

## Study of the Structure of Artichoke (*Cynara scolymus* L.) Flowers

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### ABSTRACT

This study reports the arrangement of the flower head and the structure of the floral organs of the artichoke (*Cynara scolymus* L.) cultivar 'Bayrampaşa', which is a very popular variety in Turkey. The structure of flower of this cultivar was investigated under a stereomicroscope, light microscope, and Scanning Electron Microscope (SEM). Additionally, the flower development stages were examined. The flower heads contain multiple flowers attached to a single receptacle. The flowers on the flower head are arranged in the form of a disc. The maturation of the flowers starts from the outer part of the head and continues towards the center. The number of flowers per head was determined to be 800-1200. The flowers exhibited hermaphroditism, and one flower was found to have five rudimentary petals, five stamens, and one pistil. The ovary was the inferior type. The style was of the closed type and was very long. The stigma was very long and had two portions, with one portion shorter than the other. The stamen had one theca with two lobes. The pollen was lifted up and distributed at the outer sides of the stigma, which is the secondary pollen presentation system. The pollen grains of this cultivar were circular in shape and trizonocolporate. The pollen surface was echinate with spin projections. The pollen grain length was 54.33 µm and the spin length was 5 µm in the examined cultivar. The different stages of flower development were seen in the flower head. The flowers were divided into four different stages of development and they have Measuremented in these stage.

**Keywords:** Floral organs, Flower stage, Flower morphology, Scanning electron microscope.

### INTRODUCTION

Artichoke (*Cynara scolymus* L.) is an herbaceous perennial plant belonging to the *Asteraceae* family. Artichokes are particularly widespread in the Mediterranean Basin, where the climate is characterized by warm summers and mild winters (Bekheet and Sota 2019; Falco *et al.*, 2015; Negro *et al.*, 2012; Salata *et al.*, 2013; Sekara *et al.*, 2015). Turkey production is 39,477 tons in 2018 (Tuik, 2020). World production is 1,678,872 tons in 2018 (Faostat, 2020). The globe artichoke is widely distributed all over the world, especially in the South Europe (Italy, Spain, France, and Greece), Middle

East (Turkey, Israel), North Africa (Egypt, Tunisia), South America (Argentina, Chile), United States and in China (Zeipiņa *et al.* 2015). There are two main types of artichoke cultivated in Turkey: "Sakız" and "Bayrampaşa". While the mastic type is produced for fresh consumption due to its early feature, "Bayrampaşa", which is a late variety, is generally produced for industry (canned food) (Abak, 1987). Flower head of artichoke is considered a healthy food due to low fat and cholesterol, while being a rich source of fiber, vitamins and minerals (Nouraei *et al.*, 2016).

Artichokes have a long history as herbal medicines and have been used in folk medicine since Roman times. The artichokes,

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whose head parts are evaluated as vegetables, form the main flower. The head formed at the end of the artichoke's flower shoot (stalk) expands and forms a flower tray. The edible part of the plant is limited to the fleshy leaves (bracts) and the receptacle of a large immature inflorescence. If an artichoke head is not harvested in the eating stage, the head begins to flower. The inflorescence (capitulum or head) consists of a very long peduncle (up to 180 cm), a receptacle where flowers are inserted, and external bracts (Falco *et al.*, 2015). The flowers on the floral receptacle are arranged in the form of a disc. The maturation of the flowers starts from the outer part of the head and continues towards the center. The peripheral florets flower first, and during the subsequent two to three days, flowering progresses centripetally (Janick, 1994).

The artichoke, which has a foreign pollinated flower structure (allogamy), is pollinated with insects (entomophily) (Pagnotta, 2010). Flowers are proterandric (Janic 1994; Shipunov, 2018; Ryder *et al.*, 1983). Self-pollination is avoided by the proterandry of the flowers stigmatic surfaces, which mature two or three days after pollen shedding. As a reflection of its morphological variation, the stigma can play several roles such as hydration of pollen grains and nutrition of the growing pollen tubes. Because of its ability to stimulate or prevent the emission of pollen tubes, the stigma also plays a selective role in the germination of pollen grains (Pádua Teixeira *et al.*, 2018). Therefore, artichoke production in Turkey is mostly done vegetatively (Eşiyok, 2018). Although vegetative production is common, some problems are encountered in this form of production. In production with seed, such problems can be prevented and significant increases are obtained in the yield per unit area (Tekdal, 2018). Seed cultivation has great advantages in terms of enabling rapid reproduction and solving problems related to the transportation of diseases (Macua *et al.*, 2000). In Turkey, studies of the production of artichoke from seed are almost nonexistent.

Artichoke studies in the world are mostly focused on content analysis and in vitro

micropropagation (Başay and Tokuşoğlu, 2013; Dawa *et al.*, 2012; El Sohaimy, 2014; Pérez-Esteve *et al.*, 2018; Salem *et al.*, 2019; Yıldırım *et al.*, 2020). Artichoke varieties in Turkey are largely heterozygous structure; it is not possible to replicate seeds. Therefore, artichoke is usually propagated by vegetative propagation in Turkey. There are not enough studies on the flower structure and pollen morphology of artichoke in Turkey. Considering the beginning of seed breeding studies in artichoke, the purpose of this study was to describe the flower structure, flower developmental stages, and pollen morphology of artichoke in detail for one Turkey's variety.

## MATERIALS AND METHODS

The “Bayrampaşa” variety was used as the research material. “Bayrampaşa” variety is popular, especially in Marmara Region in Turkey. The study was conducted at Bursa Uludağ University (Universities place category with the GPS coordinates of 40° 13' 8.0364" N and 28° 52' 12.0036" E.), Faculty of Agriculture, Research and Experiment Field, in Turkey.

### Morphological Observations and Measurements

Phenological observations were made in an artichoke experimental field. At the beginning of the flowering stage, diameter measurements were taken from 20 heads, and the number of flowers in a head was recorded. Flower development was defined by classification into four stages. In each stage (I, II, III, IV), the flower (cm), ovarium (cm) and stigma (cm) lengths were measured in 50 flower samples.

### Stereomicroscope Observations

The flowers and flower parts of plants at different developmental stages (single flowers and floral structures) were analyzed

under a stereomicroscope (SZ6045TR, Olympus Optical Co. Ltd., Tokyo, Japan). Detailed observations were made, and some samples were photographed (Mert, 2012).

### Scanning Electron Microscopy

To observe the floral structures (style, stigma, and anther), plant samples were fixed in FAA solution fixative (10% formalin, 5% glacial acetic acid, 50% ethanol, 35% water, by volume) (Mert, 2010). Then, the samples were dehydrated in an ethanol series (70, 80, 90, and 96%) for 40 minutes at each concentration, and in two sets of 100% ethanol for 30 minutes each. The tissues were critical-point dried for 12 hours in a desiccator, mounted on aluminum stubs with two sided tape, and coated with gold palladium (BAL TEC SCD 005). The preparations were observed using a scanning electron microscope (ZEISS EVO 40) and

photographed.

## RESULTS AND DISCUSSION

### Head Development

The “Bayrampaşa” variety of artichoke head were studied in detail and it was observed that the flower heads contained multiple flowers attached to a single receptacle (Figure 1-A). Also, Lo Bianco *et al.* (2012) stated that the artichoke head is a compound inflorescence where hundreds of florets are inserted in the fleshy receptacle; outer flowers are the oldest in development. Similarly, Gominho *et al.* (2018) stated that *C. cardunculus* flowers (‘florets’) are aggregated in inflorescences called capitula or heads and heads are usually arranged in corymb-like groups and are cyathiform to ovate. A similar study states that artichokes



**Figure 1.** (A) Head with indefinite number of flowers on a common receptacle, and (B) flowers. b= Bracte, c= Carolla, o= Ovary, p= Pappus, stg= Stigma, sty= Style.



have tiny flowers collected in a cup- or saucer-like head (capitulum) that is surrounded on the lower side by bracts (modified small leaves). The flowers are small and tightly packed, often with tubular narrow (disc) flowers in the center and sometimes with longer, flattened (ray) flowers along the edge, like in a sunflower (Anonymous, 2016). In the present study, it was found that there were 6 rows of fleshy bract leaves on the edges of the artichoke head and 800-1,200 disc florets per capitulum in the middle. Ekbiç (2005) reported that flowering artichoke heads comprise 2-3 rows of fleshy bract leaves at the edges and, depending on the variety, 600-1,200 disc florets in the middle.

### Floral Structure

In the “Bayrampaşa” artichoke variety, the flowers exhibit hermaphroditism, and each flower has five rudimentary petals, five stamens, and one pistil. Single florets assume a tubular shape, with a calix transformed into pappus, rudimentary petals, and thin anthers adnate to the pistil, which expands from the anther cone with its violet stigmatic tissue, as described by Bello *et al.* (2013) and Shipunov (2018) (Figure 1-B). The ovary is inferior, and there are many simple bristles (pappus) crowning the ovary, as stated by Süslü *et al.* (2010) and Rossi *et al.* (1992).

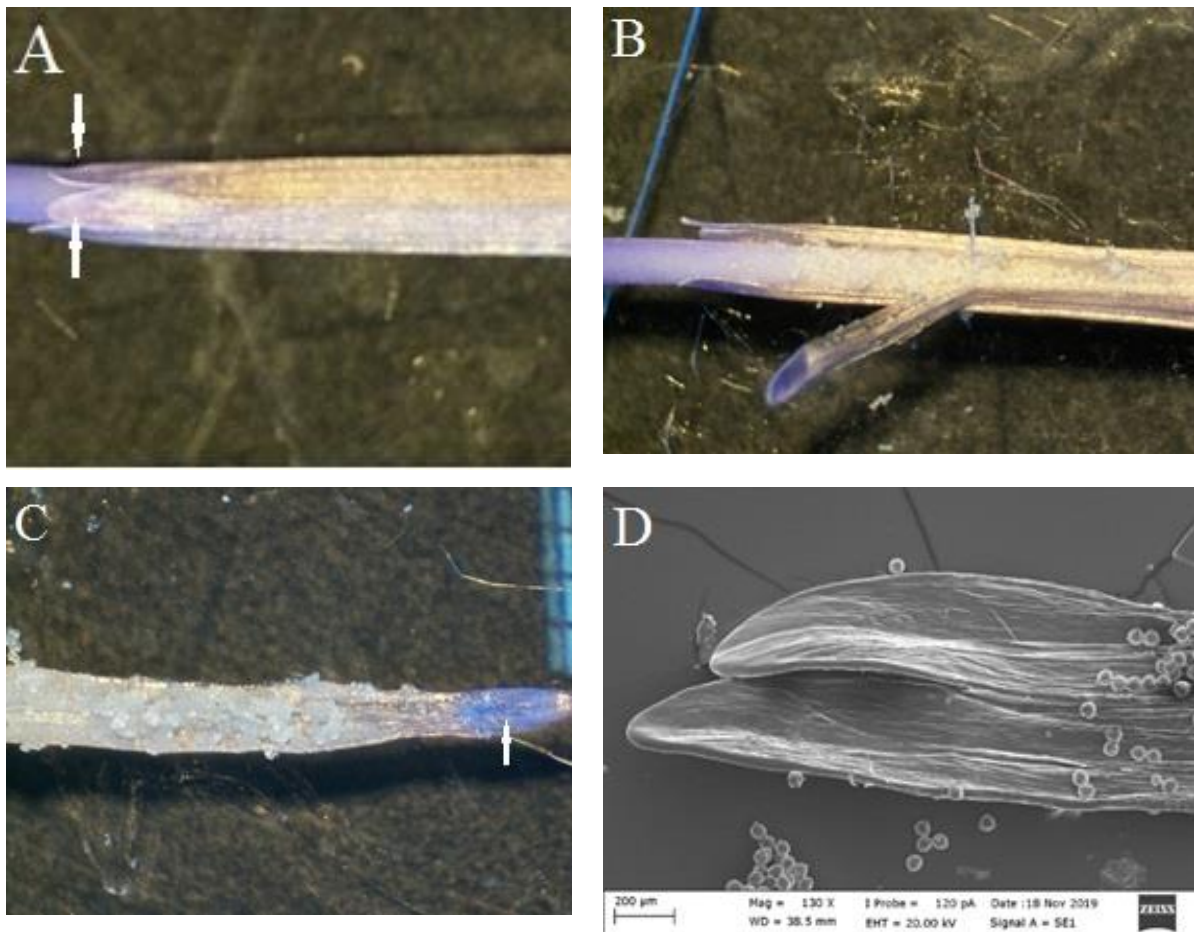
### Stamen Structure

In the “Bayrampaşa” artichoke variety, the stamens are arranged around the style and other flower organs and consist of two morphologically distinct parts, the anther and filament. The average number of anthers per flower is five, as stated for *Asteraceae* by Duistermaat (1996) and for artichoke by Keleş and Eti (2005). These anthers are attached to each other, forming a tube-shaped structure, but the filaments are separate (Figure 2-A). This structure is

known as the syngensis stamen type. It was found that the anthers were thin and long in shape, the tips of the anthers were similar to leaves and violet, with no pollen production in these parts [Figure 2 (A-D)]. The anthers dehisce by longitudinal slits during mature bud phase and hence the florets are protandrous (Figure 2-B). In particular, the introrse anthers cohere into a tube as a precondition for the typical mechanisms of secondary pollen presentation. Thus, the distribution of pollen from the anthers is different. Erbar and Leins, (2015) who examined the secondary pollen presentation system in the *Asteraceae* family, said that; the pollen is lifted up and distributed at the outer sides of the stigmas, which is the secondary pollen presentation. The secondary pollen presentation system functional appeared to be evolved to enhance the efficiency and accuracy of pollen exportation and/or pollen reception. Rao *et al.* (2017), who examined the secondary pollen presentation system in the *Asteraceae* family, stated that the secondary pollen presentation mechanism do not insulate completely from the occurrence of self-pollination and, hence, the flowers set fruit and seed through self-pollination and cross-pollination.

### Style Structure

The style is of the closed type and is very long and white. The style elongates within the anther tube (Figures 2-A and -C). The style and stigma point of separation are distinct in color (Figure 2-C). The epidermal cells of the style were observed by SEM. The epidermal cells at the point of separation differentiate into papillae cells. As shown in Figure 3-A, the transition is wavy. The epidermal cells are disordered and irregularly shaped (Figure 3-B). In fact, epidermal cell structures in different types of styles were determined (Ki Hong *et al.*, 2012). Duarte *et al.* (2006) stated that the style is composed of a single layer of epidermis with a thick, lipid, and sugar-rich



**Figure 2.** (A) The anther tube and the thin and long anthers, (B) The anthers are introrse when they dehisce by the surface next to the center of the flower, and (C, D) Opened anthers. The tips of the anthers are similar to leaves (arrows).

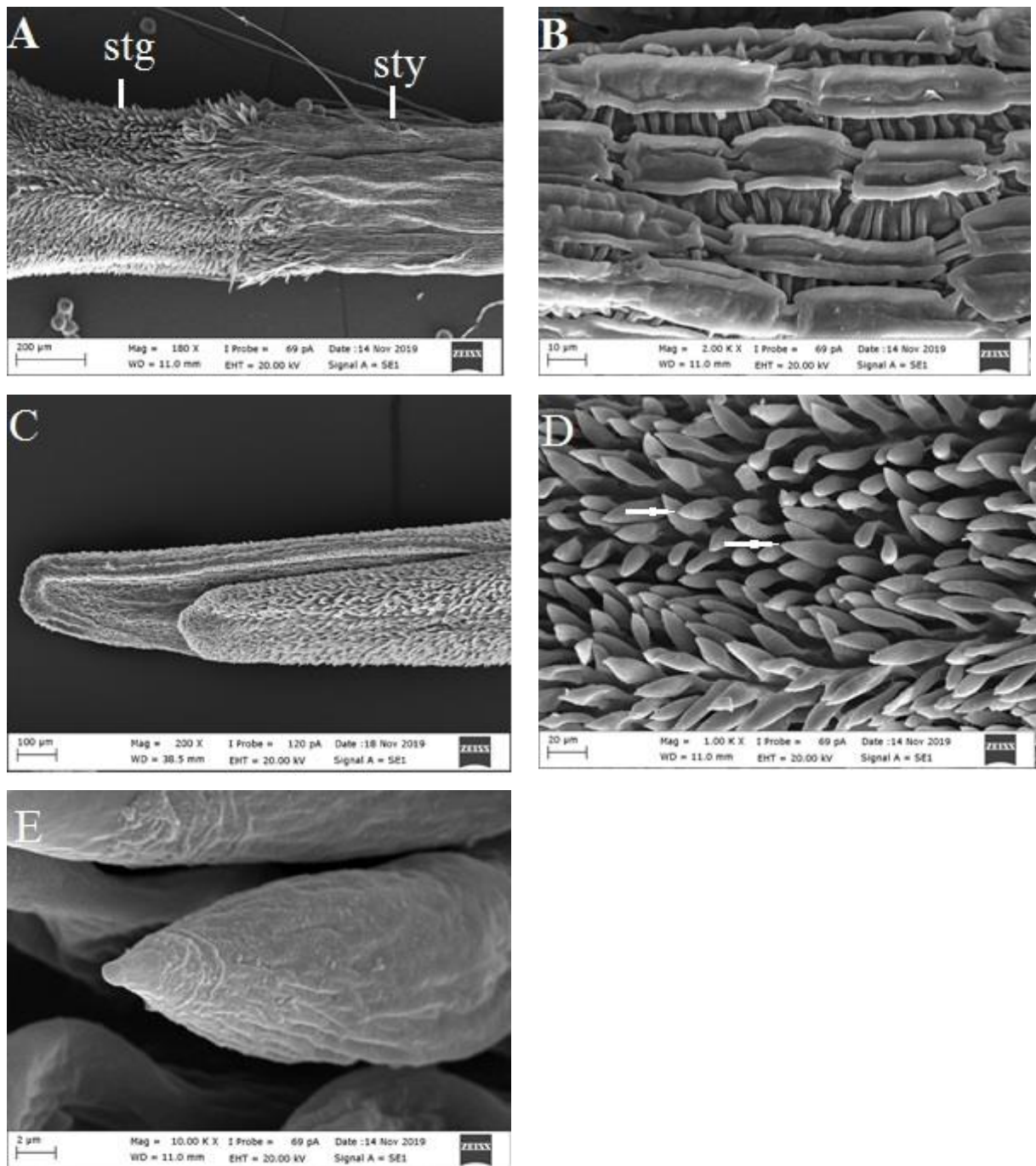
cuticle. The cells of the transmitting tissue occupy the central core of the stigma–style complex and become roundish in cross section and elongated in longitudinal section. Transmitting tissue cells are connected both in transverse and longitudinal walls, by plasmodesmata. Apical appendages are an adaptation to protect the pollen from humidity and insect predators, until the stigma and style push it up for pollen presentation (Angulo *et al.*, 2018; Stuessy *et al.* 1996).

### Stigma Structure

The stigma is very long and of the papillae type [Figure 3 (C-E)]. The stigma has two

longitudinal grooves because it consists of two portions, with one portion shorter than the other (Figure 3-C). The stigma has numerous sweeping hairs (Figure 3-D). As seen with the electron microscope, the epidermic papillae are unicellular, slightly rough and sharp (Figure 3-E). According to Torres *et al.* (2007), the pollen presenter is the sterile tissue (i.e. sweeping hairs) covering the stigmas. Dadpour *et al.* (2012) stated that, in mature flowers, the external surface of the stigma was covered by fine papillae, and a crown of hairs was visible at the stigma base. Allen *et al.* (2011) studied the stigma surface of *Helianthus* and various other *Asteraceae* species using electron microscopy, and reported that papillae may play important roles in the recognition of





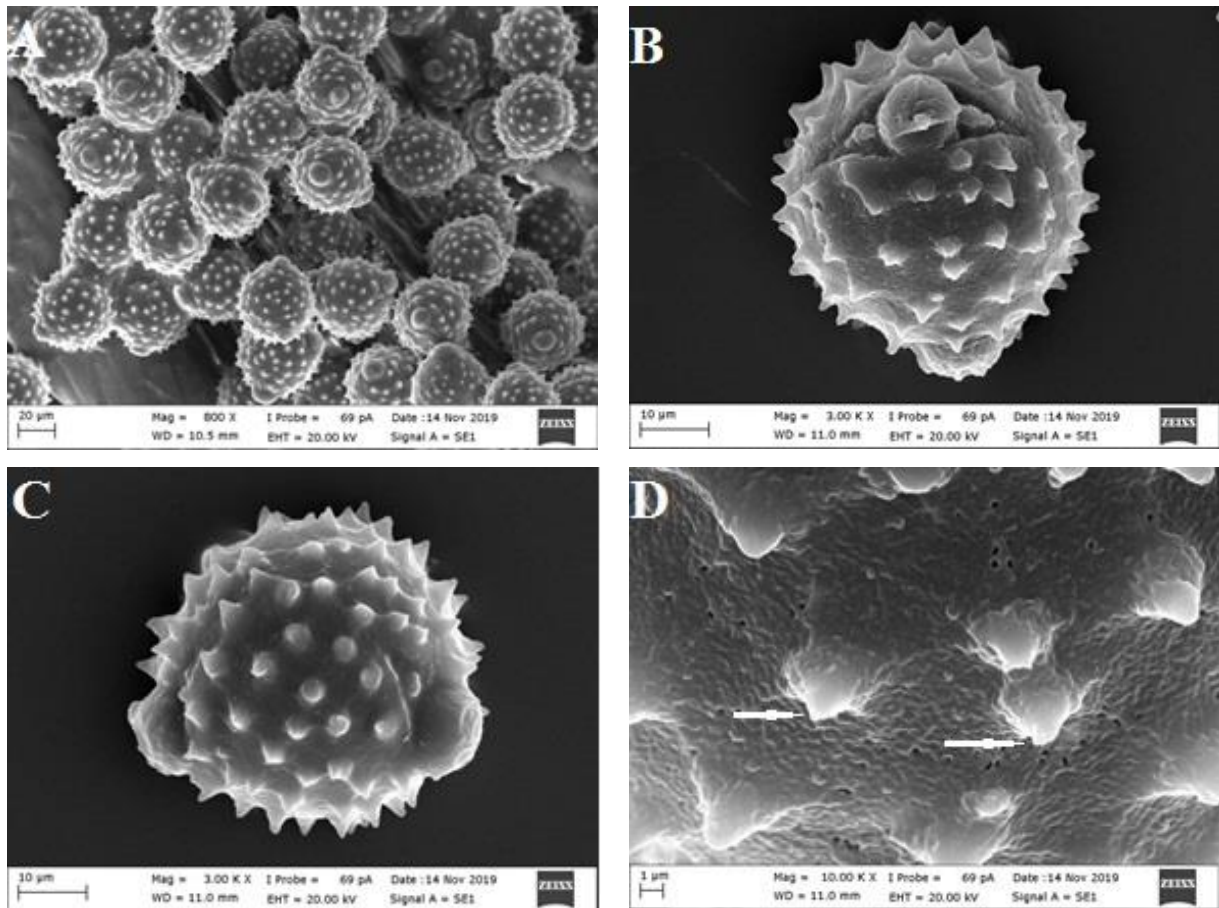
**Figure 3.** Scanning Electron Micrograph (SEM) images of style and stigma of artichoke. (A) Detail of the style branch showing the stigmatic surface and the beginning of the sterile appendage covered by sweeping hairs, (B) Epidermal cells of style, (C) the stigma, and (D, E) Papillae cells (arrows). sty= Style, stg= Stigma.

pollen grains. As a result of their work in *Cynara cardunculus*, Duarte *et al.* (2006) stated that the stigmatic papillae are unicellular, matchstick shaped, and show an increase in vacuolar volume throughout flower development.

### Pollen Surface Morphology and Dimensions

The pollen grains are circular and trizonocolporate with subdivision of the surface area into three equal parts [Figure 4 (A-C)]. The pollen surface is rough and the pollen grains have echinate exines. There are also many spines on the pollen surface. The

spines are approximately 5  $\mu\text{m}$  in length and have a sharp end (Figure 4-D). The average pollen length (polar) of the “Bayrampaşa” artichoke variety is 54.33  $\mu\text{m}$ . Lo Bianco *et al.* (2012) found that the diameter of the pollen grains of globe artichoke [*Cynara cardunculus* subsp. *scolymus* (L.) hegi] varied along the length between 43.01 and 53.42  $\mu\text{m}$ . Ahmad *et al.* (2013) noted that the pollen grains of the Family *Asteraceae* are mainly tricolporate, echinate, and the size and colpus number varies significantly. Furthermore, the *Asteraceae* family possesses zonocolporate pollen grains. The character of the pollen spines is also a diagnostic character in the family. Our results are in accordance with these results.

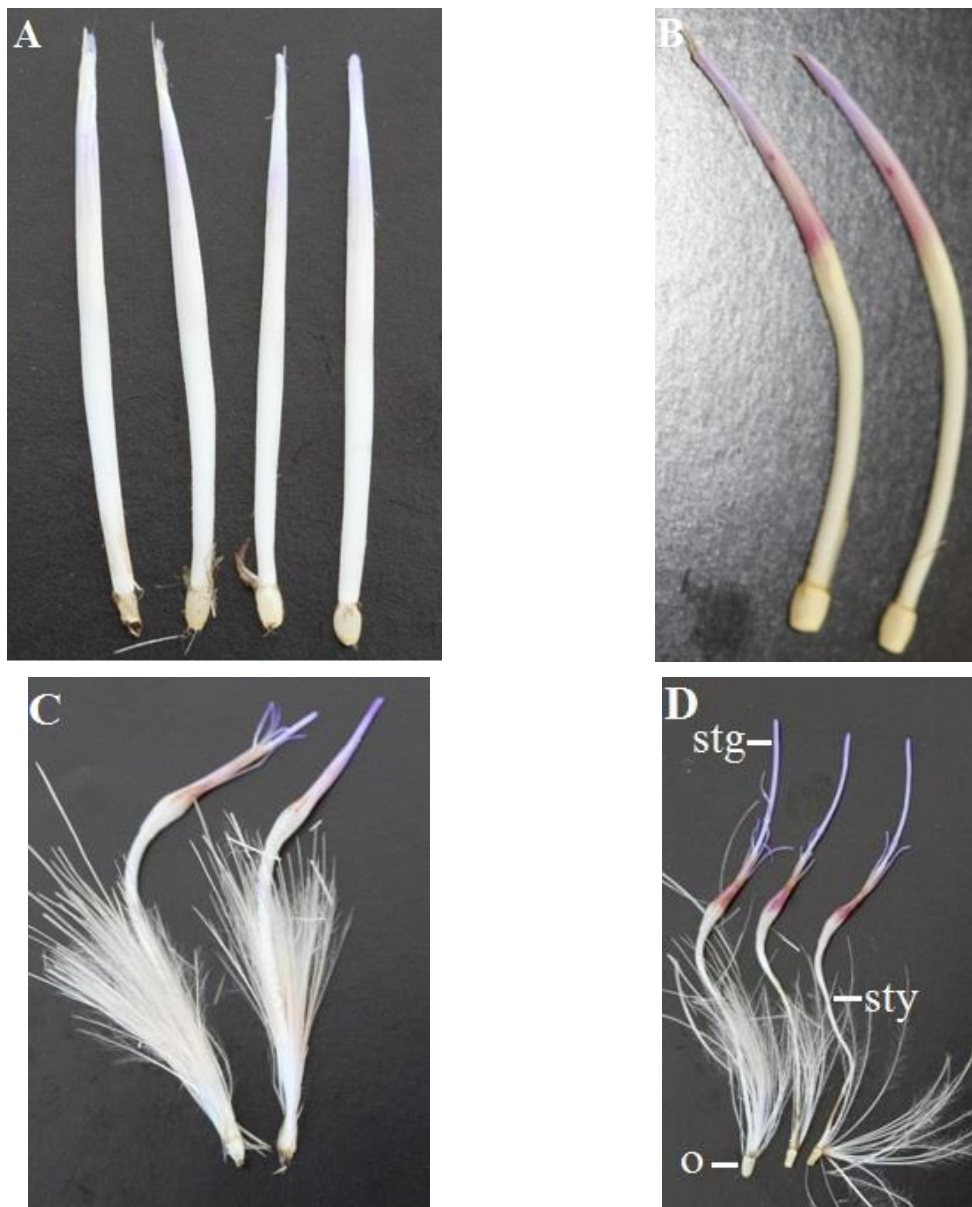


**Figure 4.** Scanning Electron Micrograph (SEM) images of pollen grains of artichoke (A-D). (B) Equatorial view of pollen grain, (C) Polar view of pollen grain, and (D) Surface view of grain and view of spines (arrows).

### Development Stages of Flowers

Investigations into the flower heads revealed flowers at different stages of development. The peripheral florets flower first, and during the subsequent two to three days, flowering progresses centripetally, as reported by Janick (1994) and Lo Bianco *et al.* (2012). In this study, the flowers in a

head were divided into four different stages of development. The developmental stages of the artichoke flowers are given in Table 1. The petals of the flowers in stage I are closed and white (Figure 5-A). The average flower length is 3.02 cm, and the ovary length is 0.18 cm (Table 2). Preparations under the stereomicroscope showed that considerable amount of pollen grains are left in the open anthers and development stage



**Figure 5.** Flowers at different stages of development. (A) At the stage of I, (B) Petals of the flowers turned blue purple color at the stage II, (C) Petals separate from the tip and open at the stage III, and (D) Flower development completed at the stage IV. o= Ovary, stg= Stigma, sty= Style.



**Table 1.** Developmental stages of artichoke flowers.

Developmental stages	Description
I.	Petal leaves are closed
II.	Coloration of petals; slight spacing at the tip
III.	Separation of the petals; the female organs and male organs become evident
IV.	Completion of full flower development

**Table 2.** Measurements from the developmental stages of artichoke flowers.

Stages	Ovary length (cm)	Stigma length (cm)	Flower length (cm)
I	0.18	unclear	3.02
II	0.25	1.44	3.14
III	0.26	2.17	6.74
IV	0.29	3.33	8.35

female organs. In stage II, the petals of the flowers turn blue-purple in color, and the tip slightly opens (Figure 5-B). In this stage, the average flower length is 3.14 cm, ovary length is 0.25 cm, and stigma length is 1.44 cm (Table 2). In stage III, the petals separate from the tip and open, and anthers are observed adhering to each other around the style. Additionally, a blue-purple stigma develops upwards from the petals (Figure 5-C). In this stage, the average flower length is 6.74 cm, ovary length is 0.26 cm, and stigma length is 2.17 cm (Table 2). It could be seen on the flowers in the stage IV that the petals turn brownish, and the fully developed stigma turns purple (Figure 5-D). At this stage, the average flower, ovary and stigma lengths are 8.35, 0.29, and 3.33 cm, respectively (Table 2). Pollen can be seen on the stigma in this stage.

In the study, it was understood that the pollen was mature in all 4 stages, but stigma was found to be at the accepting stage of pollen in the 4th period.

### CONCLUSIONS

The pollen morphology was examined for the first time in the “Bayrampaşa” variety, which is specific in Turkey. At the Same time the flower structure was evaluated. Artichoke is usually propagated vegetatively in Turkey. In this study, in order to get rid of the problems caused by vegetative

propagation, we aimed to shed light on breeding studies for seed production by examining the flower structure of the artichoke.

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## بررسی ساختار گل‌های کنگر فرنگی (*Cynara scolymus* L. آرتیشو)

س. باسای

### چکیده

در این پژوهش، چیدمان سرگل و ساختار اندام های گل کنگر فرنگی (*Cynara scolymus* L. آرتیشو) رقم "Bayrampaşa" که رقمی بسیار محبوب در ترکیه است بررسی شد. ساختار گل این رقم با استریومیکروسکوپ، میکروسکوپ نوری، و میکروسکوپ الکترونی روبشی (SEM) بررسی شد. افزون بر این، مراحل رشد گل هم بررسی شد. سرگل حاوی چندین گل است که به یک نهنج (receptacle) متصل شده اند. گل های سرگل به صورت دیسک چیده شده اند. کامل شدن گل ها از قسمت بیرونی سر شروع می شود و به سمت مرکز ادامه می یابد. در هر راس، تعداد 1200-800 گل وجود داشت. گلها هرمافرودیتیسیم (hermaphroditism) را نشان میدادند و یک گل دارای پنج گلبرگ ابتدایی، پنج پرچم و یک مادگی بود. تخمدان از نوع تحتانی (inferior) بود. خامه (style) از نوع بسته و بسیار بلند بود. کلاله بسیار بلند بود و دو قسمت داشت که یک قسمت از قسمت دیگر کوتاهتر بود. پرچم یک theca دو پارگل (lobe) داشت. گرده به شکل بالا رفته (lifted up) در بخش های بیرونی کلاله پراکنده بود، که سامانه گرده ثانویه را نشان میداد. در این رقم، دانه های گرده کروی شکل و به صورت (trizonocolporate) بود. سطح گرده با برجستگی های چرخشی خاردار (echinate with spin projections) بود. طول دانه گرده در رقم مورد بررسی 54.33 میکرومتر و طول خار 5 میکرومتر بود. مراحل مختلف رشد گل در سرگل مشاهده شد. گلها به چهار مرحله مختلف رشد تقسیم بندی و شناسایی شدند.