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Flower Biology and the Effects of Different Chemicals on Pollen Germination of Some Early Sweet Cherry Cultivars

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HIGHLIGHTS

- Flower biology of early sweet cherry cultivars.
- Fruit set and ovule structure.
- Pistil morphology of cherries.
- Pollen and fruits.

Abstract: Sweet cherry fruit is a tasty and valuable product for consumers. In order to increase the export share of cherry, which is also very important in export, it is beneficial to grow with cherry varieties that mature at different times. The cherries offered to the market in the early period will be more attractive. In this study, morphological and biological features of pistils of early-maturing 'Cristalina', 'Early Lory', 'Prime Giant', fruit set rates and pollen germination status and some chemical applications were investigated. As a result, fruit sets of cultivars were 17.6-28.6% in two years. Significant differences were observed in pistil morphology of the cultivars and 'Cristalina' had shorter pistil (14.35-14.51 mm) and style (11.47-11.65 mm) lengths than the other cultivars. Greater deformation was observed in primary ovules of 'Early Lory' right after anthesis. There were not significant differences in pollen germination ratios of the cultivars, but boric acid treatments improved pollen germination ratios of all cultivars. Boric acid application increased pollen germination with 21%. This was followed by IAA (8%), GA_3 (5%), KNO_3 (4%). It was concluded based on present findings that in orchard establishment with the early cultivars, flower biology should momentously be assessed.

Keywords: *Prunus avium* L.; anthesis; pistil; style; ovule; fruit set.

INTRODUCTION

Horticulture plants are adding value of earth's diversity and fundamental to all life. They include high content of non-nutritive, nutritive, and bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic acids, and as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals [1,2]. Worldwide, annually 2.443.407 tons cherry are produced and about 627.132 tons of that production come from Turkey [3]. With such a production quantity, Turkey is the leading cherry producer of the world. However, recent decreases in unit-area yields and exports have fallen Turkey behind Chili (118.316

tons) and China (81.627 tons) in cherry exports. According the recent data, Turkey was able to export 79.789 tons cherry [4]. To improve exports, it is quite significant to work with cherry cultivar ripening at different periods. Early-harvested ones may find buyer at quite high prices since the quantity of production is low. These cherries able to find buyer at high prices should also have desired fruit sizes to be unrivalled in export. Therefore, any factors able to influence fruit size should be taken into consideration in culture of those early cherries. Of these factors, pollination and fertilization are the primary ones [5]. Therefore, several researches have long been conducted on reproductive biology of various fruit species and types [6-12]. For an efficient fertilization and productivity, fertilization characteristics of the cultivars should definitely be analysed in cherry orchards and the most appropriate cultivar pattern should be set up accordingly [13]. In cherry flowers, anther upper surface is higher than stigma surface. Growth of filaments take place before blooming. Pistil development is completed until balloon stage [14]. It was indicated in some previous studies that relative position of anthers and stigmas influenced the behavior of pollinators in apricot, peach and nectarine [15]. Temperature accelerate blooming, but such a case is not directly proportional to pistil development and usually results in reduced fruit set [16,17]. Flower pistil is composed of stigma, style and ovary. Pollination is a process started with the advent of pollen into stigma and water absorption. Then pollen moves to ovary through style [18]. According to Nyéki and coauthors morphological structures and sizes of flower organs are genetically controlled [19]. Morphological characteristics also have functional importance. For instance, large ovary positively influences fruit set in sour cherry, but long style negative influence [20]. According to Nyéki (1980) the relations between the lengths of male and female organs influence fruit set in stone-fruits and fruit set greatly decreased in quite short female organ [21]. In some cases, pollen tube develops slowly and egg losses vigour during this slow phase of growth [22]. For fertilization to be realized, initially pollination should be realized, then germinated pollen over the stigma should generate a style to reach ovary and ovule [23]. For sure, inherent nutrients and hormones are used while pollination and fertilization. IAA plays a significant role in pollen-pistil interaction and has the greatest level at stigma and dissipate into vascular tissue following the germination of pollen [24]. However, these hormones and nutrients are not sufficient all the time and mostly insufficient [22,25]. For pollen to be germinated, there should be sucrose, boron, calcium, potassium, magnesium and gibberellic acid and indole acetic acid-like growth regulators in the growing conditions. Therefore, several researchers worked with some minerals and plant growth regulators under both in vivo and in vitro conditions [6,7,22,25-27].

This study was carried out in order to contribute to the interpretation of productivity levels of early sweet cherry cultivars in practical cultivation and laboratory studies and fruit sets with open pollination were conducted to determine fertilization biology of ‘Early Lory’, ‘Prime Giant’ and ‘Cristalina’ commonly grown in sub-tropic regions of Turkey, western and southern coastline consists with warm winters and quite hot summers, for early production. Early ripening of these varieties and superior quality characteristics compared to local varieties increase their market values, therefore they are preferred in sub-tropic regions. For this purpose, pistil morphology and biological characteristics, germination capacity of pollen and effects of some plant growth regulators and minerals on germination of pollens were investigated.

MATERIAL AND METHODS

Plant material

Experiments were conducted in 2017 and 2018. ‘Early Lory’, ‘Cristalina’ and ‘Prime Giant’ early sweet cherry cultivars grafted on Gisela 6 rootstocks in the trial orchard established in 2012 of Fruit Research Institute (Egirdir, Isparta-Turkey) were used as the plant material of the experiments. ‘Cristalina’ comes to the first harvest maturity among these cultivars with a few days. It has large, dark red fruits with high marketing quality. Early Lory is early, medium-large, forming a homogeneous fruit on the tree, dark red, sweet and quality. Prime Giant has the largest fruits and is an early variety that matures a few days later than other varieties.

Pistil morphology

Pistils were removed from the receptacles and their fresh and dry weights were weighed with a precise scale (±0.001 g) and the difference between fresh and dry weight was considered as moisture content (%). Pistil and style lengths (mm), ovary and stigma diameters (mm) were measured with a digital caliper (±0.01 mm) [16].

Ovules

A day after anthesis, 10 pistil samples were taken from each cultivar in both years. Samples were fixated into FAA (90cc 70% ethyl alcohol + 5cc glacial acetic acid + 5cc formaldehyde) solution. In histological works, ovaries were subjected to preparations, they were cross-sectioned, their development was assessed under microscope and their images were taken. In preparations, microwave radiation-supported paraffin technique was used [28]. Following the preparation, samples were cut with a rotary microtom at 10 micrometer thickness and they were opened at 50 °C water bath. Then cross-sections were put over the glass slides and dried in an oven at 65 °C.

Development of embryo sac and ovule were determined in accordance with the method specified by Cerovic and Micic [29]. The embryo sacs reached to 4 and 8 nuclei stage were accepted as fully developed. Safranine staining technique was used to monitor the tissues. Development status of primary and secondary ovules a day after anthesis was investigated and imaged under microscopy.

Fruit set ratio of the cultivars

Fruit set ratio of open pollinating flowers were determined at harvest period as indicated by Rodrigo and Herrero [16] and Alburquerque and coauthors [30] in 4 replicates with 50 flowers in each replicate. In replicates, flowers were counted at balloon stage and labelled. After fruitlet drops unfertilized, fruit counts were followed until harvest maturity. Since the early sweet cherry varieties were studied, the harvest maturity fruit counts were the fruit set values. Percentage fruit set ratios were calculated with the obtained values.

Effects of chemical treatments on pollen germination

To obtain pollen germination of the cultivars, 100 flowers at balloon stage were collected from each cultivar and different sections of the trees and they were immediately brought to laboratory. Anthers of the flowers were removed over white papers, then anthers of the cultivars were placed into petri dishes and kept under 75 W lamp for a night to burst. Pollens in petri dishes were transferred to small bottles and bottles were shaken to remove pollens from anthers. These bottles were kept in desiccators in a fridge until use in analyses. Pollen germination medium was prepared by using 100 mL distilled water, 1 g agar and 10 g sucrose and this medium was used as control treatment. Chemical treatments were implemented through supplementing control treatment with 10 ppm GA_3, 50 ppm KNO_3, 5 ppm IAA and 25 ppm H_3BO_3 separately. Growth media were poured in petri dishes hot and pollens were seeded into media before the freeze of media. About 12 hours after seeding, counts were performed under Nikon-Eclipse 80i microscope at 10X magnification and mean germination ratio of pollen grains of each flower was determined. Experiments were conducted in randomized block design with 4 replications.

Statistically evaluation

The samples were collected in randomized block design with four replicates of each kind and one tree per repetition from orchard. Resultant data were subjected to variance analysis with SAS-JUMP statistical software. Effects of the cultivars and chemicals were assessed and significant differences were tested with LSD multiple comparison test at $P<0.05$ level.

RESULTS and DISCUSSION

Fruit set ratio of the cultivars

When the fruit set ratios of sweet cherry cultivars for two years were compared to each other, it was observed that ‘Cristalina’ and ‘Prime Giant’ had greater fruit set ratios than the ‘Early Lory’. Statistical assessments on 2017 data revealed significant differences between fruit set ratios of the cultivars ($P<0.05$). Considering an average 20-25% fruit set ratio was acceptable for optimum productivity in sweet cherry cultivars, it was observed that ‘Cristalina’ and ‘Prime Giant’ reached to sufficient productivity levels in both years, but ‘Early Lory’ had slightly low yield level in 2017 (Table 1). Sweet cherry has a great place in agricultural export of Turkey and establishment of orchards with early cultivars is quite a significant issue for sub-tropic regions. In orchard establishment with early cultivars, fertilization biology, incompatibility and flowering dates of the cultivars should definitely be taken into consideration. With regard to fruit set ratios of the present cultivars, ‘Cristalina’ and ‘Prime Giant’ had greater fruit set ratios than ‘Early Lory’. Pistils play the primary role in fertilization of the cultivars. Flower productivity is represented by fruit set ratios [31]. It is not expected that all fruits over a tree will turn into fruit. For instance, a fruit set ratio of 5% is sufficient in

commercial apple culture [32]. Differences in fruit sets are generally attributed to development of ovules and nutritional status of the flowers. Besides, nutrient levels of flowers and leaves are also closely related to productivity [33-37]. Several physiological factors also influence fruit set ratios [38].

Table 1. Fruit set ratios of early sweet cherry cultivars (±SE).

Cultivar	Fruit Set (%)			
	2017	LSD Group	2018	LSD Group
'Cristalina'	28.58±1.13	a*	23.58±1.43	
'Early Lory'	17.58±1.06	b	22.84±1.26	*ns*
'Prime Giant'	20.85±4.08	ab	26.10±3.38	
P value	0.0412		0.4886	

ns non significant; *Means indicated with different letters are significantly different (*P<0.05*).

Pistil morphology

Measurements on ovaries of pistils of the cultivar flowers revealed that there were significant differences in pistil fresh weights in 2017 (P<0.05) and the greatest fresh weight was obtained from 'Prime Giant' (13.69 mg). 'Prime Giant' had greater moisture content than the other two cultivars in both years of the experiments (Table 2). 'Cristalina' had the shortest pistil length in both years. Similar findings were also observed in style lengths and the differences in style lengths were found to be significant (P<0.05).

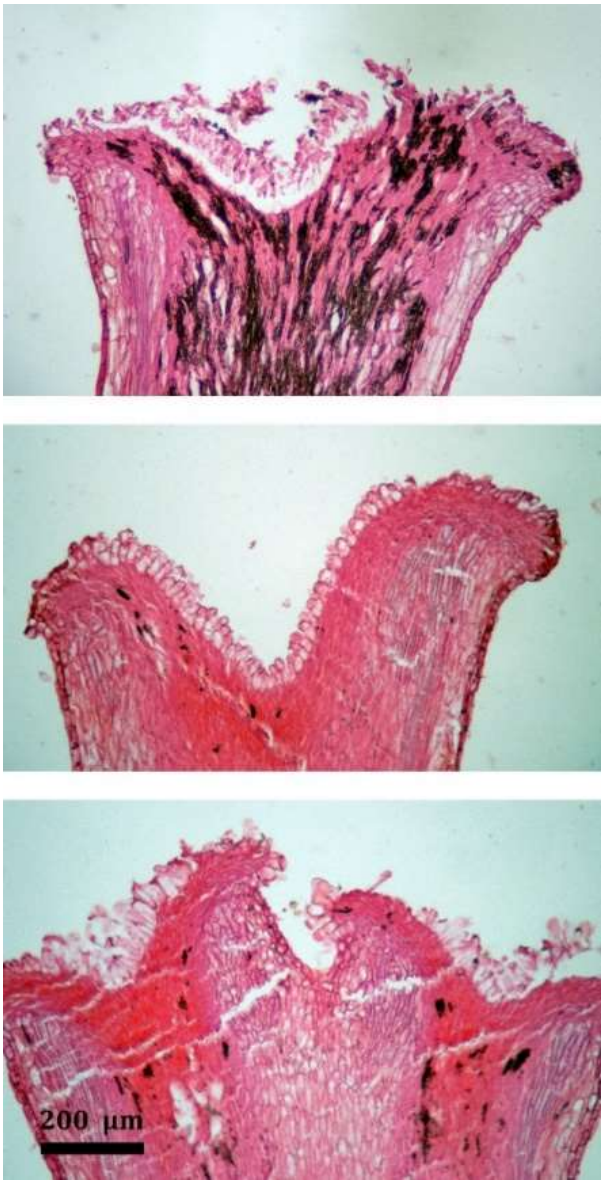

Figure 1. Stigma cross-sections. From top to bottom; 'Cristalina', 'Early Lory', 'Prime Giant'. 10X, Bar=200µm.

The differences in ovary diameters of the cultivars were not found to be significant. 'Prime Giant' had greater stigma diameter than the others (Table 4). Additionally; 'Cristalina' had smoother stigma surface, 'Early Lory' had greater lobe formation over the mid-section of the stigma. 'Prime Giant' had wider stigma and the stigma had sectional appearance (Figure 1). With regard to pistil moisture contents, 'Prime Giant' had greater moisture content than the others in both years of the experiments. Considering the pistil lengths, 'Cristalina' had lower values in both years (Table 3). Similar results were observed in style lengths and the differences in style lengths of the cultivars were found to be significant (Table 3). With regard to stigma diameters, 'Prime Giant' had wider stigmas than the others (Table 4). Stigma is located on top of style, style is located right beneath it and ovary is located at the bottom. According to Nyéki and coauthors morphological structures and sizes of these components are genetically controlled [19]. Morphological characteristics also have functional significance. For instance, larger ovary positively and longer style negatively influence fruit set in sour cherry [20]. Nyéki indicated that the relations between male and female organ lengths influenced fruit set of stone-fruits and fruit set quite decreased in quite short pistils [21].

Table 2. Pistil fresh weights and moisture contents (±SE).

Cultivar	Pistil fresh weight (mg)				Pistil moisture content (%)			
	2017	LSD Group	2018	LSD Group	2017	LSD Group	2018	LSD Group
'Cristalina'	12.23±0.66	b*	12.15±0.45		80.22±0.64	b*	80.23±0.43	b*
'Early Lory'	12.18±0.33	b	12.41±0.43	*ns*	80.45±0.24	b	80.69±0.43	b
'Prime Giant'	13.69±0.52	a	13.46±0.37		81.94±0.52	a	81.73±0.59	a
P value	0.0490		0.1068		0.0015		0.0180	

ns non significant; *Means indicated with different letters are significantly different (*P<0.05*).

Table 3. Pistil and style lengths (±SE).

Cultivar	Pistil Length (mm)				Style Length (mm)			
	2017	LSD Group	2018	LSD Group	2017	LSD Group	2018	LSD Group
'Cristalina'	14.35±0.10	b*	14.51±0.23	b*	11.47±0.21	b*	11.65±0.16	b*
'Early Lory'	17.28±0.55	a	17.09±0.52	a	14.37±0.27	a	13.90±0.55	a
'Prime Giant'	16.77±0.60	a	16.35±0.58	a	14.01±0.36	a	13.70±0.58	a
P value	0.0004		0.0014		<.0001		0.0009	

ns non significant; *Means indicated with different letters are significantly different (*P<0.05*).

Table 4. Ovary and stigma diameters (±SE).

Cultivar	Ovary Diameter (mm)				Stigma Diameter (mm)			
	2017	LSD Group	2018	LSD Group	2017	LSD Group	2018	LSD Group
'Cristalina'	2.10±0.06		2.11±0.09		1.29±0.18	a*	1.23±0.18	
'Early Lory'	2.29±0.12	*ns*	2.24±0.14	*ns*	0.91±0.07	b	1.06±0.13	*ns*
'Prime Giant'	2.23±0.15		2.33±0.08		1.34±0.12	a	1.24±0.15	
P value	0.1873		0.1165		0.0157		0.3322	

ns non significant; *Means indicated with different letters are significantly different (*P<0.05*).

Ovules

Microscopic assessments were conducted on ovary samples of the cultivars taken a day after anthesis and development of ovules were evaluated. A recess or a problem was not observed in development of embryo sacs of both the secondary and the primary ovules of the cultivars a day after anthesis. It was observed that embryo sacs completed their differentiation and achieved 4-8 nuclei stage. In 'Cristalina', normal development of ovules was observed a day after anthesis (Figure 2). In 'Early Lory', high deformation was observed in primary ovules (Figure 3). Deformation in nucellus tissue of the primary ovules results in loss of fertilization ability. Such a case then influences overall fruit set. An abnormal development was not observed in primary ovules of 'Prime Giant' (Figure 4).

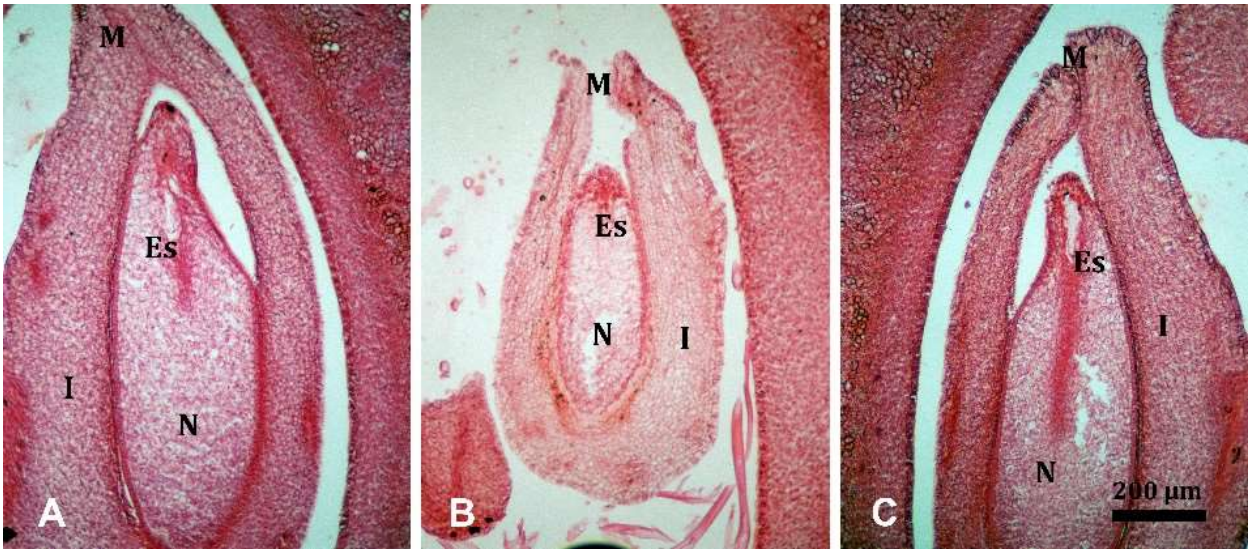

Figure 2. Status of primary (A, C) and secondary (B) ovules of 'Cristalina' a day after anthesis. Es embryo sac, I integuments, N nucellus, M micropyle. 10X, Bar=200µm.

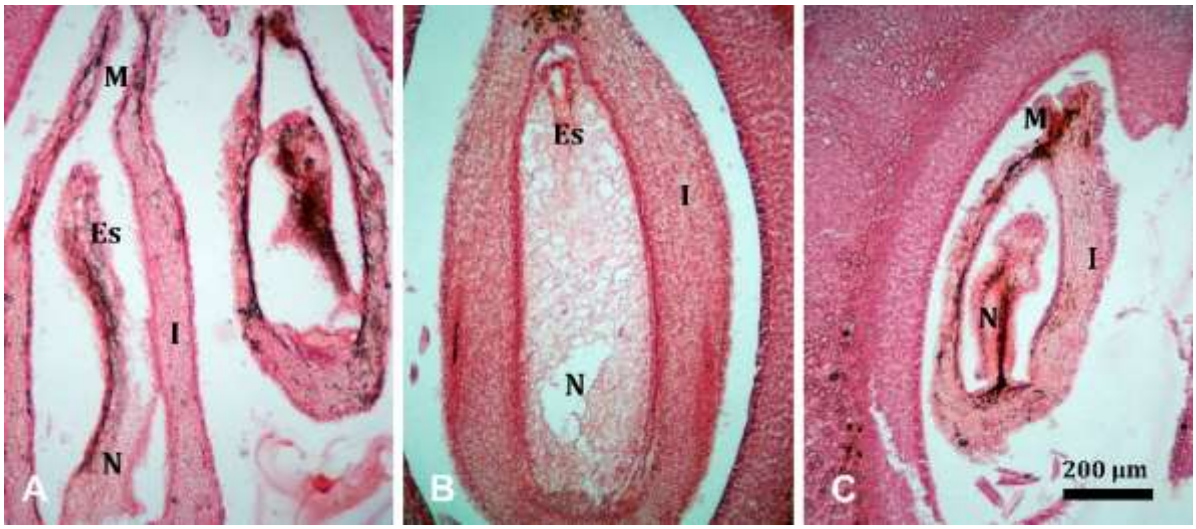

Figure 3. Status of primary (left ovule in A and B) and secondary (right ovule in A and C) ovules of 'Early Lory' a day after anthesis. Es embryo sac, I integuments, N nucellus, M micropyle. 10X, Bar=200µm.

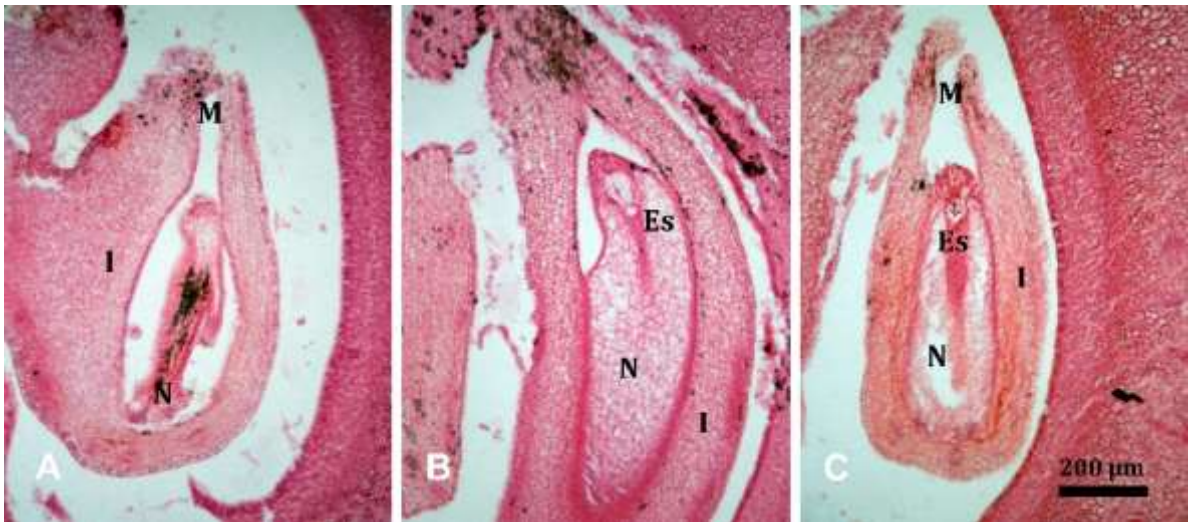

Figure 4. Status of primary (B) and secondary (A, C) ovules of 'Prime Giant' a day after anthesis. Es embryo sac, I integuments, N nucellus, M micropyle. 10X, Bar=200µm.

In present study, normal development was observed in ovary development of ‘Cristalina’ a day after anthesis. High deformation was observed in primary ovules of ‘Early Lory’. Deformation of nucellus tissue of primary ovules resulted in loss of fertilization capability. Such a case may then influence overall fruit set. Despite shorter pistils of ‘Cristalina’, normal ovule development resulted in greater productivity. An abnormal development was not observed in ovules of ‘Prime Giant’. There are two ovules in ovary of stone-fruits. However, only one of these ovules has a capability of fertilization. The one with a fertilization capability or the functional one is called as primary ovule and undeveloped one is called as secondary ovule [39]. According to Williams; in “Strong Flower” stigma receptivity, development of embryo sac and cell division of unfertilized ovules take longer time [40]. Life of apple ovule at 11 °C could be 11-12 days [41]. Pistil functionality of sour cherry may take 2-3 days [42], however, about 2-25% of ovules degenerated until anthesis [43]. In sour cherry, ovules are generally inactivated about 3-6 days after anthesis [18]. Such a duration is 1-5 days [44] or 13 days [45] in sweet cherry based on cultivar and temperature. Differences in cultivars may influence the vigor of ovule. Such a case is especially effective on functionality and vigor of embryo sac right after anthesis [46].

Effects of chemical treatments on pollen germination

Cultivars were subjected to germination tests in 1% agar and 10% sucrose media for 12 hours and germination ratios varied between 13.81-31.81% in the first year and between 13.58-22.45% in the second year. Plant growth regulator and chemicals supplemented to growth media influenced germination ratios differently (Table 5). The greatest germination ratio in all three cultivars and in both years was obtained from H_3BO_3 treatments. According to variance analysis, only the treatments were found to be significant in the first year and both treatments and cultivar x treatment interactions were found to be significant in the second year ($P<0.05$) (Table 5). In the first year, the greatest germination ratio (39.58%) was obtained from H_3BO_3 treatment and the lowest from the control treatment (21.32%). The other treatments were not significantly different from the control treatment. In the second year of the experiments, boric acid treatments yielded greater germination ratios than the other treatments and the control group. With boric acid treatments a germination ratio of 42.74% was achieved in ‘Cristalina’, 35.98% in ‘Early Lory’ and 42.63% in ‘Prime Giant’. As compared to the control groups, about 27% greater germination was observed in ‘Cristalina’, 16% in ‘Early Lory’ and 29% in ‘Prime Giant’ (Table 5).

With regard to germination capability of pollen grains, ‘Early Lory’ had greater germination ratios than the other cultivars. Improvement of germination capacity of pollen is a significant issue for fertilization. In this sense, among the plant growth regulators and chemicals, H_3BO_3 treatments were found to be prominent and the greatest germination ratios were obtained from H_3BO_3 treatments of all cultivars. Thusly, Bolat and Pirlak reported that boric acid treatments had positive effects on germination of pollen in some apricot cultivars [7]. It was also indicated in several studies that boric acid treatments yielded quite well outcomes for germination of pollen [22,25,27,47].

Table 5. Effects of growth regulators and chemicals on germination of pollen at the end of 12 hours of germination (±SE).

Cultivar	Treatment	Pollen germination (%)			
		2017	LSD Group	2018	LSD Group
'Cristalina'	GA_3	32.16±3.95		24.47±1.53	cde*
	H_3BO_3	43.01±5.17		42.74±3.88	a
	IAA	33.68±3.95		32.51±2.75	bc
	KNO_3	27.08±2.92		21.71±2.95	defg
	Control	18.33±10.67		15.42±5.42	fg
'Early Lory'	GA_3	25.60±3.45		17.40±2.23	efg
	H_3BO_3	38.99±5.51		35.98±3.41	ab
	IAA	22.94±3.01	*ns*	28.01±3.09	bcd
	KNO_3	23.00±1.61		19.59±1.33	efg
	Control	31.81±2.55		22.45±1.99	def
'Prime Giant'	GA_3	22.70±3.84		21.35±3.93	defg
	H_3BO_3	36.72±2.57		42.63±0.65	a
	IAA	26.75±2.86		19.11±2.09	efg
	KNO_3	28.55±5.06		23.40±1.97	def
	Control	13.82±2.49		13.58±1.65	g
Cultivar mean					
'Cristalina'		30.85		27.37	
'Early Lory'		28.47	*ns*	24.68	*ns*
'Prime Giant'		25.71		24.01	
	Treatment mean				
	GA_3	26.82	b*	21.07	c*
	H_3BO_3	39.58	a	40.45	a
	IAA	27.79	b	26.54	b
	KNO_3	26.21	b	21.56	c
	Control	21.32	b	17.15	c
P value					
Cultivar		0.215		0.172	
Treatment		0.0003		<.0001	
Cultivar*Treatment		0.180		0.029	

ns non significant; *Means indicated with different letters are significantly different (*P<0.05*).

CONCLUSION

It was observed in this study that 'Cristalina' and 'Prime Giant' had greater fruit set ratios than 'Early Lory'. 'Cristalina' had lower values for pistil and still lengths. 'Cristalina' and 'Prime Giant' had normal ovule development a day after anthesis. High deformation was observed in primary ovules of 'Early Lory'. Besides environmental conditions, biological characteristics such as style length, stigma diameter and ovule development influenced fruit sets of the cultivars. Significant differences were not observed in germination ratios of the cultivars, but boric acid treatments improved pollen germination ratios of all cultivars. In this sense, among the minerals, boric acid treatments could be recommended to improve fruit set ratios in practical fruit culture. Present investigations on flower biology of 'Cristalina', 'Early Lory' and 'Prime Giant' may constitute bases for further researches to be conducted on early sweet cherry cultivars. 'Cristalina' and 'Prime Giant' regarding their floral characteristics were advised as suitable for the subtropical regions of Turkey.

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Conflicts of Interest: "The author declare no conflict of interest."

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