Seed characteristics of *Withania somnifera* (Solanaceae)

Balkrishna Ghimire, Bimal Kumar Ghimire\(^1\) and Kweon Heo\(^*\)

Department of Applied Plant Science, Kangwon National University, Chuncheon 200-701, Korea

\(^1\)Department of Applied Life Science, Konkuk University, Seoul 143-701, Korea

(Received 18 April 2011; Accepted 18 May 2011)

가지과 *Withania somnifera*의 종자 형질

Balkrishna Ghimire · Bimal Kumar Ghimire\(^1\) · 허 원\(^*\)

강원대학교 농업생명과학대학 식물자원융합공학과, \(^1\)건국대학교 응용생명과학과

ABSTRACT: The seed characteristics of *Withania somnifera* were studied using light and scanning electron microscopy in order to determine the specific features of this species. The seed color is yellow, and the seed shape is reniform measuring between two to three millimeters. The seed of *W. somnifera* is exarillate and albuminous. The seed coat type is exotestal. The seed coat develops from a single integument. The young seed coat consists of single-layered exotesta, multi-layered mesotesta and single-layered endotesta. However, parenchymatous mesotesta layers are completely compressed at maturity. Therefore, the seed coat was represented by sclerenchymatous exotesta. The primary sculpture on the seed surface is reticulate, and cells are irregular in shape with undulating anticlinal walls. In addition, the seed surface has several characteristic holes between the anticlinal walls.

Keywords: exotesta, sclerenchymatous cells, seed coat, Solanaceae, *Withania somnifera*

적 요: 가지과 *Withania somnifera* (L.) Dunal의 종자 해부학적 특성을 알기 위하여 희석현미경과 주시전자 현미경을 사용하여 관찰하였다. 종자의 색깔은 노랑색이고, 종자의 형태는 신장형이며, 크기가 2~3 mm 정도이다. 종자는 종무를 가지고 있지 않았으며, 배유가 잘 발달한 유배유증자를 나타냈다. 종피는 외종피외층으로 구성되는 단주피성 이었으며, 한 종의 외종피외층, 여러 종의 외종피중층, 그리고 한 종의 외종피내층으로 구성되었다. 그러나 종자가 완질히 성숙했을 때는 외종피중층이 입착되었다. 외종피외층은 특징적으로 파상형의 후백세포로 발달하였다. 종피의 표면은 방향구조를 가지고, 수중 벽구조는 파상형으로 발달하였고, 표면에 여러 개의 구멍이 발달하는 특징이 관찰되었다.

주요이: 외종피, 후백세포, 종피, 가지과, *Withania somnifera*

*Withania somnifera* (L.) Dunal (Solanaceae) commonly known as ‘ashwagandha’, ‘winter cherry’ or ‘Indian ginseng’ is an erect branching under-shrub, widely distributed in all dry parts of subtropical India. The plant also found in Congo, South Africa, Egypt, Morocco, Jordan, Pakistan and Afganistan (Saldanha and Nicolson, 1978). It is an important plant in indigenous medical system for over 3,000 years and extract of leaf, bark and root of this plant are used for multipurpose medicinal agent. It stimulates immune system and also believes to improve memory. It has anti-tumors, anti-inflammation, anti-cancer and anti-stress effect (Haripriya et al., 2010). Some indigenous people of South Africa apply this plant for the treatment of sexually transmitted infections, asthma and also used as an anti-inflammatory agent (Van Wyk et al., 1997).

Among various embryological characters, integument and seed coat structures have diagnostic value at generic or infrageneric level as well as familial or higher taxonomic level (Eliens, 1985; Tobe et al., 1987; Tobe, 1989). In many cases, it has been possible to separate tribes (Whaiffin and Tomb, 1972), genera (Mulligan and Bailey, 1976), sections and even species (Chuang and Heckard, 1972; Ehler, 1976; Hill, 1976; Sveevey et al., 1977; Karcz et al.,

*Author for correspondence: laurus@kangwon.ac.kr*
2005). Likewise, seed morphology and seed surface pattern have also been useful for phylogenetic relationship in wide variety of angiosperm families (Esau, 1953; Mohana, 1974; Corner, 1976; Bartholot, 1981, 1984; Taktajian, 1991). Recently, Kong et al. (2011) reported about the seed morphology of Solanaeaceae in Korea. Various studies on Solanaeaceae have shown that seed morphological characters are of considerable significance in systematics, both generic and specific level (Zhang et al., 2005). Therefore, we have studied seed coat development and detailed sculpture of seed surface of W. somnifera.

Materials and Methods

Seeds of W. somnifera collected from plant of greenhouse at Kangwon National University. They were fixed in FAA (5 parts formalin: 5 parts glacial acetic acid: 90 parts 50% ethanol) and preserved in 50% ethanol. Seeds were dehydrated with ethanol series and embedded in Technovit 7100 resin. Embedded materials were sectioned 4 to 5 μm thickness with disposable knife. Serial sections were attached on slide glass and stained with 0.1% Toludine blue O. All stained slides were mounted with Entellan. All prepared slides with different stages were observed with BX-50 light microscope (Olympus Co., Japan). Photographs were taken by digital camera system attached to microscope.

For scanning electron microscopic observations, several mature seeds were dehydrated through ethanol series. After dehydration, seeds were immersed in carbon dioxide for critical point drying before coating with platinum. All samples were coated with platinum by Hitachi E-1010 ion-sputter device. The equipment used to take picture was a Hitachi S-3500N scanning electron microscope.

Results and Discussion

Fruits of W. somnifera are globose berries and orange color at maturity. The fruit bears large number of seeds within it. Mature seeds are albuminous and exarillate (Fig. 1D, E). They are small almost equal in length and width (about 2-3 mm), flattened, reniform shaped or what disoid and yellow in color.

Ovule is unisegmic. Therefore, seed coat completely lacks tegmen layer. At early stage of development, the integument is made up of five to six layers and at the fully organized embryo sac stage at the time of fertilization it becomes about seven to eight layers (Fig. 1A). The integument in mature ovule of Solanum muricatum Aiton comprised an outer epidermis, five to seven parenchymatous layers and an inner epidermis (Kopcinska et al., 2002). The innermost layer of integument was differentiated in to endothelium, which is a common characteristic in Solanaeaceae just before or during the embryo sac becomes four-nucleate stage (Cooper, 1931; Lee and Cooper, 1958; Mohan, 1970a, b; Erdelska, 1985; Kopcinska et al., 2002). After fertilization, the cells of endothelium increase in size and are visually differentiated from the rest of the integument layer which later represents the endotesta of seed coat (Fig. 1B). By the time the zygote is formed and endosperm starts to organize, there is a significant change is marked in the integument which now possesses about 12 to 13 layers of cells. This process exceeds further with the expansion of endosperm and visual separation of layer also happens. During this period, outer epidermal cells increase in size, become highly vacuolated and lignified by losing cytoplasmic substances. Inner epidermal cells become elongated and are stretched by developing endosperm, whereas multilayered mesoderm with prominent vacuole remains in between outer and inner epidermis. Thus, young seed seems to have one layered exotesta, multiple layered parenchymatous mesotesta and one layered endotesta (Fig. 1B). As we found in this study seed coat is highly multiplicative, the number of parenchymatous layers increased initially, but later these parenchymatous layers was compressed gradually and disappeared completely in mature seed coat. The number of mesotesta layers starts to decrease in the differentiating the testa of seed with the development of embryo and endosperm. Significant change in this process has been observed in Solanum muricatum when embryo becomes heart-shaped, testa contained just a few layers of parenchyma and the layer of degenerated parenchyma remnants was wider (Kopcinska et al., 2004). As in W. somnifera and Solanum muricatum, disintegration of the middle parenchyma was also common in S. macroanthum Roem. & Schult. (Mohan, 1970a) and S. demissum Lindl. (Beamish, 1955) and it is belief that these integrating parenchyma is a source of nutrients for the young developing endosperm and embryo (Kopcinska et al., 2004).

The seed coat is an exotestal type. The mature seed coat comprises of exotesta, mesotesta and endotesta though the exotesta is the most noticeable and conspicuous structure among them (Fig. 1C). The layer of seed coat in Solanaeaceae is multiplicative or not and usually reduced to exotesta and endotesta with crushed mesotesta (Corner, 1976). In W. somnifera, the exotestal cells of mature seed are thick walled, lignified, irregular shaped in cross section and highly tanniferous (Fig. 1C, D). With the development of endosperm, the mesotesta becomes compressed and it almost collapsed at maturity which is only represented by structures filled with dark stained substances (Fig. 1C). The inner epidermal cells stretched tangentially with the extension of endosperm and the cells are empty and elongated at mature
Fig. 1. Development of seed coat and seed morphology of *Withania somnifera*. A. Longitudinal section of mature embryo sac with integument; B. Transverse section of young seed coat; C. Transverse section of mature seed coat; D. Longitudinal section of mature seed; E. Scanning electron microscopic view of seed shape with hilum; F. Magnified seed surface architecture. Abbreviations: end, endosperm; ent, endotesta; ext, exotesta; hi, hilum; it, integument; mi, micropyle; mst, mesotesta. Scale bars: A, B, C = 10 μm; D, 400 μm; E = 1 mm and F = 500 μm.

seed stage (Fig. 1C). Same results were found by Dnyansagar and Cooper (1960) in *Solanum phureja* Juz. & Bukasov as middle parenchyma cells crushed at maturity whereas the endothelium did not disappear and it's small, rectangular thick-walled cells
formed the inner epidermis of the testa. However, Kopcinska et al. (2004) explained that outer epidermis is the only layer which retained its cellular structure in mature seed of *S. muricatum* but inner epidermis and middle parenchymatous layers completely disorganized.

Scanning electron microscopic view of mature seed revealed that the primary sculpturing is reticulate and has prominent outer anticlinal cell walls which are fused (Fig. 1E, F). The seed coat has concave, shallow testal cells with undulating anticlinal walls and characteristic hole through the bottom thickening (Fig. 1F). Similar pattern of exocutal cells was described in *Physalis* species by Axelius (1992) even though he found some variation in several species in distribution and pattern of holes in the thickenings. The epidermal cells of mature seeds in *W. somnifera* are irregular, polygonal or oblong with sinuate cell margin (Fig. 1E, F). Zhang et al. (2005) found two basic types of cell shape and the degree of sinuosity of the cell walls; (1) polygonal to sub-rounded in shape, isodiametric; lateral walls straight: *Atropa*, *Mandragora*, *Physcochlaena trifundibularis*, and *P. macrophylla*, and (2) cells irregular in shape, not isodiametric or occasionally isodiametric; lateral walls sinuate, sinuous or sinuate in the member of tribe Hyosycamae such as *Atropa*, *Atropa*, *Archychysconus*, *Hyoscyamus*, *Physcochlaena orientalis*, *P. physalooides*, *P. praetela*, *P. ureolata*, *Przewalska*, and *Scopolia*. The epidermal cells of *W. somnifera* have deep wedge-shaped lumen that the upper part of lumen often larger than the base which is due to wall thickening since basal wall is thicker than upper part wall. Secondary sculpture in anticlinal wall of exocutis is not so consistent in different species of Solanaceae and it varies from smooth to cover with granular, laminar, or tuberculate projection in tribe Hyosycamae (Zhang et al., 2005). In this study, we found smooth secondary sculpture in anticlinal walls (Fig. 1F). This structure also reported in Korean Solanaceae such as *Scopolia lutescens*, *Solanum species*, and *Tubocapsicum anamatum* (Kong et al., 2011).

In conclusion, seed coat type of *W. somnifera* is entirely exocutal and mature seed coat consists of sclerenchymatous exocutis associated with crushed mesocutis and endocutis. Mesocutis was multiplicative in young seed but totally degenerated at maturity of the development of embryo and endosperm. Seed surface is reticulate in sculpture with undulating anticlinal wall thickening. Also, it has several characteristic holes between anticlinal walls.

**Acknowledgements**

We are thankful to the Bio-Herb Research Center of Kangwon National University for giving permission to collect research materials and also supporting experimental instruments. Also, we appreciate two anonymous reviewers for their valuable comments and suggestions.

**Literature Cited**


Seed characteristics of *Withania somnifera* (Solanaceae)


