ANNALS OF THE UNIVERSITY OF CRAIOVA

Series: ✓*Biology*
✓*Horticulture*
✓*Food products processing technology*
✓*Environmental engineering*

Vol. XXXI (LXVII) - 2026

THE BEHAVIOR OF THE 'MAMAIA' GRAPEVINE CULTIVAR GRAFTED ON THE 'SO4', 'KOBER 5BB' AND 'FERCAL' ROOTSTOCKS IN THE PROCESS OF FORCING THE GRAFTED VINES

Antonie (Cioata) Mihaela[1,2], Ranca Aurora[2], Botu Mihai[3]
[1]University of Craiova, Doctoral School of Engineering of Animal and Plant Resources (IRAV), Romania
[2]Research Station for Viticulture and Oenology Murfatlar, Romania
[3]University of Craiova, Faculty of Horticulture, Craiova, Romania
* *Correspondence author. E-mail*: mihaela.cgc@gmail.com

Keywords*: grapevine grafts; rootstocks; methods of stratification, callus formation.*

ABSTRACT

The paper presents the callusing behavior of the 'Mamaia' grape cultivar grafted on 'SO4', 'Kober 5BB' and 'Fercal' rootstocks during the forcing process at the Murfatlar Viticulture and Winemaking Research and Development Station. The callus yield of the vines was monitored for each cultivar-rootstock combination following the use of two stratification methods in sawdust and in water. Three rootstock cultivars were tested, taking into account that the soils in the Murfatlar vineyard require special attention to tolerance to active limestone, a characteristic to which the three rootstocks behave differently. Unifactorial and bifactorial analysis of variance, as well as the Tukey HSD test, showed that 'SO4' and 'Kober 5BB' rootstocks produced significantly higher and statistically equivalent callus yields compared to 'Fercal', regardless of the stratification method used, which had no significant effect on yield. The aim is to identify the optimal cultivar-rootstock combination for the production of certified, high-quality planting material from the 'Mamaia' cultivar.

INTRODUCTION

The forcing of grafted vines is a technological stage in the multiplication process in which the grafted cuttings, immediately after the mechanical welding between the rootstock and the scion, are introduced into rooms with controlled temperature and humidity, in order to bring the vines out of vegetative dormancy and to accelerate and uniform the formation of callus tissue at the grafting point between the scion and the rootstock (Dejeu, 2010). Forcing in air-conditioned rooms reduces the callus period to 15-21 days and reduces losses caused by variable thermal conditions in common storage spaces (Corbean et al., 2009). The yield of this stage directly determines the quantity of quality grafted vines that reach the nursery field and ultimately, the number of certified grafted cuttings delivered to winegrowers.

The 'Mamaia' cultivar was obtained at Murfatlar research center through controlled cross between the 'Merlot' x 'Muscat Ottonel', being officially homologated in 1991. In favorable years, the 'Mamaia' cultivar accumulates a high sugar content (210-230 g/l), acidity of 3.8 - 4.7 g/l H2'SO4', an average grape weight of 331 g and a yield of 3.14 kg/plant and 14 t/ha (Ranca et al., 2022). It ensures high, constant, healthy and quality yields, due to both its oenological potential and the presence of

aroma in the must and wine. It is suitable for cultivation in an organic system. It is a cultivar for high-quality aromatic red wines (13.8 % vol. alcohol), non-reducing extract of 27.0 g/l, polyphenols 620-980 mg/l and good color intensity (1.690).



Figure 1. Grapes from the ‘Mamaia’ cultivar

The choice of rootstock influences both the success of the grafting itself and the subsequent behavior in the plantation, the rootstock being an essential component of the vine's adaptation to the soil and climate conditions of a given area (Ollat et al., 2016). ‘SO4’ (Oppenheim Selection no. 4, from the *Berlandieri x Riparia* group) has medium to high vigor, good affinity for grafting with most cultivars and moderate tolerance to active limestone in the soil. ‘Kober 5BB’, also from the *Berlandieri x Riparia* group, is distinguished by high vigor, good drought resistance and excellent affinity for grafting, but its tolerance to active limestone remains more limited, which can favor ferritic chlorosis on strongly calcareous soils (Ungureanu 2007). ‘Fercal’ was created specifically for lands with high active limestone content, tolerating percentages of over 40 %, but its affinity for grafting varies depending on the graft cultivar and, in some cases, requires additional attention when grafting (Țârdea & Rotaru, 2003).

The vigor: ‘Kober 5BB’ is a rootstock with very vigorous vegetative growth, a fact confirmed both in the Romanian ampelographic literature (Stroe, 2012) and in French studies on the rootstock-vigor relationship (Tandonnet et al., 2008), which measured the long-term effect of nitrogen and water on the vigor conferred by rootstocks from the *Berlandieri x Riparia* group, a category that also includes ‘SO4’. Very high vigor is an advantage in poor soils or weakly vigorous cultivars, but becomes a disadvantage for cultivars prone to millerandage and coulure, where excessive rootstock vigor affects crop quality (Keller 2010). ‘SO4’ is intermediate, with medium to high vigor. The ‘Fercal’ offers high vigor but is more balanced than the ‘Kober 5BB’, which is why it is often recommended where quality, not just quantity, is the goal (Galet, 2000).

Limestone and drought resistance: ‘Fercal’ was specifically developed for highly calcareous soils and is considered tolerant to iron (Fe) deficiency, unlike the Riparia x Rupestris rootstocks (such as 3309C or 101-14), which are considered very sensitive to ferric chlorosis (Khalil et al., 2024). In a comparative experiment by the same authors, chlorophyll concentration decreased by 75 % in 3309C, but only by 57 % in ‘Fercal’, under the same iron deficiency regime (Khalil et al., 2024) - a concrete difference, not just a quality label. ‘Kober 5BB’ follows, with an IPC

(chlorinating power index) of approximately 40, while 'SO4' tolerates slightly less active limestone (IPC ~30), although the difference with Kober is not dramatic (Stroe 2012, Țârdea & Rotaru 2003). In terms of drought resistance, 'SO4' is the weakest of these three rootstocks, which is why Romanian scientific literature clearly recommends irrigation during long periods without precipitation. 'Kober 5BB' tolerates both drought and excess moisture. 'Fercal' was created specifically for areas that are both calcareous and prone to drought - a combination of factors similar to that found in the Murfatlar area. Early selection of rootstocks for drought resistance, as a research methodology, was documented as early as the 1980s by Carbonneau (1985), a work that remains a methodological reference for tests of resistance to water stress in grapevines.

Resistance to *Phylloxera* and other diseases: all three rootstocks have a solid root resistance to *Phylloxera*, inherited from the American parent species (Stroe & Indreaș, 2010). 'Kober 5BB' has a resistance described as high, as well as a good tolerance to nematodes (*Meloidogyne incognita, Meloidogyne hapla*). 'SO4' performs almost as well and has, in addition, a good tolerance to vine borer. 'Fercal', being a complex hybrid with a European component ('Cabernet Sauvignon') in the genealogy, confirmed only in 2008 by SSR molecular markers (Laucou et al., 2008), after decades of uncertainty regarding its exact origin, is considered slightly more vulnerable compared to the pure American rootstocks. Research on golden flavescens (Caudwell et al., 1994) explicitly studied the ability of the 'Fercal' rootstock to transmit this disease, a sign that its specific sensitivities have been closely followed in the French literature. 'Kober 5BB' is suitable for poor soils, where high vigor and firm resistance to *Phylloxera* are required, with caution for cultivars prone to beading. 'SO4' provides a good balance in ordinary soil conditions, but requires irrigation in dry years. 'Fercal' is recommended on plots with very high active limestone content and dry climate, where the other two rootstocks would develop chlorosis, at the cost of a somewhat more demanding rooting process in the nursery row (Khalil et al. 2024).

The soil in the Murfatlar area is a carbonate chernozem, formed on loess deposits, with a high content of active limestone (ONVPV, 2021). This particularity makes the choice of rootstock a factor that can determine the longevity and vegetative state of the plantation once established.

The present study aims to compare the three rootstocks mentioned above, from the point of view of the yield obtained from forcing the 'Mamaia' cultivar vines grafted onto them, in order to provide a practical benchmark in choosing the optimal combination for the production of certified planting material.

The objectives pursued were: determining the callus rate for each cultivar-rootstock combination; establishing the percentage of cuttings rooted and successfully transferred to the nursery field; Statistical analysis of the data recorded for the three experimental variants was performed, in accordance with the principles of zoning grapevine cultivars on specific pedoclimatic areas (Oșlobeanu et al., 1991).

MATERIAL AND METHODS

Biological material: the 'Mamaia' cultivar was used as graft, the graft cords being harvested from the certified mother plantation of SCDVV Murfatlar during the vegetative dormancy period, with a diameter of 7-9 mm and good wood maturation. As rootstocks, 'SO4' cords from the research station's own mother plantation, 'Kober 5BB' rootstock cuttings from a certified plantation, privately owned, located in Ionești,

Vâlcea County, and 'Fercal' rootstock cuttings, with cords from the University of Iași, were used, kept under the same conditions, by stratification at 2-4 °C and relative humidity of 85-90 %, wrapped in polyethylene foil.

Location of the experiment and working conditions: the experiment was located in the grafting complex within Murfatlar research center, using own forcing installation. In December 2025, the 'Mamaia' graft cuttings were harvested and disinfected, wrapped in foil and stored in the complex's cold storage at 2-4 °C and 70-80 % humidity. The rootstock cuttings were harvested and processed in January 2026 and stored until grafting process under the same conditions as the graft cuttings.

Grafting and forcing technology: grafting was performed mechanically, using Omega tabletop grafting machines. Immediately after grafting, once the grafted cuttings were received and checked for quality, they were treated with a solution to stimulate callus at the grafting point, after which the upper third of the cutting was paraffined. The cuttings were stratified in plastic boxes with wet sawdust and in plastic boxes with water, where only the base of the cutting was covered with water. After grafting, the boxes were placed in the forcing chamber, where a temperature of 26-28 °C was maintained at the grafting point and a relative humidity of 90-95%, for 12-16 days, with daily monitoring of the callus stage. After callus formation, the cuttings were removed from the sawdust and from the boxes in which they had been standing in water, sorted, re-treated, and planted in the field on drip-tape trays covered with black polyethylene mulch.

Experimental scheme and monitored indicators: the experiment included three variants, corresponding to the three rootstocks (V1 - 'Mamaia' / 'SO4', V2 - 'Mamaia' / 'Kober 5BB', V3 - 'Mamaia' / 'Fercal') organized in 5 repetitions of 100 grafted cuttings for each stratification variant: in boxes with wet sawdust and in boxes with water.

The yield of callused cuttings (with circular, well-formed callus) (figure 2), was noted, determined upon removal from the forcing chamber by examining the callus ring at the grafting point; the data obtained were statistically interpreted to establish the most efficient graft-rootstock combination using analysis of variance (unifactorial ANOVA), with rootstock as the experimental factor, and the differences between the means of the variants are tested using the Duncan test or the Tukey test, at a significance threshold of $p<0.05$ (Perry & Sabbatini, 2013). For indicators expressed as percentages, an angular transformation (arcsine) is recommended before applying ANOVA, to satisfy the assumption of normality of the distribution.

RESULTS AND DISCUSSIONS

The tables below present the values for each cultivar-rootstock combination obtained in the 5 repetitions of 100 cuttings, both for the sawdust stratification variant and for the water stratification variant.

In the case of sawdust stratification, the 'Mamaia' /'SO4' combination gave the best average percentage value of cuttings callused according to the standard (96.6 %), followed by the 'Mamaia' /'Kober 5BB' combination (95.2 %), (table 1).

In the case of water stratification, the 'Mamaia' /'Kober 5BB' combination gave the best average percentage value of callus cuttings according to the standard (97.6 %), followed by the 'Mamaia' /'SO4' combination (97.0 %), (table 2).

Table 1

Callus yield on cultivar / rootstock combinations - sawdust stratification variant
(5 repetitions x 100 cuttings)

Combination	Repetitions	Cuttings on the forcing	Cuttings with normal callus	Percentage of standard callused cuttings (%)
'Mamaia' / 'SO4'	R1	100	95	95
	R2	100	97	97
	R3	100	95	95
	R4	100	97	97
	R5	100	99	99
	Total cuttings	500	483	x
	Average percent	x	x	96.6
'Mamaia' / 'Kober 5BB'	R1	100	94	94
	R2	100	97	97
	R3	100	96	96
	R4	100	99	99
	R5	100	90	90
	Total cuttings	500	476	x
	Average percent	x	x	95.2
'Mamaia' / 'Fercal'	R1	100	91	91
	R2	100	85	85
	R3	100	93	93
	R4	100	95	95
	R5	100	79	79
	Total cuttings	500	443	X
	Average percent	x	x	88.6

Table 2

Callus yield on cultivar/rootstock combinations - water stratification variant
(5 repetitions x 100 cuttings)

Combination	Repetitions	Cuttings on the forcing	Cuttings with normal callus	Percentage of standard callused cuttings (%)
'Mamaia' / 'SO4'	R1	100	97	97
	R2	100	98	98
	R3	100	99	99
	R4	100	93	93
	R5	100	98	98
	Total cuttings	500	485	x
	Average percent	x	x	97.0
'Mamaia' / 'Kober 5BB'	R1	100	97	97
	R2	100	98	98
	R3	100	97	97
	R4	100	99	99
	R5	100	97	97
	Total cuttings	500	488	x
	Average percent	x	x	97.6
	R1	100	91	91

‘Mamaia’ / ‘Fercal’	R2	100	85	85
	R3	100	93	93
	R4	100	90	90
	R5	100	89	89
	Total cuttings	500	448	x
	Average percent	x	x	89.6

Table 3 presents the descriptive statistics of callus yield for each rootstock, in the sawdust and water stratification variants. In both variants, ‘Fercal’ recorded the lowest average yield and the highest variability between replicates, while ‘SO4’ and ‘Kober 5BB’ performed similarly.

Table 3

Descriptive statistics of callus yield, per rootstock and stratification method

Rootstock	Sawdust (%)	Water (%)	General average (%)	Standard deviation
‘SO4’	96.6	97.0	96.8	1.93
‘Kober 5BB’	95.2	97.6	96.4	2.67
‘Fercal’	88.6	89.6	89.1	4.82

The one-way analysis of variance (rootstock as experimental factor), on arcsine transformed data (table 4) indicated statistically significant differences between the three rootstocks, in both stratification variants. The *Tukey HSD* test (table 5) showed that the difference between ‘SO4’ and ‘Fercal’ was statistically significant in both variants, as was the difference between ‘Kober 5BB’ and ‘Fercal’ in the water variant; in the sawdust variant, the difference between ‘Kober 5BB’ and ‘Fercal’ approached the significance threshold without exceeding it. In none of the variants was the difference between ‘SO4’ and ‘Kober 5BB’ statistically significant, so that the two rootstocks can be considered equivalent in terms of callus yield for the ‘Mamaia’ cultivar.

Table 4

One-way ANOVA for the effect of rootstock on callusing yield, by stratification variant

Source of variation	df	F (sawdust)	p (sawdust)	F (water)	p (water)
Rootstock	2	5.11	0.025	19.10	<0.001
Residual	12	—	—	—	—

Table 5

Tukey's HSD test for pairwise comparisons between rootstocks, by stratification variant

Comparison	Sawdust variant (p)	Water variant (p)
‘SO4’ vs ‘Kober 5BB’	0.840	0.919
‘SO4’ vs ‘Fercal’	0.027	<0.001
‘Kober 5BB’ vs ‘Fercal’	0.074	<0.001

To determine whether the stratification method influences the callusing yield of the ‘Mamaia’ cultivar, the data from Tables 1 and 2 (arcsine-transformed) were processed through a two-way analysis of variance, with rootstock and stratification method as experimental factors (Table 6). The rootstock effect remained strongly

significant, confirming the results obtained separately for each stratification variant above, while the effect of stratification method and the rootstock × method interaction were not statistically significant, showing that the ranking of the three rootstocks remained the same regardless of the stratification method used.

Table 6

Two-way ANOVA for the effects of rootstock and stratification method on callusing yield

Source of variation	df	F-value	p-value
Rootstock	2	17.41	<0.001
Stratification method	1	1.16	0.293
Rootstock × Method	2	0.37	0.693
Residual	24	—	—

The direct comparisons carried out for each rootstock separately (independent-samples *t-test*, Table 7) confirm this conclusion: the difference between sawdust and water stratification was not statistically significant for any of the three rootstocks. The choice between the two stratification methods can therefore be made on practical grounds (cost, availability of materials, nursery working space), without compromising the callusing yield, regardless of the rootstock used.

Table 7

Independent-samples *t-test* comparing the sawdust and water stratification methods, by rootstock

Rootstock	t-value	df	p-value
'SO4'	-0.40	8	0.697
'Kober 5BB'	-1.41	8	0.196
'Fercal'	-0.17	8	0.872

Correlating the experimental results with the specialist literature. The results obtained largely confirm the known trends in the general behavior of the three rootstocks. 'Kober 5BB' delivered high callusing yields in both stratification variants, supported by its vigor and good grafting affinity with most *Vitis vinifera* cultivars; it remains to be seen, however, in the subsequent stages of propagation, whether early signs of iron chlorosis appear on the carbonate soil at Murfatlar, where its tolerance to active lime is lower than that of the other two rootstocks. 'SO4', as expected, occupied a position similar to 'Kober 5BB', confirming its status as a compromise rootstock for moderately calcareous soils. 'Fercal', although bred specifically for land rich in active lime, recorded the lowest and most variable callusing yield in both stratification variants, confirming the more temperamental grafting affinity reported in the specialist literature for this rootstock (see Introduction); its long-term advantage, related to active-lime tolerance, remains to be assessed in the later stages of the planting, after it is established in the field.

CONCLUSIONS

Based on the data recorded for both stratification variants and the descriptive and inferential statistics the 'Mamaia' cultivar recorded the highest and most consistent callusing yields when grafted onto the 'SO4' and 'Kober 5BB' rootstocks, which were statistically equivalent to each other in both stratification

variants. ‘Fercal’ lagged behind in both variants, with a lower mean yield and the greatest variability between replicates.

The analysis of variance (one-way ANOVA, on arcsine-transformed data) confirmed, for each stratification variant separately, that the differences between the three rootstocks are not due to chance. *Tukey's HSD* test showed that the difference between ‘SO4’ and ‘Fercal’ is statistically significant in both variants, as is the difference between ‘Kober 5BB’ and ‘Fercal’ in the water variant; in the sawdust variant, the difference between ‘Kober 5BB’ and ‘Fercal’ approaches the significance threshold without exceeding it. In neither variant is the difference between ‘SO4’ and ‘Kober 5BB’ statistically significant, so the two rootstocks can be considered equivalent in terms of callusing yield for the ‘Mamaia’ cultivar.

The two-way analysis (rootstock × stratification method) showed that the stratification method does not significantly affect the callusing yield of the ‘Mamaia’ cultivar, and the interaction between rootstock and stratification method is likewise non-significant (confirmed by the rootstock-level *t-tests*). Consequently, for propagating the ‘Mamaia’ cultivar under the carbonate-soil conditions of the Murfatlar vineyard, the use of the ‘SO4’ or ‘Kober 5BB’ rootstocks are recommended, both offering high and consistent callusing yields regardless of the stratification method chosen; ‘Fercal’ is recommended for use with reservations, having a lower and more variable yield, with its long-term advantages related to active-lime tolerance still to be evaluated in the later stages of the planting.

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