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Rooting Abilities in *Vitis vinifera* L.: Total Lipid and Fatty Acids Evolution in Perle Noir Cutting

Henda Cheikhrouhou*, Manel Zrida, Bechir Ezzili

Center of Biotechnology Borj Cedria, Hammamlif, Tunisia
Email: *hendacheikh@gmail.com

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Abstract

Rooting ability in *Vitis vinifera* L. is the phenomena studied. The evolution of the total lipids contents and fatty acids amount of cuttings during the rooting were determined. Total lipid extraction was carried out by an extraction solvent consisting of a mixture of chloroform and methanol. Methylation of fatty acids was carried out by the boron trifluoride methanol complex. Their analysis is performed by gas chromatography. We have obtained a content of merithallus lipids equivalent with then in bibliography. In other hand 3 fatty acids were obtained: eicosapentaenoic acid, oleic acid and docosanoic acid. Among the studied of rooting abilities, we observed decreased levels of total lipids and in the essential fatty acids: eicosapentaenoic acid C20:5 n-3 and oleic acid C18:1 n-9. Minor fatty acids cuttings experimental have a variable evolution. Lipids and certain fatty acids may be markers of rooting in the vine.

Keywords

Lipids, Cutting, Fatty Acids, Rooting, Perle Noir, *Vitis vinifera* L.

1. Introduction

The majority of wine grapes cultivar were reproduced through vegetative propagation [1] [2]. Research on adventitious rooting in grape vine appeared in French and North Africa after phylloxera epidemics have destroyed the extensive plantings of “own-rooted” *V. vinifera* L. Genotypes. However, Grapevine of North America was resistant to this epidemic. The first programs were genetic: *Vitis vinifera* was crossed with *Vitis berlanderi*, with *Vitis Berlanderi*, *Vitis Riparia* and *Vitis Rupestris*, hybrids were obtained for wine production. But the wine

*Corresponding author.

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produced by these direct hybrids producers was often foxy. There was even an analytical test to find if a wine contains the product of the HPD. Restrictions imposed by the governing boards of wine and wine grape "VPQRD" rule out genetic modification of conventional wine grape genotypes, whether by traditional breeding methods or by molecular genetic approaches [3]. The direct producer hybrids obtained from *Vitis vinifera* L. and North American species and therefore have no place in the wine growing areas classified as "AOC". They are no longer wanted in the wine growing areas not classified. Reference [4] proposed the grafting of *V. vinifera* L. cuttings into a resistant rootstocks. Therefore, stocks and wine quality were given by *Vitis vinifera* L. and resistance to roots phylloxera attacks was given by *V. riparia* or *Vitis rupestris*. But North American grapevine species were difficult to propagate and rooting potentiality was poor [5]. Genotypes were crossed with rooted Vitis species such as *V. rupestris* and *V. riparia*. North American hybrid was obtained from *V. berlanderi* × *V. rupestris*, *V. berlanderi* × *V. riparia* and recalcitrant to root genotypes appeared [6]. The first program was to determine rooting potential in *Vitis vinifera* L., North American species and rootstocks and also the possible state of growth regulator [5]. At the same time reference [8] tried to find the relationship between cutting sugars and rooting in the vineyard. [7], used vitro culture to determine cytological and histological origin of adventitious roots. A review on rhizogenesis phenomenon in woody plants [9] showed that the intrinsic function of any fragment is multiple interactions in continual movement that crisscross the entire plant. Rooting appears dependent on several factors among which there are endogenous factors: bud dormancy or not, leaf and apex. These various organs involved through growth substances (auxin, cytokinin, etc.) or their metabolites (sugar, polyphenols, etc.) and external factor: temperature, hygrometry and fog can affect rooting phenomenon.

For [9], interest in adventitious root formation by woody plants may have actually slowed progress in adventitious root research for two reasons:

1) The extraction of biochemical's, proteins and other constituents of physiological importance from woody cuttings is challenging at best and there is always uncertitude regarding the effectiveness of extraction procedures.

2) The slow growing nature of woody taxa complicates effort to achieve consistent and repeatable experiments.

Among the metabolites of the cutting in rooting, phenolic compounds and lipids and fatty acids have few researched in relation to rooting. Reference [11] addresses the type of correlation between phenolic compounds and vine rhizogenetic potentiality by analyzing somme phenolic compounds in the *Carignan merithallus* during bud dormancy and end of dormancy. Authors noted a negative correlation between coumarin and rhizogenetic potential of carignan vine. In contrast, positive correlations were found with naringin and andsyringic acid. Reference [10] studied the effect of cutting date and position of carignan (*V. vinifera*) shoot on the ability and fatty acid composition. Despite that the samples was take during the bud dormancy period, the number of roots was evolved in teeth of saw, weight and percentage of roots was increased in the studied cuttings. Contrary to the rooting ability (root number, weight and percentage), there was no reproducible gradient of total lipids according to the cutting date. The major fatty acids were palmitic, oleic and linoleic acids according to the cutting date and position of carignan stem.

The purpose of this study is twofold:

1) Analyze lipids and fatty acids cuttings contained a non-dormant bud.

2) Follow the evolution of these compounds in the heart of rooting in two different conditions: total darkness and darkness/light.

2. Materials and Methods

Chloroform/methanol hexane were obtained from Merck (Darmstadt, Germany). Boron trifluoride were purchased from Sigma-Aldrich (St Quentin Fallavier, France), Milli-Q (Millipore) water was prepared using a Sarterius-arium 611 system.

2.1. Plant Material

Grapevine (*Vitis vinifera* L. cv. Perle noir) was collected in the fields of GOVPF (Groupement Obligatoire Des Viticulteurset Producteurs De Fruits), grafted on Richter 99 resulting from mass selection of Tunisia. The vines are planted in the region of Belli in the Governorate of Nabeul. The elevation is 10˚25', longitude 36˚37'W. The height is 20 meters from sea level the vineyards are planted with spacing of 3 meters between rows and are North African Goblet ductwork. Fertilization and control various operations and treatment of vineyards are made in commercial standards.

A cutting with 4 merithallus and one bud is used. The length of the cutting was standardized to 20 cm. Only the bud rank 8 is retained. Buds rank 5, 6, and 7 are eliminated. Portions of stems can form roots and can be covered with calluses. This phenomenon is described by some authors who have studied rhizogenesis [8].

Packages of 33 cuttings are our basic experimental unit. They were made from individual strips. The weight of long cuttings is not uniform even if their length is the same. Each used cutting was weighed before planting. Cuttings were selected neighbor weight factor to eliminate reservation. 6 lots of 33 cuttings obtained from this genotype are available in half-bottles filled with tap water (**Table 1**).

Cuttings are exposed to identical greenhouse conditions at CBBC (relative humidity 60%, temperature 18˚C ± 2˚C) but two different photoperiods: 3 lots are exposed to daylight (12 hours light and 12 hours darkness) and 3 others are affected by total darkness. After 6 weeks of culture, we appreciate rooting.

2.2. Extraction Process: Lipid Analysis Extraction and Analyze of Fatty Acids

Thirty shoots were dried and reduced out in fine powder. On these powders, the lipid analysis according to Cuvelier *et al*. [12] is carried out. The extraction of the total lipids of the stems of the vine was carried out according to the following technique: twenty milligrams of dry matter (DM) is fixed at warmer water (5 ml) during 5 min in which the goal is to decontaminate the endogenous enzymes such as the phospholipidases. The mixture is then crushed in a mortar with solvent of extraction made up of chloroform/methanol. The homogenate is left at rest during 24 h. Two phases appear: a higher phase aqueous non lipid is eliminated and chloroform lower phase containing the total lipids is recovered using a Pasteur pipette. This phase is evaporated and dried under nitrogen current. The dry residue obtained is dissolved in hexane.

2.3. Determination of Fatty Acids

The total extract is introduced into a crew tube. One adds 0.5 ml of C17:0 (Standard), 0.5 ml by the complex boron trifluoride methanol. We agitate 1 min then we make it in pause of 2 min and we recover the higher phase of hexane. These methylated extract are then analyzed with the help of gas chromatography (CPG). The methyl esters of *i.e*. HP the total fatty acids are analyzed by CPG using a chromatograph HP Innovax length 30 m, of diameter 0.25 mm and thickness of film interns 0.25 m. The column is maintained at an isothermal temperature of 210˚C during all the duration of the analysis. The methyl esters of the fatty acids at the same time according to the length of their carbonaceous chains and their degrees of non-saturation. Each fatty acid is represented by a peak, the identification of fatty acids was obtained according to their times of retention **Table 2**). Surface of the peaks are proportional to the quantities of the corresponding fatty acids. The percentage of the fatty acids is calculated starting from surfaces of the peaks which represent it on the chromatogram: % of an fatty acid = ($S1/\Sigma S1$ à n) × 100.

S1: surface peak corresponding to the fatty acid No.1: ΣS1 à n: the sum of surfaces of all the peaks of a chromatgram corresponding to the totality of the mixture fatty acids.

Table 1. Tap Water composition.

Elements	Concentration mg/L
K	6.5
Ca	144.5
Mg	36.5
Cl	362
NO_3	0
SO_4	400
P	Traces
Fer	0.9

Table 2. Retention time of fatty acids in Perle noir merithallus.

N°	Fatty acids		Rentention times (mn)
1	C14:0	Myristic acid	8.524
2	C15:0	Pentadecyclic acid	8.996
3	C16:0	Palmitic acid	10.516
4	C16:1 n-9	Acid hypogeic	14.487
5	C16:1 n-7	Palmitoleic acid	15.046
6	C17:0	Standard interne	22.681
7	C17:1	Heptadecanoic acid	29.251
8	C18:0	Stearic acid	35.910
9	C18:1 n-9	Oleic acid	38.591
10	C18:1 n-7	Vaccenic acid	41.740
11	C 18:2 n-6	Linoleic acid	42.782
12	C20:1	Gadoleic acid	44.621
13	C20:2 n-6	Dehydro-linolenic	46.937
14	C20:3 n-6	Dehydro-gamma-linolenic	49.935
15	C20:4 n-6	Arachidonic acid	51.287
16	C20:5 n-3 (EPA)	Acid timnodonic	61.728
17	C22:0	Behenic acid	66.709
18	C22:1	Erucicacic	73.662
19	C22:4 n-6	Adrenic acid	75.462
20	C22:5 n-3	Clupadonic acid	77.384
21	C24:0	Lignoceric acid	78.989
22	C22:6 n-3 (DHA)	Docosahexaenoic acid	87.252
23	C24:1	Nervonic acid	90.141

3. Results and Discussions

3.1. Rooting in Perle Noir

After 6 weeks rooting in cuttings is clear (**Figure 1**). Some cuttings presented roots, another roots and cals and some one only cals.

3.2. Quantitative Results

Analysis by gas chromatography of Perle noir stems extracts identified 23 peaks. They are eluted according to their retention times (**Table 2**).

These peaks were identified by co-chromatography with witnesses fatty acids. These fatty acids are present in significant quantities and variables. They are saturated as myristic acid (C14:0), palmitic (C16:0), stearic (C18:0), behenic acid (C22:0) and lignoceric acid (C24:0), as monounsaturated the palmitoleic acid (C16:1), oleic (C18:1), gadoleic (C20:1), erucic acid (C22:1) and nervonic acid (C24:1) and polyunsaturated acids as linoleic (C18:2 n-6); dihydrom-gamma-linolenic acid (C20:3 n-6), arachidonic (C20:4 n-6), timnodonic (C20: 5 n-3 (EPA)), adrenic (C22:4 n-6), clupanodonic (C22:5 n-3) and docosahexaenoic (C22:6 n3(DHA)).

Gas chromate gramphy analysis of Perle noir stems extracts presented 23 peaks eluted according to their retention times (**Table 2**).

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Figure 1. Left roots, in right cals obtained in our experience.

(C16:0), stearic acid (C18:0), behenic acid (C22:0) and lignoceric acid (C24:0., The monounsaturated fatty acids regrouped palmitoleicacid (C16:1), oleic acid (C18:1), gadoleicacid (C20:1), erucic acid (C22:1) andnervonic acid (C24:1). There is also polyunsaturated fatty acids as linoleic (C18:2 n-6); dihydrom-gamma-linolenic acid (C20:3 n-6), arachidonic (C20:4 n-6), timnodonic (C20:5 n-3 (EPA)), adrenic (C22:4 n-6), clupanodonic (C22:5 n-3) and docosahexaenoic (C22:6 n-3 (DHA)).

3.2.1. Analysis of Total Lipid

Total lipid contents are shown in **Table 3**. The levels of total lipids obtained are higher than those obtained by Darné and Tamargo Madero [13] for Cabernet Sauvignon vine grafted on SO_4. Note that we have worked on table genotype while the authors indicated above analyzed grape cuttings wine. In addition Tunisian (Grombalia) climate is different from French climate (Bordeaux).

According to **Table 3**, the variability of the results is not very important. Whatever the variety and illumination condition, total lipids levels decreased about one third of the initial lipids content at the end of all the experiment. We will study the qualitative and quantitative composition of fatty acids.

3.2.2. Elemental Composition of Fatty Acids

Essential fatty acids of the cutting intact before use in the laboratory are as follows: C18:1 n-9 (33.31%), the C20:5 n-3 (37.3%), and C22:0 (7.3%). These results are interesting and have never been treated earlier. Darné and Tamargo Madero (1979) found relatively high amount of C18:2 and C18:3 but, in our vines C18:3 was absent. The amount of the saturated fatty acid C16:0 and C18:0 was low compared to Darné and Tamargo Madero results. C18:1 represent 37% and it is higher about 3 times than results found by Darné and Tamargo.

Minor fatty acids are noted, it is the C16:1 n-9 and C16:1 n-7 presumably cis and trans. C20 fatty acids are found in low levels that never exceed 0.1%. This group is composed of C20:1, C20:2 n-6, C20:3 n-6, C20:4 n-6. The most important content of fatty acids consists of two varieties fatty acid C20:5 n-3 (EPA). It represents the major fatty acid cuttings.

A fatty acid of very long chain C20:0 is about 7.5% and another C22:6 n-3 (DHA) is 1.62% are found. It should be noted that long-chain fatty acids never exceeds 0.8% and consist of C22:1, C22:4, C22:5, C24:0 and C24:1. These fatty acids C20, C22 and C24 would they not specific varieties studied?

We can conclude that our experimental cuttings contain initially 13.2% saturated fatty acids palmitic acid 5% and 7% béhémique acid, 40.8% monounsaturated fatty acids with 35%, 5% 42.3% oleic acid and polyunsaturated fatty acid whose acid timnodonic 37.3%. Changes in fatty acids in our experimental cuttings obey some changes that are not the same in light and darkness.

From **Table 4**, there is an increase of some fatty acids after rooting both in light and darkness as the C24:1, C22:6 n-3DHA, C24:0, the C22:5 n-3, C22:1, C20:1, C18:1 n-7, C17:1, C16:1 n-7, C16:0 and C15:0.

Are they fatty acids rooting? **Table 4** shows that there are lower levels of such fatty acids C18:1 n-9 and

Table 3. Changes in the total lipid content mg/g DM versus time in Perle noir *Vitis vinifera* L.

	t0	t1		t2	
		Darkness	Light	Darkness	Light
Perle noir	11,100 ± 587.98	7650 ± 489.99	7200 ± 0	3950 ± 685.98	3700 ± 525.66

Table 4. Evolution of contents of fatty acids cuttings of Perle noir *Vitis vinifera* L. (%TL) in total darkness and light versus times (Darkness: Light).

Fatty acids	t0	t1		t2	
		Darkness	Light	Darkness	Light
C14:0	0.4769 ± 0.0040	2.5082 ± 0.0400	0.5770 ± 0.005	0.4882 ± 0.005	0.4772 ± 0.003
C15:0	0.0810 ± 0.0007	0.3343 ± 0.0060	0.2102 ± 0.001	1.1122 ± 0.02	0 .9867 ± 0.008
C16:0	4.7574 ± 0.0600	14.1877 ± 0.700	8.7026 ± 0.080	19.9813 ± 0.512	15.6089 ± 0.523
C16:1 n-9	2.8930 ± 0.0300	4.3670 ± 0.0050	2.3738 ± 0.030	5.3245 ± 0.062	2.4588 ± 1.002
C16:1 n-7	2.7127 ± 0.0350	7.9311 ± 0.0070	8.2779 ± 0.060	13.2373 ± 0.343	13.4880 ± 0.962
C17:0	2.6148 ± 0.0400	0.7411 ± 0.0080	3.2460 ± 0.040	0.3405 ± 0.002	4.9861 ± 0.522
C17:1	0.2954 ± 0.0020	2.3932 ± 0.0040	0.2040 ± 0.001	3.0468 ± 0.012	0.3421 ± 0.002
C18:0	0.3840 ± 0.0020	0.4851 ± 0.0050	0.1417 ± 0.001	0.2955 ± 0.002	0.4328 ± 0.003
C18:1 n-9	33.3160 ± 0.980	20.4171 ± 0.900	32.4254 ± 1.510	20.3270 ± 1.05	23.8265 ± 1.522
C18:1 n-7	0.9118 ± 0.0070	2.2101 ± 0.0030	3.5971 ± 0.040	1.8555 ± 0.004	3.6243 ± 0.521
C18:2 n-6	2.3672 ± 0.0240	4.1202 ± 0.0050	1.0459 ± 0.008	1.1463 ± 0.003	1.11O4 ± 0.006
C20:1	0.1297 ± 0.0030	0.3325 ± 0.0010	0.6905 ± 0.006	0.6338 ± 0.0009	0 .7459 ± 0.002
C20:2 n-6	0.1336 ± 0.0010	0.2094 ± 0.0010	0.0642 ± 0.0008	0.3293 ± 0.0005	0.0823 ± 0.0002
C20:3 n-6	0.0812 ± 0.0004	0.1039 ± 0.0010	0.0923 ± 0.0009	0.2453 ± 0.0005	0.2235 ± 0.001
C20:4 n-6	0.1158 ± 0.0050	0.0880 ± 0.0010	0.0368 ± 0.0002	0.6269 ± 0.0005	0.1368 ± 0.0003
C20:5 n-3 (EPA)	37.3046 ± 1.300	24.7483 ± 1.005	25.7877 ± 0.984	12.4686 ± 0.529	13.5439 ± 0.965
C22:0	7.3328 ± 0.500	5.71710 ± 0.070	5.93452 ± 0.672	4.8317 ± 0.05	7.43267 ± 0.852
C22:1	0.77316 ± 0.008	0.99238 ± 0.007	0.49435 ± 0.005	2.8836 ± 0.005	0.6943 ± 0.001
C22:4 n-6	0.45943 ± 0.003	1.60351 ± 0.003	0.53406 ± 0.004	1.7318 ± 0.001	0.4141 ± 0.001
C22:5 n-3	0.34089 ± 0.002	0.60991 ± 0.004	0.59968 ± 0.006	1.0477 ± 0.002	2.1134 ± 0.004
C24:0	0.25838 ± 0.002	0.57244 ± 0.005	0.50148 ± 0.004	0.8205 ± 0.001	0.8054 ± 0.001
C22:6 n-3 (DHA)	1.62161 ± 0.009	4.79042 ± 0.020	4.04386 ± 0.04	6.8390 ± 0.223	7.1325 ± 0.511
C24:1	0.07539 ± 0.001	0.18580 ± 0.002	0.22504 ± 0.001	0.3864 ± 0.002	0.4326 ± 0.002

C20:5 n-3 (EPA) majority in the idle state after rooting there are probably partial consumption these monounsaturated fatty acids and 70% polyunsaturated fatty acids and fatty acid accumulation-type C16:0, C16:1, C22:5 and C22:6. In the darkness, there is an increase of some fatty acids more than the light, one can note the C16:0, C16:1 n-9, C20:2 n-6, C20:4 n-6 , the C22:1 and the C22:4 n-6. The C16:0 can be derived from the degradation of the C22:0, C22 partially: 0 can give the C16:0 or desaturate giving the C22:1 and the C22:4 n-6. The increase in C22:4 n-6, which is mostly in the dark shows that the roots probably involves desaturation of fatty acids like C22:0 C22:1 and C22:4, this is due to desaturation desaturase that works better in darkness than light. Is it a decarboxylation in some cases or in other cases isomerization?

In light, there is an increase of C18:1 n-7 (isomer of C18:1 n-9), C17:0, C16:1 n-7, C20:1 and C22:5 n-3. The

C17:0 come from the decarboxylation of the C20:0 likely, whereas C18:1 n-7 come from an isomerization of C18:1 n-9. The C20:1 would likely come from the isomerization of C20:5. The C22:5 n-3 would likely come from the isomerization of C22:0.

To summarize, the rooting is accompanied by new fatty acids obtained by saturation or desaturation, carboxylation or decarboxylation and isomerization of the initial fatty acids. Some of these reactions would be realized better in total darkness and other light and characterize lipid metabolism probably rooting for these varieties of vines.

4. Conclusion

Essential fatty acids found in this work at t0 are: C18:1, C20:5, C22:0. During rooting, the amount of these fatty acids decreases while other fatty acids as C16:0, C16:1, C22:6 and C18:1 appear. We showed that C18: 1 n-9 (which represents 33% before rooting essential fatty acids not present more than 23% at the end of experiment) was decreased from 30%. With regard to this phenomenon, contents of the C18 isomer: 1 n-7 increased by 262%; it was of 1% before rooting and reached 3.62% after rooting. We can think that the transformation of C18:1 n-9 in C18 of isomer: 1 n-7 in any event, even if the transformation is incomplete, reaction of isomerization of C18:1 of its isomer when the rooting is suspected.

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