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Physiological Responses and Gene Expression Profiling of Drought Tolerance in Two Almond Tree Genotypes

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Abstract: Climate change increases drought events, resulting in lower agricultural productivity. Almond trees (*Prunus dulcis* (Mill.) D.A. Webb) are drought-tolerant; however, prolonged periods of water deficit can affect plant growth and productivity. The physiological and molecular responses of two almond genotypes, Filippo Ceo and Tuono, growing in the Apulia region (Italy) under well-watered and drought conditions were evaluated. Results indicate that cv. Filippo Ceo is more drought-tolerant than cv. Tuono, exhibiting higher RWC values, good levels of photosynthetic pigments, and high proline accumulation. This behavior is confirmed by gene expression analysis, which revealed upregulation of drought-responsive genes, indicating a more effective adaptation to stress of cv. Filippo Ceo. In contrast, cv. Tuono experienced greater oxidative damage and diminished physiological response. These findings indicate that cv. Filippo Ceo could be a promising cultivar for drought-prone areas, with implications for targeted breeding and agronomic strategies to improve almond tree production under water-stressed conditions.

Keywords: *Prunus dulcis*; climate change; drought adaptation; water stress

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1. Introduction

Climate change is leading to a hotter, more arid world. As temperatures rise and rainfall patterns become increasingly variable, many areas are face warmer and longer dry periods [1]. The consequences for agriculture are severe, as crops cannot carry out normal physiological and metabolic processes. In particular, water deficit is one of the most important and yield-decreasing environmental stresses for plants and seriously impacts crop productivity [2]. Decreases in water availability affect the development of plants and yields, sometimes even leading to crop failures.

Drought-tolerant plants have developed various strategies to cope with water stress. Some of them involve reducing water loss by increasing stomatal resistance, enhancing water absorption by forming deep and extensive root systems, and osmolyte accumulation [3]. Moreover, one of the primary cellular reactions to water scarcity is an extensive change in gene expression, resulting in specific control of physiological and biochemical reactions to the stress [4]. Several genes directly linked to stress responses have been identified, including those encoding dehydrin (DHN) and late embryogenesis abundant (LEA) proteins, which protect cells from water deficit. These proteins play a role in protecting proteins and membranes, thus preventing damage from drought stress [5]. Cell water homeostasis also relies on the regulation of genes that encode aquaporins (AQPs). These are membrane channel proteins that facilitate water movement and play a critical role in maintaining cell water balance [6]. Additionally, under drought stress conditions, members of transcription factor (TF) families such as NAC (named derived from three proteins, petunia NAM (no apical meristem), *Arabidopsis thaliana* ATAF1/2 (*Arabidopsis thaliana* activation factor 1/2), and CUC2 (cup-shaped cotyledon)), WRKY (named after the conserved WRKYGQK motif), MYB (myeloblastosis-related proteins), and Zn-FINGER (zinc finger proteins) can modulate plant transcriptome adjustments to recover cell homeostasis and enhance drought tolerance in the field [7]. Thus, TFs are key actors in the regulation and plants' physiology remodeling.

The almond tree, *Prunus dulcis* (Mill.) D.A. Webb, is known for its remarkable drought tolerance, allowing it to thrive even in water-scarce conditions [8]. Italy produces around 20,000 to 25,000 tons of almonds annually, having approximately 60,000 hectares dedicated to almond orchards. This area has gradually increased due to the demand for high-quality European-grown almonds [1]. The Apulian region, in particular, contributes approximately 20–25% of Italy's total almond production [9], a share that grows as more farmers transition to almond cultivation. This shift is driven not only by the crop's profitability but also by its lower water requirements and resistance/tolerance to *Xylella fastidiosa*, which has devastated the Apulian landscape, causing irreversible damage to Salento's olive groves and crippling an entire agricultural sector [10,11]. Thus, almond cultivation has emerged as a viable, sustainable alternative, providing a partial solution to offset the significant losses faced by olive growers in the region [12].

Among the traditional Apulian almond varieties, Filippo Ceo and Tuono stand out for their high quality, good yield, and resistance/tolerance to several diseases. Filippo Ceo is particularly valued for its high productivity, with medium to large trees that produce oval-shaped almonds with a pointed apex [13]. Instead, Tuono is appreciated for its self-fertility and excellent fruit characteristics. The trees have low vigor with an open canopy and flowers from mid to late season, and bear fruit on mixed shoots and spurs, resulting in a moderate yield. Tuono's almonds are medium-sized and amygdaloidal, with an early-ripening, long, elliptical kernel [13].

The overall objective of this study was the characterization of cvs. Filippo Ceo and Tuono, two almond genotypes commonly grown in the Apulia region, focusing on physiological and molecular aspects linked to drought response. Understanding the traits underlying drought tolerance in these almond cultivars could be crucial for breeding drought-tolerant varieties and for the targeted exploitation of genetic resources.

2. Materials and Methods

2.1. Site and Experimental Trial

The experiment was conducted in the summer of 2023 on an 18-year-old commercial almond orchard site in Veglie (Lecce province, Apulia region, Italy). The research was performed on the Apulian almond cultivars Filippo Ceo and Tuono, both grafted on the rootstock GF-677.

The orchard was established on sandy soil with a 6 m × 4 m tree spacing. The environmental data concerning temperatures and moisture evapotranspiration water were provided by ARIF (Regional Irrigation and Forestry Agency) Apulian weather station close to the experimental field (Lat. 40°16′26″ N Lon. 18°00′49″ E, altitude 38 m above sea level) (Figure 1).

Both cultivars underwent two contrasting irrigation regimes. "Well watered" plants were irrigated weekly starting from June according to local farming practices, whereas the "Drought" plants did not receive irrigation throughout the study. Irrigated and non-irrigated blocks were at least 10 m apart to avoid possible interference. The experiment followed a randomized block design, with five replications (n = 5) for each condition.

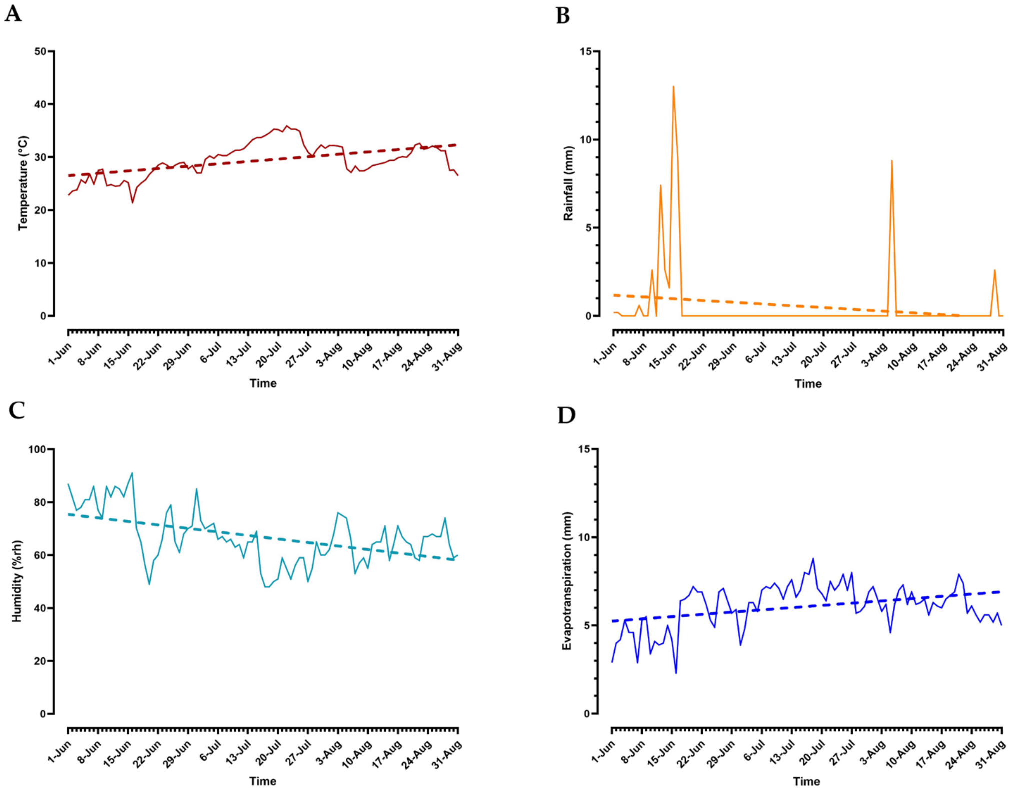

Figure 1. Temporal variation in temperature, rainfall, humidity, and evapotranspiration from 1 June 2023 to 31 August 2023 provided by ARIF (Regional Irrigation and Forestry Agency of Apulia). (**A**) Temperature (red) (**B**) rainfall (orange), (**C**) humidity (cyan) and (**D**) evapotranspiration (blue) with corresponding trend lines over time (dotted lines).

2.2. Biometric Measurements

Biometric data on the growth of whole fruits and shoots were obtained from adult trees of the cultivars of interest for each condition considered. Measurements were taken every two weeks from April to August. A digital caliper was used to determine the diameter and length of the fruits, while a millimeter ruler was employed to measure the longitudinal growth of the shoots. For each tree, at least 30 fruits and 10 shoots were labeled and monitored, uniformly distributed within the canopy to ensure adequate representativeness. The collected data were statistically analyzed to evaluate the growth dynamics as a function of time.

2.3. Physiological Index Determination

2.3.1. Relative Water Content (RWC)

To test the leaf water condition, ten leaves from each tree were collected in late August and placed in containers that were closed on-site. The following parameters were measured in the laboratory: fresh weight (FW), turgid weight at maximum turgor (TW) (measured after immerging the leaf petioles in deionized water at 4 °C for 24 h and darkness), and dry weight (DW) (after drying at 80 °C) [14]. Relative water content (RWC) was determined according to the following formula: RWC (%) = (FW − DW)/(TW − DW) × 100.

2.3.2. Biomolecules Determination

A total of 50 mg of frozen tissue was homogenized using a bead mill homogenizer for 30 s, keeping the samples cold throughout the process. The homogenates were then centrifuged at 10,000× g for 10 min at 4 °C. The resulting supernatant was diluted 1:1 with 80% cold ethanol for the analysis of photosynthetic pigments (Chl*a*, Chl*b*, Chl*a*/*b*, and carotenoids) and malondialdehyde (MDA), following the protocol described by López-Hidalgo et al. [15].

Chlorophylls and carotenoids were quantified using a JASCO V-550 UV/VIS spectrophotometer (JASCO Corporation, Tokyo, Japan), with absorbance measured at 470, 649, and 664 nm. Each measurement was performed in triplicate. Pigment concentrations were calculated according to the equations provided by Lichtenthaler [16].

For MDA determination, absorbance was recorded at 440, 532, and 600 nm, following the methods described by Hodges et al. [17]. As with the pigment measurements, each absorbance reading was repeated three times.

The free proline content was evaluated using the method by Bates et al. [18]. In brief, 0.5 g of control and stressed plant tissue were homogenized in 10 mL of 3% sulfosalicylic acid, and the homogenate was filtered through Whatman filter paper. Thereafter, 2 mL filtrate was combined with 2 mL acid ninhydrin and 2 mL glacial acetic acid in a test tube for 1 h at 100 °C, stopping the reaction by cooling the samples on ice. Next, 4 mL of toluene was added to the mixture, which was then vortexed to separate the organic and aqueous phases. Thus, the absorbance of the organic phase was read at 520 nm using a JASCO V-550 UV/VIS spectrophotometer (JASCO Corporation, Tokyo, Japan), with toluene as the blank, and proline concentration (expressed as μmol g^{-1} FW) was calculated in comparison with a standard curve of L-proline.

2.4. Total RNA Isolation, cDNA Synthesis, and Real-Time PCR Analysis

Total RNA was extracted from leaf samples based on a C-TAB protocol according to the method of Gambino et al. [19]. RNA samples were treated with DNase I (Promega, Madison, WI, USA) their absorbance was read at 260 and 280 nm to quantify RNA concentration and purity. According to the manufacturer's instructions, cDNA synthesis was carried out using TaqMan® Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA) with oligo (dT) 18 as a primer. RT-PCR was performed by SYBR Green fluorescent detection using a Real-Time PCR thermal cycler (ABI PRISM 7900 Sequence Detection System, Applied Biosystems, Foster City, CA, USA). The PCR program was as follows: 2 min at 50 °C and 10 min at 95 °C, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. In addition, the melting curve analysis was performed after PCR to check for the formation of non-specific PCR products and primer dimers. Three biological and three technical replicates were analyzed. According to De Pascali et al. [12], the primers used were designed for drought stress-related genes and transcription factors (TFs): *DHN*, *LEA*, *NAC*, *MYB*, *WRKY*, and *Zn-FINGER*. To standardize the results, the relative abundance of the actin gene (*Actin*) was used as an internal control. Relative gene expression levels were calculated by using the $\log_2 2^{-\Delta\Delta Ct}$ method [20,21]. The amplification efficiency of the target amplification was evaluated for each primer pair, and the corresponding value was used to calculate the fold changes (FCs) with the following formula: FC = $(1 + E)^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{target} - Ct_{UBQ})_{conditiont} - (Ct_{target} - Ct_{UBQ})_{Control}$.

2.5. Statistical Analysis

The data from biometric, RWC, and biomolecule analyses were assessed for normal distribution through a Shapiro Wilk test and subjected to two-way ANOVA, followed by the Tukey HSD (honestly significant difference) post hoc test ($p < 0.05$). Gene expression levels were subjected to multiple t-test analyses (FDR = 5%). Analyses were carried out using GraphPad Prism version 9.4.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Biometric Data

Biometric data of the two varieties, assessed from April to August, are reported in Figure 2, including two months (April and May) prior to water stress treatment.

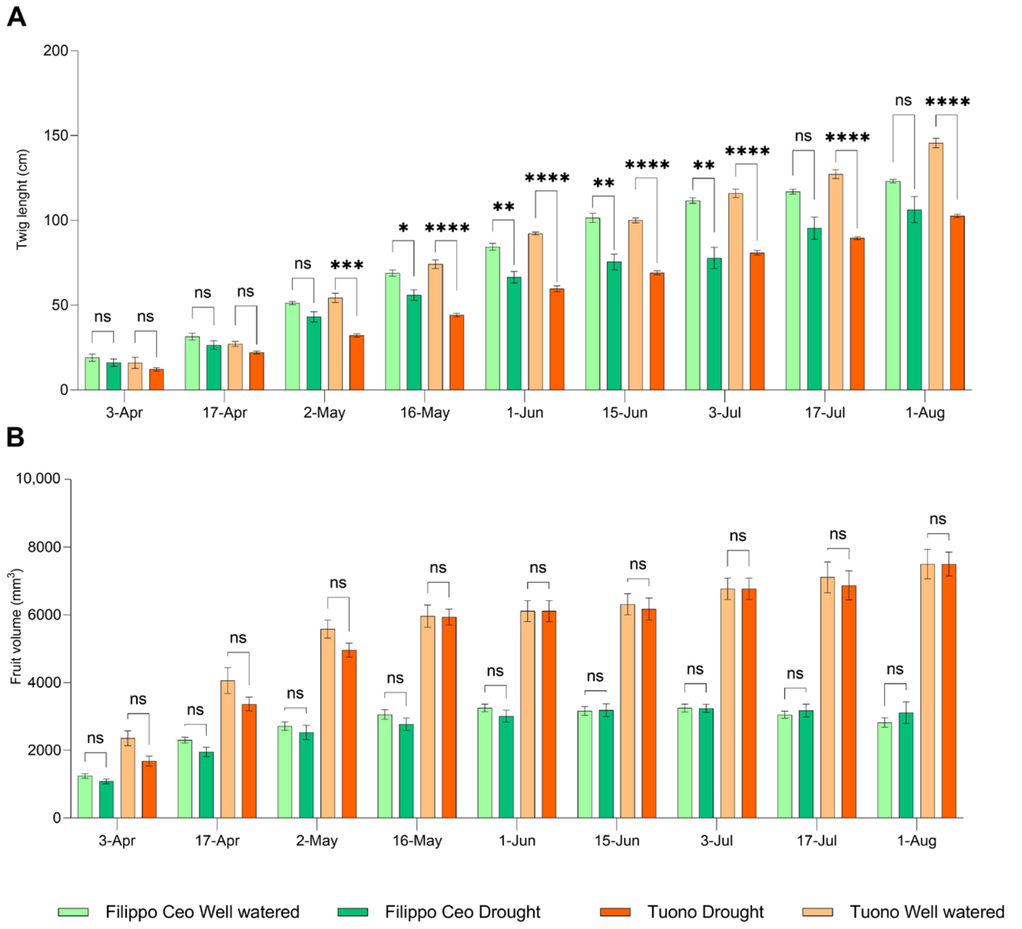

Figure 2. Growth dynamics of twigs and fruits in cvs. Filippo Ceo and Tuono under “Well watered” and “Drought” conditions. (**A**) Twig length (cm) over time for cvs. Filippo Ceo and Tuono. (**B**) Fruit volume (mm^3) over time for cvs. Filippo Ceo and Tuono. A two-way ANOVA test was conducted to identify differences between the two conditions, well watered and drought, at each time point. The threshold for statistical significance was set at $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), $p \leq 0.0001$ (****), and ns (not significant, $p > 0.05$).

Water stress negatively affects twigs growth in both plant varieties, with well-irrigated plants developing longer shoots compared to those under drought conditions (Figure 2A). This highlights the crucial role of water availability in promoting vegetative growth. However, the difference between irrigation conditions is less pronounced in Filippo Ceo than in Tuono. Conversely, drought does not appear to significantly impact fruit growth in either cultivar (Figure 2B). Fruit volume continues to increase at a steady rate regardless of irrigation conditions, with no significant differences between well-irrigated and drought-treated plants.

3.2. Physiological Parameters

The 3-month drought treatment decreased the leaf water content of the two cultivars (Table 1). However, cv. Tuono showed a more pronounced reduction (5.9%) than cv. Filippo Ceo (2.17%).

Table 1. Physiological and biochemical parameters in cvs. Filippo Ceo and Tuono leaves under "Well-watered" and "Drought" conditions. Measured parameters include relative water content (RWC), chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), Chl*a*/Chl*b* ratio (Chl*a*/*b*), carotenoid, proline, and malondialdehyde (MDA) contents. Data are presented as mean ± standard error (SEM). Different letters, in the same columns, indicate significant differences between conditions based on Tukey's post hoc test.

Conditions	Leaf RWC (%)	Chl*a* (mg/g FW)	Chl*b* (mg/g FW)	Chl*a*/*b* Ratio	Carotenoid (mg/g FW)	Proline (µmol/g FW)	MDA (µmol/g FW)
Filippo Ceo Well watered	95.83 ± 0.70 a	6.85 ± 0.04 a	2.23 ± 0.09 a	3.07 ± 0.11 c	1.48 ± 0.40 c	0.56 ± 0.03 b	9.93 ± 0.43 c
Filippo Ceo Drought	93.75 ± 1.05 b	5.59 ± 0.12 b	1.44 ± 0.45 b	3.89 ± 1.14 bc	2.62 ± 0.13 b	1.93 ± 0.35 a	11.40 ± 0.45 b
Tuono Well watered	92.60 ± 2.28 b	6.79 ± 0.19 a	1.54 ± 0.11 b	4.42 ± 0.35 ab	3.40 ± 0.05 a	0.93 ± 0.03 b	9.92 ± 0.14 d
Tuono Drought	87.14 ± 2.94 c	5.18 ± 0.09 c	0.93 ± 0.09 c	5.57 ± 0.67 a	3.62 ± 0.16 a	1.98 ± 0.67 a	12.44 ± 0.39 a

The drought stress reduced the content of chlorophyll *a* (Chl*a*) and chlorophyll *b* (Chl*b*) in the two cultivars, although with greater intensity in cv. Tuono. Specifically, Chl*a* content decreased by 23.71%, while Chl*b* content decreased by 39.61% compared to the well-watered plants; meanwhile, in cv. Filippo Ceo, the decreases were 18.39% and 38.20%, respectively (Table 1). The greater reduction in chlorophyll levels of cv. Tuono indicates that this cultivar may be more sensitive to drought, which affects its ability to maintain photosynthetic capacity. Interestingly, the carotenoid content exhibited the opposite trend, increasing in response to drought stress in both cultivars. However, there was a greater increase in cv. Filippo Ceo (77.03%) than in cv. Tuono (6.47%) (Table 1).

Proline is one of the well-known osmo-protectants that accumulate in plants as a response to stress. Under drought, both cultivars significantly accumulated proline, with an increase of 242.38% in cv. Filippo Ceo and 112.66% in cv. Tuono (Table 1). However, statistical analysis indicated that while drought had a very pronounced effect, cultivar differences were not statistically significant, implying that proline accumulation is a common response rather than a distinguishing factor between the two.

Malondialdehyde (MDA) is a stress marker of lipid peroxidation and oxidative stress, which increases when plants experience cellular damage due to drought. In the current study, cv. Tuono exhibited a 25.40% increase in MDA value compared to cv. Filippo Ceo (14.80%) in response to drought (Table 1). In cv. Tuono, the higher levels of MDA and the greater carotenoid accumulation point towards the possibility that this variety is more vigorously able to resist oxidative damage compared to Filippo Ceo.

Overall, both cultivars showed significant physiological and biochemical responses to drought stress, though their responses varied in intensity. Cv. Tuono appeared to be more affected by drought, with a higher decrease in relative water content (RWC) and chlorophyll content, and higher oxidative damage, as indicated by MDA. In contrast, cv. Filippo Ceo displayed enhanced water-holding capacity and lowered pigment degradation. Notably, both cultivars exhibited increased carotenoid and proline contents, highlighting their common adaptation mechanisms to drought.

3.3. Gene Expression Analysis

Our results show that all the genes considered are upregulated under water stress conditions but with distinct expression patterns (Figure 3). Moreover, the stress condition influenced gene regulation differently in the two almond tree cultivars.

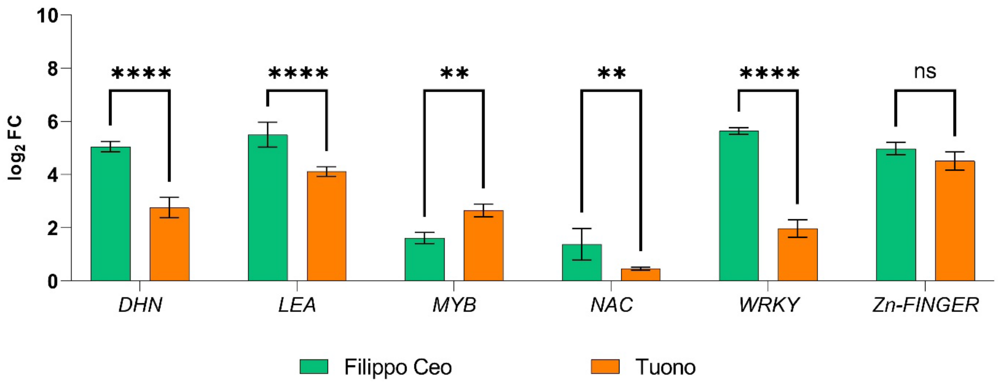

Figure 3. Differential expression ($\log_2$ fold change) of genes associated with drought responses: *DHN*, *LEA*, *NAC*, *MYB*, *WRKY*, and *Zn-FINGER* in leaves of Filippo Ceo (green) and Tuono (orange) almond cultivars. Statistical analysis was performed using a one-way ANOVA followed by Tukey's post hoc test. Significance levels are indicated as follows: $p \leq 0.01$ (**), $p \leq 0.0001$ (****), and ns (not significant, $p > 0.05$).

In cv. Filippo Ceo, all the genes examined, except for the gene encoding for the transcription factor MYB, exhibit higher expression levels than in cv. Tuono, with a more robust transcriptional response to water stress (Figure 3). This could imply a more active stress response and adaptation to stress in this variety. The expression profiles of *DHN* and *LEA*, well-known drought-related genes, show significantly higher expression in cv. Filippo Ceo than in Tuono. The *DHN* and *LEA* genes exhibit 1.83- and 1.35-fold higher values in Filippo Ceo than in Tuono, respectively. Interestingly, the *MYB* gene follows the opposite trend, with a 1.63-fold higher value in cv. Tuono than in cv. Filippo Ceo. The expression profile of *NAC* is low in both cultivars, but cv. Filippo Ceo exhibits an increase of 2.97-fold in transcripts compared to Tuono. cv. Filippo Ceo also displays a significantly higher expression of *WRKY* (2.85-fold) compared to cv. Tuono, indicating differences in the regulation of stress responses. Lastly, the *Zn-FINGER* gene displays similar expression levels in both cultivars (Figure 3). Such findings highlight various regulation mechanisms in the two cultivars, which may contribute to variations in their stress responses and overall adaptability, with a more significant jump in the response of cv. Filippo Ceo.

4. Discussion

Our study presents a scenario of physiological and molecular adaptations employed by two cvs of almond tree Filippo Ceo and Tuono, occurring in Apulia region (Italy).

The physiological and molecular data analysis reveals significant differences in drought responses between the two almond cultivars, suggesting distinct adaptation strategies. Biometric data show that drought impacts vegetative growth, especially in the Tuono cultivar, while fruit growth remains unaffected in both cultivars. These data highlight the significant variability among varieties of almond trees in their ability to adapt to soil water scarcity and their differing capacities to cope with drought stress during fruit development [22].

Concerning physiological parameters, cv. Filippo Ceo leaves have a higher RWC than cv. Tuono when subjected to drought, indicating its higher water retention capacity. This could have been due to the presence of either a more efficient root system or improved stomatal regulation, as indicated by Seleiman et al. [23], and other authors [24] who demonstrated that drought-tolerant almond cultivars possess improved water-use efficiency. Additionally, the content of chlorophyll (*a* and *b*) was significantly reduced in stressed cv. Tuono, indicating greater susceptibility to pigment breakdown, which negatively impacts the efficiency of photosynthesis. The finding concurs with [2], who reported a strong association between drought sensitivity and chlorophyll breakdown in Mediterranean tree crops. Interestingly, both almond cultivars exhibited increased carotenoid concentrations, with a higher rise in cv. Filippo Ceo, suggesting a more intense antioxidant reaction to oxidative stress induced by drought, as noted by Chandrasekaran and co-workers. [25]. Carotenoids are well documented for their role in photoprotection and oxidative stress mitigation, functioning as scavengers of reactive oxygen species (ROS) to prevent lipid peroxidation and maintain membrane stability [26,27].

Proline accumulation, a key osmo-protectant response under stress conditions, was detected in both cultivars without significant variation, indicating that it is a conserved and fundamental mechanism for drought tolerance [28]. Plants effectively reduce their osmotic potential by accumulating proline, which helps maintain cell turgor and delays stomatal closure during water deficit. This response has been widely reported in various woody species—including poplar [29], oak [30], eucalyptus [31], pine [32], and olive [10]—which are known to accumulate proline under drought conditions. This strategy supports maintaining photosynthetic activity and nutrient assimilation, thereby sustaining growth and development even under prolonged water stress [33]. The higher MDA content in cv. Tuono compared to cv. Filippo Ceo indicates more oxidative membrane damage, and poor resistance against oxidative stress, as observed in other sensitive cultivars under water shortage conditions [34]. These data imply that almond tree cultivars produce a set of responses under drought, with cv. Filippo Ceo appearing to have a slight edge in maintaining water balance and photosynthetic pigment content.

DHN and *LEA* genes are widely recognized as significant molecular markers for plant abiotic stress responses [35]. Their proteins play a crucial role in maintaining cells by stabilizing membranes, preventing protein aggregation, and retaining intracellular water in water-limited conditions. The elevated expression of *DHN* and *LEA* genes typically indicates the onset of protective mechanisms that enhance drought tolerance [36]. In parallel, transcription factors such as NAC, WRKY, MYB, and zinc finger proteins orchestrate complex regulatory networks by regulating stress-responsive genes involved in osmotic adjustment, antioxidant defense, and cell homeostasis [37,38]. The induction of these transcription factors under drought stress underscores their central role in coordinating adaptive responses. The gene expression analysis revealed significant upregulation of drought-related genes in both cultivars, albeit with some variation in induction levels. Most notable was the higher expression of *DHN* and *LEA* genes in cv. Filippo Ceo, which play a fundamental role in protecting cells from water stress, in agreement with observations in drought-tolerant *Prunus* species [12,39]. These results indicate that cv. Filippo Ceo is probably more efficient in activating cellular protection mechanisms than cv. Tuono. Conversely, the *MYB* gene was more highly expressed in cv. Tuono, suggesting that this cultivar can use an alternative regulation mechanism based on single transcription factors. MYB transcription factors exhibited significant upregulation under drought conditions, suggesting their potential involvement in drought signaling pathways [40].

However, the increased expression of the *WRKY* gene in cv. Filippo Ceo indicates that this cultivar is more efficient in activating drought stress signaling pathways, as observed in other drought-tolerant fruit trees [25]. Numerous WRKY proteins have been identified as key players in plant responses to drought stress [41]. In rice, overexpression of the *OsWRKY11* gene has been associated with improved drought tolerance and a higher survival rate of green plant tissues [42]. Similarly, in *Arabidopsis thaliana*, the *WRKY25* and *WRKY33* genes respond to both osmotic and oxidative stress conditions [43].

The expression profile of the *NAC* gene exhibits low levels in both cultivars. NAC transcription factors play a crucial role in plant stress responses, development, and secondary metabolism [44]. The observed variation between cultivars may result from differences in stress adaptation mechanisms or genetic background.

Similarly, the *Zn-FINGER* gene shows comparable expression levels in both cultivars. Zinc finger proteins are essential for transcriptional regulation, stress responses, and developmental processes in plants [45]. The *Zn-FINGER* genes could be under strict regulatory control, ensuring a stable expression profile despite genetic variation.

Growing evidence supports the role of specific transcription factors in regulating proline biosynthesis, suggesting a potential interplay in almond trees as well.

In particular, the NAC transcription factor family has been implicated in regulating proline metabolism through ABA-independent pathways. For example, ANAC055 promotes drought tolerance in *Arabidopsis* by inducing the expression of *P5CS1*, a key gene encoding Δ^1-pyrroline-5-carboxylate synthetase, the rate-limiting enzyme in proline biosynthesis. Under drought conditions, suppressing the histone methylase CAU1 leads to an upregulation of *ANAC055*, thereby enhancing proline accumulation [46].

Similarly, WRKY transcription factors modulate proline accumulation through ABA-dependent signaling. For instance, *At*WRKY57 in *Arabidopsis* and *Os*WRKY11 overexpression in *Oryza sativa* increases proline content and drought tolerance [42,47].

Moreover, the MYB family of TFs has also been demonstrated to directly regulate genes in the proline biosynthetic pathway. In *Arabidopsis*, *At*MYB2 enhances proline levels under osmotic stress by activating P5CS [48]. Likewise, in rice, *Os*MYB48-1 modulates proline accumulation under drought conditions [49]. Overall, the data reported here indicate that cv. Filippo Ceo is more drought-tolerant than cv. Tuono at both the physiological and molecular levels. The ability to maintain higher RWC, retain chlorophyll, and express genes related to cellular protection more intensely suggests that cv. Filippo Ceo is better suited for cultivation in water-stressed conditions. Conversely, although the Tuono variety is typically resistant to water stress, it has shown greater sensitivity than Filippo Ceo. This highlights the importance of implementing targeted agronomic practices, such as precise irrigation and the application of biostimulants, to improve its drought tolerance [50]. Our results are in accordance with previous studies demonstrating that drought-tolerant almond cultivars exhibit more robust antioxidant responses and higher gene expression for osmotic adjustment [51]. Furthermore, research on Mediterranean crops highlights the selection of resistant cultivars as a key point to ensure production under climate change conditions [52].

5. Conclusions

This study confirms cv. Filippo Ceo as a highly promising genotype due to its intrinsic drought tolerance, whereas cv. Tuono may benefit from targeted agronomic practices to enhance its performance under water-limited conditions. Key physiological markers—such as higher relative water content (RWC), more excellent retention of chlorophyll pigments, lower malondialdehyde (MDA) levels, and increased carotenoid accumulation—distinguished Filippo Ceo as more tolerant to drought stress.

At the transcriptional level, Filippo Ceo exhibited stronger upregulation of drought-responsive genes, particularly *DHN*, *LEA*, *WRKY*, and *NAC*, supporting its more effective activation of protective and regulatory pathways. In addition, the work emphasizes the significance of selecting drought-resistant varieties to ensure the sustainability of almond production in increasingly arid Mediterranean climates.

To better address future climate challenges, further research will focus on an in-depth analysis of the physiological and molecular mechanisms of these cultivars, aiming to enhance their productivity and gain a deeper understanding of their responses to prolonged drought conditions.

Author Contributions: M.D.P. and A.L.: conceptualization; M.D.P. and M.V.: methodology; M.D.P. and M.V.: investigation; L.D.B. and A.L.: resources; M.D.P. and M.V.: writing—original draft; L.D.B. and A.L.: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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

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