IN VITRO PERFECTED PROPAGATION BIOTECHNOLOGY OF *PRUNUS SERRULATA* SPECIES

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Abstract
The results of this study are original because in vivo rooting of microshoots is not reported in both national and international scientific literature for this species. In vivo rooting capacity of 'Kanzan' cherry microshoots was studied according to nutrient substrate, microshoots height and rooting stimulant. From the interaction of the three factors (A×B×C), the 18 experimental variants have revealed that the best in vivo rooting capacity (84%) was when 2.5 to 4.5 cm height microshoots were planted in peat mixed with perlite (1:1) and treated with Radistim.

Keywords: 'Kanzan', microcuttings, percentage of in vivo rooting

1. INTRODUCTION

Taking in account the fact that Romania is involved in a green spaces modernizing process and dendrological product range diversification by new species and varieties introduction, it requires implementation of complex species and varieties breeding programs, quality and productivity improvement to limit the importation of seedlings.

Due to its multiple features *Prunus serrulata* presents a practical interest, both nationally and internationally, it outlines the need to clarify the rapid propagation material production technology by *in vitro* cultures, highlighting in this paper the *in vivo* rooting morphogenetic potential of 'Kanzan' cherry microshoots to improve the micropropagation technology.

2. MATERIAL AND METHOD

The microshoots transfer from the multiplication phase to the *in vivo* rooting phase followed the next methodological operations:

A) Preacclimatization

- out of the growth chamber, the polyethylene film is removed from the culture vessels, to remove the cover and then, this is put back and a few holes are made (Figure 1.A). Thus the prepared culture vessels are covered with wet gauze and held in preacclimatization for 48 hours
- after 48 hours, the protective film is removed and the florets are put in a tray
- the microshoots are individualized with the knife and the basal leaves are removed (Figure 1.B)
- the microshoots are maintained in boxes covered with polyethylene foil (to prevent dehydration) until they are planted in the substrate in the acclimatization room and ensure the necessary conditions (Figure 1.C);

B) Microshoots rooting preparation

- for planting support were used alveolar pallets, each with 21 holes/pallet and raised tables (Figure 1.D);
- before planting, the microshoots were treated with rooting stimulants;
- in each socket has planted a cutting and on tables in rows planting was done in 2 × 7cm;
- after planting the alveolar pallets are passed in the acclimatization room on raised tables, covered with foil and gauze, in a tunnel system;

C) Microshoots rooting

- air humidity was maintained at approx. 80%, handmade with a fine spray;

- one treatment with Topsin 0.1% was effectuated, to prevent installation of pathogens favored by high humidity;
- after completing the first two weeks, relative humidity is gradually reduced and illumination rate is raised by removing the foil and gauze;
- *in vivo* rooting takes about 30 days.

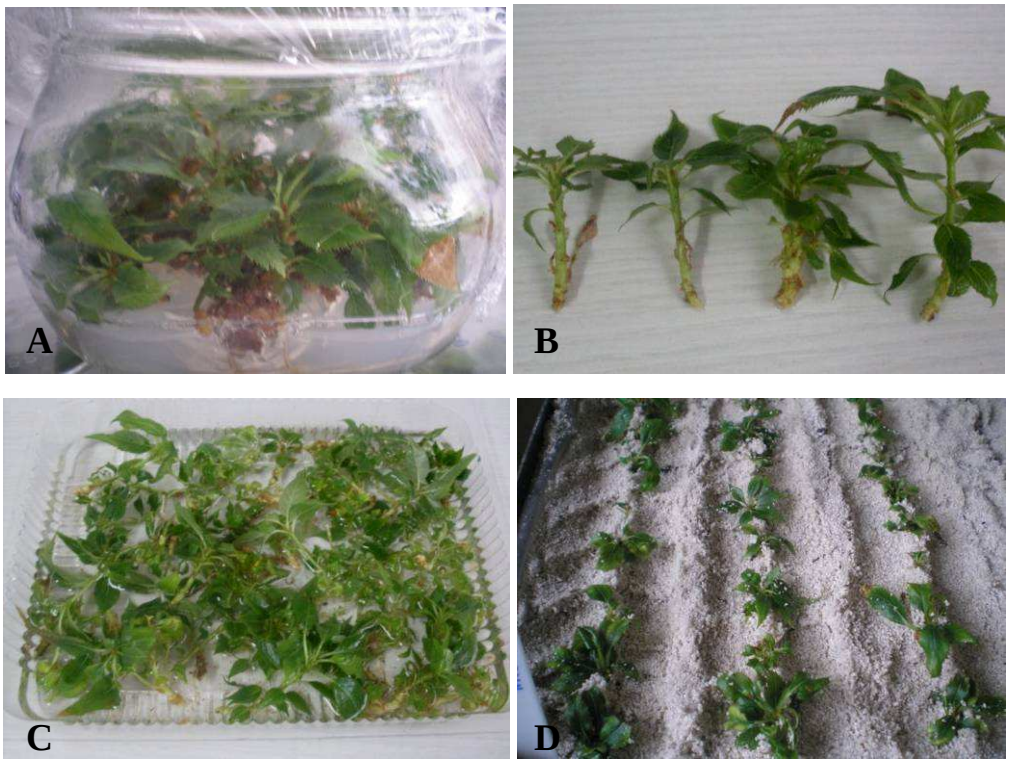


Figure 1. Preparation for in vivo rooting: A – multplied vitroplants; B – microshoots individualization; C – microshoots kept in water; D– substrate planting (original)

For *in vivo* rooting of the microshoots three nutrient substrates were tested (Table 1).

Table 1. Substrates tested for in vivo rooting

Substrate composition	Ratio	pH
Black peat + Perlite	1:1	6,5
Black peat	1	6,0
Perlite	1	7,0

Were organized trifactorial experiences to study the influence of nutrient substrate, microcuttings height, rooting stimulant and *in vivo* rooting capacity. The experiences were 3×2×3 type, with a total of 18 variants, in three repetitions, 10 plantlets/repetition and a total of 540 plantlets/experience.

Variable factors:

A. culture substrate with three graduations:
- A1 – black peat + perlite;
- A2 – black peat;
- A3 – perlite;

B. microcuttings height with two graduations**:**
- B1 – up to 2.5 cm;
- B2 – between 2.5 and 4.5 cm.

C. rooting stimulant with three graduations:
- C1 – Radistim powder;

C2 – ANA 0.1% solution;
C3 – Untreated.

For the statistical calculations was used analysis of variance – the F test (Fisher's exact test). The values of differences were compared calculated values (obtained from the calculation variant differences with average experience), with limit differences for all levels of significance (5% DL, 1% DL and 0.1% DL), establishing the significance of differences between variants (Jităreanu G., 2006).

3. RESULTS AND DISCUSSIONS

The *in vivo* rooting percentage of microshoots ranged between 4 and 84, the highest one - 84% - for the V.4, followed by 77.33% for V.5 (Table 2).

Table 2. In vitro rooting capacity of 'Kanzan' cherry microcuttings

Variant	% *in vivo* rooting
V1:A1.B1.C1	46.67
V2:A1.B1.C2	40
V3:A1.B1.C3	2.67
V4:A1.B2.C1	84
V5:A1.B2.C2	77.33
V6:A1.B2.C3	20
V7:A2.B1.C1	26.67
V8:A2.B1.C2	17.33
V9:A2.B1.C3	4
V10:A2.B2.C1	61.33
V11:A2.B2.C2	46.67
V12:A2.B2.C3	13.33
V13:A3.B1.C1	42.67
V14:A3.B1.C2	34.67
V15:A3.B1.C3	4
V16:A3.B2.C1	72
V17:A3.B2.C2	64
V18:A3.B2.C3	22.67

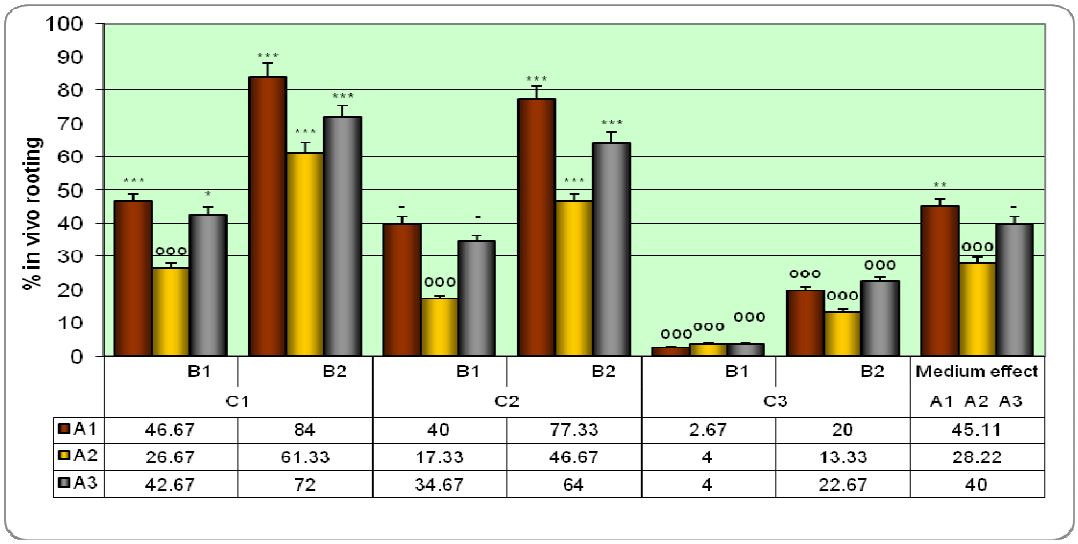
a) Rhyzogenic potential depending on culture substrate according for different heights of microcuttings and rooting stimulants

In Figure 2 is presented the influence of the three factors (A×B×C) on the studied indicator. Analyzing the values of the mean effect, for the three rooting substrates, we find that the highest *in vivo* rooting efficiency is provided by peat with perlite mixture (1:1), where the rooting percentage reaches 45.11%.

'Kanzan' cherry microcuttings obtained at *in vivo* rooting phase the highest percent, 84% respectively, when is used peat and perlite mixture for planting (1:1), the microcuttings had 2.5 to 4.5 cm in height and their base is covered with Radistim.

On the second place, with 72%, are the microcuttings planted in perlite substrate, with 2.5 to 4.5 cm in height and base treated with Radistim.

The lowest value of the studied indicator, 4% respectively, is obtained when used peat as nutrient substrate, microcuttings have 2.5 cm in height and no rooting stimulant.



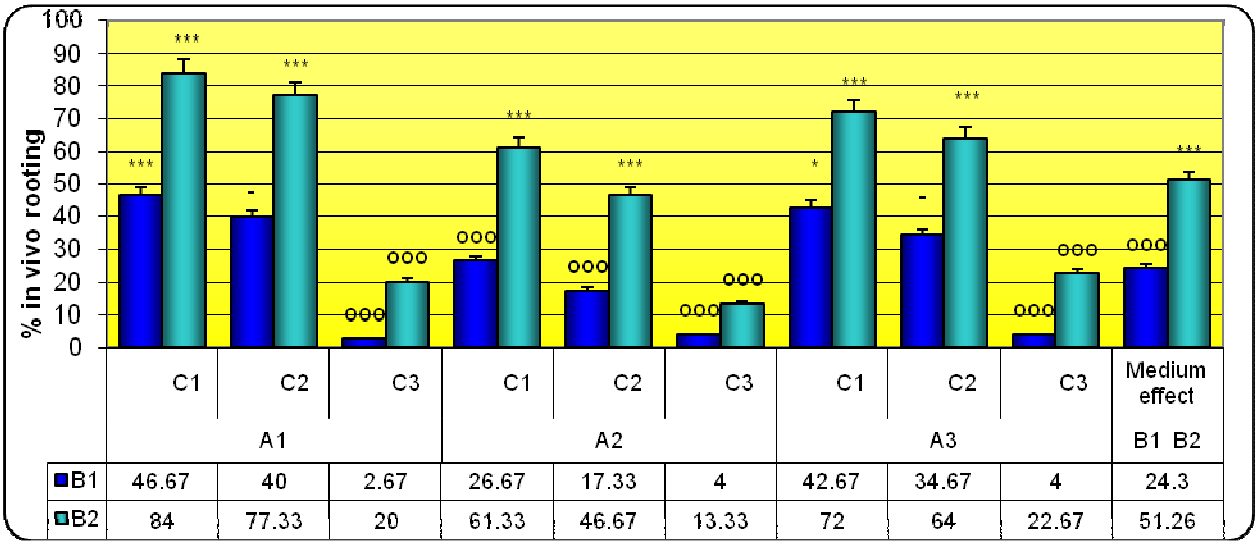
	B1 C1	B2 C1	B1 C2	B2 C2	B1 C3	B2 C3	Medium effect
A1	46.67	84	40	77.33	2.67	20	45.11
A2	26.67	61.33	17.33	46.67	4	13.33	28.22
A3	42.67	72	34.67	64	4	22.67	40

Figure 2. In vivo rooting percent variation depending on culture substrate for different microcuttings heights and rooting stimulants

b) Rhyzogenic potential depending on microcuttings height for different rooting stimulants and culture substrates

The statistical analyze of rooted microcuttings number (%), depending on their height, for the studied rooting stimulants shows that for the average effect of the highest values were obtained when using lengths of 2.5 to 4.5 microbutaşilor cm with 51.26%, differences from other graduations being statistically assured (Figure 3).

Lower percentages were obtained using microcuttings of 2.5 cm (24.3%), the differences being statistically assured.



	A1 C1	A1 C2	A1 C3	A2 C1	A2 C2	A2 C3	A3 C1	A3 C2	A3 C3	Medium effect
B1	46.67	40	2.67	26.67	17.33	4	42.67	34.67	4	24.3
B2	84	77.33	20	61.33	46.67	13.33	72	64	22.67	51.26

Figure 3. In vivo rooting percent variation depending on microcuttings lenght for different rooting stimulants and culture substrates

c) Rhyzogenic potential depending on rooting stimulant for different substrates and different microcuttings heights

Rooting stimulants influence on different levels the process of *in vivo* rooting (Figure 4). The results highlight that Radistim is a good rooting stimulant, with a mean effect on *in vivo* rooting of 55.56%, with differences statistically assured from C3 graduation.

Between the rooting stimulants: 0.1% Radistim and ANA, with 55.56% and 46.67% respectively rooted microcuttings, there are no statistical differences.
Analyzing the interaction between the factors, rooting stimulants and culture substrate, it can be noted a similar trend as the mean effect, but at different scales.
The lowest values of the studied indicator are obtained for C3 graduation, where there were 11.11% rooted microcuttings, the differences being statistically assured from the other factor C graduations.

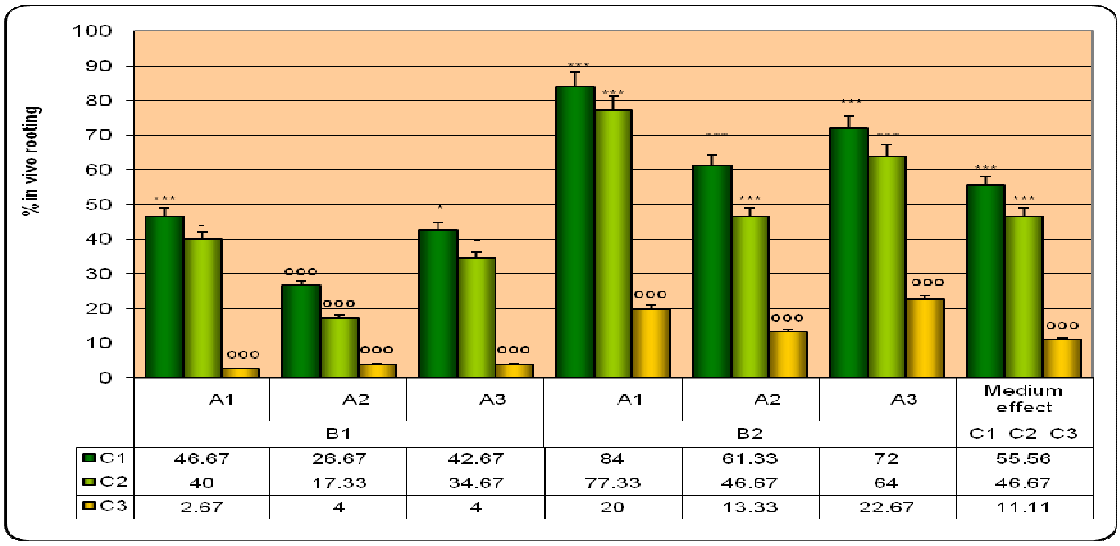


Figure 4. In vivo rooting percent variation depending on rooting stimulant for different rooting substrates and microcuttings height

d) Rhyzogenic potential depending on culture substrate for different rooting stimulants and different microcuttings heights
Analyzing the results obtained regarding the percentage of rooted microcuttings depending on culture substrate for different rooting stimulants (Figure 5), we note that for the mean effect are 45.11% rooted microcuttings on peat and perlite mixture substrate on equal proportions (Figure 6). Differences from substrates A.2 and A.3 in statistically assured. The perlite substrate has values of 40% rooted microcuttings, whose differences are statistically assured.
The worst results were recorded for A3 substrate, with 28.22% rooted microcuttings, statistically assured compared to other substrates.

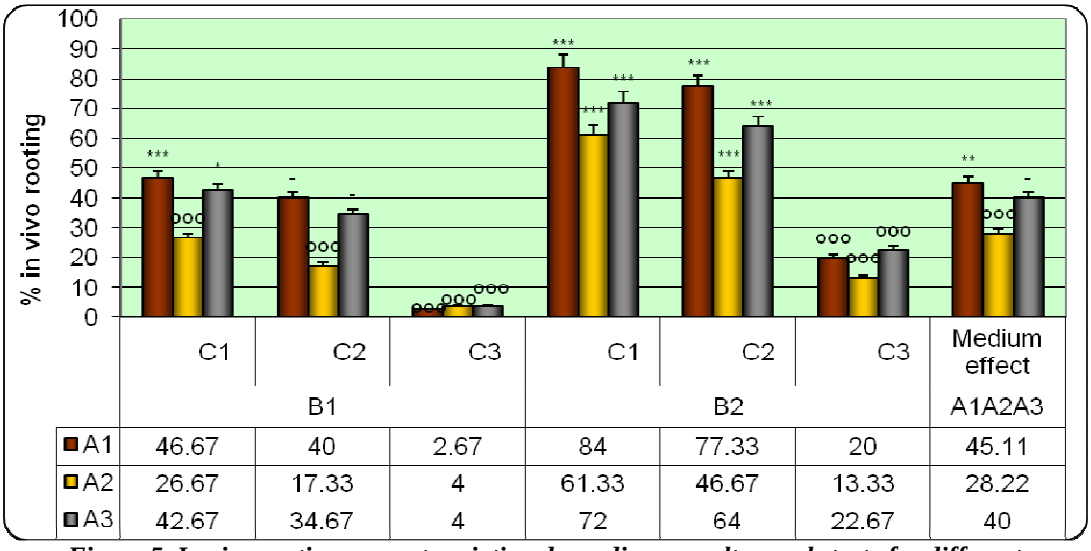


Figure 5. In vivo rooting percent variation depending on culture substrate for different rooting stimulants and microcuttings heights

Figure 6. Rooted microcuttings in mixture of peat and peat and perlite

e) Rhyzogenic potential depending on microcuttings height for different culture substrates and rooting stimulants

From the interaction between microcuttings height factor and culture substrate (B×C) it can be noted that 2.5 to 4.5 cm microcuttings and grown on A1 substrate A1 were obtained the best results, namely 51.3 rooted microshoots. Differences were statistically assured (Figure 7). The lowest values of the mean effect of B factor B were noted for microshoots up to 2.5 cm, 24.3% rooted microshoots respectively.

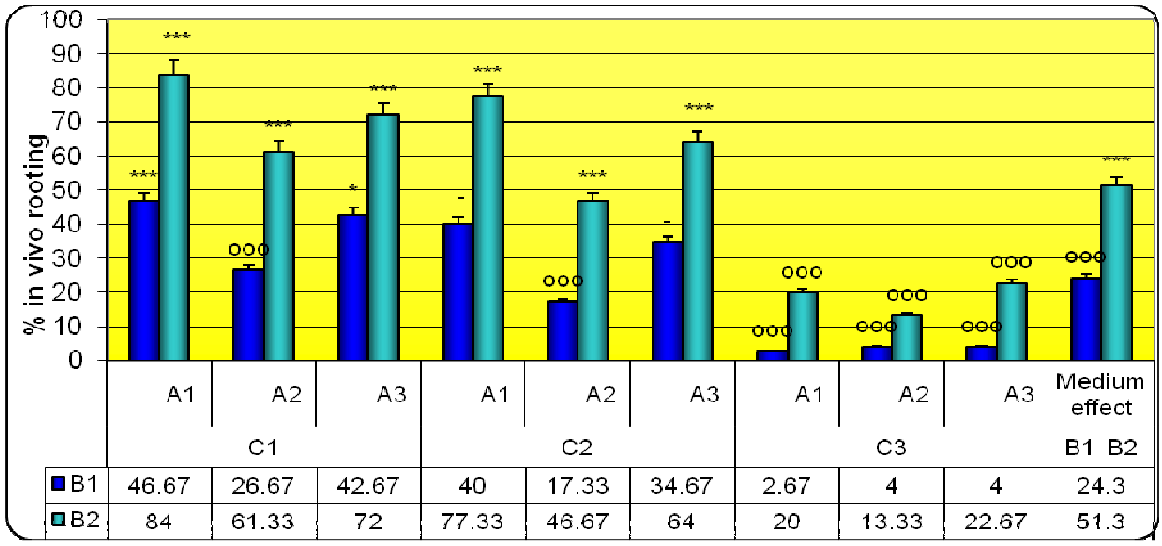
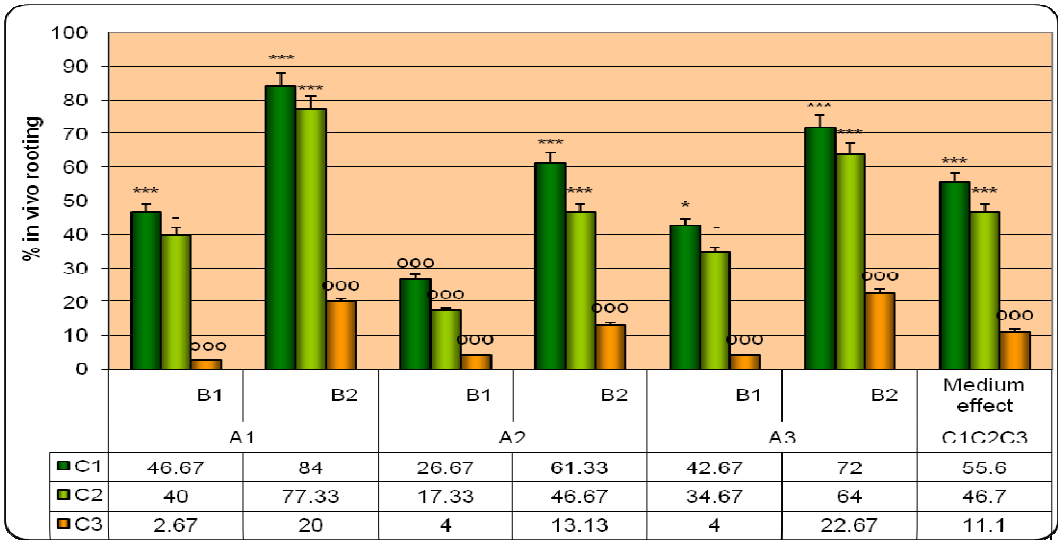


Figure 7. In vivo rooting percent variation and depending on microcuttings height for different culture substrates and different stimulants

f) Rhyzogenic potential depending on rooting stimulant for different microshoots heights and culture substrates

Rooting stimulant influence relative to the mean effect of microcuttings height showed that Radistim, applied to 2.5 to 4.5 cm microcuttings, provides the highest percentage of rooted microcuttings, namely 55.6%, different from 11.1%, as it was obtained when there was no root treatment, the differences being statistically assured (Figure 8). In order, follow graduations C2 and

C3, with 46.7% and 11.1% rooted microcuttings. The worst results - 11.1% - were registered for C3 graduation, with untreated microcuttings.



	B1	B2	B1	B2	B1	B2	Medium effect
	A1		A2		A3		C1C2C3
C1	46.67	84	26.67	61.33	42.67	72	55.6
C2	40	77.33	17.33	46.67	34.67	64	46.7
C3	2.67	20	4	13.13	4	22.67	11.1

Figure 8. In vivo rooting percent variation depending on rooting stimulant for different microcuttings heights and culture substrates

3. CONCLUSIONS

The undertaken study to establish the *in vivo* rooting capacity of 'Kanzan' cherry microcuttings led to a series of observations that allow us to state the following conclusions:

1. For *in vivo* rooting, 'Kanzan' cherry microcuttings had the highest percentage, 84%, when was used a mixture of peat and perlite (1:1), heights from 2.5 to 4.5 cm and Radistim treatments.
2. The second *in vivo* rooting percentage, 72% respectively, was when microcuttings were planted in perlite substrate, with a height of 2.5 to 4.5 cm and treated with Radistim.
3. Using peat substrate, with microcuttings of up to 2.5 cm and 2.5 to 4.5 cm, and without any root stimulants, 1% ANA or Radistim, were obtained the lowest *in vivo* rooting percentages, between 4 and 61.33.
4. *In vivo* rooting can be successfully applied when microcuttings have 2.5 to 4.5 cm in height, on a substrate with perlite and peat mixture and are treated with Radistim.
5. To avoid loss of biological material, at the end of the multiplication phase, only microcuttings with more than 2.5 cm are being transferred for in vivo rooting and those who have less will be reinstated on a multiplication medium.

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