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The Influence of Green Works on Seyve-Villard 18402 Grapes Quality, Vine with Biological Resistance

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Abstract. The reason which *Vitis* hybrid direct producers and many *Vitis* intraspecific hybrid cultivars are increasingly studied in recent times, is their resistance to diseases and pests, which is superior than noble vines. The influence of some green works on grapes quality in Seyve-Villard 18402 cultivar it's presented in this paper. The applied green works were: shoots thinning, shoots tipping, removal of sylleptic shoots and partial defoliation. The studies were carried out in 2012, in the experimental field from the didactic collection of UASVM Cluj-Napoca were focused on quality items such as: acidity, sugar content, pH level, weight of 100 grapes and carotenoids (lutein and β-carotene) content. The experience was organised on 3 variants having 3 replicates each, as it follows: V_1 – without green works, V_2 – shoots thinning, shoots tipping, V_3 – shoots thinning, shoots tipping, removal of sylleptic shoots and partial defoliation. Registered results show that the biggest values of acidity (5.7 g/l H_2SO_4), sugar content (237.6 g/l), pH level (5.3), and medium weight of 100 grape berries (211.3 g) were obtained within V_3 – shoots thinning, shoots tipping, removal of sylleptic shoots and partial defoliation. Carotenoids content was also determined. Using high-performance liquid chromatography (HPLC) technique, lutein and β-carotene were identified. Their dosage was made using calibration curves for carotenoid standards. Most parameters studied had the highest values in the V3, which were made 4 green works.

Keywords: Seyve-Villard 18402, *Vitis* intraspecific hybrid, green works, grape quality, carotenoids

INTRODUCTION

Throughout the world approximately the 80% of all grapes are used in winemaking and 13% are consumed as table grapes. The *Vitis vinifera* grapes are commonly used for wine production around the world, especially in the Europe, but in Nord and South America species such as *Vitis riparia*, *Vitis labrusca*, *Vitis aestivalis*, *Vitis rotundifolia*, *Vitis rupestris* and many *Vitis* intraspecific hybrid cultivars are also used in wine-making (Yang *et al.*, 2009). The reason which *Vitis* hybrid direct producers and many *Vitis* intraspecific hybrid cultivars are increasingly studied in recent times, is their resistance to diseases and pests, which is superior than noble vines. In Romania, like in gratest part of Europe, just *Vitis vinifera* grapes are used for wine production, but since a new law appeared in 2010 (GD,769) some hybrids and *Vitis* intraspecific hybrid cultivars (Garonnet, Seyve-Villard 18402, Chambourcin) can be used in wine industry.

During the growing of the vine some green works can be applied in order to regulate the processes of growth and fruition thus completing the dry fruiting cuttings. Most of the green works influenced especially the quality of the yield (grapes), making them to be used on a large scale in table grape culture (Bucur Georgeta, 2011). Lately, to obtain top quality wines, in some areas, these green works are applied on grape wine cultivars (white or red). Partial defoliation is one of the most used special green works (Reynolds *et al.*, 1996). Older research (Condei, 1974 cited by Oșlobeanu *et al.*, 1980) shows that the leaves which are removed by partial defoliation in ripening phenophase influence in a negative way, both the quantity and content sugars of the berry grapes. Sun exposure grapes lead to improved chemical composition of grapes and wine. However, excessive exposure is in the detriment of quality, especially in hot and moderate climates, leading to chemical loses (Dry, 2009). Watts *et al.* (1991) studied the effect of partial defoliation on the Cabernet Sauvignon grape peel color, and their sugars content, depending on the period in which this green work is performed. The anthocyanin content, the sugar level and berry grape volume increase with delay partial defoliation. Poni *et al.* (2009) states that removing basal leaves before flowering helps to avoid the occurrence of mold. Cantacuzene (2007) argues that in the case of Pinot Noir cultivar, in cold regions of California, thinning shoots gives better results compared with partial defoliation. In the ecoclimatic conditions of Târnave Vineyard, Romania, Ciobanu Florentina *et al.* (2012) studied the influence of some special green works on the quality and quantity of yield, applied to 4 grape wine varieties. The results indicate that the green works have great influence especially on crop quality.

Striegler *et al.* (2012) studied at the Northon cultivar, in Missouri, the effect of sunlight exposure of the grapes, which was controlled by green works such as thinning shoots, partial defoliating, bound and directing shoots. Thus it has been shown that all clusters which have been shadowed had the least amount of soluble solids substances, glucose and fructose and high potassium and malic acid content. After Reynolds *et al.* (2005), earlier thinning shoots increased titratable acidity and soluble solid compounds in wine and must of Pinot noir cultivar. The application of these work later prevented timely maturation of fruits and also the accumulation of solid soluble substances, the anthocyanins and phenols content. In New Zealand, shoots tipping is used to allow better lighting and easier penetration of pesticides in the grape bunches area (Petrie *et al.*, 2003). At the variety Baco noir, removing of sylleptic shoots led to an increase in the yield of grapes. Also, research is recommended to remove all sylleptic shoots, except those who may be detained for rebuilding of vine (Byrne and Howell, 1978).

Grapes contain a wide range of chemical substances (sugar, organic acids, vitamins) and also phytochemicals which are responsible for the sensory characteristics of wines (Waterhouse, 2002). Antioxidant activities of grapes are due to presence of antioxidant components such as flavonoids and carotenoids. The major carotenoids (85% of the total) are β-carotene and lutein. Both the qualitative and quantitative profiles of carotenoids in grapes are affected by factors such as plant variety, stage of maturity, soil characteristics and viticulture practices (Maria Mendes-Pinto, 2009).

The aim of this paper was to study the influence of some green works on grapes quality in Seyve-Villard 18402 (SV 18402) cultivar and to determine the qualitative and quantitative profile of carotenoids.

MATERIALS AND METHODS

1. Plant material and cultivation system

One red grape cultivar ‘Seyve-Villard 18402’, vine with biological resistance, was tested in 2012, under conventional culture system. Were applied chemical control treatments for disease and pests and used chemical fertilizers (complex NPK).

The experimental field was located in the Didactic collection of UASVM Cluj-Napoca Cluj County, Transylvania, Romania. The vine plantation was organized with 1.1 x 1.8 m distance between plants and rows. All vines were established in 1984 on their own roots with a planting density of 5050 vines/ha. Vines were pruned according to the Lenz-Moser system (bilateral cordon) and were grown on speliers, in similar climatic conditions. The design of the experiment was adapted to the conditions of the existing plantation, resembling a completely random block design.

The experiment was organised on 3 variants having 3 replicates each, as follows: V_1 – without green works, V_2 – shoots thinning, shoots tipping, V_3 – shoots thinning, shoots tipping, removal of sylleptic shoots and partial defoliation. Obtained data were gathered and processed on 3 variants and on 3 replicates within each variant, each replication including 5 vines.

2. Grape samples

All the grape samples were collected in 3^{rd} decade of september at the technological maturity of grapes. The grape berries (aproximatively 200) were randomly taken from each sample from different parts of various clusters and processed in the same day into laboratory.

Half of the grapes were used for the classical chemical analysis and the other were stored at – 20° C until further analysis.

3. Classical chemical analysis on grapes

First time weight of 100 grapes was measured with electronical balance. Next the grape berries were crushed to determine sugar content (expressed as g/l) of grape juice using a portable refractometer and correlation tables. Total acidity (g/l H_2SO_4) was determined, diluting the juice with distillate water and titrating with 0.1 Na OH to the fenolftalein endpoint. Also pH level of juice was measured by pH meter.

4. Chemicals

Methanol, ethyl acetate, petroleum ether, diethyl ether, triethylamine, sodium chloride, anhydrous sodium sulphates were purchased from Sigma Chemical Co. The purity of carotenoid standards, ß-carotene and lutein, was estimated by registering their UV–Vis spectra and by an individual HPLC run. The ß-carotene and lutein were found to be 96% and 97.5% pure, respectively. Solvents used for carotenoid analysis (ethyl acetate, acetonitrile, petroleum ether) were purchased from Merck.

5. Sample preparation and carotenoids quantification

Total carotenoids were extracted from 5 g fresh grapes using a mixture of methanol/ethyl acetate/petroleum ether (1:1:1, v/v/v). The residue was re-extracted twice with the same mixture of solvents, following the procedure described by Breithaupt and Schwack (2000). The extracts were combined and partitioned in a separation funnel, successively with water, diethyl ether and saturated saline solution. The ether phase was evaporated to dryness under vacuum, using a rotatory evaporator at 35 ˚C. The samples were kept under nitrogen, at -20 ˚C until further utilization. HPLC separation for individual carotenoids were carried out on Waters 990 controller system with PDA detector, Kontron pompe, using a reversed phase YMC C30 column (25cm x 4.6 mm), 5 μm. The mobile phase was a mixture of metanol:terţ-butil-metil-eter: water (81:15:4) (solvent A) and terţ-butil-metil-eter:metanol: water (90:7:3) (solvent B). The gradient started with 1 % B to 55 % B from 0 to 50 min and continued up to 52 min with 60% B. After min 52 the solvent B concentration decrees from 60% to 1% in two minutes (from 52 min at 54 min). The flow rate was 1 ml/ min. All chromatograms were monitored at 450 nm.

The carotenoids were identified using parallel HPLC runs with carotenoid standards as well as by recording the UV–Vis spectra and their comparison with known carotenoid spectra (Britton, 1995). Calibration curves were made using different concentrations of pure lutein and ß-carotene. The linear regression factors of the calibration curves were higher than 0.965 in all cases. The individual carotenoids content were determined according to the calibration curves and the results were expressed as μg carotenoid / kg fresh material.

6. Statistical analysis

The data were expressed as mean ± standard deviation (SD) from three replicates for each sample. In order to determine the significant differences between values, analysis of variance ANOVA were performed.

RESULTS AND DISCUSSIONS

1.Classical chemical analysis on grape

Weight of 100 grape berries, with acidity and concentration in sugars, is an important factor in determine of full maturation of the grapes in order to their harvest (Dejeu, 2004). In the experiment the SV 18402 grapes and berries were harvested and weighed at full maturity. As shown in Tab. 1 the weight of 100 berries have different values of the studied variants (165.0 g, 171.6 g and 211.3 g) with a mean of experiment among the three variants of 182.6 g. Compared with the average of experiment (considered as a control), two of the variants, V1 = 165.0 g and V2 =171.6 g, there were no differences statistically, while in the V3 (4 green works) was obtained the highest value of 211.3 g, significantly positive compared to control.

The same situation is in the case of sugar, where V3 (4 green works) recorded the highest value of 237.6 g/l, significantly positive compared to control. The other variants V1= (193.5 g/l and V2= 212.1 g/l) obtained nonsignificant differences compared with mean of experiment. These values are comparable with those recorded by ‘Fetească neagră’ at Odobești (232.2 g/l) and by ‘Băbească neagră’ at Uricani (199.0 g/l) Romania (Dobrei et al., 2008). Magarino et al. (2006) determined the sugar content of Cabernet sauvignon between 225 and 241 g/l in Spain, and Oliveira et al. (2006) reported at a few redwine cultivars from Portugal values between 195-232 g/l.

Tab.1

Weight of 100 grape berries, sugar content of grape juice, acidity and pH, SV 18402 cultivar, Cluj-Napoca, 2012

Nr. crt.	Variant	The average weight of 100 berries (g)	The average sugar content (g/l)	The average level of acidity (g/l H_2SO_4)	The average level of pH
1	V1-without green works	165.0 ns	193.5 ns	3.1 o	3.1 o
2	V2- two green works	171.6 ns	212.1 ns	4.1 ns	3.6 ns
3	V3- four green works	211.3 *	237.6 *	5.7 *	5.0 **
Mean of experiment (Mt.)		182.6	214.4	4.3	4.0
DL 5%	=	28.0	22.0	1.1	0.8
DL 1%	=	46.3	36.4	1.8	1.3
DL 0,1%	=	86.7	68.1	3.4	2.5

In case of acidity, V1- without green works registred the lowest value 3.1 g/l H_2SO_4, and V3 – three green works, 5.7 g/l H_2SO_4. Hülya Orak (2007) obtained in Turkey for 16 red grape cultivars values from 3.31 to 9.53 g/l H_2SO_4. The pH value in the analyzed variants were changed between 3.1 (V1) and 5.3 (V3). Oliveira et al. (2006) found the pH level between 3.1 ('Sousão') and 4.0 ('Touriga Franca') in Douro Region, and was similar to our findings for 'Seyve-Villard 18402'.

2. Qualitative and quantitative analysis of carotenoids, using high performance liquid chromatography (HPLC)

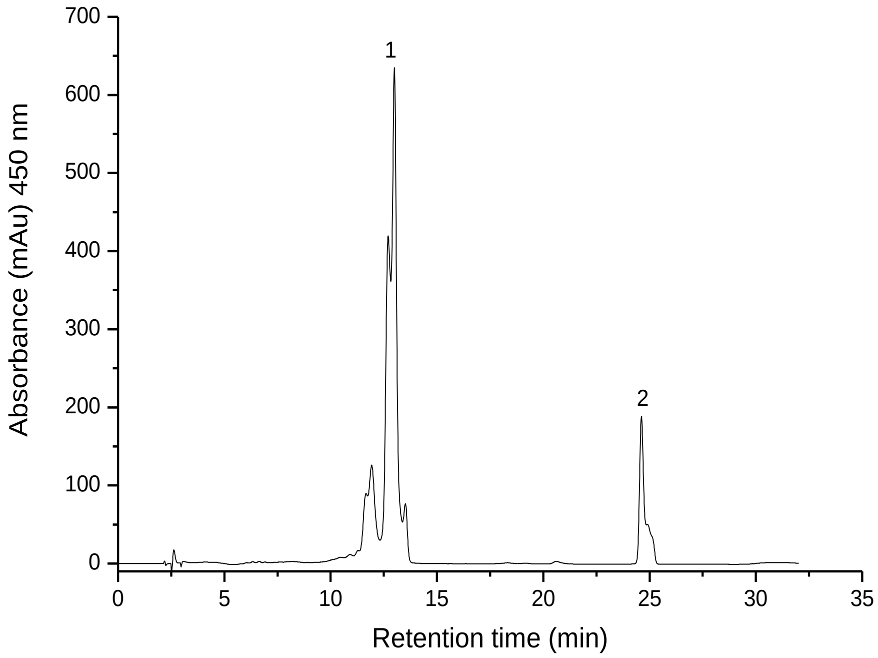

Fig. 1. HPLC chromatogram of the carotenoids separated from fresh grapes, 1- lutein and 2- β-carotene.

The main carotenoids extracted from redwine grapes cultivar 'Seyve-Villard 18402' were lutein and β-carotene. HPLC chromatogram of unsaponified carotenoids extract from grapes was recorded at 450 nm and it is shown in the Fig. 1.

The presence of carotenoids in grape berries has been extensively reported in the literature. The most important carotenoids (85% from all the carotenoids present) are ß-carotene and lutein, the rest of them being neochrome, neoxanthin, violaxanthin, luteoxanthin, flavoxanthin, zeaxanthin, and *cis*-isomers of lutein and ß-carotene (Mendes-Pinto *et al.*, 2004).

Generally, the highest level of carotenoids occured in grapes from hot viticultural regions. However, at maturity, grapes expose to sunlight seem to have lower carotenoid concentration than shaded grapes (Bureau *et al.*, 1998 a). The carotenoids are involved in the aroma of wine. It was reported thet norisoprenoids could come from the direct degradation of carotenoids such neoxanthin, violaxanthin, lutein and ß-carotene (Mordi *et al.*, 1991).

In the analysis performed in biochemistry laboratories of USAMV Cluj-Napoca, carotenoids were separated and their concentrations were determined. In Tab. 2 are presented the data regarding the carotenoid concentrations in analyzed samples.

Tab. 2

The carotenoids concentration from 'Seyve-Villard 18402' grapes samples, Cluj-Napoca, 2012

Nr. crt.	Samples analyzed SV - 18402	Lutein (µg/kg)	ß-caroten (µg/kg)	Ratio Lutein/ ß-carotene
1	V1-without green works	570.96±28	232±16	2.46
2	V2- two green works	712±46	256.8±23	2.77
3	V3- four green works	698±36	314±21	2.22

Data presented in Tab. 3 shown that the lutein content have different values between 571.0 and 712.0 µg/kg with a mean of experiment among the three variants of 660.3 µg/kg. Thus V_2- two green works, recorded the highest value 712 µg/kg, but with nonsignificant differences compared with mean of experiment. In case of β-caroten, the highest value is registred at V3- four green works (314.0 µg/kg), significantly positive compared to control.

Tab. 3

Lutein and β-caroten (µg/kg) content Cluj-Napoca, 2012

Nr. crt.	Variant	Lutein (µg/kg)	β-caroten (µg/kg)
1	V1-without green works	571.0 o	232.0 o
2	V2- two green works	712.0 ns	256.8 ns
3	V3- four green works	698.0 ns	314.0 *
Mean of experiment (Mt.)		660.3	267.6
	DL 5% =	83.4	31.1
	DL 1% =	138.0	51.5
	DL 0,1% =	258.2	96.3

Oliveira *et al.* (2006) studied the carotenoid profile in different varieties of grapes, obtaining the level of lutein concentrations between 295-1044 mg / kg, and for ß-carotene between 168-910 mg / kg. Also Bunea *et al.* (2012) determined for red table grapes values between 662-690 mg / kg. For ß-carotene concentrations had lower values 229-265 mg / kg.

The range between lutein and ß-carotene, in grapes, are very different depending on the viticultural region and the type of variety analyzed. In some cultivars (Sauvignon, Pinot Noir and Merlot) they showed a lutein level almost double than ß-carotene, in others the concentration of these two carotenoids are similar or higher ß-carotene level (Crupi, Milella and Antonacci, 2010).

CONCLUSION

In ecoclimatic conditions of UASVM Cluj-Napoca, the study on red grape cultivar 'Seyve-Villard 18402', shown that the majority of parameters analyzed recorded highest values for V3 – shoots thinning, shoots tipping, removal of sylleptic shoots and partial defoliation. These results indicate that the application of a greater number of green works leading to a better quality of grapes.

REFERENCES

1. Bucur, G.M. (2011). Viticultură. Departamentul pentru Învățământ la distanță. UASVM București.

2. Byrne, M.E. and G.S. Howell (1978), Initial response of Baco Noir grapevines to pruning severity, sucker removal and weed control. Am J Enol Viticult. 39(3). 29:192-8.

3. Bunea, C.I., N. Pop, A. Babeş, C. Matea, F.V. Dulf and A.Bunea. (2012). Carotenoids, total polyphenols and antioxidant activity of grapes (*Vitis vinifera*) cultivated in organic and conventional systems**.** Chem Cent Journal. 6:66.

4. Bureau, S.M., A.J. Razungles, R.L. Baumes and C.L. Bayonove (1998 a). Effect of vine or bunch shading on the carotenoid composition in Vitis vinifera L., berriesI. Syrah grapes. Vitic Enol Sci 53(2):64-71.

5. Breithaupt, D.E. and W. Schwack. (2000). Determination of free and bound carotenoids in paprika (*Capsicum annuum* L.) by LC/MS. Eur Food Res Technol 211(1): 52–55.

6. Britton, G., S. Liaasen-Jensen and H. Pfander. (1994) Carotenoids: Spectroscopy .Volume 1B. Basel•Boston•Berlin: Birkhäuser Verlag.

7. Cantacuzene, N. (2007). Leaf removal strategies for Pinot Noir. Practical Winery Vineyard Sep/0ct, 62-65.

8. Ciobanu, F., N. Pop, M.Iliescu, A. Babeş, C.I. Bunea, A.E. Ardelean, M.L. Lung and F. Tripon (2012b). Special green works influence on yield quantity and quality at grape varieties for flavoured and semi-flavored wines from S.C.D.V.V. Blaj, Bulletin UASVM, Horticulture, 69:1, 110-115.

9. Crupi, P., R.A. Millela and D. Antonacci (2010). Simultaneous HPLC-DAD-MS (ESI^+) determination of structural and geometrical isomers of carotenoids in mature grapes. J Mass Spectrom (Special Issue: Food Chemistry). 45: 971- 980.

10. Dejeu, L. (2004). Viticultura practică. Editura Ceres. București.

11. Dobrei, A. , L. Rotaru and S. Morelli (2008). Ampelografie. Editura SOLNESS. Timișoara

12. Dry, P. (2009). A brief history of bunch exposure. ANZ Wine Ind Journal. Nov/Dec.(in press).

13. Hülya Orak, H., (2007). Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of red grape cultivars and their correlations. Scientia Horticulturae. 111:235-241.

14. Magarino, S.P., M.L. Gonzales and S. Jose (2006). Polyphenols and clour variability of red wines made from grapes hervested at different ripeness grade. Food Chem. 197-208.

15. Mendes-Pinto, M.M., A.C. Silva Ferreira, M. Beatriz, P.P. Oliveira and P. Guedes de Pinho. (2004). Evaluation of Some Carotenoids in grapes by Reversed-and Normal-Phase Liquid Chromatography: A qualitative Analysis. J Agr Food Chem. 52 (10): 3182-3188.

16. Mendes-Pinto, M.M. (2009). Carotenoi brekdown products the- norisoprenoids-in wine aroma. Archives of Biochemistry and Biophysics. 483:236-245.

17. Mordi, C.R., J.C. Walton, G.W. Burton, L. Hughes, K.U. Ingold and D.A. Lindsay (1991) Exploratory study of ß-carotene auto-oxidation. Tetrahedron Lett. 32:4203-6.

18. Oliveira, C., A. Barbosa, A.C. Silva Ferreira, J. Guerra and P.G. De Pinho (2006). Carotenoid Profile in Grapes Related to Aromatic Compounds in Wines from Douro Region. J Food Sci.71(1): S1-S7.

19. Oşlobeanu, M., M. Oprean, I. Alexandrescu, M. Georgescu, P. Baniţă and L. Jianu (1980). Viticultură generală şi specială. Editura Didactică şi Pedagogică. Bucureşti.

20. Petrie P.R., M.C.T. Trought, G.S. Howell and H.G. Buchan (2003). The effect of leaf removal and canopy height on whole-vine gas exchange and fruit developement of Vitis vinifera L. Sauvignon blanc. Functional Plant Biology. 30(6): 711:717.

21. Poni, S., F. Benizzoni, S. Civardi and N. Libelli (2009). Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red Vitis vinifera cultivars. Aust. J. Grape Wine Res. 15:185-193.

22. Reynolds, A., D.A. Wardle and A.P. Naylor (1996). Impact of training system, vine spacing and basal leaf removal on Riesling, berry composition, canopy microclimate and vineyard labour requirements. Amer. J. Enol. Vitic. 47:63-76.

23. Striegler K.R., E. Bergmeier, J. Harris and Jogajah Satisha (2012). Influence of cluster exposure to sun on fruit composition of Norton grapes (*Vitis estivalis* Michx) in Missouri. Int. J. Fruit Sci. 12 (4):410-426.

24. Watts J.E., J. Hunter and O.T. De Villiers (1991). The Effect of Partial Defoliation on Quality Characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon Grapes. II. Skin Color, Skin Sugar, and Wine Quality. Am J Oenology and Viticulture, 42 (1):13-18

25. Waterhouse, A.L. (2002). Wine phenolics. Annals of The New York Academy of Sciences.957: 21- 36.

26. Yang, J., T.E. Martinson and R.H. Liu (2009). Phytochemical profiles and antioxidant activities of winw grapes. Food Chem 116: 332-339.

27. ***, 2010. Romanian Govern Decision. No. 769