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PHENOLOGICAL CHANGES OF SHOOT CARBOHYDRATES AND PLANT GROWTH CHARACTERISTICS IN *VITIS LABRUSCA* L. GRAPE

Bülent KÖSE[1], Hüseyin ÇELIK[2]

[1]Ondokuz Mayıs University, Samsun Vocational School,
Plant and Animal Production Program, Samsun, Turkey
[2]Ondokuz Mayıs University, Agricultural Faculty,
Department of Horticulture, Samsun, Turkey

Corresponding author email: bulentk@omu.edu.tr

Abstract

Stored carbohydrates are used to supply energy in the next season for shoot growing, flowering and ripening in grapevines. Carbohydrates are transfers from storage organs to growth areas following the bud burst in spring. The present study was carried out to determine the effect of different rootstocks on growth characteristics and phenological changes of shoot carbohydrates in 10 years-old Vitis labrusca L. grapes. The grapevines are grafted on 140Ru, 5BB and 5C rootstocks. In the experiment; changes of sugar, starch and total carbohydrate contents in annual shoots were investigated in different phenological stages. While the highest sugar and total carbohydrate content of shoots were determined in veraison period on 5BB grafted grapevines, starch content was high in the harvest on 140Ru. While the highest mean and total leaf areas were calculated in grapevines grafted on 140Ru rootstock, shoot length and diameter was the highest on 5BB rootstock. Leaf chlorophyll content was the highest in blooming period on 5C grafted grapevines.The heavier fruit clusters, berries and total yield per vine were obtained on 5BB grafted vines. In this study, 5BB rootstock was found to be favorable in terms of shoot carbohydrate content and growth characteristics for Vitis labrusca L. grape.

Key words*: carbohydrate, growth, rootstocks, Vitis labrusca L.*

INTRODUCTION

Rootstocks have significant effects on the growth; shoot development, yield, nutrient uptake and resistance to phylloxera of grapevines (Verma et al., 2010; Jogaiah et al., 2013; Rafaat et al., 2013).
Rootstocks also play essential roles in dry matter partition of root, stem, shoot and fruit (Loescher et al., 1990; Somkuwar, 2012). Therefore, grapevine growth and development are closely related to cultural practices performed throughout the growing season.
Rootstocks influence sugar and starch reserves of grapevines (Smith, 2004; Jogaiah et al., 2013). For instance, in 'Merzifon Karasi' grapes, the highest root carbohydrate content was observed in grapevines grafted on 5C rootstock and the highest stem and shoot soluble carbohydrate contents were determined in grapevines grafted on 110R and 5C rootstocks (Köse et al., 2014).
Carbohydrates are responsible for the shoot development, root and stem diameter growth, flower bud development, graft union and fruit set in various plant species (Caspari et al., 1998; Göktürk et al., 2005; Loescher et al., 1990). Carbohydrates throughout the spring season started to be used rapidly with the initiation of growth.
During to development of grapevine, shoot development slows down especially during the fruit set period and carbohydrates promptly start to accumulate in shoots (Weaver, 1976). Carbohydrate accumulation then slows down with the recess in fruit size and shoot growth almost ceases at the end of summer (Van der Zijpp and Creagh, 2011).
In 'Shiraz' grapes, carbohydrate concentration decreased in all tissues in veraison period and increased again in the harvest period (Smith et al., 2009).
Carbohydrates transport following the bud burst in spring to support shoot development and initiation of flowering varies mostly based

on species, fruit load and climate conditions (Bates et al., 2002; Holzapfel and Smith, 2012; Zufferey et al., 2012). Soluble sugars in upper-soil organs have significant contributions to the total carbohydrate content (Holzapfel and Smith, 2012; Zufferey et al., 2012). While starch concentration decreases in the dormancy period, sugar concentration increases.

The highest starch levels in shoot, stem and root are commonly observed at the end of growing season (Winkler and Williams, 1945). Such a chance is related to winter-resistance and increased conversion of starch into sugar against cold damage (Hamman et al., 1996).

The experiment was conducted to investigate the growth characteristics and phenological changes of shoot carbohydrate accumulation in *Vitis labrusca* L. grape grafted on different rootstocks.

MATERIALS AND METHODS

The research was conducted in the experimental vineyard of Ondokuz Mayıs University, Turkey. The vineyard located at 41°21’52 N latitude and 36°11’29 E longitude with an altitude of 195 m and a distance of about 2.8 km from the Black Sea coast of Turkey. The experiment was conducted from December 2012 to October 2013, in vines of the 10-years *Vitis labrusca* L. grapes were grafted on 140Ru, 5BB and 5C rootstocks.

The grapes have a foxy flavor, thick slip skins and a distinct aroma, and are consumed as table grapes, in marmalades and pickles, or as juice according to the local needs of all coastal areas of the Black Sea Region of Turkey. Short pruned grapevines were trained onto a high double cordon with 3x1.5 m spacing. Grapevines were not irrigated, not fertilized and the only supplementary water received from rainfall.

Shoot sugar, starch and total carbohydrate contents were determined at modified Eichhorn-Lorenz phenological stages (Coombe, 1995). Shoot length (cm), diameter (mm), mean and total leaf area (cm^2) were measured from pre-bloom to harvest.

Shoot length and diameter was measured at three selected shoots of each grapevine. Shoot diameter was measured on between two and three nodes of shoots with digital compass. Leaf areas were calculated non-destructively $[-1.41 + 0.527*(W^2) + 0.254*(L^2)]$ according to Elsner and Jubb (1988). Leaf width (W) and leaf length (L) were used to calculate leaf areas.

The analysis of TSSC (Total Soluble Solids Content) readings was performed by a digital refractometer (Atago Co. I. Japan).

Grape cluster and berries were weighted by digital scale (0.1 g accuracy) at harvest.

Titratable acidity was used by titration with 0.1 N NaOH at pH 8.3, using phenolphthalein as indicator.

Air temperature, relative humidity and precipitation data were obtained from Turkish State Meteorological Service, Samsun Regional Office. Monthly average temperature, relative humidity and precipitation were calculated from the daily records (Figures 1 and 2).

Experiment area has clay soil type. Physical characteristics of experimental vineyard are provided in Table 1.

Table 1. Physicochemical properties and nutrient contents of the vineyard soil

Soil Properties		Nutrient Element Contents	
Clay %	62,12	Toplam N, %	0.212
Silt %	20,09	P, mg kg^{-1}	42.77
Sand%	17,12	K, me 100 g^{-1}	1.07
Texture class	SC*	Ca, me 100 g^{-1}	28.39
pH, sat. Ext.	7.1	Mg, me 100 g^{-1}	8.96
EC, (dS m^{-1})	0.69	Fe, mg kg^{-1}	12.02
OM, %	4.33	Mn, mg kg^{-1}	23.63
Lime ($CaCO_3$), %	0.85	Zn, mg kg^{-1}	1.82
CEC, meq 100g^{-1}	30.05	Cu, mg kg^{-1}	1.11

* Soil-clay

Chlorophyll analysis

Chlorophyll a (Ch_a) and chlorophyll b (Ch_b) concentrations were determined using the method described by Lichtenthaler and Wellburn (1983). Fresh leaves (0.1 g) were placed into 8 ml of 80% acetone, and filtered through Whatman No. 2 filter paper.

After absorbance was measured in a UV-visible spectrophotometer (Pharmacia LKB-Novaspec II model spectrophotometer, UK) at 646, 663 and 470 nm, Ch_a and Ch_b (mg/g FW) were calculated according to the following equations:

Chlorophyll a (Ch_a) = $12.25\ A_{663} - 2.798\ A_{646}$

Chlorophyll b (Ch_b) = $21.5\ A_{646} - 5.1\ A_{663}$

Total Chlorophyll = Chlorophyll a (Ch_a) + Chlorophyll b (Ch_b)

Carbohydrate analysis

The samples taken between the 2nd and 3rd nodes of the shoots during the bud burst (E-L 02), pre-blooming (E-L 15), full blooming (E-L 23), veraison (E-L 35), harvest (E-L 38), after harvest (E-L 43) and dormancy (E-L 47) periods were dried in an oven at 60 ^{o}C for 48 hours.

Plant tissue samples were pulverized in a mill (IKA, Staufen, Germany) with a 40-mesh screen for analysis and 200 mg of dust was used for the extraction.

Soluble sugars were extracted twice with 8 ml of 80% ethanol at 60°C for 30 minutes (Candolfi and Koblet, 1990).

Soluble sugar content was determined with the anthrone method as described by Scott and Melvin (1953).

Starch was extracted twice with 8 ml of 1 M perchloric acid, one hour each time at 60°C and measured by the same method.

Absorbance readings were made at 620 nm with a spectrophotometer.

Glucose was used as a standard for the analysis of soluble sugars and starch.

Statistical analysis

Complete randomized block design with 3 replications were used in the study and the mean measurements were calculated. Growth characteristics and carbohydrate content were measured in four grafted vines of each replication per rootstock. The data were analyzed by two-way analysis of variance (ANOVA) to test main effects and interactions between for phenological stages and rootstocks. Data analysis was performed using SPSS 16.0 for Windows. Results studied were presented as means and a pooled standard error of mean (SEM). Differences among means were detected using Duncan's multiple range tests at significance levels of (p <0.01).

RESULTS AND DISCUSSION

Mean monthly temperature (°C), relative humidity (%) and precipitation (mm) values belonging to the long term and experiment year for phenological stages were are in Figures 1 and 2. According to long term data, mean temperatures in bud burst period were calculated as 11.4 ^{o}C. In blooming, mean temperatures were calculated as 20.3 ^{o}C.

The highest average temperatures were measured during veraison to harvest. Thereafter, temperatures decreased.

In contrast to average temperatures, relative humidity did not vary substantially throughout the season. In bud burst period, the monthly precipitation was determined as 56.5 mm. According to long term climate data, total precipitation was calculated as 274.7 mm from bud burst to harvest period. Minimum precipitation was measured in the veraison and harvest period.

The month precipitation was determined as 32.0 mm in June and 40.1 mm in July. During the growth season, total precipitation was calculated as 223 mm from bud burst to harvest period (Figure 1).

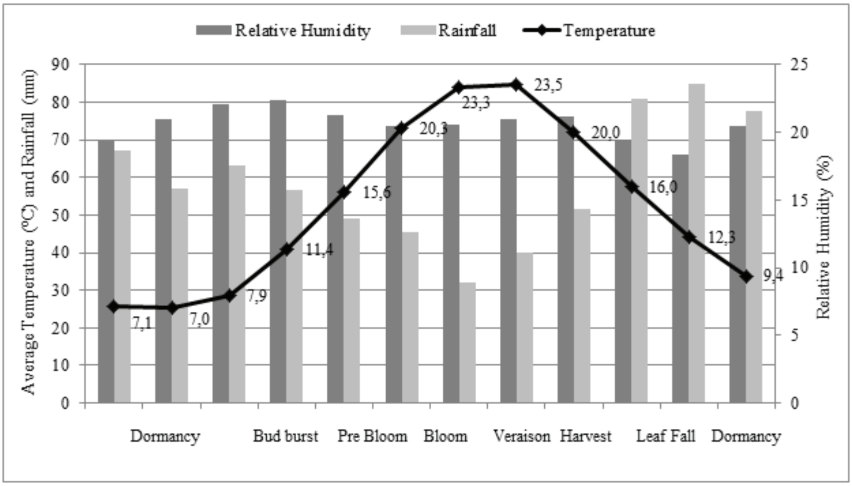

Figure 1. Changes of monthly mean temperature (°C), precipitation (mm) and relative humidity (%) throughout the growth seasons according to long term data (1960 to 2014).

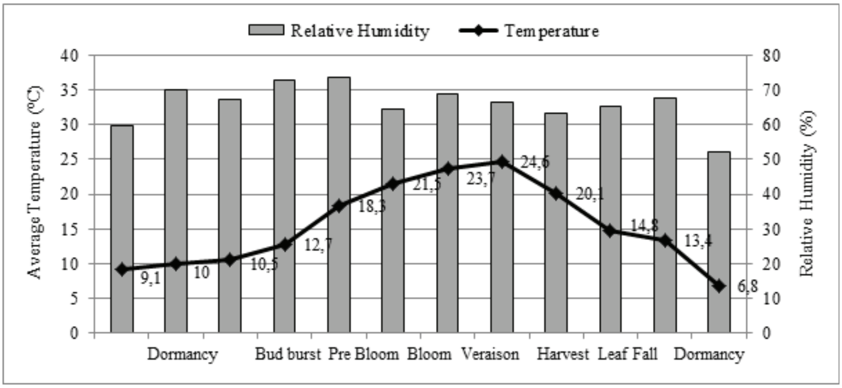

Figure 2. Changes of monthly average temperature (°C) and relative humidity (%) from bud burst to dormancy period in the experiment year.

In the experimental season, mean temperatures in the bud burst period were calculated as 10.5 °C. In blooming, mean temperatures were calculated as 21.5 °C. The highest average temperatures were measured during veraison as 24.6 °C. After the veraison stage, temperatures decreased gradually. Relative humidity was calculated respectively as 64.6% in blooming and 63.2 % in harvest (Figure 2).

Changes of sugar, starch and carbohydrates of annual shoots

Phenological changes in sugar, starch and total carbohydrate contents of annual shoots of *Vitis labrusca* L. grapes grafted on 140 Ru, 5BB and 5C rootstocks are presented in Figure 3, 4 and 5. Significant differences were observed in sugar contents of the annual shoots between rootstocks and phenological periods (p<0.01). Sugar content of shoots was the highest in veraison stage. The lowest sugar level was obtained at the blooming and harvest stages. The highest sugar contents were determined in grapevines grafted on 5BB rootstock in pre-blooming (18.03 mg L^{-1}) and veraison stages (24.32 mg L^{-1}). The lowest sugar contents were measured in grapevines grafted on 5C rootstock during the harvest (4.79 mg L^{-1}) and leaf fall period (4.90 mg L^{-1}) (Figure 3).

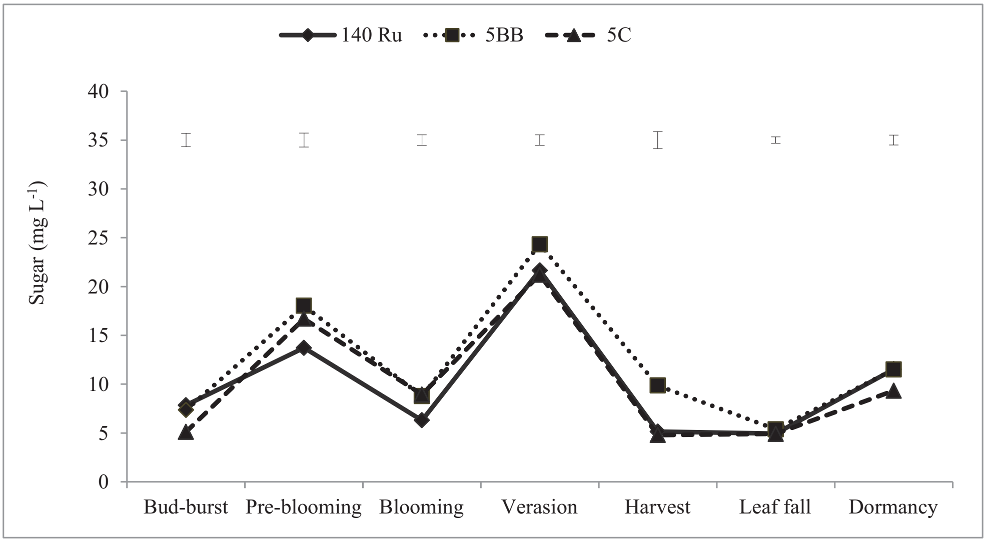

Figure 3. Seasonal changes of shoot sugar contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

In the study, the highest sugar content in annual shoots of grapes was identified as veraison stage. In general, sugar content of annual shoots at the end of growing season was higher in grapevines grafted on 140Ru rootstock than the other rootstocks. While sugar content of annual shoots of the grapevines grafted on 140Ru and 5BB rootstocks were almost at similar levels in budburst and dormancy periods, the sugar content of annual shoots of the grapevines grafted on 5C rootstock was lower in dormancy period than in previous period of budburst.

This situation may have stemmed from the earlier sugar conversion into starch in grapevines grafted on the 5C (Figure 3). Previous researchers indicated the reasons for such decreases as the high rate consumption of assimilation products during the blooming period (Dokoozlian, 2000; Lebon et al., 2008; Candolfi and Koblet, 1990) and the use of some of the sugar in sugar accumulation of the fruit and the conversion of sugars into starch for storage and usage in the development of new shoots in the subsequent spring (Winkler et al., 1974). A slight increase was determined in the sugar content of the shoots during the dormancy period. Such an increase may have resulted from the conversion of stored starch into sugar again for cold resistance in the dormancy period. Similar findings on conversion of stored starch in to against cold damage into sugar were also reported by previous researchers (Winkler and Williams, 1945; Hamman et al., 1996). Smith et al. (2009) reported that carbohydrate contents in entire tissues of grapevines decreased until blooming period and increased again between veraison and harvest periods.

Significant differences were also obtained in terms of starch content of annual shoots between rootstocks and phenological stages ($p<0.01$). In budburst stage, the highest starch content of shoots was determined in grapevines grafted on 140Ru rootstock (Figure 4).

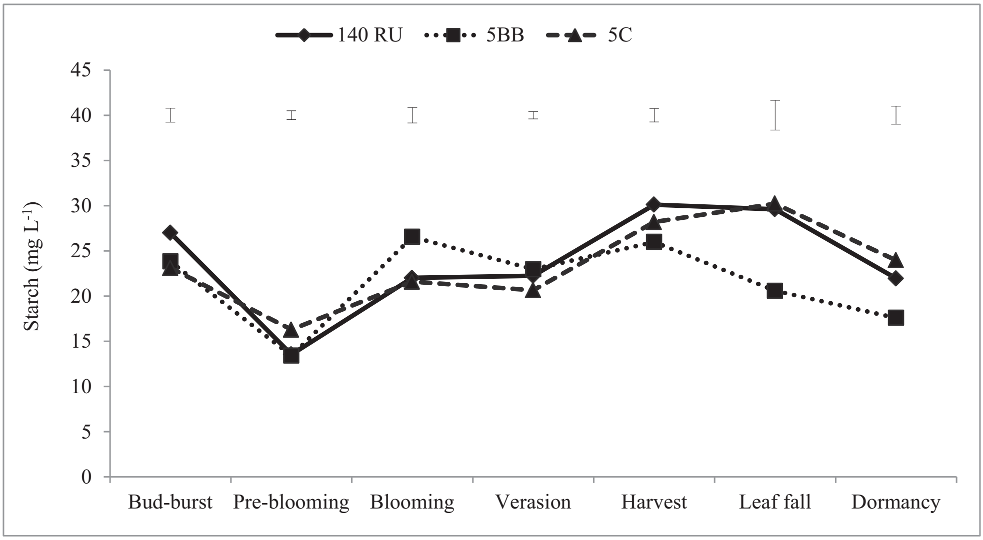

Figure 4. Seasonal changes of shoot starch contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

The starch contents of shoots were calculated as 26.99 mg L^{-1} on 140Ru, 23.81 mg L^{-1} on 5BB and 23.10 mg L^{-1} on 5C grafted grapevines in the bud burst stage.

The starch contents of new developed shoots were quite less on all rootstocks in pre-blooming period. During this period, newly developed leaves were still in consumer position and thus had relatively low assimilation capacity (Zamski and Schaffer, 1996).

The amount of starch continued to increase from pre-blooming to harvest period. In blooming period, the grapevines grafted on 5BB rootstock had the highest shoot starch content (Figure 4).

During the dormancy period, starch content of annual shoots was higher in grapevines grafted on 5C rootstock than in grapevines grafted on 5BB and 140 Ru rootstocks (Figure 4). Scholefield et al. (1978) indicated that sugar synthesized in vine leaves was converted into sugar following the harvest and stored in roots and stems to be used in shoot development of the subsequent spring.

Starch is the most significant carbohydrate providing winter resistance of xylem, especially of the roots of grapevines (Weyand and Schultz, 2006). Starch and soluble sugars are two main stored carbohydrates in grapevines (Mc Artney, 1998).

Entire leaves over the shoots are photosynthetic storage sources (Hunter and Visser, 1998). Therefore, active post-harvest leaves play significant roles in grapevines for sufficient storage of carbohydrates in roots to be used in the shoot development and flowering of the subsequent spring (Loescher et al., 1990).

Carbohydrates stored in vine canes are the indicators of grapevine health and vigor of the previous season (Balasubrahmanyam et al., 1978). Significant differences were observed in the total carbohydrate contents of annual shoots between rootstocks and phenological periods ($p<0.01$).

The amount of carbohydrate in bud burst period was higher in grapevines grafted on 140Ru than in grapevines grafted on 5BB and 5C rootstocks (Figure 5).

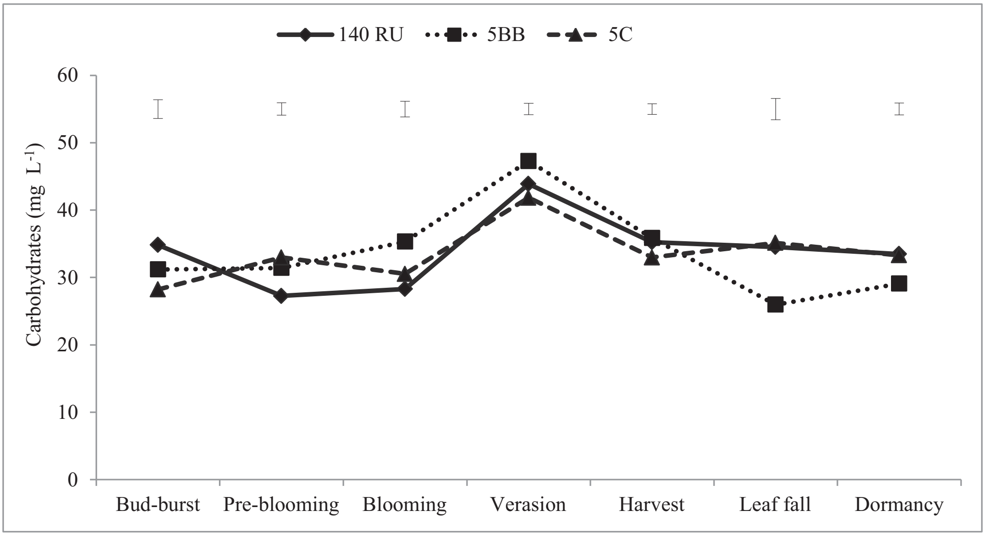

Figure 5. Seasonal changes of shoot total carbohydrate contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

In bud burst stage, carbohydrate contents were calculated as 34.84 mg L^{-1} on 140Ru, 31.18 mg L^{-1} on 5BB and 28.22 mg L^{-1} on 5C (Figure 5).

Total carbohydrate content of annual shoots in pre-blooming stage was higher in grapevines grafted on 140 Ru and 5BB rootstocks. However, carbohydrate content of annual shoots between blooming and harvest stages was higher in grapevines grafted on 5BB rootstock than the other grapevines (Figure 5). The highest mean carbohydrate content (41.56 mg L^{-1}) was calculated in grapevines grafted on 5BB rootstock at veraison stage.

Following the veraison period, decreases were observed in carbohydrate contents of annual shoots on all rootstocks. Shoot carbohydrate contents from the budburst to end of growth period were higher in grapevines grafted on 140Ru and 5BB rootstocks than grafted on 5C.

The carbohydrate contents of shoots increased and reached maximum level at the veraison stage. Thus, various researchers also reported increasing carbohydrate contents parallel to the recess in the shoot growth and increase in leaf area (Loescher et al., 1990; Kozlowski, 1992; Lakso et al., 2007). After veraison period, decreases were observed in shoot carbohydrate contents. Since new developed shoots exhibit rapid growth throughout pre-blooming period, carbohydrate accumulations of shoots were quite low. During fast growth period, grapevines use their own reserves and photosynthesis products are used for such a rapid growth and development.

During this period, it is understood that grapevine uses its own reserves as well as produced assimilates for rapid growth and development. Since carbohydrates are used for new shoot development between dormancy and blooming periods, decrease is observed in these periods. In this regard, Smith et al. (2009) stated that an increase in carbohydrates was expected until the end of the growing season. The carbohydrate level of annual shoots obtained in the dormancy period was close to the values in the bud burst period (Figure 5).

Storage carbohydrates are very important for the best shoots growth, flowering and fruit set. Also it is important to support of the storage reserves of grapevines to be used in a subsequent period as well as the formation of flower bud initiation. It was shown that accumulation of carbohydrates in shoots continuing after flowering to harvest. Carbohydrate transport and storage are related to photosynthesis capacity of grapevine canopy (Smith et al., 2009), stored carbohydrates decrease until fruit set (Wolstenholme and Whiley, 1997). Several researchers indicated varying annual carbohydrate contents of grapevines (Kliewer and Nassar, 1966; Winkler and Williams 1945).

Changes of leaf chlorophyll contents and growth characteristics

The changes in total leaf chlorophyll contents of grapes grafted on 140 Ru, 5BB and 5C rootstocks in blooming, veraison and harvest periods are presented in Figure 6.

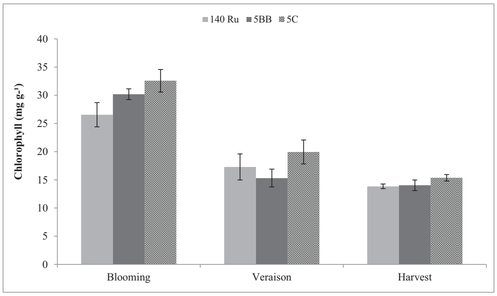

Figure 6. Seasonal changes of total chlorophyll contents of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.05$)

Significant differences were obtained in total chlorophyll contents in phenological periods ($p<0.01$) and rootstocks ($p<0.05$). The highest total chlorophyll content of grapevine leaves was calculated in blooming period. Besides, total chlorophyll content of grapevines grafted on 5C rootstock was higher than the other rootstocks in all three periods. In blooming period, leaf chlorophyll content was measured as 32.57 µg ml^{-1} for 5C, 30.19 µg ml^{-1} for 5BB and 26.58 µg ml^{-1} for 140 Ru. The total chlorophyll levels of the leaves decreased with the increase in leaf ages (Figure 6). Decreases were observed in the chlorophyll contents from veraison to harvest periods between all rootstocks. The lowest chlorophyll contents were determined in the harvest period in the all three rootstocks. The chlorophyll levels were relatively close to each other in the harvest period (Figure 6). In the study, the highest chlorophyll contents of leaves were determined in blooming stage. Poni et al. (1994) stated that leaf chlorophyll content was related to leaf age. Thus photosynthetic capacity of leaves was decreasing with increasing of leaf age.

Significant differences were obtained in terms of the mean and total leaf areas between rootstocks and phenological stages ($p<0.01$). Rapid increases were determined in mean and total leaf areas of grapevines grafted on all rootstocks until veraison period (Figures 7 and 8).

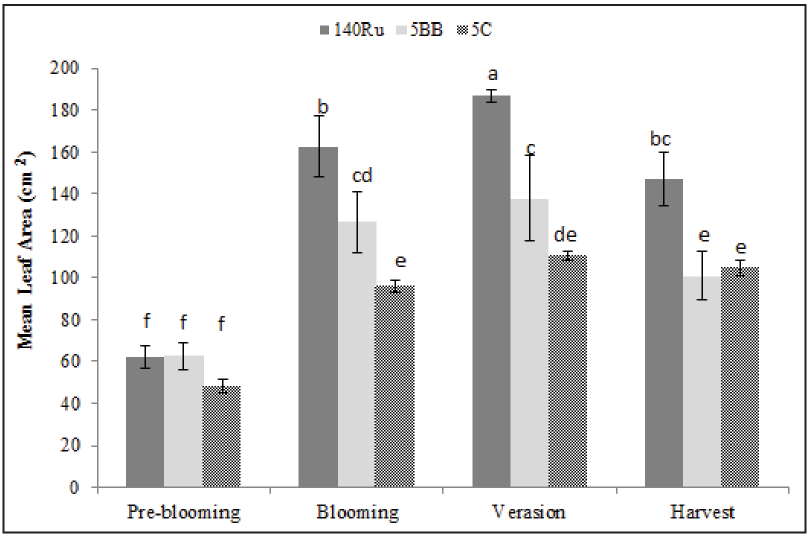

Figure 7. Seasonal changes of shoot mean leaf area (cm^2) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

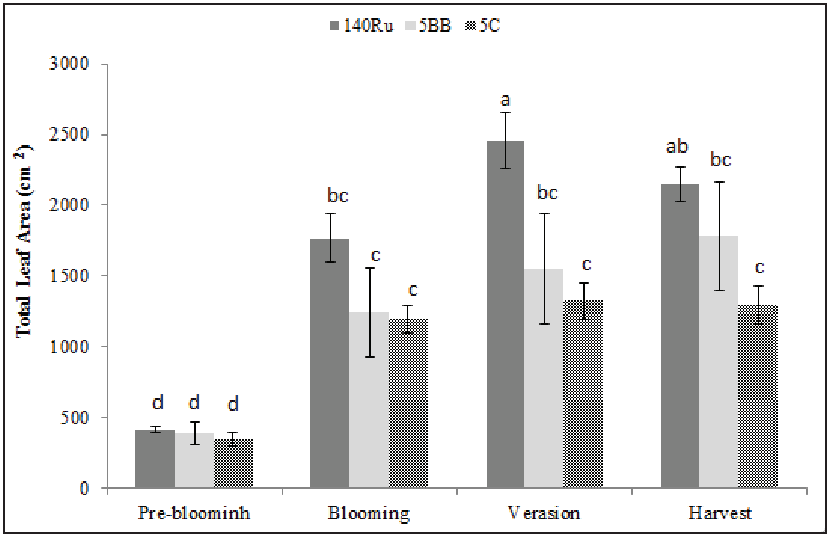

Figure 8. Seasonal changes of total shoot leaf area (cm^2) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal p<0.01; rootstocks p<0.01)

The highest total and mean leaf areas were observed in grapevines grafted on 140Ru rootstock. In veraison period, the highest mean leaf area was calculated as 187.0 cm^2 for 140Ru, 137.8 cm^2 for 5BB and 110.7 cm^2 for 5C. The total leaf areas in veraison period were measured as 2457.7 cm^2 for 140Ru, 1553.5 cm^2 for 5BB and 1326.0 cm^2 for 5C (Figure 8). In the study, the mean leaf areas of grapevines in blooming period reached to more than half of the maximum size they reached in veraison period. Maximum mean leaf area was calculated in veraison stage. The coincidence of increasing carbohydrate contents to the same period indicate the full photosynthetic capacity of the grapevine leaves.

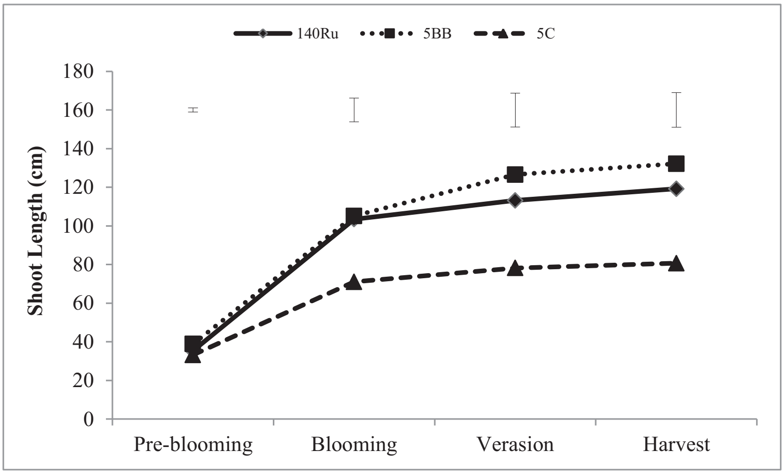

Figure 9. Seasonal changes of shoot length (cm) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal p<0.01; rootstocks p<0.01)

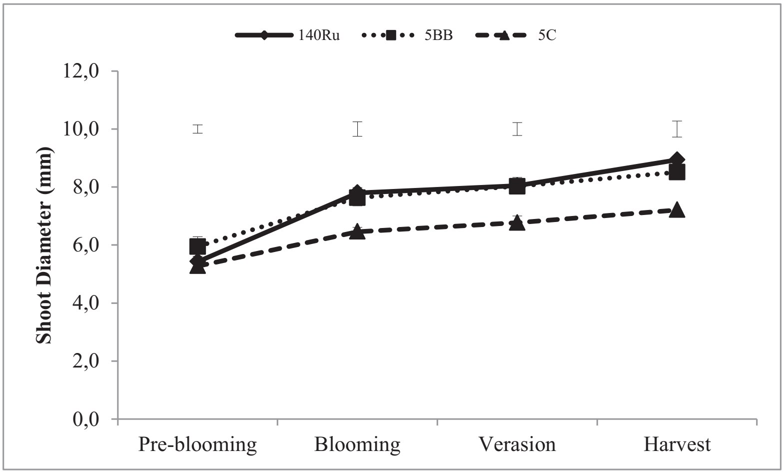

Figure 10. Seasonal changes of shoot diameter (mm) of *Vitis labrusca* L. grape grafted on 140 Ru, 5BB and 5C rootstocks (Seasonal $p<0.01$; rootstocks $p<0.01$)

A rapid increase was observed in shoot diameter and shoot length of grapevines grafted on all rootstocks until blooming period and that an increase slowed down after that period. After blooming, it was slowed down the growth of the shoot diameter and shoot length (Figures 9 and 10). Previous researchers also indicated that grapevines needed a significant amount of energy from the bud burst in spring to the veraison period (Hale and Weaver, 1962; Koblet, 1977) and new shoot development was dependent on self-reserves of the grapevines until a couple leaves reached at least half-size of the maximum leaf size (Hale and Weaver, 1962; Koblet, 1969; Winkler et al., 1974). Earlier studies revealed that photosynthetic products started to be transported to other organs when the grapevine leaves reached 30-50% of the maximum size (Koblet, 1977; Yang and Hori, 1980) or 50-75% of the maximum size (Koblet, 1969).

In the research, shoot diameter and shoot lengths showed significant differences in terms of between rootstocks and phenological stages ($p<0.01$). The changes in the shoot diameter and shoot lengths of grapes grafted on different rootstocks between pre-blooming and harvest periods are presented in Figure 9 and 10. At the end of the growth period, the greatest shoot length (132.2 cm) was determined in grapevines grafted on 5BB rootstock and the lowest value (80.6 cm) was seen in grapevines grafted on 5C rootstock (Figure 9). With regards to shoot diameters at the end of the period, the values were respectively observed as 8.9 and 8.5 cm in grapevines grafted on 140 Ru and 5BB rootstocks and as 7.2 cm in grapevines grafted on 5C rootstock (Figure 10). Current results revealed that 5C rootstock exhibited slower grow than 140Ru and 5BB rootstocks.

Yield and cluster characteristics

In the study, yield and some cluster characteristics are evaluated in Table 2. Total soluble solids content (TSSC-^{o}Brix), cluster weight and yield per vine showed significant differences as compared to rootstocks. While higher soluble solids were found on 5C grafted grapevines, mean cluster weight, berry weight and yield per vine was at on 5BB grafted grapevines (Table 2). Soluble solids content was calculated as between 19.1 and 20.6 ^{o}Brix. The highest TSSC was obtained 20.6 ^{o}Brix on 5C grafted grapevines. Cluster weight changed between 161.5 and 218.0 g. The heavier clusters and berries were produced on 5BB grafted grapevines. Total yields per vine were calculated between 8.3 and 12.3 kg according to rootstocks. The highest total yield per vine was obtained as 13.5 kg on 5BB grafted grapevines. Although 5C grafted grapevines were more successful in terms of soluble solid content, the heavier fruit clusters, berries and total yield per vine were produced on 5BB grafted grapevines (Table 2).

Table 2. The effect of rootstocks on yield and cluster characteristics of *Vitis labrusca* L. grape

Rootstocks	TSSC (°Brix)	Titrable Acidity (g/L)	Cluster Weight (g)	Berry Weight (g)	Yield (kg/vine)
140 Ru	19.1	8.7	202.6	3.3	12.3
5BB	19.6	9.2	218.0	3.6	13.5
5C	20.6	9.5	161.5	3.2	8.3
SEM	0.196	0.210	4.419	0.101	3.881
Rootstocks	**	ns	**	ns	**

(SEM; Standart Error of Means, ** $p<0.01$; ns: non-significant)

CONCLUSIONS

This study showed that stored carbohydrates consume for shoot growth, blooming and fruit set from blooming to veraison periods. The use of storage carbohydrates have been observed extensively until flowering period. Since the storage carbohydrates are very important for the shoots growth, flowering and fruit set, the cultural practices especially summer pruning which will be made before veraison stage are important for the accumulation of carbohydrates. In the study, 5BB rootstock was found to be suitable for shoot carbohydrate accumulation and growth characteristics of *Vitis labrusca* L. grape.

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