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Anther structure and pollen development in *Jurinea kilaea* Azn. (Asteraceae)

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Abstract. In this study, anther wall structure and the embryological features of male gametophyte development in *Jurinea kilaea* from Asteraceae family are described for the first time. Capitula of different sizes containing young flower buds of *J. kilaea* was collected from Tekirdağ, Saray - Kastro coast in July 2022 – 2024. Anthers separated according to their sizes under a stereo microscope were passed through arising alcohol series and embedded in Hisstore. Toluidine blue O solution was used to stain the sections. Slides were examined with light microscope and photographed by an Olympus E330 camera. In *J. kilaea*, anthers are tetrasporangiate. Anther wall consists of the outermost epidermis, the endothecium, the middle layer and the innermost tapetum layer. Tapetum cells appear to have 1 or 2 nuclei. Tapetum is plasmodial type and, tapetum cells begin to degenerate towards the end of the tetrad phase. Microsporogenesis and pollen mitosis are generally regular. Asynchrony is observed during meiosis in young anther loci. Generally, decussate type tetrad was observed. Rarely pentads were also observed. Cytoplasmic channels were observed between microspores at different stages of microsporogenesis. The mature pollen grains of *J. kilaea* are generally composed of three nuclei and have a normal structure. However, there have been instances where pollen grains exhibit an abnormal structure. Pollen sterility ratio was found to be 12.1%.

Keywords: *Jurinea kilaea,* Asteraceae, anther wall, microsporogenesis, pollen development.

INTRODUCTION

The Asteraceae family is one of the largest flowering plant families, has 12 subgenera and 43 tribes, which contain ca. 24,000–30,000 species placed within 1,600 genera (Susanna et al. 2019; Bona 2020; Rolnik and Olas 2021). It is represented in Turkey with a total of 1438 taxa, of which 152 genera, 1230 species, 133 subspecies and 75 varieties (Yıldırımlı 1999). Therefore, Asteraceae family needs more data about the additional taxonomic characters such as anther and pollen development (Çetinbaş and Ünal 2015).

The Asteraceae family members are widely distributed in the world, except for the Antarctic region, especially in tropical and subtropical semi-arid regions

such as the Mediterranean Region, Mexico and South Africa, in the forested regions of Africa, South America and Australia, in the prairies and in bush formations (Heywood 1978). They are annual, biennial or perennial herbs; rarely shrubs, trees or climbing woody plants; their tissues may or may not carry latex (Saday 2005).

Its most well-known taxa are daisies, dandelion, lettuce, endive, and artichokes. For ages, people have been using plants from the Asteraceae family for their nutritional and medicinal benefits. The majority of the family's members share a similar chemical makeup despite their great diversity. For instance, all species are good sources of inulin, a naturally occurring polysaccharide with potent prebiotic qualities. They also possess potent antibacterial, anti-inflammatory, and antioxidant qualities in addition to diuretic and wound-healing capabilities (Rolnik and Olas 2021). Family Asteraceae as a sustainable planning tool in phytoremediation and its relevance in urban areas was studied by Nikolic and Stevovic (2015). Kartal (2016) examined calcium oxalate (CaOx) crystals in the tissues and organs of eighteen species in the Cardueae tribe (Asteraceae).

Jurinea which is represented by about 300 species on earth, is mainly distributed in Central Asia, the Mediterranean basin, Iran, and Turkey (Szukala et al. 2019). In Turkey *Jurinea* is represented by 18 species in Turkey. 6 of these species are endemic to Turkey. The distributions of the endemic taxa are as follows: *J. brevicaulis* and *J. cadmea* have local distribution; *J. alpigena, J. ancyrensis* and *J. cataonica* have regional, and *J. pontica* has larger distribution. The distribution of the species according to the phytogeographic regions is as follows: 10 species of Irano-Turanian; 2 types of Mediterranean; It is an element of 5 types of Euxine (2 types of Balkans, 1 type of Caucasian, 1 type of Turkey in Europea-Thrace) (Davis 1975). They are usually herbaceous plants that can live for several years. Despite the richness of its species, only a few analyses on a regional scale have so far attempted to clarify the phylogenetic relationships within *Jurinea* (Doğan et al. 2010; Szukala et al. 2019; Bona 2020)

Jurinea kilaea is a rhizomatous plant that grows in the foredunes of Turkey (Avcı et al. 2015). *J. kilaea* has been studied more systematically (Gülez et al. 2007; Özhatay et al. 2013; Özhatay and Öztekin 2015; Kutbay et al. 2017; Tuncay and Akalın 2018; Sürmen et al. 2019; Uslu and Keçeli 2019; Ağır et al. 2016; 2017; 2021; Valcheva et al. 2020; Karaduman and Sağıroğlu 2021). Phylogenetic analysis of *Jurinea* (Compositae) species in Turkey based on ITS sequence data was performed. These include *Jurinea kilaea* (Doğan et al. 2010). Pappus and achene characteristics of *J. kilaea* is studied by Bona (2020). Morphological features of *J. kilaea* were studied by Saday in 2005. There are also biochemical studies with *J. kilaea*. The antioxidant activity and capacity of *J. kilaea* were investigated. Phenolic, flavonoid substance, antioxidant and antimicrobial properties were studied (Kılıç 2020). Fatty acid and amino acid profiles of *J. kilaea* was studied by Taç and Özcan (2019).

Although there are many systematic studies on Asteraceae, there are very few cytoembryological studies. Some of these were made by Sun and Ganders (1987) on nine gynodioecious taxa of Hawaiian bidens, by Meriç et al. (2004) on *Helianthus annuus* L., by Yurukova-Grancharova and Dimitrova (2006) on *Crepis bithynica* Boiss., by Li et al. (2010) on *Chrysanthemum morifolium* Ramat., by Kaur et al. (2010) on *Inula cuspidata* C.B. Clarke, by Liu et al. (2012) on *Ambrosia artemisiifolia* L., by Kaur et al. (2019) on some species of *Lactuca* L., by Gupta et al. (2017) on 45 species of Asteraceae from Parvati Valley in Kulu district, India, and by Chehregani and Salehi (2016) on *Achillea tenuifolia.* Palynological studies were carried out by Wortley et al. (2012), Chehregani and Salehi (2016) and Gupta et al. (2017).

In this study, *Jurinea kilaea* Azn, which is not endemic but can be considered endangered nationally and worldwide, was studied cyto-embryologically for the first time. This study will contribute to systematic and cyto-embryological studies on the Asteraceae family.

MATERIAL AND METHODS

As the study material, capitulums (8-13 mm) containing young flower buds (1,5 -8 mm) of *Jurinea kilaea* was collected from Tekirdağ, Saray - Kastro coast in July 2022 and July 2024. After being fixed in Carnoy's fixative (1acetic acid: 3 absolute alcohol), it was washed in 96% ethanol and stored in 70% ethyl alcohol. Anthers (1-6 mm) separated according to their sizes under a stereo microscope were passed through arising alcohol series and embedded in Hisstore (Leica, Historesin-embedding kit) according to the manufacturer's instructions (http://www.leicabiosystems.com/specimen-preparation/consumables/mounting-edia-section-adhesive/details/product/historesin-1/). Sections of 4 μm thickness were taken with a tungsten carbide blade on a Leica RM2255 model rotary microtome. Sections were kept in 0.5% Toluidine blue O solution (O'Brien et al. 1964) prepared in 0.1 M phosphate buffer (pH 6.8) for 2 minutes, washed in distilled water for 30 seconds and dried in air. It was closed with Entellan and made into a continuous preparation (Kartal 2015). Pollen taken at the time of flowering of *J. kilaea* was stained with Aniline blue (Merck). It was left at room temperature for half an hour. 1000 pollens were

evaluated by counting whether they were stained or not. Slides were examined with an Olympus CX31 microscope and were photographed by an Olympus E330 camera.

RESULTS

Androecium

Androecium of *Jurinea kilaea* consists of 5 stamens. Anther structure is caudate type. Anthers are united, tube-shaped, purple, basifix, filaments are free and white. In *J. kilaea*, anthers are tetrasporangiate. Pollen sacs are interconnected with connective tissue containing a vascular bundle (Figure 1). When the anthers mature, the microsporangia split open from their stomium.

Anther wall

The anther wall was also examined during pollen development in *J. kilaea*. Young anther wall consists of the outermost epidermis, the endothecium, the middle layer and the innermost tapetum layer. Endothecium thickenings are not seen in the young anther wall. Epidermis and endothecium cells are almost cubic in shape, and they have large nuclei relative to the cell size. Under the endothecium, there is a middle layer consisting of a very thin, single layer of flat cells. Tapetum cells appear to have 1 or 2 nuclei. The number of cells undergoing nuclear division is high. Asynchrony is observed during meiosis in young anther loci. The first stages of meiosis can be seen in one locus, and the tetrad stage can be seen in the other locus (Figure 1a).

Layers, the epidermis and the endothecium, remain intact in the mature anther wall. The epidermis layer consists of a single row of flat cells. It is seen that the cells of the endothecium layer are highly developed and increase in size compared to the other layers. It is also observed that fibrous thickenings develop in the cells of the endothecium layer. It is seen that the middle layer, which consists of a very thin and flat single layer of cells in the young anther, is completely degenerated in the mature anther. Tapetum is plasmodial type and, tapetum cells begin to degenerate towards the end of the tetrad phase. Then tapetal remnants from the degenerating tapetum are seen in the mature anther locus The asynchronization between loci seen in the young anther also disappears in the mature anther (Figure 1b).

Microsporogenesis

In this study, microsporogenesis stages in *J. kilaea* were examined for the first time. In sections taken from the anther during the interphase phase, the nuclei of the pollen mother cells are of similar size and the nucleoli are conspicuous. There is no callose wall around the pollen mother cells (Figure 2a). During the leptotene stage, callose wall begins to form around the microspore mother cells (Figure 2b). In the zygotene stage, the chromatin material is in a loose state (Figure 2c). In pachytene, homologous chromosomes come together. The nucleus is pulled towards the edge of the cell. This phase is also called the bouquet phase (Figure 2d). During the diplotene phase, the lengths of bivalent chromosomes begin to shorten. Callose wall formation is completed at

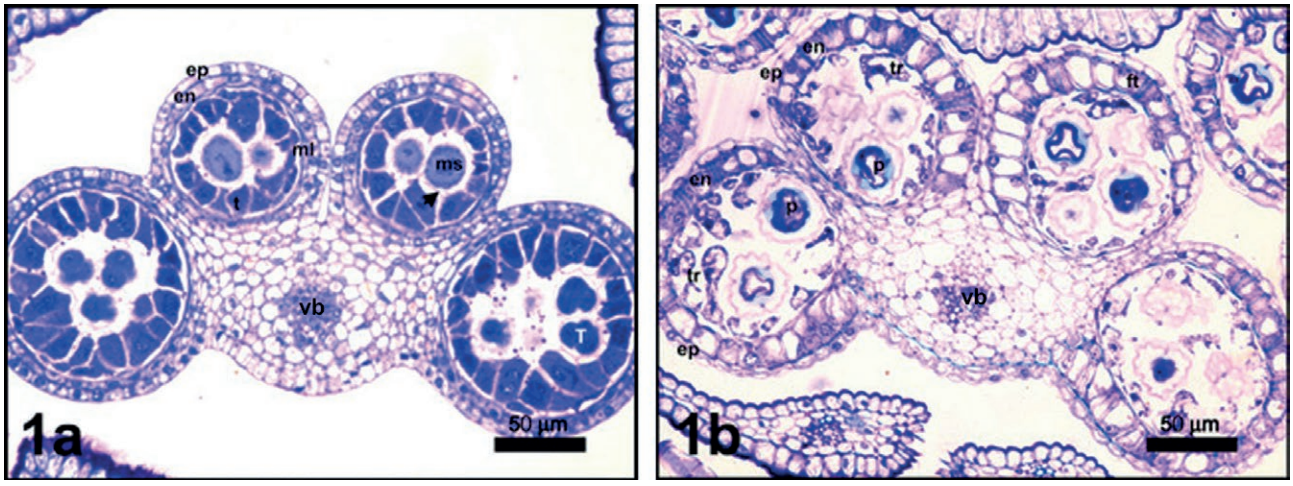

Figure 1. Anther structure in *J. kilaea.* (a) young anther; (b) mature anther (arrowhead, callose; en, endothecium; ep, epidermis; ft, fibrous thickenings; ml, middle layer; ms, microspore; p, pollen; T, tetrad; t, tapetum; tr, tapetal remnants; vb, vascular bundle).

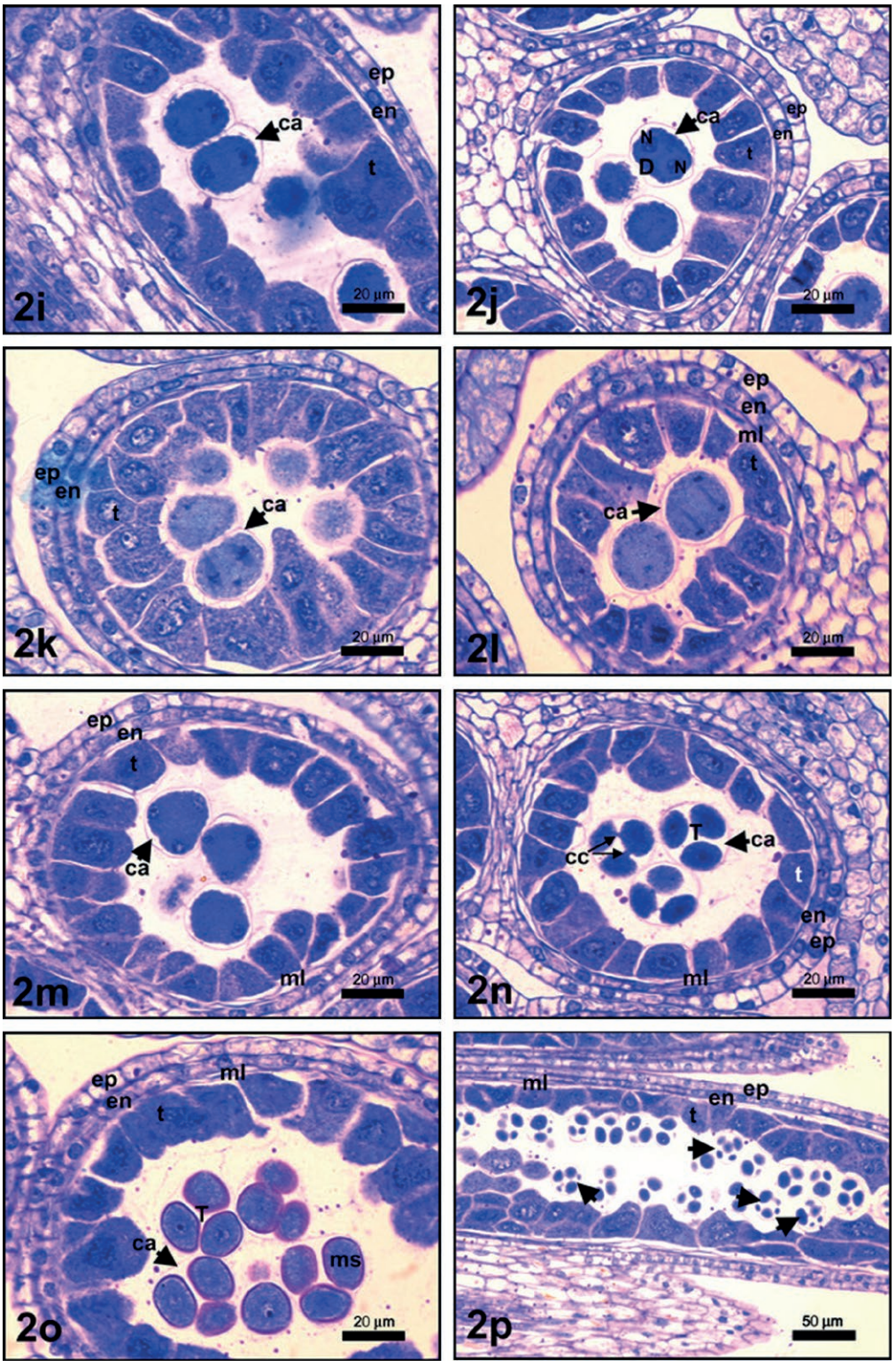

Figure 2. Microsporogenesis in pollen mother cells of *J. kilaea*. (a) interphase; (b) leptotene; (c) zygotene; (d) pachytene (bouquet stage); (e) diplotene; (f) diakinesis; (g) metaphase I; (h) anaphase I; (i) telophase I; (j) dyad phase; (k) metaphase II; (l) anaphase ll; (m) telophase II; (n) early tetrad phase; (o) late tetrad phase; (p) anomalies in tetrad phase (arrowheads, tetrad anomalies; ca-arrowheads, callose; cc; cytoplasmic channels; D, dyad; en, endothecium; ep, epidermis; ml; middle layer; ms, microspore; N; nucleus; Nu; nucleolus; PMC, pollen mother cell; t, tapetum; T, tetrad).

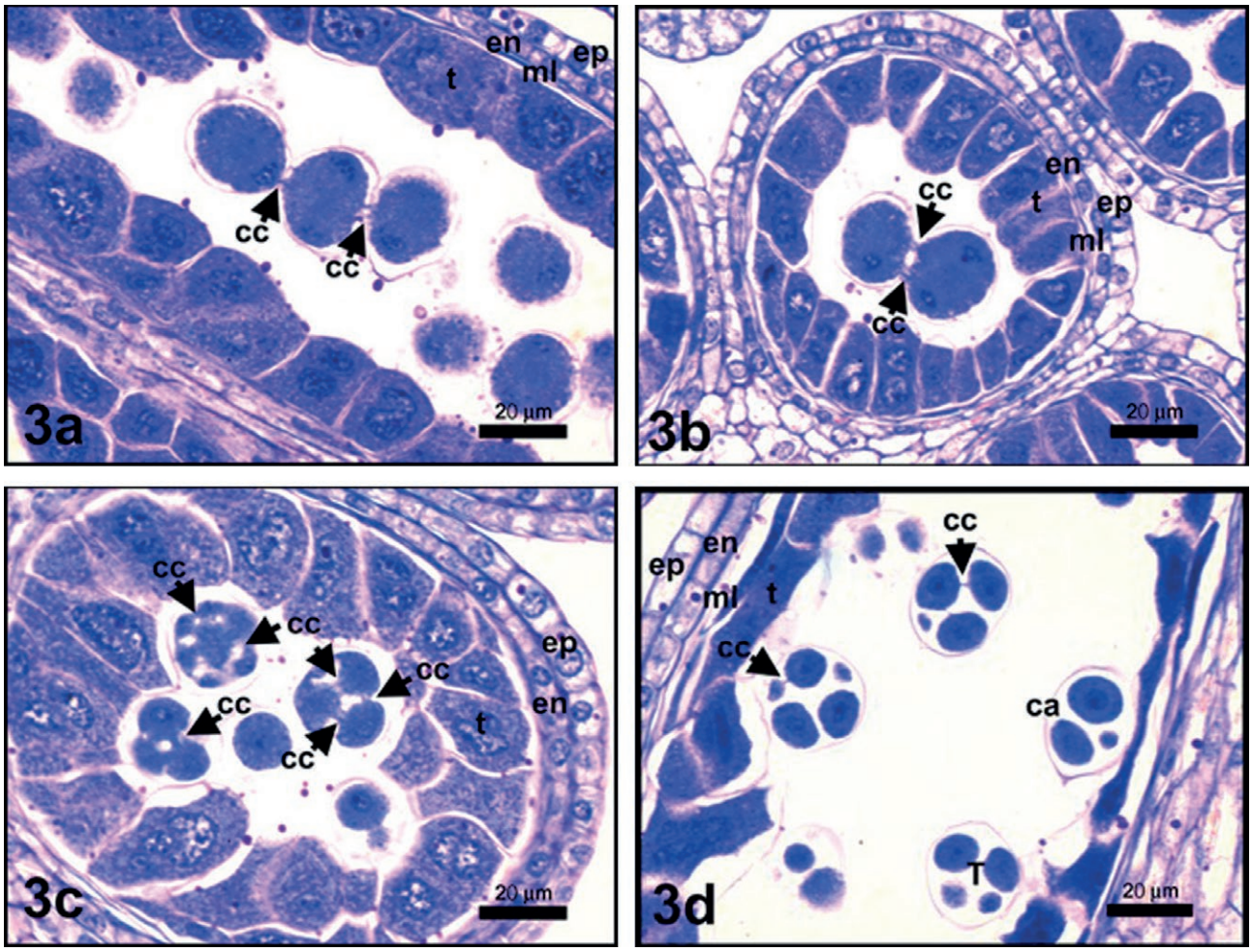

Figure 3. Cytoplasmic channels in some phases of microsporogenesis in *J. kilaea*. (a) early telophase I; (b) early dyad phase; (c) early tetrad phase; (d) late tetrad phase (ca, callose; cc-arrowheads, cytoplasmic channels; en, endothecium; ep, epidermis; ml, middle layer; t, tapetum; T, tetrad).

this stage (Figure 2e). During the diakinesis phase, chromosome shortening continues (Figure 2f). In metaphase I, the chromosomes line up on the equatorial plate and appear to be connected by spindle fibers (Figure 2g). In anaphase I, homologous chromosomes are pulled to the poles (Figure 2h). In telophase I, chromosomes lose their dense shape and return to their thread-like form. An intermediate lamella begins to form between the two nuclei, from the middle of the cell to the edges (Figure 2i). Dyad occurs with successive type of cytokinesis (Fig. 2j). In metaphase II, chromosomes align on the equatorial plate (Figure 2k). In anaphase II, sister chromatids are pulled to the poles with the help of spindle fibers (Figure 2l). It is observed that in telophase II, nuclei 4 are formed at the edges. Cytokinesis is simultaneous type at tetrad phase (Figure 2m). Cytoplasmic channels were seen between microspores in the early tetrad phase. There is a well-developed callose wall around the tetrads (Figure 2n). In later stages, the callose wall around the microspores breaks down and the microspores are released (Figure 2o). Generally, decussate type tetrad was observed. In *J. kilaea*, pentads as well as tetrads were observed in the anther locus - arrowheads (Figure 2p). Cytoplasmic channels were observed between microspores at different stages of microsporogenesis, such as telophase I (Figures 3a, 3b), early tetrad (Figure 3c) and tetrad stage (Figure 4d).

Microgametogenesis

In J. kilaea, it is observed that the microspores released after the callose wall degeneration in the tetrad stage are tricolpate and the intine layer begins to form

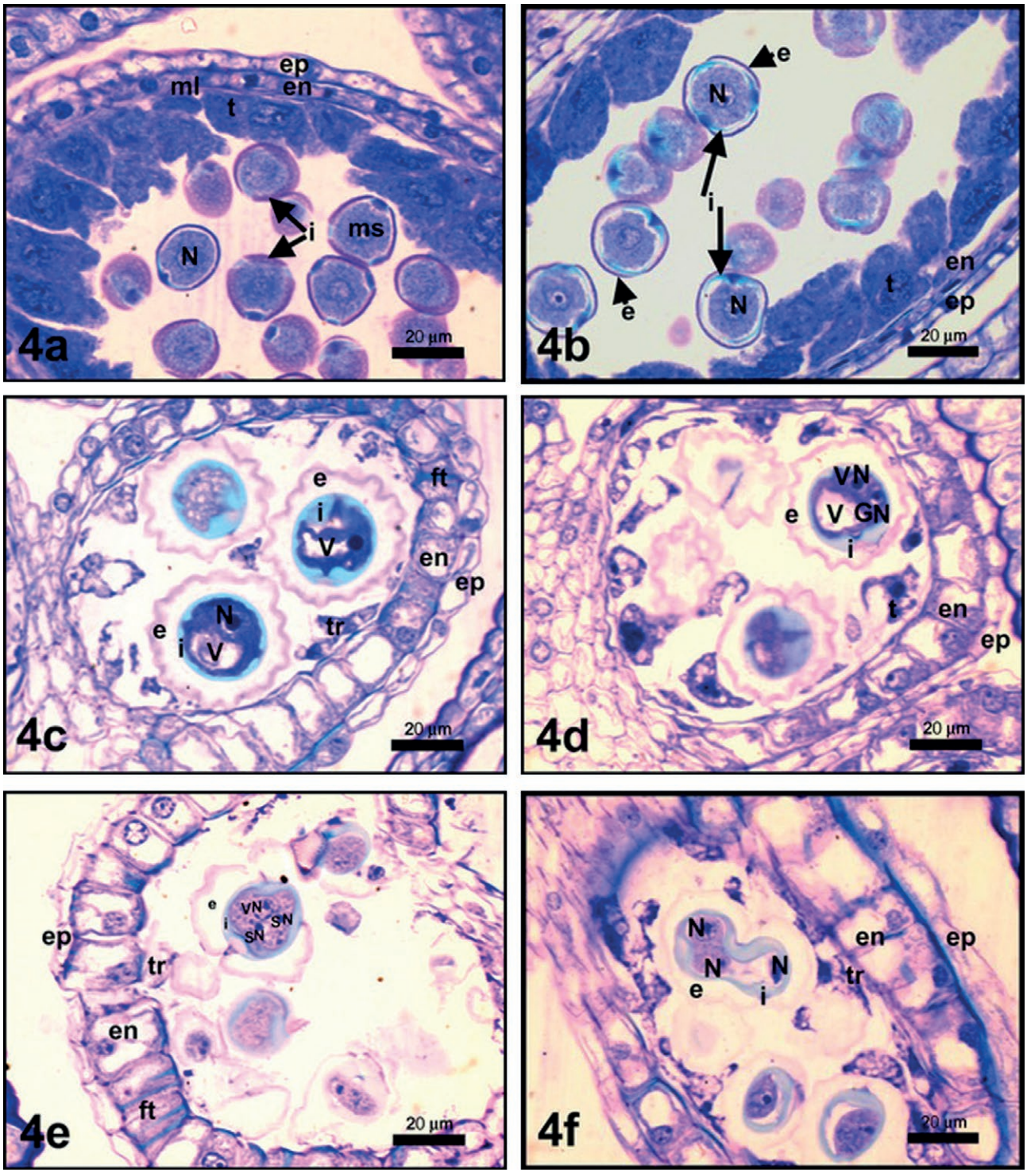

Figure 4. Pollen development in *J. kilaea*; (a) one-nucleated microspore phase after callose deposition; (b) wrinkled microspore phase (c) vacuolated microspore phase; (d) two-celled pollen phase, (e) three-celled mature pollen, (f) abnormal shaped pollen (en, endothecium; ep, epidermis; e; exine; ft, fibrous thickenings; gn, generative nucleus; i; intine (arrows); ml, middle layer; ms microspore; N, nucleus; sn, sperm nucleus; t, tapetum; tr, tapetal remnants; V, vacuole; vn, vegetative nucleus).

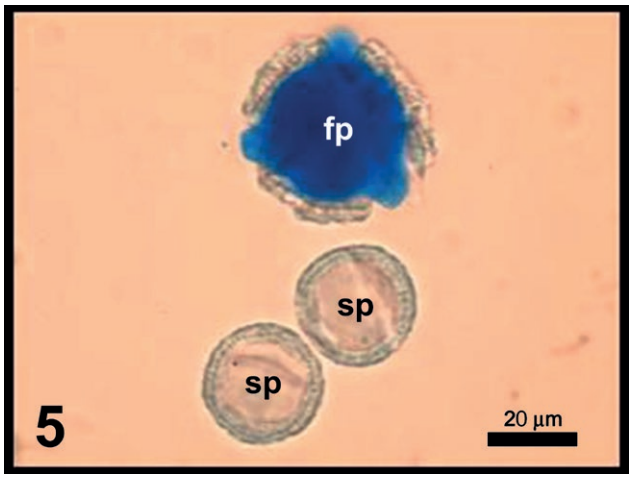

Figure 5. Pollen viability in *J. kilaea* (fp, fertile pollen; sp, sterile pollen).

around them (Figure 4a). Then, it is seen that the exine layer begins to form over the intine (Figure 4b). After the formation of the exine layer is completed in the single-nucleated stage, a vacuole that covers most of the cell is formed and the nucleus migrates to the pole where pollen mitosis will occur (Figure 4c). After first mitosis vegetative and generative nuclei are formed (Figure 4d). As the generative nucleus undergoes mitosis, sperm nuclei are formed and the three-nucleated mature pollen grain in *J. kilaea* completes its development (Figure 4d). Pollen grains of *J. kilaea* generally have a normal structure, and very rarely pollen grains with an abnormal structure have been observed (Figure 4e).

Pollen viability

Pollen viability in *J. kilaea* was examined using a light microscope. It was observed that pollens were generally stained well. Pollens were stained with aniline blue (Merck) prepared in lactophenol were considered fertile, others were considered as sterile pollen grains (Figure 5). 1000 pollens were counted. Pollen sterility rate was determined as 12.1%. Mature pollens are tricolpate and trinucleated.

DISCUSSION

In this study, the anther wall structure, and the developmental stages of the male gametophyte in *Jurinea kilaea*, which grows naturally on the coast of Tekirdağ-Saray, were examined. The embryological studies on *J. kilaea* and the Asteraceae family, especially the genus *Jurinea*, are quite limited so the findings regarding the anther wall structure and microsporogenesis are discussed with the characteristics of other species belonging to this family.

In *J. kilaea*, the androecium consists of 5 stamens, as in *Aster subulatus* Michx., *Kalimeris indica* (Linn.) Sch.-Bip., *Heteropappus arenarius* Kitamura, *Erigeron annuus* (Linn.) Pers. (Ao et al. 2009), male fertile and sterile *Chrysanthemum morifolium* Ramat. (Li et al. 2010), *Ambrosia artemisiifolia* L. (Liu et al. 2012), *Helianthus annuus* L. (Çetinbaş and Ünal 2015) from Asteraceae. Anthers of *J. kilaea* are united, tube-shaped, purple, basifixed, filaments are free and white. They are structurally like anthers of *H. annuus* (Çetinbaş and Ünal 2015). They differ only in color. Anther appendage of *J. kilaea* is apiculate like *Ainsliaea latifolia* (D. Don) Schultz-Bipontinus (Shekhar and Pandey 2009) and *Ainsliaea qianiana* (Shi et al. 2011) from Asteraceae family. When the anthers mature, the microsporangia split open from their stomium.

Anthers are tetrasporangiate in *J. kilaea*. The pollen sacs are interconnected with connective tissue containing a vascular bundle as in *C. bithynica* (Yurukova-Grancharova and Dimitrova 2006), *A. subulatus, K. indica, H. arenarius, E. annuus* (Ao et al. 2009), *C. morifolium* (Li et al. 2010), *A. artemisiifolia* (Liu et al. 2012).

In *J. kilaea*, the young anther wall, as in *Bidens cervicata* Sherff (Sun and Ganders 1987), *C. bithynica* (Yurukova-Grancharova and Dimitrova 2006), *A. subulatus, K. indica, H. arenarius, E. annuus* (Ao et al. 2009) *C. morifolium* (Li et al. 2010), *A. artemisiifolia* (Liu et al. 2012) *H. annuus* (Meriç et al. 2004; Çetinbaş and Ünal 2015) consists of epidermis, endothecium, intermediate layer and tapetum, consisting of single-row cells from outside to inside. In the young anther, the cell sizes of the epidermis and endothecium layers are similar. Fibrous thickenings of the endothecium have not yet developed in the early stage. In the mature anther, transverse expansion occurs in the epidermis cells. The epidermis remains intact until the end of pollen development in *J. kilaea*.

The endothecium layer has single-nucleated and rather larger cells than the epidermis in mature anther. It contains fibrous thickenings in the mature anther wall of *J. kilaea* as in *C. bithynica* (Yurukova-Grancharova and Dimitrova 2006) and *C. morifolium* (Li et al. 2010). In *A. subulatus, K. indica, H. arenarius, E. annuus* (Ao et al. 2009), *A. artemisiifolia* L. (Liu et al. 2012), *H. annuus* (Çetinbaş and Ünal 2015) no thickenings were seen in endothecium layer.

In the young anther of *J. kilaea*, the middle layer consists of a very flattened single layer of cells between the

endothecium and tapetum, as in *B. cervicata* (Sun and Ganders 1987), *C. bithynica* (Yurukova-Grancharova and Dimitrova 2006), *C. morifolium* (Li et al. 2010), *A. artemisiifolia* L. (Liu et al. 2012), *H. annuus* (Meriç et al. 2004; Çetinbaş and Ünal 2015). In *J. kilaea*, the middle layer is not seen in the mature anther because it degenerates at the end of microsporogenesis - the beginning of pollen mitosis. In *B. cervicata*, the middle layer becomes vacuolated immediately after differentiation and appears more flattened with further development of the anther wall. It is not evident before the formation of the plasmodial tapetum (Sun and Ganders 1987). In *C. bithynica* the middle layer is generally degenerates towards the end of prophase I of meiosis in PMCs, but occasionally stains darkly during metaphase I – anaphase I, degenerating parts of this structure can be observed (Yurukova-Grancharova and Dimitrova 2006). In *C. morifolium*, the middle layer flattened during meiosis and was still observable at the late mononuclear pollen grain stage but degenerated at the binucleate stage (Li et al. 2010). In *H. annuus*, the middle layer disappears when the pollen mother cells reach the tetrad stage (Çetinbaş and Ünal 2015).

There is a tapetum layer under middle layer in the innermost part of the anther wall. Some studies have summarized the different functions that tapetum cells can perform regarding the development of the pollen grain (Pacini et al. 1985). Some of these are the production and release of callase enzyme; transfer of polysaccharides into the locule provides the energy required during microsporogenesis and microgametogenesis cell divisions by the hydrolysis of these polysaccharides. In addition, from the synthesis of exine precursors to the formation of viscin threads; Tapetal cells are also responsible for the formation of a membrane resistant to acetolysis, the formation of orbicules or Ubisch bodies, the synthesis of sporophytic proteins, the production of trypsin, which covers the pollen grains and consists of a fibro-granular and a lipidic component, and the development of pollenkitt (Gotelli et al. 2023).

Although tapetum cells carry a single nucleus in the early stages of microsporogenesis, most of them have 2 or more nuclei in later stages. Normal mitosis, secondary nuclear divisions and nuclear fusion were observed in the tapetum cells of the anther wall in *J. kilaea*. Tapetum cells of *J. kilaea* usually have one or two nuclei. Polyploidy was observed in the tapetum cells of *Achillea tenuifolia*, from the Asteraceae family (Chehregani and Salehi 2016). The tapetum cells of *A. subulatus, K. indica, H. arenarius, E. annuus* (Ao et al. 2009) have uni- or binucleated, as in *J. kilaea*.

Programmed cell death is a physiological cell death that selectively destroys cells that are no longer needed or have no function. Although programmed cell death has been studied primarily and mostly in animal cells, it has been shown to also occur in plant cells in recent years. The occurrence of programmed cell death in the cells of the tapetum layer, which is the innermost layer of the anther wall, has been examined in studies conducted with transmission electron microscopy. While entering from the tetrad stage to the free microspore stage, it was shown that the tapetum cells lost their geometric shape and microtubules disappeared in the cytoplasm, in *Tillandsia albida*, *Lobivia rauschii* by Papini et al. (1999). According to many ontogenetic palynologists, the tetrad stage is very important in determining the exine pattern (Gabarayeva et al. 2019). It was observed that the tapetum cells began to degenerate at the same stage in *J. kilaea*.

It was observed that microsporogenesis stages were generally regular in pollen mother cells (PMC) in *J. kilaea*. Simultaneous type of cytokinesis was observed in *C. bithynica* (Yurukova-Grancharova and Dimitrova 2006), *A. subulatus*, *K. indica*, *H. arenarius* (Ao et al. 2009), *C. morifolium* (Li et al. 2010), *H. annuus* (Çetinbaş and Ünal 2015), *Galinsoga quadriradiata* Ruiz & Pav. (Kolczyk et al. 2015), *Ambrosia trifida* (Gabarayeva et al. 2019), as generally seen in dicots. *In E. annuus,* successive cytokinesis was observed in the dyad stage and simultaneous cytokinesis was observed in the tetrad phase (Ao et al. 2009). Two types of cytokinesis were observed *in J. Kilaea,* as in *E. annuus*. Successive type of cytokinesis was observed in *A. artemisiifolia*, as in monocots (Liu et al. 2012). As seen in many angiosperms, the callose wall in the PMCs of *J. kilaea* begins to form in the leptotene and disintegrate in the tetrad phase.

Asynchrony is observed during meiosis in young anther loci of *J. kilaea* as in *K. indica*, *E. annuus* (Ao et al. 2009). In one locus, the first stages of meiosis were observed, and in the other, the tetrad stage was observed in *J. kilaea*. The loci where the early stages of meiosis are seen are smaller in size than the loci where the tetrad stages are seen. It is thought that the asynchrony occurring in the anther loci is related to the transmission of nutritional materials.

In the early stages of microsporogenesis, although the cells were surrounded by the cell wall and there was callose accumulation, connections between the pollen mother cells were seen. These connections are called cytoplasmic/cytomictic channels. They represent a different type of cell wall channels from plasmodesmata. They do not have an internal structure like desmotubules and have relatively large openings compared to them (Baquar and Husain 1969; Mursalimov et al. 2010; Kolczyk et al. 2015). Cytoplasmic channels provide inter-

cellular exchange of nutrients, water, ions, various macromolecules and metabolites according to their reaction to environmental stimuli in plants (Wang et al. 2006).

Cytomixis has been considered as an abnormality in previous studies due to occurrence of pathology (Morisset 1978) or traumatic injury of plants (Takats 1959). Recently, it is generally accepted as a normal but rare cytological phenomenon. According to Mursalimov et al (2013), the absolute majority of cytomixis cases are recorded in microsporogenesis of angiosperms. Cytomixis has been described in more than 400 plant species belonging to 84 families. Among the Asteraceae family, this process has been observed in *Helianthus* (Whelan 1974), *Artemisia* (Malik and Kumari 2010), *Galinsoga* (Kolczyk et al. 2015) and *Ambrosia* (*Gabarayeva* et al. 2019). This is the first report of the genus *Jurinea*. Cytoplasmic channels are seen between cells in the stages starting from the leptotene stage of meiosis I prophase and ending with the tetrad stage in *J. kilaea*.

In *J. kilaea*, decussate type tetrads are generally formed at the end of microsporogenesis. Besides these, rarely pentads were also seen. In *H. annuus* (Çetinbaş and Ünal 2015), *E. annuus, H. arenarius* (Ao et al. 2009), *C. morifolium* (Li et al. 2010), *Achillea tenuifolia* (Chehregani and Salehi 2016) pollen mother cells produce tetrahedral microspore tetrads. *H. arenarius* (Ao et al. 2009) also produce decussate type tetrads as in *J. kilaea*. PMC of *C. Bithynica* generally produce also tetrahedral, rarely isobilateral tetrads (Yurukova-Grancharova and Dimitrova 2006).

Mature pollens of *J. kilaea* are tricolpate and trinucleated with echinate exine as in *C. bithynica* (Yurukova-Grancharova and Dimitrova 2006). In *J. kilaea*, sterile pollen grains are transparent, smaller in size than normal pollen grains, and have a smooth structure because the exine layer is undeveloped. Sterile pollen grains of *C. bithynica* are of normal size (Yurukova-Grancharova and Dimitrova 2006). In *A. subulatus, K. indica, H. arenarius and E. annuus 3-celled pollen grains were seen* (Ao et al. 2009). Pollens of *Allittia, Lorandersonia* and *Pembertonia*, classified in Astereae, are also echinates (Wortley et al. 2012).

Pollen viability was investigated in some *Lactuca* species (Asteraceae) by Kaur et al. (2019). In *L. orientalis*, 54-62% sterile pollen grains were observed because of chromosome bridges and nondisjunction during microsporogenesis. In *L. serriola*, *L. dissecta*, *L. dolichophylla and L. macrorhiza* 100% fertile pollen was produced because of the normal distribution of bivalents (Kaur et al. 2019). In *J. kilaea*, the sterile pollen rate was determined as 12.1%. Cytoplasmic channels that seen during microsporogenesis and chromosome transitions occurred in those channels might be the reason for this sterility rate in *J. kilaea*.

In conclusion, the anther wall structure, pollen development and pollen viability of *J. kilaea*, which grows naturally in a very limited area in Turkey and Bulgaria, were examined for the first time. The data obtained from this study will contribute to the cytological and embryological features used in the taxonomy of the Asteraceae family in recent years.

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