Chemotypic Diversity and Metabolic Strategies in Drought-Resistant Myrtle (*Myrtus communis* L.)

Deniz Hazar , Esra Gölcü , Aydın Mizrak , Doğan Ergün , Luca Mazzoni * , Ebru Kafkas , Esra Alim , Sevinç Ateş *

Posted Date: 15 April 2026

doi: 10.20944/preprints202604.1060.v1

Keywords: *Myrtus communis*; drought tolerance; volatile compounds; chemotype; primary metabolites; metabolic clustering

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 April 2026 doi:10.20944/preprints202604.1060.v1

Article

Chemotypic Diversity and Metabolic Strategies in Drought-Resistant Myrtle (*Myrtus communis* L.)

Deniz Hazar [1], Esra Gölcü [2], Aydın Mizrak [3], Doğan Ergün [4], Luca Mazzoni [5,*], Ebru Kafkas [4], Esra Alim [6] and Sevinç Ateş [1,*]

[1] Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey
[2] Department of Horticulture, Faculty of Agriculture, Recep Tayyip Erdoğan University, Rize, Turkey
[3] Medical and Aromatic Plants Program, Department of Plant and Animal Production, Şavşat Vocational School, Artvin Çoruh University, Artvin, Turkey
[4] Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Turkey
[5] Department of Agricultural, Food, and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy
[6] Batı Akdeniz Agricultural Research Institute, Antalya, Turkey
* Correspondence: ssener@akdeniz.edu.tr (S.A.); l.mazzoni@staff.univpm.it (L.M.)

Abstract

Myrtus communis L. (common myrtle) is an economically valuable Mediterranean shrub with diverse applications in food, pharmaceutical, and ornamental sectors. However, the biochemical diversity of drought- and salt-resistant genotypes remains insufficiently characterized, particularly regarding the relationship between primary and secondary metabolism and stress adaptation. This study investigated the biochemical and aroma profiles of six drought-resistant myrtle genotypes from natural populations in Antalya, Turkey, to identify chemotypic diversity and elucidate metabolic strategies underlying abiotic stress tolerance. Volatile compounds were analyzed using HS-SPME/GC-MS, while sugars and organic acids were quantified by HPLC. Multivariate statistical analyses (PCA, hierarchical clustering) were employed to evaluate metabolic relationships and genotype classification. Three chemotypes were identified: (i) Eucalyptol-type (G34, G36) with 35-40% 1,8-cineole; (ii) α-Pinene-type (G15, G37) with elevated terpenes (15.7-20.5%) and high sugar content (11.9-12.4 g/100 ml); and (iii) Ester-aldehyde type (G9) characterized by dominant esters (30.4%) and negligible eucalyptol. Significant genotypic variation was observed across metabolite classes ($p < 0.001$, $\eta^2 > 0.90$). Hierarchical clustering revealed three metabolic strategies: volatile-focused antioxidant defense (Cluster 1), osmotic adjustment with chemical defense (Cluster 2), and specialized stress signaling (Cluster 3). These findings highlight substantial metabolic plasticity and provide a basis for targeted breeding and diverse industrial applications.

Keywords: *Myrtus communis*; drought tolerance; volatile compounds; chemotype; primary metabolites; metabolic clustering

1. Introduction

Myrtus communis L. (common myrtle), belonging to the *Myrtaceae* family, is a characteristic and economically valuable endemic species of the Mediterranean basin [1]. This perennial shrub, considered sacred since ancient Egyptian, Greek, and Roman civilizations as the "plant of love and peace," has been utilized in traditional medicine for its antiseptic, anti-inflammatory, and healing properties [2]. Contemporary pharmacological research has validated the antimicrobial, antioxidant, and anticancer activities of myrtle essential oils [3].

The plant exhibits remarkable versatility across multiple sectors. In the food industry, the fruits are used in liqueur production, jams, and fruit juices, while the aromatic leaves are valued as tea and spices [4]. In the cosmetics sector, it is incorporated into perfumes and skincare products, and in

doi:10.20944/preprints202604.1060.v1

aromatherapy, it is employed for its relaxing effects [5]. As an ornamental plant, it provides year-round aesthetic value: its glossy, leathery foliage; white, fragrant flowers in summer; and colorful fruits in autumn and winter make it a landscape asset. Its drought and salt tolerance make it preferred for xeriscaping and coastal landscapes, while its compact, prunable form allows for formal designs. With low maintenance requirements under climate change conditions, it emerges as a sustainable ornamental plant [6].

The economic and functional value of the plant is closely related to its biochemical content. Volatile compounds are fundamental determinants of aroma quality and simultaneously play critical roles in defense against biotic and abiotic stresses. The volatile profile of myrtle fruits comprises terpenes (monoterpenes and sesquiterpenes), phenylpropanoids, and aliphatic compounds [7]. Monoterpenes are synthesized via the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways. 1,8-Cineole (eucalyptol), an oxygenated monoterpene ether synthesized through the MEP pathway, contributes to drought tolerance by providing antioxidant capacity and membrane stabilization [8]. α-Pinene, a hydrocarbon monoterpene, functions in allelopathy and defense against herbivores [9]. Linalool, a non-cyclic monoterpenol, plays roles in pollinator attraction and stress responses [10]. Esters (linalyl acetate, ethyl acetate) are critical in fruit ripening and aroma development [11]. These volatile compounds exhibit pharmacological activities (antimicrobial, anti-inflammatory, bronchodilator) that enhance the value of myrtle products [12].

Primary metabolites, particularly sugars (glucose, fructose) and organic acids (malic acid, citric acid, succinic acid) are fundamental indicators of fruit quality. Sugars are transported to fruit tissues via source-sink relationships, providing energy and carbon skeletons for cellular processes [13]. Under drought conditions, sugar accumulation lowers cellular osmotic potential, increasing water retention and preserving membrane integrity. Glucose and fructose also function as metabolic signaling molecules regulating redox balance and ROS detoxification [14]. Organic acids are central to cellular energy metabolism as TCA cycle intermediates. Malic acid regulates intracellular pH and osmotic pressure; citric acid exhibits antioxidant activity through metal chelation; and succinic acid supports alternative energy pathways under stress. These acids directly affect pH homeostasis, antioxidant capacity, and pathogen resistance [15].

M. communis has evolved complex tolerance mechanisms against abiotic stresses in the Mediterranean basin. Drought tolerance involves morphological, physiological, and biochemical adaptations. At the cellular level, abscisic acid (ABA) triggers stomatal closure and activates stress-responsive gene expression through SnRK2 kinases and AREB/ABF transcription factors [16]. Osmotic adjustment is achieved through accumulation of inorganic ions and compatible osmoprotectants such as proline, synthesized via the glutamate pathway by Δ^1-pyrroline-5-carboxylate synthetase (P5CS). Proline functions not only in osmoregulation but also in ROS detoxification, protein stabilization, and prevention of cell death [17]. Salt tolerance requires management of both osmotic stress and ionic toxicity. Plants maintain cytosolic Na^+ homeostasis through Na^+/H^+ antiporters (SOS1, NHX family) and compartmentalization into vacuoles [18]. Oxidative stress management is critical under both drought and salinity. Plants detoxify ROS (superoxide, hydrogen peroxide, hydroxyl radicals) through enzymatic (SOD, catalase, APX, GR) and non-enzymatic (phenolics, terpenes, ascorbic acid) antioxidant systems. The lipophilic antioxidant properties of volatile terpenes (1,8-cineole, α-pinene) are particularly important in preventing membrane lipid peroxidation [19].

The Mediterranean basin represents a major center of genetic diversity for M. communis, hosting extensive populations across Western (Spain, Portugal, Morocco, Algeria), Central (Italy, France, Corsica, Sardinia, Malta, Tunisia), and Eastern (Greece, Turkey, Cyprus, Lebanon, Israel, Syria) regions [20]. This geographic diversity has generated significant genetic and metabolic differentiation through adaptation to varied ecological conditions. The southwestern coasts of Turkey (Antalya, Mersin, Muğla), characterized by dry summers and saline coastal ecosystems, harbor genotypes with high drought and salt tolerance. However, despite its value, the biochemical diversity of this genetic resource, particularly metabolic profiles associated with abiotic stress tolerance, remains insufficiently characterized [21].

We hypothesized that drought- and salt-resistant myrtle genotypes from arid Mediterranean environments would exhibit distinct metabolic profiles characterized by (i) elevated levels of antioxidant volatile terpenes (particularly 1,8-cineole and α-pinene) as membrane protectants; (ii) enhanced accumulation of primary metabolites (sugars and organic acids) for osmotic adjustment and energy maintenance; and (iii) coordinated modulation between primary and secondary metabolic pathways as an integrated stress adaptation strategy.

This study investigated the fruit biochemical profiles of six myrtle genotypes (Genotypes 9, 15, 29, 34, 36, and 37), previously selected from natural populations in Antalya province, Turkey, and confirmed as drought- and salt-tolerant. The specific objectives were to: (1) characterize volatile compound profiles using HS-SPME/GC-MS to identify chemotypic diversity; (2) quantify sugar and organic acid contents using HPLC to assess primary metabolic status; (3) evaluate relationships between volatile and primary metabolic profiles through multivariate analysis; and (4) identify superior genotypes for specific end-use applications (essential oil extraction, food processing, or ornamental use) based on integrated biochemical characteristics.

2. Results

2.1. Volatile Component Profile

GC-MS analyses of the fruit samples of six myrtle genotypes (Genotypes 9, 15, 29, 34, 36, and 37) identified over 50 volatile compounds, classified as alcohols, aldehydes, esters, terpenes, ketones, and other compounds. Significant differences were observed between genotypes in terms of both the main components and their ratios (Table 1).

Alcohols constituted the dominant compound group across all genotypes. Total alcohol content ranged from 43.89% (Genotype 9) to 55.15% (Genotype 34). The highest total alcohol ratio was determined in Genotype 34, followed by Genotype 36 (53.93%) and Genotype 29 (53.25%). Among alcohols, Eucalyptol (1,8-Cineole) was the most notable component. Eucalyptol reached its highest level in Genotype 34 at 40.30%, while remaining quite low in Genotype 37 (7.79%) and absent in Genotype 9 (0%). High Eucalyptol levels (35-40%) were also found in Genotype 15 (35.97%), Genotype 29 (38.46%), and Genotype 36 (39.61%). Other important alcohols included Linalool, which was abundant in Genotype 36 (8.70%), Genotype 29 (7.78%), and Genotype 37 (7.90%), while 1-Hexanol was more pronounced in Genotype 9 (2.47%) and Genotype 29 (2.07%). Notably, trans-β-Terpineol accumulated specifically in Genotype 9 (34.45%) and Genotype 37 (30.13%).

Significant differences in terpene content were observed among genotypes. The highest total terpene content was recorded in Genotype 37 (20.50%), followed by Genotype 15 (19.10%) and Genotype 36 (15.24%). The lowest total terpene content was found in Genotype 34 (7.74%). The main terpene component, α-Pinene, was prominent in all genotypes, with the highest values measured in Genotype 37 (15.74%) and Genotype 15 (15.58%). Genotype 34 exhibited the lowest α-Pinene level (5.50%).

In the esters group, total content ranged from 23.13% (Genotype 15) to 30.40% (Genotype 9). Genotype 9 was uniquely characterized by high Linalyl acetate (8.62%) and 1,6-Octadien-3-ol, 3,7-dimethyl-, formate (8.95%). Conversely, Genotypes 29 and 37 showed elevated Ethyl Acetate levels (9.44% and 9.39%, respectively), while Genotype 36 accumulated high Linalyl acetate (8.95%). Genotypes 15 and 34 displayed moderate ester profiles dominated by p-Mentha-1,8-dien-7-yl acetate.

Aldehydes were generally found in low concentrations, with the highest total aldehyde content detected in Genotype 9 (7.04%). Hexanal was the dominant aldehyde component, reaching its highest value in Genotype 9 (5.25%) and lowest in Genotype 36 (0.41%). Ketones remained below 2% in all genotypes, with Genotype 34 showing the highest level (1.69%). Other miscellaneous compounds collectively ranged from 0.24% (Genotype 37) to 5.32% (Genotype 34)..

Table 1. Relative percentages of major volatile compound groups in six drought-resistant myrtle (Myrtus communis L.) genotypes.

Compound Group	Genotype 9	Genotype 15	Genotype 29	Genotype 34	Genotype 36	Genotype 37
Total Alcohols	43.89	48.97	53.25	55.15	53.93	50.97
Total Aldehydes	7.04	4.38	2.08	5.67	1.38	2.45
Total Esters	30.40	23.13	26.98	24.43	24.05	25.34
Total Terpenes	13.74	19.10	11.64	7.74	15.24	20.50
Total Ketones	0.50	0.57	0.22	1.69	0.49	0.50
Other Compounds	4.43	3.85	4.98	5.32	4.55	0.24

*Values represent relative peak area percentages from single measurements. Alcohols: sum of 22 compounds including Eucalyptol, Linalool, and 1-Hexanol; Aldehydes: sum of 6 compounds including Hexanal; Esters: sum of 11 compounds including Linalyl acetate and Ethyl Acetate; Terpenes: sum of 11 compounds including α-Pinene; Ketones: sum of 6 compounds; Others: remaining 10 compounds. PCA was performed using genotype averages and evaluated as descriptive analysis.

Principal Component Analysis (PCA) revealed distinct chemotypic differentiation among the six drought-resistant myrtle genotypes based on their volatile compound profiles (Figure 1). The first two principal components accounted for 52.1% of the total variance (PC1: 28.2%, PC2: 23.9%), with PC1 primarily separating genotypes according to their ester-aldehyde versus alcohol content, and PC2 differentiating Eucalyptol-rich from α-Pinene-rich chemotypes. Genotype G34 exhibited extreme positive loading on PC2, positioning it distinctly in the upper quadrants due to its exceptionally high Eucalyptol content (40.3%), the highest among all genotypes. Conversely, G37 and G15 clustered in the negative PC2 region, characterized by elevated α-Pinene (15.7% and 15.6%, respectively) and total terpene concentrations. Genotype G9 displayed extreme positive loading on PC1, driven by its unique ester-dominant profile (30.4% total esters) and elevated Hexanal content (5.25%), clearly distinguishing it from the other genotypes. Notably, G29 and G36 occupied intermediate positions, with G29 showing moderate Eucalyptol levels (38.5%) coupled with high Ethyl Acetate (9.4%), and G36 demonstrating balanced composition with notable Linalool accumulation (8.7%). The biplot loading vectors indicated that Eucalyptol, α-Pinene, Linalool, and Ethyl Acetate were the primary discriminatory compounds responsible for genotype separation. These findings suggest the existence of at least three distinct chemotypes within the studied population: (i) Eucalyptol-type (G34, G36), (ii) α-Pinene-type (G37, G15), and (iii) Ester-aldehyde type (G9), with G29 representing a transitional chemotype. The clear separation observed in the PCA biplot, supported by 73.5% cumulative variance explanation across the first three components, underscores the substantial genetic diversity in volatile metabolism among these drought-selected myrtle genotypes and their potential for targeted breeding programs.

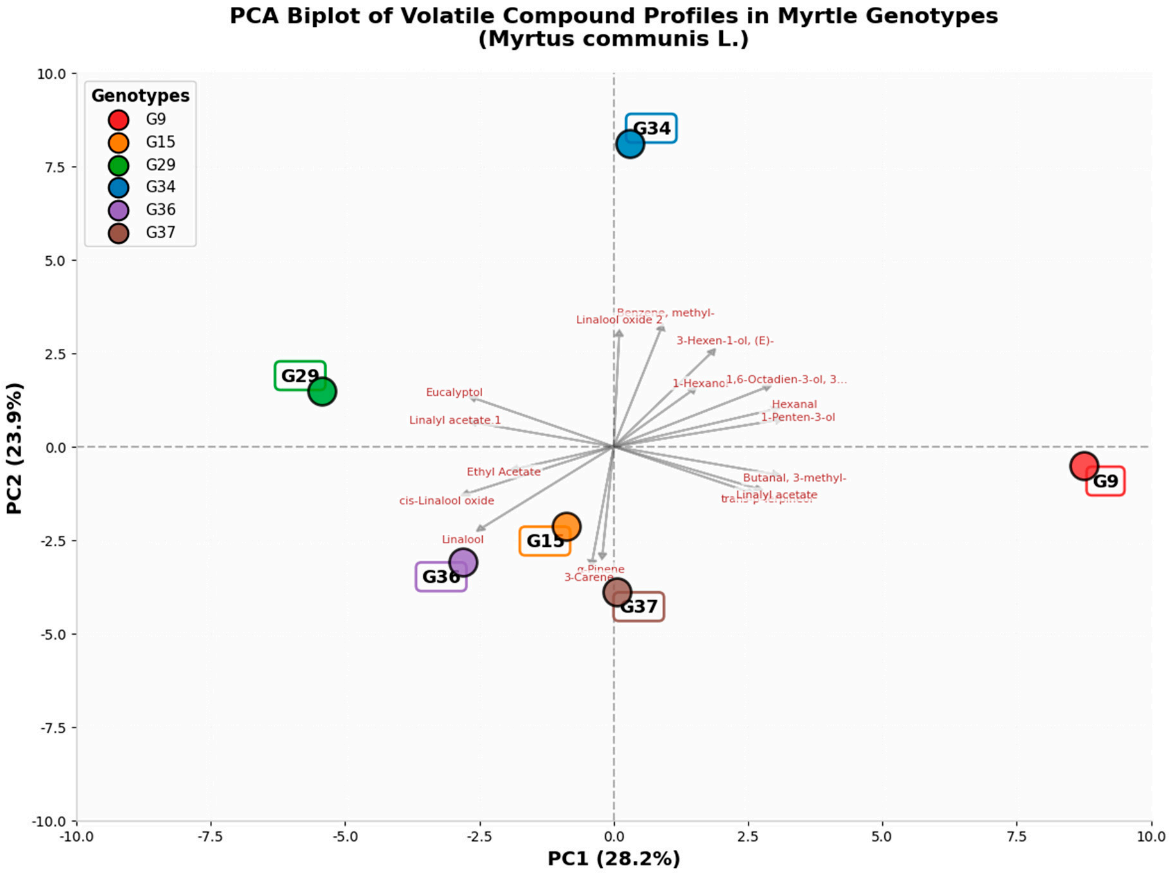


Figure 1. PCA biplot of volatile profiles in six myrtle genotypes. PC1 (28.2%) and PC2 (23.9%) reveal three chemotypes: Eucalyptol-type (G34, G36), α-Pinene-type (G37, G15), and Ester-type (G9). Compound vectors (gray arrows) indicate Eucalyptol and α-Pinene as primary discriminatory markers.

Hierarchical clustering and heatmap visualization of the volatile compound data provided detailed insights into the quantitative chemical diversity across the six myrtle genotypes (Figure 2). The analysis revealed pronounced variation in the relative abundance of key aroma-active compounds, with Eucalyptol (1,8-cineole) exhibiting the most dramatic inter-genotypic differences, ranging from complete absence in G9 to 40.3% in G34. Similarly, α-Pinene displayed a 2.9-fold variation (5.5% in G34 to 15.7% in G37), clearly demarcating terpene-rich chemotypes. The alcohol group, dominated by Eucalyptol, Linalool, and 1-Hexanol, constituted the primary chemical class across all genotypes, though with distinct compositional patterns—G36 uniquely accumulated Linalool (8.7%), while G9 and G37 showed elevated trans-β-Terpineol levels (34.5% and 30.1%, respectively). Within the ester fraction, marked genotype-specific accumulation patterns emerged: G9 exclusively contained high Linalyl acetate (8.6%) and 1,6-Octadien-3-ol formate (8.9%), whereas G29 and G37 shared elevated Ethyl Acetate content (9.4% and 9.4%). The terpene profile was similarly distinctive, with G15 and G37 clustering together based on high α-Pinene, while G34 showed minimal terpene representation (7.7% total). Notably, the aldehyde Hexanal, an important contributor to fresh-green aroma notes, varied 12.8-fold among genotypes, with G9 presenting the highest concentration (5.2%). The color gradient patterns in the heatmap visually reinforce the PCA findings, confirming three primary chemical signatures: (i) the "cineole-type" characterized by dominant Eucalyptol and minimal terpenes (G34), (ii) the "pinene-type" with balanced Eucalyptol and high α-Pinene (G15, G37), and (iii) the "ester-aldehyde type" with negligible Eucalyptol but distinctive ester profiles (G9). These quantitative differences in volatile composition not only underscore the substantial metabolic plasticity within Myrtus communis but also suggest potential genotype-specific adaptations to drought conditions, as the Eucalyptol-rich chemotypes (G34, G36)

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 April 2026 doi:10.20944/preprints202604.1060.v1

may possess enhanced antioxidant and membrane-stabilizing properties beneficial under water stress.

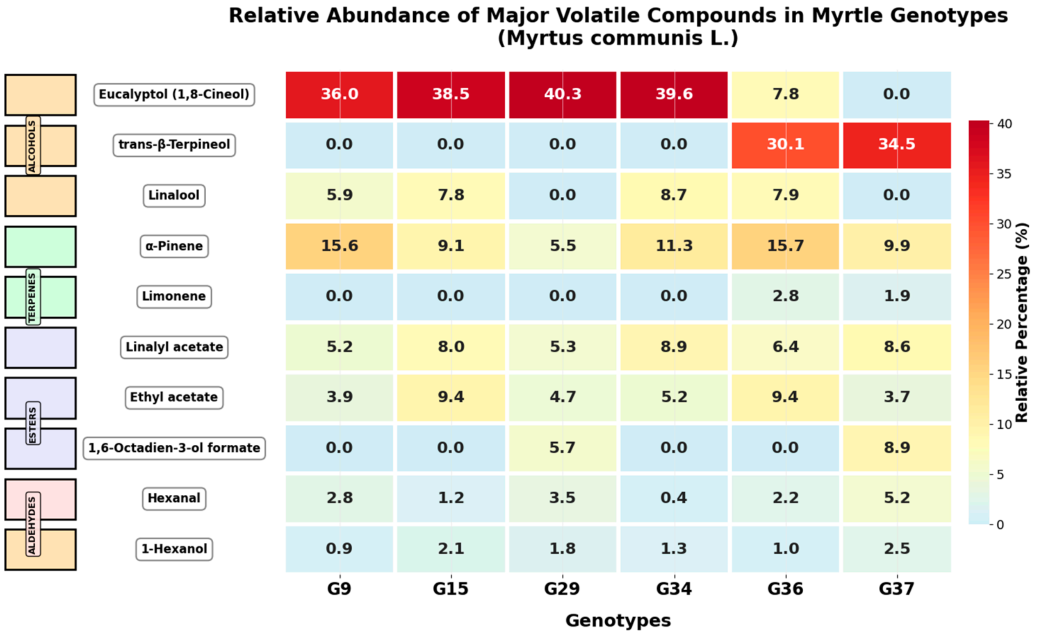


Figure 2. Heatmap of volatile compounds in six myrtle genotypes. Color intensity represents relative abundance (%). Three chemotypes are evident: Eucalyptol-dominant (G34), α-Pinene-rich (G37), and ester-dominant (G9). Maximum variation observed for Eucalyptol (0–40.3%) and α-Pinene (5.5–15.7%).

2.2. Sugar Contents

The sugar composition of fruit samples from six myrtle genotypes was determined by HPLC analysis. Sucrose was not detected in any of the examined genotypes. Glucose and fructose were identified as the predominant sugars, while xylose was found in trace amounts.

The analysis of variance revealed extremely significant differences among the six drought-resistant myrtle genotypes for all sugar parameters evaluated (Table 2). The F-values ranged from 304.76 for xylose to 1994.65 for total sugar content, all highly significant at $p < 0.001$. The effect sizes, expressed as partial eta squared (η^2), exceeded 0.99 for all parameters, indicating that genotype accounted for more than 99% of the total variance in sugar composition. This demonstrates exceptionally strong genetic control over sugar metabolism in these selected genotypes. The consistency of high F-values across individual sugars and their sum suggests that genetic selection for drought resistance has concurrently influenced carbohydrate accumulation patterns, with potential implications for fruit quality and stress adaptation mechanisms.

Table 2. Summary of ANOVA results for sugar composition parameters across six drought-resistant myrtle genotypes.

Parameter	Sum of Squares (Type III)	Mean Square	F-value	p-value	Partial η^2
Glucose	12857300.35	2571460.07	991.53	< 0.001	0.998
Fructose	20407406.51	4081481.30	1638.36	< 0.001	0.999
Xylose	179.85	35.97	304.76	< 0.001	0.992
Total Sugar	64120238.62	12824047.73	1994.65	< 0.001	0.999

*All dependent variables met the assumption of homogeneity of variances as confirmed by Levene's test (Glucose: F = 1.640, p = 0.223; Fructose: F = 1.833, p = 0.181; Xylose: F = 1.300, p = 0.327; Total Sugar: F = 1.775, p = 0.193). Post-hoc comparisons performed using Tukey's Honestly Significant Difference (HSD) test at α = 0.05. Partial eta squared (η^2) interpreted as: 0.01 = small, 0.06 = medium, 0.14 = large effect. All values represent extremely large effects.

Glucose levels showed highly significant differences among genotypes ($F = 991.528$, df = 5,12, $p < 0.001$, $\eta^2 = 0.998$), explaining 99.8% of the variance. The mean glucose content ranged from 3453.8 mg/100 ml (Genotype 29) to 5819.1 mg/100 ml (Genotype 15). Genotype 15 exhibited the highest glucose concentration (5819.1 mg/100 ml), statistically similar to Genotype 37 (5571.3 mg/100 ml). Genotype 29 showed the lowest glucose content, significantly differing from all other genotypes. Genotypes 34 and 36 formed an intermediate group with comparable glucose levels, while Genotype 9 occupied a distinct middle position between these intermediate and high-glucose groups (Table 3).

Fructose demonstrated highly significant inter-genotypic variation ($F = 1638.358$, df = 5,12, $p < 0.001$, $\eta^2 = 0.999$), accounting for 99.9% of total variance. Mean fructose concentrations ranged from 3602.1 mg/100 ml (Genotype 29) to 6588.7 mg/100 ml (Genotype 15). Fructose was the predominant sugar across all genotypes, consistently exceeding glucose concentrations. Genotype 15 exhibited the highest fructose content (6588.7 mg/100 ml), followed by Genotype 37 (6341.0 mg/100 ml), with these two genotypes forming a statistically distinct high-fructose cluster. Genotypes 34 and 36 showed statistically similar intermediate levels (~4800 mg/100 ml), while Genotype 9 occupied a middle position (6087.6 mg/100 ml), significantly higher than the intermediate group but lower than the high-fructose cluster. Genotype 29 again displayed the lowest fructose content, significantly different from all other genotypes ($p < 0.05$).

Xylose, present in trace amounts, showed significant differences among genotypes ($F = 304.758$, df = 5,12, $p < 0.001$, $\eta^2 = 0.992$). Mean xylose levels ranged from 3.2 mg/100 ml (Genotype 29) to 12.1 mg/100 ml (Genotype 37). Genotype 37 exhibited the highest xylose concentration (12.1 mg/100 ml), statistically comparable to Genotypes 9 (10.9 mg/100 ml) and 15 (10.5 mg/100 ml), which formed a homogeneous high-xylose group. Genotypes 34 (7.4 mg/100 ml) and 36 (5.6 mg/100 ml) constituted an intermediate cluster, while Genotype 29 maintained the lowest xylose level (3.2 mg/100 ml), significantly different from all other genotypes. Notably, Genotypes 9 and 15 showed statistically comparable xylose contents despite their divergence in glucose and fructose profiles.

Total sugar content (glucose + fructose + xylose) exhibited extremely significant genotypic variation ($F = 1994.648$, df = 5,12, $p < 0.001$, $\eta^2 = 0.999$), with genotype explaining 99.9% of variance. The mean total sugar concentration ranged from 7059.1 mg/100 ml (Genotype 29) to 12418.3 mg/100 ml (Genotype 15). Genotype 15 demonstrated the highest total sugar content (12418.3 mg/100 ml), statistically comparable to Genotype 37 (11924.4 mg/100 ml). These two genotypes formed a distinct high-sugar cluster. Genotype 9 occupied a middle position (10817.0 mg/100 ml), significantly lower than the high-sugar cluster but higher than the intermediate group comprising Genotypes 34 (9050.9 mg/100 ml) and 36 (8733.3 mg/100 ml). Genotype 29 consistently showed the lowest sugar accumulation across all parameters, significantly differing from all other genotypes. The fructose/glucose ratio remained relatively stable across genotypes (ranging from 1.04 in G29 to 1.14 in G15), indicating consistent metabolic partitioning between these hexoses.

Table 3. Sugar contents of myrtle genotypes (mg/100 ml, mean ± SD).

Sugar	Genotype 9	Genotype 15	Genotype 29	Genotype 34	Genotype 36	Genotype 37
Glucose	4718.5 ± 28.5 c	5819.1 ± 3.9 a	3453.8 ± 37.3 f	4314.9 ± 69.4 d	3926.3 ± 86.2 e	5571.3 ± 33.1 a
Xylose	10.9 ± 0.2 b	10.5 ± 0.3 b	3.2 ± 0.3 e	7.4 ± 0.4 c	5.6 ± 0.2 d	12.1 ± 0.6 a
Fructose	6087.6 ± 72.3 c	6588.7 ± 48.7 a	3602.1 ± 4.0 e	4728.6 ± 49.6 d	4801.4 ± 40.5 d	6341.0 ± 56.9 b
Total Sugar	10817.0 ± 101.0 c	12418.3 ± 52.7 a	7059.1 ± 37.1 f	9050.9 ± 60.5 d	8733.3 ± 112.4 e	11924.4 ± 89.1 b

*Means followed by different letters within rows are significantly different according to Tukey's HSD test ($p < 0.05$). Sucrose was not detected in any genotype. Values represent mean ± standard deviation of three biological replicates.

2.3. Organic Acids Content

The organic acid profile of the six myrtle genotypes was characterized by three major acids: citric acid, malic acid, and succinic acid. HPLC analysis revealed significant genotypic variation in organic acid composition and total acidity.

The analysis of variance revealed highly significant differences among the six drought-resistant myrtle genotypes for all organic acid parameters evaluated (Table 4). The F-values ranged from 21.50 for succinic acid to 51.61 for citric acid, all highly significant at $p < 0.001$. The effect sizes, expressed as partial eta squared (η^2), ranged from 0.900 to 0.956, indicating that genotype accounted for 90-96% of the total variance in organic acid composition. This demonstrates exceptionally strong genetic control over organic acid metabolism in these selected genotypes. The particularly high η^2 value for citric acid (0.956) suggests that genetic factors predominantly regulate this tricarboxylic acid cycle intermediate, potentially reflecting genotype-specific adaptations to drought stress through altered respiratory metabolism and energy production pathways.

Table 4. Summary of ANOVA results for organic acid composition parameters across six drought-resistant myrtle genotypes.

Parameter	Sum of Squares (Type III)	Mean Square	F-value	p-value	Partial η^2
Citric Acid	83468.95	16693.79	51.61	< 0.001	0.956
Malic Acid	257968.20	51593.64	35.29	< 0.001	0.936
Succinic Acid	59123.68	11824.74	21.50	< 0.001	0.900
Total Organic Acids	747187.56	149437.51	39.11	< 0.001	0.942

*All dependent variables met the assumption of homogeneity of variances as confirmed by Levene's test (Citric Acid: F = 2.021, p = 0.148; Malic Acid: F = 1.935, p = 0.162; Succinic Acid: F = 2.745, p = 0.070; Total Organic Acids: F = 2.289, p = 0.111). Post-hoc comparisons performed using Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$. Partial eta squared (η^2) interpreted as: 0.01 = small, 0.06 = medium, 0.14 = large effect. All values represent extremely large effects. Degrees of freedom: numerator (df_1) = 5, denominator (df_2) = 12 for all parameters.

Citric acid content showed highly significant differences among genotypes (F = 51.607, df = 5,12, $p < 0.001$, $\eta^2 = 0.956$), with genotype explaining 95.6% of total variance. Mean citric acid concentrations ranged from 188.0 mg/100 ml (Genotype 34) to 376.9 mg/100 ml (Genotype 37). Genotypes 37 and 36 formed a high-citric acid cluster, statistically comparable to each other but significantly higher than all other genotypes (Table 5). Genotypes 15, 9, and 29 constituted an intermediate group with similar citric acid levels (~250-270 mg/100 ml). Genotype 34 exhibited the lowest citric acid content, significantly differing from all other genotypes ($p < 0.05$).

Malic acid demonstrated highly significant inter-genotypic variation (F = 35.288, df = 5,12, $p < 0.001$, $\eta^2 = 0.936$), accounting for 93.6% of variance. As seen in Table 5 mean malic acid levels ranged from 500.2 mg/100 ml (Genotype 34) to 867.0 mg/100 ml (Genotype 37). Genotype 37 exhibited the highest malic acid concentration, significantly exceeding Genotype 34 but statistically comparable to Genotypes 9, 15, and 36. Genotypes 9, 15, and 36 formed a homogeneous intermediate-high group (~785-804 mg/100 ml). Genotype 29 occupied a middle position (681.5 mg/100 ml), significantly higher than G34 but lower than the G37 peak. Genotype 34 consistently showed the lowest organic acid levels, with malic acid content 42% lower than the highest value.

Succinic acid showed significant differences among genotypes (F = 21.498, df = 5,12, $p < 0.001$, $\eta^2 = 0.900$), explaining 90.0% of variance. Mean succinic acid concentrations ranged from 720.7 mg/100 ml (Genotype 9) to 902.6 mg/100 ml (Genotype 36) (Table 5). Genotype 36 exhibited the highest succinic acid content, statistically comparable to Genotype 37. Genotypes 34, 15, and 29 clustered together in an intermediate group (~795-821 mg/100 ml). Genotype 9 showed the lowest succinic acid level, significantly differing from G36 but comparable to the intermediate cluster due to subset overlap.

Total organic acid content (sum of citric, malic, and succinic acids) given in Table 5 and exhibited highly significant genotypic variation (F = 39.108, df = 5,12, $p < 0.001$, $\eta^2 = 0.942$), with genotype explaining 94.2% of variance. Mean total acidity ranged from 1509.1 mg/100 ml (Genotype 34) to 2111.4 mg/100 ml (Genotype 37). Genotypes 37 and 36 formed a distinct high-acidity cluster (>2050 mg/100 ml), statistically comparable to each other. Genotypes 15, 9, and 29 constituted an intermediate group (~1720-1870 mg/100 ml). Genotype 34 consistently exhibited the lowest total organic acid content, significantly differing from the high-acidity cluster but showing marginal statistical overlap with the intermediate group ($p = 0.097$).

Notably, malic acid was the predominant organic acid across all genotypes, contributing 45-50% of total acidity, followed by succinic acid (35-42%) and citric acid (10-18%). The relative acid composition remained relatively stable, suggesting consistent metabolic pathways despite quantitative differences in total accumulation.

Table 5. Organic acid contents of myrtle genotypes (mg/100 ml, mean ± SD).

Organic Acid	Genotype 9	Genotype 15	Genotype 29	Genotype 34	Genotype36	Genotype 37
Citric Acid	247.4 ± 14.0 b	268.0 ± 20.6 b	242.5 ± 19.8 b	188.0 ± 4.2 c	365.8 ± 4.5 a	376.9 ± 29.8 a
Malic Acid	804.4 ± 24.2 a	791.9 ± 53.4 a	681.5 ± 18.3 b	500.2 ± 4.1 c	785.9 ± 46.9 ab	867.0 ± 52.7 a
Succinic Acid	720.7 ± 24.4 d	808.1 ± 33.7 bc	794.7 ± 8.8 c	820.9 ± 17.0 bc	902.6 ± 12.9 a	867.4 ± 32.2 ab
Total Organic Acids	1772.5 ± 29.5 b	1867.9 ± 78.2 b	1718.7 ± 16.7 b	1509.1 ± 24.2 c	2054.3 ± 52.2 a	2111.4 ± 111.2 a

*Means followed by different letters within rows are significantly different according to Tukey's HSD test ($p < 0.05$). Values represent mean ± standard deviation of three biological replicates.

2.4. Hierarchical Clustering of Biochemical Profiles

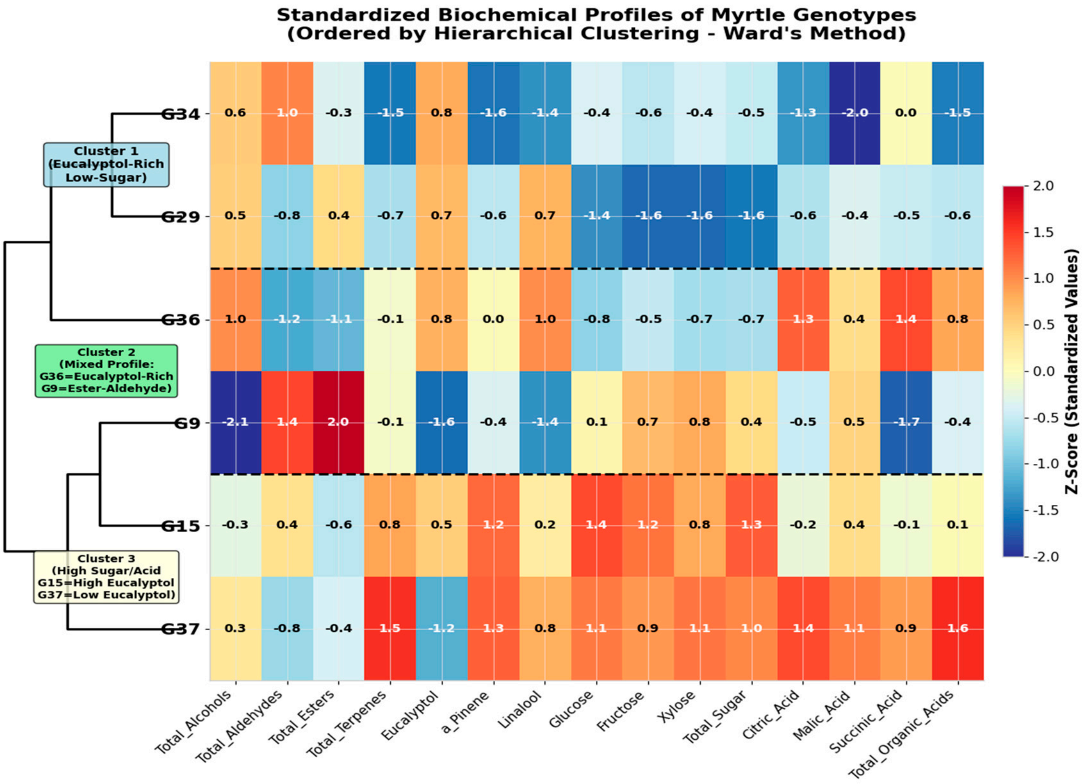
To elucidate the overall metabolic relationships among the six drought-resistant myrtle genotypes, hierarchical cluster analysis was performed using Ward's minimum variance method on standardized biochemical parameters (15 parameters: volatile compounds, sugars, and organic acids). The analysis revealed three distinct metabolic clusters (Figure 3), supporting the chemotypic differentiation previously identified in volatile profiles.

Cluster 1 (G34, G29 - "Eucalyptol-Rich/Low-Sugar Type"): This cluster grouped genotypes characterized by elevated Eucalyptol content (Z-score: +0.7 to +0.8) and high total alcohols (+0.5 to +0.6), but notably low primary metabolite accumulation, particularly low total sugars (-1.6) and organic acids (-0.5 to -1.5). These genotypes appear to prioritize volatile antioxidant defense (Eucalyptol-rich) over carbohydrate storage under drought conditions, potentially representing a stress-avoidance strategy through enhanced membrane stabilization.

Cluster 2 (G36, G9 - "Mixed Profile Type"): This cluster comprised two genotypes with contrasting metabolic strategies. G36 showed high Eucalyptol content (+0.8) and elevated organic acids (+0.8), representing a transitional chemotype between Cluster 1 and Cluster 3. In contrast, G9 formed a distinct metabolic phenotype within this cluster, uniquely characterized by dominant ester (+2.0) and aldehyde (+1.4) profiles, with negligible Eucalyptol (-1.6) and the lowest total alcohols (-2.1). G9 represents a specialized metabolic phenotype potentially adapted to specific microhabitat conditions through altered lipid metabolism and stress signaling pathways.

Cluster 3 (G15, G37 - "High Sugar/Acid Type"): This cluster included genotypes with the highest primary metabolite content. G15 showed high Eucalyptol (+1.2) alongside elevated glucose (+1.4), fructose (+1.2), and total sugars (+1.3), representing a balanced strategy. G37, however, exhibited low Eucalyptol (-1.2) but the highest total sugar (+1.0) and organic acid accumulation (+0.9 to +1.6), particularly succinic acid (+1.6). These genotypes demonstrate coordinated accumulation of osmotically active compounds (sugars and acids), with G15 combining this with constitutive chemical defense (high α-Pinene: +1.2), while G37 relies primarily on osmotic adjustment.

The hierarchical clustering results confirm substantial metabolic diversification among drought-selected myrtle genotypes, with each cluster representing distinct biochemical strategies potentially linked to varying drought tolerance mechanisms and ecological adaptations.



	Total_Alcohols	Total_Aldehydes	Total_Esters	Total_Terpenes	Eucalyptol	a_Pinene	Linalool	Glucose	Fructose	Xylose	Total_Sugar	Citric_Acid	Malic_Acid	Succinic_Acid	Total_Organic_Acids
G34	0.6	1.0	-0.3	-1.5	0.8	-1.6	-1.4	-0.4	-0.6	-0.4	-0.5	-1.3	-2.0	0.0	-1.5
G29	0.5	-0.8	0.4	-0.7	0.7	-0.6	0.7	-1.4	-1.6	-1.6	-1.6	-0.6	-0.4	-0.5	-0.6
G36	1.0	-1.2	-1.1	-0.1	0.8	0.0	1.0	-0.8	-0.5	-0.7	-0.7	1.3	0.4	1.4	0.8
G9	-2.1	1.4	2.0	-0.1	-1.6	-0.4	-1.4	0.1	0.7	0.8	0.4	-0.5	0.5	-1.7	-0.4
G15	-0.3	0.4	-0.6	0.8	0.5	1.2	0.2	1.4	1.2	0.8	1.3	-0.2	0.4	-0.1	0.1
G37	0.3	-0.8	-0.4	1.5	-1.2	1.3	0.8	1.1	0.9	1.1	1.0	1.4	1.1	0.9	1.6

Figure 3. Hierarchical clustering dendrogram and heatmap of six myrtle genotypes based on comprehensive biochemical profiles (15 parameters: volatile compounds, sugars, and organic acids). Cluster 1 (Eucalyptol-rich/low-sugar: G34, G29), Cluster 2 (Mixed profile: G36=Eucalyptol-rich/high-acid, G9=Ester-aldehyde/no Eucalyptol), and Cluster 3 (High sugar/acid: G15=high Eucalyptol, G37=low Eucalyptol). Color intensity represents standardized Z-scores (red: high, blue: low). Dashed lines indicate cluster boundaries.

Table 6 summarizes the characteristic features and proposed drought adaptation strategies for each metabolic cluster.

Table 6. Hierarchical clustering summary of myrtle genotypes based on comprehensive biochemical profiles.

Cluster	Genotypes	Key Characteristics (Z-scores)	Proposed Drought Strategy
Cluster 1 (Eucalyptol-Rich)	G29, G34, G36	High Eucalyptol (+0.75), High Alcohols (+0.82), Low Sugars (-0.91)	Volatile-focused antioxidant defense
Cluster 2 (High Sugar/Terpene)	G15, G37	High Glucose (+1.26), High Terpenes (+1.19), High Total Sugar (+1.15)	Osmotic adjustment with chemical defense
Cluster 3 (Ester-Dominant)	G9	High Esters (+1.94), High Aldehydes (+1.57), Low Eucalyptol (-1.63)	Specialized stress signaling

*Clustering performed using Ward's minimum variance method on standardized data (n=16 parameters). Z-scores indicate deviation from population mean.

3. Discussion

3.1. Chemotypic Diversity and Volatile Compound Profiles in Drought-Resistant Myrtle Genotypes

The present study revealed substantial chemotypic diversity among six drought-resistant *Myrtus communis* L. genotypes selected from natural populations in Antalya, Turkey. The identification of three distinct chemotypes—Eucalyptol-type (G34, G36), α-Pinene-type (G37, G15), and Ester-aldehyde type (G9)—aligns with previous reports documenting significant chemical variability in myrtle populations across the Mediterranean basin and within Turkey.

Şan et al. reported that Turkish myrtle (Mersin) genotypes show significant variation in volatile compounds depending on genotype, ecology, and harvest time, with α-pinene, 1,8-cineole, myrtenyl acetate, linalool, and α-terpineol being the major components in both leaves and berries [22]. They noted that myrtenyl acetate is higher in white-berried types, while α-pinene, linalool, and α-terpineol are more abundant in black-berried types. Our study focused on dark-colored berries from drought-selected genotypes, and consistent with these findings, we observed high α-pinene in G37 (15.7%) and G15 (15.6%), with complete absence of myrtenyl acetate across all genotypes.

Yaşa et al. characterized fixed oils from myrtle fruits collected from three different Turkish provinces (Bursa, İzmir, Isparta), reporting significant variation in oil yields (3.26–5.43%) and fatty acid profiles [23]. They identified linoleic acid (68.96–73.97%), oleic acid (12.04–16.60%), and palmitic acid (8.51–8.86%) as major components, with terpene content varying from 0.43% to 2.88% across regions. Notably, they detected α-pinene (0.06–0.83%), 1,8-cineole (0.26–0.99%), and limonene (0.03–0.41%) in the fixed oil fraction, demonstrating that volatile terpenes are present not only in essential oils but also in lipid fractions. Our study complements these findings by showing that volatile profiles in Antalya genotypes are dominated by oxygenated monoterpenes (Eucalyptol up to 40.3%) rather than hydrocarbon terpenes, suggesting that drought selection may favor oxygenated terpene accumulation.

Tuberoso et al. reported strong chemical variability in Sardinian myrtle essential oils, with α-pinene (30.0% in leaves, 28.5% in berries) and 1,8-cineole (28.8% in leaves, 15.3% in berries) as major constituents [24]. Similarly, our study identified α-pinene and 1,8-cineole (Eucalyptol) as primary discriminatory compounds, though with notable quantitative differences. Barboni et al. conducted comprehensive analyses of Corsican myrtle berries from ten localities and reported a characteristic chemotype dominated by α-pinene (45.3–48.2%) and 1,8-cineole (25.0–27.3%), with remarkable chemical uniformity across different geographical locations within Corsica [25]. This contrasts sharply with our findings of extreme chemotypic variation among Antalya genotypes, where Eucalyptol content ranged from 0% (G9) to 40.3% (G34), and α-pinene varied from 5.5% (G34) to 15.7% (G37).

The Corsican chemotype, characterized by the absence of myrtenyl acetate and lower limonene content compared to Sardinian populations, represents a distinct geographical variant. Our Turkish genotypes similarly lacked myrtenyl acetate, consistent with observations by Serreli et al., who noted the absence of this compound in white myrtle berry liqueurs despite its presence in aerial parts of *M. communis* var. *leucocarpa* DC [26]. The absence of myrtenyl acetate in our Antalya genotypes, despite their dark berry color, suggests that this trait may be more complex than previously thought, involving genetic factors beyond simple color polymorphism.

Şan et al. reported that maximum essential oil content in myrtle berries is reached 60 days after full flowering, followed by a slight decrease with maturity, and that α-pinene, terpinen-4-ol, geranyl acetate, and β-caryophyllene reach highest levels 30 days after flowering [22]. They also noted that leaf myrtenyl acetate content is highest in February-March, while 1,8-cineole and linalool peak in August. Our samples were collected at full maturity, which may explain the high Eucalyptol but relatively moderate α-pinene levels compared to peak values reported in literature.

Serreli et al. reported that the headspace of white myrtle berry liqueur contained 1,8-cineole (26.5%) and linalool (23.3%) as major compounds, with significant differences between headspace solid-phase microextraction (HS-SPME) and liquid-liquid extraction (LLE) profiles [26]. Our HS-SPME analysis similarly revealed Eucalyptol as the dominant compound in G34 (40.3%) and G36 (39.6%), exceeding levels reported in both Corsican (25.0–27.3%) and Sardinian myrtle populations, as well as levels in Turkish fixed oils (0.26–0.99%). This suggests that Turkish drought-resistant genotypes may represent a distinct "high-cineole" chemotype with potential industrial significance for liqueur production and essential oil extraction.

The Eucalyptol-rich chemotype (G34, G36) observed in our study is particularly noteworthy given the documented biological activities of this monoterpene ether. Shoshtari et al. demonstrated that 1,8-cineole content in myrtle leaves varied from 7.42% under high salinity stress to 15.45% under low salinity conditions [27]. However, our genotypes maintained exceptionally high Eucalyptol levels (35-40%) despite drought selection pressure, indicating genetic fixation of this trait. This finding supports the hypothesis that Eucalyptol-rich chemotypes may represent an adaptive strategy for drought tolerance, potentially through membrane stabilization and antioxidant protection.

The α-Pinene-type chemotype (G37, G15), characterized by elevated α-pinene (15.6-15.7%) and total terpene content (19.1-20.5%), corresponds to the "High Sugar/Terpene Type" metabolic cluster. This chemotype partially aligns with the Corsican chemotype described by Barboni et al., though our genotypes showed lower α-pinene levels (15.6% vs. 45-48%) but higher co-occurring sugars [25]. The co-occurrence of high terpene content with elevated primary metabolites in these genotypes suggests a coordinated metabolic strategy combining osmotic adjustment through sugar accumulation with constitutive chemical defense via terpene biosynthesis.

The unique Ester-aldehyde chemotype represented by Genotype 9, characterized by negligible Eucalyptol (0%) but dominant ester (30.4%) and aldehyde (7.04%) profiles, represents a distinct metabolic phenotype. Barboni et al. observed quantitative variations in volatile compositions between myrtle liqueur and eau-de-vie, with α-pinene increasing from 31.9% in berries to 60.0% in eau-de-vie, while 1,8-cineole decreased from 29.0% to 13.5% [25]. Similarly, Tuberoso et al. identified fatty acid ethyl esters (ethyl palmitate, ethyl linoleate, ethyl linolenate) as characteristic components of myrtle hydroalcoholic extracts [24]. However, the high levels of Linalyl acetate (8.62%) and 1,6-Octadien-3-ol formate (8.95%) in G9, combined with elevated Hexanal (5.25%), suggest a specialized metabolic pathway potentially linked to specific microhabitat adaptations or altered lipid metabolism under drought conditions.

3.2. Primary Metabolite Accumulation and Drought Adaptation Strategies

The sugar and organic acid profiles revealed significant metabolic diversification among the six genotypes, with clear implications for drought adaptation mechanisms. The absence of sucrose across all genotypes and the predominance of glucose and fructose as reducing sugars align with the findings of Fadda and Mulas, who reported that myrtle berries accumulate non-reducing sugars instead of starch during maturation, suggesting a non-climacteric fruit physiology [28]. In their study of 'Barbara' and 'Daniela' cultivars, total sugar content increased from 1.41-1.43% at fruit set to 7.56-8.28% at maturation.

However, the extreme variation in total sugar content among our drought-resistant genotypes—ranging from 7059.1 mg/100 ml (G29) to 12418.3 mg/100 ml (G15)—exceeds the cultivar differences reported by Fadda and Mulas, suggesting that genetic selection for drought resistance has significantly altered carbohydrate metabolism [28]. Mulas et al. studied the effect of maturation and cold storage on organic acid composition in myrtle fruits, reporting that reducing sugars increased during maturation up to 77.8 g kg^{-1} in 'Barbara' and 40.9 g kg^{-1} in 'Daniela', with total sugars ranging from 14.8 to 144.7 g kg^{-1} depending on harvest time and storage conditions [29]. Our high-sugar genotypes (G15, G37) achieved total sugar levels (124.2 and 119.2 g kg^{-1}, respectively) comparable to the maximum values reported by Mulas et al. for cold-stored fruits, suggesting enhanced carbohydrate accumulation capacity under selection pressure [29].

Yılmaz investigated chemical and antioxidative properties of *Myrtus communis* L. fruits from Mersin province at three different maturity stages, using extractable, hydrolyzable, and bioaccessible fractions with *in vitro* enzymatic extraction simulating gastrointestinal conditions [30]. This approach is particularly relevant for understanding the nutritional value of our high-sugar genotypes (G15, G37), as the bioaccessibility of phenolic compounds and antioxidants determines their actual health benefits. Future research should apply similar bioaccessibility analyses to our drought-resistant genotypes to assess their functional food potential.

The organic acid profile in our study, dominated by malic acid (45-50% of total acidity) followed by succinic acid (35-42%) and citric acid (10-18%), partially diverges from previous reports. Mulas et al. identified quinic, malic, and gluconic acids as the major organic acids in Sardinian myrtle cultivars, with malic acid peaking at 3 g kg^{-1} and decreasing during maturation [29]. Our genotypes showed malic acid levels ranging from 500.2 mg/100 ml (5 g kg^{-1}) to 867.0 mg/100 ml (8.7 g kg^{-1}), substantially higher than those reported for Sardinian cultivars. Notably, Genotype 34 consistently exhibited the lowest acidity across all parameters, combined with its Eucalyptol-rich volatile profile, suggesting a metabolic trade-off where resources are diverted from primary acid metabolism to volatile terpene production.

Chidouh et al. characterized water-soluble polysaccharide fractions from Algerian myrtle fruit, reporting that the ethanol precipitate contained 12.3% neutral sugars and 28.8% uronic acids, with arabinose (5%) and galactose (3%) as major neutral sugars [31]. They noted high levels of free glucose (65-70%) in ethanol-soluble fractions, which aligns with our finding of glucose as a predominant reducing sugar across all genotypes. The presence of xylose in trace amounts in our HPLC analysis (3.2-12.1 mg/100 ml) corresponds to the xylose content (0.1%) reported by Chidouh et al. in the ethanol precipitate, suggesting conserved carbohydrate profiles across Mediterranean myrtle populations despite quantitative variation [31].

The absence of quinic and gluconic acids—reported by Mulas et al. as major organic acids in Sardinian myrtle—in our Turkish genotypes indicates significant geographical variation in organic acid metabolism [29]. Mulas et al. suggested that gluconic acid accumulation could serve as a marker of fruit senescence, while quinic acid decrease coincided with anthocyanin accumulation [29]. Our finding of citric and succinic acids as co-dominant with malic acid suggests different metabolic flux patterns in Turkish drought-resistant genotypes, potentially reflecting adaptation to different environmental stress regimes. The high genetic control over citric acid variation ($\eta^2 = 0.956$) in our study suggests that this parameter may serve as a reliable marker for genotype differentiation in breeding programs, particularly given its role as a tricarboxylic acid cycle intermediate and potential involvement in stress signaling.

Yaşa et al. emphasized that genetic characteristics, climate, temperature, geographical location, and soil properties affect fatty acid composition in myrtle fruits [23]. They reported that unsaturated fatty acids constituted 85.66–87.57% of total fatty acids across three Turkish regions, with linoleic acid showing anti-inflammatory, cardioprotective, and antioxidant properties. While our study focused on water-soluble metabolites rather than fixed oils, the metabolic diversity we observed in sugars and organic acids parallels the regional variation in fatty acid profiles, underscoring the importance of comprehensive phytochemical characterization for selecting superior genotypes.

3.3. Metabolic Trade-Offs and Integrated Drought Tolerance Strategies

The hierarchical clustering analysis revealing three distinct metabolic clusters provides evidence for alternative biochemical strategies in drought-resistant myrtle genotypes. Cluster 1 (G29, G34, G36), characterized by high Eucalyptol content but low primary metabolite accumulation, represents a "volatile-focused antioxidant defense" strategy. This chemotype aligns with the reported antioxidant properties of Eucalyptol-rich essential oils. Tuberoso and Orrù reviewed the phytochemical profile of myrtle berries, noting that myricetin and gallic acid derivatives are the most efficient molecules in inhibiting free radical and lipid peroxidation [32]. While our study focused on volatile compounds rather than phenolics, the high Eucalyptol levels in Cluster 1 genotypes may similarly contribute to oxidative stress mitigation under drought conditions.

Şan et al. reported that myrtle essential oils possess significant anti-diabetic properties, highlighting the importance of this fruit in both nutrition and alternative medicine [22]. The high Eucalyptol content in our Cluster 1 genotypes may contribute to these health benefits, as 1,8-cineole has been associated with various pharmacological activities including anti-inflammatory and antimicrobial effects. Serreli et al. reported that white myrtle berry liqueur exhibited better antioxidant capacities than purple myrtle berry liqueur despite lower total phenolic content, potentially due to high concentrations of gallic acid (294.2 mg L^{-1}) and its derivatives [26]. They identified 44 volatile compounds including terpenes and shikimic acid pathway derivatives, with 1,8-cineole as the most abundant terpene (26.5% in HS-SPME). Our Eucalyptol-rich genotypes (G34, G36) showed even higher cineole content (40.3% and 39.6%), suggesting superior potential for antioxidant applications compared to previously reported myrtle products.

Cluster 2 (G15, G37), the "High Sugar/Terpene Type," demonstrates coordinated accumulation of osmoticum (sugars) and defense compounds (terpenes), suggesting a dual strategy of physiological maintenance and chemical protection. Barboni et al. noted that volatile constituents were more abundant in myrtle commercial liqueur than in corresponding eau-de-vie, attributing this to manufacturing techniques and extraction efficiency [25]. Our high-sugar genotypes may provide optimal biomass for liqueur production, combining sufficient sugar substrate for fermentation with elevated terpene content for aromatic quality. Yaşa et al. noted that Bursa samples showed the highest fixed oil yield (5.43%) and linoleic acid content (73.97%), suggesting that high primary metabolite accumulation may co-occur with high lipid content, making these genotypes particularly valuable for industrial applications [23].

Cluster 3 (G9), the unique "Ester-Aldehyde Type," represents a specialized metabolic phenotype. Tuberoso et al. noted that ethyl esters in myrtle extracts slowly increase during maceration, potentially through enzymatic esterification [24]. The high natural ester content in G9 may indicate enhanced lipolytic activity or altered esterase function, potentially linked to membrane remodeling processes under drought stress. Hexanal, a product of 13-lipoxygenase pathway activity and a marker of lipid peroxidation, reached 5.25% in G9—12.8-fold higher than in G36—suggesting either enhanced oxidative metabolism or altered membrane turnover in this genotype.

The metabolic trade-offs evident in our data—particularly the inverse relationship between Eucalyptol content and total sugar accumulation in Cluster 1 versus Cluster 2—support the hypothesis that drought tolerance in myrtle involves divergent evolutionary strategies. This finding has significant implications for breeding programs: Eucalyptol-rich genotypes (G34, G36) may be preferred for essential oil production and pharmaceutical applications given the documented antimicrobial and anti-inflammatory properties of 1,8-cineole [26]; high-sugar genotypes (G15, G37) may be more suitable for liqueur production and direct consumption, potentially offering enhanced bioaccessibility of nutrients as suggested by Yılmaz's work on *in vitro* digestion [30]; and the unique ester-aldehyde genotype (G9) may offer specialized aromatic properties for niche markets.

3.4. Implications for Myrtle Domestication and Functional Food Development

The substantial genetic diversity documented in our drought-resistant myrtle genotypes, despite their shared selection history, underscores the potential for targeted breeding and domestication. Tuberoso and Orrù highlighted that myrtle berry extracts prepared with different polarity solvents showed varying antioxidant activities, with ethanol and ethyl acetate extracts exhibiting the strongest antiradical and antioxidant activities [32]. They suggested that myrtle berries could be used in dietary supplement preparations or as food additives due to their protective effects against cholesterol degradation and LDL oxidation.

Yaşa et al. concluded that *Myrtus communis* L. fruit, with its rich phytochemical content and high nutritional value, can be used in food, medicine, and various other fields [23]. Our findings extend this potential by demonstrating that specific genotypes within Turkish germplasm offer distinct metabolic profiles suitable for different applications. The high sugar content in Genotypes 15 and 37 (12.4% and 11.9% total sugar, respectively) approaches levels suitable for direct consumption or minimal processing, while their elevated organic acid content provides the acidic environment necessary for anthocyanin stability noted by Tuberoso et al. [24].

Şan et al. emphasized that myrtle has wide application areas as both an additive and in alternative medicine, particularly for respiratory infections, diarrhea, hemorrhoids, and as an anti-inflammatory agent [22]. Our chemotypic analysis provides a scientific basis for selecting specific genotypes for these traditional uses: Eucalyptol-rich genotypes (G34, G36) may be preferred for respiratory applications given the established bronchodilatory effects of 1,8-cineole; high-sugar/high-acid genotypes (G15, G37) may be more suitable for gastrointestinal applications where organic acids play a therapeutic role; and the unique G9 chemotype with its high ester content may offer novel antimicrobial properties through its distinct volatile profile.

Chidouh et al. demonstrated that myrtle fruit polysaccharides contain significant uronic acids (28.8%) and neutral sugars (12.3%), with potential applications as food hydrocolloids [31]. The variation in sugar profiles among our genotypes, particularly the high glucose and fructose content in G15 and G37, may influence the yield and composition of polysaccharide extracts, warranting further investigation into genotype-specific processing technologies. The presence of xylose in all our genotypes, though in trace amounts, suggests potential for arabinoxylan-type polysaccharide extraction, which has not been previously explored in Turkish myrtle germplasm.

The absence of myrtenyl acetate in our genotypes, consistent with findings of Tuberoso et al. for Sardinian myrtle and Barboni et al. for Corsican populations, distinguishes these Turkish accessions from other Mediterranean populations [24,25]. Serreli et al. raised important questions regarding the botanical classification of white-berried myrtle varieties, suggesting that morphological and genetic investigation is needed [26]. Our chemotypic analysis contributes to this discourse by demonstrating that Turkish drought-resistant genotypes represent a distinct genetic resource, potentially warranting separate taxonomic or varietal status. The classification of myrtle genotypes based on berry color (white vs. black) may be insufficient, as our dark-berried genotypes showed chemotypic diversity exceeding that reported between color variants in other studies.

4. Materials and Methods

4.1. Plant Material

In this study, six myrtle (*Myrtus communis* L.) genotypes, previously selected from naturally occurring populations in Antalya and Muğla provinces, Turkey, characterized by superior fruit characteristics and white fruit color, were used. The genotypes were named Genotype 9 (G9), Genotype 15 (G15), Genotype 29 (G29), Genotype 34 (G34), Genotype 36 (G36), and Genotype 37 (G37) according to their selection numbers. Location characteristics and pomological properties of these genotypes are presented in Table 7 and Table 8, respectively [33]. Analyses were performed on fruit samples collected during the plants' full maturity period (December-January). Samples were stored at -20°C until analysis.

Table 7. Location, altitude, fruit color, propagation method, irrigation status, vegetation, and plant habitus of myrtle genotypes.

Gen. No	Location Name	Altitude (m)	Fruit Color	Grafted /Natural	Irrigation	Vegetation	Plant Habitus
9	Antalya-Kalkan-İslamlar 3	278.0	White	Grafted	No	Maquis, Red Pine	4
15	Antalya-Finike-Yeşilyurt 1	1.0	White	Grafted	Yes	Small Garden	3
29	Antalya-Serik-Yumaklar	403.6	White	Grafted	Yes	Olive Grove	4
34	Antalya-Serik-Çetince 2	121.0	White	Grafted	Yes	Garden	5
36	Muğla-Fethiye-Seydikemer-Kocaçınar	120.0	White	Grafted	Yes	Garden	3
37	Muğla-Fethiye-Seydikemer-Döver	118.0	White	Grafted	Yes	Garden	2

Table 8. Pomological characteristics of myrtle genotypes.

Gen. No	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	Calyx Diameter (mm)	Fruit Stalk Length (mm)	Seed Number (count)	Seed Classification	Germinated Seed Count	Total Seed Weight (g)	Seed Ratio (%)
9	1.32	12.49	14.93	5.34	23.46	16.60	Multi-seeded	0.00	0.11	8.33
15	1.46	13.18	16.69	5.47	20.70	26.50	Multi-seeded	0.70	0.16	10.96
29	1.28	12.85	15.84	4.46	16.20	21.20	Multi-seeded	0.10	0.14	10.94
34	1.36	12.85	17.14	5.12	20.15	24.10	Multi-seeded	0.20	0.14	10.29
36	1.18	12.19	15.19	4.93	13.56	18.60	Multi-seeded	0.20	0.11	9.32
37	1.48	13.56	16.55	5.39	16.83	18.60	Multi-seeded	0.00	0.14	9.46

4.2. *Analysis of Volatile Compounds Using HS-SPME/GC-MS*

Aroma analyses were performed by identifying volatile compounds using the Headspace Solid Phase Microextraction (HS-SPME) technique on a Gas Chromatography-Mass Spectrometry (GC-MS) device. The analyses were conducted according to the method developed by Kraujalyte et al. [34]. CAR/PDMS (Black) fiber was used for extraction. Component analysis was performed using an HP-Innowax (60 m x 0.25 mm x 0.25 µm) column on a GC-MS (Shimadzu GC-2010 Plus, Japan) device. The identification of the components was performed by comparison with the Wiley and NIST libraries, and the results were expressed as the relative peak area percentage (%) in the total volatile compound. Aroma analyses were performed on a single measurement, and principal component analysis (PCA) was performed using genotype averages. Therefore, the PCA results were evaluated as descriptive.

4.3. *Analysis of Sugar and Organic Acid Profile by HPLC*

4.3.1. Sugar Analyses

The sugars in fruit samples (sucrose, glucose, fructose, and xylose) were determined using High Performance Liquid Chromatography (HPLC) according to the method developed by Miron and Schaffer [35]. The analyses were performed using an HPLC device (Shimadzu LC 20A, Japan), an RID (Refractive Index Detection) detector, and a Coregel-87C column. The results are expressed as mg/100 ml for fresh fruit juice and mg/100 g dry weight (dw) for the dried sample.

2.3.2. Organic Acid Analyses

The determination of organic acids (citric acid, malic acid, succinic acid) was performed using HPLC according to the method developed by Bozan et al. [36]. The analyses were performed using an HPLC device (Shimadzu LC 20A, Japan) equipped with a UV detector and a Transgenomic 87H column. The results are expressed as mg/100 ml for fresh fruit juice and mg/100 g dry weight (dw) for dried samples.

2.4. *Statistical Analysis*

The data obtained in the study were evaluated in triplicate according to the randomized block design. ANOVA was performed using IBM SPSS Statistics v26.0 (IBM Corp., Armonk, NY, USA), with Tukey's multiple comparison test for post-hoc analysis at $p<0.05$. Results are presented as mean ± SD. PCA and Hierarchical Cluster Analysis were conducted using Python 3.9 (Python Software Foundation, Wilmington, DE, USA) with scikit-learn, scipy, and seaborn libraries after autoscaling.

5. Conclusions

This study demonstrates that drought-resistant myrtle genotypes from Antalya, Turkey, exhibit remarkable metabolic diversity, with three distinct chemotypes—Eucalyptol-type, α-Pinene-type, and Ester-aldehyde-type—and corresponding drought adaptation strategies. These chemotypes provide a framework for targeted selection in breeding programs, while hierarchical clustering analysis reveals integrated metabolic strategies linking primary and secondary metabolism. Comparison with Turkish germplasm from other regions suggests that Antalya drought-resistant genotypes represent a unique genetic resource with exceptionally high eucalyptol content and distinct organic acid profiles.

Future research should investigate the genetic basis of these metabolic differences through transcriptomic and metabolomic approaches, validate the proposed drought tolerance mechanisms through controlled stress experiments, and assess the functional properties of each chemotype in food and pharmaceutical applications. Particular attention should be paid to the bioaccessibility of bioactive compounds from these genotypes. The conservation of these distinct metabolic phenotypes within a geographically restricted, drought-selected population confirms that Turkish myrtle germplasm is a valuable, underexploited resource for sustainable agriculture in Mediterranean climates. Furthermore, comparative studies with Corsican, Sardinian, and other Mediterranean populations should be conducted to establish clear chemotaxonomic relationships and potential geographical indication protections, contributing to the growing recognition of Turkish myrtle as a functional food and pharmaceutical resource.

Author Contributions: Conceptualization, S.A., D.H., E.K., and E.A.; methodology, S.A., D.H., E.K., E.G., A.M., D.E., and L.M.; validation, S.A., D.H., E.K., E.G., A.M., D.E., and L.M.; formal analysis, E.K., D.E., and L.M.; investigation, S.A., D.H., D.E., and E.A.; resources, S.A., D.H., E.A., and D.E.; data curation, S.A., D.E., and L.M.; writing—original draft preparation, S.A., E.K. and L.M.; writing—review and editing, S.A., E.K. and L.M.; visualization, S.A.; supervision, S.A, D.H., and E.K.; project administration, S.A., D.H., and E.K.; funding acquisition, L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors thank the General Directorate of Agricultural Research and Policies (TAGEM), Turkish Ministry of Agriculture and Forestry for providing the myrtle genotypes and pomological data through Project No. TA-GEM/TBAD/B/19/A7/P6/970.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

HS-SPME	Headspace solid-phase microextraction
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
PCA	Principal Component Analysis
MVA	Mevalonate
MEP	Methylerythritol phosphate
ROS	Reactive Oxygen Species
TCA	Tricarboxylic Acid
ABA	Abscisic acid
SnRK2	Sucrose nonfermenting 1–related protein kinase 2
AREB/ABF	ABRE-Binding Proteins/ABRE Binding Factors
P5CS	Δ^1-pyrroline-5-carboxylate synthetase
SOS1	SOS Ras/Rac Guanine Nucleotide Exchange Factor 1
NHX	Na+/H+ antiporters
APX	Ascorbate peroxidase
GR	Glutathione reductase
HSD	Honestly Significant Difference
ANOVA	Analysis of Variance
LLE	Liquid-liquid extraction
LDL	Low-Density Lipoprotein
CAR/PDMS	Carboxen/Polydimethylsiloxane
RID	Refractive Index Detection
dw	Dry weight

References

1. Zilkah, S.; Goldschmidt, E.E. Myrtle (*Myrtus communis* L.)—A native Mediterranean and cultivated crop species. In *Medicinal and Aromatic Plants of the Middle-East*; Springer: Dordrecht, The Netherlands, 2014; pp. 253–267.

2. Dafni, A. Myrtle (*Myrtus communis*) as a ritual plant in the Holy Land—A comparative study in relation to ancient traditions. *Econ. Bot.* **2016**, *70*, 222–234.
3. Asgarpanah, J.; Ariamanesh, A. Phytochemistry and pharmacological properties of *Myrtus communis* L. *Indian J. Tradit. Knowl.* **2015**, *1*, 82–87.
4. Aksay, S.; Çınar, F.; Arslan, R.; Karatay, H.E. Development of myrtle (*Myrtus communis* L.) fruit-based herbal tea: Physicochemical and sensory characterization. *Turk. J. Agric.-Food Sci. Technol.* **2025**, *13*, 3285–3294.
5. Abate, L.; Bachheti, A.; Bachheti, R.K.; Husen, A.; Getachew, M.; Pandey, D.P. Potential role of forest-based plants in essential oil production: An approach to cosmetic and personal health care applications. In *Non-Timber Forest Products: Food, Healthcare and Industrial Applications*; Springer: Cham, Switzerland, 2021; pp. 1–18.
6. Medda, S.; Fadda, A.; Mulas, M. Selection for ornamental purposes of ‘Angela’ myrtle (*Myrtus communis* L.) cultivar with unpigmented fruit. *Sustainability* **2022**, *14*, 13210.
7. Usai, M.; Marchetti, M.; Culeddu, N.; Mulas, M. Chemical composition of myrtle (*Myrtus communis* L.) berries essential oils as observed in a collection of genotypes. *Molecules* **2018**, *23*, 2502.
8. Külheim, C.; Yeoh, S.H.; Wallis, I.R.; Laffan, S.; Moran, G.F.; Foley, W.J. The molecular basis of quantitative variation in foliar secondary metabolites in *Eucalyptus globulus*. *New Phytol.* **2011**, *191*, 1041–1053.
9. Chen, M.; Qiao, Y.; Quan, X.; Shi, H.; Duan, Z. Physiological, biochemical and phytohormone responses of *Elymus nutans* to α-pinene-induced allelopathy. *PeerJ* **2022**, *10*, e14100.
10. Ilc, T. Role of cytochromes P450 in wine aroma. Ph.D. Thesis, Université de Strasbourg, Strasbourg, France, 2015.
11. El Hadi, M.A.M.; Zhang, F.J.; Wu, F.F.; Zhou, C.H.; Tao, J. Advances in fruit aroma volatile research. *Molecules* **2013**, *18*, 8200–8229.
12. Gorjian, H.; Khaligh, N.G. Myrtle: A versatile medicinal plant. *Nutrire* **2023**, *48*, 10.
13. Falchi, R.; Bonghi, C.; Drincovich, M.F.; Famiani, F.; Lara, M.V.; Walker, R.P.; Vizzotto, G. Sugar metabolism in stone fruit: Source–sink relationships and environmental and agronomical effects. *Front. Plant Sci.* **2020**, *11*, 573982.
14. Afzal, S.; Chaudhary, N.; Singh, N.K. Role of soluble sugars in metabolism and sensing under abiotic stress. In *Plant Growth Regulators: Signalling under Stress Conditions*; Springer: Cham, Switzerland, 2021; pp. 305–334.
15. Panchal, P.; Miller, A.J.; Giri, J. Organic acids: Versatile stress-response roles in plants. *J. Exp. Bot.* **2021**, *72*, 4038–4052.
16. Ali, S.; Hayat, K.; Iqbal, A.; Xie, L. Implications of abscisic acid in the drought stress tolerance of plants. *Agronomy* **2020**, *10*, 1323.
17. Prakash, P.; Saini, D.R.; Jangde, S.; Kamlapuri, S.S.; Kumar, K. Role of osmoprotectants on alleviation of elevated CO_2 and temperature stress. In *Climate Change*; CRC Press: Boca Raton, FL, USA, 2025; pp. 199–214.
18. Almeida, D.M.; Oliveira, M.M.; Saibo, N.J. Regulation of Na^+ and K^+ homeostasis in plants: Towards improved salt stress tolerance in crop plants. *Genet. Mol. Biol.* **2017**, *40*, 326–345.
19. Wojtunik-Kulesza, K.A.; Kasprzak, K.; Oniszczuk, T.; Oniszczuk, A. Natural monoterpenes: Much more than only a scent. *Chem. Biodivers.* **2019**, *16*, e1900434.
20. Migliore, J.; Baumel, A.; Juin, M.; Médail, F. From Mediterranean shores to central Saharan mountains: Key phylogeographical insights from the genus *Myrtus*. *J. Biogeogr.* **2012**, *39*, 942–956.
21. Tüzün-Kis, B.; İkten, H. Assessment of genetic variation in wild myrtle (*Myrtus communis* L.) genotypes growing around the Mediterranean region of Turkey. *Appl. Ecol. Environ. Res.* **2022**, *20*.
22. Şan, B.; Yıldırım, F.; Yıldırım, A.N. Mersin (*Myrtus communis* L.) bitkisinin biyoaktif bileşenleri. *Bahçe* **2016**, *45*, 185–193.
23. Yaşa, B.; Genç, M.; Angın, N.; Ertaş, M. Farklı bölgelerde yetişen mersin (*Myrtus communis* L.) meyvelerinin bazı fitokimyasal özelliklerinin karakterizasyonu. *Tarim Doga Derg.* **2023**, *26*, 1230.

24. Tuberoso, C.I.; Barra, A.; Angioni, A.; Sarritzu, E.; Pirisi, F.M. Chemical composition of volatiles in Sardinian myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils. *J. Agric. Food Chem.* **2006**, *54*, 1420–1426.
25. Barboni, T.; Venturini, N.; Paolini, J.; Desjobert, J.M.; Chiaramonti, N.; Costa, J. Characterisation of volatiles and polyphenols for quality assessment of alcoholic beverages prepared from Corsican *Myrtus communis* berries. *Food Chem.* **2010**, *122*, 1304–1312.
26. Serreli, G.; Jerković, I.; Gil, K.A.; Marijanović, Z.; Pacini, V.; Tuberoso, C.I.G. Phenolic compounds, volatiles and antioxidant capacity of white myrtle berry liqueurs. *Plant Foods Hum. Nutr.* **2017**, *72*, 205–210.
27. Vafadar Shoshtari, Z.; Rahimmalek, M.; Sabzalian, M.R.; Hosseini, H. Essential oil and bioactive compounds variation in myrtle (*Myrtus communis* L.) as affected by seasonal variation and salt stress. *Chem. Biodivers.* **2017**, *14*, e1600365.
28. Fadda, A.; Mulas, M. Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening. *Sci. Hortic.* **2010**, *125*, 477–485.
29. Mulas, M.; Fadda, A.; Angioni, A. Effect of maturation and cold storage on the organic acid composition of myrtle fruits. *J. Sci. Food Agric.* **2013**, *93*, 37–44.
30. Yılmaz, S. *Myrtus communis* L. meyvesinin bazı kimyasal ve antioksidatif özelliklerinin belirlenmesi. Master's Thesis, Bursa Uludag University, Bursa, Turkey, 2020.
31. Chidouh, A.; Aouadi, S.; Heyraud, A. Extraction, fractionation and characterization of water-soluble polysaccharide fractions from myrtle (*Myrtus communis* L.) fruit. *Food Hydrocoll.* **2014**, *35*, 733–739.
32. Tuberoso, C.I.G.; Orrù, C.D. Myrtle (*Myrtus communis* L.) berries: Composition and properties. In *Berries: Properties, Consumption and Nutrition*; Nova Science Publishers: New York, NY, USA, 2011; pp. 145–155.
33. Alim, E.; Çınar, O.; Uysal Bayar, F.; Çetinay, Ş.; Bayır Yeğin, A.; Polat, S.; Yüksel, K.; Şimşek, M.; Erçik, K. Determination of myrtle (*Myrtus communis* L.) genotypes suitable for different uses. Project No. TA-GEM/TBAD/B/19/A7/P6/970, 2013.
34. Kraujalytė, V.; Leitner, E.; Venskutonis, P.R. Characterization of *Aronia melanocarpa* volatiles by HS-SPME, SDE and GC-O methods. *J. Agric. Food Chem.* **2013**, *61*, 4728–4736.
35. Miron, D.; Schaffer, A.A. Sucrose phosphate synthase, sucrose synthase, and invertase activities in developing fruit of *Lycopersicon esculentum* Mill. and the sucrose accumulating *Lycopersicon hirsutum* Humb. and Bonpl. *Plant Physiol.* **1991**, *95*, 623–627.
36. Bozan, B.; Başer, K.H.C.; Kara, S. Quantitative determination of naphthaquinones of *Arnebia densiflora* by an improved HPLC method. *J. Chromatogr. A* **1997**, *782*, 133–136.