

# Embryo, Seed coat and Pericarp Development in *Abeliophyllum distichum* Nakai (Oleaceae): A Rare and Endemic Plant of Korea

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**Abstract** - *Abeliophyllum distichum* is a monotypic taxon of Oleaceae and endemic to Korea. A comprehensive study on embryogeny and fruit and seed coat ontogeny in *Abeliophyllum* was carried out via microtome and light microscopy. The fertilization occurs during mid- to late April and embryo matures by early July. The embryo development follows the general fashion from globular embryo – transition embryo – heart shaped embryo – torpedo embryo – walking-stick embryo to mature embryo. The pericarp clearly differentiates into three histological zones: exocarp, mesocarp, and endocarp. The young seed comprises 10-12 cells thick seed coat and the mature seed coat comprises an exotesta, 6-8 mesotesta and an endotesta. Any crystals, phenolic-like compounds, idioblasts, and the sclereids are not found in pericarp as well as seed coat. An overall development confirms Solanade type of embryogenesis in *Abeliophyllum*. The endocarp becomes more prominent in mature fruit and all the layers of endocarp are highly lignified. On the basis of mechanical layer the seed coat is of exotestal type.

**Key words** - *Abeliophyllum distichum*, Embryogeny, Pericarp, Seed coat

## Introduction

*Abeliophyllum distichum* Nakai (Oleaceae) commonly known as ‘White Forsythia’ is a monotypic genus and endemic to Korea. The plant with beautiful pale pink or white flowers grows as an under-story shrub in mixed deciduous woodland, often under *Pinus densiflora*, *Quercus myrsinaefolia* and *Q. acutissima* (Kim and Maunder, 1998). It is closely allied to *Forsythia* but differing in having flattened broadly winged fruits in contrast with capsular fruits of *Forsythia*. The first description of this plant was made by Nakai (1919) who had collected it in the Chinsan hills, lies in middle part of Korean peninsula. The populations of this plant are only found in three localities in central Korea although several more meanwhile extinguished populations have been recorded (Kim and Maunder, 1998). Natural populations of the taxon are geographically isolated from each other and have been critically affected by human collection and environmental disturbances (Kang *et al.*, 2000). The restricted geographical

distribution and apparent rarity were commented upon by Cotton (1948): ‘Its distribution was confined to an exceedingly small area; if it had not been brought into cultivation it was not only species but also genus which might easily have become extinct’. Despite Cotton’s fears (1948) *Abeliophyllum* has not become extinct; however it is close to extinction and qualifies for the IUCN Category of ‘Critically Endangered’, indicating a high risk of extinction in the near future (Kim and Maunder, 1998). Now, *Abeliophyllum* is cultivated in a number of Korean collections including the Chollipo Arboretum, Hantaek Botanic Garden, Keochungsan Botanic Garden, Kwanak Arboretum, Korea National Arboretum (previously named as Kwangnung Arboretum), and the Milim Botanic Garden (Anonymous, 1991). Anti-inflammatory and anti-oxidant chemicals has been reported from the leaf and flower extract of *A. distichum* (Ahn and Park, 2013; Park *et al.*, 2014).

On the basis of cladistic analysis of Korean Oleaceae Lee and Park (1982) reported that *Abeliophyllum* closely related with *Fontanesia* and is believed to have originated from the same ancestor (monophyly). Kim *et al.* (2000) compared nuclear ITS sequence data of *Abeliophyllum* with closely

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related genera of Oleaceae *Forsthia* and *Fontanisia* and found that nuclear ITS data strongly supports the sister group relationship of *Abeliophyllum* and *Forsythia* rather than the monophyly of *Abeliophyllum* and *Fontanisia*. In his original description of *Abeliophyllum*, Nakai (1919) relates it taxonomically to *Fontanisia*, but it resembles *Forsythia* in chromosome number (Taylor, 1945), vegetative appearance, and the lamellated pith. However, the solitary pendulous ovules in the ovary, which forms a compressed, suborbicular fruit with a wing around the edge, relate it morphologically to *Fontanisia*.

Rohwer (1993) carried a preliminary survey of the fruit and seed of the Oleaceae, but his report lacked the detailed study of fruit and seed of *Abeliophyllum*. Recently, Ghimire and Heo (2014) carried the embryological study of *Abeliophyllum distichum* and also compared the mature seed coat structure of the species with *Forsythia* and *Fontanisia*. However, their report still lacked detailed embryogenesis and ontogeny of seed coat and pericarp. Our primary objective in this study is to demonstrate and document the development of embryo, seed coat, and pericarp of *Abeliophyllum distichum*. We attempts to provide a detailed embryogenesis, comprehensive seed and fruit anatomical features of monotypic Korean endemic genus that might useful for the purpose through phylogenetic analyses on that basis.

## Materials and Methods

### Specimens

Fruit at different developmental stages were collected from the Rare and Endemic Plant Garden, Korea National Arboretum, Pocheon Korea. About 10-15 fruits were collected every week starting from late April till the late July, 2014 and fixed in FAA solution at least for one week and preserved in 50% ethanol.

### Light microscopy

For anatomical study preserved plant materials (seeds and fruits) were dehydrated with ethanol series (50%, 70%, 80%, 90%, 95%, and 100%). After complete dehydration, the materials were passed through alcohol + Technovit combinations (3:1, 1:1, 1:3, and 100% Technovit) and then embedded in

Technovit 7100 resin. Embedded materials were then sectioned. Serial sections of 4-6  $\mu\text{m}$  thickness were cut with Leica RM2255 rotary microtome (Leica Microsystems GmbH, Germany) using disposable blades, stuck onto a slide glass, and dried with an electric warmer for 12 hours. Dried slides were stained with 0.1% Toluidine blue O for 60 to 90 seconds, rinsed with running water and again dried with an electric slide warmer for more than six hours to remove water. The stained slides were then mounted with Entellan (Merck Co., Germany) and permanent slides were observed under the AXIO Imager A1 light microscope (Carl Zeiss, Germany). Microscopic photos were taken by AxioCam MRc5 attached camera system. Multiple image alignment was done using Photoshop CS4 for Windows 2007. None of the image alteration facilities of Photoshop have been used to change the original images captured by camera.

## Results

### Embryo development

The flower of *Abeliophyllum* is bicarpellary, each carpel bearing a single pendulous ovule (Fig. 1A). Fertilization occurs during mid- to late April. The fertilization results the diploid zygote at the micropylar end and triploid polar nuclei at the center of mature embryo sac (Fig. 1B). The first division of the zygote is always transverse and forms terminal and basal cells (Fig. 2A). The terminal cell and basal cell both divide transversely to give rise to a four-celled proembryo (Fig. 2B). Two terminal cells now divide by vertical walls oriented at right angles to each other to give rise to octant, while two basal cells divide transversely to produce four celled suspensor initial (Fig. 2C). The subsequent division of octant resulted globular embryo – transition embryo – heart shaped embryo – torpedo embryo – walking-stick embryo to mature embryo, while subsequent division of suspensor initial give rise long suspensor (Figs. 2D-I). The overall development confirms Solanade type of embryogenesis in *Abeliophyllum*.

### Fruit wall development

Fruit of the *Abeliophyllum* is bilocular circular samara. Each locule bearing single seed is separated by a septum (Figs. 3A-C). The pericarp clearly differentiated into three

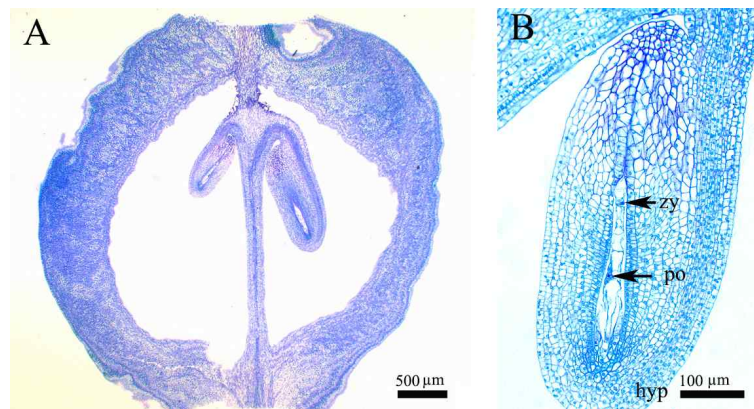


Fig. 1. Longitudinal section (LS) of young fruit of *A. distichum*. (A) LS of young fruit with two pendulous ovule. (B) LS of mature ovule showing zygote and polar nuclei. Abbreviations: zy, zygote; po, polar nuclei.

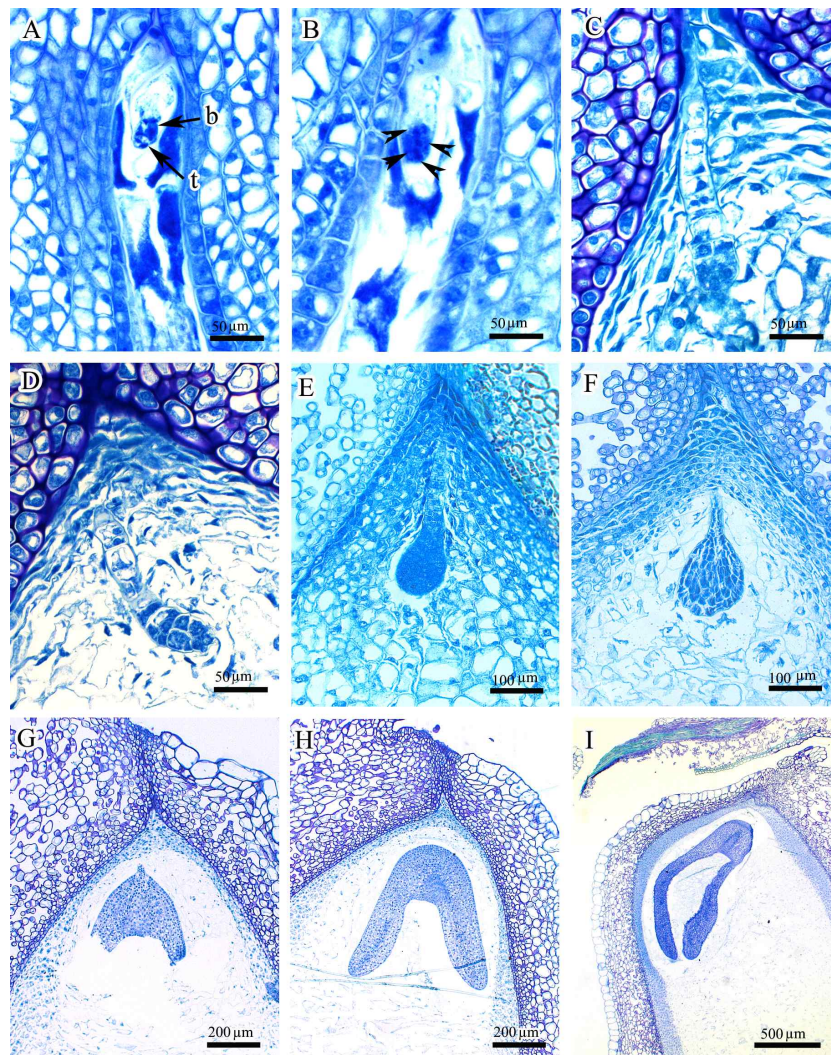


Fig. 2. Longitudinal section (LS) of seed at different developmental stages. (A) Two celled proembryo. (B) Four celled proembryo. (C) Eight celled proembryo. (D) 16-celled proembryo. (E) Globular embryo. (F) Transition embryo. (G) Herat embryo. (H) Torpedo embryo. (I) Walking-stick embryo. Abbreviations: b, basal cell; t, terminal cell.



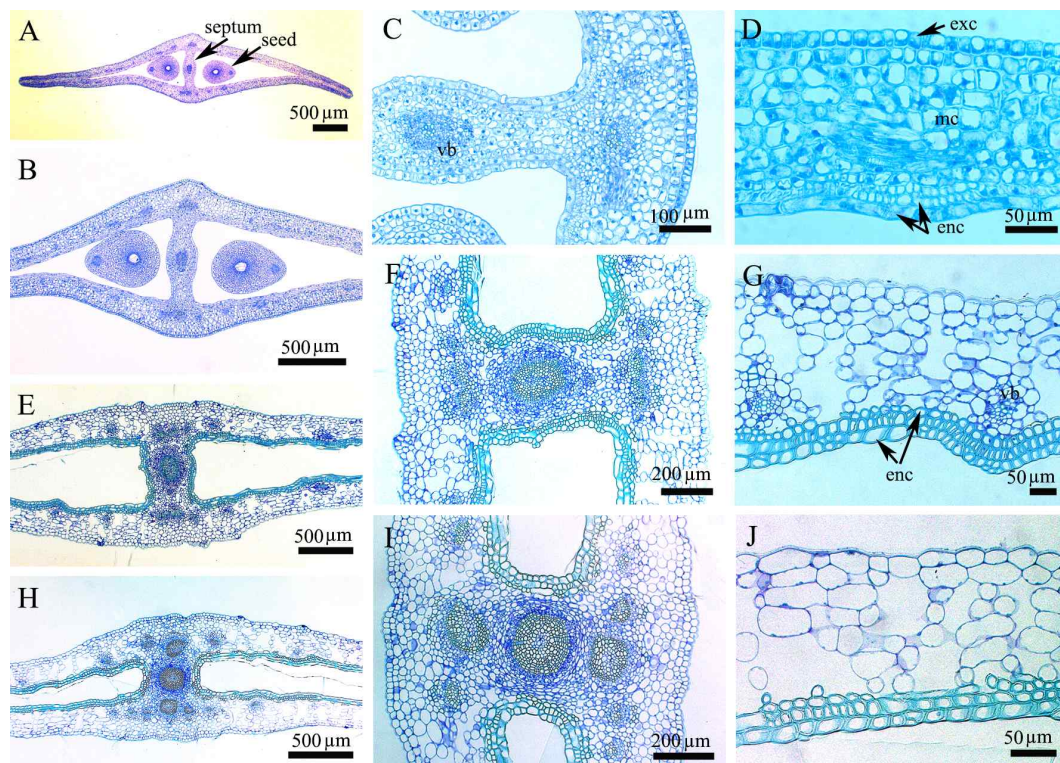


Fig. 3. Transverse section (TS) of fruit of *A. distichum* at different developmental stages. (A-D) TS of young fruit, (A) Whole fruit. (B) Part through septum. (C) Septum. (D) Pericarp. (E-J) TS of premature fruit. (E) Part through septum. (F) Septum. (G) Pericarp. (H-J) TS of mature fruit. (H) Part through septum. (I) Septum. (J) Pericarp. *Abbreviations:* enc, endocarp; exc, exocarp; mc, mesocarp; vb, vascular bundle.

histological zones: exocarp, mesocarp, and endocarp developing correspondingly from outer epidermis, mesophyll, and inner epidermis of the carpel. In young fruit the pericarp is 10-12 cell layered thick (Figs. 3C and D). The exocarp is represented by single epidermis with rectangular cells (Fig. 3E). Mesocarp is 6-8 layered comprising thin walled parenchymatous cells. The endocarp is 2-3 layered and the cells of innermost layer, which attached with locule, are tangentially elongated and cutinised. In mature fruits exocarp cells are highly cutinized and with thick cell wall (Figs. 3E-J). The parenchyma cells of the mesocarp are turn out to be irregular in shape and size. Some of them are under protoplasm-dissolving process or even complete disintegration as a result many intercellular spaces developed in that region (Figs. 3G and J). The phenolic-like compounds idioblasts and the sclereids are completely absent. Numerous vascular bundles developed in mesocarp (Figs. 3E-I). The endocarp becomes more prominent in mature fruit and all the layers of endocarp are highly lignified

(Figs. 3G and J).

### Seed coat development

Ovule is unitegmic, thus the entire seed coat originated from the single integument. At the time of fertilization the integument is 10-12 cells thick. The inner epidermal layer of the integument develops into an endothecium which later develops into endotesta. The young seed comprised same number of seed coat layer as in the fully matured embryo sac (Figs. 1B, 4A and C). In young seed, the exotesta composed of thick walled and relatively large rectangular or bedded cells (Figs. 4A and B). Except in the raphal portion, which is exceptionally thick, the mesotesta is 8-10 cell layered comprising compactly arranged elongated or polygonal parenchymatous cells. Several changes occur in all the three regions when seed matures (Figs. 4C-H). The endotestal cells compressed tangentially and become more longer than the wider (Figs. 4C-F). The mesotestal cells undergo protoplasm-

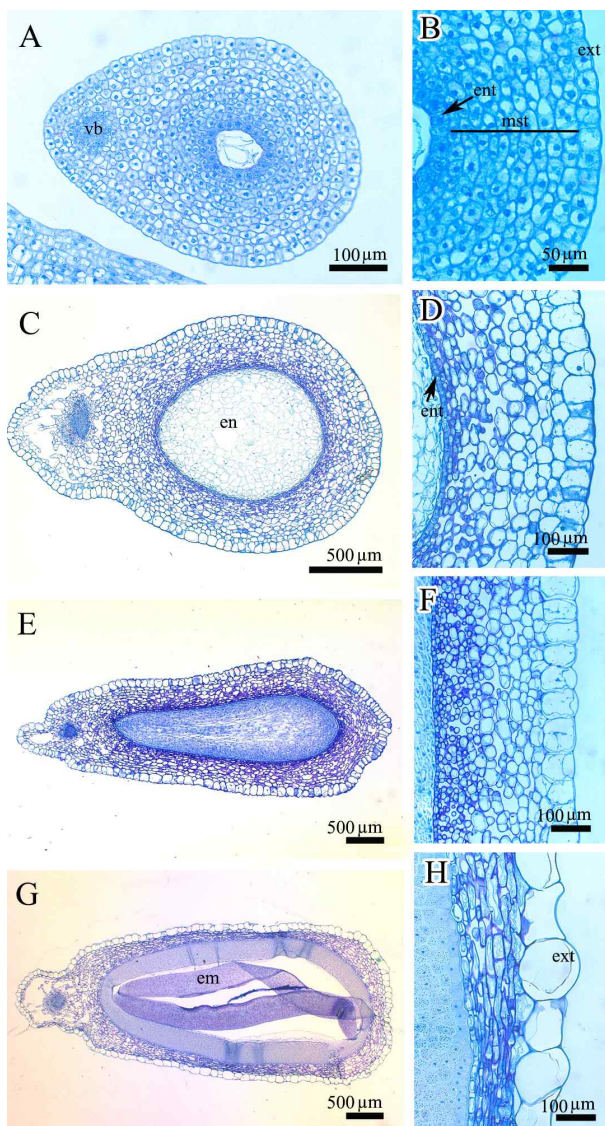


Fig. 4. Transverse section (TS) of seed of *A. distichum* at different developmental stages. (A) TS of young seed full. (B) Seed coat. (C-F) TS premature seeds. (C and E) Whole seeds. (D and F) Seed coat. (G and H) TS mature seed. (G) Whole seed. (H) Seed coat. *Abbreviations:* en, endosperm; ent, endotesta; ext, exotesta; mst, mesotesta; vb, vascular bundle.

dissolving process which starts from inner layer. Due to this shrinkage of mesotestal cells numerous intracellular spaces are formed (Figs. 4E and F). Simultaneously, considerable increment in size of exotestal cells occurs. By way of seed matures tangential elongation of cells in all three regions occurs and it happens more strongly in mesotesta. The mature seed comprises an exotesta, 6-8 mesotesta and an endotesta (Figs. 4G and H). The exotesta is well developed in terms of

maturity and the cells are thick walled, rounded or polygonal with convex outer and inner periclinal wall (Fig. 4H). The mesotestal cells become elongated and compressed tangentially whereas endotesta crushed in many places.

## Discussion

Except for the embryogenesis, detailed embryology of the *Abeliophyllum distichum* has been documented by Ghimire and Heo (2014). After overall comparison they confirmed that *Abeliophyllum* is similar to other genera of Oleaceae in many embryological features, having some distinct features such as the mode of anther wall formation, formation of a nucellar cap, and formation of obturator and hypostase. In this study, we presented the comprehensive embryogenesis starting from zygote to full matured embryo in *A. distichum*. We correspondingly documented the detailed ontogeny and structure of seed coat and pericarp.

The distyly flower in *Abeliophyllum distichum* was already reported in previous studies (Kang *et al.*, 2000; Hong and Han, 2002). We also found both pin and thrum flower in the study populations. This distyly nature of flower facilitates pollen exchange in different individuals. Studies on heterostyly-incompatibility of *A. distichum* revealed that illegitimate pollination (pin  $\times$  pin or thrum  $\times$  thrum) resulted in only 1.3 % fruit set, while legitimate pollination (pin  $\times$  thrum or vice versa) showed 30.8 % (Ryu *et al.*, 1976). Flowers open during late March to early April and last for less than a week. Since micro- and megasporogenesis was out of our interest, only mature ovules after anthesis were collected. Our first collection was of third 23<sup>rd</sup> April 2014, and observation suggested that fertilization almost over at that time and even ovary already took the shape of fruit. In fact, division of zygote has already been started because we found only few zygotes from first collection. Zygote develops into embryo via various proembryonal stages: globular embryo – transition embryo – heart shaped embryo – torpedo embryo – walking-stick embryo to mature embryo.

In Oleaceae, embryogeny may be Carryophyllad, which is reported in *Ligustrum ovalifolium* (Soueges, 1942) or Solanad as in *Jasminum* (Maheshwari, 1975). *Abeliophyllum*, *Jasminum*, and *Ligustrum* represent three different tribes in Oleaceae:

Forsythieae, Jasmineae and Olea respectively and the phylogenetic study has confirmed that Forsythieae is closer with Jasmineae than Olea (Wallander and Albert, 2000). This study confirms the Solanad type of embryogeny in *Abeliophyllum* that resembles with *Jasminum* rather than *Ligustrum*. However, it has been suggested that mode of embryo development is one of the most variable embryological characters within angiosperm and possibly only consistent below the generic level (Tobe, 1989). Thus, the similar mode of embryogeny found in *Jasminum* species and *A. distichum* does not validate the relationship of the two tribes nevertheless this feature parallelly evolved in *Jasminum* and *Abeliophyllum*.

Kim and Maunder (1998) reported that the *Abeliophyllum distichum* produces very little seeds in wild populations. Recently, Gimire and Heo (2014) found similar result in cultivated plants, that embryo development is rare in the species and only few fertilized zygotes develop into mature seeds. The observation of this study is very much similar to the previous results. All the research samples for this study were collected from three cultivated population of Korea National Arboretum (KNA). In the study populations both the morphs were equally present and plenty of fruits were set, however, the survival of embryo until the mature seed was scarce. In early July, we found only few mature seeds with living embryo from one population while in other two populations almost all the seeds were with perished embryo. The degeneration of embryo might be related with some physiological process however is beyond of our interest. This little production of fertile seed in wild habitat and possible sexual incompatibility in cultivated population lead this plant for the IUCN category of critically endangered indicating high risk of extinction in near future.

Fruit develops as a complex unity, other floral parts besides the carpel may be involved in fruit formation, and much has been discussed about the participation of ovarian accessory tissues in the fruit concept (Roth, 1997; Spjut, 1994; Judd *et al.*, 2002). Besides ovarian characteristics, the texture, the thickness, and the stratification of the pericarp can be considered in order to establish the fruit types (Spjut, 1994). Fruit of *Abeliophyllum distichum* is circular samara that developed from bicarpellary suborbicular and laterally

compressed syncarpous ovary. Three pericarp regions: exocarp, mesocarp, and endocarp develop from three corresponding regions: epidermis, mesophyll, and endodermis of the ovary. The exocarp is very similar with the epidermis of the ovary comprising only one layer. The mesocarp is multilayered formed by monotypic cells. Two different mesocarp (outer parenchymatous and inner lignified) zones have been described in *Fraxinus* species (Andrew, 2008). During the development of peicarp in *A. distichum* numerous intercellular spaces are formed in mesocarp. This is rather usual incident as also reported in outer mesocarp of *F. velutina* and *F. biltmoreana* (Andrew, 2008).

The most noticeable event during the pericarp development is the transformation of endocarp and development of vascular bundle in septum. The endocarp is structurally the most elaborated part during the pericarp development in *A. distichum*. In the beginning, it is 1-2 layered and the cells are parenchymatic, during the development both periclinal and anticlinal divisions occur resulting 3-4 layered endocarp in mature fruit and the cells are intensely lignified. Mechanically it is perhaps the most significant part of pericarp which protects seeds in *Abeliophyllum*. On the other hand, increment in size with noteworthy development of xylem takes place in vascular bundles in septum. Altogether there are more than half a dozen of vascular bundles in the septum region, with an extraordinarily large one in the middle, provided with full mechanical support to the mature fruit.

On the basis of mechanical layer, Ghimire and Heo (2014) described the exotestal seed coat in *Abeliophyllum distichum* and the result of this study is not different either. Three regions of seed coat: exotesta, mesotesta, and endotesta are very much identical in young seeds. As the seed matures gradual development occurs in exotesta, however, mesotesta and endotesta slowly distorted. In mature seeds, the exotestal cells are well developed thick walled, large, and roundish or barrel shaped and therefor protective function of the seed coat is adequately taken over by this part. Ghimire and Heo (2014) compared mature seed coat of *Abeliophyllum*, *Forsythia* and *Fontanesia* and found similar seed coat structure in former two. In other Oleaceae, the seed coat types similar to that of *Abeliophyllum* and *Forsythia* have been observed in *Syringa pекinensis*, *Phillyrea angustifolia*, *Fraxinus excelsior* and



*Ligustrum quihoui* (Rohwer, 1993). Interestingly, these four genera all belong to the tribe Oleace.

In conclusion, this is by far the first report for the comprehensive embryogeny of critically endangered *Abeliophyllum distichum*. The result clearly indicates the Solanade type of embryo development in *A. distichum*. Ontogeny of pericarp shows that endocarp is highly lignified and acts as protective layer in fruit. Similarly, in seed coat the layer that provides mechanical support and protection is exotesta. The crystals, sclereids, and idioblastic cells are totally absent in mature pericarp and seed coat.

## Acknowledgement

This study was financially supported by the project ‘Studies on the Establishment of SeedBank base for the Asian network’ Korea National Arboretum, Pocheon, Korea.

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(Received 7 April 2015 ; Revised 6 June 2015 ; Accepted 9 June 2015)