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# An investigation of floral development in some *Polygala* taxa of Türkiye

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## Absract

*In this study, the flower development of* Polygala anatolica Boiss. & Heldr., P. monspeliaca L. *and* P. vulgaris L., *species belonging to the* Polygalaceae *family, which naturally grows in Türkiye, was revealed by applying histological and scanning electron microscopy techniques. Flower development begins with the variation of the apical meristem into the floral meristem. The onset of the pistil primordia preceded the pistil primordia. Sections taken from different flower buds revealed that the ovules were orthotropic (atropous), produce two integuments, were bitegmic (double integuments), and appeared crassinucellate. These data indicated that the compared species showed no embryological differences. Scanning electron microscopy and stereomicroscopy revealed the formation of numerous irregularly indented and ridged areas on the petal surface, and the cuticle density increased as developmental stages progressed. The stigma lobes appeared distinct, the stamen filaments developed within sheaths attached to the corolla lobes, and the styles elongated significantly throughout the bud developmental stages. This study provides the first study of the floral development and characteristics of three* Polygala *species in Türkiye, providing embryological data for comparative systematic studies among related taxa.*

***Keywords:*** *Flower development, embryology, Polygalaceae,* Polygala*, SEM*

[**]*This study was produced from Fatmanur Kıçar's Master's Thesis.*

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# Türkiye'deki bazı *Polygala* taksonlarında çiçek gelişiminin incelenmesi


## Öz

*Bu araştırmada, Türkiye'de doğal olarak yayılış gösteren* Polygalaceae *familyasına bağlı* Polygala vulgaris L.*,* P. monspeliaca L. *ve P. anatolica* Boiss. & Heldr. *türlerinin çiçek gelişimi, histolojik ve taramalı elektron mikroskobu teknikleri uygulanarak ortaya konulmuştur. Çiçek gelişimi, apikal meristemin floral meristeme dönüşümüyle başlamıştır. Erkek organ taslaklarının başlangıcı, dişi organ taslağından önce başlamıştır. Farklı çiçek tomurcuklarından alınan kesitlerle tohum taslaklarının anatrop şekilde teşekkül ettiği, iki integüment taşıdığı bitegmik (çift integümentli) olduğu ve tohum taslaklarının krassinusellat tipte göründüğü belirlenmiştir. Bu verilerin sonucunda karşılaştırılan türlerin embriyolojik olarak farklılık göstermediği belirlenmiştir. Taramalı elektron mikroskobu ve stereo mikroskobu incelenmelerinde petal yüzeyinde çok sayıda düzensiz girintili çıkıntılı bölümlerin oluştuğu ve gelişim evreleri tamamlandıkça kütikula yoğunluğunun arttığı tespit edilmiştir. Stigma parçalarının ayrı göründüğü, stamenlerindeki filamentlerinin korolla loplarına yapışık kılıf içinde geliştiği ve tomurcukların gelişim evreleri boyunca stilusların büyük ölçüde uzadığı gözlemlenmiştir. Çalışma sonucunda, Türkiye'deki 3* Polygala *türünün çiçek gelişimi ve özellikleri ilk defa çalışılarak akraba taksonlar arasında karşılaştırmalı sistematik çalışmalara embriyoloji açısından veri sağlanmıştır.*


***Anahtar kelimeler:*** *Çiçek gelişimi, embriyoloji, Polygalaceae,* Polygala*, SEM,*

## 1. Introduction

Polygalaceae is a family of herbaceous, shrubby, rarely small trees or climbing and ascendent plants with 29 genera and 554 species [1,3,28]. The Polygalaceae family, which is widespread worldwide especially in tropical regions, is distributed throughout the world except for New Zealand and the South Pole of the South Pacific islands and the parts of America close to the North Pole [2-5].

*Polygala* L. species grown in Türkiye have been found to be used for therapeutic purposes in Anatolia. In Elazığ and its surrounding areas, the roots of *Polygala pruinosa* Boiss. subsp. *pruinosa* are used to help with expectoration and sweating; while the aboveground parts of *Polygala anatolica* Boiss. & Heldr., excluding the roots, are known to reduce inflammation, act as a diuretic, increase breast milk, and aid sweating in the Aegean region [6-8].

In the family Polygaleceae, the inflorescences may be axillary or terminal. They usually have a racemose inflorescence [9]. This is also a character shared with the family Fabaceae [10,24]. The calyx-corolla is irregular. Sepals are 5 and free, the inner 2 large and petaloid, the outer 3 small and sepaloid. Petals are 3, ± fused, and developed with a staminal tube. Stamens are monadelphous, 8 in number. Anthers open with pores. Capsule is 2-celled, flattened, usually winged. Seeds are arillate, 1 per locule, and may be hairy or glabrous [11].

The floral morphology of the genus *Polygala* shows that zygomorphic flowers have one bract and two bracteoles. The five-pointed, petaloid calyxes contain two long wings that enclose both the corolla and the reproductive organs of the flower. The median of the

three petals, which meet at the base, is winged and bears a crested fringe and crest that encloses both the anther and stigma (Figure 1). The filaments fuse along their length and attach to the base of the winged petal. The two-lobed stigma and style fuse to form the bicarpellate gynoecium. A septum divides the ovary into two locules, each containing a single bazal attached ovule [12].


Figure 1. Crest structure in *Polygala* [13].

Flower bud formation develops as a process starting from physiological differentiation and concluded in morphological differentiation [14]. The formation of flower-bearing buds is a process that requires the meristematic apex of the bud, which is undifferentiated in the early stages of growth, to transform into a flower-bearing structure.
In this study, systematic botanical information and embryological and micromorphological comparative research were aimed for the species *P. anatolica* Boiss. & Heldr. *P. monspeliaca* L. and *P. vulgaris* L. belonging to the Polygalaceae family, which naturally grows in Türkiye.

## 2. Materials and methods

The aim of this study was to investigate the floral developmental characteristics of three *Polygala* species selected based on their taxonomic characteristics. Three taxonomically distant species within the genus, *P. anatolica*, *P. monspeliaca*, and *P. vulgaris* were selected for this study based on their diagnostic characters.
The locality information (coordinates, altitude, habitat) of the collected samples was recorded and the samples were photographed in their habitats (Figure 2a-c). Taxa, sample numbers, and locality information of the studied samples are given in Table 1.

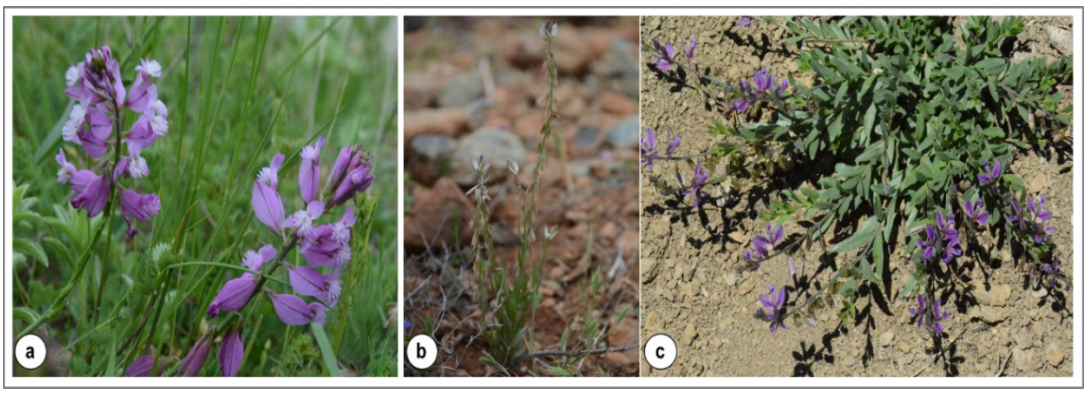


Figure 2a-c. General views of *Polygala* taxa. a**)** *P. anatolica,* b) *P. monspeliaca* and c) *P. vulgaris*

Table 1. Taxon name, sample numbers and locality information of the studied samples

| | |
|---|---|
| ***P. anatolica*** | A3 Sakarya: Geyve around, Çamlık Village, *Pinus nigra* opening, N40º 26′ 35.4, E30º 22′ 47, 640 m, 18.06.2021, *A. A. Dönmez 20856 – E. O. Karahan* |
| ***P. monspeliaca*** | C1 Muğla: Datça, Amazon Bay, serpentine, scrub, N36º-48′-12.91- E 028º-03′-41.2, 6m, 16.04.2021, *A. A. Dönmez 20797 – E. O. Karahan* |
| ***P. vulgaris*** | A8 Erzurum:Oltu, Maden road Duralar Village, *Juniperus* clayey slopes, N40°-29′-29.9, E 41º-56′-20.9, 25.7.2020, *A. A. Dönmez 20743- Y. Kaya* |

### *2.1. LM examinations*

Bud and flower samples of various sizes were collected to study flower bud formation and development of the identified *Polygala* species. Sections of the flower buds were made using the paraffin method [15]. Longitudinal sections of 10–12 µm thickness were taken from the bud samples using a Leica SM2000R microtome. The sections were stained with Safranin fast green. Paraffin-embedded sections were examined under a light microscope (LM), and photographs were taken using a Leica DFC 290 camera microphotography instrument.

### *2.2. SEM examinations*

In this study, the floral characters (floral surface characteristics, cuticle density and folds, and corolla surface ornamentation) of the identified taxa were examined micromorphologically. The aim was to identify features not detectable with light microscopy using Scanning Electron Microscopy (SEM) and to assist in resolving taxonomic problems that arise when distinguishing genera.

Flower buds collected from the field were used for micromorphological examination. The flower surface was first examined under a stereomicroscope and fixed on an aluminum object holder (stap) with double-sided carbon fiber tape [16]. The entire surface of the fixed samples was coated with 12.5-15 nm gold. Examination and photography were performed using a Tescan brand GAIA3 GMU (model 2016) 117-0302 Scanning Electron Microscope (SEM) at Hacettepe University Advanced Technologies Application and Research Center (HUNITEK). Photographs were saved to digital media in "bmp" format with 768 x 918 pixel dimensions.

## 3. Results

In sections taken from the flower buds of *P. anatolica*, *P. monspeliaca*, and *P. vulgaris* the developmental processes from the beginning of the apical meristem to the maturation of the reproductive organs were examined in 4 stages; 1. Flower bud differentiation, 2. Corolla/Petal differentiation, 3. Male organ differentiation, and 4. Female organ differentiation.

### *3.1. Polygala anatolica*

In the first stage, the bud axis is elongated and forms a pointed projection (Figure 3a). Two bracts begin to appear on either side of the leaf axils, and a distinctly convex apical meristem is observed. In the early developmental stages (stages 1 and 2), the stigma and style are fused (Figures 3b-c). In the second stage, the flower parts, which continue to differentiate within the flower bud, begin to surround the bud. The bracteoles surrounding the floral organs surround the ring-shaped tissue (Figure 3d).

In the second stage, the style is elongated. The filaments are attached to the corolla lobes and are enclosed in a U-shaped staminal sheath (Figure 3e-f). In the third stage, the floral primordia expand, and the stamen primordia begin to enlarge and develop (Figure 3g). Filament differentiation is followed by anther formation. In the mature stages (stages 3 and 4), the ovary becomes prominent, taking on a cordate appearance (Figure 3h-i). The ovary is found to be divided into two locules, each containing a single apically attached ovule. It has a relatively long style. Eight stamens are identified, with more pronounced anther slits. The stamens are found to fuse at the base, forming a sheath. The anthers are observed to be bisporangiate.

In the fourth stage, the two carpel primordia elongate and grow, forming a parenchymatic style. The ovary emerges beneath the carpel primordia. Two ovules are observed in the ovary. The pistil parts are prominent. The style begins to elongate upward (Figures 3j-l). Two integument primordia are observed to differentiate from the base. The ovule develops from the placenta of the ovary and is homogeneous. The tissue of the ovule is parenchymatic (Figure 3m). The funiculus, micropyle, and female gametophyte are present in the same position and vertically. In the 3rd and 4th stages, in the sections where the mature female gametophyte was observed (Figure 3n), it was observed that the ovule primordiums emerged by budding from the cells in the placenta wall.
In the flower of *P. anatolica*, similarly shaped anticlinal and periclinal cells were observed on the petal surface, and the prominence of these cells and the density of the cuticle increased as development continued. The protrusions, which completely cover the surface, are more concentrated towards the tips. Irregular reticulate folds are present on the cuticle surface. Images of the petal surface of *P. anatolica* at all identified stages are shown in Figures 3o-r.

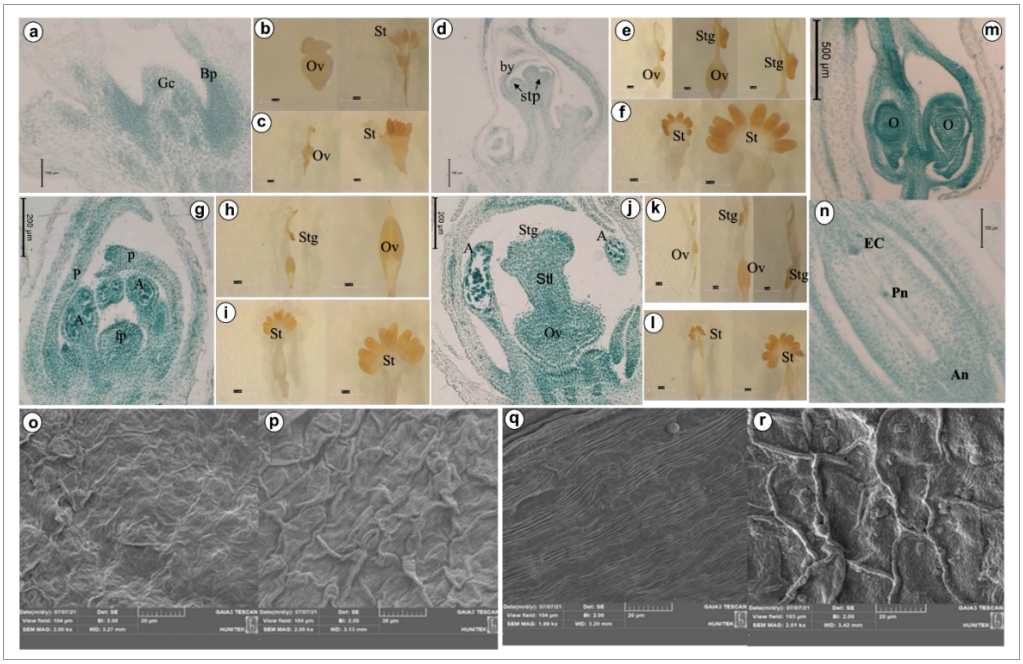


Figure 3. *P. anatolica*, a-c. flower bud development, d-f. petal differentiation, g-i. stamen differentiation, j-l. pistil differentiation, m. ovule, n. mature female gametophyte, o. 1st stage p. 2nd stage q. 3rd stage and r. petal micromorphology at 4th stage (SEM).

*Abbreviations:* Gc: Growth cone, fp: Floral primordium, Bp: Bud primordium, by: Bract leaf, stp: Stamen primordium, A: Anther, o: ovule, ov: ovarium, P: petal, Stg: Stigma, Stl: Style, St: Stamen, EC: Egg cell, Pn: Polar nucleus, An: Antipod cells.

### *3.2. Polygala monspeliaca*

In the first stage, the first primordiums begin to emerge from the flower bud. Flower initiation begins with the differentiation of the apical meristem into a rounded projection (Figure 4a). The apical meristem expands, flattens, and transforms into the floral meristem, which will form the primordia of the floral organs. At this stage, two bracts begin to appear on either side of the leaf axils, and a distinctly convex apical meristem is observed. The apex of the flower bud gradually begins to take on a pointed, circular shape. In the first and second stages of *P. monspeliaca*, the ovary structures are not fully developed (Figures 4b-c). The lower stigma lobe is observed to be larger than in *P. anatolica* and *P. vulgaris.* Eight stamens are present. The lower parts of the filaments are fused and ciliated above.

In the second-stage flower bud, the floral parts begin to surround the bud. Bracteoles surrounding the floral organs are surrounded by a ring of tissue (Figure 4d). Filaments are attached to the corolla lobes and are enclosed in the staminal sheath (Figure 4e-f). The stamen primordia continue to grow and develop (Figure 4g). In the mature stages (3rd and 4th stages), the ovary becomes distinct. The style is narrow and curved, the upper stigma is short and curled like a hook, and the lower stigma is long (Figure 4h-i). The carpel primordia elongate and grow, forming a parenchymatic style. The ovary appears beneath the carpel primordia. Two ovules are observed in the ovary. The pistil parts are prominent. The style begins to elongate upward. The anthers appear transparent in the fourth stage. The filaments are fused at the base (Figure 4j-m). The ovule's tissue is parenchymatic (Figure 4n). The funiculus, micropyle, and female gametophyte in the ovule are located vertically and in the same position. Mature female gametophytes were observed in the 3rd and 4th stages (Figure 4o).

Anticlinal and periclinal cells were also identified on the petal surface. The prominence of these cells and the density of the cuticle increased as development continued. These cells were observed to be elongated rectangular in shape. Irregular reticulate folds were present on the cuticle surface. These folds were identified along the longitudinal axis of the petal. Images of the petal surface of *P. monspeliaca* at all identified stages are shown in Figures 4p-s.

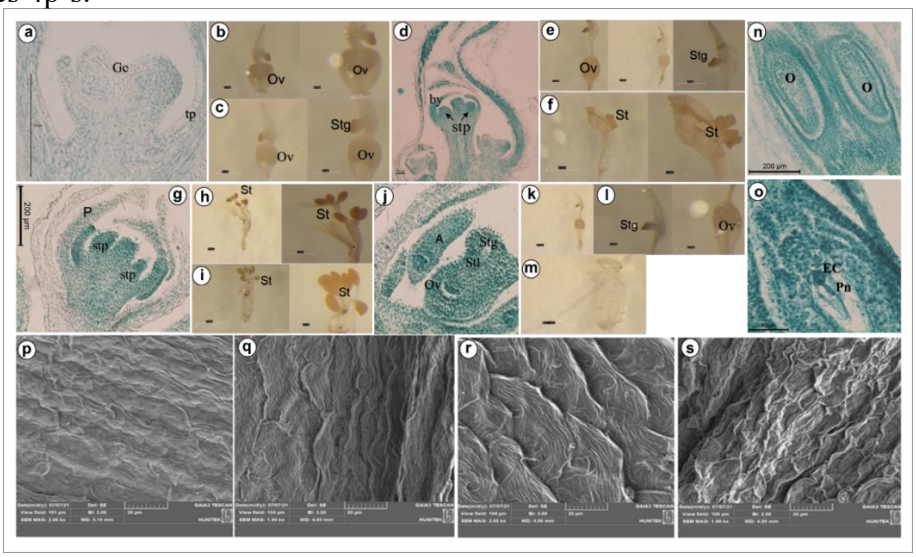


Figure 4. *P. monspeliaca*, a-c. flower bud development, d-f. petal differentiation, g-i. stamen differentiation, j-m. pistil differentiation, n. ovule, o. mature female gametophyte, p. 1st stage q. 2nd stage r. 3rd stage and s. petal micromorphology at 4th stage (SEM).

*Abbreviations:* Gc: Growth cone, tp: Bud scale, by: Bract leaf, stp: Stamen primordium, A: Anther, o: ovule, Ov: Ovarium, P: petal, Stg: Stigma, Stl: Style, St: Stamen, EC: Egg cell, Pn: Polar nucleus.

### *3.3. Polygala vulgaris*

In the first stage, the flower buds begin to swell, and the bud axis within the flower buds becomes elongated and widened (Figure 5a). In *P. vulgaris*, in the early developmental stages (stages 1 and 2), the stigma and style are fused. The ovary is not fully developed. The style is not yet evident in the first stage (Figures 5b-c). In the second stage, the flower parts, which continue to differentiate within the flower bud, begin to surround the bud. The bracteoles surrounding the floral organs are surrounded by a ring of tissue (Figure 5d). In the second stage, the style begins to elongate. The stamens are monadelphous (all the stamens are present together). Eight stamens were observed. The filaments are attached to the corolla lobes and are found to fuse within the U-shaped staminal sheath (Figures 5e-f). In Stage 3, the floral primordia expand, and slight swellings begin to appear in the intermediate. Two stamen primordia then differentiate into a protrusion on either side of the floral meristem. The stamen primordia begin to enlarge and develop (Figures 5g-i). Filament differentiation is followed by anther formation. In the 4th stage, two carpel primordia appear on the floral meristem. In subsequent stages, two more carpel primordia grow and elongate, forming a parenchymatic style. The ovary appears beneath the carpel primordia. Two ovules are observed within the ovary. The pistil parts have become apparent. The style has begun to elongate upward (Figure 5j-l).

In flower buds, two integument primordiums have been observed to differentiate from the base. The ovule is formed from the placenta of the ovary and is homogeneous. Sections show that the ovules are bitegmic (double integumented), bearing two integuments. The texture of the ovule is parenchymatic (Figure 5m). The funiculus, micropyle, and female gametophyte are located in the same position and vertically. Consequently, in later stages of integument development, mature ovules, based on the appearance of the micropyle, are identified as anatropous ovules. In sections of stages 3 and 4, where the mature female gametophyte has formed (Figure 5n), ovule primordiums are observed to emerge by budding from cells in the placenta wall. As the three-layered ovular primordia differentiate through periclinal divisions, some cells in the nucellus project anteriorly. The archespore cell divides periclinally to form the primary parietal cell and primary sporogenous cells. The primary parietal cell undergoes periclinal divisions in the subdermatogenous region of the outer layer of the ovular primordia. Consequently, the ovules appear crassinucellate in sections.

In all stages of *P. vulgaris* flower buds, cells with projections slanted in different directions (anticlinal) and parallel to the cell surface (periclinal) were observed on the petal surface. The prominence of the cells and the density of the cuticle gradually increased from stage 1 to stage 4. These cells were generally observed to have rectangular shapes. Irregular reticulate folds were observed on the cuticle. Images of the petal surface in all stages of *P. vulgaris* are shown in Figures 5o-r.

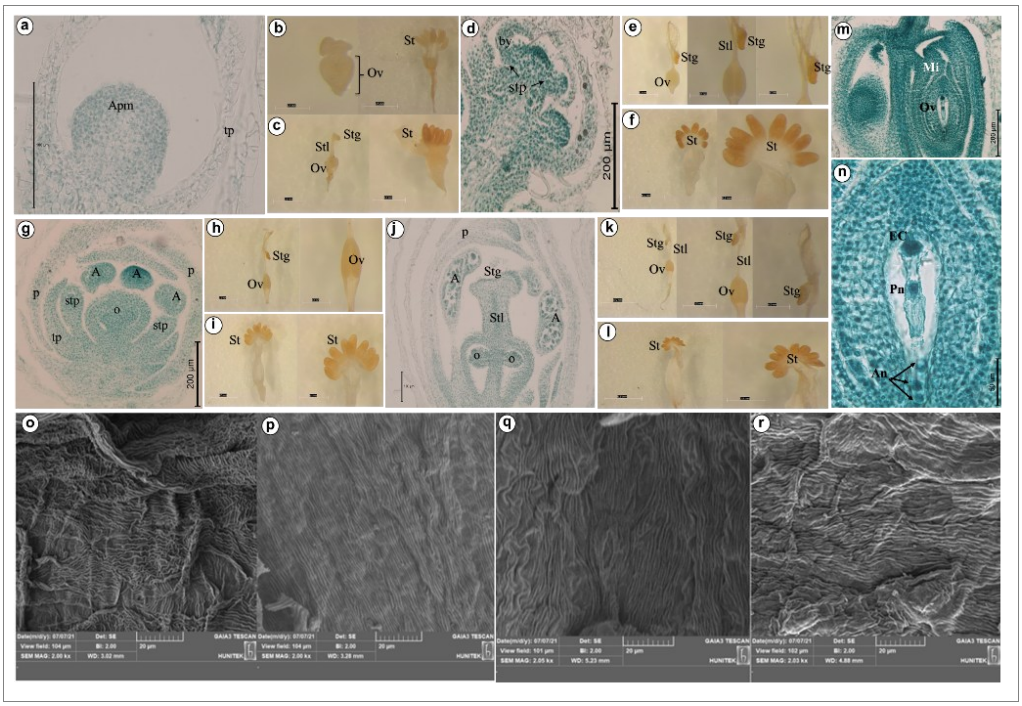


Figure 5. *P. vulgaris*, a-c. flower bud development, d-f. corolla/petal differentiation, g-i. stamen differentiation, j-l. pistil differentiation, m- ovul, n. mature female gametophyte, o. 1st stage p. 2nd stage q. 3rd stage and r. petal micromorphology at 4th stage (SEM).

*Abbreviations:* Apm: Apical meristem, tp: Bud scale, fp: Floral primordium, stp: Stamen primordium, A: Anther, o: ovule, ov: ovarium, P: petal, Stg: Stigma, Stl: Style, St: Stamen, EC: Egg cell, Pn: Polar nucleus, An: Antipod cells.

## 4. Discussion

The development of flower buds at different stages was examined in sections taken from *P. anatolica*, *P. monspeliaca* and *P. vulgaris* which naturally occur in Türkiye. The species were evaluated embryologically based on the studies conducted. This study aims to contribute to the literature and compare the relationships between floral organ characteristics of the species, as no embryological research has been conducted on *Polygala* species in Türkiye.

In the first stage of flower development, the bract of the inflorescence was observed to be visible. In the second stage, the floral primordium expanded to produce a transversely elongated primordium. As growth continued, these two points, where the primordiums became pointed on either side, were observed to be the bracteoles at the time of initiation. Bracteoles are the first organs to develop. Elongation of the bracteoles is followed by the formation of the pistil primordium, which surrounds a central cavity in the middle portion of the carpel primordium. This central cavity is visible within the young ovary in longitudinal section. This is then followed by the formation of the floral organs. Continued growth of the pistil primordia produces locules as the ovary wall differentiates upon closure of the carpels. Locules arise from the widening of the spaces between the pistil primordium. In each locule, the ovule primordium swells outward, creating an oval shape. The style and stigma develop from the apical segments of the pistil primordia. The carpel primordium grows upward and fuses along its lateral margins to form a large cylindrical style covered by the stigma primordium. At the final stage, a complete pistil with stigma is observed. The ovaries of the studied *Polygala* species were found to

develop into anatropous, bitegmic, and crassinucellate ovules, exhibiting apical placentation.

Our results appear to be supported by studies conducted by various researchers on species belonging to the Polygalaceae and Fabaceae families. Furthermore, a comparison of the embryological characteristics of the three studied species revealed no differences. Indeed, Netolitzky (1926), Davis (1966), and Corner (1976) reported that the ovules were crassinucellate and bitegmic [17-19].

In a study examining ovule ontogeny in *P. vulgaris, P. myrtifolia* L., and *P. chamaebuxus* L., W. Verkerke and F. Bouman (1980) reported that the ovule primordia of *Polygala* are trizonate and of dermal origin. In *P. vulgaris*, the integuments are parenchymatic, but W. Verkerke and F. Bouman interpreted the parenchymal tissue of *P. vulgaris* as being reduced in *P. myrtifolia* [20].

In another study examining the structure of the ovule and seed of the genus *Xanthophyllum* Roxb. of the Polygalaceae family, W. Verkerke (1985) reported that the ovule primordia are trizonate, the outer integument is of dermal origin, and the ovary in the ovules is orthotropic (atropous) [21].

In an embryological study on the *Lotus glaber* Mill. species belonging to the Fabaceae family, Beatriz G. Galati, Sonia Rosenfeldt and Graciela M. Tourn (2006) reported that the mature ovule is bitegmic (double integument), with two integument primordiums emerging from the base and the ovule carrying two integuments [22].

Three taxa belonging to the genus *Polygala* were examined morphologically and micromorphologically using scanning electron microscopy (SEM) and stereomicroscopes. These studies were conducted to support our embryological results, to compare the relationship between morphological and micromorphological features with embryological features, and to further clarify the link between embryology and floral morphology by investigating the development of the species' floral parts.

The flower buds of the species *P. vulgaris, P. monspeliaca*, and *P. anatolica*, which we divided into four different stages, were micromorphologically examined for perianth characteristics, cuticle density and folds, and corolla surface ornamentation. The analyses revealed the morphological impact of differences in specific developmental stages. The petal surface was observed to consist of numerous irregularly indented and protruding segments. No significant changes were observed on the petal surfaces of *P. anatolica* and *P. monspeliaca* in the early (stages 1 and 2) and mature (stages 3 and 4) stages. Cuticle density was observed to increase as the buds completed their developmental stages. No study was found that could compare our results on flower surface characteristics in the genus *Polygala* or other species of the family Polygalaceae. It appears that our results are supported by studies conducted by various researchers on species from the Polygalaceae and Fabaceae families.

In our study, it was concluded that the stigma lobes of the flower buds of *P. vulgaris* are physically separate from each other. These results are similar to the studies conducted by Bello et al. (2010) on *Polygala chamaebuxus* L., *Monnina salicifolia* Ruiz & Pav., *Polygala calcarea* F.W.Schultz, *Polygala serpyllifolia* Hose, and *Polygala vulgaris*. However, the same study reported that the stigma lobes of *Securidaca longepedunculata*

Fresen., *Securidaca retusa* Benth. *Bredemeyera floribunda* Willd., *Barnhartia floribunda* Gleason, *Diclidanthera bolivarensis* Pittier, *Salomonia cylindrica* (Blume) Kurz, *Atroxima afzeliana* (Oliv. Ex Chodat) Stapf, and *Xanthophyllum clovis* (Steenis ex Meijden) Meijden are in close contact with each other [23].

In our study, it was observed that the styles of *P. vulgaris* and *P. anatolica* were relatively long. Westercamp examined the flower structure of *P. amara, P. chamaebuxus, P. major* Jacq, *P. vulgaris* subsp. *oxypera* (Rechb.) Schübl. & G.Martens, and *P. vulgaris* and reported that their styles were more or less long [24].

Studies have shown that *P. vulgaris, P. monspeliaca* and *P. anatolica* have eight stamens and that the stamens are grouped together. The stamens are seen to be monodelpheus. Comparing with studies in the literature, Royen & Steenis (1952) reported that the genus *Eriandra* P.Royen & Steenis has seven to ten stamens. Styer (1977) reported that the genus *Diclidanthera* Mart. has eight to ten stamens [25- 26].

It was determined that the filaments of the stamens of the studied species were attached to the corolla lobes and fused within the U-shaped staminal sheath. Similarly, when we look at the literature, Milby (1976) stated that the filaments of the monadelphous type stamens were fused at the base in his studies on the flower anatomy of *Polygala* [12].
In our studies on the flower buds of *P. vulgaris, P. monspeliaca* and *P. anatolica*, it was determined that the ovary is divided into two locules, each containing a single apically attached ovule. In his study on *P. speciosa* Sims, Payer (1851) reported that the bicarpellate gynoecium surrounded two ovules, one per locule [27].

As a result of all these observations and research, the embryological, morphological, and micromorphological study of species provides significant benefits in plant systematics. There is limited information in the literature regarding the embryogenesis of individuals of the genus *Polygala*. No embryological studies have been conducted on European or Asian taxa of this genus, which is distributed worldwide. This study on the embryogenesis of this genus, which naturally occurs in Türkiye, will fill a significant gap in the literature. Embryological, morphological, and micromorphological studies of *Polygala* species in Türkiye, which lacks embryological data, will be crucial in revealing similarities and differences among species. It is expected that this study will benefit all future studies on the genus *Polygala*, and that the results will contribute to the embryological and systematic implications of the genus *Polygala*.

## 5. Conclusion

The aim of this study was to provide systematic botanical information and a comparative embryological and micromorphological study for *P. vulgaris*, *P. monspeliaca*, and *P. anatolica* species of the Polygalaceae family that naturally occur in Türkiye.

According to the information obtained in the study:

The relationship between morphological and embryological characteristics of some *Polygala* species collected from the field during flowering was determined.

The embryological development of the target species was studied comparatively in field samples.

Morphological and micromorphological studies using scanning electron microscopy (SEM) and stereomicroscopes investigated the development of the species' floral parts,

further clarifying the relationship between embryology and floral morphology and providing new data on the genus' reproductive characteristics.

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