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ACTA BIOLOGICA CRACOVIENSIA Series Botanica 63/1: 21–29, 2021
10.24425/abcsb.2020.131675

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POLSKA AKADEMIA NAUK
ODDZIAŁ W KRAKOWIE

EMBRYOLOGICAL STUDIES ON SILENE *MURADICA* SCHISCHK. (CARYOPHYLLACEAE) – A GYNODIOECIOUS SPECIES FROM TURKEY

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Received February 4, 2021; revision accepted March 18, 2021

In this study, female gametophytes of *Silene muradica*, which is a gynodioecious species, were examined histologically. Buds and blossoms of *S. muradica* were used as the research material. They were collected in the Sivas province (Turkey) in July 2019, and fixed with ethanol:acetic acid solution (3:1, v/v). Flower parts were dissected under a stereo microscope. They were dehydrated in rising alcohol series and then embedded in Historesin. The sections were taken by a rotary microtome and stained with 0.5% Toluidine blue O. The ovary of *S. muradica* has three carpels and a single chamber, the ovules are arranged on a central column. The mature ovule is of the campylotropous type, crassinucellate and bitegmig. The megaspore mother cell undergoes regular meiotic division and forms a linear megaspore tetrad after meiosis. The development of the embryo sac is monosporic. The chalazal megaspore is functional and the others degenerate. The mature embryo sac is eight-nucleated and of the Polygonum type. The synergid cells and the egg cell are completely surrounded by the cell wall. Antipodal cells are temporary cells, which degenerate immediately after fertilization. Before fertilization, polar nuclei are fused in the central cell and form the secondary nucleus. The endosperm development is of the nuclear type. Nucellar tissue is permanent and forms perisperm in mature seeds. The embryo development is of the Caryophyllad type. In this study, the development of the female gametophyte of *S. muradica*, which was determined to be a gynodioecious species, was reported for the first time.

Key words: *Silene muradica*, Caryophyllaceae, embryo sac, gynodioecious, Turkey.

INTRODUCTION

The genus *Silene* L. (Caryophyllaceae), represented by about 700 species divided into 44 sections, is widely distributed throughout the Northern hemisphere (Siroky et al., 2001). The South Balkan Peninsula and Turkey are two of the main centers of diversity for the genus (Greuter, 1995). The genus is one of the largest genera of flowering plants in the world and the most species-rich taxa in Turkey (Meric and Guler, 2013). *Silene* is represented by 136 species in the flora of Turkey and 40% of these species are endemic (Coode and Cullen, 1967; Davis et al., 1988; Tan and Vural, 2000). *Silene muradica* Schischk. was collected from the Malazgirt district (Turkey) by B.K. Schischkin in 1916, named in 1920 and introduced to the world of science (Coode and Cullen, 1967). The species is distributed in the Nigde, Nevsehir, Sivas, Malatya, Elazig, Tunceli, Erzincan, Mus, Van and Antalya provinces of Turkey (Coode and Cullen, 1967). It was considered to be endemic to Turkish flora for 99 years. However, in 2019, a record was reported from Razi village of Iran borders near the Yüksekova district of Hakkari province (Turkey) (Heidari Rikan et al., 2019).

Gynodioecy is a situation in which female and hermaphrodite individuals appear together in the same population. In gynodioecious species, both male and female organs of hermaphroditic individuals are functional, whereas female flowers typi-

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PL ISSN 1898-0295

cally produce dysfunctional anthers and no viable pollen grains. Gynodioecy in angiosperms is considered to occur in approximately 7% of species and 2% of genera (McCauley and Bailey, 2009; Dufay et al., 2014; Casimiro-Soriguer et al., 2015). It is known that there are many taxa which have gynodioecious individuals in the Caryophyllaceae family like *Arenaria, Dianthus, Silene, Stellaria,* etc. (Shykoff et al., 2003; Stone and Olson, 2018). Sexual system rates of 98 species belonging to the subgenera *Silene* and *Behenantha* of the *Silene* genus were found to be 58.2% for hermaphroditism, 14.3% for dioecy, 13.3% for gynodioecy and 12.2% for gynodioecy–gynomonoecy (Casimiro-Soriguer et al., 2015). Also, one gynomonoecious and one andromonoecious species have been identified (Casimiro-Soriguer et al., 2015). Stone and Olson (2018) reported that female individuals of gynodioecious *Silene vulgaris* produced twice as many seeds as hermaphrodite individuals when there was a sufficient pollinator in the environment. However, Delph et al. (1999) reported that female individuals of *Silene acaulis* produced an equal number or fewer seeds than hermaphrodite ones.

Although the *Silene* genus contains about 700 species in the world, studies on reproductive biology are very few. In this limited number of studies, the following problems have been examined: embryo and endosperm development of *Silene conoidea*, pollen wall ontogeny of *Silene pendula*, cytochemical properties of pollen and tapetum ontogeny of *Silene dioica*, ultrastructure of the endosperm and perisperm of *Silene alba* and the female and male gametophyte development of *Silene involucrata*, an arctic species (Cook, 1909; Heslop-Harrison, 1963; Audran and Batcho, 1981; Mohana Rao et al., 1988; Kellmann-Sopyla et al., 2017). However, studies on the reproductive biology of the genus *Silene*, which includes hermaphrodite, dioecious, gynodioecious, gynomonoecious and andromonoecious species, are quite limited.

Scientific data on *Silene muradica* are also very limited. The chromosome number of the species was determined as 2n = 24 (Martin et al., 2015). In addition, pollen properties were examined by Yıldız (2001). The literature review shows that there are no studies on embryology of this species. The aim of this study was detailed examination of the development of the embryo sac and embryo of *S. muradica*, known as endemic to the flora of Turkey until 2019, using light microscopy.

MATERIALS AND METHODS

The buds and flowers of various sizes of *S. muradica* were collected in the villages of Kayadibi and Menşurlu (39°29′09.6″ N, 36°43′11.4″ E, 1375 m, Sivas, Turkey) in July 2019. The samples were fixed in the field with ethanol (absolute): acetic acid (glacial) solution (3:1, v/v) for 24 hours and then stored in 70% ethyl alcohol. The anthers and ovaries were dissected under a stereo microscope. They were treated in rising ethyl alcohol series and embedded in the Historesin (Historesin Embedding Kit, Leica Biosystems Heidelberg, Germany). The embedded blocks were cut into 4 μm thick sections, using a rotary microtome (RM 2255, Leica Biosystems, Nussloch GmbH, Germany) with a tungsten carbide blade (Leica). The sections were stained in 0.5% Toluidine Blue O solution for 2 minutes and washed in distilled water for 30 seconds and then dried in air (O'Brien et al., 1964, modified). They were closed with Entellan (Merck). Observation and photography were carried out using a digital camera which was attached to an Olympus CX21 light microscope (Tokyo, Japan).

RESULTS

Silene muradica was found to be a gynodioecious species (coexistence of male sterile female plants and hermaphrodite plants in the population). Androecium in male-sterile female flowers contains ten stamens. The anther initially appears as a mass of unorganized cells surrounded by epidermis (Fig. 1a). In the advanced stages of development, the anther shrinks with the degeneration of the cells under the epidermis (Fig. 1b). Gynoecium consists of one compound pistil of three carpels with a superior ovary, a three-lobed style and a stigma. In the ovary of *S. muradica*, there are 18–20 developing ovules which are attached to the central axis (free-central placentation). The ovules are of the campylotropous type, crassinucellate and bitegmic. There are two cell lines in the outer integument and the inner integument. The micropyle is formed by the inner integument (Fig. 2).

One of the subepidermal cells at the tip of the ovule differentiates to form the megaspore mother cell (MMC). The MMC appears deep within the

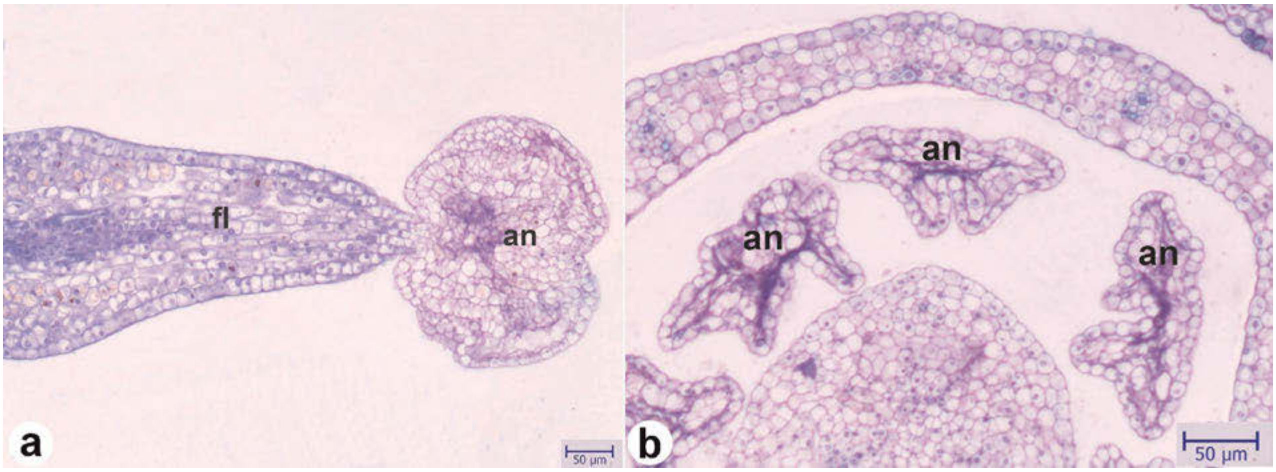

Fig. 1. Male sterile anthers of a female flower of *Silene muradica*. (**a**) anther longitudinal section at the initial stage of development (**b**) anther cross section at an advanced stage of development. an – anther, fl – filament.

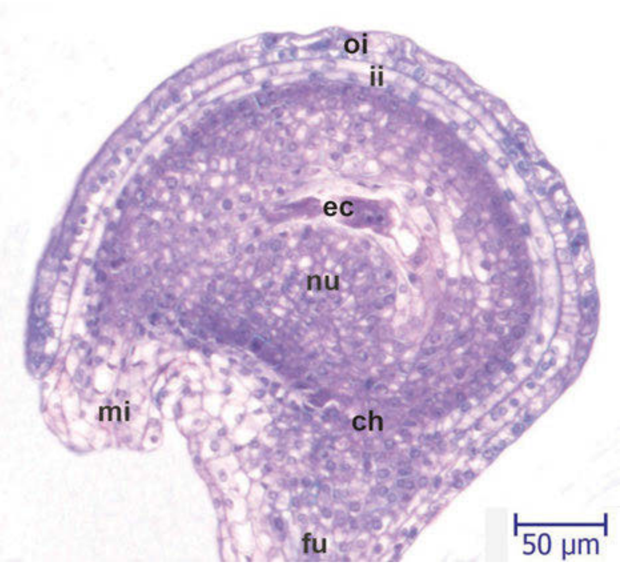

Fig. 2. Campylotropous ovule of *S. muradica*. ch – chalaza, ec – embryo sac, fu – funiculus, ii – inner integument, mi – micropyle, nu – nucellus, oi – outer integument.

nucellus tissue (crassinucellate type), and - with a large volume and large nucleus - it is easily distinguished from the other cells (Fig. 3a). As the outer integument starts to become apparent, the first meiotic division begins in the MMC. The volume of the MMC increases during prophase I of meiosis (Fig. 3b). The stages of the first meiotic division, metaphase I, anaphase I and telophase I, occur regularly, and than cytokinesis is seen between two nuclei (Figs. 3c,d). The second phases of meiosis, metaphase II, anaphase II and telophase II, occur regularly (Fig. 3e). As a result of meiotic division of the MMC, a linear tetrad of megaspores occurs (Fig. 3f). Thus, megasporogenesis of *S. muradica* is completed.

After megasporogenesis, three megaspores on the micropylar side are degenerated, while the functional megaspore on the chalazal side undergoes the mitotic division (Fig. 4a). The two nuclei are formed at the end of the first mitotic division of the functional megaspore. They move towards the opposite poles and form a large central vacuole between them (Fig. 4b). The nuclei in the poles undergo the second mitotic division and give the embryo sac with four nuclei (Fig. 4c). After the third mitosis, eight nuclei are formed in the common cytoplasm of the embryo sac. Then the egg cell, synergid cells, antipodal cells, and central cell are surrounded by the cell wall (Fig. 4d). Thus, a Polygonum type embryo sac is formed.

In *S. muradica*, a mature female gametophyte (embryo sac) is of the Polygonum type with seven cells and eight nuclei. It shows clear polarization. The mature embryo sac contains an egg apparatus (one egg cell and two synergid cells), three antipodal cells and a central cell with two polar nuclei (Fig. 4d). The polar nuclei come to the middle of the central cell and fuse before fertilization with sperm and form the secondary nucleus (Figs. 5a,b). During fertilization, one of the sperm nuclei combines with the egg cell to form the zygote, while

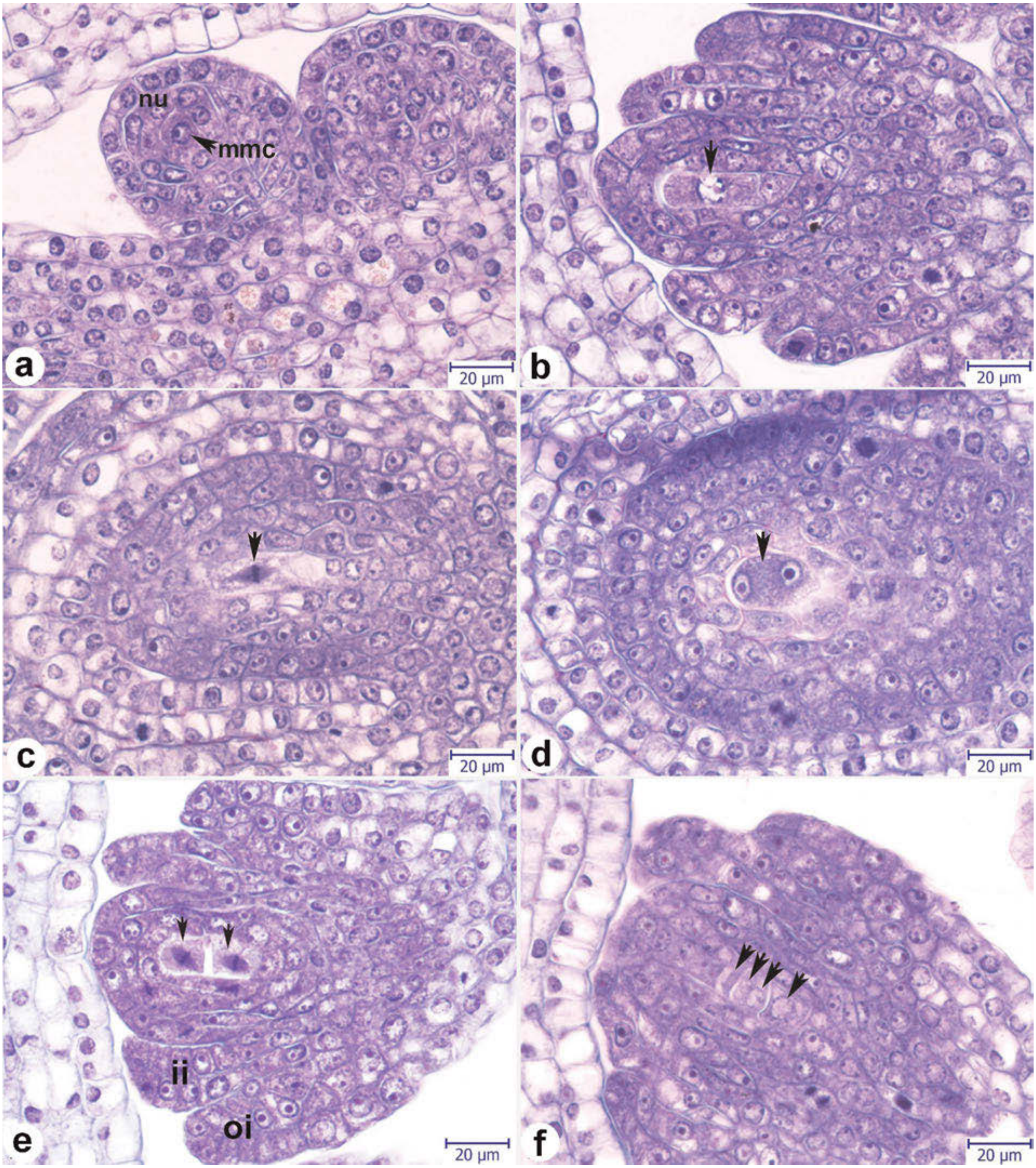

Fig. 3. Megasporogenesis in *S. muradica*. (**a**) megaspore mother cell (arrow) (**b**) diakinesis stage of meiotic prophase (arrow) (**c**) metaphase stage of the first meiotic division (arrow) (**d**) telophase stage of the first meiotic division (arrow) (**e**) metaphase stage of the second meiotic division (arrows) (**f**) linear tetrad of megaspores (arrows). ii – inner integument, mmc – megaspore mother cell, nu – nucellus, oi – outer integument.

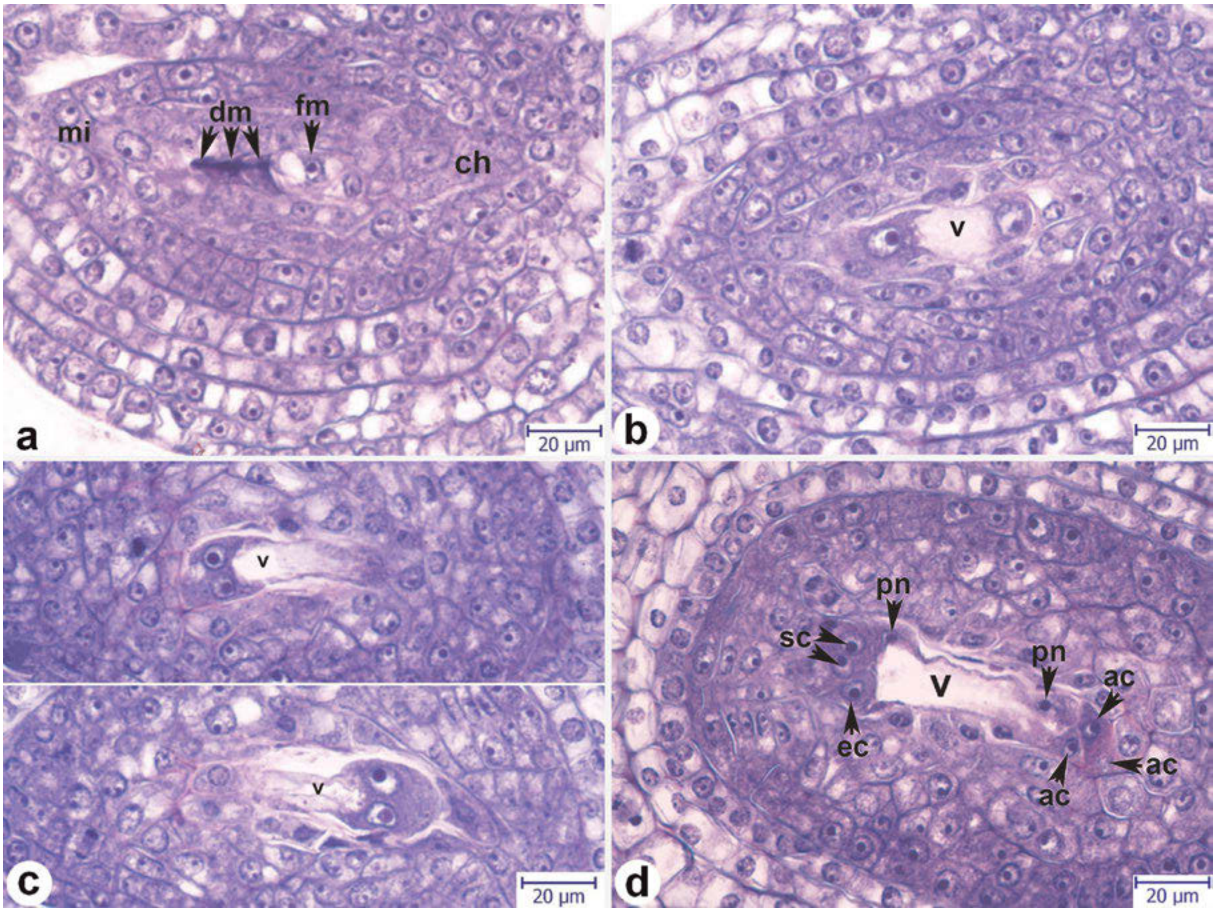

Fig. 4. Megagametogenesis in *S. muradica*. (**a**) chalazal functional megaspore and three degenerated megaspores (arrows) (**b**) two-nucleate stage of megagametogenesis (**c**) four-nucleate stage of megagametogenesis (the photos are taken from two consecutive sections of the same embryo sac) (**d**) eight-nucleate embryo sac. ac – antipodal cell, ch – chalaza, dm – degenerated megaspores, ec – egg cell, fm – functional megaspore, mi – micropyle, pn – polar nucleus, sc – synergid cell, v – vacuole.

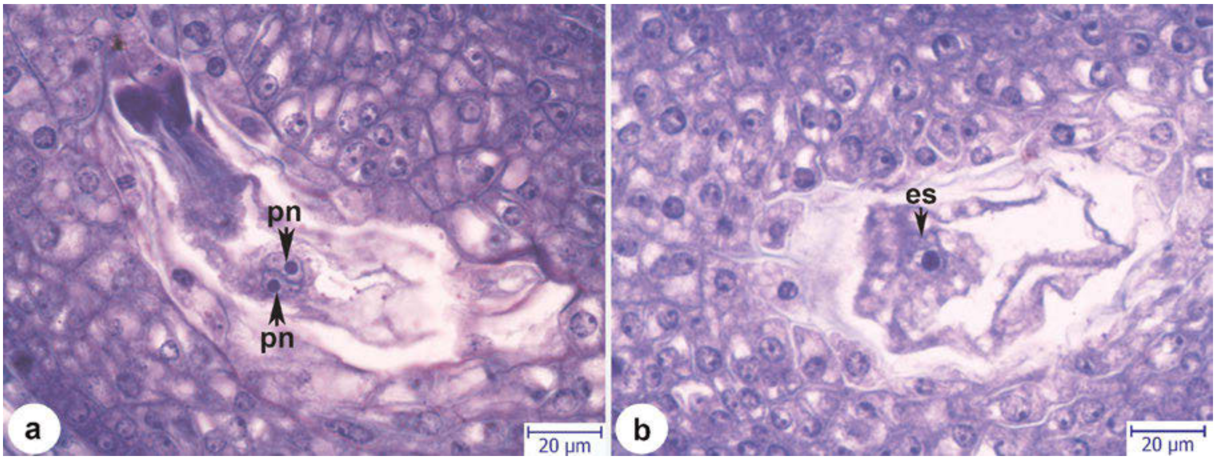

Fig. 5. Fusion of polar nuclei in *S. muradica*. (**a**) polar nuclei that are next to each other (**b**) embryo sac secondary nucleus. es – embryo sac secondary nucleus, pn – polar nucleus.

the other combines with the secondary nucleus and forms the primary endoperm nucleus (Fig. 6a). The primary endosperm nucleus begins to divide before the zygote (Fig. 6b). Endosperm development is of the nuclear type (Fig. 6c), and embryo development is of the Caryophyllad type. The suspensor is six-eight celled, and its basal cell is enlarged and pear-shaped. The basal cell is very active metabolically, and its nucleus is polyploid (Fig. 6d). Cell wall ingrowths which are darkly stained are seen at the micropylar end of the basal cell (Fig. 6d). In the developing seed, the endosperm is gradually absorbed by the embryo, and the nucellar tissue is permanent. In the mature seed, the radicle (embryonic root) is surrounded by a small amount of cellular endosperm at the micropylar side, and there is a huge number of perisperm cells in the center (Figs. 7a,b).

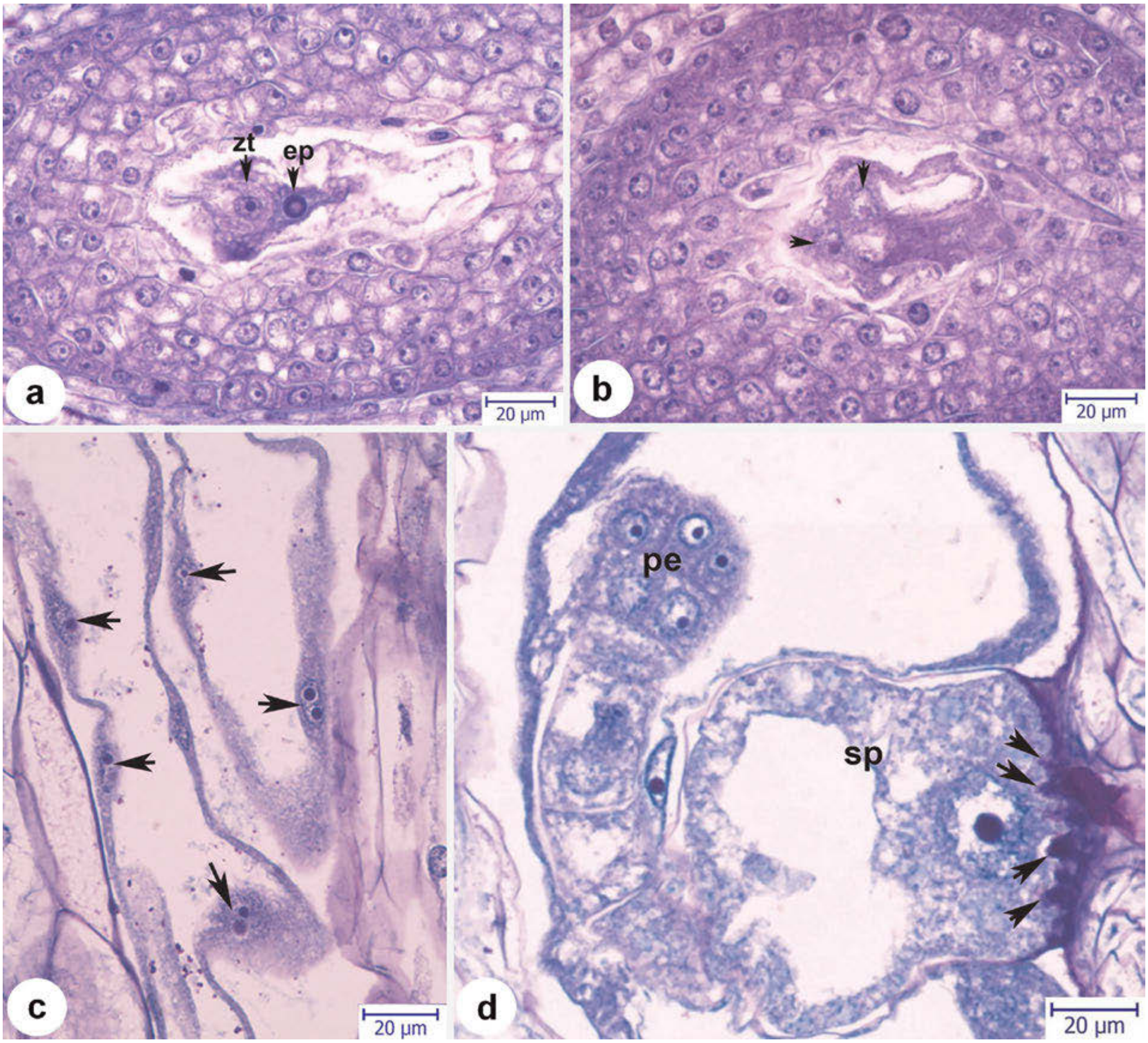

Fig. 6. Development endosperm and embryo in *S. muradica*. (**a**) zygote and primary endosperm nucleus (**b**) the first division of nuclear primary endosperm nucleus (**c**) nuclear endosperm (arrows show endosperm nuclei) (**d**) proembryo and suspensor (arrows indicate cell wall ingrowth). ep – primary endosperm nucleus, pe – proembryo, sp – suspensor, zt – zygote.

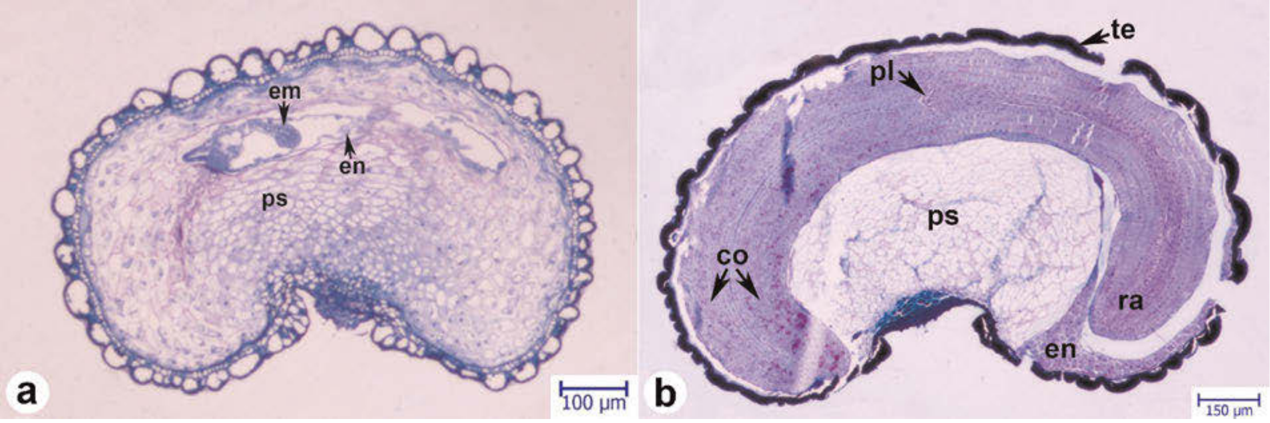

Fig. 7. Seeds of *S. muradica*. (**a**) developing seed (**b**) mature seed. co – cotyledons, em – embryo, en – endosperm, pl – plumule, ps – perisperm, ra – radicle, te – testa.

DISCUSSION

In this study, the reproductive structures of *Silene muradica* which is a gynodioecious species, were investigated histologically. In a male-sterile female flower of *S. muradica*, the anther initially appears as a mass of unorganized cells surrounded by epidermis, then it shrinks with the degeneration of the cells under the epidermis and does not produce pollen. In the *Silene* genus, mitochondrial genes cause cytoplasmic male sterility (CMS), resulting in gynodioecy. In the gynodioecious *Silene* species, the gender is often determined by a combination of mitochondrial-encoded CMS and nuclear restorer genes (Garraud et al., 2011; Sloan et al., 2012).

S. muradica shows the characteristics of the Caryophyllaceae family in terms of ovule and embryo sac development (Davis, 1966). In the superior ovary, 18–20 centrally attached ovules are developed. The developing ovules are of the campylotropous type, crassinucellate and bitegmic. Kellmann-Sopyla et al. (2017) reported that arctic *Silene involucrata* had ovules of the anatropous type. Also, Wang et al. (2017) found that the ovules of *Myosoton*, *Stellaria media* and *S. pallida* are of the amphitropous type. However, in previous studies, it was reported that the *Silene* genus had the campylotropous or hemianatropous type ovules (Davis, 1966; Kubitzki, 1993; Endress, 2011). In all studies related to the ovule of the genus *Silene*, it has been reported that the ovule is bitegmig and the micropyle is formed by the inner integument as in *S. muradica* (Davis, 1966; Kubitzki, 1993; Endress, 2011; Kellmann-Sopyla et al., 2017; Wang et al., 2017).

In *S. muradica*, the MMC cell appears in the depth of the nucellus (crassinusellate type). This feature was also mentioned in other studies on the genus *Silene* (Davis, 1966; Kubitzki, 1993; Endress, 2011; Kellmann-Sopyla et al., 2017; Wang et al., 2017). Likewise, crassinucellate type ovules have been reported in other species of the Caryophyllaceae family (*Myosoton, Stellaria, Dianthus*, etc.) (Buell, 1952; Wang et al., 2017). Embryo sac development in *S. muradica* is of the monosporic Polygonum type. The megaspore on the chalaza end is functional and undergoes three mitotic divisions in a row, eventually forming a mature eight-nucleated and seven-celled embryo sac. Similar findings have been reported in *Myosoton, Stellaria, Dianthus* species and *Silene involucrata* (Buell, 1952; Davis, 1966; Endress, 2011; Kellmann-Sopyla et al., 2017; Wang et al., 2017). Endosperm development in *S. muradica* is of the nuclear type. In the mature seed, the endosperm cells take up very little space at the tip of the mature radicle. The nucellar tissue is permanent in mature seeds and filled with nutrients to form perisperm. Mohana Rao et al. (1988) stated that nuclear endosperm develops in *Silene alba* and that the endosperm takes up less space than perisperm and is loaded with nutrient transfer from perisperm rather than storing food for the developing embryo.

Embryo development in *S. muradica* is of the Caryophyllad type (Maheshwari, 1950). Cook (1909) reports that embryo development in *Silene conoidea* is of the Caryophyllad type. In *S. muradica*, the suspensor is six-eight celled, and its basal cell is enlarged. The wall ingrowths in the basal cell are seen on the micropylar side. Similarly, the wall ingrowths in the basal cell are reported in *Stellaria media* by Newcomb and Fowke (1974). Although there are very few studies on the embryology of the genus *Silene* (Cook, 1909; Mohana Rao et al., 1988; Kellmann-Sopyla et al., 2017), when these studies were evaluated, it was found that *S. muradica* has the general characteristics of the Caryophyllaceae family in terms of its embryological features.

CONCLUSION

During this study, the ovule, female gametophyte and embryo development of *S. muradica* were examined for the first time and the obtained findings were compatible with those for the species previously studied in the Caryophyllaceae family. Thus, a contribution was provided to the information about the embryology of the family. Also, it was revealed for the first time that *S. muradica* is a gynodioecious species.

AUTHORS' CONTRIBUTIONS

CK: preparation of the samples; observation and photography of the slides; writing of the article, MT: collecting of the plants. The authors have declared that there is no conflict of interest.

ACKNOWLEDGEMENT

This work was supported by the Scientific Research Fund of Trakya University, Turkey (Project number: TUBAP–2019/152).

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