EFFECTS OF PLANT GROWTH REGULATORS ON MICROPROPAGATION OF GISELA 6 (*PRUNUS CERASUS* × *P. CANESCENS*) CHERRY ROOTSTOCK

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ABSTRACT

Gisela 6 (*P. Cerasus* × *P. Canesens*) is an economically important cherry rootstock of as increases the effectiveness and productivity of cherry plantations. Given the characteristics of Gisela 6, developing efficient micropropagation protocols allowing mass production under *in vitro* conditions would be important. The effect of various concentrations of growth regulators on *in vitro* micropropagation of rootstock Gisela 6, specifically the effect of BAP for new shoot regeneration and the effect of IBA for rhizogenesis induction is here evaluated. The use of initial explants required lateral shoots originating from virus-free 'mother plants.' The micropropagation coefficient was very high in all concentrations of BAP, but the best results were obtained in BAP 1 mg l^{1-} where the biometric parameters gave the highest values. Also, the rhizogenesis response was highly affected by the concentration of IBA. The highest rooting index was noticed in IBA 2 mg l^{1-} resulting where were observed the highest values for all the monitored biometric parameters, such as rooting percentage, the root number, and the length of the roots. Optimizing this micropropagation protocol enhances the possibility of further developing cherry plantations in our country.
Keywords: *micropropagation, rootstock Gisela 6, rooting, cytokinin, auxin*

1. INTRODUCTION

Gisela 6 is an economically important rootstock as it greatly increases the effectiveness and productivity of cherry plantations. In addition, it adapts very well to the cherry cultivars tested so far, gives good fruit quality, has small plant height, increases planting density, reduces production costs, etc. Moreover, this rootstock adapts very well to different soil types, has bacterial

resistance (Chenglin *et al.*, 2019), and tolerance to viruses. Certified virus-free rootstocks (Saponari *et al*., 1999; Savino *et al*., 2007) ensure genetic stability and create robust root systems to increase post-planting survival in the field, while dwarf or semi-dwarf rootstocks are used to create suitable high-density planting systems (Long and Kester 2010).

In our country, farmers' demand for seedlings grafted on Gisela 6 is increasing, especially in the Korça region, where this rootstock is adaptable to soil and climatic conditions. The industrial production of plant species with commercially significant products now has a valuable commercial future thanks to the use of tissue culture techniques for plant multiplication. But the optimization of the process from the establishment of in vitro culture to large-scale micropropagation and greenhouse production continues to be a problem. The first step is the optimization of a micropropagation protocol under laboratory conditions, with a possibility to further extend this application for large-scale production (Kitto 1997).

In vitro propagation of vegetative cherry rootstocks is an effective method, and is determined by the interaction of several complex factors (Nacheva and Gercheva 2009), such as the composition of nutrient media (water, macro-micronutrients, sugar, hormones, vitamins, etc.), lighting and temperature. Moreover, since it is a technique that produces homogeneous plant material with genetic stability, micropropagation is being widely used to mass-produce plant species of economic importance (Kongjika *et al*., 2002).

There are many reports about the application of *in vitro* techniques, especially for the micropropagation of Gisela 6. The different results provided inform about the adaptability of this rootstock under certain physic-chemical and climatic conditions. Buyukdemirci (2008) tried to optimize a micropropagation protocol testing various types and concentrations of PGRs (IBA, NAA, BAP). Fidanci *et al.* (2008) mentioned the effective use of IBA at 0.5 and 1 mg l^{-1} for rhizogenesis induction.

In this regard, it would be of great interest to optimize a micropropagation protocol for the mass production of Gisela 6, which Albanian farmers can further use. Something like this would help them get these rootstocks faster and easier, and would certainly reduce the cost of transport.

The present investigation study aims to enhance *in vitro* production of this rootstock by optimizing the micropropagation protocol testing various types of BAP and IBA for enhancing specifically in vitro regeneration and rhizogenesis induction.

2. MATERIAL AND METHODS

Plant materials collection and sterilization: For the micropropagation of the cherry rootstock - Gisela 6, the plant material (lateral buds) was taken in the mini-screenhouse of the collection of the fruit trees in the Experimental

Base, ATTC Vlora. The explants were taken from new branches, and their inoculation took place in April 2020 at the *in vitro* laboratory of ATTC. For stabilizing aseptic cultures, the isolated lateral shoots (1.5-2 cm), were left for 30 min. in running water. After, were immersed in ethanol 70% for 10 min, followed by disinfection with NaOCl (sodium hypochlorite) 10% for 25 min. The final step at this stage was rinsing three times with sterilized distilled water.

PGRs combination in each micropropagation stage: the sterilized explants were inoculated in MS nutrient medium (Murashige and Skoog 1962) where four different concentrations (0.5 mg l^{1-}; 1 mg l^{1-}; 1.5 mg l^{1-}; 2 mg l^{1-}) of BAP (6-benzylaminopurine) and IBA (indole 3-butyric acid) were tested in various stages of micropropagation, specifically BAP for *in vitro* regeneration via subcultures, and IBA for rhizogenesis induction. The regeneration response in the subculture stage was evaluated and compared between treatments and with the control variant (BAP-free medium). The nutrient medium was supplemented with sucrose 3% and agar 0.7% in all cases. All the manipulations were performed under aseptic conditions using a laminar flow cabinet.

Growth chamber conditions: the incubation conditions in the growth chamber were as follows: photoperiod parameters 16 hours/light and 8 hours dark, temperature 24 ± 1°C, and phosphorescent lighting 3500 lux.

Data evaluation and Statistical Analysis: In all stages of micropropagation, the data were obtained after eight weeks. During the proliferation and subculture stage, the number and length of shoots were evaluated. Meanwhile, the number and length of roots were evaluated for the rhizogenesis stage. In all cases, morphometric parameters were also observed and evaluated. Data analysis for all experimental tests was done by analysis of variance ($P < 0.05$) Test (ANOVA). Data are presented as averages accompanied by a standard error and standard deviation. Mean comparisons were made using the Tukey-Kramer test. Statistical analysis was realized with the statistical program JMP version 16.0.

3. RESULTS AND DISCUSSIONS

In vitro regeneration stage

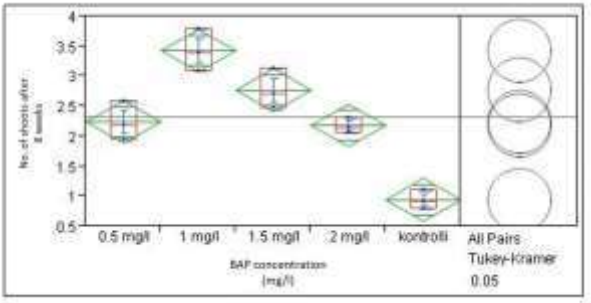
In the proliferation phase, the effect of different concentrations of the cytokinin BAP on shoot number regeneration for Gisela 6 was studied. Cytokinins are PGRs that inhibit apical dominance and stimulate lateral bud development (Salisbury *et al*., 1992; Taiz *et al*., 2006). The role of cytokinins is influential when combined with auxins in various ratios to stimulate growth and morphogenesis. The best concentration of BAP in the proliferation stage resulted at 1 mg l^{-1} where after eight weeks, were observed the highest values of shoots number (3.43) and shoots length (3.65 cm) (Fig.1). Similar results on

BAP effect at 1 mg l^{-1} for lateral shoots development were also reported for *Prunus* genus in MS nutrient medium (Tabachnik & Kester., 1977; Kamali *et al.*, 2001).

Fig. 1: Gisela plant 6 in proliferation phase BAP (1 mgl^{-1}).

Cultivation under different concentrations of BAP shows high differences in the biometric parameters monitored (number and length of shoots). The optimal concentration of BAP resulted at 1 mg l^{-1}, where the shoot number and length values were specifically 3.43 and 3.65 cm. This result shows a positive correlation between BAP concentration at $1mgl^{-1}$ and the number of shoots. It was reported that as the concentrations of BAP increase by more than 1mg l^{-1}, the proliferation values of the explants decrease due to the high concentrations of cytokinins in the nutrient medium stimulating the formation of small buds, which fail to develop further. The data about the impact cytokinins on the in vitro shoots regeneration of Gisela 6 underwent statistically the analysis of variance (ANOVA), and the results are significantly different (Graph. 1 and 2).

Concentrations of BAP (mgl^{-1})	Mean
Control	0.95 c
0.5 mgl^{-1}	2.25 b
1 mgl^{-1}	3.43 a *
1.5 mgl^{-1}	2.78 ab
2 mgl^{-1}	2.19 b
Mean	2.32



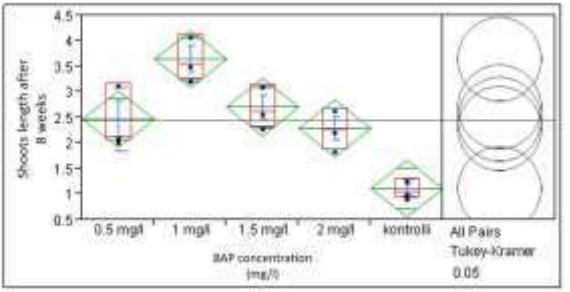
* a = highest level of truthfulness and b lowest level of truthfulness for DMV = 2.792013 for alpha @ = 0.05 according to the Tukey-Kramer test.

Graph. 1: Box plots (variances, standard deviation and mean) for number of shoots according to concentrations (mgl^{-1}) of BAP after 8 weeks.

Analysis of variance on estimating the number and length of shoots in the test according to BAP concentrations in the proliferation phase shows statistically proven differences for the level of authenticity P = 0.05 according to the Tukey-Kramer test. The changes for the shoots number parameter are illustrated in Graphic 1, where BAP concentrations have statistically proven variability for the probability level (P = 0.05), represented by blue circles and BAP concentrations (0.5 mg l^{-1} and 2 mg l^{-1}) and the control variant (BAP-free medium), are below the overall mean value 2.32.

Analysis of variance on the estimation of shoot length in the test according to BAP concentrations in the proliferation phase also shows statistical differences for the level of authenticity P = 0.05 according to the Tukey-Kramer test (Graph. 2) for probability level (P = 0.05). In this case, BAP at 2 mg l^{-1} and the control variant for the shoot length parameter are below the overall mean value 2.44.

BAP concentration (mg / l)	Mean
Control	1.11 c
0.5 mgl – 1	2.47 b
1 mgl – 1	3.65 a *
1.5 mgl – 1	2.71 ab
2 mgl – 1	2.29 b
Mean	2.44



*a = highest level of truthfulness and b lowest level of truthfulness for DMV = 2.792013 for alpha @ = 0.05 according to the Tukey-Kramer test.

Graph. 2: Box plots (variance, standard deviation and mean) for shoot length according to BAP concentrations (mgl^{-1}).

Cytokinins stimulate cell division and lateral buds' formation, thus promoting shoots proliferation (Dobranszki and Silva 2010). The influence of cytokinins in tissue and organ culture depends on the nutrient medium, species, and explant age (Thorpe *et al*., 2008). Bud proliferation and elongation are stimulated by combining cytokinin BAP with auxin ANA (0.1 mgl^{-1}) (Rodriguez *et al*., 1993; Scarpa *et al.*, 2000; Damiano *et al*., 2008).

De Oliveira *et al.*, 2010 reported that at BAP concentrations higher than 1mgl^{-1}, the number of shoots decreases, probably related to the reducing effect of high concentrations of BAP, which may cause callus formation in tissue cultures, thus decreasing shoots proliferation Similar effects have been reported in (Abbott *et al*., 1976; Welander, 1985; Saponari *et al*., 1999; Pruski *et al*., 2000) in the proliferation of the *Prunus* species. Damiano *et al*., (2008) have reported similar results.

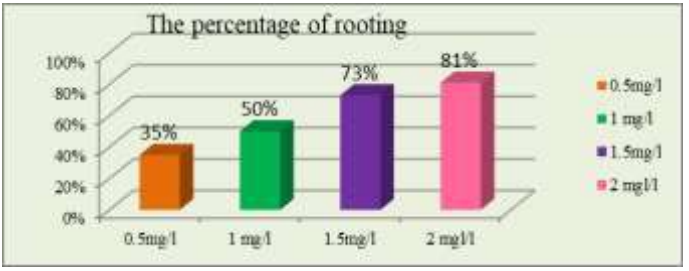
Rooting phase

Once mass production during the subculture stage occurred, the obtained plantlets were transferred in a rhizogenesis induction medium where different concentrations of the IBA auxin were tested for rooting response of the shoots.

The observation and measurements for the rhizogenesis induction process were made after 20 days of culture. Based on the analysis of variance, statistically significant differences (Table 3 and 4; Diagram 3 and 4) for different concentrations of IBA are observed in the rooting phase. The control variant (free-IBA medium) resulted without no root formation. Meanwhile, the highest rooting index (81%) was obtained on rooting media containing IBA at 2 mg l^{-1} (Fig. 2). This concentration allows for the highest number of roots (3.9) and root length (4.3 cm). The rooting percentage for all IBA concentrations is in Graphic 3 plotted, whereas the mean values and the

statistical analyses are plotted in Graphic 4 for the roots number parameter and in Graphic 5 for the roots' length parameter.

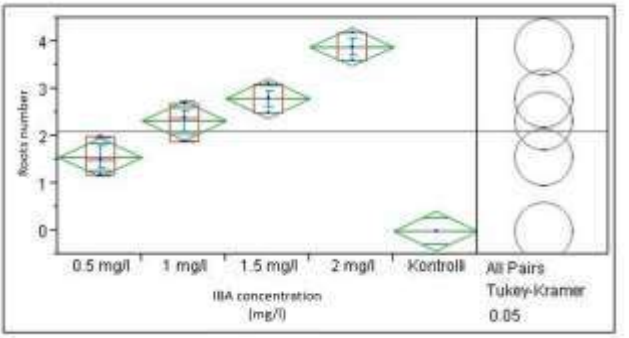
Fig. 2: Rooting phase **a)** at AIB 1 mg l^{-1} **b)** at AIB 2 mg l^{-1}
c) Acclimatization phase AIB 2 mg l^{-1}.



Graphic 3. Gisela 6 rooting rate in different IBA concentration.

Analysis of variance on the estimation of the number of roots in the test according to IBA concentrations in the rooting phase shows that there are statistically significant differences for the level of authenticity P = 0.05 according to the Tukey-Kramer test. Comparisons of the means for the studied treatments are presented by different letters illustrated in Graphic 4.

AIB concentration (mgl $^{-1}$)	Mean
Control	0 d
0.5 mgl – 1	1.56 c
1.0 mgl – 1	2.33 bc
1.5 mgl – 1	2.8 b
2.0 mgl – 1	3.9 a *
Mean	2.12

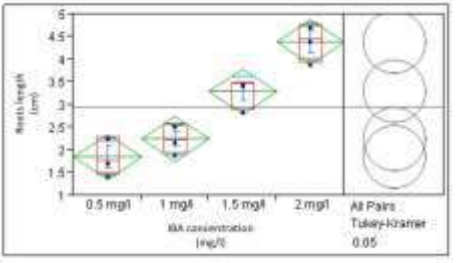


*a = highest level of truthfulness and b lowest level of truthfulness for DMV = 2.792013 for alpha @ = 0.05 according to the Tukey-Kramer test.

Graphic 4. Box plots (variances, standard deviation and mean) for the number of roots according to AIB concentrations (mg l^{-1}).

Analysis of variance on estimating the roots length and means comparisons for the studied treatments in different concentrations of IBA, also present significant differences on the obtained values in different classes according to the Tukey-Kramer test. These changes are illustrated in Graphic 5, where IBA doses show variability for the probability level (P = 0.05). It is observed that the roots length values obtained in MS medium supplemented with IBA at 0.5 mg l^{-1} and 1 mg l^{-1}, are below the overall mean value 2.95.

AIB concentration (1mgl $^{-1}$)	Mean
0.5 mgl – 1	1.86 c
1 mgl – 1	2.25 b
1.5 mgl – 1	3.30 b
2 mgl – 1	4.39 a *
Mean	2,955



*a = highest level of truthfulness and b lowest level of truthfulness for DMV = 2.792013 for alpha @ = 0.05 according to the Tukey-Kramer test.

Graphic 5. Box plots (variances, standard deviation and mean) for root length according to AIB concentrations (mg l^{1})

Other authors have reported similar results, for example, in the rooting of GF-677 rootstocks at IBA concentrations at 2 mg l^{-1} (Sepahvand *et al.*, 2012),

of the species *Prunus avium* L. (Shatnawi *et al*., 2007), *Junglas regia* L. shoots (Olate *et al*., 2009), in the rooting of plum (Hossain *et al*., 2003), etc.

Acclimatization phase

Gisela 6 rootstock plants grown *in vitro* were transferred to peat and perlite pots in the acclimatization greenhouse, under *in vivo* autotrophic conditions, high light intensity and intense water stress. Rooted plants resulted in high survival (78%).

4. CONCLUSIONS

Plant micropropagation is a means to address plantlets of high quantity. The method has nowadays become crucial for commercial applications, especially for Gisela 6 rootstock, an *important cherry rootstock of Central Europe.* The data of this study on the hormonal effect on the micropropagation and rooting of the Gisela 6 cherry rootstock resulted in several conclusions. The exogenous application of cytokinins and auxins, is effective respectively, on the obtaining of a high micropropagation coefficient and *in vitro* rhizogenesis of new plantlets. During the proliferation stage, the highest number of shoots was recorded in MS culture medium supplemented with BAP at 1mgl-1, thus stimulating growth and morphogenesis. In the rooting phase, the highest rooting index (81%) was recorded in MS medium supplemented with IBA at 2 mgl-1, resulting in the most optimal concentration compared to other treatments. Optimization of this micropropagation protocol for mass production of Gisela 6 rootstock is a means to address further development of cherry plantations in our country.

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