A study of Anther development in *Azalea alexander* L.

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**ABSTRACT**

*Azalea alexander* L. is belonging to Ericaceae family. In this study, anther development and microsporogenesis of *Azalea alexander* L. were considered. The flowers, in different developmental stages, were collected, fixed in Formalin -glacial acetic acid-alcohol (FAA), stored in 70% ethanol, embedded in paraffin and then, sliced at 7-10 μm by rotary microtome and stained by Eozine and hematoxylin. The results indicated that anther walls development followed the dicotyledonous type and were tetrasporangiate with composed of epidermal layer, endothecium layer, middle layers and tapetum layer. Microspore tetrads are tetrahedral tapetum layer was secretory type. Pollen grains are tricolporate. The nucleus is divided by the mitosis into two nuclei, small generative and large vegetative nuclei that's why they are called bi-nucleated pollen grain.

**INTRODUCTION**

*Azaleas alexander* L. are one of the most important of garden plants. The majority of naturally occurring species are native to the areas in western and central China. *Azaleas* belong to family of Ericaceae and genus of Rhododendron. The genus Rhododendron belongs to tribe Rhodoreae. [1,3].

They require acid soils and protection from the cold and wind. Appropriate soil for this plant is peat and soil’s drainage must be excellent [20]. It's flowers in the different colors are red, violet, white, pink and yellow. Few other evergreen shrubs produce such a spectacular floral display in March and April. The leaves are deciduous or perennial it is a shrub whose drainage must be excellent [20].

It has 8 stamens with oblong anther. Often *Azaleas* are planted near foundations, steps and decks to soften architectural features [1].

Notably, there is not enough information about anther structure and male gametophyte developmental stages of *Azalea alexander* L. Development of male gametophyte involves a series of occurrences to produce and release mature pollen grains from anther.

The main aim of this paper was to investigate a detailed study on microsporogenesis of *Azalea alexander* L., for improving the knowledge of microsporogenesis developmental events and for evaluating of taxonomic relationship among *Azalea* species.

**MATERIAL AND METHODS**

In this research flowers from natural population were collected at different developmental stages between 8:00 - 9:00 AM, from Chalus in north of Iran.

Flowers were fixed in FAA (Formalin-glacial acetic acid-alcohol). After the fixation process; the samples were dehydrated during alcohol series (15, 30, 50, 70), and embedded in paraffin after the process of paraffin saturation in toluene. And sections with a thickness of 7-10 μm using a rotary microtome. Staining was carried out with Eozine and Hematoxylin [23,9].

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Several sections for each anther developmental stages were investigated with a Zeiss Axiostar plus light microscope. Many samples were studied before each stage and photomicrographs were made from the most effective ones.

**Result:**

Each pollen sac was concluded of peripheral cells forming an undifferentiated walls and a mass of uniformed cells (archeousporial cells) (fig1A).

Each young anther was consisted of 4 pollen sacs (tetrasporangiate) with connective tissue in the center (Fig 1C).

From the cross section we see that the anther wall consists of layers from outer to inner: epidermis, endothecium, middle layer and layer of tapetum (Fig. 1D).

Taptum layer *Azalea alexander* L. was secretory type in this development stage. microspore mother cells (Microsporocytes), were also detectable (Fig1B).

Microspores and pollen grains are produced from pollen mother cells (Microsporocytes), PMCs within loculus anthers of the flower. Microsporocytes are recognizable by their large volume, dense cytoplasm, and conspicuous nuclei. The single cell layer of tapetum surrounding the anther loculus is first recognizable at the early Microsporocyte stage.

Large volume, dense cytoplasm and one or two large nuclei are evident in the tapetal cells at this point. Each microspore mother cell undergoes meiosis; during which M.M.C undergo successive type.

**Fig. 1:** A: Archeospore cells; B:Microspore mother cells(M.M.C) C:Tetrasporangiate anther with connective tissue (Con) D:Each anther contains four layers: epidermis (Epi), endothecium (End), middle layer (Mid) and tapetum (Ta) E: Pollen grains in dyad stage  F: A dyad cell with callosic wall (C.W)
Fig. 1G: Terahedral tetrads (Tet) in the anther loculus H: callosic wall in a tetrad cell I: adult anther with endothecium layer (End) J: endothecium (End) layer are tangentially elongated K: pollen grain with Exin (Ex) and Intin (In), nucleus (Nu) In early stage of meiosis, Microsporocytes separate with callose walls; The first nuclear division of meiosis (meiosis I) is accompanied by cell wall formation resulting in the formation of two haploid cells in a dyad (fig1 E,F).

After meiosis II numerous tetrads of microspores appear, tetrad shape is tetrahedral and the wall formation taking place after each stage of meiosis.
Then the wall surrounding the each microspore cells dissolve and the microspores in the pollen sac are released.

The mitotic division of the microspore is unequal. Therefore, a darker generative nucleus and a lighter vegetative nucleus appear. So pollen grains are two-celled at the time of shedding. Exin (Ex) and Intin (In) are formed in this stage (fig 1K).

when pollen grains become mature, the tapetal cells were degenerated, In meiotic division, the middle layers cells start a degeneration process. After the tetrads are formed, the innermost of the middle layers begins to degenerate and all of them become flattened during the free microspore stage and have degenerated completely at the mature pollen grain stage. Pollen grain is tricolpated.

Discussion:
Results obtained from this research show that Developmental stages of Azalea alexander L. anther wall followed the dicotyledonous type, which was composed of an epidermal layer, an endothelial layer, one middle layer and tapetum which is consistent with Davis 1966 and bagheri 2014. The tapetum was secretory that was similar with the findings of Chehregani et al., [8] and bagheri [2] for Lepidium vesicarium L and Lepidium sativum L for Azalea alexander L. The anther tapetum is the main supplier of nutrients and cell wall precursors for developing pollen grains [21].

A sharp correlation was observed between division in microspore mother cells (M.M.Cs) and development of anther’s tapetum. That was reported for other dicotyledonous plant [6,13,10].

The microspores at releasing time are vacuolated and they have peripheral nucleus. The nucleus is then divided during mitosis into two nuclei, a small generative nucleus, and large vegetative nucleus, hence the name bi- nucleated pollen grain, which is in accordance with findings of chehregani and jafari [8,16].

REFERENCES