

## INFLUENCE OF CARBOHYDRATE CONTENT ON GRAFTING IN TABLE GRAPE VARIETIES GELU AND PAULA

### INFLUENȚA CONȚINUTULUI DE HIDRAȚI DE CARBON ASUPRA CALUSĂRII LA ALTOIRE LA SOIURILE PENTRU STRUGURI DE MASĂ GELU ȘI PAULA

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**Abstract.** *The factors that determine the formation of the callus at the grafting point, respectively the percentage of vines capable of being planted in the nursery, are of genetic, technological and ecological nature. Thus, the genetic nature of the two symbionts is crucial to the success of grafting. In the present work determinations were made regarding the behavior of table grape varieties Gelu and Paula 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<sub>4-4</sub> (12.02%). In the Vinifera varieties the same indicator was higher for Paula grape variety (14.32%) and for Gelu variety (13.07%). As a result of the grafting and forcing, the following aspects resulted: the percentage of vines suitable for planting was the highest in the Paula variety grafted on Crăciunel 2, respectively 99%, and in the Gelu variety grafted on Ripariagloire, of 98%. 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 Paula / Crăciunel 2 variant, at 76%. Gelu / Crăciunel 2 variant resulted in obtaining the most vines (7%) that formed root primordia at the intermediate node as well.*

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

**Rezumat.** *Factorii care condiționează formarea calusului la punctul de altoire, respectiv procentul de vițe apte de a fi plantate în școala de vițe sunt de ordin genetic, tehnologic și ecologic. Astfel, natura genetică a celor doi simbioți este hotărâtoare pentru reușita prinderii la altoire. În lucrarea de față s-au făcut determinări privind comportarea soiurilor pentru struguri de masă Gelu și Paula altoite pe trei portaltoi Riparia Gloire, Berlandieri x Riparia Selectia Oppenheim 4-clona 4 Blaj și Berlandieri x Riparia Selectia Crăciunel 2. S-a constatat că cel mai ridicat conținut total în hidrați de carbon l-a avut portaltoiul Riparia gloire (13,49%), urmat de Crăciunel 2 (12,78%) și SO<sub>4-4</sub> (12,02%). La soiurile vinifera același indicator a fost mai mare la soiul Paula (14,32%) și Gelu (13,07%). În urma altoirii și forțării au rezultat următoarele aspecte: procentul de vițe apte pentru plantare a fost cel mai mare la soiul Paula altoit*

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pe Crăciunel 2, de 99%, iar la soiul Gelu, altoit pe Riparia gloire, de 98%. Vișele cu sudură complet formată la punctul de altoite, mugurele pornit în vegetație și primordii de rădăcini a fost în procent maxim, la varianta Paula/Crăciunel 2, în procent de 76%. Varianta Gelu/Crăciunel 2 a dus la obținerea celor mai multe vișe care au format primordii de rădăcini și la nodul intermediar (7%).

**Key words:** carbohidrați, calus, rădăcini primordiale

## INTRODUCTION

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 particularly 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).

## MATERIAL AND METHOD

Research was carried out at the Research and Development Station for Viticulture and Winemaking (SCDVV) in Iasi in 2019. Two varieties of table grapes, Gelu and Paula, obtained at SCDVV Iași, were grafted on three rootstocks (Riparia Gloire, Berlandieri x Riparia Selectia Oppenheim 4-clone 4). And Berlandieri x Riparia Selectia Crăciunel 2).


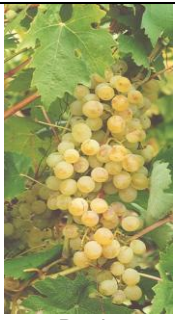
Using the two varieties taken into consideration, respectively the three rootstocks on which the varieties were grafted, two series of determinations were made.

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
 Gelu	Free fecundation of local grape variety Coarnă neagră and irradiation with X rays of its seeds	Calistru Gheorghe Damian Doina	1999
 Paula	Intraspecific sexual hybridation of Bicane x Aromat de Iași	Calistru Gheorghe Damian Doina	1997

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:

$$\% \text{ Soluble sugars (glucose)}: \frac{E_c - E_a}{E_b} \times 50 \left[ \frac{\mu\text{g}}{\text{ml}} \right]$$

$$\% \text{ Starch (glucose)}: \frac{E_d - E_a}{E_b} \times 50 \left[ \frac{\mu\text{g}}{\text{ml}} \right]$$

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

50 = concentration of standard glucose solution, in  $\mu\text{g} / \text{mL}$ .

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:

$$\% \text{ sugars (glucose)} = Z \frac{200 \times 4}{m \times 10^6} \times 100 [\%]$$

$$\% \text{ starch (glucose)} = A \frac{500}{m \times 10^6} \times 100 [\%]$$

Z = the sugar content of the extract calculated in  $\mu\text{g} / \text{mL}$ ;

A = starch content of extract calculated in  $\mu\text{g} / \text{mL}$ ;

4 = factor for the dilutions performed during the determination;

500 = volume of starch extract in mL;

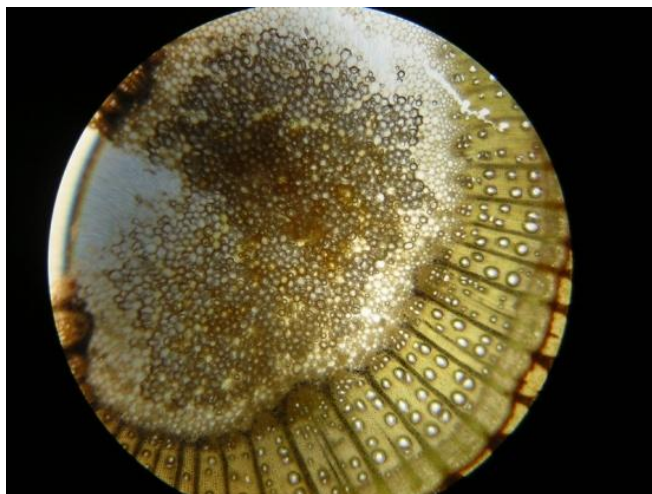
200 = volume of filtrate from which sugars are determined in mL;

m = mass of dry planting material 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.



**Fig. 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
Selecția Oppenheim 4-clona 4 (SO <sub>4-4</sub> )	12.02	9.58	2.44	52.33
Berlandieri x Riparia Selectia Crăciunel 2	12.78	9.79	2.99	52.17

Regarding the results obtained regarding the total carbohydrate content in the string rootstocks (tab. 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<sub>4-4</sub>), 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**

<b>Rootstock</b>	<b>Total carbohydrate (%)</b>	<b>Soluble sugars (%)</b>	<b>Starch (%)</b>	<b>Rope moisture (%)</b>
Gelu	14.32	8.99	5.33	55.60
Paula	13.07	9.47	3.60	56.04

After analyzing the carbohydrates from the rootstock varieties, the content of the carbohydrates from the rootstock varieties was also analyzed. Of the two varieties taken into consideration, the best result was obtained at the table grape variety Gelu, which recorded a total carbohydrate content of 14.32% (8.99%, representing the percentage of soluble sugars), respectively 5.33% starch). The other variety, Paula, registered a lower result compared to the Gelu variety, having a total carbohydrate content of 13.07% of which 9.47% represents soluble sugars and 3.60% 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% - 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, the Paula / Crăciunel 2 variant recorded the best result (99%), while for the Gelu variety, the best variant was Gelu / Riparia Gloire (98%). From the vines that are suitable for planting, the percentage of grafting was analyzed, here the best results were obtained by the Paula / Crăciunel 2 variant (76%), and Gelu / Riparia Gloire (71%) respectively.

The percentage of vines with the bud that entered vegetation was a good one with values of 71% (Gelu / Riparia Gloire), respectively 76% (Paula / Crăciunel 2). For both varieties taken into consideration, the percentage of vines where the bud did not start vegetation was analyzed, here the variants Gelu / SO4-4 and Gelu / Crăciunel 2 having equal values (30%), while for the other variety, the variant with the highest percentage of buds that did not enter vegetation was Paula / SO4-4 (29%).

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	Callus complete	No callus	Base	Node 2
Gelu	Riparia Gloire	98	71	27	19	45	34	98	2	96	2
	Selecția										
	Oppenheim 4-clona 4 (SO4+)	95	65	30	38	30	27	95	5	92	3
	Berlandieri x Riparia Selectia Crăciunel 2	97	67	30	21	37	39	97	3	90	7
Paula	Riparia Gloire	98	70	28	23	35	40	98	2	95	3
	Selecția										
	Oppenheim 4-clona 4 (SO4+)	97	68	29	35	37	25	97	3	91	6
	Berlandieri x Riparia Selectia Crăciunel 2	99	76	23	28	30	41	99	1	94	5

\* 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 grafting (%) - calves with complete callus formed at the grafting point, with buds started in vegetation and with root primordia

The degree of callus forming was another parameter analyzed, here the best results were obtained by the variant Paula / Crăciunel 2 (99%) and Gelu / Riparia Gloire (98%), these having fully formed callus. All the variants were classified according to diameter too, according to specialized literature (7-8.5 mm; 8.6-10 mm; 10.1-12 mm), the results obtained being very varied for each particular variant.

The last parameter analyzed was the one regarding the location of the roots on the grafted vines, following the place where the grafted vines formed root primordia. All the variants taken into account had a high percentage of occurrence of root primordia at the base of grafted vines, compared to their formation at the second node where the highest percentage was obtained in the Gelu / Craciunel 2 (7%) and Paula / SO4- variants. 4 (6%).

### CONCLUSIONS

1. 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%).
2. The physiological humidity of the analysed canes was within normal limits, both in the scion and in the rootstocks (52-56%), which showed that the graft material was kept under proper conditions.
3. The callus formation for different diameters shows that the best ratio was at Riparia Gloire and Selecția Crăciunel 2 with the diameters of 8.6-10 mm, respectively 10.1-12 mm, and at the rootstock SO4-4 at the diameters of 7-8.5, respectively 8.6-10 mm.
4. The percentage of vines with the most numerous basal roots was registered at the Riparia Gloire rootstock (95-96%).

### REFERENCES

1. Balasubrahmanyam V.R., Eifert J., Diofasi L., 1978 - *Nutrient reserves in grapevine canes as influenced by cropping levels*. Vitis 17, p. 23–29.
2. 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.
3. Loescher W.H., Mccamant T., KELLER J.D., 1990 - *Carbohydrate reserves, translocation, and storage in woody plant roots*. HortSci. 25, p. 274–281.
4. 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), p. 563-550.
5. 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), p. 218-222.
6. Țârdea C., Rotaru, Liliana, 2003 - *Ampelografie vol. 1*. Editura "Ion Ionescu de la Brad", Iași.
7. Țârdea C., Rotaru, Liliana, 2003 - *Ampelografie vol.2*. Editura "Ion Ionescu de la Brad", Iași.