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Evaluation of multiplication potential of cold stored shoot cultures of selected fruit genotypes

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Abstract. We presented the results about preserving *in vitro* selected fruit genotypes by cold storage strategy and the capacity for following shoot multiplication. The investigation included five fruit genotypes: plum 'Požegača', cherry rootstocks ('Gisela 5' and 'Tabel Edabriz'), raspberry 'Meeker', and blackberry 'Čačanska Bestrna'. The best results were achieved with shoots of the plum cultivar 'Požegača' which can be successfully stored at 5°C up to 10 months, without subculturing producing viable shoots. Furthermore, a significant increase in the multiplication index (13.0) of this genotype was noticed especially in shoots cultivated for 21 days under standard growing conditions, followed by storage at 5°C in total darkness for six months. 'Gisela 5' and 'Tabel Edabriz' cherry rootstocks were successfully preserved for three months in the same manner, and the plants (100%) well recovered. After being stored in cold conditions, the multiplication index of shoots in both rootstocks exhibited an increase ranging from 1.7 to 4.8 times compared to those grown under standard conditions. The viability rate of *in vitro* shoots of the raspberry cultivar 'Meeker' in cold conditions was also high (>67%). In this genotype, as well as in both cherry rootstocks, slow growth storage was accompanied by slow nitrogen uptake from the medium. In addition, the multiplication potential of encapsulated raspberry 'Meeker' and blackberry 'Čačanska Bestrna' and cold stored (5°C, darkness) shoot tips was evaluated. Encapsulated shoot tips of 'Čačanska Bestrna' (18.8%) and 'Meeker' (6.3%) survived the monthly cold storage period and exhibited regrowth. Shoots of selected genotypes were capable of multiplication in successive subcultures after cold storage, and often the multiplication indexes were higher compared to the control shoots that were consistently grown under standard conditions in the growth chamber. These results represent a significant contribution to the revitalization and further development of the national fruit gene bank.

Key words: Fruit tree germplasm, *in vitro* cold storage, encapsulation, survival, multiplication

Introduction

Plant gene banks under *in situ*, ex situ and *in vitro* conditions have been established in most countries worldwide. Genetic resources, specifically gene banks maintained under *in situ* conditions, facilitate the work of breeders; however, they require extensive land areas and incur considerable costs. Moreover, these plants are directly exposed to diseases, pests, and other external abiotic stress factors.

The establishment of a modern germplasm collection necessarily requires the utilisation of *in vitro*

techniques for the preservation of plant/fruit species. These techniques are considered significant alternatives to traditional germplasm preservation under field conditions. *In vitro* technology is employed for the conservation of the genetic resources of temperate fruit crops. As early as 1983, it was ranked as the third priority by the IBPGR Advisory Committee for *in vitro* storage (Stushnoff & Fear, 1985).

In addition to cultivated fruit species and cultivars, a significant number of wild and related species have been documented in Serbia. Recognizing the need for conservation and exchange of germplasm with other countries, the 'Fruit Gene Bank of Yugoslavia' was established in 1987/88 during the time of ex-Yugoslavia (Paunović et al., 1996). This gene bank played a crucial role in investigating, collecting, conserving, and exchanging genetic resources. It housed active collections of diverse fruit germplasm. From 1989 to 1991, a comprehensive study and selection of genotypes from 14 different fruit species were carried out under varying ecological conditions. The project involved the participation of 137 scientists from 19 fruit research institutions. During this period, 1,087 autochthonous genotypes, 2,505 introduced genotypes, and 459 fruit hybrids (including pome, stone, shell, soft, and subtropical fruit species) were identified and conserved under *in situ* conditions. Regrettably, the breakup of Yugoslavia and limited financial resources had a detrimental impact on the gene bank, leading to the extinction of numerous active collections, particularly those containing autochthonous fruit species and cultivars.

The Fruit Research Institute in Čačak possesses a substantial collection of 773 genotypes from various fruit species. Currently, our breeders are actively studying numerous local varieties, with a particular focus on clonal selection of autochthonous apple, plum, and sour cherry cultivars that display desirable traits such as pest and disease resistance, extended shelf life, and more. These promising outcomes undeniably highlight the need to continue our ongoing efforts and establish a gene bank in Serbia. The establishment of such a bank would involve developing protocols for the preservation of this exceptionally valuable germplasm. In the early 1990s, the technique of 'slow growth storage' was successfully implemented for the short- and medium-term preservation of various fruit species, including *Fragaria* × *ananassa* (Wilkins et al., 1988; Reed, 1992), *Prunus cerasus* (Borkowska, 1986; 1990), and *P. persica* (Leva et al., 1992). This preservation method allows for the development of what is known as 'stock plant material', which can be propagated as needed. It involves reducing the metabolic activity of *in vitro* cultures by modifying the composition of the nutrient medium and adjusting the cultivation conditions for *in vitro* shoots (Lambardi & Ozudogru, 2013). By increasing the time period between consecutive culture transplantations, the duration of each subculture is extended. This approach enhances the safety of conservation by reducing disruptions to the culture system and minimising the risk of contamination during the subculture process (Benelli et al., 2022). Recent reports have also highlighted the application of slow growth storage in the conservation of temperate fruit germplasm, demonstrating its effectiveness in *Malus* spp. (Monticelli et al., 2020; Kabylbekova et al., 2021), *Prunus* spp. and *Prunus* interspecific hybrids (Sota & Kongjika, 2014; Gianní & Sottile, 2015; Arbeloa et al., 2017; Ozudogru et al., 2017), *Pyrus* spp. (Sedlak et al., 2019; Turdiyev et al., 2020), *Rubus* spp. and *Ribes* spp. (Turdiyev et al., 2020).

Since its establishment, the Tissue Culture Laboratory at the Fruit Research Institute in Čačak has successfully utilised the cold storage technique, performed at +5°C in complete darkness, to create *in vitro* collections of different temperate fruit tree species. These collections are intended for short- and medium-term preservation, exchange, and potential rapid propagation when circumstances require it. This technique has proven effective for preserving vegetative rootstocks for cherry and sour cherry such as 'Gisela 5' (*P. cerasus* × *P. canescens*) and 'Tabel Edabriz' (*P. cerasus*) (Ružić & Cerović, 1999; Ružić et al., 2015a). It has also been successfully applied to indigenous plum (*P. domestica*) cultivars including 'Požegača', 'Sitnica', and 'Crvena Ranka' (Ružić & Cerović, 1990; Ružić et al., 2012; Vujović et al., 2020), myrobalan plum (*P. cerasifera* Ehrh.) (Ružić et al., 2015b), and apple (*Malus* × *domestica* Borkh.) (Ružić et al., 2016). Furthermore, investigations have been conducted on the preservation of encapsulated shoot tips using 'synthetic seed' technology under cold conditions, particularly within the *Rubus* genus (Ružić et al., 2011).

The aim of this investigation was to examine the multiplication capacity of *in vitro* shoot cultures and encapsulated shoot tips following cold storage at +5°C in complete darkness, through successive subcultures.

Materials and Methods

Plant material used in the experiments. Highly valuable genotypes were selected for the introduction of *in vitro* methods into fruit preservation. These genotypes were either required to have fully developed protocols or to be studied from the perspective of their preservation capacity and their introduction into modern repositories such as plant gene banks.

'Požegača', a large-scale cultivated plum believed to originate from Asia, used to be a major plum cultivar in Serbia with 38% coverage. However, due to its susceptibility to plum pox virus, it is sparsely grown nowadays. Intensive efforts are being made to work on clonal selection of this cultivar, particularly focusing on self-fertile large-fruited selections.

'Gisela 5', a cherry rootstock, is an interspecies hybrid of *Prunus cerasus* and *Prunus canescens*. It is widely recognized as a leading low-vigorous rootstock that effectively limits tree growth while promoting high yield per tree, large fruits, and overall tree health.

'Tabel Edabriz' is a cherry rootstock, selected by INRA and Ctifl in the late 1980s as one of the first dwarfing rootstocks for sweet cherry orchards in France. It is a clone of *Prunus cerasus*. Trees grafted on Tabel Edabriz exhibit early fruiting and good crop yields.

'Meeker' is a raspberry cultivar derived in the USA from the cross of 'Willamette' and 'Cuthbert'. The fruit is medium-sized and rich in ellagitannins, making it the best source of ellagic acid.

'Čačanska Bestrna' is a blackberry cultivar developed in Serbia through the crossbreeding of 'Dirksen Thornless' and 'Black Satin'. It is a highly vigorous cultivar with very large, shiny black, long-cylindrical berries. The taste is sweet and aromatic, with a pronounced flavour.

Establishment of aseptic cultures of analysed genotypes. Using the standard method of surface sterilisation for initial explants, aseptic cultures were established (Ružić & Cerović, 1990; Ružić et al., 1993; 2011). Shoots of all five genotypes were then regenerated on medium containing mineral salts and vitamins according to Murashige & Skoog (1962) (MS medium), along with 2 mg l^{-1} of 6-benzyladenine (BA), 0.5 mg l^{-1} of indole-3-butyric Acid (IBA), 0.1 mg l^{-1} of gibberellic acid (GA_3), 7 g l^{-1} of agar, and 20 g l^{-1} of sucrose. After the aseptic cultures were successfully established, the shoots were further multiplied using full-strength MS medium with varying hormonal content based on the specific genotype. The shoots underwent *in vitro* multiplication through several subcultures and were subsequently utilised for setting up the trials.

Cold storage (CS) of plum, cherry rootstocks and raspberry. Plum 'Požegača', cherry rootstocks 'Gisela 5' and 'Tabel Edabriz', and raspberry 'Meeker' were stored at +5°C in total darkness. To determine the optimal preconditioning timing for shoots intended to be stored, the plantlets were subcultured for 7, 14, and 21 days prior to transferring them into a cold chamber. The following media were used:

– Plum: full-strength MS medium with 1 mg l^{-1} BA, 0.5 mg l^{-1} IBA, and 0.1 mg l^{-1} GA_3.

– Cherry rootstocks: full-strength MS medium with 1 mg l^{-1} BA, 0.5 mg l^{-1} naphthalene acetic acid (NAA), and 0.1 mg l^{-1} GA3.

– Raspberry: full-strength MS mineral salts and vitamins with a doubled content of $FeSO_4$ x 7 H_2O and 0.5 mg l^{-1} BA.

All media contained agar at 7 g l^{-1} and sucrose at 20 g l^{-1}. After being kept in cold storage, the cultures were transferred to a growth chamber for 3–7 days. Subsequently, viable shoots were transplanted onto fresh medium for multiplication. The multiplication parameters, including the multiplication index, length of the axial and lateral shoots, and number of leaves on the axial and lateral shoots, were determined at various time points. These included before cold storage, immediately after cold storage, and during successive subcultures after cold storage.

The cultures were grown under standard growth conditions before and after the CS, which included a room temperature of 23°C ± 1°C, a 16/8-hour photoperiod (light/dark), and a light intensity of 54 µmol $m^{-2}s^{-1}$.

In the case of cherry rootstocks and raspberry, the total nitrogen content in the medium was determined at various time points, including immediately after autoclaving (control), after 10 (only in raspberry) and 21 days of culturing shoots under standard growth conditions, and upon cold storage. The samples of media were frozen and kept at -20°C until analysis, which was performed according to the Kjeldahl procedure (Sarić et al., 1986).

Encapsulation of blackberry and raspberry shoot tips. A technique described by Lisek & Orlikowska (2004)

was used to encapsulate shoot tips of blackberry 'Čačanska Bestrna' and raspberry 'Meeker' in calcium alginate, followed by cold storage at 5°C in total darkness. Sodium alginate (3%) was dissolved in water containing 4% glucose or 3% sucrose, or in MS medium without growth regulators which contains 4% glucose or 3% sucrose. The encapsulated shoot tips were placed in sterile Petri dishes, sealed with Parafilm, and stored at 5°C in darkness for one, two, and three months. After the specified period, the beads with explants were transferred to appropriate multiplication media and maintained under standard growth conditions for 21 days. For the regrowth of blackberry and raspberry explants, the following media compositions were used, respectively: MS mineral and organic composition with BA 1 mg l^{-1}, IBA mg l^{-1}, and GA_3 0.1 mg l^{-1}; MS mineral and organic composition with doubled Fe resource ($FeSO_4$ x 7 H_2O) and BA 1 mg l^{-1}. The regrowth capacity of the stored explants and the *in vitro* multiplication of shoots in three successive subcultures after cold storage were evaluated. The multiplication parameters were compared to those of the control group (unencapsulated shoots grown under regular *in vitro* conditions).

For each treatment in all experiments, twenty culture vessels with five uniform shoots (or explants) and two replications were used. The data were analysed using ANOVA followed by Duncan's Multiple Range Test for mean separation.

Results and Discussion

CS of plum, cherry rootstocks and raspberry. The cold storage technique proves beneficial when the preservation of a collection under sterile and controlled conditions is of utmost importance. It is particularly suitable in situations that demand rapid propagation, as this technique allows the development of 'stock plant material' that can be propagated as needed. Additionally, cold storage enables the conservation of germplasm in a smaller space, reducing costs and eliminating risks associated with field conditions.

A very high multiplication index of 'Požegača' shoots was obtained after 6 and 10 months of cold storage, as indicated in Table 1. While some shoots exhibited etiolation, no evidence of necrosis was observed even after 10 months. The multiplication index varied depending on the duration of shoot growth under standard conditions before being placed in cold storage. Significantly the highest multiplication index was achieved in shoots cultivated for 21 days under standard growing conditions, followed by storage at 5°C in total darkness for six months (13.8). According to Ružić & Cerović (1990), a 21-day subcultivation period proved to be the most favourable before placing *in vitro* shoots in a cold storage temperature regime. As for shoot length, the highest value of this parameter was observed after a 10-month cold storage period in shoots that were previously cultivated for 7 days under standard conditions (Table 1).

Table 1. The multiplication parameters of plum 'Požegača' assessed with respect to the duration of subculture under standard conditions prior to cold storage (CS) and the duration of CS

Tabela 1. Parametri multiplikacije sorte šljive Požegača u zavisnosti od trajanja subkulture u standardnim uslovima pre skladištenja i dužine skladištenja na hladnom (HS)

	Multiplication index/*Indeks multiplikacije*		Length of shoots/*Dužina izdanaka* (mm)	
Duration of subculture before CS *Trajanje subkulture pre HS*	After 6 months of CS *Posle 6 meseci HS*	After 10 months of CS *Posle 10 meseci HS*	After 6 months of CS *Posle 10 meseci HS*	After 10 months of CS *Posle 10 meseci HS*
7	6.9 b*	4.1 b	7.0 c	22.0 a
14	6.5 b	6.8 b	9.0 bc	16.0 ab
21	13.0 a	5.8 b	11.0 bc	14.0 bc
	p = 0.05		p = 0.05	

*Mean values of each parameter organizated in column pairs followed by the same letter are not significantly different according to Duncan's Multiple Range Test/*Prosečne vrednosti za svaki parametar organizovane u parovima kolona koje su praćene istim slovom nisu statistički značajno raličite prema Dankanovom testu višestrukih intervala*

After a three-month maintenance period in cold storage, the multiplication index of shoots in both investigated rootstocks showed an increase of 1.7 to 4.8 times compared to those grown under standard conditions (Table 2). The multiplication index was better in 'Gisela 5' genotype when shoots were 21 days in CS, as well as shoot lenght of these genotypes was higher after 3 months in CS in comparson to 'Tabel Edabriz' genotype. The shoots exhibited etiolation, and sporadic necrosis was observed on the leaves in the base section. The survival rate was exceptionally high, reaching 100%. Upon transferring the cultures from the cold chamber to standard conditions, the shoots promptly developed and regained their morphology and capacity for multiplication, along with the greening of the leaves.

Upon a three-month maintenance period in cold storage, the shoots of raspberry 'Meeker' appeared very long and etiolated, with a relatively high survival rate of 67.27%. The shoots displayed viable, etiolated stems, but there was evidence of necrosis on leaves in the base section. As a result, the number of viable leaves in shoots kept in cold storage was significantly lower compared to those grown under standard conditi-

Table 2. The multiplication parameters of 'Gisela 5' and 'Tabel Edabriz' shoots after being grown for 21 days under standard conditions (SC) and after 3 months of cold storage (CS)
Tabela 2. Parametri multiplikacije izdanaka vegetativnih podloga za kalemljenje Gisela 5 'and Tabel Edabriz' posle 21 dan gajenja u standardnim uslovima (SU) i posle 3 meseca skladištenja na hladnom (HS)

	Multiplication index/*Indeks multiplikacij*e		Shoot length (mm)/*Dužina izdanaka*	
Genotype *Genotip*	21 days in SC *21 dan u SU*	3 months in CS *3 mecesa HS*	21 days in SC *21 dan u SU*	3 months in CS *3 mecesa HS*
'Gisela 5'	3.2 a*	5.5 a	13.3 a	28.1 a
'Tabel Edabriz'	1.5 b	7.2 a	9.6 a	17.1 b

*Means followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test (p = 0.05)/*Prosečne vrednosti za svaki parametar u svakoj koloni koje su praćene istim slovom nisu statistički značajno raličite prema Dankanovom testu višestrukih intervala (p = 0,05)*

Table 3. The multiplication parameters of raspberry 'Meeker' shoots after being grown for various periods under standard conditions (SC), preserved for 3 months in cold storage (CS), and upon removal from CS
Tabela 3. Parametri multiplikacije izdanaka sorte maline Meeker posle gajenja u standardnim uslovima (SU) tokom različitih vremenskih intervala, posle 3 meseca skladištenja na hladnom (HS) i 21 dan nakon hladnog skladištenja

Treatments *Tretman*	Multiplication index *Indeks multiplikacije*	Length of axial shoot *Dužina osovinskog izdanka* (mm)	Length of axillary shoots *Dužina aksilarnih izdanka* (mm)	No of viable leaves of axial shoot *Br. vijabilnih listova osovinskog izdanka*	No of viable leaves of axillary shoots *Br. vijabilnih listova aksilarnih izdanaka*
10 days under SC *10 dana u SU*	2.0 b*	8.0 c	6.0 b	7.0 a	4.7. a
21 days under SC *21 dan u SU*	2.2 b	8.0 c	6.0 b	7.4 a	5.0 a
3 months in CS *3 mececa HS*	2.2 b	18.0 a	8.0 a	2.9 c	2.5 b
21 days upon removal from CS *21 dan nakon HS*	2.9 a	12.0 b	6.0 b	4.2 b	4.5 a

*Means followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test (p = 0.05) /*Prosečne vrednosti za svaki parametar u svakoj koloni koje su praćene istim slovom nisu statistički značajno raličite prema Dankanovom testu višestrukih intervala (p = 0,05)*

ons. However, the multiplication index was at the same level compared to standard subculturing after 10 and 21 days, while the lengths of axial and lateral shoots were even higher (Tab. 3). Additionally, the multiplication index and length of axial shoots in this genotype were 1.3 and 1.5 times higher, respectively, in the first subculture after cold storage compared to those grown constantly under standard conditions (Table 3).

According to Ružić et al. (2003), raspberry 'Meeker' did not exhibit desirable multiplication capacity on the media used. Therefore, the observed increase in both the multiplication index and vitality of shoots during maintenance in cold storage, as observed in this study, was crucial for successful micropropagation. The tendency of the multiplication index to increase after cold storage was also observed in other cultures, such as sour cherry cultivars (Borkowska, 1990; Petrevica & Bite, 2003), and certain peach cultivars (Leva et al., 1992), among others. This occurrence in microplants is likely stress-induced due to the absence of dormancy, as noted by Borkowska (1996). This further supports the justification for short-term maintenance of raspberry cultures at low temperatures. However, some raspberry cultivars have shown relatively high sensitivity to long-term maintenance at low temperatures, leading to changes in cultural behaviour and a significant decline in multiplication rates (Popescu et al., 2004).

Undoubtedly, nitrogen is one of the essential elements for the mineral nutrition of plants, both *in vivo* and *in vitro*. Nitrogen serves multiple physiological roles, indicating its direct or indirect involvement in various plant processes. In the Murashige & Skoog (1962) medium, nitrogen is one of the most abundant elements, with a concentration of 840 mg l^{-1}. The authors considered nitrogen, along with potassium, to be particularly effective in *in vitro* conditions. McCown & Sellmer (1987) also emphasised the significant stimulating effect of nitrogen on *in vitro* growth. Given that the availability of nitrogen is often limited in many *in vitro* cultures, this paper aims to determine the optimal duration of maintenance of cherry rootstocks and raspberry shoots in cold storage, considering the nitrogen uptake from the medium (Table 4).

Rootstock 'Gisela 5' exhibited a nitrogen uptake of 26.6% from the medium after 21 days of culturing under standard conditions, which increased to 45.4% after three months of cold storage. On the other hand, 'Tabel Edabriz' showed a lower nitrogen uptake of 16% from the medium after 21 days under standard conditions and 38.9% after three months of cold storage. In the case of raspberry 'Meeker', the nitrogen up-

Table 4. The uptake of nitrogen (%) from the medium during different periods of shoot growth under standard conditions (SC) and after cold storage (CS), in relation to the fresh (control) medium, in raspberry and cherry rootstocks

Tabela 4. Količina N (%) preuzeta iz medijuma posle različitih perioda gajenja u standardnim uslovima (SU) i posle skladištenja na hladnom (HS) u odnosu na njegovu količinu u svežem medijumu (kontrola) kod maline i vegetativnih podloga za kalemljenje

	'Gisela 5'		'Tabel Edabriz'		'Meeker'	
Medium origin *Poreklo medijuma*	Quantity of N *Sadržaj N* (g)	N taken up from the medium *Količina N preuzeta iz medijuma* (%)	Quantity of N *Sadržaj N* (g)	N taken up from the medium *Količina N preuzeta iz medijuma* (%)	Quantity of N *Sadržaj N* (g)	N taken up from the medium *Količina N preuzeta iz medijuma* (%)
Fresh medium (control) *Svež medijum (kontrola)*	2.82	–	2.75	–	2.74	–
10 days under SC *Posle 10 dana u SU*	n/p *n/s*	–	n/p *n/s*	–	2.51	8.4
21 days under SC *Posle 21 dan u SU*	2.07	26.6	2.31	16.0	1.64	40.2
3 months in CS *Posle 3 meseca HS*	1.54	45.4	1.68	38.9	1.84	32.8

n/p – not performed/*n/s – eksperiment nije spoveden*

take during cold storage was also relatively low, with only 32.9% of nitrogen absorbed compared to the initial nitrogen quantity in the medium (Table 4). Both sweet cherry rootstocks demonstrated significantly lower nitrogen uptake from the medium throughout the cold storage period compared to standard *in vitro* cultivation conditions. Ružić et al. (2000) reported that 'Gisela 5' absorbed 88% of nitrogen after 40 days of growth under standard *in vitro* conditions. The low uptake of nitrogen from the medium suggests that the addition of nitrogen to the medium is unnecessary when storing raspberry 'Meeker' and cherry rootstocks under cold conditions. It can be assumed that the initial nitrogen content in the MS medium is sufficient for the growth and development of these species, as a substantial quantity of this element remained in the medium even after three months, and the growth and development of plants were not hindered. The slow growth was accompanied by a slow uptake of nitrogen from the medium.

Based on our observations, it is evident that the response to *in vitro* cold storage differs among species and cultivars due to their genetic specificities. In a study by Klavina et al. (2003), it was reported that raspberry cultures, when exposed to cold storage conditions, displayed lower cold resistance compared to cherries. These variations were also reflected in the enzyme activities observed.

Encapsulation of blackberry and raspberry shoot tips. Regarding blackberry 'Čačanska Bestrna' and raspberry 'Meeker', 18.8% and 6.3% of plants, respectively, survived the one-month encapsulation period and exhibited regrowth. However, viable explants were not obtained after three months of cold storage in both genotypes, and only desiccated and dried beads, along with necrotic explants, were observed. In comparison to results obtained for other fruit species, such as the 'M.26' apple rootstock, which achieved over 80% conversion (Standardi & Piccioni, 1997), or *Actinidia*, with conversion rates of over 50% for unipolar explants (Adriani et al., 2000), our results can be described as relatively poor. It is important to note that these are pioneering results obtained in our laboratory and in the country as a whole. In both genotypes regrowth was observed only in alginate beads that contained MS medium with 3% sucrose and lacked growth regulators. The research conducted by Watt et al. (2000) supports the assumption that the presence of sugar in the beads improves the survival of stored explants.

Upon removing the encapsulated explants from cold storage, the multiplication index of shoots regrown from these explants significantly decreased from the first to the third subculture in blackberry. However, it remained comparable to the multiplication index of unencapsulated shoots (Table 5). In contrast, raspberry exhibited a significant increase in this parameter towards the third subculture (Table 6). Similar findings were reported by Lisek & Orlikowska (2004), who observed a generally higher multiplication rate in strawberry during the second subculture after cold storage of encapsulated shoot tips compared to non-stored cultures, whereas raspberry multiplication was lower in the second subculture. In the third subculture, shoot multiplication in both species was similar to that in non-stored cultures (Lisek & Orlikowska, 2004).

Table 5. The multiplication parameters from 3 successive subcultures after retrieval of encapsulated shoot tips of blackberry 'Čačanska Bestrna' from cold storage (CS)

Tabela 5. Parametri multiplikacije u 3 uzastopne subkulture nakon hladnog skladištenja (HS) inkapsuliranih vrhova izdanaka sorte kupine Čačanska bestrna

Subculture after CS *Sukultura posle HS*	Multiplication index *Indeks multiplikacije*	Length of axial shoot *Dužina osovinskog izdanka* (mm)	Length of axillary shoots *Dužina aksilarnih izdanaka* (mm)
I	8.0 a*	8.3 ab	5.0 b
II	5.0 b	7.8 b	5.2 b
III	4.8 b	8.2 ab	5.2 b
Control/*Kontrola*	5.0 b	9.0 a	6.6 a

*Means followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test (p = 0.05) / *Prosečne vrednosti za svaki parametar u svakoj koloni koje su praćene istim slovom nisu statistički značajno različite prema Dankanovom testu višestrukih intervala (p = 0,05)*

Table 6. Multiplication parameters from 3 successive subcultures after retrieval of encapsulated shoot tips of raspberry 'Meeker' from cold storage (CS)
Tabela 6. Parametri multiplikacije u 3 uzastopne subkulture nakon hladnog skladištenja (HS) inkapsuliranih vrhova izdanaka sorte maline Meeker

Subculture after CS *Sukultura posle HS*	Multiplication index *Indeks multiplikacije*	Length of axial shoot *Dužina osovinskog izdanka* (mm)	Length of axillary shoots *Dužina aksilarnih izdanaka* (mm)
I	2.0 b*	6.0 c	5.0 a
II	2.0 b	7.2 b	5.0 a
III	4.0 a	7.0 b	5.5 a
Control/*Kontrola*	2.2 b	8.7 a	5.0 a

*Means followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test (p = 0.05)/ *Prosečne vrednosti za svaki parametar u svakoj koloni koje su praćene istim slovom nisu statistički značajno raličite prema Dankanovom testu višestrukih intervala (p = 0,05)*

Conclusion

Our results have demonstrated that *in vitro* propagated plum, cherry rootstocks, and raspberry can be successfully stored under common cold conditions (at +5°C, in darkness) to achieve a high survival rate.

The low uptake of nitrogen from the medium suggests that there is no need to add this element to the medium when kept under chilling conditions. It was observed that slow growth is accompanied by a slow uptake of nitrogen from the medium.

Short-term maintenance in cold storage can be employed to improve multiplication rates and shoot length by replacing the natural dormancy period.

The encapsulation method used in this study requires further development or experimentation, as it yielded a low conversion rate.

These findings provide a solid foundation for the development of a standardised protocol for the maintenance of *in vitro* fruit germplasm in cold storage. This protocol could contribute to the revitalization and further development of the national fruit gene bank.

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PRAĆENJE KAPACITETA ZA MULTIPLIKACIJU IZDANAKA RAZLIČITIH VOĆAKA NAKON KONZERVACIJE PRIMENOM TEHNIKE HLADNOG SKLADIŠTENJA *in vitro*

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Rezime

U ovom radu su prikazani rezultati *in vitro* čuvanja kultura voćaka korišćenjem strategije hladnog skladištenja i sposobnosti izdanaka za multiplikaciju nakon konzervacije. U istraživanja je bilo uključeno pet genotipova različitih vrsta voćaka: sorta šljive Požegača, vegetativne podloge za trešnju i višnju Gisela 5 i Tabel Edabriz, sorta maline Meeker i sorta kupine Čačanska bestrna. Sorta šljive Požegača je uspešno čuvana do 10 meseci, dajući izdanke sa visokim indeksom multiplikacije koji je bio posebno izražen kod izdanaka uzgajanih 21 dan u standardnim uslovima, a zatim skladištenih na +5°C u potpunom mraku tokom šest meseci. Podloge za kalemljenje trešnje i višnje Gisela 5 i Tabel Edabriz su takođe uspešno očuvane na isti način tokom tri meseca, a stopa preživljavanja *in vitro* izdanaka je iznosila 100%. Nakon skladištenja u hladnim uslovima, indeks multiplikacije izdanaka kod vegetativnih podloga Gisela 5 i Tabel Edabriz je bio povećan od 1,7 do 4,8 puta po redosledu u odnosu na izdanke uzgajane u standardnim uslovima. Stopa preživljavanja *in vitro* izdanaka maline sorte Meeker je takođe bila visoka (iznad 67%) u hladnim uslovima. Kod ovog genotipa, kao i kod obe vegetativne podloge, spor rast je pratio sporu apsorpciju azota iz podloge. Vrhovi izdanaka sorte maline Meeker i kupine Čačanska bestrna su takođe bili inkapsulirani u kalcijum-alginatne kuglice i čuvani u hladnim uslovima (na +5°C, u potpunom mraku). Samo 18,8% vrhova izdanaka sorte Čačanska bestrna i 6,3% vrhova izdanaka sorte Meeker, koji su bili inkapsulirani u kalcijum-alginatne kuglice, preživeli su jednomesečni period hladnog skladištenja i dali regeneraciju vijabilnih izdanaka u *in vitro* uslovima. Međutim, izdanci su bili sposobni za multiplikaciju u sukcesivnim subkulturama nakon regeneracije, a indeksi multiplikacije su bili veći u odnosu na kontrolne izdanke koji su konstantno gajeni u standardnim uslovima u komori za gajenje *in vitro* biljaka.

Ključne reči: germplazma voćaka, *in vitro* skladištenje na hladnom, inkapsulacija, preživljavanje, multiplikacija.

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