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# OPTIMIZAREA UNOR FACTORI ÎN VEDEREA REGENERĂRII PRIN EMBRIOCULTURĂ LA UNELE GENOTIPURI DE PRUN ȘI CIREȘ

# OPTIMIZATION OF SOME FACTORS IN ORDER TO REGENERATE USING EMBRYO CULTURE IN SOME PLUM AND CHERRY GENOTYPES

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**Abstract**

**In last years, it has been observed that the germination rate of the resulting stones in hybrid combinations is very low. Starting from this problem, was checked a different solution than classic to obtain hybrid plants, solution that offers and analysis that gives as many explanations for this situation. The biological material used was represented by 1,140 plum hybrids stones (resulted from different hybrid combinations - 'Andreea x Romanța', 'Pitestean x Andreea', 'Tita x Jojo', and open pollination of 'Stanley', 'Jojo', 'Anna Spath', 'Tamaioasă de Bistrita', 'Mildora' cvs.) and 815 sweet cherry hybrids stones resulted from open pollination of 'Skeena', 'Superb', 'Stella', 'Kordia', 'Severin' cvs. The method used for regeneration was embryo culture with 2 variants of culture medium based on Lee Fossard components and hormonal balances, which included IBA, BAP and GA3. In plum, 40.35% of the stones were unsuitable for inoculation due to dehydration, which also led to a very small percentage of regenerated plants of only 3.97%, and in sweet cherry, was recorded 61.96 % dehydrated stones and the germination percent was 13.23%.**

**Cuvinte cheie:** prun, cireș, embrio cultură, mediu de cultură, sâmbure.
**Key words:** plum, sweet cherry, embryocultura, culture medium, stone.

## 1. Introduction

Embryo culture has a very important role in fruit tree species breeding programs. It is particularly used when poor embryo development occurs due to abortion or genetic compatibility between interspecific hybrids (Ramming, 1990, Sharma et al., 1996).

*In vitro* development of embryos however is influenced by various factors (Li et. al, 2018). In addition to the influence of the parents, the developmental stage of the embryo (Dulic et al., 2016), the composition of the culture medium (Khoshandam, et. al, 2017), in many cases, the germination rate was also affected by the size of the embryo (Arbeloa et. al, 2006; Ognjanov et al., 1994, Sundouri, et al., 2014). The cold treatment is also an important factor as well as the treatments with stimulators carried out in the same period on the stones (Sulusoglu, 2012). However, seeds could be successfully germinated without cold treatment by isolating embryos from the cotyledon. In this way, the time required to obtain the plants could be shortened (San, 2014). Also according to other authors, apricot, peach and cherry stones did not germinate on the culture medium without prior stratification.

The purpose of this study is to identify the causes of obtaining a very low germination rate by the classical method and regeneration by embryo culture of plum and sweet cherry hybrid stones.

## 2. Material and methods

**The biological material** used was represented by 1,140 plum hybrids stones (resulted from different hybrids combinations - 'Andreea x Romanța', 'Pitestean x Andreea', 'Tita x Jojo', and open pollination of 'Stanley', 'Jojo', 'Anna Spath', 'Tamaioasă de Bistrita', 'Mildora' cvs.) and 815 sweet cherry hybrids stones resulted from open pollination of 'Skeena', 'Superb', 'Stella', 'Kordia', 'Severin' cvs.

**The culture substrate** was represented by culture media variants based on Lee&Fossard (1977) macroelements, microelements and vitamins Lee&Fossard (1977) with different hormonal balances:

Variant 1: macroelements Lee&Fossard; microelements Lee&Fossard; vitamins Lee&Fossard; hormonal balance: IBA 5 mg/l, BAP 20 mg /l, GA3 2 mg/l; chelat (NaFeEDTA); agar 6 g/l; sucrose 20 g/l.

Variant 2: macroelements Lee&Fossard; microelements Lee&Fossard; vitamin Lee&Fossard; hormonal balance: IBA 5 mg/l, BAP 20 mg /l; chelat (NaFeEDTA); agar 6 g/l; sucrose 20 g/l.

**Disinfection of the biological material**, consist of two sterilization options, one of them being applied in two stages:

Variant 1: immersion with stirring in tap water + dish detergent + Tween 80 = 5 minutes; cleaning in tap water - immersion in alcohol 96% (w/v) = 2 min; immersion in $Ca(OCl)_2$ (Ca hypochlorite) = 3 min; immersion with stirring in bidistilled and sterile water = 3 x 5 minutes.

Variant 2:

*Stage 1:* immersion with stirring in tap water + dish detergent + Tween 80 = 5 minutes; cleaning in tap water -immersion in alcohol 70 vol. %; immersion in Ca(OCl)2 6% (w/v)= 10 minutes; immersion with stirring in bidistilled and sterile water = 3 x 5 minutes.

*Stage 2:* after removing the endocarp: immersion in alcohol 96 vol. %= 1 minute; immersion in $Ca(OCl)_2$ 6 % (w/v)= 5 minutes; immersion with stirring in bidistilled and sterile water = 3 x 5 minutes.

## 3. Results and discussions

After removing the endocarp, it was found that the stones were in a pronounced state of dehydration both in plum (Fig. 1. a, b) and sweet cherry (Fig. 2). In this situation, the stones that were the least dehydrated were chosen (if there were better specimens). Analyzing figure 3, it can be seen that in plums the percentage of split stones varied from 18.88% in the case of hybrids stones from open pollination of 'Stanley' cv., rising to very high values of 49.75% for hybrids stones from 'Tita x Jojo' combination, 51.98% for the hybrids stones from 'Piteștean x Andreea' combination and a maximum of 52% at hybrids stones from open pollination of 'Mildora' cv. The average for all analyzed hybrids stones was 40.35% dehydrated stones.

For sweet cherry (Fig. 4) the lowest dehydration value was at hybrids stones from open pollination of 'Skeena' cv. and the highest 71% at hybrids stones from open pollination of 'Kordia' cv. The hybrids stones studied had an average of 61.96 % dehydrated stones. Pronounced dehydration made it difficult or even impossible to remove the skin from the stone. During the establishment of the embryo culture, was tried to hydrate the stones by keeping them for different times in the last immersion with distilled and sterile water (sometimes the double-distilled and sterile water was refreshed) but following this treatment the stones became gelatinous, which also made it heavy or impossible to remove the skin.

In cases where the removal of the integument and inoculation on the culture medium was successful, an increased number of infections were found (Table 1 and 2).

From the total number of stones inoculated to 680 pieces of plum, 505 were infected, so a share of 74.12%. Of the total stones with infections, 345 were disinfected by V1 and 159 were disinfected by the V2 method. For sweet cherry were inoculated 310 stones, our observation established that 137 were found with infections: 87 sterilized by V1 and 50 sterilized by V2.

Disinfection variant V2 proved to be more effective both in the case of plum stones infections (33.55%) and sweet cherry stones infections (36.50%). The disinfection variant V1 which was done only before endocarp removal was weaker recording 72.78 % infections in plum and 63.50 % infections in sweet cherry.

The evolution of plum embryos on the two variants of culture medium was different (Table 3). In the plum, referring to the stones that remained without infections, it was found that on V1 the average of germination stones was 14.78 embryos and on V2 they germination was 16.39 embryos. The best results were recorded in case of hybrids stones obtained from open pollination of 'Tamâioasă de Bistrita' cv. where all embryos started on V2 (100%) compared to V1 with only 25%. In the case of hybrids stones from open pollination of 'Stanley' cv. and from 'Andreea x Romanța' combination each registered 33.33% embryos which started to grow compared to V2 which did not register embryo growth. Hybrids stones obtained from 'Pitestean x Andreea', 'Tita x Jojo' combinations and from open pollination of 'Jojo', 'Anna Spath', 'Mildora' cvs. did not start growing on V2.

On the culture medium of variant 1 which presents in addition to variant 2 GA3 gibberellic acid, although the results are weaker in terms of the number of embryos germination/hybrids stones population, a greater number of stones with embryos germination recording positive results also in the case of hybrids stones obtained from 'Pitestean x Andreea' combination - 33.33%, from open pollination of 'Anna Spath' cv. - 25% and 'Jojo' cv. - 4.35%. If we relate the number of germination stones 27 to the number of stones with which the experience was established, it is found that in plum out of 680 stones only a percentage of 3.97% reached the germination stage.

Another important observation was that to plum the growth did not take place on the same axis at the hypocotyl and epicotyl. At the cotyledonary node, a bend occurred and the hypocotyl and epicotyl became parallel, which made it extremely difficult to plant the few seedlings obtained. The plant had the appearance of the letter "U" lying down and sticking the root into the substrate attracted the aerial part after it, and conversely trying to put the aerial part vertically pulled the root out of the substrate.

In the case of sweet cherry (Table 4), from 173 stones remaining without infections inoculated on the two variants of the culture medium, it was found that only 41 embryos started to grow, so on the V1 variant of the medium there was an average of 27.12 % embryos started to grow and V2 an average of 16.36 % embryos started to grow. Another remark would be that embryos from all hybrids stones started growing on the variant V1 and on the variant V2 the hybrids stones from open pollination of ‘Superb’ cv. did not register embryos that started growing. Regarding the hybrids stones factor, it can be shown that a good behavior recording the best results were in the case of hybrids stones resulted from open pollination

of 'Kordia' cv. (41.67% on V1 and 50% on V2), 'Severin' cv. (44.44% on V1). Poor results were recorded on V2 by hybrids stones obtained by open pollination of 'Severin' cv. (6.67%), on V2 by hybrids stones from open pollination of 'Superb' (17.50%), 'Skeena' (19.23%) and 'Stella' (20%) cvs. And in the case of the sweet cherry, if we compare the number of 41 stones which started to grow, to the total number of 310 stones, we find that only 13.23% of the stones have started to grow on the culture medium. The appearance of the resulting seedlings was normal (Photo 5).

## 4. Conclusions

The biological material used, even if it came from fruits harvested at the optimal time from the point of view of ripening, did not show viability and maturity for optimal germination, which would lead to obtaining vigorous and uniform plants: natural causes or storage conditions.

In the analyzed stones there was no cotyledonary mass (a small amount of dry substance), so at the time of harvesting the cotyledons were not fully formed, they had no starch reserves from which to feed the embryo, even if it had formed.

It is purposed analysis and interpretation of results correlated with environmental factors (temperature, the moment of installation of high temperatures and their duration; the agricultural technique applied to the trees in which hybrid combinations are done and the storage conditions of the hybrid stones, in order to detect the optimal moment for harvesting the hybrids fruits and the favorable storage conditions).

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**Tables and figures**



Fig. 1 a, b. Plum stones

Fig. 2. Sweet cherry stones

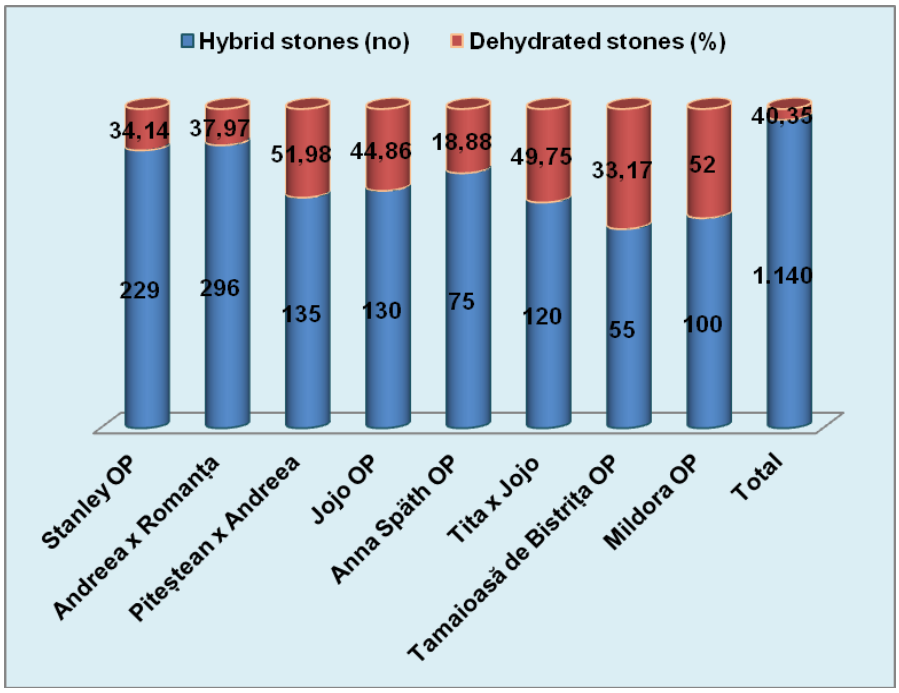


**Fig. 3. Quality of the plum hybrids stones**

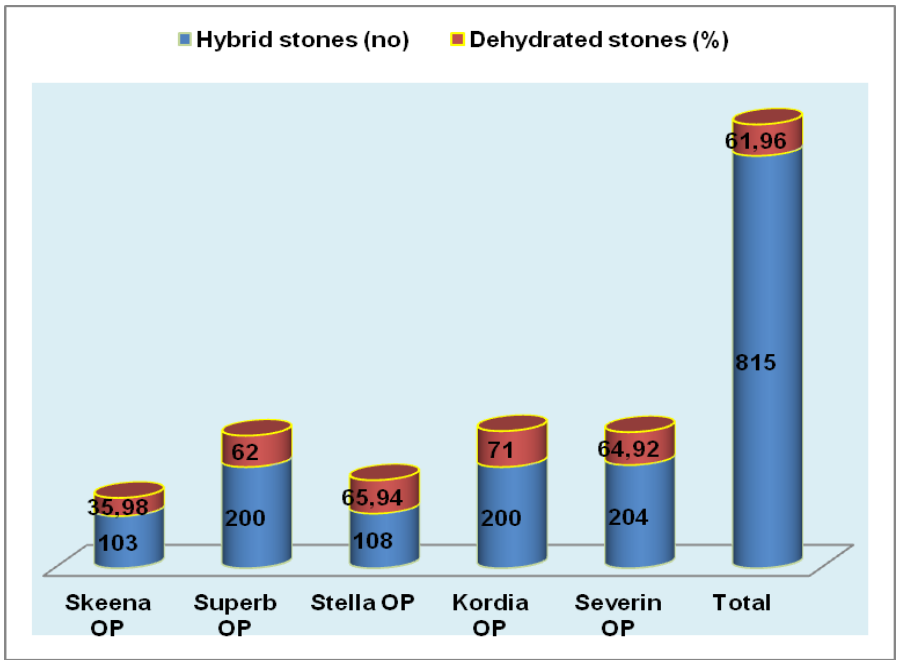


**Fig. 4. Quality of the sweet cherry hybrids stones**

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**Table 1. The influence of disinfection methods of the plum biological material**

| No. | Combination | No. inoculated stones | Infections (no.) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | | V1 | | V2 | |
| | | | no. | % | No. | % | No. | % |
| 1 | Stanley OP | 152 | 120 | 78.94 | 77 | 64.17 | 43 | 35.83 |
| 2 | Jojo OP | 72 | 32 | 44.45 | 22 | 68.75 | 10 | 31.25 |
| 3 | Anna Spath OP | 61 | 51 | 83.61 | 37 | 72.55 | 14 | 27.45 |
| 4 | Tămâioasă de Bistrița OP | 37 | 27 | 73.97 | 16 | 59.26 | 11 | 40.74 |
| 5 | Mildora OP | 48 | 40 | 83.33 | 31 | 77.50 | 9 | 22.50 |
| 6 | Andreea x Romanța | 184 | 146 | 79.35 | 103 | 70.55 | 43 | 29.45 |
| 7 | Pitești an x Andreea | 65 | 32 | 49.23 | 21 | 65.63 | 11 | 34.38 |
| 8 | Tita x Jojo | 61 | 56 | 93.33 | 38 | 67.86 | 18 | 32.14 |
| | **Total** | **680** | **504** | **74.12** | **345** | **72.78** | **159** | **33.55** |

**Table 2. The influence of disinfection methods of the sweet cherry biological material**

| No. | Combination | No. inoculated stones | Infections (no.) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | | V1 | | V2 | |
| | | | no. | % | No. | % | No. | % |
| 1 | Skeena OP | 66 | 33 | 50.00 | 20 | 60.61 | 13 | 39.39 |
| 2 | Superb OP | 76 | 32 | 42.11 | 19 | 59.38 | 13 | 40.63 |
| 3 | Stella OP | 37 | 17 | 45.95 | 11 | 64.71 | 6 | 35.29 |
| 4 | Kordia OP | 58 | 30 | 51.72 | 21 | 70.00 | 9 | 30.00 |
| 5 | Severin OP | 73 | 25 | 33.33 | 16 | 64.00 | 9 | 36.00 |
| | **Total** | **310** | **137** | **44.19** | **87** | **63.50** | **50** | **36.50** |

**Table 3. Plum embryos evolution**

| No. | Combination | Culture media variant | No. stones without infections | Stones germination | |
|---|---|---|---|---|---|
| | | | | No. | % |
| 1 | Stanley OP | V1 | 26 | 4 | 15.38 |
| | | V2 | 6 | 2 | 33.34 |
| 2 | Jojo OP | V1 | 23 | 1 | 4.35 |
| | | V2 | 17 | 0 | 0.00 |
| 3 | Anna Spath OP | V1 | 4 | 1 | 25.00 |
| | | V2 | 6 | 0 | 0.00 |
| 4 | Tămâioasă de Bistrița OP | V1 | 8 | 2 | 25.00 |
| | | V2 | 2 | 2 | 100.00 |
| 5 | Mildora OP | V1 | 6 | 0 | 0.00 |
| | | V2 | 2 | 0 | 0.00 |
| 6 | Andreea x Romanța | V1 | 20 | 0 | 0.00 |
| | | V2 | 18 | 6 | 33.33 |
| 7 | Pitești an x Andreea | V1 | 27 | 9 | 33.33 |
| | | V2 | 6 | 0 | 0.00 |
| 8 | Tita x Jojo | V1 | 1 | 0 | 0.00 |
| | | V2 | 4 | 0 | 0.00 |
| | **Average** | **V1** | **115** | **17** | **14.78** |
| | **Average** | **V2** | **61** | **10** | **16.39** |
| | **Total** | | **176** | **27** | 15.34 |

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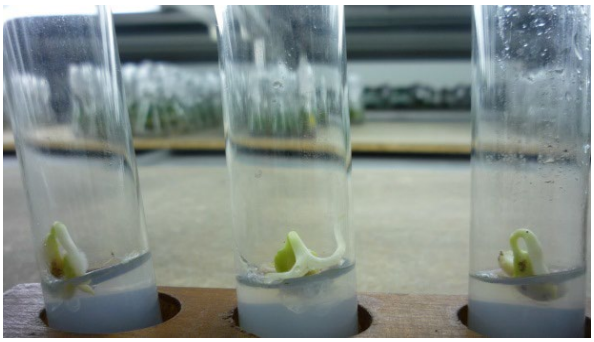

**Fig. 4. Growing plum seedlings**

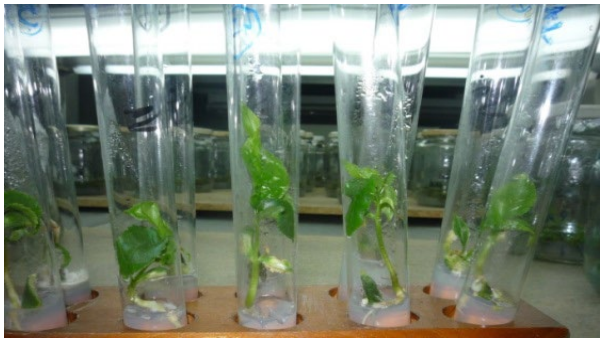

**Fig. 5. Growing sweet cherry seedlings**

**Table 4. Sweet cherry embryos evolution**

| No. | Combination | Culture media variant | No. stones without infections | Stones germination | |
|---|---|---|---|---|---|
| | | | | No. | % |
| 1 | Skeena OP | V1 | 26 | 5 | 19.23 |
| | | V2 | 7 | 2 | 28.57 |
| 2 | Superb OP | V1 | 40 | 7 | 17.50 |
| | | V2 | 4 | 0 | 0.00 |
| 3 | Stella OP | V1 | 10 | 2 | 20.00 |
| | | V2 | 10 | 3 | 30.00 |
| 4 | Kordia OP | V1 | 24 | 10 | 41.67 |
| | | V2 | 4 | 2 | 50.00 |
| 5 | Severin OP | V1 | 18 | 8 | 44.44 |
| | | V2 | 30 | 2 | 6.67 |
| | **Average** | **V1** | **118** | **32** | **27.12** |
| | **Average** | **V2** | **55** | **9** | **16.36** |
| | **Total** | | **173** | **41** | **23.69** |