Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 2, 2022
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

USE OF DIFFERENT HORMONES ON *IN VITRO* PROPAGATION OF 'GISELA 5' CHERRY ROOTSTOCK

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Abstract

Gisela 5 is one of the most important dwarfing rootstocks for cherry (Prunus avium) in Central Europe, recommended in very high density plantations. It was obtained by crossing Prunus cerasus 'Schattenmorelle' x Prunus canescens and it is known for its high precocity, dwarfing and productivity. 'Gisella 5' is propagated clonally, using greenwood, soft or hardwood cuttings. In vitro cultures were established using vegetative shoots of 'Gisela 5', and after sterilization and inoculation of explants, multiplication mediums containing various concentrations of macroelements and phytohormones as BAP and GA3 were tested. Rooting capacity of the explants was observed on mediums with different salt concentrations and auxins as IBA, IAA and NAA.

Key words*: auxines, 'Gisela 5', in vitro, micropropagation, rooting, rootstock.*

INTRODUCTION

'Gisela 5', a hybrid between *Prunus cerasus* and *Prunus canescens,* is one of the most important rootstocks for high density cherry (*Prunus avium)* plantings, in terms of the capacity of dwarfing, precocity and production (Zimmerman, 1994; Long & Kaiser, 2010).

Micropropagation of 'Gisela 5' cherry rootstocks maintains the advantages of clonal propagation, while adding on the benefits of micropropagation: possibility to produce high number of plants, production of pathogen-free planting material, all year-round production, genetic fidelity to mother plants.

Most used and efficient cytokinin used in micropropagation of 'Gisela 5' cherry rootstock is BAP, in concentrations of 0.3-0.5 mg/l, depending on the mineral composition of the medium used (Clapa et al., 2013; Borsai et al., 2020). BAP may be used alone, or in combination with gibberellins and auxins (Buyukdemirci, 2008; Thakur et al., 2016; Sharma et al., 2017). Clapa et al. (2013) efficiently propagated 'Gisela 5' cherry rootstock using MS medium supplemented with BAP in concentration of 0.3 mg/l and recommended the use of whole shoots as explants, explants no older than 1.5 or 2 months for both multiplication and rooting stages.

Borsai et al. (2020) recommended the use of MS or DKW medium with 0.3-0.5 mg/l BAP and 4 g/l agar for multiplication stage. Fallahpour et al. (2015), concluded that best results were obtained on WPM and DWK supplemented with 2 mg/l BAP, resulting in the highest percentage of shoot multiplication and number of shoots

When comparing different types of mediums, Nacheva and Gercheva (2009) concluded that sorbitol in the multiplication medium encourages the growth of the lateral buds and the further development of the shoots, when added in a proportion of sucrose: sorbitol 2: 1 or sorbitol: sucrose 1: 2. Most used hormones used in the process of rooting are IBA, in concentrations of 0.5-2 mg/l (Buyukdemirci, 2008; Fallahpour et al., 2015; Borsai et al., 2020), followed by NAA (Tariverdi et al., 2007). The mineral composition of the medium is also an important factor in rooting, as combinations of different concentrations of IBA or NAA with MS, DKW or WPM minerals give different results. Regarding the rooting stage, Fallahpour et al. (2015), recommends the use of WPM supplemented with 2 mg/l IBA, obtaining the

highest rooting percentage (93.70%), when compared to MS (53.1%) and DKW (14.0%). Tariverdi et al. (2007) obtained rooting percentages between 36.03% and 90.83% by supplementing the medium with different concentrations of NAA, the highest percentage being reached at a concentration of 6 mg/l NAA.
High rooting percentages of 94.74% were obtained on DKW medium supplemented with 1 mg/l IBA (Borsai et al., 2020). In the same study, Borsai et al. (2020) concluded that efficient *ex vitro* rooting of the shoots ca be obtained with floating perlite and 1 mg/l IBA with a percentage of rooting and survival of 96.15%. Sharma et al., 2017, established 100% rooting percentage on MS medium with normal salts concentration and 0.5 mg/l IBA, while rooting percentages were lower on mediums with IAA and NAA. Buyukdemirci (2008), observed that when using half strength MS in rooting stage, only the concentration of nitrates should be decreased. Potassium phosphate, magnesium sulphate, calcium chloride and microelements play an important role in rooting and decreasing MS nitrates resulted in the production of more roots per shoot. Thakur et al. (2016), concluded that the percentage of rooting can be increased by using two-step procedure for the rooting stage, with explants being immersed in liquid half-strength MS medium supplemented with 0.5 mg/l IBA, for 24 hours, in dark conditions and then cultured on hormone-free half strength MS.
Micrografting on Gisela 5 rootstock is possible and can be utilized for propagation of cherry threes with successful results (Exadaktylou et al., 2007).
The aim of this research was to develop and efficient procedure of multiplication and rooting of 'Gisela 5', using green wood nodal segments as starting plant material.

MATERIALS AND METHODS

Herbaceous green wood shoots of 'Gisela 5' were collected from Istrița Fruit Research & Development Station of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, in the period between April and June. Nodal segments with at least one bud were cut and sterilized with 70% ethanol for 25-60 seconds, 0.075-0.1% $HgCl_2$ for 7-8 minutes, followed by three rinses with sterile distilled water. Concentrations of disinfection solution and exposure time depended on the period of time when the plant material was collected, the material collected in the last period of the study requiring higher concentration and longer exposure times for successful disinfection. Explants were inoculated on hormone-free MS medium and transferred after two weeks of MS medium supplemented with hormones.

Multiplication

Media used for the multiplication stages was prepared accordingly to Table 1, with pH adjusted to 5.75 before sterilisation in the autoclave at 121°C and at an atmospheric pressure of 1.1 Bar for 20 minutes.

Table 1. Culture mediums used for multiplication stage

Basal salts	Hormones concentration		Agar concentration (g/l)	Sucrose concentration (g/l)
	BAP (mg/l)	GA3 (mg/l)		
DKW	1	0	7	30
DKW	1	0.1	7	30
MS	1	0	7	30
MS	1	0.1	7	30

Rooting

Apical shoots obtained on medium with added GA3, about 15-20 mm in length, were cultivated on MS medium with the concentrations of halved (X/2) or quartered (X/4) supplemented with 1 mg/l NAA, accordingly to Table 2.

Both media used for the rooting stage were prepared accordingly to Table 2, with pH adjusted to 5.75 before sterilisation in the autoclave at 121°C and at an atmospheric pressure of 1.1 Bar for 20 minutes.

Table 2. Culture mediums used for rooting stage

Basal salts concentration	NAA concentration (mg/l)	Agar concentration (g/l)	Sucrose concentration (g/l)	Activated charcoal concentration (g/l)
X/2	1	7	25	1
X/4	1	7	25	1

Acclimatization of explants

Acclimatization was carried 60 days after explants were transferred to the rooting mediums. The rooted shoots were removed from *in vitro* environment and the agar was carefully washed off the roots.

First 2-3 leaves from the bottom part of the explants were removed in order to reduce evapo-transpiraton off shots and roots were shorted to a maximum length of 5-6 centimeters. The shoots were placed in small pots, in sterilised substrate composed of peat, fine sand and perlite. The pots were covered with glass covers in order to keep the humidity high, and they were removed gradually, in order to help adapt the rooted shoots to the normal humidity level.

RESULTS AND DISCUSSIONS

Multiplication

Best results in term of shoot growth were recorded with the mediums supplemented with GA3, with 8.55 mm average shoot length on DKW and 8.31 mm average shoot length on MS (Figure 2). Highest number of shoots per explant were recorded on MS medium supplemented with GA3, average 10 shoots/explant, then DKW mediums, with no significant difference between them, 5.67 shoots/explant on the medium with GA3 and 5.60 shoots/explant on medium only with BAP and the lowest number of shoots per explant, 4.43, was recorded on MS supplemented only with BAP (Figure 3).

Figure 1. 'Gisela 5' explants in multiplication stage

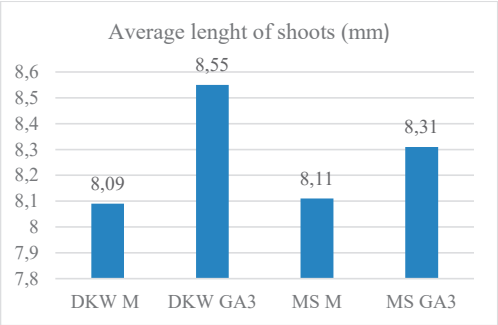

Figure 2. Average length of shoots (mm) on the four variants of mediums used for multiplication

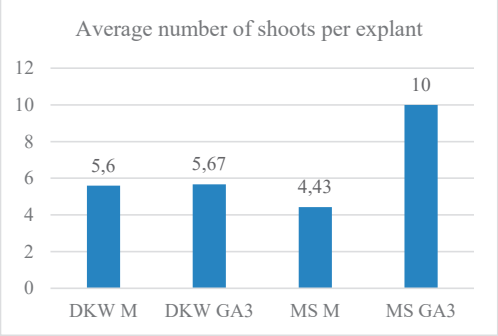

Figure 3. Average number of shoots per explant on the four variants of mediums used for multiplication

Regarding the number of leaves, best results were obtained on MS medium supplemented with GA3, 13.35 leaves/shoot, followed by MS medium with BAP only, 8.55 leaves/shoot. DKW medium showed similar results, 7.40 leaves per shoot on DKW with BAP and GA3 and 7.39 on medium DKW only with BAP (Figure 4).

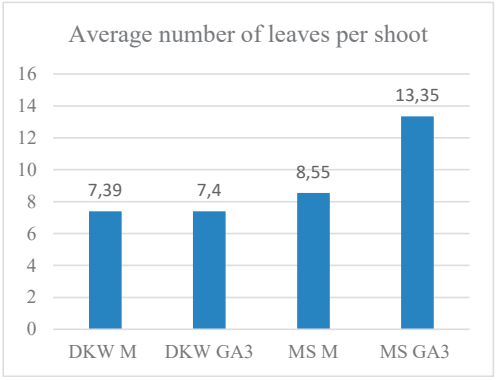

Figure 4. Average number of leaves per shoot on the four variants of mediums used for multiplication

Rooting and acclimatization

High percentages of rooting were achieved on both rooting mediums, with 83.33% on X/4 + 1 mg/l NAA and 76% on X/2 + 1 mg/l NAA.

Figure 5. Roots of shoots cultivated on X/4 medium

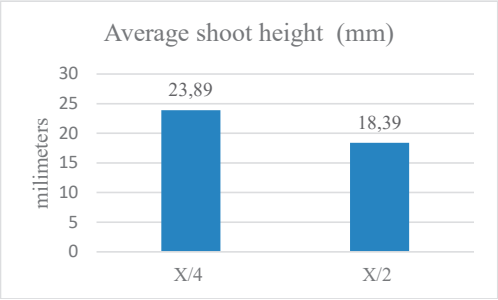

Figure 6. Average shoot height on the two variants of rooting medium

Medium with quartered concentration of salts (X/4) gave better results in terms of average height (23.89 mm on X/4 and 18.39 mm on X/2) (Figure 6), average leaf number (7.83 leaves on X/4 and 5.78 leaves on X/2) (Figure 7)

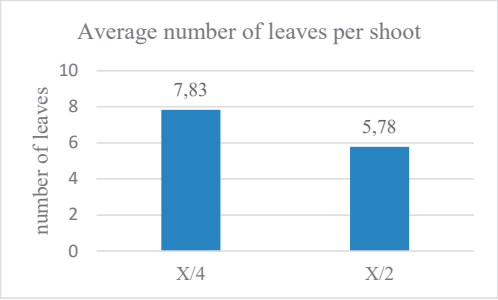

Figure 7. Average number of leaves/shoot on the two variants of rooting medium

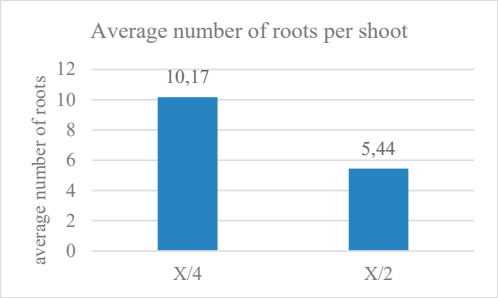

Figure 8. Average number of roots/ shoot on the two variants of rooting medium

Figure 9. Rooted shoot grown on X/4 medium, before acclimatization

Regarding root development and growth, medium X/4 showed better results in term of average number of roots (10.17 roots on X/4 and 5.77 roots on 5.44) (Figure 8). Average length of roots was slightly higher on X/2 (73.62 mm), compared to X/4 (71.67 mm), but we need to consider that the average number of roots on X/4 was much higher (Figure 10).

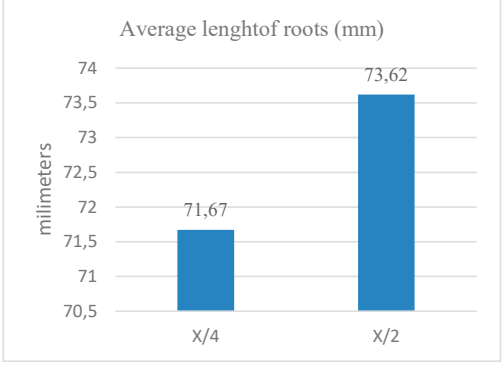

Figure 10. Average length of roots on the two variants of rooting medium

CONCLUSIONS

'Gisela 5' cherry rootstock can be successfully propagated using green wood shoots as a starting material.

Best results for multiplication stage were obtained on DKW medium supplemented with 1 mg/l BAP and 0.1 mg/l GA3. Rooting was successfully achieved on medium MS with quartered macro and microelements concentration, supplemented with 1 mg/l NAA and 3 g/l activated charcoal.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education, the National Council for the Financing of Higher Education, within the CNFIS-FDI-2021-0430, acronym DECIS.

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