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Pollen and Anther Development in *Onobrychis schahuensis* Bornm. (Fabaceae)

Abdolkarim Chehregani, Nayereh Tanaomi and Massoud Ranjbar
Laboratory of Plant Cell Biology, Department of Biology, Bu-Ali Sina University, Hamedan, Iran

Abstract: Young buds and flowers of *Onobrychis schahuensis* were removed at different stages of anther development and sectioned with micotome after preparation. The anther and pollen development were studied with light microscopy after staining. Results showed that some ultrastructural characters of anther and pollen grains were different from other Fabaceae members. The undifferentiated anther is ovoid-shaped and tetrasporangiated. The anther wall development follows the dicotyledonous type, which is composed of an epidermal layer, an endothelial layer, one middle layer and tapetum. The tapetum is secretory and its cells are uniseriate and uninucleate but some of them are binucleate. The microspore tetrads are tetrahedral. Pollen grains are ovate, tricolporate and shed at a bicellular stage.

Key words: Development, *Onobrychis schahuensis* Bornm., Fabaceae, anther, pollen

INTRODUCTION

The genus *Onobrychis* belongs to tribe Hedysareae and with nearly 130 species is mainly distributed in the northern temperate regions but centre of its diversity is in the eastern Mediterranean area and west Asia; a few taxa are cultivated as fodder or ornamentals (Lock and Simpson, 1991; Yakovlev *et al*., 1996; Mabberley, 1997). In Flora Iranica Rechinger (1984) treated 77 species under 9 sections viz., *Dendrobrychis* (7 species), *Lophobrychis* (5), *Onobrychis* (14), *Laxiflorae* (3), *Anthyllium* (7), *Afghanicae* (3), *Insignes* (3), *Heliobrychis* (21), *Hymenobrychis* (12), with two species remaining unassigned. Morphological characters of the male gametophyte and details of microsporogenesis can be used in systematic studies for defining the circumscription of taxones. There are some taxonomically important characters such as variability in the number of anther layers, tapetum type, the arrangement of tetrads within the callosic wall, the number of pollen pores or colpes, bi or tricellular pollen grains and the number of tapetum nuclei (Liu and Huang, 1999, 2003; Galati *et al*., 2006; Gotelli *et al*., 2006).

There are two major types of tapetum, the more primitive secretory type, considered to be the prevalent type in the majority of plants and the amoeboid type that extends to the microspores in the anther locule for presumable direct delivery of tapetal contents (Furness and Rudall, 2001).

Also four types of anther wall development were described by Davis (1966) based on the secondary parietal layers: Basic type (type I), Dicotyledonous type (type II), Monocotyledonous type (type III) and Reduced type (type IV). In general, one specific type of the anther wall development is found in each family. However, some families possess two types of anther wall development, such as the Commelinaceae having type I and III (Hardy *et al*., 2000) and the family Solanaceae having type I and II (Carrizo, 2002).

The development of the male gametophyte involves a series of events culminating in the production and release of mature pollen grains from anthers (Mc Cormick, 1993, 2004). Because having a few studies on microsporogenesis (Liu, 1997; Liu and Huang, 1999), there is not any information about *O. schahuensis* microsporogenesis. The present paper is the first report and is an attempt to understand the male gametophyte development in *O. schahuensis* and its taxonomic significance.

MATERIALS AND METHODS

Young floral buds were collected from field plants in native growing area. The voucher specimen is deposited at the Bu-Ali Sina University Herbarium (ICN 10998) and labeled as follows: Iran, prov. Kermanshah, 15 km from Javanrod to Tazeabad, Alt. 1250 m. The flowers and buds in different stages of development were removed at 4.5.2006, fixed in FAA70 (formalin, glacial acetic acid and 70% ethanol, 5:5:90 v/v), stored in 70% ethanol, embedded in paraffin and sectioned at 7-10 μm with a Micro DC 4055 microtome. Staining was carried out with PAS (Periodic Acid Schiff) according to protocol suggested by Yeung (1984) and contrasted with Meyer's Hematoxylin. Several sections for each anther developmental stage were studied under a Zeiss Axiostar Plus light microscope. For each stage, at least 20 flowers were studied and photomicrographs were made from the best ones.

Corresponding Author: Abdolkarim Chehregani, Laboratory of Plant Cell Biology, Department of Biology, Bu-Ali Sina University, Hamedan, Iran

RESULTS AND DISCUSSION

The anther and pollen development were studied with light microscopy. The undifferentiated anther is ovoid-shaped and tetrasporangiated (Fig. 1). The anther wall consists of epidermis, endothelium, one middle layer and tapetum that is secretory type (Fig. 1a-h). The microsporangial wall follows the dicotyledonous type of ontogeny (Davis, 1966). The outer secondary parietal layer gives rise to two layers, the outermost forming the

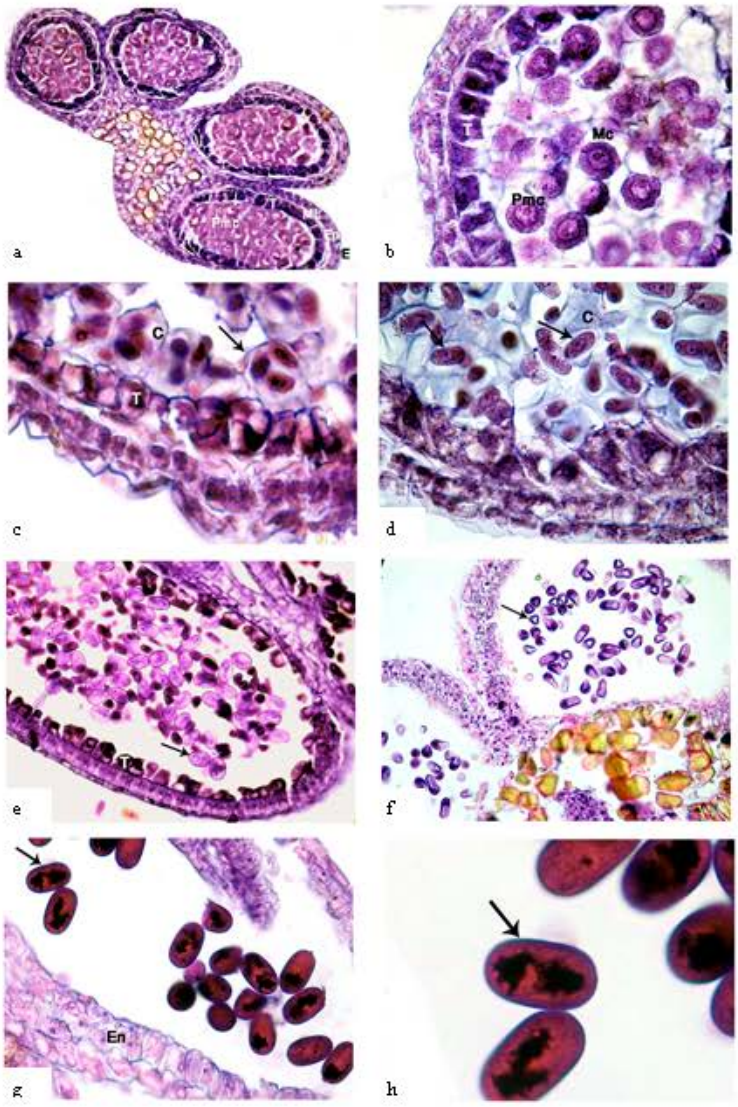

Fig. 1: (a) Cross section of anther at early microspore mother cells stage with epidermis, endothecium, middle layer and tapetum. Note that the microspore mother cells are tightly appressed and fill the locular space. (b) Section of a single locule showing late microspore mother cells. Note that the microspore mother cells are separated and each is surrounded by a callosic coat. The locule is lined by a well-differentiated tapetum (T). (c) Section through tetrahedral tetrad; callosic wall are visible that surrounding early tetrads. (d) Anther at the late tetrad stage. Note the nucleus at the center (arrows) and callosic wall. (e) Anther at the free young microspore stage. A central vacuole appears in young free microspores and pushing the nucleus towards the peripheral regions (arrow). (f) Anther at the late stage of pollen grain. The pollen grains are ovate in length, triangular in cross and 3-colporate (arrow). (g) Anther at the mature pollen grain stage (arrow). Note the absence of tapetum and the mature endothecium. (h) Mature releasable bicellular pollen grain (arrow). C: callosic wall; E: epidermis; En: endothecium; Mc: microspore mother coat; Ml: middle layers; Pmc: microspore mother cells; T: tapetum

endothelium and the innermost, the middle layer. The inner layer directly functions as tapetum. The tapetal cells are uninucleate but some of them are binucleate and have a dense cytoplasm.

The early microspore mother cells are uninucleate and poorly vacuolated (Fig. 1a). Then late microspore mother cells become separated and each of them is surrounded by a callosic coat (Fig. 1b). In this time tapetum is well-differentiated. When microspore mother cells enter the phase of meiotic division, the four haploid microspores become enclosed in a thick callosic wall. At the early tetrad stage, the microspores contain a big nucleus at the center (Fig. 1c).

Cytokinesis is simultaneous and the arrangement of microspores is tetrahedral (Fig. 1c,d). The microspore tetrads are still within the callosic walls during the late tetrad stage (Fig. 1d). Once released from the tetrad, the microspores enlarge. A central vacuole appears in them, pushing the nucleus towards the periphery and tapetum degeneration is visible well (Fig. 1e). The pollen grains are tricolporate (Fig. 1f). As a result, the mitotic division of the microspore is unequal. Therefore, a small generative cell and a large vegetative cell appear so pollen grains have two-celled at the time of shedding (Fig. 1g, h). In this time tapetum degenerate completely and we can see endothelium and epidermis.

Morphological characters of the male gametophyte and details of microsporogenesis can be used in systematic studies for defining the circumscription of the genus. In general, one specific type of the anther wall development is found in each family. The anther wall development in the Faboideae was reported as Dicotyledonous type (Davis, 1966; Prakash, 1987), including the genera *Trifolium* (Hindmarsh, 1964), *Pisum* and *Lens* (Biddle, 1978), *Indigofera* (Ashrafunnisa and Pullaiah, 1995), *Desmodium* (Buss *et al*., 1969), *Lotus* (Galati *et al*., 2006) and based on our results, in the present study of *O. schahuensis* are also Dicotyledonous type.

Until now all the studied members are uniform in showing a simultaneous cytokinesis of pollen mother cells. Tapetum layer is secretary type in most studied Fabacian plants (Liu and Huang, 1999; 2003; Gotelli *et al*., 2006). However, in *Indigofera* and *Rhynchosia* (Oomman, 1971), *Uraria* (Liu and Huang, 1999), in *Dumasia miaoliensis* (Liu and Huang, 2003) and in *Macroptilium arenarium* (Gotelli *et al*., 2006), the anther wall consists of an epidermal layer, an endothelial layer, two middle layers and a tapetal layer, that belongs to Basic type (type I). Present results showed that in *O. schahuensis* is also accordance to Basic type and The arrangement of microspores within the callosic walls is tetrahedral, as the same other Fabacean member (Galati *et al*., 2006).

Present results showed that the pollen grains are ovate and tricolporate in *Onobrychis schahuensis* but in some species of Fabaceae, such as *Dumasia miaoliensis* 6-porate pollen grains was reported (Liu and Huang, 2003). In many species the generative cell divides before pollen release into two identical sperm cells (tricellular pollen grains), while in others of this division only occurs after pollen germination (bicellular pollen grains). Pollen grains of all the studied members such as *Uraria* (Liu and Huang, 1999), *Dumasia miaoliensis* (Liu and Huang, 2003), *Lotus* (Galati *et al*., 2006) and based present results, *O. schahuensis* are bicellular. The tapetal cells are uninucleate in *Uraria* (Liu and Huang, 1999), *Dumasia miaoliensis* (Liu and Huang, 2003), *Macroptilium arenarium* (Gotelli *et al*., 2006), but our results indicated that in *O. schahuensis* there are both bi and uninucleate tapetal cells.

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