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INFLUENCE OF CORBOHYDRATE CONTENT ON GRAFTING IN WINE GRAPE VARIETIES 'AROMAT DE IAȘI' AND 'GOLIA'

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Abstract

In the present work determinations were made regarding the behavior of wine grape varieties 'Aromat de Iași' and 'Golia', respectively Chasselas doré variety, that was taken as control, grafted on three rootstocks Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 Blaj and Berlandieri x Riparia Selection Crăciunel 2. It was found that the highest total content of carbohydrates had the rootstock Riparia gloire (13.49%), followed by Crăciunel 2 (12.78%) and SO_{4-4} (12.02%). In the Vinifera varieties the same indicator was higher for 'Golia' grape variety (15.64%) and for 'Aromat de Iași' variety (14.97%), compared to the control variety Chasselas doré (13.83%). As a result of the grafting and forcing, the following aspects resulted: the percentage of vines suitable for planting was the highest in the 'Golia' variety grafted on Riparia Gloire, respectively 98%, and in the 'Aromat de Iași' variety grafted on Crăciunel 2, of 96%. The vines for which the grafting point was complete, where the buds entered vegetation and the root primordia was in maximum percentage were found at the 'Golia'/SO_{4-4} variant, at 74%.

Key words: *callus, carbohydrates, primordial roots.*

INTRODUCTION

In grafted vines, the root development and healing of the graft union are particulary affected by the water content and by carbohydrates stored in scions and rootstock. The accumulation, transformation and translocation of carbohydrates in individual parts of the grapevine have been described in the literature by various authors (Koblet, 1969, 1975; Schaefer, 1978, 1986a, 1986b; Koblet and Perret, 1990; Koblet et al., 1993; Candolfi-Vascocnelos et al., 1994, 1995; Warmund et al., 1986; Schaefer and Schropp, 1987; Schumann and Schaefer, 1988; Vršič, 1996) examined various effects of individual procedures for the cultivation of grafted vines on the level of substances stored in the grafted vines.

Rootstock plays a role in the partitioning of biomass between root, shoot, trunk and fruit. Not only are carbohydrates stored in vine canes evidence of the health and vigor of the previous season's growth (Balasubrahmanyam et al., 1978), in many plant species, root carbohydrates are responsible for shoot development, growth in stem and root diameters as well as new root length, flower bud initiation and growth, and fruit set (Loescheret et al., 1990).

In grafted vines, the root development and healing of the graft union are particulary affected by the water content and by carbohydrates stored in scions and rootstock. The accumulation, transformation and translocation of carbohydrates in individual parts of the grapevine have been described in the literature by various authors and examined various effects of individual procedures for the cultivation of grafted vines on the level of substances stored in the grafted vines (Vršič et al., 2009).

High carbohydrates and levels of specific plant hormones are required for successful callus formation (Hunter et al., 2004), but little work describing relationships between the two has been conducted. Starch is directly involved in callus formation and vegetative growth of rootstocks during callusing (Hunter et al., 2004). Rootstock cultivars affect starch levels in scions to differing degrees and also vary with respect to starch depletion during callusing, which impact time required for callus development (Phillips et al., 2015).

MATERIALS AND METHODS

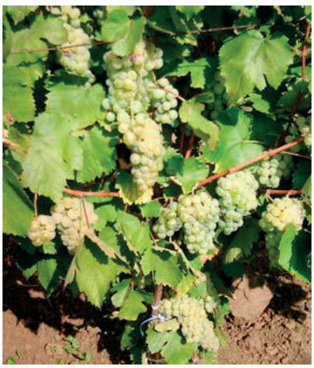
Research was carried out at the Research and Development Station for Viticulture and Winemaking (SCDVV) in Iasi in 2019. Two varieties of wine grapes, 'Aromat de Iași' and 'Golia', obtained at SCDVV Iasi, respectively Chasselas doré variety taken as control (Table 1), were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selection Oppenheim 4-clone 4 and Berlandieri x Riparia Selection Crăciunel 2).

Using the two varieties taken into consideration, respectively the three rootstocks on which the varieties were grafted, two series of determinations were made:

Determinations regarding the total carbohydrate content of the rootstock and scion canes (soluble sugars and starch) by the chemical method with the anthrone reagent and a representation of the starch in the shoots by the colorimetric method based on the staining reaction of the starch with Lugol reagent.

Determinations on several growth parameters after the end of the process of forcing of the grafted cuttings: the percentage of vines fit for planting, the percentage of successful grafting, the percentage of vines with eyes not provided in the vegetation, the callus formation on diameter of the grafted calves, the degree of callus formation of the grafted vines and the location of the roots on the grafted cuttings.

Table 1. Studied biological material

Grape variety	Genitors	Author	Year of homologation
Aromat de Iași 	Free fecundation of Tămâioasă românescă seeds	Dănulescu Dumitru	1980
Golia 	Intraspecific hybridation of Sauvignon x Șarbă	Dănulescu Dumitru	1999

Chasselas doré (control) 	Ancient grape variety with uncertain origin. It is supposed to be Swiss.	Unknown	Cultivated since the 11th century

Carbohydrates were determined by the chemical method with the anthrone reagent. The sugars were extracted with ethyl alcohol, respectively starch with perchloric acid, under certain conditions, and treated with anthrone reagent.

When the sugars were extracted, chopped chunks in pieces under 0.5 cm were dried in the oven at 65°C to a practically constant mass. The material thus dried was finely milled to the destruction of the cell structure and then passed through the mill once more. An amount of 0.5 g of the prepared material was weighed to the nearest 0.01 g, mixed with about 0.5 g of sand and 5 ml of ethyl alcohol and stirred by mixing until a homogeneous mass was obtained. To the obtained mixture were added 40 ml of water and then introduced into a polyethylene centrifuge tube. It was centrifuged for 20 minutes at 8000 rpm. The liquid consisting of water and alcohol was poured into a 200 ml graduated flask, and over the remaining sediment in the centrifuge tube 5 ml of warm ethyl alcohol was poured and mixed with a glass wand, until homogenized. Then 20 ml of warm ethyl alcohol were added and centrifuged for 20 minutes. The liquid containing the alcohol was poured over that obtained at the first centrifugation, and the operations were repeated two more times. The flask with the solutions obtained after all the centrifugations is filled to the mark with water. From the obtained basic solution 100 ml were pipetted into a 200 ml volumetric flask and 1 ml of lead acetate solution was added. After 5 minutes it was filled up to the mark with water. After another 10 minutes the solution was filtered and 100 ml of the filtrate was pipetted into another 200 ml volumetric flask. The excess of lead acetate was neutralized by the addition of 1 ml of sodium acid phosphate solution. After 5 minutes, the mixture was made with water and then stirred vigorously. 40 ml of the solution were taken and centrifuged, and the clear and transparent liquid constitutes the sugar extract.

The mixture remaining in the centrifuge tube after the extraction of sugars was mixed with 5 ml of water until a homogeneous mass was obtained and 6.5 ml of perchloric acid solution were added dropwise. The mixture was stirred continuously for 15 minutes then diluted with water and centrifuged again for 20 minutes. The liquid was decanted into a 500 ml graduated flask and the treatment was repeated with perchloric acid and water as well as centrifugation twice more. The liquid was decanted and introduced into the same balloon which was brought to the mark with water. The extract thus obtained represents the starch from the rootstock and from the scion canes respectively.

The calculation, expression and interpretation of the results of the total carbohydrate content (soluble sugars and starch) in the extract are expressed as glucose and calculated with the formulas:

% Soluble sugars (glucose): $\frac{Ec-Ea}{Eb} x\ 50 \left[\frac{\mu g}{ml}\right]$

% Starch (glucose): $\frac{Ed-Ea}{Eb} \times 50 \left[\frac{\mu g}{ml}\right]$

Ea, Eb, Ec, Ed = extinctions of solutions a, b, c, d (average of the 3 determinations);

50 = concentration of standard glucose solution, in µg/ml.

The content of soluble sugars, respectively of starch of the planting material expressed as glucose and related in percentage to the dry planting material at 65°C, is calculated with the formula:

% sugars (glucose) = Z $\frac{200\times4}{m\times10^6}$ x 100 [%]

% starch (glucose) = A $\frac{500}{m\times10^6}$ x 100 [%]

Z = the sugar content of the extract calculated in µg/ml;

A = starch content of extract calculated in µg / ml;

4 = factor for the dilutions performed during the determination;

500 = volume of starch extract in ml;

200 = volume of filtrate from which sugars are determined in ml;

m = mass of dry planting material at 65°C, taken for determination.

RESULTS AND DISCUSSIONS

For a representation of the starch in ropes by the colorimetric method that is based on the staining reaction of the starch with Lugol reagent, the analyzed parts are represented by annual elements of the freshly harvested kernel (fruit ropes). The starch that accumulates in the woody tissues of the annual cords, gives them resistance to the low temperatures in winter and constitutes the nutritional reserve for starting the buds/winter eyes in the spring of next year.

At the end of the resting period, canes were harvested for both rootstock varieties and scion varieties through which cross sections were made using the microtome. These sections were treated with Lugol reagent and allowed for a period of time to dry thus obtaining preparations that were analyzed under a microscope (Figure 1).

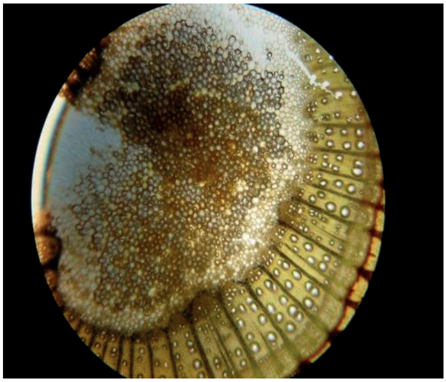


Figure 1. Aspects of the starch content accumulated in the ropes of rootstock and grape varieties

Table 2. Carbohydrates from the ropes of rootstock varieties

Rootstock	Total carbohydrate (%)	Soluble sugars (%)	Starch (%)	Rope moisture (%)
Riparia Gloire	13.49	10.29	3.20	52.71
Selection Oppenheim 4-clone 4 (SO_{4-4})	12.02	9.58	2.44	52.33
Berlandieri x Riparia Selection Crăciunel 2	12.78	9.79	2.99	52.17

Regarding the results obtained regarding the total carbohydrate content in the string rootstocks (Table 2), the best result was registered at the rootstock Riparia Gloire (13.49%) of which the percentage of soluble sugars was 10.29%, and the starch percentage was 3.20%.

The other two rootstock varieties had results close to each other, Berlandieri x Riparia Selection Crăciunel 2 having a percentage of 12.78% total carbohydrates, of which soluble sugars 9.79% and starch 2.99%, and Selection Oppenheim 4-clone 4 (SO_{4-4}), 12.02% carbohydrate of which 9.58% represents the percentage of soluble sugars and 2.44% the percentage of starch. Both varieties had inferior results to the rootstock Riparia Gloire.

Analyzing these results it can be seen that all three varieties of rootstocks are suitable for grafting because the material with a total carbohydrate content (sugars, starch) below 12% is rejected from propagation, the varieties taken into operation having values above that which would have led to the removal from graft.

Table 3. Carbohydrates from the ropes of grape varieties

Scion	Total carbohydrate (%)	Soluble sugars (%)	Starch (%)	Rope moisture (%)
Aromat de Iași	14.97	9.56	5.41	53.74
Golia	15.64	11.38	4.26	56.50
Chasselas doré (control)	13.83	8.63	5.20	57.36

After analyzing the carbohydrates from the rootstock varieties, the content of the carbohydrates from the scion varieties was also analyzed (Table 3). Of the three varieties taken into consideration, the best results were obtained by 'Golia' grape variety, which recorded a total carbohydrate content of 15.64% (11.38%, representing the percentage of soluble sugars , respectively 4.26% starch), followed by the other variety, 'Aromat de Iași', having a total carbohydrate content of 14.97% of which 9.56% represent soluble sugars and 5.41% starch. Both varieties recorded higher values compared to the control variety Chasselas doré (13.83%, of which 8.63% soluble sugars and 5.20% starch).

After it was found that the material used fulfills the necessary conditions for being grafted, it was prepared for grafting. They were grafted using a pedal operated device, by uniting the two partners through a joining tip, in the form of the letter omega, they were covered in paraffin, they were layered and forced into vegetation. The process lasted 21 days in which the temperature was raised in the first days (30°C) and then gradually reduced (25°C), the humidity was between 68% and 91%, in the absence of light on the entire duration of the cycle, due to the fact that the crates were covered with a canvas of geotextile material, over which lay a 5 cm layer of sawdust. After the forcing process was completed, the grafted vines were acclimatized for 5 days. After the completion of the forcing, the grafted vines were analyzed following a series of parameters whose results are presented in table 4.

As for the percentage of vines suitable for planting, 'Golia'/Riparia Gloire variant recorded the best result (98%), while in the 'Aromat de Iași' variety, the best association was at the grafting on the Crăciunel 2 rootstock (96%), both varieties having better results compared to the best variant obtained in the control variety, Chasselas doré/Riparia Gloire (94%).

From the vines that are suitable for planting, the percentage of a good grafting was also analyzed. The best results were obtained by the 'Golia'/SO_{4-4} variant (74%), respectively 'Aromat de Iași'/Riparia Gloire (72%), while in the control variety, the best result was Chasselas doré/Riparia Gloire (68%).

The percentage of vines with the bud that entered vegetation was good, with values of 74% ('Golia'/Riparia Gloire), respectively 72% ('Aromat de Iași'/Crăciunel 2), compared to 68% (Chasselas doré/Riparia Gloire). For both varieties taken into consideration, the percentage of vines where the bud did not enter vegetation was analyzed. The variants 'Golia'/Crăciunel 2 (21%) and 'Aromat de Iași'/Riparia Gloire (23%) had lower values compared to Chasselas doré/Riparia Gloire (26%), the control variety.

The degree of callus formation was another parameter analyzed, here the best results were obtained by the variant 'Golia'/Riparia Gloire (98%) and 'Aromat de Iași'/Crăciunel 2 (96%), these having fully formed callus. The control variety, Chasselas doré/Riparia Gloire (94%) recorded the best results, having a lower value compared to the results obtained in the two varieties taken into consideration. Callus formation on different diameters was also tracked here. The grafted vines were calibrated on the three diameters found in the specialized literature (7-8.5 mm; 8.6-10 mm; 10.1-12 mm), the results obtained being very varied for each variant, both in the varieties taken into operation and in the control variety.

The last parameter analyzed was the one regarding the location of the roots on the grafted vines, namely the place where the grafted vines formed root primordia. All the variants had a high percentage of root primordia occurring at the base of grafted vines, compared to their formation at the second node, where the highest percentage was obtained in 'Aromat de Iași'/SO_{4-4} (7%) and 'Golia'/SO_{4-4} (5%) while the control variety Chasselas doré/Riparia Gloire, had lower values, namely 2%.

Table 4. Results obtained after the completion of the forcing process

Scion	Rootstock	*Vines suitable for planting (%)	**Percentage of grafting (%)	Vines where the buds did not enter vegetation (%)	Diameter of callus (%)			Degree of callus (%)		Root placement (%)	
					7-8,5 mm	8,6-10 mm	10,1-12 mm				
Aromat de Iași	Riparia Gloire	95	72	23	20	26	49	95	5	91	4
	Selection Oppenheim 4-clona 4 (SO_{4-4})	95	70	25	34	31	30	95	5	88	7
	Berlandieri x Riparia Selection Crăciunel 2	96	71	25	30	32	34	96	4	92	4
Golia	Riparia Gloire	98	70	28	30	35	33	98	2	95	3
	Selection Oppenheim 4-clona 4 (SO_{4-4})	96	74	22	38	32	26	96	4	91	5
	Berlandieri x Riparia Selection Crăciunel 2	93	72	21	32	38	23	93	7	89	4
Chasselas doré (control)	Riparia Gloire	94	68	26	29	42	23	94	6	92	2
	Selection Oppenheim 4-clona 4 (SO_{4-4})	92	67	25	37	30	25	92	8	91	1
	Berlandieri x Riparia Selection Crăciunel 2	91	66	25	29	36	26	91	9	90	1

*Percentage of vines suitable for planting (%) - vines with complete callus formed at the point of grafting, with buds started in vegetation and with root primordia + vines with complete callus formed at the point of grafting, primordia of root formed, without buds started in vegetation.
**Percentage of grafting (%) - calves with complete callus formed at the grafting point, with buds started in vegetation and with root primordia.

CONCLUSIONS

The total content in carbohydrates was maximal in the case of the rootstock Riparia Gloire (13.49%), which was also reflected on the high percentage of grafted vines that developed a root system (98%).

The physiological humidity of the analysed canes was within normal limits, both in the scion and in the rootstocks (52-57%), which showed that the graft material was kept under proper conditions.

The callus formation for different diameters shows that the best ratio was at Riparia gloire and Selection Crăciunel 2 with the diameters of 8.6-10 mm, respectively 10.1-12 mm, and at the rootstock SO_{4-4} at the diameters of 7-8.5 mm, respectively 8.6-10 mm.

The percentage of vines with the most numerous basal roots was registered at the Riparia gloire rootstock (95-96%).

REFERENCES

Balasubrahmanyam, V.R. & Eifert, J. & Diofasi, L. (1978). *Nutrient reserves in grapevine canes as influenced by cropping levels*. Vitis 17, 23-29.

Candolfi-Vasconcelos, M.C. & Candolfi, M.P. & KOBLET, W. (1994). *Retranslocation of carbon reserves from the woody storage tissues into the fruit as a response to defoliation stress during the ripening period in Vitis vinifera* L. Planta, 192, 567-573.

Candolfi-Vasconcelos, M.C. & Candolfi, M.P. & KOBLET, W. (1995). *Rücktransport von Reserven aus Holz und Wurzeln in die reifenden Trauben*. Schweiz. Z. Obst- & Weinbau 132, 148-149.

Hunter, J.J. & Volschenk, C.G. & Le Roux, D.J. & Adams, L. (2004). *Plant material quality: A compilation of research.* ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa.

Koblet, W. (1969). *Wanderungen von Assimilaten in Rebtrieben und Einfluß der Blattfläche auf Ertrag und Qualität der Trauben*. Wein-Wiss. 24, 277-319.

Koblet, W. (1975). *Wanderungen von Assimilaten aus verschiedenen Rebenblättern während der Reifephase der Trauben.* Wein-Wiss. 30, p. 241-249.

Koblet, W. & Perret, P. (1990). *Beziehung zwischen Triebwachstum, Wurzelentwicklung und Assimilatwanderung in Topfreben.* Wein-Wiss. 45, 93-96.

Koblet, W. & Candolfi-Vasconcelos, M.C. & Aeschimann, E. & Howell, G.S. (1993). *Influence of defoliation, rootstock, and training system on 'Pinot noir' grapevines. I. Mobilization and reaccumulation of assimilates in woody tissue.* Vitic. Enol. Sci. 48, 104-108.

Loescher, W.H. & Mccamant, T. & Keller, J.D. (1990). *Carbohydrate reserves, translocation, and storage in woody plant roots.* HortSci. 25, 274-281.

Phillips, N. & Reynolds, A. & Di Profio, F. (2015). *Nonstructural Carbohydrate Concentrations in Dormant Grapevine Scionwood and Rootstock Impact Propagation Success and Vine Growth.* HortTechnology, 25(4), 563-550.

Schaefer, H. (1978). *Der Proteinstoffwechsel der Jungreben in der Rebschule.* Weinberg & Keller 25, 331-351.

Schaefer, H. (1986a). *Zum Stoffwechsel von jungen Pfropfreben in der Rebschule mit unterschiedlicher Veredlungsaffinität und Wüchsigkeit*. Wein-Wiss. 41, 102-116.

Schaefer, H. (1986b). *Zum Stoffwechsel von jungen Pfropfreben in der Rebschule mit unterschiedlicher Veredlungsaffinität und Wüchsigkeit.* Wein-Wiss. 41, 250-263.

Schaefer, H. & Schropp, A. (1987). *Stoffwechselunterschiede in gut und schlecht wachsenden Reben nach der Veredlung.* Wein-Wiss. 42, 330-341.

Schumann, F. & Schaefer, H. (1988). *Über den Einfluß unterschiedlichen Laubschnitts in Rebschulen auf den Stoffwechsel der Pfropfrebe*n. Wein-Wiss. 43, 22-28.

Vršič, S. (1996). *The importance of stored substances in rootstocks and scions and assimilation area for the growth of grafted vines*. Diss. Univ. Ljubljana.

Vršič, S. & Pulko, B. & Valdhuber, J. (2009). *Influence of Defoliation on Carbohydrate Reserves of Young Grapevines in the Nursery.* Europ.J.Hort.Sci., 74(5), 218-222.

Warmund, M.R. & Starbuck, C.J. & Lockshin, L. (1986). *Growth, cold hardiness, and carbohydrate content of 'Vidal blanc' grapevines propagated by hardwood vs. softwood cuttings.* Am. J. Enol. Vitic. 37, 215-219.