



Nucellus deformation in sweet cherry primary ovules at anthesis

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Abstract

Low yield is the main problem in many sweet cherry growing areas. Yield occurs with effective fruit set under favourable conditions. Development of floral organs at anthesis must be completed to realize sweet cherry fruit set. Especially, developmental stage of sweet cherry ovules at anthesis is related with fruit set. In this study, nucellus of primary ovules at anthesis was studied for two consecutive years (2009-2010) in '0900 Ziraat' sweet cherry variety and its clones (4503, 4218, 3501, 3503 and 3201) grafted on Gisela 5 and Mazzard seedlings rootstocks. Nucellus not completed in the interior of integuments was observed at anthesis stage. It was found that there was a statistically significant difference between the nucellus deformations of '0900 Ziraat' and that of its clones. In addition, rootstocks were found to be partially effective on nucellus deformations.

Keywords: Sweet cherry, primary ovule, nucellus, deformation

Introduction

Flower bud formation must occur in order to mention about the productivity of fruit trees. At the end of flower formation, ovule and embryo sac must be functional at anthesis for fruit set. Ovule maturity at anthesis has proven to be an important factor in fertilization process and fruit set (Albuquerque et al., 2002).

Many factors affect the development of ovule, such as temperature (Beppu and Kataoka, 2011; Egea and Burgos, 1995), nutrition (Detzel et al., 2000; Flore and Layne, 1999; Pipitone et al., 1994) and plant growth regulators (Albuquerque et al., 2006; Beppu et al., 2005; Sanzol and Herrero, 2001) in fruit trees. In addition to these factors, ovule development is different between species and also among cultivars in same species (Ruiz et al, 2010; Mert and Soylu, 2007; Ruiz and Egea, 2007). Ovule maturity is associated with the number of nuclei in embryo sac at anthesis. Embryo sacs must contain at least 4 nuclei in apricot (Albuquerque et al., 2002) and sour cherry (Furukawa and Bukovac, 1989) and 2-4 nuclei in plum (CaiZhen et al., 2010) at anthesis. Developmental stage of ovule is explained also with nucellus status. Mic'ic' and Đuric' (1998) reported an atrophy of ovules in plum flowers starting from chalazal region of the nucellus from

the beginning of balloon stage. According to Zeller (1960), ovules in the apple flowers that opened in axillary position were smaller and the integuments did not cover the nucellus. These flowers are characterized by low fertilization capability (Bubán, 1996). Degeneration of ovule is another problem for fertilization process in addition to nucellus abnormalities. Early degeneration of the ovule is affected by environmental factors while some of ovule abnormalities have a genetic base (Sanzol and Herrero, 2001). Degeneration of ovule tissues is associated with the accumulation of callose in the ovule. The translocation of metabolites to nucellus is interrupted by callose accumulation at the chalazal region of nucellus. This status occurs before morphological ovule degeneration (Rodrigo and Herrero, 1998; Pimienta and politico, 1982). Senescent of ovule in fruit trees plays a significative role in EPP (effective pollination period) and it is an important phenomenon for fruit set (Sanzol and Herrero, 2001). The ovule longevity is generally controlled by environmental conditions. This time was 1-5 days depending on the cultivar and temperatures in sweet cherry (Postweiler et al., 1985). Ovule longevities of '0900 Ziraat' and 'Sweet Heart' were approximately 7 and 8 days respectively from anthesis (Emre, 2011)

and 4-5 days in 'Mora di Cazzano' sweet cherry variety (Tonutti et al., 1991).

Low yield is a major problem for '0900 Ziraat', which is the most important sweet cherry variety for both Turkey and the world. Developments of '0900 Ziraat' flower primordia in buds were progressed well from differentiation stage to bud burst (Sarisu and Kankaya, 2012). Mert and Soylu (2007) reported that, although pollination and pollen tube growth were normal, '0900 Ziraat' had a lot of abnormal ovules in the flowering period. Deformation and degeneration in ovules at anthesis have a decisive influence on fertilization and after process. Ovule development is associated with the productivity of sweet cherry (Mert and Soylu, 2007; ShiPing et al., 2004). In this study, nucellus deformations at anthesis were examined in '0900 Ziraat' and its five different clones grafted on Gisela 5 and Mazzard seedlings for two consecutive years.

Materials and Methods

Plant material

This study was carried out at the Fruit Growing Research Station (37° 49' 21" N; 30° 52' 12" E and 920 m altitude; Egirdir, Isparta, Turkey). '0900 Ziraat' and its clones (4503, 4218, 3501, 3503 and 3201) which were selected with the project of "Characterization of sweet cherry cultivars and types by pomological, molecular and genetic methods" (Demirtaş et al., 2006) were examined in 2009 and 2010. These clones were grafted on Gisela 5 and Mazzard seedling rootstocks and planted in the same experimental orchard in 2000. Although '0900 Ziraat' and its clones have phenotypically similar characteristics (4218 is divided with early flowering than the others), there are genotypic differences among scion materials (Demirtaş et al., 2006).

Microscope preparations

Pistils of just opened flowers were sampled from three different trees for all combinations and immediately fixed in FAA (formaldehyde: glacial acetic acid: 70% ethanol; 5:5:90 v/v). The fixed samples were dehydrated in an ethanol-xylo series and embedded in paraffin according to Albuquerque et al., (2002). Longitudinal sections (10 µm) were taken with a rotary microtome (Leica RM 2125 RT) and stained with 1% safranin and fast green. Some of the samples were stained with 0.1% aniline blue in 0.1 N tripotassium phosphate (K₃PO₄·3H₂O) for fluorescent light microscopy and 350-400 µm light filter was used (Mert and Soylu, 2007).

Determination of nucellus size

Digital images were collected using Nikon Coolpix P6000 camera attached to the binocular Nikon Eclipse 80i microscope. The images were processed using the ImageJ (Abramoff et al., 2004). Nucellus and integument measurements were recorded from the section in which the embryo sac was present. Orientation of the embryo sac in the ovule was examined for each section; primary ovule sections were measured per tree of combination per year. In each ovary, the larger ovule was considered as the primary ovule (Rodrigo and Herrero, 1998). With these measurements, InA (area of the inner side of integument) and NuA (area of nucellus) were calculated with ImageJ (Figure 1a). NuA/InA values ranked between 0 and 1. The ratio approached to 1 when nucellus deformation decreased. NW (nucellus width) was measured as the largest while NL (nucellus length) was measured as the longest measurement of nucellus tissue (Figure 1b).

Temperature data of flowering period

Flowering period was described as between first bloom and petal fall. Air temperature was measured as hourly with HOBO U12-013 data logger (Onset, Pocasset, MA) from 1 April to 31 May in two consecutive years and average daily temperatures were calculated for the flowering period.

Statistical analysis

An analysis of variance by an LSD test allowed the determination of clones or rootstocks that significantly affected the nucellus deformation of primary ovule at anthesis.

Results

Flowering period was recorded between 20 April and 7 May in 2009 and between 10 April and 30 April in 2010. There was a difference of approximately 10 days between the two years. In the study area, 2010 flowering period temperatures were higher than 2009 (Figure 2). Average temperature was 12.06 °C in 2010 and 10.51 °C in 2009 in the flowering period. No difference was found in terms of flowering dates between scion materials except for 4218 which bloomed 4-5 days earlier than the others. Nucellus width and length differences between '0900 Ziraat' and its clones were found statistically insignificant. In general, average longitudinal dimensions of nucellus were measured as 0.75-0.96 mm whilst average widths were 0.30 to 0.37 mm (Figure 3).

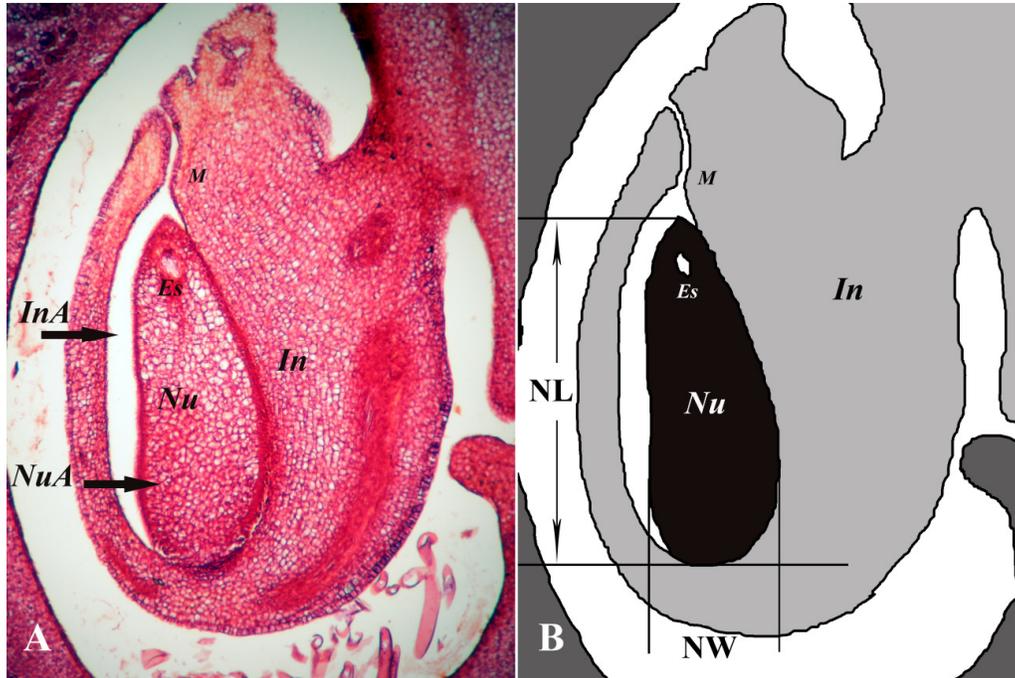


Figure 1. The measurements points of ovule. *Es* embryo sac; *M* micropyle; *Nu* nucellus; *In* integument; *InA* area of the inner side of integument; *NuA* nucellus area; *NW* nucellus width; *NL* nucellus length.

Embryo sacs in all of the primary ovules were seen as mature with the number of nuclei at anthesis. However, the nucellus did not complete in the interior of integuments (Figure 4). Tissue contractions were observed generally unilateral in the middle part of the nucellus. This deformation of nucellus was calculated as NuA and InA ratio. Although there were no significant differences in nucellus width and length, NuA/InA of nucellus were found to be significantly different.

The effects of rootstocks on NuA/InA were found statistically insignificant in 2010 (Table 1). NuA/InA on Mazzard seedlings (0.852) were higher than Gisela 5 (0.832) at a statistically significant level in 2009 ($p < 0.05$).

Table 1. NuA and InA ratios on different rootstocks

Rootstock	NuA/InA		Average
	2009	2010	
Gisela 5	0.832b*	0.853 ^{ns}	0.843
Mazzard	0.852a	0.865	0.859

*The difference between the averages shown in the same column with different letters is statistically significant ($p < 0.05$), *ns* not significant

The effects of scion on NuA and InA ratio were found statistically significant for each year ($p < 0.01$). 3503 had the highest value in two years while the lowest value was determined in '0900 Ziraat' (2009) and 3501 (2010) (Table 2).

Table 2. NuA and InA ratios on different scions

Scion	NuA/InA		Average
	2009	2010	
'0900 Ziraat'	0.792d**	0.842bc**	0.817
3501	0.820cd	0.802c	0.811
3503	0.879a	0.894a	0.886
4503	0.867ab	0.858ab	0.862
3201	0.840bc	0.881ab	0.860
4218	0.855ab	0.880ab	0.867

**The difference between the averages shown in the same column with different letters is statistically significant ($p < 0.01$)

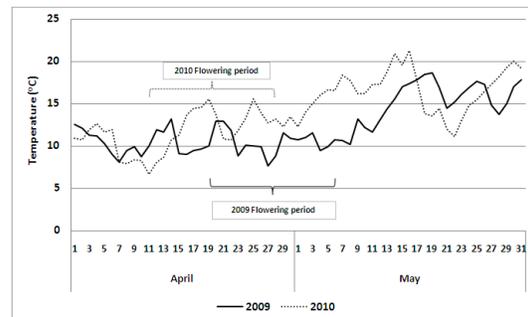


Figure 2. The daily average temperatures (between 1 April and 31 May) and flowering periods in 2009-2010.

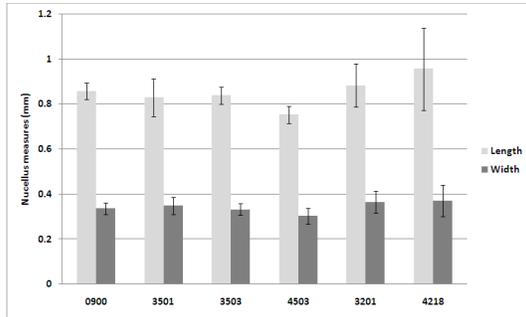


Figure 3. Nucellus measures in primary ovules. Bars=±SD

Discussion

The embryo sac development stage differences of '0900 Ziraat' and its clones were unimportant in this study. Generally, it was observed that the embryo sacs of primary ovules completed 4-8 nuclei stage at anthesis for two consecutive years. The similar developmental stage of the embryo sac at the anthesis was reported in apricot (Alburquerque et al., 2002) and sour cherry with 4-8 nuclei stage (Furukawa and Bukovac, 1989) and 2-4 nuclei in plum (CaiZhen et al., 2010). Therefore, limiting the development of the embryo sac at anthesis is not the case for '0900 Ziraat' sweet cherry variety and its clones.

Ovule development is affected by many factors. Especially, deformations of ovules are genetically controlled. Although flowers were sampled at the similar stage, deformations of nucellus were different among scions at the anthesis in this study. Mic'ic' and Đuric' (1998) reported the atrophy of ovules in plum. According to Zeller (1960), ovules in the apple flowers that opened in axillary position on the shoots were smaller and the integuments did not cover the nucellus.

Mert and Soylu (2007) reported that abnormalities of ovules were likely to be the cause of the low set in '0900 Ziraat'. Although scions were phenotypically similar (except for 4218), there were significant differences among the scions in the nucellus status at the anthesis stage. Due to the fact that scions were genotypically different (Demirtaş et al., 2006), it was considered that nucellus deformation was genetically controlled. However, although the flowering period in 2010 was hotter than that in 2009, air temperature increase was not effective on nucellus deformations in this study. Many researchers reported that the development of ovule was different between species and also among cultivars in same species (Ruiz et al, 2010; Mert and Soylu, 2007; Alburquerque et al., 2004; Stösser and Anvari, 1982). Some ovule abnormalities have a genetic base according to Sanzol and Herrero (2001).

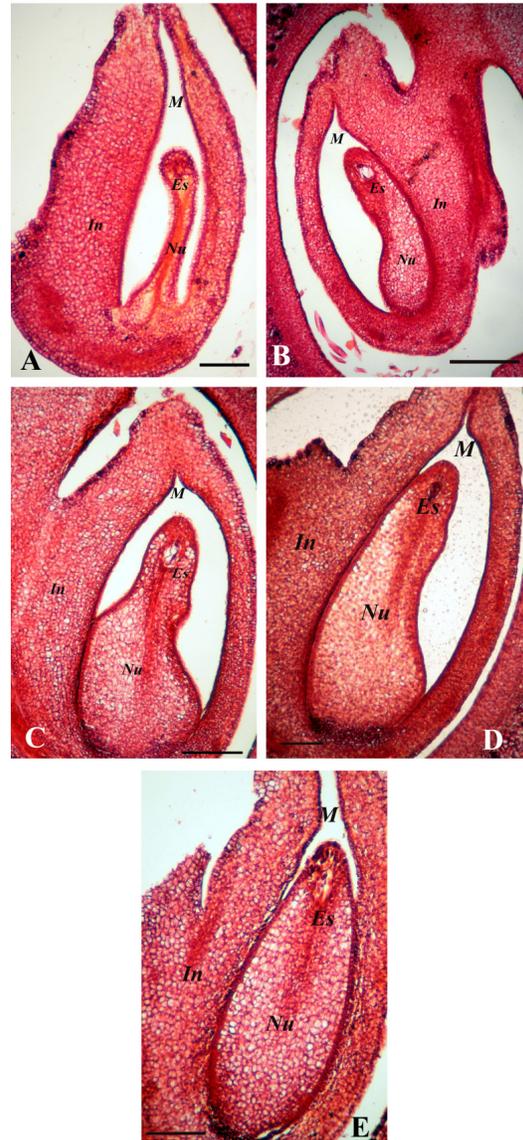


Figure 4. Deformations of nucellus at anthesis. A- NuA/InA: 30%; B- 60%; C:65%; D:70%; E:90%; Es embryo sac; M micropyle; Nu nucellus; In integument; Bar: 200 µm.

It was found to be partially the effect of rootstocks on nucellus deformation in this study. The nucellus of the apple trees grafted on M9 rootstocks is larger than that of the apple trees grafted on seedling rootstocks. The larger nucellus is favorable for the fruit set (Marro and Lalatta, 1978).

In addition to nucellus deformations, primary ovule degenerations with callose accumulation were observed at anthesis in this study. According to Postweiler et al. (1985), cherry ovule life expectancy may be 1-5 days after anthesis depending on temperature. In fruit trees, degeneration of ovule depending on the environmental conditions can begin due to the

accumulation of callose at anthesis (Sanzol and Herrero, 2001). However, formation of abnormal nucellus at anthesis was observed to be independent from degeneration process in this study.

Conclusion

There are significant differences in the deformations of primary ovule nucellus between '0900 Ziraat' and its clones at anthesis. Almost all of the embryo sacs were observed normally at anthesis. In spite of fertilization, nutrient transport to the zygote may be interrupted due to abnormal nucellus. As a result, nucellus deformation was higher in '0900 Ziraat' than the others; 3503 was found to be recommendable by virtue of having the lowest level of deformation.

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