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EFFECTS OF DWARFING GISELA 5 ROOTSTOCK ON REPRODUCTIVE POTENTIAL, VEGETATIVE GROWTH, AND PHYSIOLOGICAL FEATURES OF SOME SWEET CHERRY CULTIVARS IN HIGH-DENSITY SWEET CHERRY ORCHARDS

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Abstract
Gisela 5 rootstock is most important in terms of reducing the vigor of growth. The varieties grafted on Gisela 5 had good horticultural results in terms of yield, adaptability and dwarf growth. This study was aimed to evaluate the growth and physiological behavior of the most popular sweet cherry cultivars in Europe grafted of Gisela 5 rootstock in one of the most important fruit growing area from Romania. The rootstock – scion combinations namely Skeena, Kordia and Ferrovia were grafted on Gisela 5 dwarf rootstocks. Gisela 5 influenced significantly the trunk cross section area among all the tested cultivars ($p<0.05$). Ferrovia cultivar was the most vigorous in terms of trunk cross sectional area and total annual growth length. Total annual growth was lower for Kordia (1225.61 cm). The ratio between Chl a and Chl b seems to be constant in all grafted plants. The photosynthesis rate [μ mol (CO_2) m^{-2} s^{-1}] varied from 24.12 μ mol (CO_2) m^{-2} s^{-1} in the Kordia grafted sweet cherry variety to 25.80 μ mol (CO_2) m^{-2} s^{-1} in the Ferrovia sweet cherry cultivar. Data obtained from field measurements and laboratory observations demonstrated that the Gisela 5 rootstock is compatible with foreign sweet cherry varieties under the selected growing area and can be used to achieve high-density sweet cherry orchards.

Keywords: chlorophyll, dwarf Gisela 5 rootstock, high-density sweet cherry orchards, physiology, Prunus avium L.

1. INTRODUCTION

In Romania, annual production levels of cherries fruit are of around 70,000 tons (FAOSTAT 2012). Sweet cherry (*Prunus avium* L.), which belongs to the *Prunus* genus and is part of the *Rosaceae* family of *Rosales* order, is one of the most appreciated and popular fruits by consumer due to their quality and presence on the market during springtime, when the variety of fresh fruits is not so rich (Usenik et al., 2008; Montiel et al., 2010; Ballistreri et al., 2013).
Sweet cherry cv. Hongdeng and cv. Van grafted on *Prunus pseudocerasus* rootstock and grown in different climatic conditions were evaluate to observe shoot growth and flower bud differentiation. The conclusion of this research affirms that growth and production of fruit trees are influenced by climatic conditions (Li et al., 2010).

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Orchard management system was improved by the introduction of dwarfing cherry rootstocks in fruit growing technology. Rootstock – scion graft combination play a significant role in the vegetative growth of the trees, adaptability to environmental resources, resistance to various pathogens, sustainable of orchard, and yield and fruit quality. The authors indicate that the selection of an appropriate rootstock and scion should be pursued to achieve intensive cherry plantations, sustainability and yield efficiency (Predieri et al., 2003; Stehr, 2005; Li et al., 2010; Gyeviki et al., 2012; Ağlar and Yıldız 2014; Lanauskas et al., 2014; Popescu and Popescu, 2015).
Previous studies showed that Gisela 5 (*Prunus cerasus* L. × *Prunus canescens* Bois) positively influence the yield and dwarf growth compared to the other rootstocks (Jiménez et al., 2007; Lang, 2008; Cantín et al., 2010; Usenik et al., 2010).
Developing of high density sweet cherry orchards by using appropriate dwarf rootstocks increased disease resistance, precocity and productivity. Dwarfing rootstock and newest cultivars are an essential part of the performance technologies used in modern fruit growing (Facteau et al., 1996; Lang et al., 1997; Webster, 1998; Atkinson and Else 2001; Whiting et al., 2005; Seleznyova et al., 2008). Rootstocks are also used to improve the resistance of trees to biotic and abiotic stress (Larsen et al., 1987; Webster, 1995).
The analysis of morphological and anatomical characteristics of rootstocks is an important tool in understanding their influence on the growth of the scion (Zoric et al., 2012).
Radunić et al. (2011) reported that the training system for intensive sweet cherry orchard is a cultivation measure expressed by plantation density and canopy size which can influence fruit quality, yield and labor cost. It is unlikely for a single rootstock to have all the properties mentioned above. The selection of grafted sweet cherry trees depends on the conditions of growing areas and the objectives of producers. It is important to select an appropriate rootstock - scion combination to provide a tool for developing high density orchards in Romanian growing conditions.
The aim of this research was to determine, in the environmental conditions of one of the most important fruit growing area from Romania, the effect of rootstock-scion combinations proposed on physiology and vegetative growth of grafted sweet cherry trees in order to provide useful information to achieve modern sweet cherry orchards.
Photosynthetic capacity is an important parameter that explains the physiological activity of the plants and the potential for vegetative development and productivity in cherry trees. The effect of rootstocks on the physiological growth of cherry (*Prunus avium* L.) was evaluated using some physiological parameters such as photosynthesis rate, chlorophyll (chl) levels (chlorophyll a, chlorophyll b, total chlorophyll, chl a/b ratio) and carotenoids (car). Vegetative growth was examined using the following parameters: trunk cross-sectional area (TCSA), vegetative and flower buds, and length of shoot on *Prunus avium* L. cultivars grown in high-density sweet cherry orchards.

2. MATERIALS AND METHODS

Plant material and culture conditions

The studies were carried out at the Research Institute for Fruit Growing Pitesti – Maracineni, research institute in the field of fruit growing technologies and fruit tree breeding. The average annual temperature is around 9.7 °C and means annual rainfall totaling about 660 mm/year. The intensive cherry orchard is situated in one of the most important horticultural areas of Romania. The planting system was 4 m between rows and 2 m between trees within row plants, resulting an intensive orchard with 1,250 trees/ha. Trees canopy is trained to a spindle for intensive orchards with permanent basal branches. The orchard has a trellis system and an individual support for each tree. The space between the tree rows in the orchard is maintained by permanent grassing.

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Dwarfing sweet cherry rootstock that was tested and evaluated is represented by Gisela 5 (*P. cerasus* × *Prunus canescens* Bois). In recent years, Gisela 5 is commonly grafted with sweet cherry cultivars in Romania and other European countries. The evaluated cultivars were represented by modern foreign sweet cherry. The foreign cultivars Kordia, Ferrovia and Skeena were grafted on dwarfing rootstock Gisela 5.

Growth measurements

Regarding the assessment of rootstocks influence on vigor of cultivars, we determined the total number of shoots per tree, mean shoot length (cm), trunk cross section area – TCSA (cm^2) and trunk circumference – TC (cm). Growth measurements for shoot were taken from 10 trees per rootstock - scion combination. The number of shoots was determined by counting the number of shoots per graft combination. Trunk cross-section area (TCSA) conferred to the scion is an important tool to characterize the influence of rootstock. Trunk cross-section area (TCSA) was calculated using trunk diameter (d) and π (π is equal 3.14) function. The calculating formula for TCSA was $\pi (d^2/4)$. For each sweet cherry tree grafted trunk diameter was measured at 15 cm above the graft union. The trunk circumference was calculated using trunk diameter with the formula $\pi \times d$. Annual growing capacity and productivity potential were analyzed by determining the number of leaf buds and flower buds.

Photosynthesis rate measurement

Physiological studies were carried out when the trees were in their phenological growth and flowering stages. Leaf gas exchange parameters were measured with a portable plant CO_2 analysis package (S151 Infrared CO_2 analyzer, Qubit Biology Inc., Ontario, Canada). Photosynthesis was measured in attached leaves maintained in an assimilation chamber. The CO_2 concentration was measured with an infrared gas analyzer (IRGA). The difference between the initial CO_2 concentration in air and the air in the leaf chamber is used to measure rate of photosynthesis. During testing, the light level (PPFD) was 1800 $\mu mol\ m^{-2}\ s^{-1}$, the air temperature was 24 ± 2 °C and the ambient relative humidity was between 60 and 65%. The effects of rootstocks on net photosynthesis rate were evaluated in the leaves of the middle part of the east-oriented annual shoots. Leaf gas exchange was expressed as $\mu mol\ CO_2\ m^{-2}\ s^{-1}$.

Leaf chlorophyll and carotenoids measurements

Leaf chlorophyll and carotenoids measurements were represented by chlorophyll a (mg chlorophyll per g fresh weight), chlorophyll b ($mg\ g^{-1}$), total chlorophyll ($mg\ g^{-1}$), chl a/b ratio and carotenoids. Leaves were collected in early morning, and transported in an ice box to the laboratory for later determinations of assimilatory pigments content. Photosynthetic pigments were extracted in 80% acetone. Absorbance at 662, 646 and 440.5 was determined using a BOECO S-20VIS spectrophotometer (Boeckel & Co, Hamburg, Germany). Total chlorophyll content was calculated as the sum of chlorophyll a and chlorophyll b. The results are obtained as mg pigments per g fresh weight.

Statistical analysis

A statistical analysis was performed using analysis of variance in the SPSS 16.0 software (IBM Corporation, Armonk, New York, USA) and means of the data obtained from field measurements and laboratory observations were compared using Duncan's multiple range tests at 5% level. The results of this research are expressed as mean ± standard error (SEM).

3. RESULTS

The data of trunk cross section area (TCSA) and trunk circumference are presented in Figure 1. The influence of the Gisela 5 rootstock on the 3 foreign sweet cherry varieties Kordia, Ferrovia and Skeena recorded values of TCSA between 19.54 (Kordia) and 24.24 cm (Ferrovia). Gisela 5

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influenced significantly the trunk cross section area for Ferrovia, Kordia and Skeena sweet cherry varieties ($p<0.05$). Trunk circumference recorded lower values for Kordia cultivar and higher for Ferrovia. The data recorded in the third year after planting of sweet cherry trees grafted for trunk cross section area (TCSA) and trunk circumference demonstrated that, in particular, soil and climate conditions from one of the most important horticultural areas of Romania, foreign cultivars grafted on Gisela 5 foreign rootstock are within limits of dwarf rootstocks category and these graft combination could contribute to the development high-density cherry orchards.

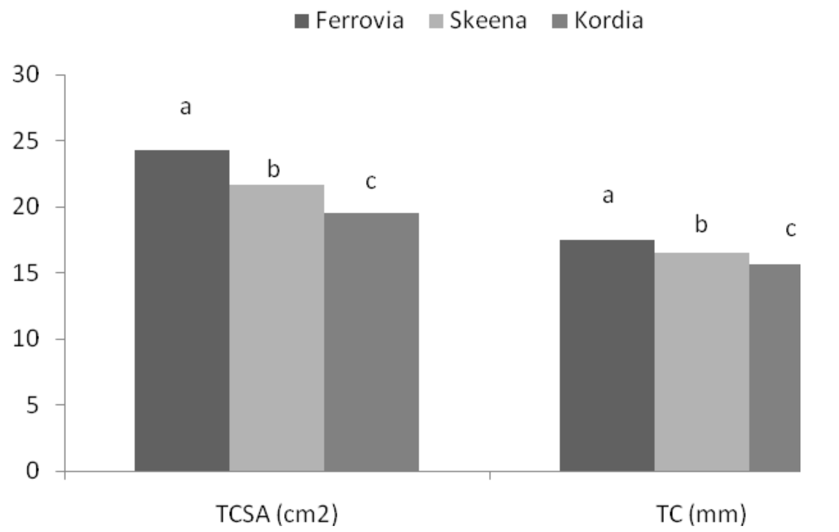

Figure 1. Effect of Gisela 5 rootstock on Trunk Cross Section Area (TCSA) and Trunk Circumference (TC)
Note. Data presented as mean ± standard error of mean (SEM). Values within a group followed by different letters are significantly different at $p<0.05$ (Duncan's test). Values followed by the same letter are not significantly different according to Duncan's multiple range test ($p<0.05$).

Mean values of total number of shoots and shoots lengths are presented in Figure 2. The number of shoots and the total length of annual increases described are parameters that can show the influence of rootstock on the annual tree growth vigor. Ferrovia has formed the largest number of branches on the tree (44) and recorded the biggest total annual growth (1,833.48 cm). Kordia has formed the lowest number of shoots, while the mean shoots lengths was highest. Total annual growth (cm) was lower for Kordia (1225.61 cm). The total number of annual shoots and the means of shoots lengths of Ferrovia, Kordia and Skeena sweet cherry varieties were significantly affected by the influence of the Gisela 5 rootstock ($p<0.05$) (Figure 2).
Reproductive and vegetative growth potential in sweet cherry trees was evaluated by determining the total number of leaf buds and flower buds. In the growth stage, interactions between rootstock × scions were statistically significant for the leaf buds, flower buds and total number of buds (Table 1). Ferrovia and Kordia cultivars grafted on Gisela 5 did not show a significant difference between the values recorded for flower buds. Three years after planting the vegetative process is very well represented by the largest number of leaf buds. The number of vegetative buds varied from 286 (Skeena) to 370 (Ferrovia), while the number of flower buds varied from 15 (Kordia) to 27 (Skeena).
In Table 2 we indicate the data recorded for total chlorophyll content (mg g^{-1}), chlorophyll a content (mg g^{-1}), chlorophyll b content (mg g^{-1}), Chl a / Chl b ratio in the leaves under the influence of Gisela 5 dwarf rootstock.

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Total chlorophyll content (mg g^{-1}) was expressed by amount of chlorophyll a and b (mg g^{-1}). In the chloroplasts of plant there are several forms of chlorophyll pigments, the most common being chlorophyll a. Different types of chlorophyll are present in varying ratios. The chlorophyll a and b concentration was reported to vary from cultivar to cultivar due the influence of the different rootstocks and photosynthetic capacity of scions. The chlorophyll a and chlorophyll b concentration was higher in Ferrovia while the lowest content of chlorophyll a and b was observed for Kordia grafted on Gisela 5. The statistical analysis using analysis of variance in the SPSS 16.0 software showed no significant differences on total chlorophyll content of Skeena and Ferovia grafted on Gisela 5. In rootstock – scion interactions the highest Chl a / Chl b ratio was observed for Kordia grafted on Gisela 5. The ratio between Chl a and Chl b seems to be constant in all grafted plants, but total chlorophyll was higher in Ferrovia and lowest in Kordia. Chlorophyll parameters were differently influenced in the rootstock - scion combination.

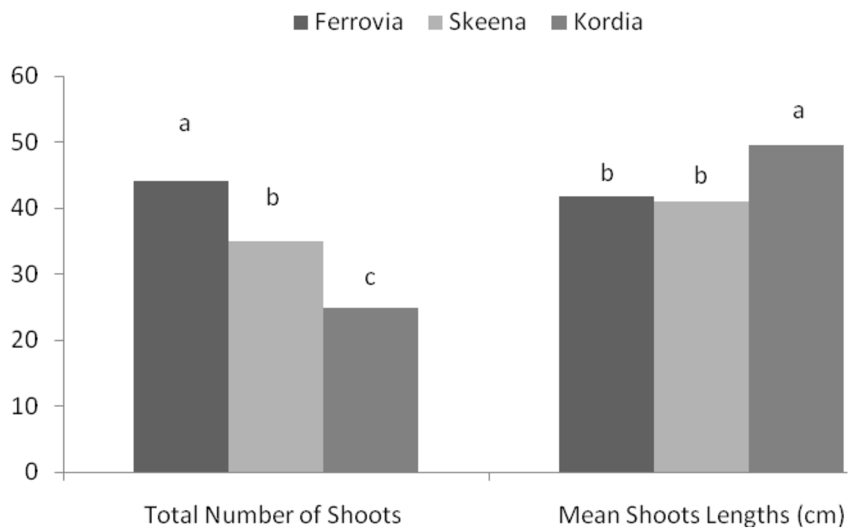

Figure 2. Effect of Gisela 5 rootstock on total number of shoots and shoots lengths

Note. Data presented as mean ± standard error of mean (SEM). Values within a group followed by different letters are significantly different at $p<0.05$ (Duncan's test). Values followed by the same letter are not significantly different according to Duncan's multiple range test ($p<0.05$).

Table 1. Effect of Gisela 5 rootstock on leaf buds, flower buds and total buds

Rootstock	Cultivar	Leaf Buds	Flower Buds	Total Buds
Gisela 5	Skeena	286 ± 2.31 b	27 ± 1.20 a	313 ± 2.03 b
	Ferrovia	370 ± 1.87 a	24 ± 0.88 b	394 ± 2.31 a
	Kordia	210 ± 2.33 c	15.00 ± 0.57 b	225 ± 3.46 c
Mean		267.71 ± 13.61	7.14 ± 1.27	272 ± 14.57

Note. Data presented as mean ± standard error of mean (SEM). Values within a group followed by different letters are significantly different at $p<0.05$ (Duncan's test). Values followed by the same letter are not significantly different according to Duncan's multiple range test ($p<0.05$).

Table 2. Total chlorophyll content (mg g^{-1}), Chlorophyll a (mg g^{-1}), Chlorophyll b (mg g^{-1}), Chl a / Chl b ratio in the leaves under the influence of dwarfing rootstocks

Rootstock	Cultivar	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Chl a / Chl b ratio	Total Chlorophyll content (mg g^{-1})

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Gisela 5	Skeena	11.00 ± 0.59 a	3.88 ± 0.58 a	2.83 ± 0.52 a	14.89 ± 0.58 a
	Ferrovia	11.20 ± 0.43 a	4.10 ± 0.60 a	2.73 ± 0.38 b	15.30 ± 0.84 a
	Kordia	10.18 ± 0.65 b	3.50 ± 1.09 b	2.91 ±1.13 a	13.68 ± 0.43 b
Mean		10.61 ± 0.90	3.74 ± 0.52	2.84 ± 0.25	14.35 ± 1.36

Note. Data presented as mean ± standard error of mean (SEM). Values within a group followed by different letters are significantly different at p<0.05 (Duncan's test). Values followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05).

The data recorded for carotenoids (mg g^{-1}), Chl a+b / Car ratio and photosynthesis rate [µmol (CO_2) m^{-2} s^{-1}] in the leaves under the effect of Gisela 5 rootstock are presented in Table 3.
The content of carotenoids (mg g^{-1}) in Ferrovia and Kordia was significantly affected by influence of Gisela 5 rootstock. The content of carotenoids measured in the leaves was higher in Ferrovia (2.43 mg g^{-1}) than in Kordia (2.05 mg g^{-1}) and Skeena (2.26 mg g^{-1}) grafted on Gisela 5 rootstock. Chl/Car ratio varied from 6.28 mg g^{-1} (Ferrovia) to 6.66 mg g^{-1} (Kordia). The differences that were observed for carotenoids pigments and Chl/Car ratio in Skeena and Kordia cultivars were not significant.
The photosynthesis rate varied from 24.12 µmol (CO_2) m^{-2} s^{-1} in the Kordia grafted sweet cherry variety to 25.80 µmol CO_2 m^{-2} s^{-1} in the Ferrovia grafted trees (Table 5). The influence of the rootstock on the photosynthesis processes of the scion was significant in Ferrovia and Kordia grafted on Gisela 5 rootstock.

Table 3. Carotenoids (mg g^{-1}), Chl / Car Ratio and Photosynthesis rate [µmol (CO_2) m^{-2} s^{-1}] in the leaves under the effect of dwarfing rootstocks

Rootstock	Cultivar	Carotenoids (mg g^{-1})	Chl a+b / Car ratio	Photosynthesis rate [µmol (CO_2) m^{-2} s^{-1}]
Gisela 5	Skeena	2.26 ± 0.33 b	6.59 ± 0.10 a	24.86 ± 1.53 b
	Ferrovia	2.43 ± 0.65 a	6.28 ± 0.19 b	25.80 ± 2.04 a
	Kordia	2.05 ± 0.30 b	6.66 ± 0.09 a	24.12 ± 2.07 b
Mean		2.21 ± 0.32	6.51 ± 0.06	24.54 ± 1.48

Note. Data presented as mean ± standard error of mean (SEM). Values within a group followed by different letters are significantly different at p<0.05 (Duncan's test). Values followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05).

4. DISCUSSIONS

Tree vigor expressed in trunk cross sectional area and flower bud production has been studied in several cultivars of perennial fruit crops (Ruiz and Egea 2008; Usenik et al., 2008; Sitarek and Bartosiewicz 2012). The sweet cherry cultivars Brooks, Cristobalina, Marvin and Ruby grafted on 'SL-64' (*Prunus mahaleb* L.) rootstock showed the highest production of flower buds per node in the different years (Montiel et al., 2010).
Gregory et al. (2013) noted that to build a sustainable production system it is necessary to understand the relation among grafted plants and specific environmental resources in order to improve the technology performance of intensive plantation.
Hrotkó et al. (2009) reported that sweet cherry trees on various rootstocks grew differently, significant differences was found in the trunk cross sectional area of trees on different rootstocks from 57.40 cm^2 to 130.73 cm^2.
The final node number, final shoot length, metamer length, TCSA increment, shoot growth cessation were determined to explain the reduced tree height influenced by rootstock-specific gene regulation in Bing sweet cherry scions grafted on Gisela 5 and Gisela 6 rootstocks (Prassinos et al., 2009). Tree height, canopy volume, trunk diameter, shoot length and trunk cross sectional area of 0900 Ziraat sweet cherry cultivar were examined to observe the effects for these growth vigor on

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the influences of four rootstocks (Gisela 5, Gisela 6, MaxMa 14 and SL 64). Observations showed that the trees grafted on SL 64 and MaxMa 14 rootstocks were more vigorous than those grafted on Gisela 5 and Gisela 6 rootstocks (Ağlar and Yıldız, 2014).

Stehr (2005) reported that Gisela 5 rootstock is recommended to growers high density systems with good results for yield per tree volume. Cantín et al. (2010) studied the influence of seven different rootstocks onto vegetative growth of Van and Stark Hardy Giant sweet cherry cultivars and among these rootstocks Gisela 5 showed the lowest annual increasing rate of TCSA for both cultivars.

Sylvia and Karina sweet cherry trees were grafted on Gisela 3, Gisela 5, Piku 4, Weiroot 72 and F 12/1 rootstocks in order to evaluate growth, productivity and fruit quality. Gisela 5, Gisela 3 and Weiroot 72 were found to be the most dwarfing for both cultivars (Sitarek and Bartosiewicz, 2012).

Gonçalves et al. (2005) have explained physiological parameters of grafted sweet cherry trees expressed by gas exchange and chlorophyll a fluorescence, chlorophyll a and b, and carotenoids.

Several reports related to photosynthesis parameters in horticultural species have been documented previously and many of these studies have examined the influence of rootstocks on physiological parameters of different perennial fruit plants (Pérez et al., 1997; Flore and Layne, 1999; Lichev and Berova, 2004; Whiting and Lang, 2004; Gonçalves et al., 2005; Malcolm et al., 2008; Cantín et al., 2010; Quentin et al., 2013; Götz et al., 2014).

Rootstocks provide a root system to the scion variety grafted onto them and affect physiological changes in the scion leaves. Rootstocks are usually used for grafting perennial fruit species and these are known to influence various physiological processes in the scion such as vigor, length of shoot and chlorophyll and carotenoids pigments.

Photosynthetic activity in sweet cherry cultivar Stella was evaluated under the influence of ten rootstocks. Net photosynthetic rate varied from 7.3 µmol CO_2 m^{-2} s^{-1} to 11.3 µmol CO_2 m^{-2} s^{-1} in the growth stage and from 5.8 µmol CO_2 m^{-2} s^{-1} to 9.4 µmol CO_2 m^{-2} s^{-1} in the flower formation phase and under the influence of Gisela 5 rootstock sweet cherry cultivar Stella recorded 10.7 µmol CO_2 m^{-2} s^{-1} in the growth stage and 7.1 µmol CO_2 m^{-2} s^{-1} in the flower formation (Lichev and Berova, 2004).

Pilarski et al. (2007) examined the content of photosynthetic pigments and their proportions in the stems and leaves of a sweet cherry tree. The authors reported that the total content of chlorophyll in leaves was 4.5 mg dm^{-2} and the chlorophyll a/b ratio was 3.8.

Pérez et al. (1997) reported that photosynthetic capacity of same sweet cherry cultivar was influenced when trees was grafted on different rootstocks. The level of chlorophyll concentration varied from 36.66 (mg g^{-1}) to 24.65 (mg g^{-1}) while the content of chlorophyll b varied from 11 (mg g^{-1}) to 7.28 (mg g^{-1}). Chl a / Chl b ratio in the leaves had almost the same values (Pérez et al., 1997).

5. CONCLUSIONS

In conclusion, our results demonstrated that cv. Kordia, cv. Ferrovia, and cv. Skeena are compatible with dwarfing rootstock Gisela 5 in the specific climate and soil of Romania and these combinations should be used to achieve high-density sweet cherry orchards.

The highest photosynthesis rates were obtained by cv. Ferrovia trees [25.80 µ mol (CO_2) m^{-2} s^{-1}] and the ratio between Chl a and Chl b seems to be constant in all grafted plants.

The most vigorous trees in terms of trunk cross sectional area and total annual growth length were observed in the Ferrovia/Gisela 5 while the less vigorous trees in Kordia/Gisela 5 cultivar – rootstock combination.

Sweet cherry (*Prunus avium* L.) physiology and the growth vigor capacities have a major impact for developing vegetative and reproductive structures. A rational choice of scion – rootstock

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combination to obtain small grafted trees that have adapted to the specific environmental conditions is required to develop commercially high-density sweet cherry culture.

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