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MICROPHENOPHASES ON SOME JUNEBEARING STRAWBERRY VARIETIES IN REGION OF SKOPJE

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Abstract

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This paper gives an overview of the results from the microfenophases on junebearing strawberry varieties: *Pocahontas, Evita, Marmalade, Belrubi, and Elsant* in Skopje region, Republic of Macedonia. The beginning of the flower bud differentiation among the studied strawberry varieties is, on average, during the first half of August when the day lasts for 13.5-14 hours. The calyx is formed during the second half of September, stamens during the first half of October, and the pistils during the second half of October. Archesporial tissue and meiosis are formed in November. The microspores are formed in December. *Pocahontas* variety has earliest flower bud initiation (FBI), followed by *Evita, Marmalade* and *Belrubi*. *Elsanta* variety has the latest FBI. The higher temperatures and a photoperiod (duration of sunshine) have positive influence on early FBI.

Key words: strawberry, differentiation, flower buds, microphenophases

Introduction

The development of the reproductive organs encompasses organogenesis, which begins with the formation of meristem in the mother plant that lasts until the development of the ripe fruit. The differentiation of the flower buds is performed in several phases: induction, initiation, differentiation and development of flower organs in the buds (microphenofases) and external changes (phenophases). The strawberry forms flower buds of mixed character whose generative elements differentiate at the peak of the growth (Micic and Gjuric, 1989). Each mixed flower bud has 2 meristem: axillary meristem (with a vegetative function) and top meristem (with a transition function from vegetative to generative phase and generative). The axillary meristem undergoes 3 vegetation periods. During the first year, spring-summer period, new daughter cells are formed in the axillary leafs. During the second year of vegetation, in spring – summer period, the daughter cells begin to form new buds. The meristem that was previously axillary becomes the top meristem of the new bud (Vitkovskii, 1984). At a certain moment, due to the influence of genetic and external factors (ecological, morphological, physiological, chemical), the vegetative buds begin to transform themselves into generative buds, thus surviving the wintertime. In the third year of vegetation, during the winter period the microsporogenesis and macrosporogenesis take place. During the spring the flowering, the fertilization and the embryogenesis take place.

The Cormophyta's organogenesis is developed in 12 phases following certain patterns (Kuperman, 1968). Isaeva uses the same division because of the specificity of the process of fruit organogenesis and divides some of the phases into subphases. Micic and Gjuric (1989) accept the scheme proposed by Isaeva and they establish a new scheme for the junebearing strawberry flower bud differentiation.

During the first and second phase, the flower bud is in a vegetative shape. Depending on the conditions the differentiation of the generative organs begins, i.e. the vegetative bud enters the generative phase (III phase). There are vegetative caps in all of the leaf buds, however only few of them will develop a central inflorescence axis with primary flower stem as well as side inflorescence axis, secondary flower stems, etc. The reasons for the transformation of the vegetative cup into a generative one are still unknown.

The knowledge of the organogenesis cycle i.e. the sequence and the dynamics of the microphenophasis and the

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phenofasis in the process of development of specific organs, represents the basis for the differentiation of specific procedures and treatments, as well as new, more intense production technologies. The objective was to influence these processes. The possibility of influencing the course of differentiation is of special interest in order to reach a higher bearing potential.

Materials and Methods

Climatic conditions

The research was conducted within the period of 2003-2005 at the strawberry production orchard that is the property of the Institute of Agriculture, municipality of Kisela Voda, Skopje. The Skopje valley is situated at 41°57'N and 21°28'S, and is surrounded by a massif of mountains. It is characterized with a modificated Continental climate under a Mediterranean climate influences. Moderate cold and humid winters, as well as hot and dry summers are typical for this region. The data about the climatic factors were collected from the Hydrometeorological station, Skopje. The average drought index 19.7 (according De Martonen) shows that the climate is semiarid. The average amount of rainfall is 425.4 mm, and the vegetation is 231.4 mm. The highest precipitation happened in 2003 (498.2 mm) and the lowest in 2004 (300.4). The average semi-annual temperature for the period 2003-2005 is 13.2°C. The lowest temperature was recorded in 2004 (12.9°C). The average photoperiod is from 1.05 to 31.07 and amounts 905.1 hours. The highest duration of the photoperiod for the same period was recorded in 2004 (984.6) and the lowest in 2003 (834.5).

Scheme of sample analysis

The research encompasses 5 junebearing strawberry varieties: *Pocahontas*, *Evita, Marmalade, Belrubi* and *Elsanta*. The analysis was performed on mixed flower buds from the mother plant, on every 10-15 days, beginning from August until January. The research concentrated on top mixed buds i.e. the central axis of inflorescence. The material was fixed in Carnoy 2, and monitored using binocular. The microphenofases were determined in compliance with the Micic and Gjuric (1994) scheme. For the monitoring of the microphenophases the anthers were taken out, colored with acetocarmin, as well as temporary preparations were set up according to the Squash method. The following next microphenophasis were studied: the flower bud initiation, the establishment of inflorescence axis, the shaping of the vegetative organs (calyx, sepal), the shaping of generative organs (anthers, carpels), and the differentiation of the archesporial tissue, the meiosis, and the appearance of young microspores.

Results

Monitoring of the microphenophasis

The results from the microphenophases research are depicted in Table 1a (period of analysis is between August and the end of September) and Table 1b (period of analysis is between October and December). In the armpits of all the leafs that are in a process of foundation one can find vegetative domes. Under the influence of some endogenous factors, flower buds will be formed in some of them. Prior to the FBI, there are changes in the induction and the initiations. The first end second phase of the organogenesis are characteristic for the bud's vegetative stage. Later, the apical meristem is being raised and broadened and acquires semi-spherical form. That is the beginning of the morphological changes to the meristem dome. These changes signify the transition from vegetative to generative stage (III phase of the organogenesis). With the climatic conditions we have, these changes were mostly noticed during the first decade of August. The forming of inflorescence axis (IV stage) is followed by the differentiation of the primary (IV') and secondary (IV'') axis. These changes usually happen during the first half of September. Their formation is acropetal. The beginnings of flowers have cylindrical form. Such form is firstly developed by the upper meristem. The main inflorescence axis takes the central place in the flower buds. Along the main inflorescence there are 6 or more beginnings of lateral buds whose degree of differentiation drops as we descend from the top to the basis of the central axis. In the armpit of the young beginnings besides the vegetative buds the primordia of the secondary peduncle is differentiated (flower stem). The differentiation degree of the primordia of the secondary peduncle is lower compared to the one found in the flower beginnings of third degree. The lateral mixed buds have the same shape like the top mixed buds; however, they are with a lower differentiation degree. In the lateral mixed buds, there are 1-2 leaves beginnings with vegetative conus and beginnings of a central inflorescence axis and a primary peduncle. The primary flower is developed from the terminal growth point, and the secondary flowers are developed from the lateral buds.

In the V phase, the beginnings of flower organs are formed and differentiated. On the top of inflorescence axis the initial in-depth is formed (Va). Intensive growth and formation of the sepal primordia (Vb) follows in the second half of September. Later on, ate the end of September and the first half of October the calyx primordia (Vc), anthers primordia (Vd) are formed.

The receptaculum develops with carpels primordia (Ve). These changes in the differentiation of the floral organs are noticed in the second half of October and until the end of November. The order of the formation of the floral organs is genetically controlled. In agro-climatic conditions that exist in Skopje, the archesporial tissue (VIa) is formed during the first half of November, and the meiosis in November (VIb). The young microspores and processes of microgametogenesis are formed in December (VIc). Parallel with the microsporogenesis the carpels are being formed. Before the deep dormancy begins the receptacle has already grown, and anthers and carpels are differentiated. The anther are ripening, developing an amber colour. The pillar and the stigma are developing in the carpels. All flower parts grow in both length and width. The peduncle continues to grow. The sepal's top parts are closing. The primordias develop high differentiation degree, which is necessary for the normal transfer to dormancy (Vf). During shorter days hormones are produced that are necessary for the plant to enter the deep dormancy. The deep dormancy begins at the end of December and beginning of January. During winter, practically all organs stop growing. Until the beginning of the vegetation process, the microgametogenesis and the macrogametogenesis take place. The pollen grain ripens and the process of development of egg cells has finished.

The quantity and the quality of these processes are different and depend on the genetic, ecological, agro technical, pomotechnical, as well as other factors. In Skopje region the FBI in junebearing strawberry varieties begins during the first half of August, when there are conditions of long day duration of 13.5-14 hours. Higher temperatures and a photoperiod from 1.05 to 31.07 have positive influence on the early differentiation.

Occurrence of the microsporogenesis

Pocahontas variety develops the FBI earliest, the *Evita, Marmalade* and *Belrubi,* follow. The *Elsanta* develops the FBI the latest. The ordering of the differentiation is determined by the degree of ripeness of the pollen grain in De-

Table 1a
Microphenophases of the strawberry variety mixed bud (period August and the end of September)

Variety	Year	Date of sample analyses (August - end of September)				
		1. Aug.	15. Aug.	31. Aug.	15. Sep.	30. Sep.
Pocahontas	2003	/	IIIa	IIIb	IV,-IV,,	Va,Vb
	2004	IIIa	IIIb	IV,-IV,,	Va,Vb	Vc,
Evita	2003	/	IIIa	IIIb	IV,-IV,,	Va,Vb
	2004	IIIa	IIIb	IV,-IV,,	Va,Vb	Vc,
Marmolade	2003	/	/	IIIa , IIIb	IV,-IV,,	Va,Vb
	2004	/	IIIa, IIIb	IV,-IV,,	Va,Vb	Vc,
Belrubi	2003	/	/	IIIa , IIIb	IV,-IV,,	Va,Vb
	2004	/	IIIa, IIIb	IV,-IV,,	Va,Vb	Vc,
Elsanta	2003	/	/	IIIa	IIIb IV,	IV, Va,
	2004	/	IIIa	IIIb, IV,	IV, Va,	Vb

Table 1b
Microphenophases of the strawberry variety mixed bud (period October - December)

Variety	Year	Date of sample analyses (October - December)					
		15. Oct.	30. Oct.	15. Nov.	30. Nov.	9. Dec.	22. Dec.
Pocahontas	2003	Vc,	Vd	Ve, Via	Vf, VIb	VIc, Vf	VId, Vf
	2004	Vd	Ve,VIa	Vf, VIb	Vic, Vf	VId, Vf	Vf
Evita	2003	Vc,	Vd	Ve,Via	Vf, VIb	Vic, Vf	VId, Vf
	2004	Vd	Ve,VIa	Vf, VIb	VIc, Vf	VId, Vf	Vf
Marmolade	2003	Vc,	Vd,	Ve,Via	Vf, VIb	VIc, Vf	VId,Vf
	2004	Vd,	Ve,VIa	Vf, VIb	VIc, Vf	VId, Vf	Vf
Belrubi	2003	Vc,	Vd,	Ve,Via	Vf, VIb	VIc, Vf	VId,Vf
	2004	Vd,	Ve, VIa	Vf, VIb	VIc, Vf	VId, Vf	Vf
Elsanta	2003	Vb	Vc	Vd Ve,	Via Vf,	VIb VIc	Vf VId
	2004	Vc	Vd Ve,	Via Vf,	VIb Vic	Vf VId	

Table 2
Microsporogenesis among the strawberry varieties

Variety	Phases of microsporogenesis			
	Archesporial tissue, %	Diads, %	Tetrads, %	Microspores with one nucleous, %
pocahontas	/	/	/	100
evita	/	5	10	85
marmolade	/	7.6	15.3	76.9
belrubi	/	36.3	9.1	54.5
elsanta	5	30	5	60
Average	1	15.7	7.8	75.3

cember (Table 2). *Pocahontas* has earliest organogenesis i.e. microsporogenesis. In the anthers, the microspores with one nucleus were formed (100%). In 15-22.1% of *Evita* and *Marmalade* cells, the meiosis took place, and in 76.9-85.0% the microspores with one nucleus were formed. In *Belrubi* variety 45.4% of cells, the meiosis took place. Within the same period, 35% from the *Elsanta's* cells were in meiosis, and 60% of them have already developed microspores.

Discussion

The timeframe and the dynamic of the process of flower bud formation are specific for each variety (Kuperman, 1968). In a moderate Continental and Mediterranean climate, the strawberry varieties initiate their flower buds in September (Paydas and Kaska, 1997; Stanchevic, 1971). In the northern and cold Russian regions, the strawberry varieties initiate their flower buds at the end of September and beginning of October (Vitkovskii, 1984). Izhar (1997) has determined one more group of strawberry varieties known as ISD (infra short days) whose flower bud initiation, flowering, ripening take place earlier compared to those of the junebearing varieties.

The beginning of the flower bud initiation differs from year to year. This means that the climatic changes influence the early or late differentiation. The early differentiation is in positive correlation with the higher temperatures and with a photoperiod that lasts from 1.05 until 31.07, and in negative correlation with the rainfalls. In 2004, higher temperatures were recorded for the period from 1.05 until 31.07, as well as a longer photoperiod for 81.9 hours (3.4 days), compared to 2003. In 2004 for the same period, the rainfall was 48.8 mm, which is less than what was recorded for 2003 (134.1 mm). As a result, the varieties have differentiated their flower buds much earlier.

Darrow (1966) determined the scope of the influence temperatures and photoperiod has on the strawberry's flower bud initiation. The author explains that during autumn, the duration of the day shortens to 11-13 hours, and the axillary buds transform into flower buds. According to Grbic and Vujanic (1974), the first changes in the vegetative meristem happen during very high mid-day temperatures, above 20^{o}C with a minimal water residue of 30 mm. The temperatures during induction and FBI (Doving and Mage, 2001) are of greater importance than the temperatures during the flowering and ripening. Better differentiation is reached during longer days with optimal temperature for FBI between 15-20^{0}C, however for the majority of the varieties the optimal temperature is 18-25^{o}C. The manner of breeding of the strawberries influences the initiation of the flower bud differentiation. Regarding Eshghi and Tafazoli (2007) higher temperatures and long day similar to those in Skopje region, have positive influence on the early differentiation and the FBI in junebearing strawberry varieties begins during the first half of August.

Conclusions

In region of Skopje, in condition of modified continental climate with Mediterranean climate influences the quality and the flow of the process of flower bud differentiation (FBI) depends on several factors, such as genetic, ecological, agro technical, pomotechnical, as well as other ones. FBI in junebearing strawberry varieties begins during the first half of August, when there are conditions of long day duration of 13.5-14 hours. *Pocahontas* variety has the earliest FBI, followed by *Evita, Marmalade* and *Belrubi. Elsanta* variety has the latest FBI. Higher temperatures and a photoperiod from 1.05 to 31.07 have positive influence on the early differentiation.

References

Darrow, G. M., 1966. The strawberry. History, Breeding and Physiology. *Holt, Rinehart and Winston*, New York, N.Y., 447 pp.

Doving, A. and F. Mage, 2001. Prediction of strawberry fruit yield. *Acta Agriculture. Scand. Sect. B, soil and plant science,* **51:** 35-42.

Eshghi S. and E. Tafazoli, 2007. Possible Role of Cytokinins in Flower Induction in Strawberry. *American Journal of Plant Physiology,* **2:** *167-174*.

Grbic, O. and D. Vujanic, 1974. Time and course of the development of flower initiatives in some cultivars of sour cherries dependent upon temperatures and precipitation. *Yournal of Jugoslav Pomology*, **27:** 9-20.

Izhar, S., 1997. Infra short day strawberry types. In: H.A.TH van der Scheer, F. Lieten, J. Dijkstra (Editors), *III International strawberry symposium*, Veldhoven, Netherlands, 01 September, 1997, Acta Hort. (ISHS) **439:** 155-160.

Jackson, D. I. and G. D. Sweet, 1972. Flower initiation in temperate woody plants. *Hort. Sciences,* **42:** 9-24.

Kuperman, F. M., 1968. Morphophysiologal properties of trees. *Ed. MGU,* Moscow, 23-139 pp. (Rus).

Micic, N. and G. Gjuric, 1989. Winter buds of the strawberry and their growth and development. *Contemporary Agriculture*, **37** (11-12): 581-590.

Micic, N. and G. Gjuric, 1994. Algorithm basis of organogenesis in fruits. *Yournal of Jugoslav Pomology,* **28** (107-108): 67-81.

Paydas, S. and N. Kaska, 1997. Differences in fruit bud formation on strawberries grown at sea level and high altitude. *Acta-horticulture,* **345:** 81-92.

Selamovska, A., 1994. Morphogenesis of flower buds on some almond cultivars. *Yearbook of the Faculty of Agriculture,* **44:** 127-135.

Selamovska, A., 2007. Differentiation and development of strawberry mixed buds in region of Skopje. In: *Andrejevic Endowment* (Ed.), Belgrade, pp. 1-90 (Ser).

Stancevic, A., 1971. Genesis, history and biological properties of strawberry. *Yournal of Jugoslav pomology,* **15:** 33-36.

Vitkovskii, V. L., 1984. Morphogenesis of fruit trees. *Kolos*, Leningrad, pp. 7-130 (Ru).

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