

## Apple Ovule Setting

N. Mičić<sup>1,2</sup>, G. Đurić<sup>1,2</sup> and B. Pašalić<sup>1</sup>

<sup>1</sup> Faculty of Agriculture, University of Banjaluka, Bulevar vojvode Petra Bojovica 1a, Banja Luka, Bosnia and Herzegovina

<sup>2</sup> Genetic Resources Institute, University of Banjaluka Bulevar vojvode Petra Bojovica 1a, Banja Luka, Bosnia and Herzegovina

**Keywords:** pro-embryo, physiological maturity, aborted seeds, fertility control

### Abstract

**A study of the correlation between the number of the set ovules and fruit retention on trees until the physiological maturity phase was carried out on the following cultivars: 'Royal Gala', 'Idared', 'Jonagold', 'Golden Delicious' and 'Granny Smith', in an eight-year-old intensive orchard system on the rootstock M9 with trees spacing 4.0×1 m. Fruit yield in the orchard during the year of study was 21-24 kg/tree. Studied apple trees were not treated with fruit thinning agrochemicals. The study of embryo development was conducted in three phases: 1) at the end of flowering; 2) during fruit shedding in June (on freshly shed fruits); and 3) at the moment of fruit physiological maturity before harvesting. Histo-cytological analysis showed normal constitution of female gametophyte and formation of pro-embryo without noticeable abortive process at the level of optical microscopy and histological sections 12 micrometers thick produced by parafine technique. Fruit analyses show that during the June fruit shedding were shed those fruits which on average had less than 30% of ovules with normally developed embryos. Furthermore, among the same cultivars observed, physiologically mature fruits set for harvesting on average had over 70% of normally developed embryos, with that in diploid cultivars it was over 75%, and in the triploid cultivar 'Jonagold' the number was above 55%. Genotype differences in the structure of the developed embryos in both shed and physiologically mature fruits were discussed in this paper as the limiting factor of apple tree yield capacity in intensive orchard management systems.**

### INTRODUCTION

Fruit development in apple and fruit retention on the tree until physiological maturity is in the function of embryo development. Control of embryo development is an important aspect of yield capacity control, where for the projected yields it is necessary that up to 30% of flowers are set, or that 18.96% of flowers give physiologically mature fruits (Đurić et al., 1997). The key issue of fruit retaining on the trees is not only reduction of the number of fruits to the estimated number per tree (thinning), but primarily is adequate pollination and fertilization (cultivar composition, pollen transfer, the high compatibility of cultivars; Cerović and Mičić, 1996; Guerrero-Prieto et al., 2009). The study of the constitution of female apple gametophyte through differentiation phases of flowering of individual flowers in inflorescence showed a complex fertility algorithm (Mičić et al., 2009) of ovules for fertilization in inflorescence and opened a question of border number of set ovules as factors of fruit retaining in the trees in intensive orchard management systems (Djurić and Mičić, 2012): rootstock greatly modifies vigour and there is great pressure of agrochemicals through mineral nutrition, through the application of phytohormones to manage growth and fruit bearing, and through intensive treatments with pesticides and herbicides, which led to numerous studies of various aspects of fruit setting (Free, 1962; DeGrandi-Hoffman et al., 1986 in Dennis, 1967; Byers, 1998; Tromp and Borsboom, 1994).

The aim of the study was to establish the limiting number of normally developed embryos as precondition in reaching the fruit maturation.

## MATERIALS AND METHODS

The issue of fruits retaining on the trees until the harvesting moment is seen as an issue of realization of yield potential and in this study it is defined through observation of limiting numbers of embryos that have to develop into fruits so the fruits would not be shed from the trees, i.e., in order to reach physiological maturity. The study of correlation in the number of set ovules and fruit retaining on the trees until physiological maturity was performed in following cultivars: 'Royal Gala', 'Idared', 'Jonagold', 'Golden Delicious' and 'Granny Smith' in an intensive orchard system situated in Prijedor (44°46'30"N; 16°41'51"E). Trees were eight years old and grafted on the rootstock M9. Studied apple trees were not treated with fruit thinning agro-chemicals.

During the phase of full flowering and 15-20 days after flowering were performed histo-cytological analyses of female gametophyte constitution and forming of pro-embryo by using standard preparation of permanent histo-cytological sections by paraffin technique: fixation by Navashin, i.e., 10 ml of 1% chromic acid + 4 ml formalin + 1 ml glacial acetic acid (Navashin, 1936) and dyeing with Delafield's hematoxylin (Hematoxylin in  $\text{AlNH}_4/\text{SO}_4/2+12\text{H}_2\text{O}$  saturated solution with addition of PA glycerine and methanol, where the dye remains for two months on the light).

During the June fruit shedding freshly shed fruits from the trees were picked, 100 fruits per studied cultivar, (20 fruits from 5 trees) and on those fruits analyses of level of embryo development were conducted. The analysis of level of embryo development was performed by classifying seeds in two groups: a) seeds with normally developed embryos for developmental phase of embryogenesis at the moment of observation; and b) aborted seeds representing a seed coat without an embryo (embryos aborted soon after setting or during the first phases of embryogenesis).

At the moment of physiological maturity of fruits, from the trees of the same cultivars were collected 100 fruits per tree in which we determined number of seeds with normally developed embryos and number of aborted seeds – remains of seed coats where embryos are not present.

## RESULTS AND DISCUSSION

In all analyzed cultivars histo-cytological analysis showed normal constitution of female gametophyte and forming of pro-embryo without any noticeable abortive processes at this level of observation (Fig. 1). The analyses of ovule constitution in fruits which are shed from the trees during June shedding are given in Table 1.

Largest average number of seeds with normally developed embryo in the shed fruit in the June shedding is observed in the cultivar 'Golden Delicious' ( $3.62 \pm 0.13$ ) and the smallest in the cultivar 'Idared'. The largest average number of seeds with empty seed coat (aborted seeds) is observed for the cultivar 'Gala Royal' (5.27) and the smallest for cultivar 'Jonagold' (3.80). Upon testing the observed differences in the numbers of normally developed embryos in the fruit shed during the June shedding the observed cultivars could be divided into two groups: 1) cultivars with more than 3 normally developed embryos: 'Golden Delicious' and 'Gala Royal'; 2) cultivars with less than 3 normally developed embryos: 'Granny Smith', 'Jonagold' and 'Idared'. Furthermore, it was observed that shed fruit had in average less than 30% of the ovules in which the embryo development was normal until the moment of shedding.

Analysis of ovule establishment in fruits which have reached the phase of physiological ripeness on the tree is presented in Table 2.

Largest possible number of seeds with normally functioning embryo, in the physiologically healthy fruit (Fig. 2), is observed in the cultivar 'Gala Royal' (8.17), and the smallest in the cultivar 'Jonagold' (5.78). The largest average numbers of seeds with empty seed coat were observed in the cultivar 'Jonagold', and the smallest in the cultivar 'Gala Royal' (2.43). Based on the significance testing of the observed differences in the number of normally developed embryos in the fruit which reached the physiological ripeness phase the cultivars could be divided into three different groups which differ from each other with high statistical significance: 1) cultivars with 80% of normally developed

embryos 'Gala Royal' and 'Golden Delicious'; 2) cultivars with 70% of normally developed embryos: 'Granny Smith' and 'Idared'; and 3) cultivars with less than 60% of normally developed embryos: 'Jonagold'. Furthermore, it is observed that the diploid cultivars in this phase have over 70% of the normally developed embryos, while the cultivar 'Jonagold', as a triploid cultivar, has under 60% normally developed embryos. Bearing in mind that all five cultivars had high yield in the year of observation, it can be concluded that 70% of the normally developed embryos in the fruit of the diploid cultivars is a limit for the sustainability in those growing conditions, while for the triploid cultivar 'Jonagold', the limit is over 50% of the normally developed embryos for the sustainability of the fruit until reaching the physiological ripeness phase.

## CONCLUSIONS

Histo-cytological analysis of the female gametophyte which was performed 15 to 20 days after the end of the flowering phase indicates normal establishment of the female gametophyte in all observed apple cultivars. The fruit of all the studied cultivars which were shed from the tree during the June shedding had in average less than 30% of ovules with normal embryo development until the moment of shedding. Research indicates that the presence of 70% of the normally developed embryos in the fruit of the studied diploid cultivars is necessary in order to secure the retention of the fruit on the tree until the physiological ripeness. The exception in that respect is cultivar 'Jonagold', which as a triploid cultivar needs presence of 50% of normally developed embryos.

## Literature Cited

- Byers, R.E. 1998. Effects of bloom-thinning chemicals on apple fruit set. *Journal of Tree Fruit Production* 2(1):13-31.
- Cerović, R. and Mičić, N. 1996. Oprašivanje i oplodnja jabučastih i koštičavih voćaka. *Jugoslovensko Voćarstvo* 30(113-114):73-98.
- DeGrandi-Hoffman, G., Hoopingarner, R. and Klomparens, K. 1986. Influence of honey bee (*Hymenoptera: Apidae*) in-hive pollen transfer on cross-pollination and fruit set in apple. *Environmental Entomology* 15(3):175-186.
- Dennis Jr, F.G. 1967. Apple fruit-set: evidence for a specific role of seeds. *Science* 156(3771):71-73.
- Đurić, G., Mičić, N., Cerović, R. and Oljača, R. 1996. Sortne specifičnosti u pomotehnici jabuke uzgajane u ubliku vretena: III Rodni potencijal i stepen njegove realizacije. X kongres voćara Jugoslavije. Uvodni referati i abstrakti, str. 168.
- Đurić, G. and Mičić, N. 2012. Female gametophyte differentiation dynamics in apple inflorescence. 2<sup>nd</sup> Global Congress on Plant Reproductive Biology. p.14. Pécs.
- Free, J.B. 1962. The effect of distance from pollinizer varieties on the fruit set on trees in plum and apple orchards. *The Journal of Horticultural Science* 37(4):262-271.
- Guerrero-Prieto, V.M., Rascón-Chu, A., Romo-Chacón, A., Berlanga-Reyes, D.I., Orozco-Avitia, J.A., Gardea-Béjar, A.A., Parra-Quezada, R. and Sánchez-Chávez, E. 2009. Short communication. Effective pollination period in 'RedChief' and 'Golden Delicious' apples (*Malus domestica* Borkh). *Spanish Journal of Agricultural Research* 7(4):928-932.
- Mičić, N., Đurić, G. and Cvetković, M. 2009. Differentiation of female gametophyte through the microphenophases of individual flowers development in apple blossom. *Agroznanje* 10(1):33-49.
- Navashin, M.S. 1936. [Методика цитологического исследования для селекционных целей]. М., "ОГИЗ-Сельхозгиз".
- Tromp, J. and Borsboom, O. 1994. The effect of autumn and spring temperature on fruit set and on the effective pollination period in apple and pear. *Scientia Horticulturae* 60(1-2):23-30.

## Tables

Table 1. Average number of normally developed embryos and aborted embryos in ovules in fruits shed from trees during June shedding.

Cultivar	Ovule constitution	
	Developed embryo	Empty seed coats
	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
1. Royal Gala	3.21±0.16	5.27±0.18
2. Idared	2.23±0.11	4.07±0.16
3. Jonagold	2.40±0.18	3.46±0.19
4. Golden Delicious	3.62±0.13	3.80±0.18
5. Granny Smith	2.74±0.16	4.85±0.16
Mean	2.84±0.18	4.29±0.19

Table 2. The average number of normally developed embryo and aborted embryo in physiologically mature fruits of observed apple cultivars.

Cultivar	Ovule constitution	
	Developed embryo	Empty seed coats
	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
1. Royal Gala	8.17±0.17	2.43±0.16
2. Idared	6.94±0.20	3.31±0.19
3. Jonagold	5.78±0.17	4.21±0.18
4. Golden Delicious	7.95±0.19	2.61±0.17
5. Granny Smith	6.97±0.20	4.00±0.19
Mean	7.16±0.17	3.31±0.16

## Figures

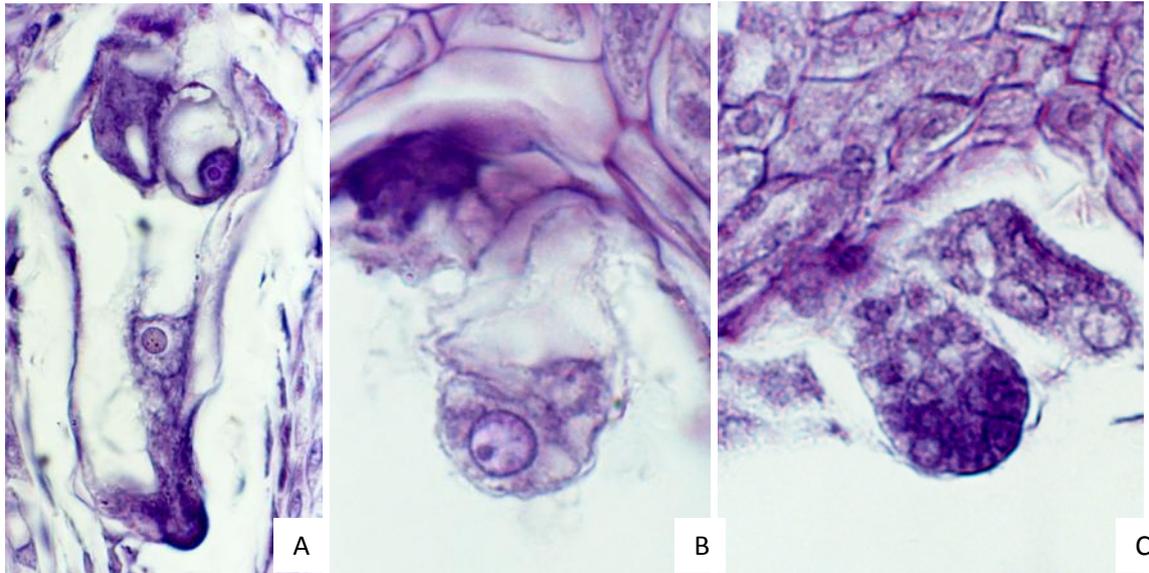


Fig. 1. Fertility control by use of analyses of female gametophyte (A – egg cell with synergids and central nucleus of embryo sac), pro/embryo development (B – first zygote division with noticeable hypophysis region) and first phases of embryogenesis (C – pro-embryo before reaching globular development phase): histological analyses at this level of observation do not indicate abortive processes in studied cultivars.

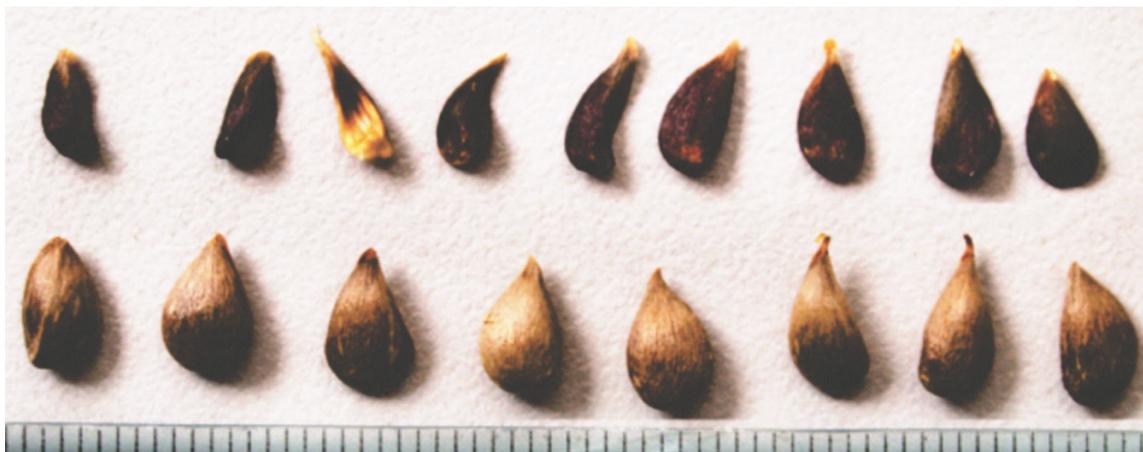


Fig. 2. Seeds with normally developed embryos (below) and aborted seeds (above).