

Changes of phenolic compounds in Carignan merithallus (*Vitis vinifera* L.) during bud dormancy and end of dormancy phase: correlation with rhizogenesis

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Received 29 August 2011; revised 17 September 2011; accepted 21 October 2011.

ABSTRACT

The aim of the present study is to address the type of correlation that may exist between phenolic compounds and vine rhizogenetic potential by analyzing some phenolic compounds in the Carignan merithallus. Phenolic compounds were analyzed by HPLC in the young shoots (or merithallus) of Carignan vine (*Vitis vinifera* L.) and we established a correlation between the studied compounds and the rhizogenetic potential of shoots during the phases of bud dormancy and end of dormancy, taking into account the position effect on shoots. This work was carried out for the first time on this type of vine. Among the studied phenolic compounds, we observed a negative correlation between coumarin and rhizogenetic potential of Carignan vine. In contrast, positive correlations were found with naringin and syringic acid. Obtained results confirmed the fact that the action of phenolic compounds is complex and might be qualified as cofactors that interact with auxin on rhizogenesis.

Keywords: Phenolic Compounds; HPLC; Carignan Shoots; Rhizogenesis

1. INTRODUCTION

Polyphenols, a group of substances with a broad spectrum of physiological activities, are spread in plants used in traditional and modern medical systems. Phenolics have been shown to have a role in tissue browning, flavor, and color characteristics of the derived products [1]. An understanding of phenolic composition in fresh fruit and the factors that affect phenolic compounds are critical in the design of products and storage conditions [2].

Many studies have been undertaken on the phenolic acid compositions of various fruits and their related cultivars such as apples and pears [3,4], *Pyrus* [5,6], pome and stone fruit [7], *Diospyros* [8], carrots [9], and *Prunus* [10].

Phenolic compounds are active biological molecules having one or more benzene rings with one or more hydroxyl functions [11]. At the plant level, phenolic compounds contribute to development, cell multiplication, reproduction, differentiation, flowering, and lignification. Their content in plants depends on many genetic, physiological and environmental parameters [12].

Phenolic compounds accumulate mainly in the cell membrane (lignin and some flavonoids) and in vacuoles where soluble phenols such as chlorogenic acid, anthocyanins, flavonols and tannins are stocked [13]. These compounds can participate also in the phenomenon of rhizogenesis. Some authors reported that such contribution might be positive [14-17]. However, other studies showed no or negative effects of these compounds on rhizogenesis [18-21].

The purpose of this work was to establish the correlation type that may exist between phenolic compounds and vine rhizogenetic potential by analyzing some phenolic compounds in the Carignan merithallus.

2. EXPERIMENT

2.1. Plant Material

The influence of the sampling date on the rhizogenetic potentiality of vine merithallus has been previously studied [22], Carignan vines grown in double Guyot at the experimental station of the Obligatory Grouping of Wine Growers and Fruit Producers (GOVPF) in Baddar (Northeast Tunisia). In this study, vine shoots of various Carignan genotypes were sampled monthly on five specific dates that coincide with the phenomenon of bud dormancy and end of dormancy [23]. The first sampling

took place on September 13, 2005, the second on October 13, 2005, the third on November 13, 2005, the fourth on December 13, 2005 and the last on January 13, 2006.

Thirty merithallus of Carignan vine were sampled from various rows and positions according the convention of friml [24]. Samples were dried and reduced to a fine powder prior to phenolic compound analysis.

2.2. Polyphenols Analysis

2.2.1. Determination of Total Phenolic Compounds

Analysis of total polyphenols in Carignan merithallus samples was carried out using Folin-Ciocalteu reagent [25]. In alkaline medium, this reagent is reduced to tungsten and molybdenum oxides, giving a blue color in the presence of polyphenols. Briefly, 125 μ L of diluted extract (methanol 80%) was mixed with distilled water (500 μ L) and 125 μ L of Folin-Ciocalteu reagent. Then, the mixture was agitated, paused for 3 min before the addition of 1250 μ L of $\text{CO}_3(\text{Na})_2$ at 7%. Finally, the mixture's volume was adjusted by distilled water to 3 mL. After 90 min in darkness, absorbance was determined at a wavelength of 760 nm. The standard curve was prepared using gallic acid at 50, 100, 200, 300, 400, and 500 mg/L. Total polyphenol content was expressed as mg of gallic acid equivalent per g dw.

2.2.2. Solvent Extraction

Solvent extraction was performed according to the method described by [26]. Merithallus sample (2.5 g dw) was mixed with 15 mL water, agitated for 30 min and kept for 24 h at 4°C in total darkness. The mixture was then filtered on ash-free filter paper (Whatman n 4) before storage at 4°C for subsequent analyses.

2.2.3. HPLC Analysis

Phenolic compounds were identified and quantified using RP-HPLC coupled to a UV-visible detector and equipped with a specific C_{18} column: Hypersil ODS (250 \times 4.6 mm, 4 μ m). The mobile phase consisted of acetonitrile (solvent A); and a mixture of HPLC-grade water

and sulphuric acid at 0.2% (solvent B). The gradient program was set up for the mixture (A)-(B) as follows: 15% - 85% for 0 - 12 min, 40% - 60% for 12 - 14 min, 60% - 40% for 14 - 18 min, 80% - 20% for 18 - 20 min, 90% - 10% for 20 - 24 min, and 100% - 0% for the last 24 - 28 min. Injected volume was 20 μ L and peaks were examined at 280 nm. Peaks were characterized by their retention times and corresponding compounds were identified as compared to retention times of pure standards. All chemical analyses were conducted in triplicate.

2.3. Statistical Analysis

All determinations were performed in triplicate and the results are expressed as means values \pm standard deviations (SD). The data were subjected to statistical analysis using statistical program package STATISTICA [27]. The one-way analysis of variance (ANOVA) followed by Duncan multiple range test were employed and the differences between individual means were deemed to be significant at $P < 0.05$. Correlation coefficients were calculated based on coumarin, naringin and syringic acid percentages at different rhizogenetic potential levels.

3. RESULTS

3.1. Total Polyphenols

The total mean content of polyphenols in merithallus as function of sampling date is presented in **Table 1**. The total polyphenol content is expressed as mg of gallic acid equivalent per g of dry weight ($\text{mg GAE}\cdot\text{g}^{-1}$ dw). Total content of phenolic compounds in Carignan merithallus was the highest in November, but showed almost a constant variation for the rest of sampling period (**Table 1**). By considering the merithallus position on Carignan shoots (apical, central and basal), results showed a great variation in total polyphenol content (**Table 2**). This variation was accentuated in November and characterized by a greatest concentration of polyphenols in the apical position, which was three-fold higher than that observed in central and in basal positions.

Table 1. Variation of total phenolic compounds (T) in *Carignan merithallus* as function of sampling date ($\text{mg GAE}\cdot\text{g}^{-1}$ dw).

	September	October	November	December	January
T ($\text{mg GAE}\cdot\text{g}^{-1}$ dw)	3.77 \pm 0.76 ^b	3.30 \pm 0.64 ^d	7.63 \pm 0.17 ^a	1.93 \pm 0.94 ^e	3.55 \pm 0.26 ^c

Values are the means of triplicates \pm SD. Values in the same row with different superscript, ^{a-e} are significantly different at $P < 0.05$.

Table 2. Variation of total phenolic content in *Carignan merithallus* ($\text{mg GAE}\cdot\text{g}^{-1}$ dw) as function of their position on vine shoots.

Position	Month of the year				
	September	October	November	December	January
Apical	1.76 \pm 0.49 ^d	4.18 \pm 0.60 ^e	14.73 \pm 1.46 ^a	1.21 \pm 0.42 ^e	5.22 \pm 0.04 ^b
Central	6.06 \pm 0.36 ^a	3.06 \pm 0.01 ^d	5.64 \pm 0.84 ^b	1.32 \pm 0.68 ^e	3.27 \pm 0.84 ^c
Basal	3.5 \pm 0.01 ^a	2.65 \pm 0.01 ^c	2.52 \pm 0.53 ^{cd}	3.27 \pm 0.52 ^b	2.17 \pm 0.26 ^d

Values are the means of triplicates \pm SD. Values in the same row with different superscript, ^{a-e} are significantly different at $P < 0.05$.

3.2. Variation of Individual Phenolic Compounds

Figure 1 indicates on chromatographic profile (retention time) of phenolic compounds found in Carignan merithallus. Nine phenolic compounds (out of 12) were identified according to their respective retention time as follows: 1) gallic acid, 2) caffeic acid, 3) dihydroxyphenylacetic acid, 4) chlorogenic acid, 5) syringic acid, 6) ferulic acid, 7) naringin, 8) quercetin, 9) coumarin.

Percent distribution of analyzed phenolic compounds within shoots of Carignan merithallus as function of sampling date is presented in **Table 3**. We noted that the content of some phenolic compounds (ferulic acid and coumarin) was proportional to the variation of total content in merithallus. On the other hand, quercetin and dihydroxyphenylacetic acid contents increased with time in contrast to chlorogenic acid. Gallic acid percentage in the central part of shoots increased steadily to reach highest values in December before decreasing. For the same position, caffeic acid was highly expressed in No-

vember while the concentrations of naringin and syringic acid showed a sawtooth-shaped variation (**Table 3**). As the variation of non-identified compounds present in the chromatogram was not stable over time, only phenolic compounds that have significant correlations with the rhizogenetic potential were considered for further investigation.

3.3. Correlation between Phenolic Compounds and Rhizogenetic Potential

When addressing the variation of % coumarin and carignan rhizogenetic potential with respect to sampling date [28], we noticed that there was a strictly negative correlation for merithallus in basal position (**Figure 2**). Our investigation showed a moderately positive correlation between syringic acid and the rhizogenetic potential (**Figure 3**). There is also a positive correlation between naringin and rhizogenetic potential but not significant (**Figure 4**).

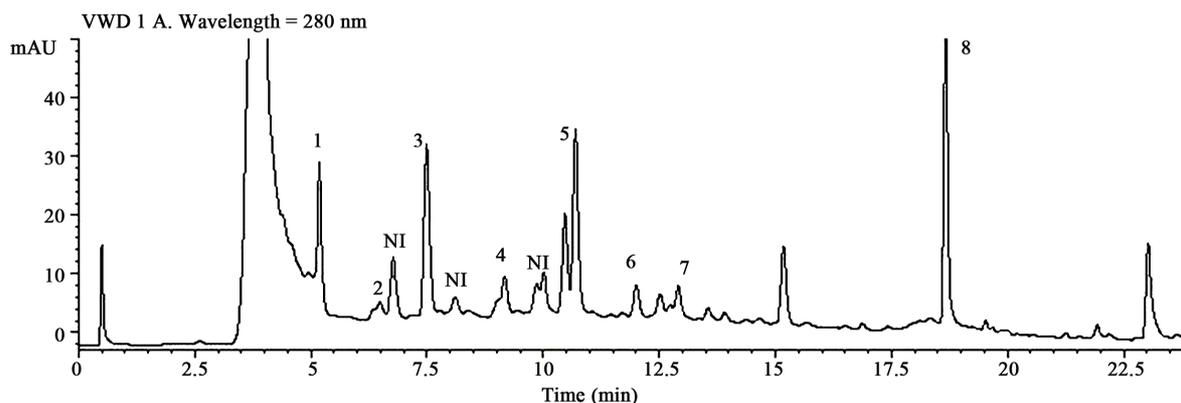


Figure 1. Chromatographic profile of phenolic compounds found in Carignan shoots. 1. Gallic acid; 2. Caffeic acid; 3. Dihydroxyphenylacetic acid; 4. Chlorogenic acid; 5. Syringic acid; 6. Ferulic acid; 7. Naringin; 8. Coumarin; NI. Non identified.

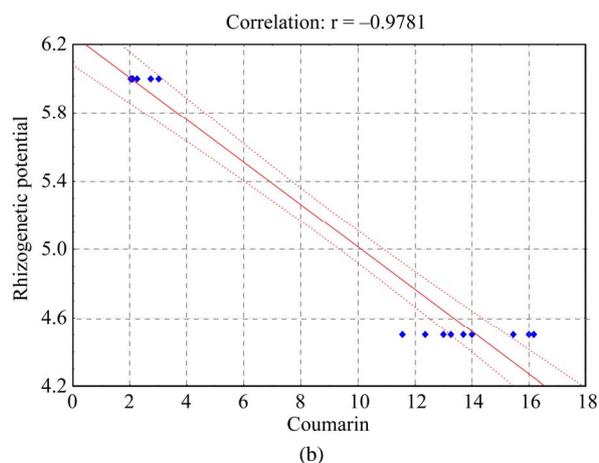
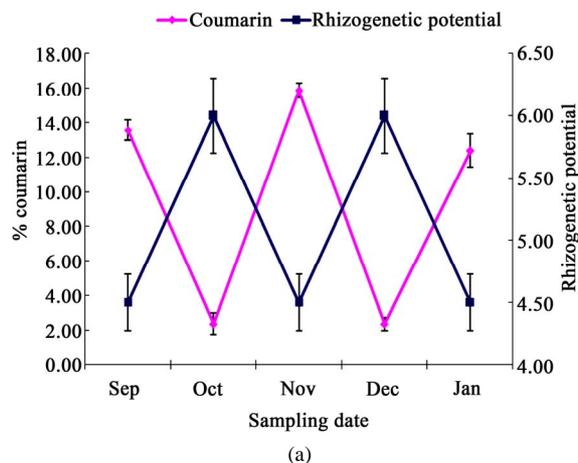


Figure 2. Evolution of the coumarin in the merithallus (a) and correlation between these percentages in basal position and the rhizogenetic potential (b).

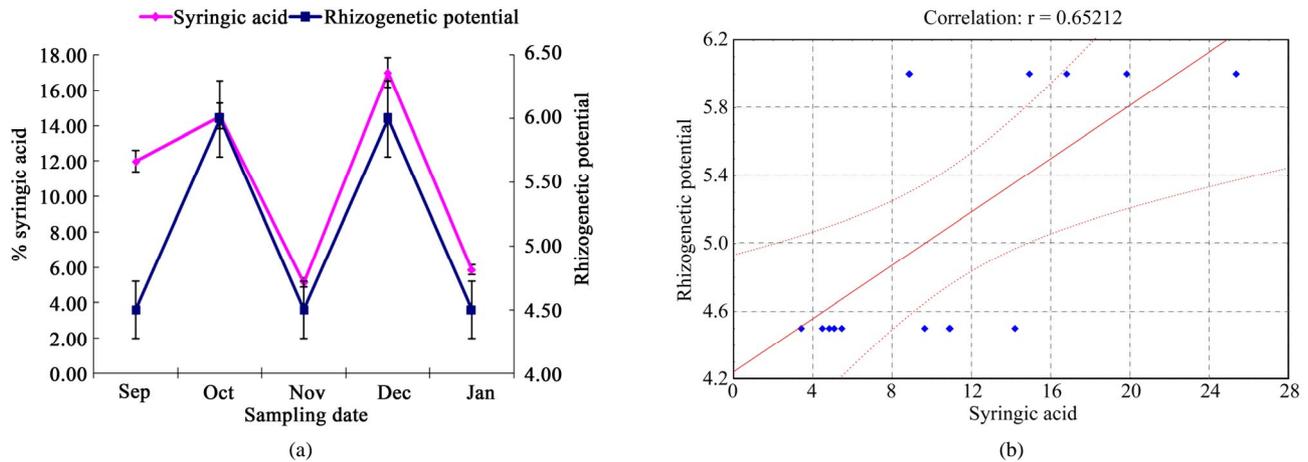


Figure 3. Evolution of syringic acid in the merithallus (a) and correlation between these percentages and the rhizogenetic potential (b).

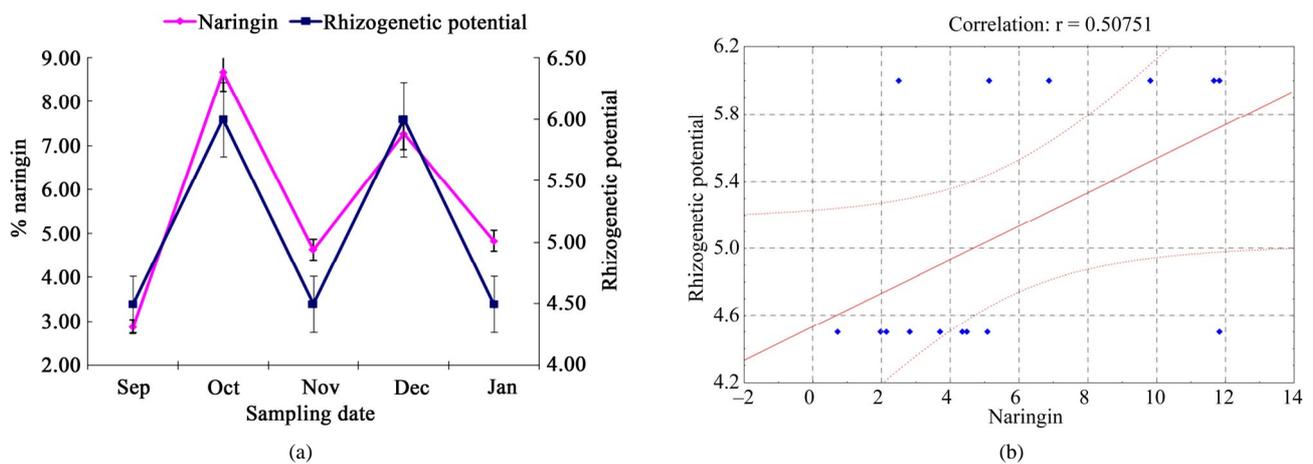


Figure 4. Evolution of the naringin of the merithallus (a) and correlation between these percentages and the potential rhizogenetic according to the date of taking away (b).

4. DISCUSSION

Interactions between phenolic compounds, organogenesis and plant growth have been studied frequently as mentioned in the introduction section. The regulation of the endogenous rate of auxin as part of the enzymatic activity of auxin-oxydase, and auxin transport are two important events.

The polarized transport of auxin has been studied by several authors [29-32]. However, this transport can be disturbed or controlled by some phenolic compounds. In terms of regulation of polarized transport, the role of some specific phenolic compounds (phytotropins) is the most evident as they compete with efflux transporters of auxin according [29].

The variation of percent coumarin in Carignan and the rhizogenetic potential as function of sampling dates [22] showed a significant negative correlation for the basal position of the merithallus on vine shoots. Syringic acid and naringin can also correlate positively with the rhizo-

genetic potential. We noticed also that the quercetin content increased during the phase of dormancy of buds and reached highest levels by January (Table 3). Darne and Atalay [33] reported that there was a direct correlation between the rhizogenetic capacity and the synthesis of phenolic compounds in vine shoots.

By studying the adventive rhizogenesis in *Pinus contorta*, Brinker *et al.* [32] noticed that the active transport of auxin is reduced during the first phase of the rhizogenesis. They also observed a reduction in the transcription of the gene which codes for the kinase-like protein, involved in the control of auxin transport. Intermediate products resulting from the biosynthesis of phenolic compounds may accumulate and create a barrier that inhibits auxin transport. Brinker *et al.* [32] indicated that treatments with auxin activate the cellular division. This hormone has a major role in the initiation and the development of adventive roots. The mechanism utilizes phenolic compounds as a barrier that prevents auxin transport. This natural barrier consists of a protein of

Table 3. Percent distribution (% w/w) of analyzed phenolic compounds within shoots of Carigan merithallus as function of sampling date.

Compounds	1	2	3	4	5	6	7	8	9	10	11
RT (min)	5.20	6.50	6.60	7.50	8.20	9.20	9.80	10.75	12.00	13.00	18.75
Apical	3.24 ± 0.34 ^h	10.46 ± 1.02 ^d	3.14 ± 0.42 ⁱ	13.30 ± 2.12 ^b	3.35 ± 0.45 ^g	37.17 ± 5.42 ^a	9.18 ± 0.87 ^e	11.08 ± 1.75 ^e	5.73 ± 0.65 ^f	2.17 ± 0.32 ^j	1.11 ± 0.13 ^k
Central	3.81 ± 0.42 ^f	3.33 ± 0.41 ^g	1.65 ± 0.17 ^j	3.92 ± 0.42 ^e	1.64 ± 0.24 ^j	35.91 ± 4.58 ^a	23.39 ± 3.13 ^b	11.22 ± 1.56 ^e	2.37 ± 0.26 ⁱ	2.90 ± 0.32 ^h	9.83 ± 0.99 ^d
Basal	4.43 ± 0.55 ^g	3.90 ± 0.40 ^h	2.11 ± 0.25 ^k	6.90 ± 0.77 ^f	25.02 ± 3.49 ^a	10.80 ± 2.17 ^e	11.56 ± 2.32 ^d	14.61 ± 1.75 ^b	2.83 ± 0.33 ^j	3.81 ± 0.43 ⁱ	13.96 ± 1.46 ^c
Apical	4.62 ± 0.58 ^h	1.89 ± 0.22 ^k	18.86 ± 3.02 ^b	16.24 ± 2.12 ^c	4.68 ± 0.54 ^g	7.75 ± 0.84 ^f	8.47 ± 0.95 ^e	20.44 ± 3.12 ^a	2.32 ± 0.28 ⁱ	12.19 ± 1.86 ^d	2.43 ± 0.32 ⁱ
Central	3.41 ± 0.40 ⁱ	3.49 ± 0.38 ^h	5.00 ± 0.65 ^g	12.06 ± 2.04 ^d	20.35 ± 3.18 ^a	17.77 ± 2.58 ^b	12.62 ± 2.13 ^c	9.10 ± 0.85 ^f	2.54 ± 0.26 ^k	2.56 ± 0.28 ^j	11.04 ± 1.53 ^c
Basal	7.18 ± 0.86 ^e	9.60 ± 1.02 ^e	3.20 ± 0.8 ^j	22.62 ± 3.45 ^a	4.09 ± 0.58 ^h	7.64 ± 0.84 ^f	11.91 ± 2.15 ^d	15.49 ± 1.79 ^b	3.68 ± 0.41 ⁱ	12.09 ± 1.59 ^e	2.45 ± 1.32 ^k
Apical	5.57 ± 0.62 ^h	12.23 ± 1.56 ^d	5.22 ± 0.62 ⁱ	21.31 ± 4.12 ^a	6.39 ± 0.75 ^f	8.06 ± 0.91 ^e	13.97 ± 1.94 ^b	5.88 ± 0.65 ^g	12.51 ± 1.35 ^c	4.66 ± 0.52 ^j	4.10 ± 0.57 ^k
Central	6.38 ± 0.59 ^f	15.10 ± 2.45 ^b	5.89 ± 0.73 ^g	9.93 ± 1.55 ^e	2.91 ± 0.36 ^j	11.97 ± 1.58 ^d	18.50 ± 2.13 ^a	5.42 ± 0.63 ^h	5.23 ± 0.60 ⁱ	5.42 ± 0.63 ^h	13.20 ± 1.59 ^e
Basale	4.73 ± 0.55 ^j	14.31 ± 1.85 ^c	5.58 ± 0.65 ^f	9.41 ± 1.87 ^e	2.76 ± 0.38 ^k	11.35 ± 1.69 ^d	19.71 ± 2.61 ^a	5.13 ± 0.68 ^g	4.95 ± 0.52 ^j	5.10 ± 0.67 ^h	16.88 ± 1.89 ^b
Apical	4.79 ± 0.58 ^h	17.91 ± 2.89 ^b	1.79 ± 0.29 ^k	11.97 ± 2.45 ^c	4.10 ± 0.51 ⁱ	8.38 ± 0.97 ^e	2.95 ± 0.36 ^j	27.24 ± 3.42 ^a	6.19 ± 0.73 ^f	10.55 ± 1.76 ^d	5.08 ± 0.64 ^g
Central	30.20 ± 4.26 ^a	7.43 ± 0.88 ^f	2.57 ± 0.31 ^j	11.24 ± 2.36 ^b	5.10 ± 0.67 ⁱ	8.85 ± 0.98 ^e	5.92 ± 0.64 ^g	9.53 ± 0.98 ^d	2.48 ± 0.32 ^k	5.52 ± 0.69 ^h	11.17 ± 1.56 ^e
Basal	5.70 ± 0.61 ^g	4.35 ± 0.45 ^h	4.01 ± 0.51 ⁱ	10.59 ± 1.98 ^e	8.68 ± 0.94 ^d	30.31 ± 4.22 ^a	7.49 ± 0.84 ^e	17.26 ± 0.83 ^b	2.05 ± 0.25 ^k	7.06 ± 0.84 ^f	2.42 ± 0.34 ^j
Apical	4.76 ± 0.51 ^h	11.85 ± 1.69 ^b	2.89 ± 0.32 ^j	31.04 ± 5.45 ^a	5.75 ± 0.66 ^g	11.49 ± 2.45 ^d	6.58 ± 0.76 ^f	9.88 ± 0.98 ^e	1.62 ± 0.18 ^k	11.66 ± 2.01 ^c	2.42 ± 0.31 ^j
Central	22.71 ± 3.02 ^b	3.30 ± 0.38 ^g	4.17 ± 0.33 ^j	35.01 ± 5.68 ^a	1.58 ± 0.22 ^j	11.07 ± 2.46 ^d	5.69 ± 0.65 ^e	11.48 ± 1.45 ^e	1.02 ± 0.14 ^k	1.84 ± 0.19 ^j	2.05 ± 0.26 ^h
Basal	0.41 ± 0.05 ^k	10.55 ± 1.45 ^c	3.52 ± 0.38 ^f	24.52 ± 3.73 ^a	2.51 ± 0.31 ^h	23.47 ± 3.19 ^b	14.50 ± 1.76 ^c	3.45 ± 0.42 ^g	2.24 ± 0.28 ⁱ	1.80 ± 0.20 ^j	12.5 ± 1.56 ^d

Values are the means of triplicates ±SD. Values in the same row with different superscript, ^{a-k} are significantly different at $P < 0.05$. Compounds: 1: Gallic acid, 2: caffeic acid, 3: NI, 4: dihydroxyphenylacetic acid, 5: NI, 6: chlorogenic acid, 7: NI, 8: syringic acid, 9: ferrulic acid, 10: Naringin, 11: Coumarin.

having a flavonolic origin [30,32] noticed that the transcription of the gene coding for this enzyme is affected during the phase of radial primordial formation.

5. CONCLUSIONS

The evolution of phenolic compounds was studied on Carignan merithallus during the phases of bud dormancy and end of dormancy. Our outcomes indicate that rhizogenesis coincides with quercetin synthesis. This phenolic compound belongs to the class of phytochemicals according to Brunn et al. [29]. We observed also a negative correlation between coumarin and rhizogenetic potential of Carignan vine. In contrast, positive correlations were found with naringin and syringic acid.

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