Effect of embryo development on *in vitro* germination of early ripening varieties in sweet cherry (*Prunus avium* L.)

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Introduction

Hybridization of *Prunus avium* L. is considered as a major path for development of new cultivars. Breeding program of *Prunus avium* L. is usually accompanied by the problem of low seed germination that is very difficult to achieve using conventional methods, especially in early ripening varieties. Therefore, in vitro embryo culture has an important role in the breeding programs. Low viability of seeds is closely related with the degree of embryo development. Also, success of embryo culture depends on several other factors such as period of stratification, selection of appropriate nutrition media, as well as occurrence of infection that may significantly affect the further development of the embryo. The aim of this work was to detemine preferable method between direct embryo culture and in-ovulo embryo culture and to what extend state of embryo development (age, size) affect germination. At the same time, infection monitoring was performed in order to determine which methodological aproach has the least infection risk.

Material and methods

Four early ripening varieties of *Prunus avium* L. , Rita, Burlat, Carmen as mother parent, were crossed with variety Early Star ,as mail parent, by hand pollination of emasculated flowers. Embryos in a different ripening stages were sampled every third day, starting 30 days after pollination. Further steps were performed in the laboratory for *in vitro* culture. Peeled seeds were washed for 30 minutes in running tap water , sterilized in 1% sodium hypochlorite, containing 0,1% Tween, for 20 min., followed by rinsing with distilled water. Sterilization was accomplished in laminar flow hood with 70% ethanol for 1 minute, followed by 3 rinses with sterile distilled water. After sterilization, embryos within seed coat were taken out from the endocarp by cracking. Embryos were put in culture in two different ways - direct embryo culture and in-ovulo embryo culture.

In direct embryo culture, isolated embryos from the seed coat, were cultivated on medium MS2 (Murashige and Skoog 1962) supplemented with BA 1mg/l, NAA 0,5 mg/l hormones, 20 g/l sucrose, 10 g/l sorbitol and 6 g/l agar. Embryos were kept in the fridge at 4°C in dark for a month.

In in-ovulo embryo culture, embryos within seed coat were cultivated on medium MS1 without hormones, supplemented with 30 g/l sucrose and 6 g/l agar. Embryos were stored in dark conditions, temperature 24-26°C, for three weeks. Thereafter, embryos were isolated from seed coat and transferred in the fridge at 4°C in dark for a month.

After stratification period, both types of cultivated embryos were transferred to the BH medium (Brooks & Hough, 1958) and placed in a chamber at the temperature of 22°C ± 1°C with 16/8 h light/dark photoperiod.

Results and disccusion

Percentage of infection successively increased with the maturity of the fruit in all varieties. Table 1. shows that significantly better results were obtained from direct embryo culture. Removing the seed coat decreased the occurrence of infection. Thus, in hybrid combination Burlat x E.star the highest infection rate of direct embryo culture amounted to 18,7%, whereas in-ovulo embryo culture the infection rate reached 91,7 %.

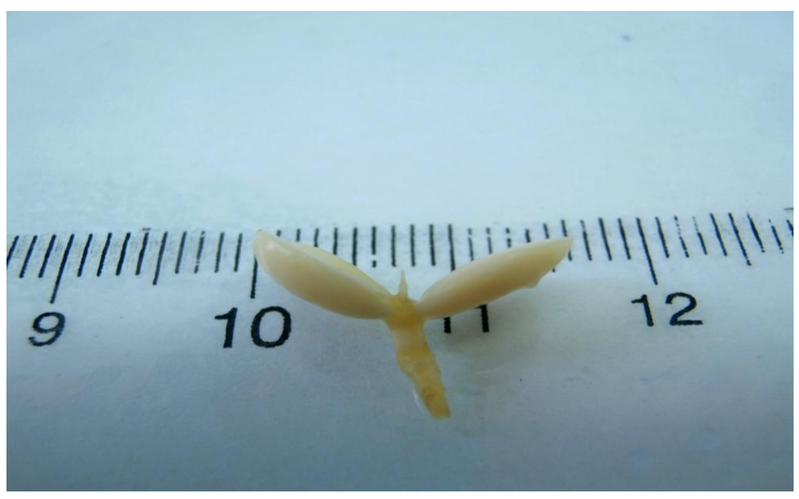
Fruit ripening and thus embryo development stage during the embryo culture establishment had a great influence on its further development. Embryos with average 1-2 mm in size had the lowest percentage of germination. Slightly larger embryos, 3-4 mm, showed significantly better results. In direct embryo culture, hybrid combination Burlat x E. Star had the highest percentage of developed embryos (66,7%) with embryos 3-4mm in length. Highest percentage of embryos developed in plants had hybrid combination Karmen x E. Star , 75%. In very early ripening variety Rita, as a mother plant, the highest germination was obtained from embryos 9-10 mm , 66,7 %. Stimutated embryo development and embryo size increase was observed after one month stratification by MS2 medium.

In–ovule embryo culture significantly increase the embryo size but percent of obtained seedlings was lower due to culture infection.

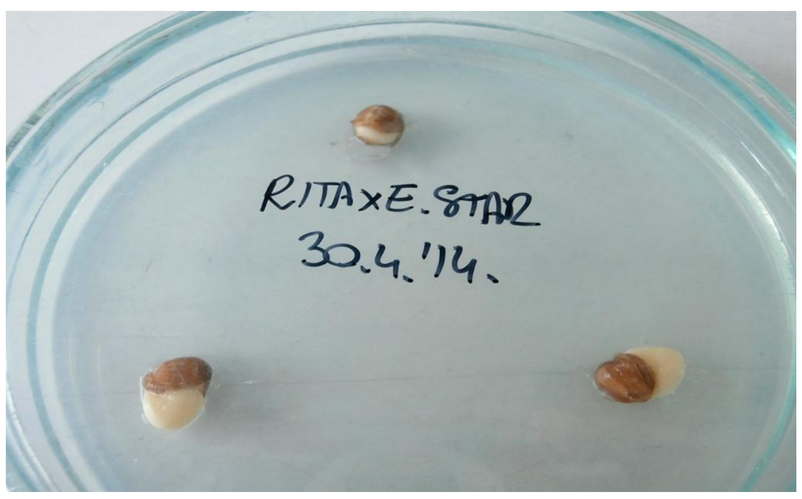
Reported embryo culture method produced strong seedlings with good transplanting survival. Although, aclimatization period was sensitive stage, 65% of hybrids were grown to planting stage under controled relative humidity. Generaly, about three weeks were sufficient to allow seedlings to harden off and start new growth.

Table 1. Effects of embryo development and occurrence of infection in embryo culture

Hybrid combination	No. of days after pollination	Ripening stage	Embryo size (mm)	Direct embryo culture						In-ovulo embryo culture				
				Infection %	Incomplete plant development(%)			Embryos developed into plants %	Embryo size after three weeks in dark conditions (mm)	Infection %	Incomplete plant development(%)			Embryos developed into plants %
					Completely undeveloped	Developed only root	Developed only shoot				Completely undeveloped	Developed only root	Developed only shoot	
Rita x Early star	31	Green	1-2	0,0	90,0	0,0	5,0	5,0	2-3	10,0	70,0	0,0	15	5,0
	34	Green-yellow	3-4	0,0	6,7	40,0	13,3	40,0	4-5	0,0	60,0	0,0	30,0	10,0
	37	Yellow	5-6	3,0	12,1	21,2	27,3	36,4	6-7	13,5	44,2	5,8	17,3	19,2
	40	Yellow-light red	7-8	10,8	13,5	0,0	37,9	37,8	8-9	25,6	25,6	20,5	5,2	23,1
	43	Light red	9-10	0,0	33,3	0,0	0,0	66,7	10-11	50,0	0,0	25,0	0,0	25,0
	46	Dark red	9-10	0,0	0,0	0,0	66,7	33,3	10-11	/	/	/	/	/
Burlat x Early Star	31	Green	1-2	0,0	100,0	0,0	0,0	0,0	2-3	0,0	25,0	12,5	37,5	25,0
	34	Green-yellow	3-4	8,3	8,3	0,0	16,7	66,7	4-5	10,7	28,6	14,3	14,3	32,1
	37	Yellow	5-6	0,0	12,5	6,2	18,8	62,5	6-7	35,0	25,0	0,0	5,0	35,0
	40	Yellow-light red	7-8	0,0	0,0	0,0	50,0	50,0	8-9	41,7	16,7	25,0	8,3	8,3
	43	Light red	9-10	0,0	5,0	5,0	55,0	35,0	10-11	42,8	7,2	15,0	15,0	20,0
	46	Red	9-10	18,7	6,3	0,0	62,5	12,5	10-11	91,7	0,0	0,0	0,0	8,3
Carmen x Early Star	31	Green	1-2	0,0	33,3	0,0	0,0	66,7	2-3	9,3	90,7	0,0	0,0	0,0
	34	Green-yellow	3-4	0,0	0,0	25,0	0,0	75,0	4-5	11,5	51,0	0,0	12,5	25,0
	37	Yellow	5-6	0,0	18,2	9,1	27,2	45,5	6-7	0,0	56,25	43,75	0,0	0,0
	40	Yellow-light red	7-8	0,0	9,1	9,1	45,4	36,4	8-9	0,0	25,0	15,0	15,0	45,0
	43	Light red	9-10	5,3	15,7	10,5	43,2	25,3	10-11	11,0	29,0	5,0	35,0	20,0
	46	Red	9-10	10,0	40,0	5,0	15,0	30,0	10-11	11,5	18,5	25,0	15,0	30,0

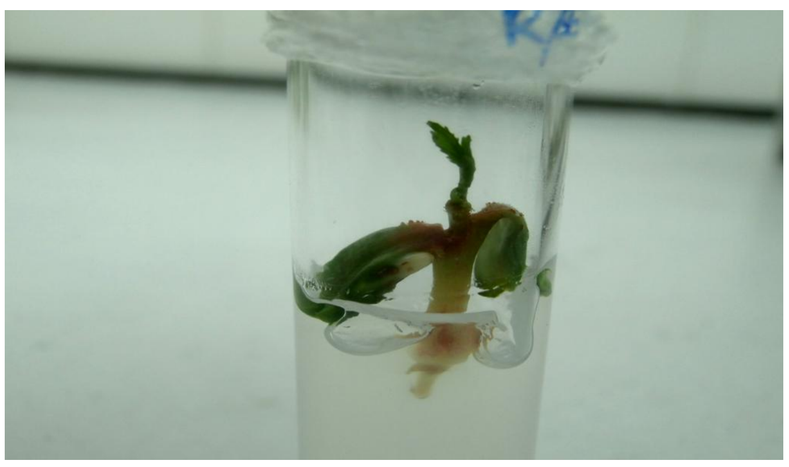


Isolated embryo one month after stratification



In-ovulo embyo culture after two week in dark conditions

Embryo on BH media after one month at +4°C



Earlz stage of embryo development *in vitro*

Fully developed plant from embryo culture

Aclimatized sweet cherry hybrids

Conclusions

Germination of seeds from *Prunus avium* L. early ripening varieties by direct embryo culture showed to be more efficient than in-ovulo embryo culture. Embryos isolation from seed coat decreases occurrence of infection. Maturity of the fruit also affects on occurrence of infection with greater infection rate in more mature fruits. Size of the embryos 3 - 4 mm in length had all seed constituents differentiated for normal germination in bipolar plant. It was generally associated with green stage of fruit development regardless variety. Our results provide an *in vitro* method for routine embryo culturing in sweet cherry with a critical embryo size between 3-4 mm to achieve high seedling recovery from direct embryo germination.