PRUNUS SERRULATA VAR. 'KANZAN' MICROSHOOTS BEHAVIOUR DURING *IN VITRO* ROOTING AND ACCLIMATIZATION PHASES

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Abstract
The paper presents aspects regarding in vitro rooting and acclimatization percentages of Prunus serrulata var. Kanzan vitroplants. During in vitro rooting stage, good results were obtained using a nutrient medium with the following composition: halved Murashige - Skoog mineral salts, Linsmaier – Skoog vitamins, gibberellic acid (GA) 0.1 mg/l, indolelacetic acid (IAA) 1.0 mg/l, activated carbon 0.3 mg/l, iron chelate 38 mg/l, 30 g/l dextrose, 7 g/l agar. The substrate recommended for acclimatization of in vitro plants is composed of black peat + perlite (1:1), at pH = 6.0.

Keywords: 'Kanzan', auxins, in vivo rooting percentage, acclimatization

1. INTRODUCTION

Japanese cherry represents „*probably the most popular subject of dendrology and ornamental horticulture*" (Kuitert and Peterse, 1999).
Prunus serrulata 'Kanzan' is an IIIrd size tree, with erect growth, then large. Is one of the most beautiful and wide spread Japanese trees (Iliescu, 2008).
Containers modern culture makes demands for Japanese cherry to be in a continuous growing, being used in various conditions: balcony, terrace, greenhouses and gardens, along with other ornamental plants.
Studies on the establishment of *in vitro* biotechnology of ornamental variety *Prunus serrulata* 'Kanzan' presented till this moment described the explants and microshoots evolution during the initiation and multiplication phase (Figure 1).

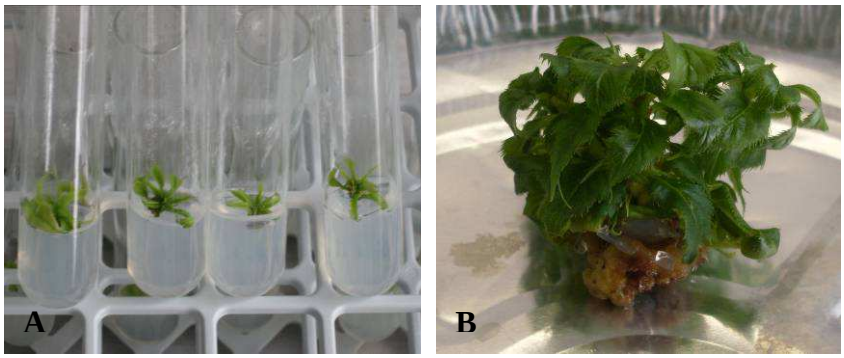

Figure 1. Microshoots obtained in the initiations and multiplication phase:
A – explants at 21 days after inoculation; B - Microshoots after 30 days after multiplication (original)

The research continued with the transfer of microshoots on rooting nutritive media, followed by their acclimatization.

2. MATERIAL AND METHOD

Development of the paper is based on the own results conducted in the laboratory of *Horticultural Plants Micropropagation, Genetic Engineering and Vegetal Biotechnologies,* from the University of Pitesti, Faculty of Sciences, undertaken between 2007 and 2009.

For *in vitro* rooting of ornamental cherry microcuttings resulted from the *in vitro* multiplication phase were tested six experimental variants (Table 1).

Table 1. Experimental variants for in vitro rooting phase

Variants	**Variable factors**	
	A: Nutrient medium	*B: Photoperiod*
V.1	A.1	B.1
V.2	A.1	B.2
V.3	A.2	B.1
V.4	A.2	B.2
V.5	A.3	B.1
V.6	A.3	B.2

The experience is bifactorial.
Variable factors:

A. The rooting nutrient medium:
- ⇨ A.1;
- ⇨ A.2;
- ⇨ A.3.

B. Photoperiodism:
- ⇨ B.1 – 14 hours photoperiodism;
- ⇨ B.2 – 12 hours photoperiodism.

For all nutrient medium variants, the basic components, macroelements, microelements, vitamins, gibberellic acid, iron chelate, dextrose, agar and activated carbon are constant and have the same concentration, the variable factor is represented by auxin's concentration (AIA) (Table 2).

Table 2. Nutrient media components tested for ornamental cherry in vitro rooting

Components mg/l	**Medium variants**		
	A1	A2	A3
M.S Macroelements	1/2n	1/2n	1/2n
M.S Microelements	1/2n	1/2n	1/2n
L.S Vitamins	n	n	n
Gibberellic acid(mg/l)	0.1	0.1	0.1
AIA(mg/l)	0.5	1.0	1.5
Activated carbon (g/l)	0.3	0.3	0.3
NaFeEDTA(mg/l)	38	38	38
Dextrose (g/l)	30	30	30
Agar (g/l)	7	7	7

Legend: M.S – Murashige & Skoog, L.S - Linsmaier & Skoog

For vitroplants acclimatization, the experience was monofactorial, being tested two nutrient substrates (Table 3).

Table 3. Nutrient substrates tested for ornamental cherry vitroplants acclimatization

Variable A factor Substrate components	**Components proportion**	**pH**
A.1. Black peat + Perlite	1:1	6.0
A.2. Red peat + Perlite	1:1	5.0

During acclimatization, the rooted shoots were passed on a substrate with light texture, treated with rooting biostimulators, ensuring high atmospheric humidity (80%) and constant temperature.

Results were recorded as a rooting percentage, respectively acclimatization and were processed statistically with Duncan test.

3. RESULTS AND DISCUSSIONS

Analyzing the interaction between the nutrient medium and photoperiodism (A x B), the results obtained have shown that the nutrient medium has a significant influence, the most effective one proved to be A.2 with 82% rooted plants, followed by nutrient medium A.3 with 74% rooted plants (Figure 2).

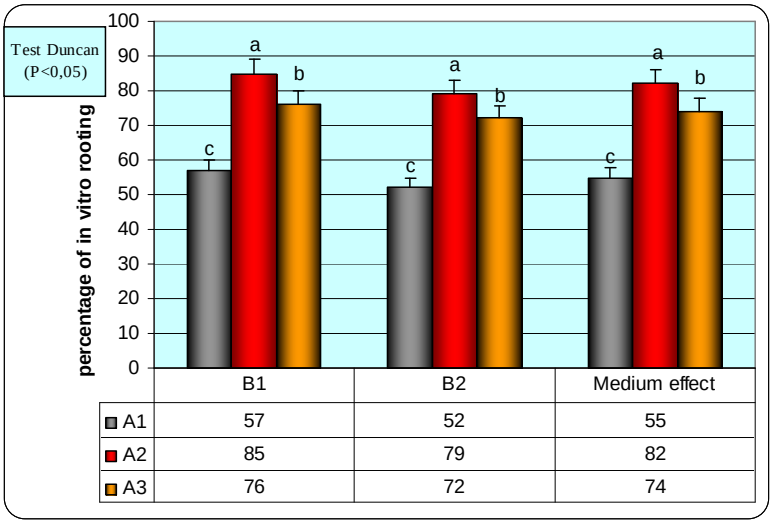

Figure 2. The variation of in vitro rooting percentage depending on the nutrient media, for different photoperiods

Interpreting the data regarding the interaction of each nutrient medium with each photoperiodism graduation it was found that for photoperiodism of 14 hours, the best results with 85.0% rooted plants obtained on nutrient medium A.2. Differences from other nutrient media are significant and provided statistically.

For the 14 hours photoperiodism, the influence of nutrient media A.1, A.2 and A.3 on the *in vitro* rooting process is greater than photoperiodism of 12 hours. In this case we can state that the nutrient media tested are more effective at 14 hours photoperiodism (Figure 3).

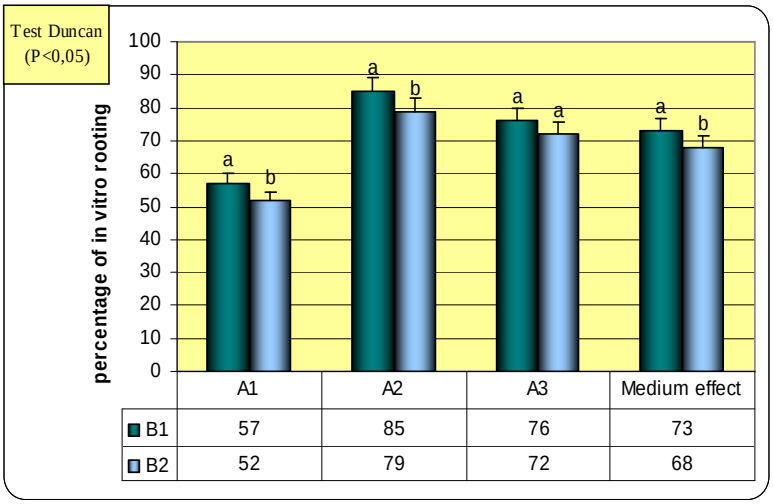

Figure 3. The variation of in vitro rooting percentage depending on the photoperiod for different nutrient media

The rooting period lasted 30 days.
Plants obtained at the end of this *in vitro* culture stage replicate all the vegetative organs of vitroplants (Figure 4).

Figure 4. In vitro rooted plant

Looking at the interaction between the two nutrient mixtures tested as a support for acclimatization of "Kanzan" variety vitroplants, as an average effect, it is found that the highest degree of acclimatization was determined by A.1 nutrient mixture.
The influence of this nutritional support is quantified to 72.0% of plants adapted, the difference from the other prescription being provided statistically (Figure 5).

Figure 5. Plantlets acclimatization on peat and perlite substrate

The explanation of obtaining the highest percentage of acclimatized plants on nutrient medium A.1 is given by the presence in the nutrient composition of black peat and perlite, proportion of 1:1, providing continuous moisture to the substrate, comparable to that of culture vessels where plant roots occurred.
Acclimatization phase was marked by the onset of plantlets active growth, emerging the first leaflets. Acclimatization period lasted 15 days.

4. CONCLUSIONS

Studies regarding the behavior of *Prunus serrulata in vitro* culture led to the following conclusions:
1. For the rooting phase results confirm that the auxins have a substantial role in triggering and sustaining the rhizogenesis process, within certain concentrations.
2. Interpreting the interaction between the nutrient medium and photoperiodism in rooting process, the variant with the highest percentage of rooting is V.3 respectively 85%, on nutrient medium with 1 mg/l IBA and photoperiod of 14 hours.

3. The greatest acclimatization degree has occurred on nutrient mixture A.1, containing black peat and perlite 1:1.

5. REFERENCES

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