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# Development of male and female reproductive organs in Moldavian dragonhead, *Trachyspermum ammi* (L.) Sprague (Apiaceae)

**Reyhaneh Saeidi, *Golnaz Tajadod, Sayeh Jafari Marandi, Maryam Peyvandi**

Biology Department, College of Bioscience, Islamic Azad University, Tehran North Branch, Tehran, Iran

*Corresponding author: Tajadodg@gmail.com

## Introduction :

Zenian *(Trachyspermum ammi* (L.) Sprague) belongs to the Apiaceae. It is an herbaceous, annual, aromatic, erect, and branched plant with umbrella inflorescences (Mozaffarian, 2013). Zenian originated from the Indian subcontinent and the Mediterranean region (Nagalakshmi et al., 2000). This species is distributed natively in Ethiopia, Egypt, and India and planted in Iran, Afghanistan, and Pakistan (Mozaffarian, 2013).

The seeds of Zenian are used in most of Iran's pharmaceutical industry in the production of anticancer, antithrombotic, antibacterial, blood pressure lowering, antitussive, and liver-protecting drugs (Esmaeili & Ghobadianpour, 2016).

Various research in flower/inflorescence development and evolution patterns (Baczynski et al., 2022; Ajani et al., 2016; Linke et al., 2003), male and female gametophyte development and embryogenesis (Zhou et al. al., 2023; Kazemivash et al., 2020; Zarini et al., 2017; Kaplan & Iris., 2016; Jonoubi et al., 2015; Sheikhbahaei et al., 2014) have been carried out. Flowers in the Apiaceae family are usually poly-symmetric, tetracyclic, and five-petaled, and the ovary is inferior and bilobed. Given this uniformity, the evolution of flowers is expected to be homogeneous among species. However, different studies have shown a high diversity of flower morphology in this genus (Ajani et al., 2016).

The literature review showed that the developmental characteristics of the reproductive organs of female plants have only been considered in a limited way in some research (Sehgal, 1965). Considering the medicinal importance of ginseng, investigating the structure of reproduction and reproduction can help increase our knowledge concerning this valuable plant's breeding, reproduction, and reproduction.

## Methods and Materials:

The seeds were obtained from the Pakan Seed Company of Isfahan (Iran) and planted in plastic pots. At the beginning of the flowering stage and a little before that, the top of the flowering stem, buds, and flowers at different ages were collected and fixed in FAA 70 solution with a volume ratio of 5:5:90. This solution included formalin, glacial acetic acid, and 70% ethanol. After that, the samples were stored in 70% ethanol. Different dilutions of ethanol were used to dehydrate the samples, and after molding in paraffin, appropriate sections were prepared to study the developmental stages. For this purpose, a DC microtome (Cut 4040, Co., Mainz, Germany) was used. Staining was done with hematoxylin-eosin according to the method of Yeung (1984). More than ten samples were studied for each evolutionary stage. A Nikon light microscope (Nikon, Tokyo, Japan) was used to examine and take photos of the sample.

## Results & Discussion:

Umbellate inflorescences, alternate, pentamerous, four-lobed flowers, and the inferior ovary comprise the main characteristic features of the Apiaceae family. The initiation sequence of flower organs is centripetal. In addition to vegetative organs, secretory ducts are also present in various reproductive parts of plants, which is one of the specialties of this family. Like other members of this family, they are schizogenic type. Asynchrony of flower development was seen in the umbels and umbellets, and the peripheral umbels appeared earlier than the middle ones. The filament is folded and bent inside the bud from

the initial stages of development until before the anthesis of the Zenian flower, which is a feature that can also be seen in other plants of this family, such as Pycnocycla nodiflora (Sheikhbahai et al., 2014). Some developmental traits (with the phylogenetic signature) of flower reproductive organs in this family are primitive (polygonum-type of the embryonic sac, anatropous ovule, secretory tapetum layer), and some of them are advanced (unitegmic and tenuinucellate ovules). These features have phylogenetic importance.

Given evolutionary insight, unitegmic and tenuinucellate ovules are a simplified type of crassinucellar, bitegumic ovules, which are usually formed in the sympetalous and partially in epipetalous families (Zhang et al., 2011; Wang & Ren, 2007), which confirms that the species of the Apiaceae family are a more evolved group than other angiosperms (Zhou et al., 2023). The similarity of the developmental characteristics of male and female gametophytes between different species such as Seseli resinosum, Bupleurum mucronatum, Cuminum cyminum, Ferula sinkiangensis, Pimpinella spp and Trachyspermu ammi (females) has been mentioned before (Kaplan & Iris, 2016). In the early stages of another development, several archaesporic cells under the epidermis create parietal and hexagonal cells with their tangential divisions. The parietal layers include the epidermis, mechanical, middle, and nutrient layers. Although the epidermis in the female plant is wrinkled and highlighted as a thin jelly, it remains on the mechanical layer until the last stages of anther maturation and flowering.

At the early stages of the anther's development, archeosporial cells are differentiated beneath the epidermis of the anther, and each of them divides into peripheral (origin of anther walls) and sporogenous (origin of MMC (microspore mother cells)) cells. The epidermis, endothecium, middle, and innermost tapetum layers comprise the main layers of anther walls. This callosic wall shows that meiosis division is imminent. Several meiosis divisions of MMC cause the formation of tetrahedral tetrads that are surrounded

by a particular callosic wall. The degeneration of the middle layer at the end of the tetrad stage has been seen in Zenian and also in other species of this family, such as white celery (Zarini et al., 2017), Korean dogtooth (Sheikhbahai et al., 2014). The tapetum layer with more than two or even three cells is seen in this species. The different size of the pollen sacs in each anther is observed and different stages of cell division in it and other pollen sacs of the same anther could be synchronous. Zhou. et al. (2023) consider the time mismatch of divisions in the anthers of Glehnia littoralis as a reproductive strategy that prolongs the pollination and cross-fertilization time. Functionally, the presence of hermaphrodite and male flowers in one plant is ubiquitous in the celery family (Ajani et al., 2016). It was also observed in the female plant. Male sterility, due to the function of the nutrient layer leads to the production of abnormal microspores or empty anthers, or it affects the anther blooming. Deficiency in the early stages of development was also observed in female plants. Some of these defects are related to limited nutrient resources available to the plant, unsuccessful pollination, and unsuccessful fertility (Zhou et al., 2023).

In female plants, pistil development is delayed compared to stamens. Stigma is broad at the base and forms two tuberous protrusions (stylopods). In the female plant, the megaspore tetrad is of the linear type, and the embryo sac is polygonum-type. The secretory duct in the ovary's wall is also often visible, which indicates the high secretory activity in the plants of this family, which are valuable as medicinal plants. Like most of the species of this family, Megaspores in the female plant are of linear type. However, in cases such as *Glehnia littoralis*, the linear and T-shaped tetrad type has been reported (Zhou et al., 2023).

## Conclusion :

Given our finding, the following features could be seen in the male phase: isostemonous stamens, secretory tapetum layer, tetrahedral microspore tetrad, spindle shape microspores, mature pollen with three-celled and three germinal furrows. Asynchrony of microspore development was observed in the anthers of the same plant flowers and the pollen sacs of the same anther. Also, the development of male and female gametophytes was not synchronized in the same flower. The ovules were anatropous, unitegmic, and tenuinucellate. The linear type of megaspore tetrad, micropillar functional megaspore, and polygonum type of embryo sac development are other characteristics of this plant.

*Keywords: Embryo sac, Microspore, Pollen grain, ovule, Tetrad*

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## Statement on ethics:

*The authors declare that this work has not been published elsewhere nor submitted to another publication simultaneously.*