all grouped in subtribe Withaninae (Olmstead et al., 1999, 2008). Morphologically, stylar heteromorphism has been observed in *Athenaea*, *Aureliana* and *Withania*, supporting the molecular evidence. Dioecy has also been noted in some *Withania* species (Hunziker, 2001; Anderson et al., 2006) but *W. somnifera* has exclusively bisexual flowers. The dioecy of *Withania* evolved from a self-compatible ancestor (Anderson et al., 2006), because self-compatibility is likely ancestral in this genus as for much of *Solanum* (Whalen and Anderson, 1981).

The number of integuments is a characteristic feature of angiosperms ovules, and bitegmic ovules are usually accepted as primitive in flowering plants as compared to unitegmic or ategmic ovules (Bouman, 1984; Cronquist, 1988; Takhtajan, 1991; Johri et al., 1992; Endress and Igersheim, 2000; Wang and Ren, 2008). The detailed embryology of several angiosperms is poorly known, and data on unitegmic ovule ontogeny are sparse (Bouman, 1984). A unitegmic ovule is a typical embryological feature of the Solanaceae family, which includes an enormous number of taxa having economic value. It is an important source of vegetable, ornamental, medicinal and other economic plants. A number of embryological revisions of Solanaceae have been published. Studies on the plants of this family apparently began in the middle of the 19th century, but detailed embryological data on solanaceous plants did not appear in the literature until the early 20th century. Soueges (1907), Palm (1922) and Sevensson (1926) described embryo sac development in *Atropa belladonna*, *Nicotiana* and *Hyoscyamus niger*, respectively, and came to the

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PL ISSN 0001-5296

same conclusion that a tetrad is formed from the macrospore mother cell and only the inner (chalazal) cell of the tetrad is functional, giving a normal 8-nucleate embryo sac of the Polygonum type. However, Nanetti (1912) in *Solanum muricatum* and Young (1922) in *S. tuberosum* invoked the Lilium type, and this was criticized later by Bhaduri (1932), who reported Polygonum-type development in *S. melongana*. Cooper (1931), Bhaduri (1932, 1935), Satina (1945), Goodspeed (1947), Parashar and Singh (1986), Villari and Messina (1996), Karihaloo and Malik (1996), Carrizo Garcia (2002) and others made significant contributions to the embryology of a variety of Solanaceae species.

*Withania somnifera* is a very important plant in Ayurvedic and indigenous medical systems. It stimulates the immune system and is believed to improve memory. Some of its embryological features, such as the formation of an endothecium in the anther wall, elongated tapetal cells, a Polygonum type of embryo sac and a cellular type of endosperm formation, have been described (Mohan Ram and Kamini, 1964). Here we present the embryology of *W. somnifera*, compare the observed characters with those of other Solanaceae, and discuss the phylogenetic relationships of *Withania*.

## MATERIALS AND METHODS

We collected flower buds, open flowers and fruits of *W. somnifera* from plants grown from seed in a glasshouse at Kangwon National University, Korea. Samples in all stages from young bud to open flower were collected every week from mid February to late March, then every two weeks in April and May 2010. The collected plant material was fixed in FAA (formalin, glacial acetic acid, 50% ethanol, 5:5:90) and preserved in 50% ethanol. Some samples were dehydrated in an ethanol series and embedded in Technovit 7100 resin (Heraeus Kulzer GmbH). Embedded materials were sectioned. Serial sections 4–5 μm thick were dried and stained with 0.1% Toluidine blue O. Some samples were also dehydrated in a t-butyl alcohol series and then embedded in Paraplast (Oxford Labware) with a melting point of 56–58°C for microtoming. Serial sections cut 6–8 μm thick with a rotary microtome were stained with Heidenhain's hematoxylin (Wako Pure Chemical), safranin (Junsei Chemical) and fast green FCF (Wako Pure Chemical) (triple staining). All stained slides were mounted with Entellan (Merck KGaA). We counted the cells in mature pollen stained with 1% acetocarmine. All prepared slides showing the various stages were observed with a BX-50 light microscope (Olympus, Japan). Photographs were taken with a digital camera (Olympus C4040Z, 4.1 MP, mpx, 3× optical zoom, Japan) attached to the microscope. Digital images in JPEG format were then processed with Photoshop CS4 for Windows 2007.

## RESULTS

### ANTHER AND DEVELOPMENT OF MALE GAMETOPHYTE

*Withania somnifera* is an evergreen under shrub 60–150 cm in height, having all parts pubescent with dendritic hairs; the inflorescence is an axillary cluster of 4–6 subsessile flowers (Fig. 1a). The flowers are bisexual, with 5 stamens and 2 fused carpels (Fig. 1b). The stamen filament is small and without any lateral appendages. The anther is tetrasporangiate (Fig. 1c). Prior to maturation the wall consists of 4 or 5 cell layers: an epidermis, an endothecium, 1 or 2 middle layers and a tapetum (Fig. 1d). The endothecium and middle layer originate from a common meristematic layer. The tapetum originates from a different cell layer of the anther primordium. The middle layer later divides periclinally and becomes a 2-layered structure (Fig. 1e). Thus, anther wall formation is dicotyledonous-type, in which only the outer secondary parietal layer takes part in the formation of the middle layer (see Davis 1966 pp. 10). The tapetum is glandular and the cells binucleate (Fig. 1e). Nuclear division in tapetal cells usually occurs before meiosis begins in microspore mother cells. Meiosis in a microspore mother cell is accompanied by simultaneous cytokinesis (Fig. 1f) and the resulting microspore tetrads are predominantly tetrahedral (Fig. 1g). During maturation the middle layers degenerate and the endothecium develops fibrous thickenings (Fig. 1h). Anther dehiscence takes place through longitudinal slits (Fig. 1h). First the suture between 2 microsporangia in an anther lobe detaches, and soon thereafter the pollen is released from the microsporangium through a common slit formed between the 2 microsporangia of the theca. Mature pollen grains are 2-celled at shedding time (Fig. 1i).

### OVULE AND DEVELOPMENT OF FEMALE GAMETOPHYTE

The ovule is anatropous and tenuinucellate (Fig. 2a). Apical dermal cells of the nucellus do not divide periclinally; thus no nucellar cap is formed. The hypodermal archesporium is 1-celled, differentiating beneath the apical epidermis of the nucellus (Fig. 2a). The archesporial cell does not undergo further division and functions directly as a primary sporogenous cell which develops into a megaspore mother cell (Fig. 2b). During this time the archesporial cell increases in volume and becomes more

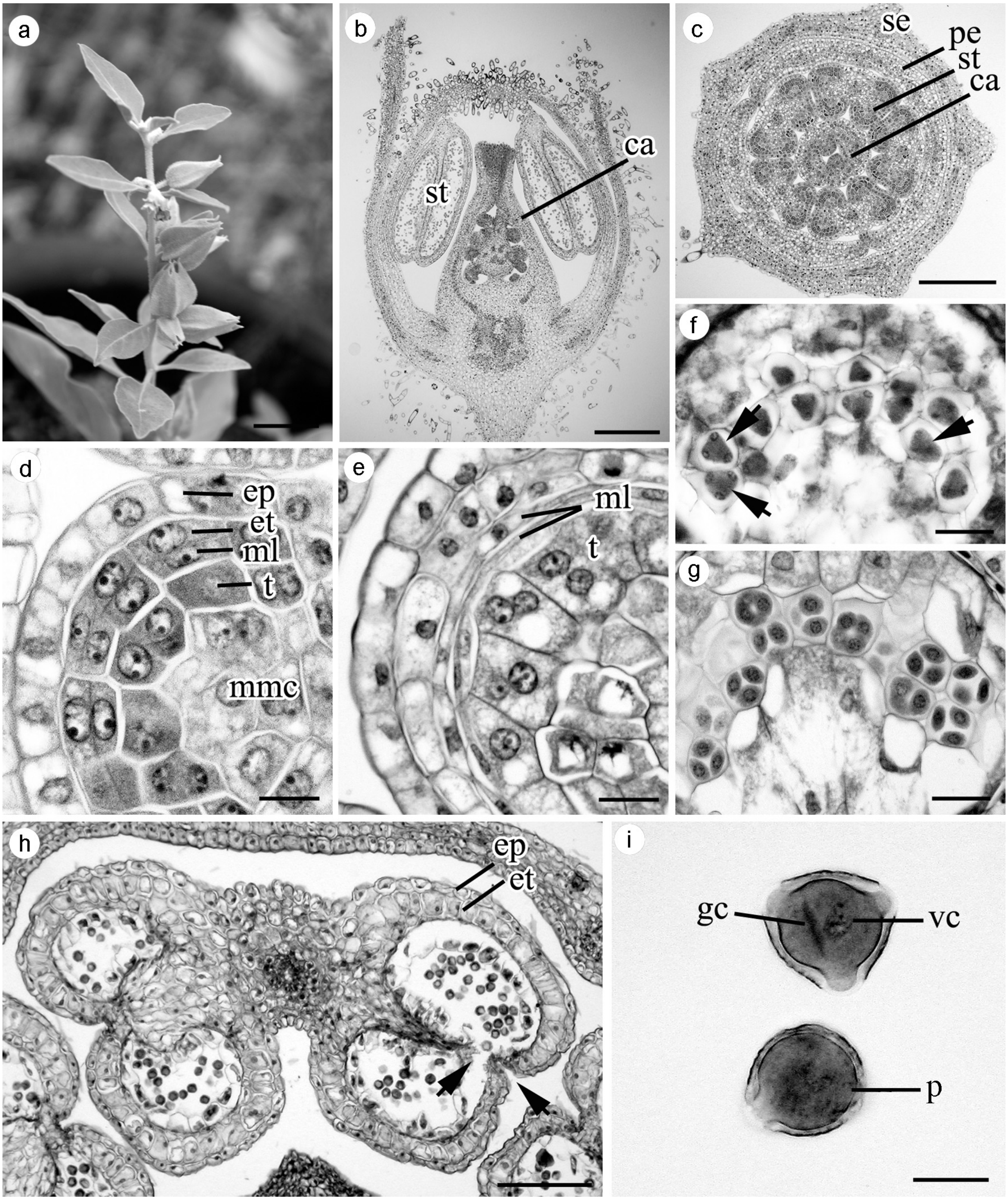

**Fig. 1.** Anther and microspore development in *Withania somnifera*. (**a**) Vegetative twig of plant with inflorescence, (**b**) Longitudinal section (LS) of young flower, (**c**) Transverse section (TS) of young flower, (**d**) TS of young anther showing dicotyledonous type of wall development, (**e**) TS of microsporangium showing middle layers and binucleate tapetal cells, (**f**) Microspores in simultaneous cytokinesis, (**g**) Microspore tetrads, (**h**) TS of mature anther before dehiscence, (**i**) 2-celled mature pollen stained with acetocarmine. an – anther; ca – carpel; et – endothecium; ep – epidermis; gc – generative nucleus in pollen; ml – middle layer; p – mature pollen grain; pe – petal; mmc – microspore mother cell; se – sepal; st – stamen; t – tapetum; vc – vegetative cell. Bars = 200 μm in (b,c); 10 μm in (d,e,f,g,i); 50 μm in (h).

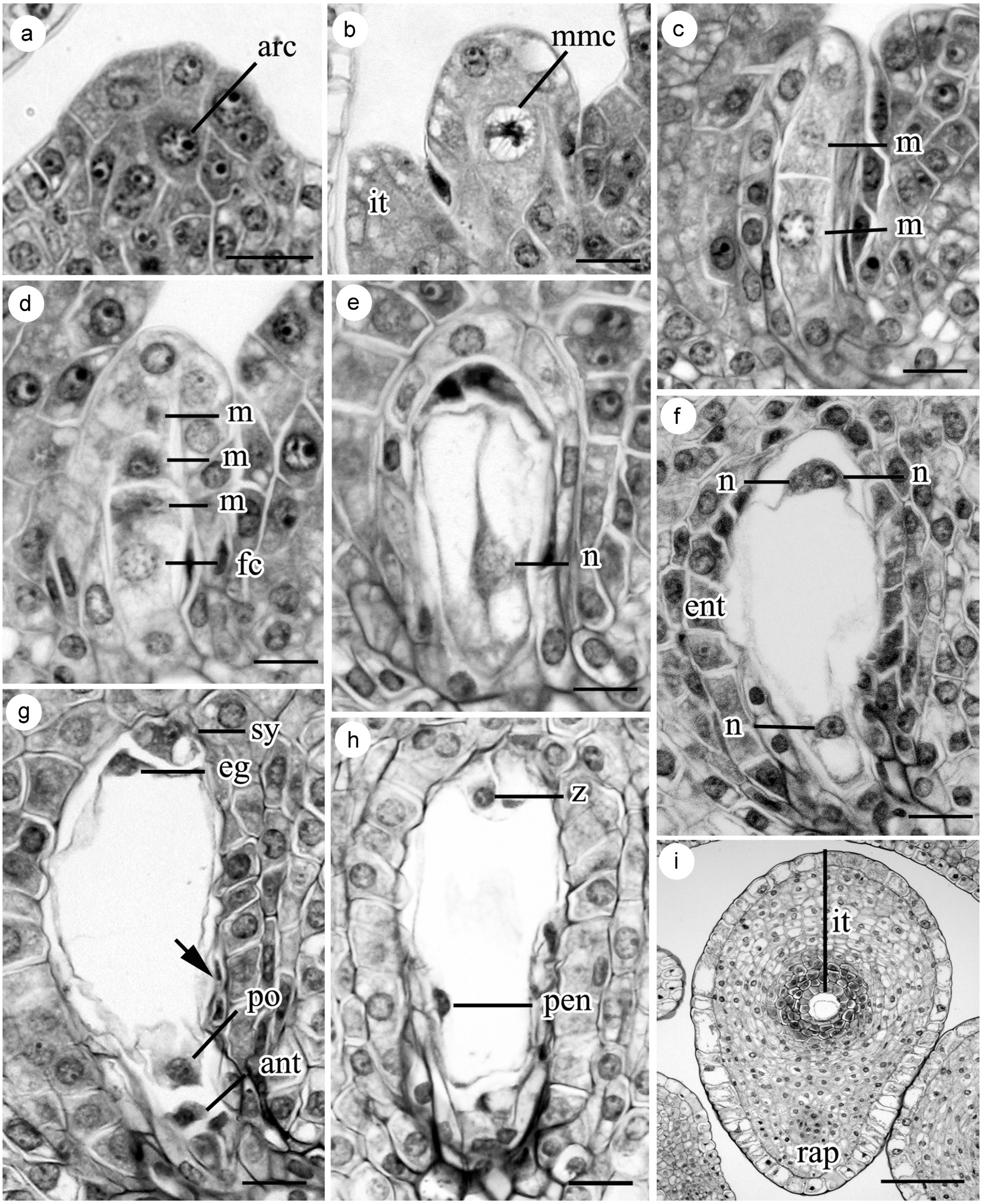

**Fig. 2.** Ovule and megagametophyte development in *W. somnifera*. (**a**) Longitudinal section (LS) of young ovule with 1-celled archesporium, (**b**) LS of young ovule with megaspore mother cell and integument, (**c**) LS of young ovule with dyad of megaspores, (**d**) LS of young ovule with linear tetrad of megaspores, showing functional megaspore, (**e**) LS of ovule at 2-nucleate embryo sac stage, one nucleus at the micropylar end appears in next section, (**f**) LS of ovule at 4-nucleate embryo sac stage, one nucleus at chalazal end appears in next section, (**g**) LS of organized mature embryo sac with synergid, egg cell, polar nucleus and antipodal cells (arrowhead indicates vestige of nucellus), (**h**) LS of young seed with zygote and primary endosperm nucleus, (**i**) Transverse section of mature ovule with multilayered integument and raphe. arc – archesporium; ant – antipodal cell; eg – egg cell; ent – endothelium; fc – functional megaspore; it – integument; m – megaspore cell; mmc – megaspore mother cell; n – nucleus of embryo sac; po – polar nucleus; rap – raphe; sy – synergid; z – zygote nucleus. Bars = 10 µm in (a–h); 20 µm in (i).

prominent. The megaspore mother cell undergoes meiosis, during which first a dyad (Fig. 2c) and finally a linear tetrad is formed (Fig. 2d). In megaspore tetrads the 3 micropylar megaspores degenerate and the chalazal megaspore becomes functional. The functional megaspore develops successively into a 2- (Fig. 2e), 4- (Fig. 2f) and 8-nucleate embryo sac (Fig. 2g). Thus the mode of embryo sac formation is of the monosporic Polygonum type, and the organized mature embryo sac is obovoid and slightly curved. It is 7-celled and 8-nucleate, with an egg cell, 2 synergids, 2 polar nuclei and 3 antipodal cells (Fig. 2g). The 2 polar nuclei fuse with each other before fertilization and form a central nucleus, which usually is positioned in the chalazal half of the embryo sac and develops into endosperm after fusion with one of the male gametes. The synergids usually are elongated, with a conspicuous vacuole and prominent nucleus. The 3 antipodal cells degenerate before or soon after fertilization. Peripheral nucellar cells appear viable up to the binucleate stage of the embryo sac; their degeneration begins during the formation of the tetranucleate gametophyte (Fig. 2f). Remnants of the nucellus are noticeable in the chalazal region even when the gametophyte becomes fully mature (Fig. 2g). No obturator is formed.

The ovule is unitegmic (Fig. 2b). The integument is dermal in origin: certain cells of the peripheral nucellar layer grow radially and undergo periclinal division. At the initial stage, the integument is 2 cell layers thick; by the dyad and tetrad stage it becomes 3- or 4-layered, and later, by the 2-4-nucleate embryo sac stage it is 5-layered or more. The mature integument is multiplicative and the innermost layer of the integument develops as an endothelium (integumentary tapetum), directly bordering the growing embryo sac (Fig. 2f). The endothelium generally differentiates at the binucleate embryo sac stage. No vascular supply was observed in the integument.

The entrance of the pollen tube to the ovule is porogamous. After fertilization the zygote is formed, usually at the micropylar end. The large primary endosperm nucleus is located in the chalazal half of the embryo sac (Fig. 2h). At the same time, the integument cells multiply and the cells of the outermost layer enlarge and become vacuolated (Fig. 2i). After division the zygote develops into the proembryo (Fig. 3a). Endosperm formation is of the cellular type (Fig. 3b,c). A hypostase also differentiates on the chalazal side in post-fertilization stages (Fig. 3c). Division of the primary endosperm nucleus is followed by the formation of a transverse wall, producing 2 unequal endosperm cells: one large cell on the micropylar side and one small cell on the chalazal side (Fig. 3b). Further divisions are also transversal until the endosperm become 4–5 celled; after this the divisions may be longitudinal, transversal or oblique (Fig. 3c). We did not follow embryogenesis in detail but data on early and late embryogenesis from this study indicate that it proceeds in the usual manner, with the formation of proembryos followed by embryos (Fig. 3a,d).

## DISCUSSION

Various inconsistent embryological characters have been observed in several genera of Solanaceae; they vary particularly in anther wall development, ovule morphology, number of archesporial cells in the nucellus, megagametophyte development, endosperm formation, seed coat morphology and seed surface sculpture (Davis, 1966; Briggs, 1992; Villari and Messina, 1996; Carrizo Garcia, 2002; Zhang, 2005).

We compared the embryological features of *Withania somnifera* with those of some other Solanaceous plants, which are listed in Table 1. Both dicotyledonous and basic types of anther wall formation are common in the Solanaceae, and neither type predominates in the family (Carrizo Garcia, 2002). However, Davis (1966) suggested that basic-type anther wall formation is less frequent and does not characterize the family, and stated that *W. somnifera*, following the basic type of wall formation, is an exception among the other members of Solanaceae following the dicotyledonous type. Against those statements, we found the dicotyledonous type of wall formation in *W. somnifera*; none of our samples showed basic wall formation. One of the middle layers is formed later by its own division but the tapetum does not take part in the formation of the middle layer. We suggest that the previous report should be revised. A multilayered endothecium has been observed in some Solanaceae such as *Nicotiana glutinosa* and *N. tabacum* (Jose and Singh, 1968) but in *W. somnifera* it is single-layered and fibrous. In *Solanum nigrum* a fibrous thickening of the endothecium was found only at the anther tip (Saxena and Singh, 1969). The tapetal cells of *W. somnifera* are glandular, as Davis (1966) reported in the same species, and these cells become binucleate. Both amoeboid and glandular types of tapetal cells have been described in *Datura stramonium* (O'Neal, 1920) the former being common. During shedding, 2-celled pollen grains generally are observed in Solanaceae (Davis, 1966), as we found in *W. somnifera*. *Capsicum frutescens* is an exception, bearing 3 cells in mature pollen (Davis, 1966). Abnormal pollen with 2–8 nuclei (Dharmaraj and Prakas, 1978) and high male sterility have been noted in some species of *Capsicum* (Raghuvanshi and Singh, 1984).

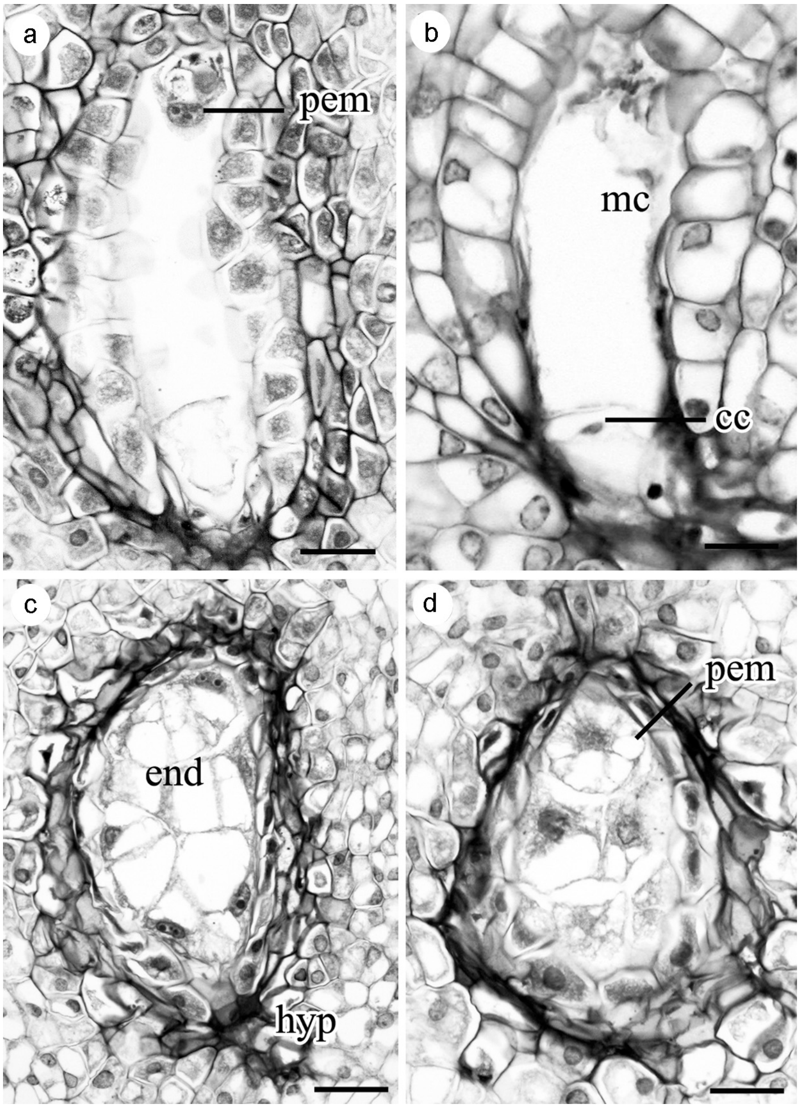

**Fig. 3.** Endosperm and embryo development in *W. somnifera*. (**a**) Longidudinal section (LS) of young seed with proembryo, (**b**) LS of young seed with one large endosperm cell at micropylar pole and one small endosperm cell at chalazal pole, (**c**) LS of young seed with many endosperm cells and hypostase at chalazal end, (**d**) LS of young seed with proembryo. cc – endosperm cell at chalazal pole; end – endosperm; hyp – hypostase; mc – endosperm cell at micropylar pole, pem – proembryo. Bars = 10 µm.

It is evident from Table 1 that the majority of anther characteristics are consistent. One of the most inconsistent features of the anther and male gametophyte is the number of nuclei in tapetal cells. The type of anther wall development, the nature of middle layer, and the number of nuclei in tapetal cells are the most inconsistent anther characteristics at tribal level and occasionally at generic level as well (Tobe, 1989). *Withania* has 2, *Capsicum* has 3 and *Atropa* has 4 nuclei in tapetal cells. These three genera represent three different tribes: respectively, Physaleae, Capsiceae and Hyoscyameae (Olmstead et al., 2008). The morphological feature distinguishing the Capsiceae tribe is the lack of terminal lobes and the presence of 10 nerves in the calyx; most Solanaceae have terminal lobes and 5 nerves in the calyx (D'Arcy, 1986, 1996). Accrescent calyces are a single character distinguishing the Physalis tribe from other Solanaceae (D'Arcy, 1996).

Ovule and female gametophyte development follow similar patterns throughout the Solanaceae. A bilocular ovary is a characteristic feature of the family. In a few samples of *W. somnifera* we observed a trilocular ovary but it occurred much less frequent-

TABLE 1. Comperision of embryological characters of *Withania somniferea* with those of other Solanaceae

| Charater | Solanoideae | | | | Nicotianoideae | Cestroideae | Solanaceae |
|---|---|---|---|---|---|---|---|
| | *Withania somnifera* | *Solanum* | *Capsicum* | *Atropa* | *Nicotiana* | *Browallia* | |
| Anther and microspores | | | | | | | |
| Number of sporangia | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Thickness of anther wall | 5 | ? | ? | 4 or 5 | 4 or 5 | ? | 5 or 6 |
| Mode of wall formation | Dicotyledonous | Dicotyledonous, Basic | Dicotyledonous | Dicotyledonous | Basic | Dicotyledonous | Dicotyledonous, Basic |
| Anther epidermis | Persistent | Persistent | ? | Persistent | Persistent | ? | Persistent |
| Endothecium | Fibrous | Fibrous | Fibrous | Fibrous | Fibrous | ? | Fibrous or not |
| Middle layers | Crushed | ? | ? | Crushed | ? | ? | Crushed |
| Tapetum | Glandular | Glandular | Glandular | Glandular | ? | ? | Glandular, amoeboid |
| Number of nuclei in tapetal cell | 2 | ? | 3 | 4 | 2 | ? | 2 |
| Cytokinesis in meiosis | Simultaneous | Simultaneous | Simultaneous | Simultaneous | Simultaneous | ? | Simultaneous |
| Shape of microspore tetrad | Tetrahedral | ? | Tetrahedral | Tetrahedral, isobilateral, decussate | ? | ? | Tetrahedral, isobilateral, decussate |
| Mature pollen | 2-celled | ? | 3 | 2 | ? | ? | 3, rarely 2-celled |
| Anther dehiscence | Longitudinal slit | Longitudinal slit | Longitudinal slit | Longitudinal slit | Longitudinal slit | ? | Longitudinal slit |
| Ovule and integument | | | | | | | |
| Ovule orientation | Anatropous | Anatropous/ Amphitropous | Anatropous | Anatropous | Anatropous | Anatropous | Hemianatropous, anatropous, campylotropous |
| Number of integuments | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Thickness of integuments | 4-5 cells | 6-10 cells | ? | | | 7 | ? |
| Vascular bundle | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Endothelium | Formed | Formed | ? | Formed | Formed | Formed | Formed or not |
| Obturator | Not formed | ? | ? | Not formed | ? | ? | Not formed |
| Nucellus and megagametophyte | | | | | | | |
| Nucellus type | Tenuinucellate | Tenuinucellate | Tenuinucellate | Tenuinucellate | Tenuinucellate | Tenuinucellate | Tenuinucellate |
| Number of archesporial cells | 1 | 1 to many | 1 | 1 | 1 | 1 | 1 to many |
| Shape of megaspore tetrad | Linear | Linear | ? | Linear | Linear | Linear | Linear, T-shaped |
| Thickness of parietal tissue | Absent | Absent | ? | ? | Absent | Absent | Absent |
| Mode of embryo sac formation | Polygonum | Adoxa | Allium | Polygonum, Allium | Allium | Polygonum | Allium, Polygonum, Adoxa |
| Antipodal cells | Ephemeral | Ephemeral | ? | ? | Ephemeral | Ephemeral | Ephemeral |
| Nucellar cap | Not formed | Not formed | ? | Not formed | ? | ? | Not formed |
| Nucellar tissue in mature ovule | Absent | Absent | ? | Absent | ? | ? | Absent |
| Hypostase | Formed | Formed | ? | ? | ? | ? | Formed |
| Fertilization, endosperm and embryo | | | | | | | |
| Path of pollen tube | Porogamous | Porogamous | ? | Porogamous | Porogamous | Porogamous | Porogamous |
| Mode of endosperm formation | Cellular | Cellular | Cellular | Nuclear | Cellular | Cellular | Nuclear, cellular, helobial |
| Type of embryo | Nicotiana* | Solanad | ? | Solanad | ? | ? | Solanad |
| References | | | | | | | |
| | This work, Mohan Ran & Kamini, 1964, Bhaduri, 1936<br>*not observed in this study | Briggs, 1993<br>Kopcinska et al., 2004<br>Walker, 1955 | Lengel, 1960<br>Dharmaraj and Prakash, 1978<br>Carrizo Gracia, 2003 | Yurukova-Grancharova et al., 2011 | Villari and Messina, 1996<br>Sehgal and Gifford, 1979 | Mohan, 1966 | Johri, 1992<br>Kopcinska et al., 2004<br>Davis 1966<br>Carrizo Gracia, 2002 |

*not observed in this study

ly than bilocular ovaries. The ovule is tenuinucellate and anatropous; this becomes clearly evident at maturity. The integument is initiated after differentiation of the archesporium, and the ovule becomes curved at the same time. Villari and Messina (1996) reported a similar situation for *Nicotiana glauca*. The integument and archesporium appear simultaneously in *Lycopersicon* (Cooper, 1931). A multilayered integument and the formation of an endothelium are very common embryological features observed in many genera of the Solanaceae (Badhuri, 1935; Dnyansagar and Cooper, 1960; Mohan, 1966; Mogensen and Suthar, 1979; Sehgal and Gifford, 1979; Briggs, 1992; Villari and Messina, 1996). In *W. somnifera* the endothelium appeared when the embryo sac entered the binucleate stage, as also described in *Browallia demissa* (Mohan, 1966).

The type of female gametophyte development seems inconsistent throughout the family. Polygonum (Mohan Ram and Kamini, 1964; Mohan, 1966; Johri et al., 1992), Allium (Mohan, 1970; Dharmaraj and Prakash, 1978; Villari and Messina, 1996) and Adoxa (Young, 1922; Davis, 1966; Govil, 1980) types of gametophyte development have been described in different species of Solanaceae. Dharmaraj and Prakash (1978) reported finding Polygonum, Allium and Adoxa types in a single species (*Capsicum annuum* var. *acuminatum*) and also suggested seasonal influences on embryo sac development. We observed Polygonum-type gametophyte development in this study. Mohan Ram and Kamini (1964) also reported it in this species, but they noted variation or deviation from Polygonum towards the Allium or bisporic type of development; we did not see such variation or deviation in the research materials we used. We noted no hooks or special beaks in synergids as found in *Browallia demissa* (Mohan, 1966) and *Solanum phureja* (Davis, 1966). The hypostase, previously described in *Solanum demissum* (Walker, 1955) and *S. nigrum* (Briggs, 1992) in this family, was also evident in *W. somnifera*.

Soueges (1907, 1920a,b) studied embryogenesis in a number of solanaceous genera and concluded that it follows an almost uniform pattern, but Bhaduri (1936) observed a number of variations in *Nicotiana plumbaginifolia*, *Petunia nyctaginiflora*, *Physalis minima* and *Withania somnifera*. The Solanad type of embryogenesis, which has been observed in many plants (Mohan Ram and Kamini, 1964; Erdelska, 1985; Johri et al., 1992; Kopcinska et al., 2004), seems to be the most common type in the family. On the other hand, Badhuri (1936) suggested the *Nicotiana* type of embryogeny in *W. somnifera*. We cannot remark on that here due to the lack of research material from all stages. Some fragmentary data from this study merely suggest that it follows the general pattern, as the zygote after division forms a proembryo which later develops into a mature embryo. All three modes of endosperm development have been reported in the family, though the cellular type is frequent and is regarded as a general characteristic of Solanaceae (Mohan Ram and Kamini, 1964; Johri et al., 1992). Nevertheless, nuclear endosperm has been observed in *Hyoscyamus orientalis*, *Salpiglossis picta*, *Scopolia atropoides* and *Schizanthus pinnatus* (see Mohan Ram and Kamini, 1964) and helobial in *H. niger* (Svensson, 1926). We found cellular-type endosperm formation in *W. somnifera*; Mohan Rama and Kamini (1964) also reported it in this species. Division of the primary endosperm cell usually is transverse until the endosperm becomes at least 4- or 5-celled; after that the divisions may be transverse, longitudinal or oblique. During early endosperm development the cells have large vacuoles which soon disappear due to accumulation of a large amount of oil and starch (Ghimire et al., 2011).

Our study confirmed the dicotyledonous type of anther wall formation in *W. somnifera*, in agreement with Carrizo Garcia's (2003) findings in *W. adpressa*: only the cells of the outer secondary parietal layer divide periclinally, and the tapetum differentiates itself directly from the inner secondary parietal layers. Subsequent cell divisions may also occur after periclinal division in the outer layer, producing 2 or 3 middle layers. On the basis of anther wall formation, *Withania* resembles *Athenaea* because both follow the dicotyledonous type, whereas *Aureliana* follows the basic type, similarly to *Tubocapsicum*, a member of the Withaninae tribe (Carrizo Garcia, 2002). The mode of anther wall formation has been considered a consistent embryological feature at generic level in angiosperms (see Tobe, 1989) and at least at species level in Solanaceae (Carrizo Garcia, 2002), except for *Solanum nigrum* (Bhandari and Sharma, 1987).

## CONCLUSION

Here we clarified the confusion about some embryological characteristics of *Withania somnifera*. The subfamilies of Solanaceae share most of the embryological features. Solanaceae shows considerable variation in its mode of embryogeny and we are still unable to outline it completely in *W. somnifera*, though it has been reported as *Nicotiana* type. The dicotyledonous type of anther wall formation, described as basic in previous reports, is new for this species. These reported embryological characters of *W. somnifera* will be of use in future taxonomic and phylogenetic studies of this taxon.

## ACKNOWLEDGEMENTS

We thank the Bio-herb Research Center of Kangwon National University for granting permission to collect research material and providing experimental instruments, and the anonymous reviewers for valuable comments.

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