

In vitro propagation of Gisela 5 (*Prunus cerasus* × *P. canescens*)

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The main purpose of the presented study was to investigate the techniques for *in vitro* propagation of Gisela 5 (a rootstock for cherry trees). Shoot tips and axillary buds were used as a source for explants. The buds were previously sterilized using two approaches. The first approach was based on dichloroisocyanuric acid and involved 23 buds, of which 95.5% survived. The second approach was based on sodium hypochlorite and involved 21 buds, of which 57.1% survived. Vital sprouts were subcultivated on the multiplication media G_1 (based on MS medium) and G_2 (based on woody plant medium). Out of 40 sprouts 119, sprouts were successfully regenerated on the G_1 medium, and 169 on the G_2 medium. The G_2 culture medium has proved to be better, since the percentage of callused and failed sprouts was lower when compared with G_1 culture medium. *In vitro* developed plants were transferred to four culture media for rooting, which differed in the content of auxines (IBA and NAA). In the culture medium with 0.5 mg/L IBA, the percentage of rooted plants was the highest (90%) compared with medium with 1 mg/L NAA which resulted in the lowest percentage (65%) of rooted plants. Rooted plants were transplanted into the growth substrate and acclimatised using two approaches. Acclimatization in the moist chamber was better when compared to the common growth chamber, since acclimatization in the moist chamber survived more plants (36.4%) compared with acclimatisation in the common growth chamber (11.5%).

Key words: Gisela 5, cherry rootstock, micropropagation, *in vitro* propagation

INTRODUCTION

Gisela 5 is known as a dwarfing rootstock for sweet cherry, developed from the cross between *Prunus cerasus* 'Schattenmorelle' × *P. canescens*. It is considered as very useful and economically important dwarfing rootstocks for intensive sweet cherry growing in temperate conditions. Production, marketing and trade rights for these rootstocks, which originate from a breeding program at the University of Giessen (Germany), are assigned to CDB (Consortium Deutscher Baumschulen), an association of German nurseries. There are very few data about micropropagation of Gisela 5 in the scientific literature.

The *in vitro* growth and development of a plant is determined by interaction of several complex factors: light, temperature, composition of the nutrient media (water, macro- and micro-elements, sugars, growth regulators, vitamins, etc.), gas exchange rate of the cultural vessels, etc (Pierik, 1997).

The aim of our study was to investigate different sterilization procedure for *in vitro* culture initiation of Gisela 5. Two nutrient media, one based on MS (Murashige and Skoog 1962) and another on the McCown woody plant medium (Loyd and McCown 1980), were compared in order to determine the medium with the best multiplication rate. Another aim of our study was to investigate different nutrient media for *in vitro* rooting of the Gisela 5 rootstock, which differed in the content of auxines (IBA and NAA) in order to determine the most suitable medium. One of the aims was also to compare two acclimatisation methods: in a common growth chamber (in a laboratory) and moist chamber (inside a greenhouse).

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MATERIALS AND METHODS

Plant Material

Shoots of Gisela 5 were collected on June 20, 2008 from the experimental field Pohorski dvor, which belongs to the Faculty of Agriculture and Life Sciences, Hoče, Slovenia. In the laboratory for tissue culture, the shoot tips and axillary buds were dissected with a scalpel and used as a source for explants (Fig. 1).

Establishment of *in vitro* culture

Half of explants were sterilised with dichloroisocyanuric acid (DICA) (Aldrich, Germany). Explants were soaked for 15 min in DICA (16.6 g/L) to which 2-3 drops of Tween 20 (a wetting agent) were added and rinsed three times in sterile distilled water. The other half of explants were immersed in 70% ethanol for 40 sec, soaked in 1% sodium hypochlorite for 15 min and rinsed three times in sterile distilled water.

Sterilised explants were placed on the initiation medium which contained the following substances: MS mineral salts supplemented with 100.0 mg/L myo-Inositol, 0.1 mg/L thiamine-HCl, 0.5 mg/L pyridoxine-HCl, 0.5 mg/L nicotinic acid, 2.0 mg/L glycine, 0.1 mg/L IBA, 1.0 mg/L BAP, 0.1 mg/L GA_3, 30 g/L sucrose and 8.0 g/L agar (Plant Agar, Duchefa). The pH was adjusted to 5.7, before autoclaving.

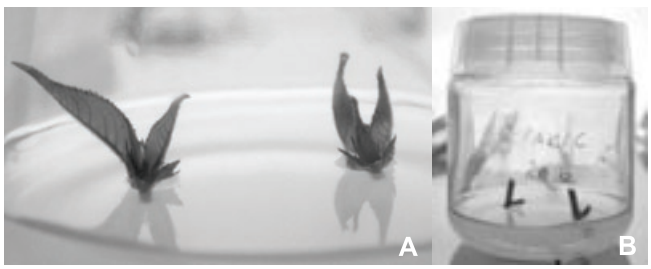

Fig. 1: Inoculated buds: shoot tips (A) and axillary buds (B) (Photo: Puster 2008)

Table 1: Number and percentage of contaminated, non vital and vital explants after sterilisation

Type of sterilisation	Number of inoculated explants	Number and percentage of contaminated explants after 12 days	Number and percentage of non vital explants	Number and percentage of sterile and vital shoots
DICA	23	2 (16.6%)	0 (0%)	21 **(91.3%)**
Sodium hypochlorite	21	0 (0%)	9 (42.9%)	12 **(57.1%)**

The cultures were maintained in growth chamber at 23°C with a 15 hours photoperiod (15.000 Lux). Every three days after inoculation, explants were checked for contamination (Table1). Contaminated and non vital explants were discarded.

MULTIPLICATION

Vital and developed shoots were transferred on two different shoot proliferation media G_1 and G_2 (Table 2). Onto each medium 40 explants were transferred. The number of shoots after 56 days was counted and the multiplication rate was calculated for each medium (Table 3).

Table 2: Shoot proliferation media G_1 and G_2

	G_1	G_2
MS	4.3 g/L	/
McCown	/	2.358 g/L
Sucrose	30 g/L	20 g/L
IBA	0.1 mg/L	/
NAA	/	0.005 mg/L
GA_3	0.1 mg/L	/
BAP	1 mg/L	2 mg/L
Glycine	2 mg/L	2 mg/L
Myo-Inositol	100 mg/L	100 mg/L
Nicotinic acid	0.50 mg/L	1 mg/L
Thiamine	1 mg/L	2 mg/L
Pyridoxine-HCl	0.50 mg/L	1 mg/L
Agar	8 g/L	8 g/L
pH	5.7	5.7

Table 3: Multiplication rate on media G_1 and G_2

Medium	Shoots		Multiplication rate
	Inoculated	Proliferated	
G_1	40	119	2.98
G_2	40	169	4.23

Rooting

For rooting, *in vitro* developed shoots (1.5-3 cm long) of Gisela were placed on four culture media for rooting (R_1, R_2, R_3, R_4), which differed in the content of auxines (IBA and NAA). All rooting media contained the following substances: half of MS mineral salts supplemented with 100.0 mg/L myo-Inositol, 2.0 mg/L thiamine-HCl, 1.0 mg/L pyridoxine-HCl, 1 mg/L nicotinic acid, 2.0 mg/L glycine, 30 g/L sucrose and 8.0 g/L agar (Plant Agar, Duchefa). Four media (R_1, R_2, R_3, R_4) differed in the content of auxines (IBA and NAA) as follows: R_1 with 1.0 mg/L IBA, R_2 0.5 mg/L NAA, R_3 0.5 mg/L IBA and R_4 1 mg/L NAA supplemented. The pH was adjusted to 5.7, before autoclaving.

On each of the four media mentioned before, 20 shoots were placed and maintained in a growth chamber at 23°C with a 15 hours photoperiod (15.000 Lux). The number of rooted plants was calculated after 14, 28, 42 and 56 days after cultivation.

Acclimatisation

All plants with properly developed roots were transferred into the growth substrate (Bio-Potgrond, Klasmann). Before the transfer, roots were carefully washed under tap water to remove all remnants of agar. The transferred plants were acclimatised using two approaches. The first approach was based on acclimatisation in an ordinary growth chamber in the laboratory and the second approach was conducted in a moist chamber inside the greenhouse.

RESULTS AND DISCUSSION

With the sterilisation procedure DICA 91.3% of sterile plants was obtained and after sterilisation with sodium hypochlorite only 57.1% sterile and vital shoots were obtained (Table 1). After the hypochlorite sterilisation procedure, a lot of explants (42.9%) died without contamination, while with DICA sterilisation all explants that were not contaminated survived and produced sterile and vital shoots. Nacheva and Gercheva (2009) obtained from 85 to 100% sterile explants after sterilisation with calcium hypochlorite. Fidanci et al. (2008) report that the most appropriate time for taking explants from Gisela 5 is from the end of April to the beginning of June. Taking explants earlier caused a high level of contamination, whereas browning increased and growth and multiplication rates were reduced, for those collected later.

The results from the testing of two different nutrient media for multiplication show that higher number of shoots were obtained on G_2 medium, based on Mc Cown woody plant medium, where the multiplication rate was 4.23

Fig. 2: Shoots obtained on G_2 medium (A), acclimatised plants (B) and developed roots (C)

compared with G_1 medium where the multiplication rate was 2.98. From 40 sprouts 119 sprouts,t were reproduce on the G_1 medium, and 169 on the G_2 medium. The G_2 culture medium has proved to be better, since the percentage of callused (32.5%) and failed sprouts (0%) were lower than in the case of G_1 culture medium with 60% of callused and 5% failed sprouts.

Table 4: Number and percentage of cultivated, rooted, non rooted and failed shoots on four rooting media

Medium	No. of shoots	Number of rooted plants after				No. and percentage of rooted plants		Number of non rooted plants		Number and percentage of failed shoots	
		14 days	28 days	42 days	56 days	No.	%	No.	%	No.	%
R_1	20	0	4	4	6	14	70	3	15	3	15
R_2	20	2	10	2	2	16	80	1	5	3	15
R_3	20	0	4	5	9	18	90	0	0	2	10
R_4	20	4	8	0	1	13	65	3	15	4	20

Nacheva and Gercheva (2009) investigated the effect of the type and the concentration of carbohydrates (sucrose and sorbitol) added to the nutrient medium with auxins (IBA, NAA and IAA) on the multiplication rate (number of shoots per plant). The best results (multiplication rate of 3.72 - 4.31 at 1.9 cm mean length of the shoots) were achieved in the variants containing 0.005 µM IBA, 20 g/L of sucrose and 10 g/L of sorbitol.

The results from the testing of four different nutrient media for rooting show that in the culture medium R_3 with 0.5 mg/L IBA, the percentage of rooted plants was the highest (90%) compared with medium R_4 with 1 mg/L NAA, where the lowest percentage (65%) of rooted plants was obtained (Table 4). On the R_3 medium, among 20 plants two were contaminated and 18 plants produced roots. On the media R_1, R_2 and R_4, vital shoots without roots have been obtained.

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