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*Original scientific paper*

# INTERACTION OF ENVIRONMENT CONDITIONS AND GENOTYPES ON EXPRESSION OF GENETIC BACKGROUND IN MICRO-PHENOPHASES OF STRAWBERRY MIXED FLOWER BUD

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The aim of this research is differentiation or micro-phenophases of reproductive organs on two junebearing strawberry (*Fragaria* x *anannassa*) varieties senga sengana and pocahontas, depending on climate conditions, rosettes ordering and cultivate manner (orchard mulched on black foil and orchard on bare soil). The beginning of differentiation of flower buds is genetic characteristic depending on climate conditions (insulations, day length, higher midday and night air temperatures from 1.05 till the beginning of differentiation, the sum of rainfalls from the beginning of May until the end of July), order of rosettes and cultivate manner The sum of effective temperatures over 10$^{o}$C from 1$^{st}$ of May till the beginning of differentiation has no influence on beginning of flower buds differentiation. First morphological changes of the apical meristem were started in the first decade of August that has coincided with the day length of 14 hours and day insulations of 9.3 hours. Micro-phenophases were undergoing almost at the same time in both varieties, only the beginning at pocahontas was 2-3 days earlier. Primary rosettes differ 10-15 days earlier than the secondary rosettes. Plants that grown on black foil had 7-10 days earlier flower bud differentiation compared to those grown on bare soil.

*Key words:* strawberry, mixed flower buds, differentiation, micro-phenophases.

## INTRODUCTION

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Organ-genesis in *Cormophyta* is going on into 12 stages. Dynamic of flower bud formation is specific for each variety. Quality and quantity of these processes is different and dependent of the ecological, agro-technical and other factors. According to SELAMOVSKA (2007) and RICHMOND (2002) the first process went in the plant is differentiation of vegetative organs, after what depending on conditions the differentiation of reproductive organs took place. Not all of the vegetative topic tissue formed flower buds, at the same time. During the growing of the plant, the relation between vegetative and generative buds changed, much outnumbered of the vegetative topics entering and reached the final cycles of organ genesis (STAVELEY, 2003).

Differentiation of flower buds passes through 4 phases: induction, initiation, differentiation and organ development of flower buds (inner-hidden changes which take place inside the bud (III-VII stage) known as micro-phenophase of differentiation; and development of flower organs outside the bud (VIII-XII stage), obviously changes known as phenophases of differentiation.

From the strawberry winter buds in spring firstly are developed the mixed buds. Order of rising and dynamic of growth and development of mixed buds is in correlation with their differentiation. The apical mixed bud is developed as first one, after what the most are developed side mixed buds from the basis to the top of the side growth. Dynamic and time of the flower bud formation is specific for each plant species.

A great number of factors influenced at the beginning of differentiation of flower buds, such as genetic control (SAKAI *et al.,* 2000; LIU, 2001), genotype (RAKITIN, 2001), the condition and plant age (NISHIYAMA and KANAHAMA, 2002; SELAMOVSKA, 2007), temperature regime (SELAMOVSKA *et al.,* 2007), length of the day (PAROUSSI *et al.,* 2002), photoperiod (NISHIYAMA and KANAHAMA, 2000), altitude (PRAPATSORN *et al*., 2005), latitude (RAKITIN, 2001), way of cultivation (PLEKHANOVA and PETROVA, 2002; SELAMOVSKA, 2007; SELAMOVSKA *et al.,* 2008a; SELAMOVSKA *et al*., 2009), time of planting (SELAMOVSKA *et al*., 2008b) type of planting material (LUTCHOOMUN, 1999), ordering of rosettes (SELAMOVSKA and RISTEVSKI, 2008), phyto hormone (OZGUVEN and YILMAZ, 2002; PAROUSSI *et al*., 2002).

Differentiation of the generative organs is recognizable in the form of elongation and expansion of apical meristem (I-II stage). Third stage could be divides into sub-stage IIIa (hidden stage of forming inflorescence) and sub-stage IIIb (elongation of cone) In the following stage differs the primary (IV') and secondary axis (IV'') of the flower. In the V stage there is evident the beginning of flower organ formation and differentiation, undergoing into six sub-stages (Va, Vb, Vc, Vd, Ve and Vf). Sporogenous tissue is formed in the VI stage and passed through several sub-stages (VIa, VIb, Vic, and VId). Micro-sporogenesis and macro-gametogenesis are undergoing starting with VIIa to VIIc and continued with stage VIIIa-VIIId. Knowing the cycles of organ genesis presented a great interest and challenge as the possibilities of acting during the differentiation in order to get as much as possible higher yield potential that was the initial step and main goal of this study.

## MATERIALS AND METHODS

**Plant material. -** Dynamic of process of differentiation of reproductive organs is recorded within 15-day intervals during the period from June to March, in two consequent years (2003/2004, 2004/2005). Object of the research were mother plants and primary and secondary rosettes of two june-bearing strawberry (*Fragaria* x *anannassa*) variety senga sengana and

pocahontas. Two types of orchards were investigated; the first one mulched on black foil and the second on bare soil, experimental station Agriculture Institute - Skopje.

**Trial scheme. -** The soil had been examined before planting. Because the soil was low quantity in humus and with high soil pH-reaction, some corrections had to be made. Rosettes had been planted on well managed soil, distance of 0.8 x 0.25 m. The apical mixed bud was used for visual orientation during the organ genesis. Rosette organ genesis was monitored and recorded in series and material used for analyses was taken in intervals of 10-15 days and was fixed in Carrnoy 2. For determination the organ genesis was used SELAMOVSKA (2007a) scheme. Photo documented development changes are shown in results.

## RESULTS AND DISCUSSION

Results of the researches showed several factors influenced on differentiation of flower buds, such as climate conditions before beginning and during the differentiation, order of rosettes and cultivate manner. First morphological changes on apical meristem (Figures 1) were started in the first decade of August (Table 1 a, 1b), when the day length is 14 hours and night length is 10 hours. Inflorescence axis usually is formed in September (Figures 2, 3). Differentiation of flower organ primordial appears in the first half of September. This stage takes place earlier in plants grown on black foil the both mother plants and primary rosettes. By the end of September and the first half of October was formed the sepal (Vb). Here should be noted that sepal is formed earlier in plants grown under black foil the both mother plants and primary rosettes. Petals and anthers (Figure 4) were formed in the second half of October (Vc, Vd). By the end of October and at the beginning of November was recorded the formation of carpel primordial (Ve) so called microsporophyll (Figure 5). First appearance of receptaculum is raised at the third decade of October (Vf).

Figure 1.

Figure 2.

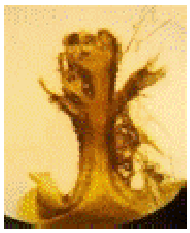

Figure 3.

Figure 4.

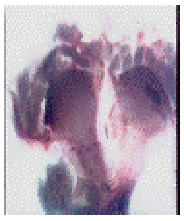

Figure 5.

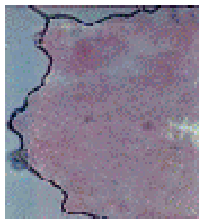

Figure 6.

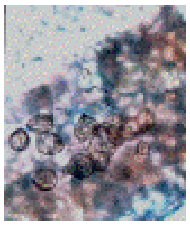

Figure 7.

Micro-sporophytes are formed in the first half of November (VIa), at the same time when the carpels of receptaculum are formed, and micro-sporogenesis took place. Archesporial tissue is formed in November (VIa) (Figure 6), afterwards in December was going meiosis (VIb). Until the winter standby was followed growth and development of formed flower primordial, until reaching a certain state of differentiation, as necessary for normal entering into standby. All flower organs were completed even at the end of December and first half of January was constituted the microspores (VIc). Until the beginning of flowering that was happened in April, micro-gametogenesis (VId) is completed (Figure 7).

*Table 1a. Micro-phenophases of strawberry organ genesis variety Senga sengana*

| Variety | Type of orchard | Year | Material | August | | | September | | January | | November | | December | | January | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 01 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 |
| senga sengana | on black foil | 2003 | MP | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb, Vf | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb, Vf | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | 2004 | MP | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc |
| | on bare soil | 2003 | MP | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc |
| | | 2004 | MP | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc |

*Table 1b. Micro-phenophases of strawberry organ genesis variety Pocahontas*

| Variety | Type of orchard | Year | Material | August | | | September | | January | | November | | December | | January | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 01 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 |
| Pocahontas | on black foil | 2003 | MP | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb, Vf | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb, Vf | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | 2004 | MP | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc |
| | on bare soil | 2003 | MP | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, Via | VIb | VIb,Vf | VIb,Vf | VIc |
| | | 2004 | MP | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | I r | | IIIa | IIIb | IV-IV" | Va | Vb, Vc | Vd, | Ve, VIa | VIb | VIb,Vf | VIb,Vf | VIc | VId |
| | | | II r | | | IIIa | IIIb | IV-IV" | Va | Vb | Vc, Vd | Ve, Via | VIb | VIb,Vf | VIb,Vf | VIc |

MP-Mother Plant, I r-Primary rosettes, II r-Secondary rosettes

In Skopje conditions, at both varieties senga sengana and pocahontas micro phenophases were going almost at the same time, with only 2-3 days earlier appearance recorded in pocahontas. Differentiation of flower organs has began almost at the same time both in mother plants and primary rosettes while in secondary rosettes it has began later after 10-15 days. Primary rosettes that were formed earlier in May and beginning of June, they finished their vegetative growth earlier, and they differentiated their flower buds earlier than secondary rosettes (Table 1a, 1b). Results of conducted researches corresponded with the references (SELAMOVSKA and NIKOLIC, 2007), according to which the order of rosettes influenced earlier differentiation of flower buds; thereby earlier formed rosettes formed flower buds earlier and plentiful. Secondary rosettes were differentiated into flower buds and flowers 14 days earlier than the tertiary ones. Differentiation of flower buds at both cultivars was evident as earlier in 2003 than in 2004. This indicated the influence of climate conditions over differentiation of flower buds; in 2003 were recorded higher quantity of insulations during the period from 1$^{st}$ of May to 31$^{st}$ of July or in total 896 hours, differs of 2004 with 814.1 hours in total). The higher an average daily (9.7 hours) and monthly insulations (299 hours) in 2003 compared to 2004 (8.9 hours daily and 271 hours monthly insulations) associated with lower quantity of rainfalls in the mentioned period (48.8 mm in 2003; 134.5mm in 2004). This fact unambiguously and clearly indicates on positive influence of insulations junebearing strawberry varieties. The drought influenced organ genesis, so in conditions of extreme aridity small numbers of flower buds were evident. Temporary missing of water-supply during the day caused inactivation of plant biochemical processes and differentiation is completely stopped (WANG and CMAP, 2000; SELAMOVSKA, 2007a). In warmer and more arid conditions, the process of organ genesis as well as the micro sporogenesis took place earlier (SELAMOVSKA, 2007). Quantity of effective temperatures over 10$^{o}$C, from 1.05 until beginning of differentiation had no influence on beginning of differentiation at mix flower buds in strawberry varieties; this data in 2003 was amounted 1075.8$^{o}$C, while in 2004 much more 1107.5$^{o}$C.

Regarding the obtained results in Skopje conditions start of differentiation is strongly dependent of the genotype, climate conditions, and day length and cultivation manner. Moderate continental and Mediterranean climate would allow to the strawberry varieties to initiate their flower buds in September. In the northern and cold Russian regions initiation of the flower buds is established at the end of September and mostly at the beginning of October (RAKITIN, 2001). During autumn the duration of the day shortens to 11-13 hours, and axillary buds are transformed into flower buds. At the infra short strawberry varieties (ISD) flower buds were started with initiation in conditions of 13.5-14 hour day duration and wide temperature diapason (10-26$^{o}$C) during the night (SELAMOVSKA, 2007b). Positive correlation has been recorded between the start of the flower buds differentiation and the start of microsporogenesis. Variety pocahontas had an earlier differentiation of flower buds and an earlier microsporogenesis than senga sengana (SELAMOVSKA *et al*., 2009).

Beginning of organ genesis and following stages took place 5-7 days earlier in plants grown on black foil (SELAMOVSKA, 2007), compared with those grown on bare soil (Table 1a, 1b). The reason for earlier differentiation of flower axis in plants grown on black foil was the higher temperature reached under the foil. According to MORENO *et al.* (2009) observed a 6°C increase in average soil temperatures using black polyethylene. Black foil as mulch has the possibility to keep the soil moisture and more hydrated media absorbs more sunlight. Therefore, black foil used as mulch had a positive influence on temperature and hydrating regime of the soil

and indirectly influenced on more intensive development of the plant as well as earlier vegetation.

## CONCLUSION

Beginning of differentiation of mix flower buds in strawberry junebearing varieties is genetic characteristic, dependent on climate conditions (insulations, day length, higher an average air temperature during the day and night that is moving from beginning of May to the end of July), order of rosettes and cultivate manner. Quantity of effective temperatures over 10$^{o}$C from 1$^{st}$ of May until beginning of differentiation had no influence on beginning of differentiation of flower buds. First morphological change of apical meristem in both varieties was started in the first decade of August. Differentiation of flower organs has began in the first half of September afterwards archesporial tissue formation has began in November. By the end of December and first half of January microspores were yet formed. Micro-phenophases in both of varieties were going almost at the same time, 2-3 days earlier at pocahontas. Primary rosettes were differentiated 10-15 days earlier than secondary. Plants grown on black foil were differentiated 7-10 days earlier than plants grown on bare soil.

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# INTERAKCIJA IZMEĐU SPOLJNE SREDINE I GENOTIPA NA GENETIČKU EKSPRESIJU MIKROFENOFAZE MEŠOVITIH CVATNIH PUPOLJAKA KOD JAGODE

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Izvod

Cilj ovog istraživanja je bio diferencijacija i analiza mikrofenofaza niz kojih prolazi razvoj reproduktivnih organa kod dvije jednorodne sorte jagoda (*Fragaria* x *anannassa*) senga sengana i pokahontas, u zavisnosti od klimatskih uslova, pojava rozeta i način obrade zemljišta (mulčirani voćnjak sa crnom folijom i voćnjak na golo zemljište). Početak diferencijacije cvatnih pupoljaka koji je genetski predodređen ovisan je i o klimatskim uslovima (insolaciju, dužinu dana, povećane srednjednevne temperature i temperature vazduha preko noći počevši od 01. Maja do početka diferencijacije, ukupna suma padavina od 01. Maja do 31. Jula), redosled rozeta i način obrade zemljišta. Temperaturna suma preko $10^oC$ od 01. Maja pa do početka diferencijacije nema uticaj na diferencijaciju. U Skopskim uslovima pojava diferencijacije je zapažena u prvu dekadu Augusta, sa dužinom dana od 14 časova i insolacijom od 9.3 časova. Kod ispitivanih sorata mikrofenofaze prolaze u istom ili sličnom vremenskom intervalu, sa ranim početkom od 2-3 dana kod sorte pokahontas. Diferencijacija primarne rozete odvija se 10-15 dana ranije kod obe sorte u odnosu na sekundarne rozete. Kod biljke koje se gaje na mulčiranom zemljištu diferencijacija cvatnih pupoljaka počinje 7-10 dana ranije u odnosu na biljke koje se gaje na ugar.

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