Alinteri J. of Agr. Sci. (2021) 36(1): 33-37
e-ISSN: 2587-2249
info@alinteridergisi.com

http://dergipark.gov.tr/alinterizbd
http://www.alinteridergisi.com/
DOI:10.47059/alinteri/V36I1/AJAS21006

RESEARCH ARTICLE

Determination of Flower Formation and Development in *Arbutus andrachne* L. with Association of Phenology

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ARTICLE INFO

Article History:
Received: 08.07.2020
Accepted: 09.10.2020
Available Online: 03.02.2021

Keywords:
Arbutus Andrachne L
Flower Primordia
Flower Biology
Strawberry Tree

ABSTRACT

Flower and floral organ development in the eastern strawberry tree (*Arbutus andrachne* L.) was investigated in this study to determine its stages and timing. After careful dissection and examination of the samples under microscopy, taken ever 10-15 days from at the end of January to the middle of April in 2019, the results concluded that flower formation completed in seven stages and that inflorescence and individual flower differentiation occurred at the end of winter. First individual flower primordia were evident 56 days before anthesis. Generative parts appeared almost 3 weeks (23 days) later and completed their initial growth in another 3 weeks (21 days). The formation of all floral organ primordia completed within the floral cup in which 5 sepals, 5 petals, 10 stamens and pistil. Individual flower primordia which were connected to the main axis by a pedicel grew on the main axis, and side branches on a second degree were connected to this main axis. Flower primordia were visible on March 2 and showed their size increased 15- and 27-fold in terms of width and length, respectively, over the course of 6 weeks.

Please cite this paper as follows:

Gökbayrak, Z. and Engin, H. (2021). Determination of Flower Formation and Development in *Arbutus andrachne* L. with Association of Phenology. *Alinteri Journal of Agriculture Sciences, 36*(1): 33-37. doi: 10.47059/alinteri/V36I1/AJAS21006

Introduction

The province of Çanakkale (Troy) not only has an old fruit cultivation history but also is an area with numerous wild fruit species. One of these naturally growing species is *Arbutus andrachne* L., belonging to the *Ericaceae* family. It is called the “sandal tree” or “Eastern or Greek strawberry tree”. This wild species is traditionally used for its health enhancing properties (Amro et al., 2013; Abidi et al., 2015). In some countries it also has a value in beverage making (Botelho et al., 2015), reforestation (Moreno et al., 2008) and as an ornamental plant (Bertsouklis and Papfotiou, 2013).

Plants can grow up to 2-3 meters in non-rainy areas.

It usually blooms in March-April in Turkey and fruits can be collected from nature in September-October. Fruits have 10-20 mm dimensions and 5-15 g weight and are attractive and have a red appearance (İslam and Pehlivan, 2016; Çolak, 2019). The number of studies on this fruit, which is not sufficiently recognized and consumed in very small amounts, is limited.

In order to cultivate *Arbutus andrachne* L. species that grow in wild forms, it is necessary to reveal its flower biology. The first step to do that is the determination of flower differentiation and flower organs. Knowing how and when a flower is formed is of great importance for growers and biologists. Flower bud formation and differentiation could be influenced by cultural practices in various species (Shen et al., 1999; Beppu and Kataoka, 2000; Engin and Ünal, 2007; Engin et al., 2010) and Salisbury and Ross (1992)

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stated that the most typical example of the developmental process being controlled by environmental stimuli is the transition of meristems from the vegetative to generative state in buds. In addition, correctly employing cultural practices (pruning, irrigation, etc.) in orchards and obtaining the desired results require the recognition of the principles of productivity in buds. Illumination on floral organ morphology and differentiation in buds over time might enable those interested parts to achieve them.

This research was carried out to establish morphological changes during flower formation and size-related growth of the floral organs in *Arbutus andrachne* L.

Material and Method

Plant Material and Study Site

The study was carried out on *Arbutus andrachne* L. species which grow naturally around Çanakkale, Turkey. Plants were located from the region (40°07'04.23''N and 26°30'53.84''E, elevation: 130 m). The research was carried out by utilizing the laboratory facilities of Department of Horticulture, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Turkey. Sampling time was from the end of January to the mid-April, 2019. Samples of buds at the axils of leaves were taken every 10-15 days at each sampling date and the phenological stages were recorded according to the G-E system in grapevine (Gökbayrak and Engin, 2019). Mean monthly temperatures were 8.4, 9.2, 11.1 and 16.3°C from January to April. During the same duration, mean monthly precipitation were 80.1, 68.2, 61.3, and 42,3 (mm).

Methods

After keeping the samples in the solution (formalin 10%, ethyl alcohol 50% and glacial acetic acid 5%) for 48 or longer hours, they were dissected using an Olympus SZX7 stereo zoom microscope (Olympus Corp., Japan) and their scales and wooly hairs were removed. Flower organs were imaged using a digital microscope camera (LC20, Olympus Corp., Japan) mountable on the microscope. Lengths and widths of flower bud and flower, including its generative parts, taken at the same phenological stage were measured in µm. The measurements were enabled by a software program (LC20-Bundle LC micro, Olympus Corp., Japan).

The works of Engin and Ünal (2007) in sweet cherry and Gökbayrak and Engin (2019) in grapevine were used as guides to assess the stages of primordial development of both flower and floral primordia. Additionally, an attempt to establish a link between the size-related growth in the *Arbutus andrachne* flowers and the floral organ was made.

Results and Discussion

Development of bud, flower and floral organ primordia was depicted in *Arbutus andrachne* L. and presented in Figure 1. Based on the observations, the flower formation in *Arbutus andrachne* L. was completed over 7 distinct stages. Stage I (morphological indication of the transition from vegetative to reproductive state) was noticeable through a rounded meristem in February 2019 (Figure 1A). This stage occurred 80 days before anthesis (DBA) corresponded to the stages 2-5 according to the G-E system in grapevine (Gökbayrak and Engin, 2019). Engin and Ünal (2007) reported that this phase is affected by annual environmental conditions, especially by temperature.

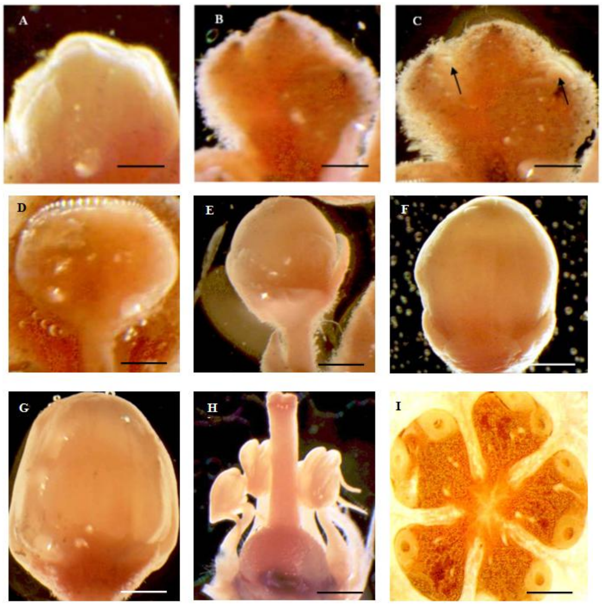

Figure 1. Microscope images of development of bud, flower and flower organs primordia in *Arbutus andrachne* at different stages. A- Stage I: phase from vegetative to reproductive with rounded meristem, B- Stage II: bud primordia rounded and enlarged, C- Stage III: floral primordium (arrows show flower primordia), D- Stage IV: flower primordia with calyx and petal, E- Stage V: the petals completely formed, F- Stage VI: all flower primordia differentiated, sepal and petal obvious and stamens and pistil blanketed under corolla, G- Stage VII: flower continued to enlarge, H- Stage VII: pistil and stamens visible after sepal, petal and some of stamen were removed, I- Stage VII: disc form in the base of the gynoecium. Bars=50 µm in (A) to (D) and 100 µm in (E) to (I).

At stage II (February 20), the apex became thickened and broadened to form an elongated dome (Figure 1B). In this phase, which was occurred 70 DBA, any observable flower primordia was present, in agreement with Watt et al. (2008), Jones et al.(2009)and Gökbayrak and Engin (2019), who expressed that flower primordia did not develop in grapevine before or during winter dormancy.

Fifty-nine DBA flower primordial formation started in the samples on March 2 (stage III) and separate flower primordia were revealed (Figure 1C). At stage IV (March 15), floral organogenesis took place 46 DBA. Calyx was formed with the initiation of 5 sepals which were fused into a cup (synsepalous) and corolla developed with five fused petals (synpetalous) (Figure 1D). Keller (2015) stated that individual sepal and petal development occurs 5 weeks during and after bud break in grapevine. This was supported with the current observations, although the duration was a bit longer. While the flower primordium itself continued to develop, the primordia of sepal and petal also extended to grow (Figure 1E).

On March 25 (stage V), 36 DBA, ten stamens and one pistil primordia started to form. Stamens and pistil were completely encased by the petals. In the samples on April 5 (stage VI), the petals first expanded 25 DBA and took a round shape. Then, narrowing at the tips they had the prolate spheroid shape (Figure 1F). Stamen and pistil primordia could be seen when sepals and petals were removed.

On April 15 (stage VII), 15 DBA, flower primordium continued to enlarge (Figure 1G). At this stage, the stamens and the pistil were complete (Figure 1H) and the style and the stigma were obvious. The pistil was longer than the stamens and two separate horns are formed at the end of each anther (Figure 1H). The ovary had 5 carpels and each carpel contained seed primordia (Figure 1I). Once the flower had all its parts developed, it flexed downward and assumed the shape of a wide bellied bell.

Although Skevchenko (2017) reported that the flowers are arranged in racemose inflorescences, *A. andrachne* L. has an inflorescence that is similar to compound panicle. Flower primordia are connected to the main axis with flower pedicels (Figure 2). Both the main axis and side branches end with a flower primordium. The main axis is dominant with respect to the side branches and is longer than the side branches. As the development continues in the buds, the main axis continues to extend from the apical end.

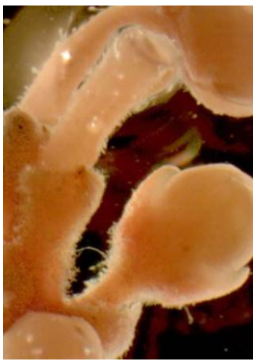

Figure 2. Microscope images of inflorescence primordia in *Arbutus andrachne* L. Bars=100 µm

Measurements taken on primordia (bud, flower and the flower parts) of *Arbutus andrachne* L. were presented given in Table 1. *Arbutus andrachne* L. buds increased their width and their length more than two-fold before initiation of flower primordia between the stages I (February 10) and III (March 2). Flower primordia increased their width and their length more than 15- and 27-fold, respectively, between the stages III (March 2) and VII (April 15). At the beginning of flower primordia formation, flower primordia were wider than it was longer, but at later phases it grew more in the lengthwise. Pedicels increased their length more than 8-fold between the stages IV (March 15) and VII (April 15). First signs of reproductive parts were discernible at the end of March (36 DBA). Anther width was limited compared with the other floral organs. Filaments, on the other hand, increased their length more than 4-fold in a period of 21 days. Gynoecium growth was remarkable and all its components grew in a short time. Total pistil length increased from 53.4 to 194 µm, ovary width from 42.9 to 121 µm, ovary length from 40.7 to 91.3 and stigma diameter from 13.1 to 35 µm in approximately 20 days. Observations on the growth rate of the generative primordia has had received a little attention. Considine and Knox (1979) and Caporali et al. (2003) presented some volume information of the pistil primordia in Gordo blanco (*Vitis vinifera* L. cv.) and *Vitisvinifera* ssp. *silvestris*, respectively. Gökbayrak and Engin (2019) also determined size related growth of floral primordia in Cabernet Sauvignon (*Vitis vinifera* L. cv.).

Table 1. Measurements (µm) of primordia (bud, flower and floral organs) in the *Arbutus andrachne* L. at each date of sampling in 2019 and corresponding stage (mean, ranges in parenthesis).

Floral stage	I	II	III	IV	V	VI	VII	Relative growth (%)
Sampling date	February 10	February 20	March 2	March 15	March 25	April 5	April 15	
Days before anthesis	80	70	59	46	36	25	15	
Bud width	97.18 (125.1 – 82.3)	174.5 (148.8 - 185.3)	218.6 (179.5 - 248.7)					224
length	73.30 (83.79 - 50.31)	146.7 (125.1 - 170.6)	178.3 (147.3 - 221.6)					243
Flower width			26.71 (13.24 - 32.83)	133.7 (114.4 - 148.2)	232.6 (180.1 - 295.9)	300.1 (217.1 – 390.2)	392.8 (370.4 - 450.3)	1507
length			17.51 (10.12 - 21.89)	108.2 (91.36 - 113.8)	278.2 (216.4 - 350.8)	330.2 (297.9 - 374. 7)	489.6 (436.5 - 560.2)	2796
stem				30.71 (15.92 - 40.84)	127.4 (90.03 - 205.8)	173.9 (157.8 – 200.3)	260.8 (214.6 - 304.2)	849
Anther width					31.22 (19.29 - 38.54)	43.29 (37.16 – 48.55)	50.67 (38.34 - 59.43)	162
length					33.65 (23.76 - 38.76)	62.38 (52.87 – 76.64)	95.99 (74.56 - 112.3)	285
Filament length					18.78 (12.87 - 29.41)	38.46 (30.02- 45.76)	80.10 (64.53 - 89.23)	426
Pistil length					53.40 (31.16 - 79.87)	110.3 (90.62 – 138.7)	193.9 (186.2 - 217.1)	363
Ovary width					42.95 (22.98 - 62.76)	97.34 (70.56- 118.7)	121.1 (90.16 - 136.7)	281
length					40.78 (32.67 - 58.39)	68.34 (53.63- 82.19)	91.36 (80.32 - 96.48)	224
Stigma diameter					13.17 (10.11 - 18.36)	19.29 (11.56 – 30.16)	35.02 (26.82 - 45.67)	265

Conclusion

Here in this study, morphological changes during flower formation were determined. A link between the floral stages, timing of morphological changes in the floral primordia and organ development was accomplished for the *Arbutus andrachne* L. The transition from vegetative to reproductive had occurred 80 days before anthesis. Appearance of the flower bud marked the formation of the flower. First individual flower primordia were observable 56 days before anthesis. Ongoing from this point, the evolution of flower primordia and floral organs was quick. The floral cup contained 5 sepals, 5 petals, 10 stamens and a pistil. The branching showed that flowers are connected with the main axis and an axillary branch with a pedicel. In order to cultivate *Arbutus andrachne* L. that grows wild in nature, it is necessary to reveal its flower biology. Appreciation of reproductive growth stages for the *Arbutus andrachne* L. is necessary for performing cultural operations and providing a consistent perception.

Acknowledgement

This work was supported by Çanakkale Onsekiz Mart University the Scientific Research Coordination Unit, Project number: FHD-2018-2523.

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