Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

# γ-tocopherol methyltransferase gene sequencing and SNP discovery associated with olive oil quality

Nadia Ayadi[1], Rayda Ben Ayed[2,3*], Sezai Ercisli[4], Melekber Sulusoglu Durul[5], Ebru Sakar[6], Ahmed Rebai[1]

[1] Laboratory of Molecular and Cellular Screening Processes, Genomics and Bioinformatics Group, Centre of Biotechnology of Sfax, PB 1177, 3018 Sfax, Tunisia
[2] Department of Agronomy and Plant Biotechnology, National Institute of Agronomy of Tunisia (INAT),43 Avenue Charles Nicolle, 1082 El Mahrajène, University of Carthage-Tunis, Tunis 1082, Tunisia
[3] Laboratory of Extremophile Plants, Centre of Biotechnology of Borj-Cédria, B.P. 901,Hammam Lif 2050, Tunisia
[4] Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum 25240, Türkiye
[5] Department of Horticulture, Faculty of Agriculture, Kocaeli University, Kocaeli 41285, Türkiye
[6] Department of Horticulture, Faculty of Agriculture, Harran University, Sanliurfa 63280, Türkiye

**ARTICLE INFO**

***Original paper***

***Article history:***
*Received: May 06, 2023*
*Accepted: July 04, 2023*
*Published: August 31, 2023*

***Keywords:***

*Olea europaea, genetic diversity, olive oil, SNP markers, tocopherols*

**ABSTRACT**

The olive tree and olive oil are among the first things that come to mind throughout history when the Mediterranean geography is mentioned. Thus the fruit of the olive is very important in the agriculture of the Mediterranean region as a source of olive oil; it is one of the basic components of Mediterranean cuisine. γ-tocopherol methyltransferase (GTMT), a key enzyme in the tocopherols biosynthesis pathway, is involved in the conversion of δ- and γ-tocopherol to β- and α-tocopherol, respectively. In fact, it plays an important role in the α-tocopherol composition and the quality of olive oil. A total of 14 olive cultivars (*Olea europaea L.*) were used in the present work. The materials were collected from diverse areas of Tunisia and to make a comparison four cultivars originating from Greece, Algeria, Morocco, and Spain were included. Young leaves of cultivars used for DNA extractions. PCR amplified the *Vte4* gene from 14 olive cultivars and verified by electrophoresis on a 2% agarose gel for each variety. DNA sequencing of the olive cultivars revealed several single-nucleotide polymorphisms (SNPs). Statistical and bioinformatics analysis draw attention to some associations between some of the single nucleotide polymorphisms (SNPs), tocopherols contents and oleic acid content. In fact, two significant associations are obtained between SUBS24 and both total-tocopherols and beta-tocopherol. Moreover, dendrograms showed a correlation between genetic diversity and chemical characteristics which makes the *Vte4* gene more interesting in terms of tocopherols levels.

**Doi:** http://dx.doi.org/10.14715/cmb/2023.69.8.17


## Introduction

The olive tree (*Olea europaea* L.) is the most precious and important tree for the people of the Mediterranean countries (1,2). It is not only the symbol of Mediterranean culture and diet but also the marker of the influence of this culture in the world. The olive tree is thus a social link that has structured the landscapes, economic activities and social life of the Mediterranean people, including Tunisia (3).

Nowadays, the enthusiasm for olive and olive oil is linked to a strong demand for quality. For this reason, the International Olive Council (IOC) is present to encourage the expansion of fair international trade in olive oil and table olives and update the commercial standards appropriate to olive products and improve quality. The social and economic importance of the olive tree for Tunisia is innumerable. This glorious tree is the main fruit species in Tunisia with approximately 1.85 million hectares (79% of the surface devoted to arboriculture and 34% of arable land) (4). Production reaches approximately 10 million tons of olives, of which 22 thousand tons are intended for the preparation of table olives according to IOC (5). Concerning the olive sector, Tunisia produces 150 thousand tons of which 97 thousand tons are intended for export, this gives Tunisia the 4th largest producer of olive oil in the world and oil exports and the first rank as a producer and exporter of the southern Mediterranean just after the European Union in terms of world production of olive oil (6).

From a consumer point of view, the most important thing to look for is the origin and the category of olive oil (7). In fact, olive oil is well known not only for its exceptional flavor but also for its impressive health properties. Indeed, it contributes to the prevention of heart disease and even to the reduction of the risk of osteoporosis and diabetes (8). All the benefits of olive oil make this product the subject of many scientific researches. In Tunisia, the olive forests stretch from north to south and contain nearly 1,500 cultivars, some of which are rare and others with similar characteristics that are difficult to distinguish. Among the main cultivars of olive trees in Tunisia we cite: the cultivar 'Chetoui' in the north and the cultivar 'Chemlali' in the center and in the south there are cultivars like "Oueslati", 'Chemchali', 'Zalmati' or 'Zarazi' (9). Virgin olive oil is

* Corresponding author. Email: raydabenayed@yahoo.fr; rayda.benayed@inat.ucar.tn

obtained from the fruit of the olive tree only by mechanical processes or other physical processes under thermal conditions which do not lead to any alteration of the oil and which have not undergone any other treatment. The International Olive Oil Council (IOC) classifies the olive oils produced under different denominations according to different parameters such as acidity, peroxide index and ultraviolet absorbance. Indeed, EVOO (Extra Virgin Olive Oil) is accepted as the best quality (with high content of tocopherols in olive oil).

The biosynthesis of tocopherols begins with the formation of the precursor molecule, homogentisate (HGA). In order to obtain the four iso-forms of tocopherols, several enzymes intervene (10). These enzymes are located in different compartments like the internal envelope or the plastoglobules (11), There are at least four main and essential enzymes participating in the biosynthesis pathway of tocopherols, in the various responses to stress and in phytohormone signaling pathways. Much more research still needs to be done to know the process of tocopherol biosynthesis in *Olea europaea.* This tocopherol biosynthesis pathway is frequently targeted in genetic engineering to make transgenic lines with the desired types and safe amounts of tocopherols to improve the antioxidant and nutritional values of important crops (12). The *Vte4* gene has a crucial role in the process of tocopherol biosynthesis. γ-tocopherol methyltransferase (GTMT), an enzyme secreted by the *Vte4* protein, is the last enzyme in the tocopherol biosynthesis pathway. It catalyzes the conversion of γ-tocopherol to α-tocopherol, the most bioactive and nutritionally significant form of vitamin E, and β-tocopherol respectively. The main function of α-tocopherol is to protect polyunsaturated fatty acids (PUFAs) from reactive oxygen species (ROS) by scavenging radicals (13). The activity of this enzyme can determine the composition of tocopherol, and therefore the activity of vitamin E. The development of plant lines with increased tocopherol content may be useful in agriculture and also in bioremediation (14).

In recent years, important success in studies in the field of plant biotechnology, in the creation of gene sources and in technical advances in the field of genomics, has been achieved. While aiming to contribute to meeting the demands of the increasing world population, the evaluation of the collections with the developing genomic technologies provides significant benefits in breeding (selection, hybridization, etc.) studies to be used for this purpose. On the other hand, in the field of plant molecular breeding, the contribution of new DNA markers such as SNP (Single Nucleotide Polymorphism) to accelerating breeding is seen as an acceptable approach today (15-19).

Tunisia is an important country for olive and olive oil production and export as well. Therefore this work concentrated on the identification of γ-tocopherol methyltransferase (GTMT) gene sequencing and SNP discovery associated with olive oil quality in fourteen native olive cultivars of Tunisia. The results are expected to offer a better vision of the constituents and quality of olive oil.

## Materials and Methods

### Plant material

The material includes 14 Tunisian olive cultivars collected in diverse areas of Tunisia and to make a comparison four cultivars originating from Greece, Algeria, Morocco, and Spain were used. Young leaves of cultivars used for DNA extractions (Table 1).

### DNA extraction

DNA was extracted from young leaves using the CTAB methods described by Fabbri et al (20). The obtained genomic DNA was dissolved in TE buffer (1X) (50 mM Tris-HCl pH 8, 1 mM EDTA pH 8) and stored at -20°C. Therefore, after DNA extraction and purification, DNA quantification was determined for each sample using the NanoDrop™ 2000c spectrophotometer (Thermo Scientific™, Waltham, MA, USA).

### Primer design, PCR amplification and sequencing of *Vte4*

The PCR primers (GTMTF: 5' TGATGATCCACCGAGACAAA 3' and GTMTR: 5' AC-CTTGTCGTCCAATCCTTG 3') were designed using the Primer3 program (21). The *Vte4* gene was PCR amplified from the 14 olive cultivars. The PCR reactions are carried out in 30 µl reaction volumes containing: 100 ng of olive genomic DNA, 2.5 µL of MgCl2 (25mM), 2 µL DNTP(10mM), 1 µL of each primer (Forward primer (10 µM), Reverse pri-

**Table 1.** Details of the SNPs detected in the *Vte4* gene from the 14 studied olive oil cultivars.

| Olive oil varieties /SNP | SUBS21 | I/D22 | SUBS22 | SUBS24 | SUBS88 | I/D143 | SUBS143 | SUBS146 |
|---|---|---|---|---|---|---|---|---|
| TounsiGf | T | I | A | A | G | I | C | G |
| ChemlaliZar | T | I | T | A | G | I | C | G |
| Besbesi | T | I | A | T | G | I | A | G |
| Jarboui | T | I | T | A | G | I | C | G |
| DhokarBeng | T | I | A | A | G | I | C | G |
| Manzanille | T | I | A | A | G | I | C | G |
| Bidhhma | T | I | A | T | G | I | C | G |
| Jemribeng | T | I | A | A | G | I | C | G |
| ZarrziZar | T | D | T | A | G | I | C | G |
| Rkhaymi | T | I | T | A | G | I | C | G |
| Chehla | C | I | T | A | G | I | C | G |
| Zalmati | T | I | A | T | G | D | C | G |
| Sigoise | T | D | T | T | G | I | C | G |
| Chetoui | T | I | A | A | G | I | C | G |

mer (10µM)), 0.5 of 5u/µL of Taq Polymerase(5u/µL), 10 µLof Taq Buffer (10X) and 3.25 µL of distilled water. PCR amplifications were performed on AB Applied Biosystems PCR GeneAmp 9700 Thermal cycler with a starting denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 30 s, 57 °C for 30 s and 72°C for 30 s, with a final elongation at 72°C for 10 min. Verification of the PCR amplification is carried out by electrophoresis on a 2% agarose gel for each cultivar. PCR products were sequenced in triplicate from either end using the same primers as reused in PCR amplification and analyzed on the AB3130XL for sequence determination.

**Sequence analysis and SNP marker discovery**

Fourteen Tunisian olive cultivars were used for the *Vte4* gene sequence. Those cultivars had different tocopherols content. Sequence alignment using ClustalW (22,23) was used for DNA polymorphisms in the *Vte4* gene sequence for fourteen olive cultivars and to determine the types and positions of SNPs. In order to confirm the possible heterozygous sequence, the outputs from the sequencer were visually inspected. The potential SNPs were resequenced to reduce false positives by cause of sequencing artifacts.

**Statistical methods**

Descriptive statistics were done by IBM SPSS statistics (version 26) and predictive analytics software (24) to check the quality of genotyping and to analyze the association of alleles. Logistic regression was used to examine the genotype/phenotype association. The relationship between the tocopherols content and the discovered SNPs was examined through different statistical means and techniques. The significance of differences at a 5% level among means of various groups for each SNP was determined by one-way ANOVA. Analysis of variance was applied to analyze the association of the studied SNPs synchronously with tocopherols content. Binary logistic regression was employed to test the associations of the studied SNPs with tocopherols content and oleic acid level separately. IBM SPSS statistics (version 26) predictive analytics software (24) was used for all analyses to determine the interactions between tocopherols contents and the studied SNPs as in rather between SNPs.

**Cluster analysis**

Cluster analysis of used olive cultivars and educated parameters (the tocopherols content and the 8 discovered SNPs) were realized using IBM SPSS statistics (version 26) predictive analytics software (24). The hierarchical cluster analysis method was used to perform the phylogenetic analyses based on the 14 *Vte4* gene sequences.

**Bioinformatics analysis**

Multiple sequence alignment of the GTMT gene sequence was accomplished by using ClustalW (22,23) with default parameters.

**Results**

***Vte4* gene sequencing, SNP discovery, and molecular characterization**

We sequenced the *Vte4* gene from fourteen Tunisian olive cultivars to obtain a wide number of SNP markers. The alignment of the nucleotide sequences of the GTMT gene showed 8 variations (Table 1) of which six variations are of the substitution type and only two correspond to insertions/deletions.

**Allelic and genotypic frequencies of the SNPs markers**

The 8 variations located in the *Vte4* gene were then statistically validated to determine allelic and genotypic frequencies. The calculation of the allele frequencies as well as the genotypic frequencies of the genetic markers studied are mentioned in the following Tables 2 and 3.

**Allelic frequencies of the 8 SNPs markers**

The allelic frequencies of each studied variation (Table 2) showed a dominance of one allele over another. In fact, for the SUBS 21 substitution, we observed a dominance of the T allele over the C allele with a frequency equal to 93%, which shows that the C allele is rare, similarly for insertion/deletion 22 where we found a dominance of the D allele compared to I with a frequency equal to 86%. For the SUBS 22 substitution, the two alleles A and T have almost the same allelic frequency. Regarding the SUBS24 substitution, we note a dominance of the A allele over the T allele with a frequency equal to 71%. The G allele shows total dominance with an allele frequency of 100% for both SUBS 88 and SUBS146. For I/D143 and SUBS 143, we notice a dominance of the I and C allele with a frequency equal to 93% which shows that the D and A alleles respectively are rare.

**Genotypic frequencies for the 8 SNPs**

For the genotypic frequencies of each studied SNPs markers, dominance of one genotype over another is observed (Table 3), except for SUBS24 in which the frequency of the heterozygous genotype is 0.5 and that of the homo-

**Table 2.** Allelic frequencies for the 8 SNPs markers.

| SNP | SUBS21 | | I/D22 | | SUBS22 | | SUBS24 | | SUBS88 | | I/D143 | | SUBS143 | | SUBS146 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | C | I | D | A | T | A | T | G | C | I | D | C | A | G | A |
| Allelic Frequencies | 0,93 | 0,07 | 0,86 | 0,14 | 0,57 | 0,43 | 0,71 | 0,29 | 1 | 0 | 0,93 | 0,07 | 0,93 | 0,07 | 1 | 0 |

**Table 3.** Genotypic frequencies for the 8 SNPs markers.

| SNP | SUBS21 | | | I/D22 | | | SUBS22 | | | SUBS24 | | | SUBS88 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TT | CC | CT | II | DD | ID | AA | TT | AT | AA | TT | AT | GG | CC | GC |
| Genotypic frequencies | 0.86 | 0.005 | 0.24 | 0.74 | 0.02 | 0.24 | 0.32 | 0.18 | 0.5 | 0.5 | 0.08 | 0.41 | 1 | 0 | 0 |

| SNP | I/D143 | | | SUBS143 | | | SUBS146 | | |
|---|---|---|---|---|---|---|---|---|---|
| | II | DD | ID | CC | AA | CA | GG | AA | AG |
| Genotypic frequencies | 0.86 | 0.005 | 0.13 | 0.86 | 0.005 | 0.13 | 1 | 0 | 1 |

zygous genotype is 0.41.

### SNP-SNP interaction

We determined eight 8 variable sites of *Vte4* polymorphism among fourteen olive cultivars. Linkage disequilibrium (LD) measures based on the chi-square statistic for testing for association between alleles of genetic function were studied. Figure 1 indicates the interaction analysis plots drawn using all 8 SNP markers. Each plot contains a Chi-Square value.

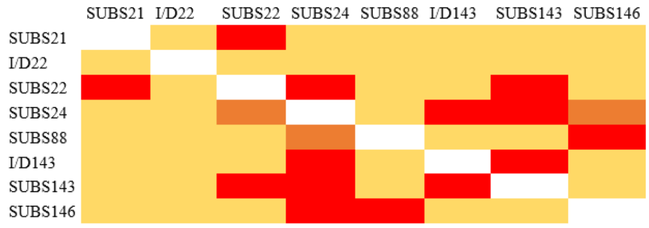


**Figure 1.** Interaction analysis between SNPs markers increasing intensity of color corresponds to an increasing interaction between SNPs.The same color indicates a similar level of statistical significance between SNPs cases. Red for a Chi-Square value > 2, Orange for a Chi-Square value between 1 and 2, and Yellow for a Chi-Square value < 1.

### Genotype-phenotype association and SNP-SNP interaction

#### *Association statistics for tocopherols content and genetic variants in Vte4 gene*

Associations between alpha, beta, gamma and total-tocopherols compositions and the 8 SNPs in the *Vte4* gene separately were investigated. Table 4 shows the mean, the P-value and Fisher's exact test. Two significant associations are obtained between SUBS24 and both Total-Tocopherols (F=4.780, P=0.049) and Beta Tocopherols (F=5.400, P=0.039) however there was no interaction between the other SNPs and the tocopherols levels.

#### *Associations statistics for oleic acid content and SNPs markers*

In order to illustrate the association between the quality of olive oil cultivars (oleic acid) and gene information, we applied the Fisher's test. Table 5 shows Fisher's exact test and significance values of the 8 SNPs markers related to the oleic acid level. The results are summarized in Table 5 and demonstrated the absence of any significant association. The following three SNPs markers showed the least P-value SUBS22 (P=0.092), SUBS88 (P=0.092) and I/D143 (P=0.093).

#### *Binary logistic regression for all SNPs markers*

Binary logistic regression was used in order to illustrate the association between olive oil parameters and SNPs markers. Table 6 shows the P-value for all SNPs markers. The results summarized in Table 6 demonstrated the absence of any significant associations between the eight SNPs markers and the studied parameters.

### Hierarchical classification

The matrice of distance constructed with SNPs markers and tocopherols content was used to plot three dendrograms which are shown in Figures 2[A], 2[B] and 2[C]. Four groups were obtained in Figure 2[A] by cutting the dendrogram at a degree of similarity equal to 15. Group 1 consists of three cultivars 'Chemlali Zar', 'Chehla and 'Rkhaymi'. This group was found in group 2 of the dendrogram (Figure 2[B]) generated by the tocopherols content, indeed this group presented olive oil cultivars with low tocopherols levels. The second group includes two cultivars 'Jarboui' and 'Dhokar beng'. We noticed that this group of cultivars also exists in group 2 of the dendrogram (Figure 2[B]). The third group contains 'Besbessi', 'Zalmati' and 'Chetoui'. We found a similarity between this group and Group 1 of the dendrogram (Figure 2[B]) and Group 2 of the dendrogram (Figure 2[C]). The fourth group comprises 'Bidhhma', 'Jemribeng', 'Tounsigf', 'Manzanille', 'Zrazzizar' and 'Sigoise'. Euclidean distances were calculated

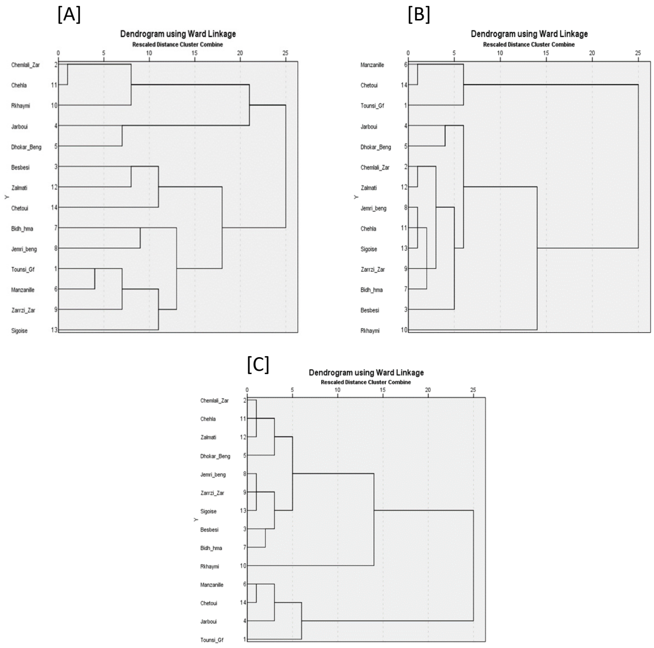


**Figure 2.** (**A**) Classification of olive cultivars based on SNPs markers using clustering by Ecludiean distance; (**B**) Classification of olive cultivars based on tocopherols content using clustering by Ecludiean distance ; (**C**) Classification of olive cultivars based on SNPs markers and tocopherols content using clustering by Ecludiean distance.

between all the olive cultivars. The matrix distance was used for cluster analysis (UPGMA) in SPSS version 26.

### Discussion

The *Vte4* gene has a crucial role in the process of tocopherol biosynthesis. γ-tocopherol methyltransferase (GTMT), a key enzyme in the tocopherol biosynthesis pathway, catalyzes the conversion of γ-tocopherol to α-tocopherol and β-tocopherol respectively. The main function of α-tocopherol is to protect polyunsaturated fatty acids (PUFAs) from ROS by scavenging radicals (13). The activity of this enzyme can determine the composition of tocopherol, and therefore the activity of vitamin E (25).

We sequenced the *Vte4* gene from 14 Tunisian olive cultivars. The alignment of the nucleotide sequences of the GTMT gene showed 8 variations. The allelic frequencies of each studied variation showed a dominance of one allele over another, however for the genotypic frequencies of each studied SNP marker, a dominance of one genotype over another is observed for SUBS24. The obser-

**Table 4.** Association results between tocopherols isoforms content and the discovered SNPs.

| SNP | Alpha-Tocopherols | | | Beta-Tocopherols | | | Gamma-Tocopherols | | | Total-Tocopherols | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **F** | ***p*** | **Mean** | **F** | ***p*** | **Mean** | **F** | ***p*** | **Mean** | **F** | ***p*** | **Mean** |
| SUBS21 | 3.385 | 0.091 | (T)34.720<br>(C)364.016 | 0.330 | 0.330 | (T)2.200<br>(C)10.255 | 0.161 | 0.695 | (T)5.500<br>(C)7.104 | 0.059 | 0.812 | (T)242.000<br>(C)279.308 |
| I/D22 | 2.060 | 0.177 | (I)171.000<br>(D)368.744 | 0.361 | 0.559 | (I)4.375<br>(D)10.564 | 0.101 | 0.756 | (I)6.185<br>(D)7.123 | 1.428 | 0.255 | (I)166.950<br>(D)294.925 |
| SUBS22 | 0.579 | 0.461 | (A)279.143<br>(T)365.036 | 0.716 | 0.414 | (A)4.925<br>(T)11.582 | 0.736 | 0.408 | (A)5.625<br>(T)7.535 | 0.204 | 0.659 | (A)304.750<br>(T)265.400 |
| SUBS24 | 0.368 | 0.555 | (A)400.233<br>(T)324.203 | 5.400 | **0.039** | (A)23.200<br>(T)5.993 | 1.753 | 0.210 | (A)9.443<br>(T)6.320 | 4.780 | **0.049** | (A)417.000<br>(T)238.364 |
| SUBS88 | 0.011 | 0.919 | (G)327.150 | 0.323 | 0.580 | (G)4.650 | 0.562 | 0.468 | (G)8.850 | 1.008 | 0.335 | (G)183.000 |
| I/D143 | 0.072 | 0.794 | (I)814.913<br>(D)454.288 | 0.055 | 0.819 | (I)6.600<br>(D)9.917 | 0.000 | 0.991 | (I)7.030<br>(D)6.986 | 0.277 | 0.608 | (I)351.000<br>(D)270.923 |
| SUBS143 | 0.247 | 0.628 | (C)324.253<br>(A)381.100 | 1.218 | 0.291 | (C)8.650<br>(A)9.852 | 3.298 | 0.094 | (C)5.935<br>(A)9.625 | 0.412 | 0.533 | (C)260.810<br>(A)316.225 |
| SUBS146 | 0.810 | 0.386 | (G)229.150 | 0.013 | 0.910 | (G)8.650 | 0.170 | 0.687 | (G)5.950 | 0.121 | 0.734 | (G)243.000 |

**Table 5.** Association results between oleic acid content and the discovered SNPs.

| SNP | Oleic acid | |
|---|---|---|
| | F | *P* |
| SUBS21 | 0.000 | 1.000 |
| I/D22 | 0.083 | 0.465 |
| SUBS22 | 0.577 | 0.092 |
| SUBS24 | 0.167 | 0.314 |
| SUBS88 | 0.577 | 0.092 |
| I/D143 | 0.571 | 0.093 |
| SUBS143 | 0.500 | 0.111 |
| SUBS146 | 0.333 | 0.175 |

F : Fisher's exact test ; P: P-value .

**Table 6.** Binary logistic regression between SNPs markers and both tocopherols isoforms content and oleic acid.

| Parameters/SNP | Alpha-Tocopherols | Beta-Tocopherols | Gamma-Tocopherols | Total-Tocopherols | Oleic acid |
|---|---|---|---|---|---|
| | **p** | **p** | **p** | **p** | **p** |
| SUBS21 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| I/D22 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| SUBS22 | .423 | .999 | 1.000 | .423 | .999 |
| SUBS24 | 1.000 | 1.000 | .999 | .999 | .999 |
| SUBS88 | 1.000 | 1.000 | .999 | .999 | .999 |
| I/D143 | .999 | .999 | .999 | .999 | .999 |
| SUBS143 | 1.000 | 1.000 | .999 | .999 | .999 |
| SUBS146 | 1.000 | 1.000 | .999 | .999 | 1.000 |

P: P-value.

vation of Figure 1 revealed that the highest Chi-square values were shown for SUBS22, SUBS24, SUBS88, I/D143 and SUBS146 meaning that these markers are the most informative markers and therefore able to distinguish between our olive cultivars. The statistical associations between alpha, beta, gamma and total-tocopherols compositions and the 8 SNPs in the *Vte4* gene showed two significant associations between SUBS24 and both Total-Tocopherols (F=4.780, P=0.049) and Beta Tocopherols (F=5.400, P=0.039) (Table 4) however there was no interaction between the other SNPs and the tocopherols levels. These two significant associations suggest a direct effect of SUBS24 on the rate of tocopherols for each cultivar and hence influenced the antioxidant parameter. In fact, SUBS24 is located in the *Vte4* gene that has a crucial role in the process of tocopherol biosynthesis. These statistical approaches aim to anticipate genomic regions with high tocopherols level (26)

In order to illustrate the association between the quality of olive oil cultivars (oleic acid) and gene information, we applied the Fisher's test. Table 5 shows Fisher's exact test and significance values of the 8 SNPs markers related to the oleic acid level. The results are summarized in Table 5 and demonstrated the absence of any significant association. The following three SNPs markers showed the least P-value SUBS22 (P=0.092), SUBS88 (P=0.092) and I/D143 (0.093). As for the binary logistic regression, the results summarized demonstrated the absence of any significant associations between the eight SNPs markers and the studied parameters.

Euclidean distances were calculated between all the olive cultivars. The matrix distance was used for cluster analysis (UPGMA) in SPSS version 26. The observation of the three dendrograms reveals that there is correlation between genetic variability and chemical characteristics (level of tocopherols). However, no correlations were observed with the geographical origin, which was also reported by Ben Ayed et al (27).

Overall, this study demonstrated that two significant associations were obtained between SUBS24 and both total-tocopherols and beta-tocopherols. Using the obtained data, we constructed three dendrograms that illustrate the family relationships that unite our cultivars. The observation of these dendrograms reveals that there is a correlation between genetic variability and chemical characteristics (level of tocopherols). However, no correlations were observed with the geographical origin. In perspective, we plan to expand the sample size of olive cultivars while increasing the number of SNP markers to be used.

## Conclusions

We analyzed the polymorphism of the 8 SNP markers located in the *Vte4* gene in a sample of olive cultivars and examined their genotype-phenotype association. Two significant associations are obtained between SUBS24 and both total-tocopherols and beta-tocopherol. Using the obtained data, we constructed three dendrograms that illustrate the family relationships that unite our cultivars. The observation of these dendrograms reveals that there is a correlation between genetic variability and chemical characteristics (level of tocopherols). However, no correlations were observed with the geographical origin. In perspective, we plan to expand the sample size of olive cultivars while increasing the number of SNP markers to be used

## Interest conflict

There is no potential conflict of interest to declare.

## Consent for publications

The author read and proved the final manuscript for publication.

## Availability of data and material

All data generated during this study are included in this published article.

## Authors' Contribution

All authors had equal roles in study design, work, statistical analysis and manuscript writing.

## Funding

This article was achieved based on the material and equipment of the Laboratory of Molecular and Cellular Screening Processes, Genomics and Bioinformatics Group, Centre of Biotechnology of Sfax which the authors thank it.

## Ethics approval and consent to participate

No human or animals were used in the present research.

## References

1. Arji I, Safari M, Hadavi I. Effects of different organic manures and chemical fertilizers on yield and yield component of olive (Olea europaea L.,) cv Zard in Kermanshah province. Agrotech Ind Crops. 2021; 1(2): 61-70. https://doi.org/10.22126/atic.2021.6514.1013.
2. Ogras SS. Comparative study of phenolic compounds in olive oils from differentgeographic regions. Turk J Agric For. 2022; 46 (3):318-26. https://doi.org/10.55730/1300-011X.3005.
3. Dervishi A, Jakše J, Ismaili H, Javornik B, Štajner N. Comparative assessment of genetic diversity in Albanian olive (Olea europaea L.) using SSRs from anonymous and transcribed genomic regions. Tree Genet Genomes. 2018; 14: 1-13. https://doi.org/10.1007/s11295-018-1269-6
4. Tunisie la verte -Huile d'Olive. Available online : http://www.tunisiaoliveoil.com/titre_etude/tunisie-la-verte/.
5. Conseil Oléicole International. Available online: https://www.internationaloliveoil.org/monde-de-lolivier/les-olives-de-table/?lang=fr.
6. Abdelhamid S, Gouta H, Gharsallaoui M, Ghrab M, Kwon YT, Yoon IS, Byun MO. A review on current status of olive and olive oil production in Tunisia. J Korean Soc Int Agric. 2013; 25(4): 351-57.
7. Torres-Ruiz F, Marano-Marcolini C, Lopez-Zafra E. In search of a consumer-focused food classification system. An experimental heuristic approach to differentiate degrees of quality. Food Res Int. 2018; 115: 177-90. http://doi.org/10.1016/j.foodres.2018.03.067.
8. Gorzynik-Debicka M, Przychodzen P, Cappello F, Kuban-Jankowska A, Marino Gammazza A, Knap N, Wozniak M, Gorska-Ponikowska M. Potential health benefits of olive oil and plant polyphenols. Int J Mol Sci. 2018; 19(3): 686. https://doi.org/10.3390/ijms19030686.
9. Tunisian Premium Virgin Olive Oils. Available online: https://www.tunolio.com/olive-varieties/.
10. Riewe D, Koohi M, Lisec J, Pfeiffer M, Lippmann R, Schmeichel J, Willmitzer L, Altmann T. A tyrosine aminotransferase involved in tocopherol synthesis in Arabidopsis. Plant J. 2012; 71(5): 850-59. https://doi.org/10.1111/j.1365-313X.2012.05035.x.

11. Soll J, Kemmerling M, Schultz G. Tocopherol and plastoquinone synthesis in spinach chloroplasts subfractions. Arch Biochem Biophys, 1980; 204(2): 544-50. https://doi.org/10.1016/0003-9861(80)90066-1.
12. Fritsche S, Wang X, Jung C. Recent advances in our understanding of tocopherol biosynthesis in plants: an overview of key genes, functions, and breeding of vitamin E improved crops. Antioxidants, 2017; 6(4): 99. https://doi.org/10.3390/antiox6040099.
13. Meena VK, Taak Y, Chaudhary R, Chand S, Patel MK, Muthusamy V, Yadav S, Saini N, Vasudev S, Yadava DK. Deciphering the genetic inheritance of tocopherols in Indian mustard (Brassica juncea L. Czern and Coss). Plants, 2022; 11(13): 1779. https://doi.org/10.3390/plants11131779.
14. Lushchak VI, Semchuk NMi Tocopherol biosynthesis: chemistry, regulation and effects of environmental factors. Acta Physiol Plant. 2012; 34(5): 1607-628. https://doi.org/10.1007/s11738-012-0988-9.
15. Ipek A, Ipek M, Ercisli S, Tangu NA. Transcriptome-based SNP discovery by GBS and the construction of a genetic map for olive. Funct Integr Genomics. 2017; 17(5):493-01. https://doi.org/10.1007/s10142-017-0552-1.
16. Chen J-D, He W-Z, Chen S, Chen Q-Y, Ma J-Q, Jin J-Q, Ma C-L, Moon D-G, Ercisli S, Yao M-Z, Chen L. TeaGVD: A comprehensive database of genomic variations for uncovering the genetic architecture of metabolic traits in tea plants. Front Plant Sci. 2022; 13:1056891. https://doi.org/10.3389/fpls.2022.1056891
17. Duran ST, Ipek A. Identification of SNP markers linked to Rf locus in carrot using GBS. Turk J Agric For, 2022; 46 (6): 898-07. https://doi.org/10.55730/1300-011X.3051
18. Yildiz M, Arbizu C. Inter-primer binding site (iPBS) retrotransposon markers provide insights into thegenetic diversity and population structure of carrots (Daucus, Apiaceae). Turk J Agric For. 2022; 46 (2):214-23. https://doi.org/10.55730/1300-011X.2972.
19. Zia MAB, Demirel U, Nadeem MA, Ali F, Dawood A, Ijaz M, Caliskan ME. Genome-wide association studies (GWAS) revealed a genetic basis associated with floral traits in potato germplasm. Turk J Agric For. 2022; 46 (1):90-103. https://doi.org/10.3906/tar-2104-37.
20. Fabbri A, Hormaza JI, Polito VS. Random amplified polymorphic DNA analysis of olive (Olea europaea L.) cultivars. J Am Soc Hortic Sci. 1995; 120 (3): 538-42.https://doi.org/10.21273/JASHS.120.3.538.
21. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. Primer3-new capabilities and interfaces. Nucleic Acids Res. 2012; 40(15): e115-e115. https://doi.org/10.1093/nar/gks596.
22. Multiple Sequence Alignment by CLUSTALW. Available online: https://www.genome.jp/tools-bin/clustalw.
23. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22 (22): 4673-680. https//doi.org/10.1093/nar/22.22.4673.
24. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.
25. Georgiadou EC, Goulas V, Ntourou T, Manganaris GA, Kalaitzis P, Fotopoulos V. Regulation of on-tree vitamin E biosynthesis in olive fruit during successive growing years: the impact of fruit development and environmental cues. Front Plant Sci. 2016; 7: 1656. https://doi.org/10.3389/fpls.2016.01656.
26. Ayed RB, Moreau F, Hlima HB, Rebai A, Ercisli S, Kadoo N, Hanana M, Assouguem A, Ullah R, Ali E. SNP discovery and structural insights into OeFAD2 unravelling high oleic/linoleic ratio in olive oil. Comput Struct Biotechnol J. 2022; 20: 1229-243. https://doi.org/10.1016/j.csbj.2022.02.028.
27. Ben Ayed R, Ben Hassen H, Ennouri K, Rebai A. Genetic markers analyses and bioinformatic approaches to distinguish between olive tree (Olea europaea L.) cultivars. Interdiscip Sci: Comput Life Sci. 2016; 8: 366-73. https:doi.org/10.1007/s12539-016-0155-x.