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# Ultraweak luminescence dynamics reveal drought-induced oxidative stress and antioxidant defense mechanisms in grapevine leaves

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# Abstract

Ultraweak luminescence (UWL) is a spontaneous photon emission derived from metabolic processes in living organisms. Despite its low intensity, UWL is closely linked to plant physiological metabolism, though its mechanisms remain poorly understood. In this study, *Cabernet Sauvignon* grapevines were used as plant material, and drought stress was simulated through controlled water-deficit treatments. We examined the associations between UWL intensity, reactive oxygen species (ROS), photosynthetic characteristics, and energy status. Drought stress caused a gradual decline in UWL intensity in grapevine leaves, alongside reduced soil and leaf water content and altered water distribution—decreased free water and increased bound water. Membrane damage was evident through elevated ROS indicators, including hydrogen peroxide ($H_2O_2$), superoxide anion radical ($O_2^{-}\cdot$) production rate, malondialdehyde (MDA) content, and relative electrolyte conductivity. Photosynthetic function deteriorated significantly, with decreased chlorophyll content, gas exchange parameters, including net photosynthetic rate (Pn), stomatal conductance (Gs), and chlorophyll fluorescence parameters, including Fv/Fm, effective quantum yield (Yield), and photochemical quenching (qP), while intercellular $CO_2$ concentration (Ci) and non-photochemical quenching (qN) increased. Energy metabolism was suppressed, with reduced ATP levels and energy charge, and increased ADP and AMP. Correlation analysis showed strong negative relationships between UWL intensity and ROS markers, and positive associations with photosynthetic traits, fluorescence metrics, ATP levels, and energy charge. These results suggest that UWL reflects drought-induced physiological stress, particularly involving photosynthesis and energy status. ROS likely influence UWL indirectly via membrane and metabolic disruption.


# 1. Introduction

Ultraweak luminescence (UWL) is a form of biologically induced photon emission that is ubiquitous across living systems. Unlike classical bioluminescence observed in fireflies or luminescent bacteria, and distinct from photoluminescence such as fluorescence, UWL originates from intrinsic metabolic processes and is emitted by all living organisms—including unicellular microbes, plants, animals, and humans. This emission is invisible to the naked eye due to its extremely low intensity—2 to 3 orders of magnitude lower than bacterial bioluminescence—corresponding to only a few to several thousand photons, typically within the wavelength range of 180 to 800 nm [1–3]. All organs, tissues, and cells of living organisms emit photons, and this ultra-weak luminescence is closely linked to a range of physiological processes, including metabolism, cell division, energy metabolism, photosynthesis, and signal transduction. As a comprehensive indicator of biological activity, UWL carries multifaceted information, making it a valuable tool for investigating the dynamic relationship between cellular metabolism and photon emission in living systems [4]. UWL was first discovered in 1923 by Soviet cell biologist Alexander Gurwitsch during his investigation of cell division in onion root tips [5]. Since then, advances in ultra-sensitive low-light imaging technologies have significantly broadened and deepened research on UWL [6, 7]. Since the 1990s, UWL has gradually been applied in agriculture, where it has been used to investigate luminescence patterns during various developmental stages and to examine its correlations with fruit quality, seed vigor, floral bud differentiation, agronomic practices, and plant stress

resistance [8–11]. Among these, studies investigating the relationship between UWL and plant stress resistance are relatively extensive. For example, Dong et al. [12] examined UWL in xerophytic species and found a correlation between luminescence intensity and drought tolerance among different tree species. Under extreme temperature conditions, cultivars with greater resistance to cold or heat exhibited higher UWL intensities; similarly, seed luminescence increased with rising temperatures [13]. Salt stress was found to suppress UWL intensity; however, seeds of salt-tolerant cultivars showed both higher germination rates and stronger luminescence compared to those of sensitive ones [14]. Despite these advances, most studies have primarily focused on the association between UWL and plant growth or development, while the origin and underlying mechanisms of UWL remain largely unexplored.

Regarding the origin of UWL, some researchers propose that its excitation is closely related to cellular processes such as cell division, energy metabolism, photosynthesis, and signal transduction. From a biochemical perspective, UWL in biological systems may originate from reactive oxygen species (ROS) [15]. When ROS accumulate in cells, hydroxyl radicals can trigger a lipid peroxidation chain reaction with polyunsaturated fatty acids in the cell membrane, generating singlet oxygen. As the excited singlet oxygen returns to its ground state, photons are emitted, resulting in UWL [16]. The origin of UWL may also be associated with energy transformation. One hypothesis suggests that UWL arises from energy-based luminescence, where a portion of the energy from high-energy compounds such as ATP is released in the form of photons [17]. However, these theories remain speculative and are not yet supported by sufficient experimental evidence. Further comprehensive studies are needed to clarify the mechanisms underlying UWL generation.

In the relationship between UWL and drought resistance, numerous studies have shown that UWL undergoes significant changes under various stress conditions and is closely associated with plant stress resistance [18]. Experiments have demonstrated that the UWL values of crop seeds and seedlings can reflect differences in drought resistance among different varieties. The greater the drought resistance, the higher the UWL intensity. Studies on crops such as soybeans and wheat under simulated drought stress conditions have found that UWL intensity varies among different varieties of the same species. Varieties with stronger drought resistance exhibit higher UWL intensity, and the stronger the drought resistance, the greater the UWL intensity [13]. The relationship between UWL and drought resistance discussed above has primarily focused on differences in UWL intensity among plants with varying drought resistance. However, there is still limited research on the biological mechanisms underlying this relationship. Therefore, '*Cabernet Sauvignon*', a drought-tolerant wine grape cultivar, was used in this study to investigate changes in leaf water metabolism, reactive oxygen species (ROS) accumulation, photosynthetic performance, energy status, and UWL intensity under drought stress. The objectives were to clarify the relationships between UWL intensity and ROS levels, photosynthetic traits, and energy metabolism, and to explore the potential sources and underlying mechanisms of UWL generation. This study provides a theoretical foundation for understanding the link between UWL and drought tolerance, as well as the physiological processes involved in UWL production.

# 2. Material and methods

## 2.1. Experimental materials and treatments

The experiment was conducted using two-year-old potted '*Cabernet Sauvignon*' grapevines grown at the research base of Inner Mongolia Agricultural University. When the grapevines had developed 14 to 16 leaves, 20 uniformly growing potted plants were selected and tagged. Ten plants were assigned to the control group (CK) and irrigated regularly according to soil moisture levels. The remaining ten were subjected to drought treatment (T1), in which they were thoroughly watered at the onset of the treatment and then received no further irrigation, thereby inducing continuous drought stress until the end of the experiment. Leaf samples were collected on days 0, 5, 10, 15, 20, 25, and 30 after treatment initiation. At each time point, 3 to 5 fully expanded, healthy leaves of uniform size were collected from the upper-middle portion of the canopy. A portion of the fresh leaves was immediately used to measure UWL intensity, water metabolism parameters, and chlorophyll content. The remaining leaves were stripped of their main veins, cut into small pieces, thoroughly mixed, flash-frozen in liquid nitrogen, and stored at −80°C for subsequent analysis of reactive oxygen species (ROS) and energy indicators.

## 2.2. Measurement parameters and methods

### 2.2.1. Ultraweak luminescence intensity

Measurement of ultraweak luminescence (UWL): The UWL intensity was measured using the BPCL-2-SH ultraweak luminescence testing system, manufactured by Beijing Jianxin Lituo Technology Co., Ltd. After turning on the device, it was preheated for 30 minutes before measurement. The high voltage was set to 1050 V, and the standard light source emission intensity was set to 20,000 counts per second. The sampling time was set to 5 seconds, with a sampling interval of 1 second. Grape leaves were sampled by punching small discs with a diameter of 1 cm using a hole puncher, which were then placed flat in the dark chamber measurement cup. Each sample was measured in triplicate, with each repetition consisting of continuous measurements taken 10 times. The measured values were averaged after subtracting the background, yielding the UWL intensity of the grape leaves.

### 2.2.2. Water metabolism

The relative soil water content was measured using a soil moisture rapid tester (TZS-ZX-G model, Zhejiang Top Yunong Technology Co., Ltd.).

Leaf water content measurement: fresh leaf samples were collected and weighed to determine their fresh weight (FW). After weighing, the leaves were immersed in deionized water and stored at 4°C for 8 hours to ensure full hydration. Subsequently, the turgid fresh weight (TW) was recorded. The leaves were then placed in an oven at 105°C for 1 hour to halt enzymatic activity, followed by drying at 80°C until a constant dry weight (DW) was achieved. The dry weight was then recorded. The formulas for calculating various leaf water content indicators are as follows:

Total leaf water content (%) = (FW − DW)/FW×100%

Relative leaf water content (%) = (FW − DW)/(TW − DW)×100%

Measurement of free water and bound water content: The complete leaves were stripped of their main veins, and uniform small discs were punched out using a hole puncher. After weighing, the discs were immediately placed into weighing bottles, denoted as $W_1$. Then, a 60%–65% sugar solution, six times the weight of the plant tissue, was added to the weighing bottle. The bottle was placed in the dark for 6 hours, during which it was gently shaken occasionally. After 6 hours, the leaves were removed, and any excess sugar solution on the surface was absorbed using wet gauze and filter paper, followed by immediate re-weighing, denoted as $W_2$. The following indicators were then calculated:

Leaf free water content (%) = $(W_1 - W_2)/W_1 \times 100\%$

Leaf bound water content (%) = Total leaf water content− Leaf free water content

Free water/ Bound water=Leaf free water content/ Leaf bound water content

## 2.2.3. Reactive oxygen species

The concentrations of hydrogen peroxide ($H_2O_2$) and the production rate of superoxide anion radicals ($O_2^{-}\cdot$) were determined using commercial assay kits (Solarbio Life Sciences, Beijing, China) in accordance with the manufacturer's instructions. Malondialdehyde (MDA) content was measured using the thiobarbituric acid (TBA) method, as described by Chen et al. [19].

Relative electrical conductivity was assessed following the method described by Chen et al. [19]. Briefly, grape leaves were thoroughly rinsed with ultrapure water, and 20 circular leaf discs of uniform size were punched using a hole punch. The discs were placed in glass test tubes containing 10 mL of ultrapure water and incubated at room temperature for 4 hours, with gentle shaking 3–5 times during the incubation. The initial conductivity of ultrapure water ($S_0$) and the conductivity after incubation with the leaf discs ($S_1$) were measured using a conductivity meter. Subsequently, the samples were boiled at 100°C for 30 minutes and cooled to room temperature before measuring the final conductivity ($S_2$). Relative electrical conductivity was calculated using the following formula: $(S_1 - S_0)/(S_2 - S_0) \times 100\%$

## 2.2.4. Photosynthetic and chlorophyll fluorescence characteristics

Chlorophyll content was measured using the 80% acetone extraction method. Gas exchange parameters, including net photosynthetic rate (Pn), transpiration rate (Tr), intercellular $CO_2$ concentration (Ci), and stomatal conductance (Gs), were measured using a CIRAS-3 portable photosynthesis system between 9:00 and 11:00 a.m. For each treatment, three uniformly growing plants were selected. From each plant, 3 to 5 fully expanded, healthy leaves of similar size were collected from the upper-middle portion of the canopy. Dust was gently wiped from the leaf surface, and measurements were taken while avoiding the main veins. All selected leaves were pre-labeled to ensure consistent measurements on the same leaves over time.

Chlorophyll fluorescence parameters were measured using a Mini-PAM fluorometer. After 20 minutes of dark adaptation using aluminum foil, the maximum quantum yield of PSII (Fv/Fm), photochemical quenching coefficient (qP), and non-photochemical quenching coefficient (qN) were recorded. Subsequently, the effective quantum yield of PSII (Yield) under light-adapted conditions was determined.

### 2.2.5. Energy

The concentrations of ATP, ADP, and AMP in grape leaves were quantified using high-performance liquid chromatography (HPLC). The analysis was performed on an ELITE Lachrom HPLC system equipped with a HITACHI UV-VIS Detector L-2400, an Autosampler L-2200, and a Column Oven L-2300. A high-speed refrigerated centrifuge (HC-2518R) was employed during sample preparation. The standards used were 5'-ATP disodium salt, 5'-ADP disodium salt, and 5'-AMP disodium salt (Sigma, USA). All other reagents were domestic chromatographic-grade chemicals, and Milli-Q ultrapure water (Millipore, USA) was used throughout the analysis. Chromatographic separation was carried out on a LaChrom-C18 column (4.6 mm × 250 mm, 5 μm; Hitachi). The mobile phase consisted of 35 mmol/L $NaH_2PO_4$ buffer (pH 6.8), delivered at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 259 nm. The column temperature was maintained at 30°C, and the injection volume was set to 10 μL using the autosampler.

The energy charge (EC) is calculated using the following formula:

$$EC = ([ATP] + 0.5[ADP])/([ATP]+[ADP]+[AMP])$$

## *2.3 Statistical analysis*

All experimental measurements were performed in triplicate. Results are presented as mean values±standard error (SE), with 95% confidence intervals. Data were organized using Excel 2016 software, line graphs were created using GraphPad Prism 8.0 software, and correlation analysis was performed using IBM SPSS Statistics 27.0 (** indicates $p < 0.01$, highly significant correlation; * indicates $p < 0.05$, significant correlation).

## 3. Results and Analysis

## 3.1. Changes in UWL intensity in grapevine leaves under drought stress

The variation in UWL intensity in grapevine leaves under drought stress is presented in Fig. 1. In the control group, UWL intensity remained relatively stable throughout the treatment period, exhibiting only minor fluctuations. In contrast, the UWL intensity in drought-stressed leaves showed a pronounced downward trend and was significantly lower than that of the control. A notable decline began on day 15 of the drought treatment and continued through to the end of the experiment (day 30), by which time

UWL intensity had decreased by 46.72% compared to the pre-treatment level. Compared with the control, UWL intensity in drought-stressed leaves was reduced by 51.67% on day 30 (Fig. 1).

In summary, UWL intensity progressively declined with increasing drought severity and duration, suggesting that UWL intensity serves as an effective indicator of physiological stress in grapevine plants

## 3.2. Changes in water metabolism in grapevine under drought stress

The changes in grapevine water metabolism under drought stress are shown in Fig. 2. As the treatment progressed, the relative soil water content in the control group fluctuated between 50% and 60%. In contrast, drought-stressed plants exhibited a pronounced and continuous decline in soil moisture, which remained significantly lower than that of the control. By the end of the treatment period (day 30), relative soil water content had dropped to just 10.01%, representing an 81.25% decrease compared to the control (Fig. 2A). Total leaf water content in both control and drought-stressed plants fluctuated between 60% and 80% throughout the experiment. However, on days 25 and 30, total water content in drought-stressed leaves was significantly lower than in the control (Fig. 2B). Relative leaf water content in the control group remained above 90% during the entire treatment period. Under drought stress, however, it showed a gradual decline and was consistently lower than in the control. On day 30, relative leaf water content decreased to 80.42%, a 13.78% reduction compared to the control (Fig. 2C). Free water content in control leaves remained relatively stable, whereas in drought-stressed plants, it gradually declined over time. By day 30, free water content had dropped to 22.23%, a significant reduction of 48.31% relative to the control (Fig. 2D). In contrast, bound water content remained steady in the control group but increased progressively under drought stress. On day 30, bound water content reached 45.43%, which was 32.97% higher than in the control (Fig. 2E). The free water to bound water ratio (FW/BW) in control leaves showed little variation throughout the treatment. However, in drought-stressed leaves, the ratio declined significantly over time, reaching 0.49 on day 30—representing a 61.13% decrease compared to the control (Fig. 2F).

In summary, drought stress markedly affected water metabolism in grapevine. Relative soil water content, total and relative leaf water content, free water content, and the free/bound water ratio all declined significantly, while bound water content increased substantially.

## 3.3. Relationship between ultraweak luminescence and reactive oxygen species in grapevine leaves under drought stress

### 3.3.1. Changes in reactive oxygen species in grapevine leaves under drought stress

The changes in ROS levels in grapevine leaves under drought stress are shown in Fig. 3. Throughout the treatment period, levels of $H_2O_2$, $O_2^{-}\cdot$ production rate, MDA content, and relative electrolyte conductivity remained relatively stable in the control group. In contrast, all ROS-related indicators in drought-stressed leaves exhibited a progressive increase and were significantly higher than those in the control. By the end of the drought period (day 30), compared to pre-treatment levels, $H_2O_2$ content had increased by 91.21%, the $O_2^{-}\cdot$ production rate by 4.98-fold, MDA content by 4.58-fold, and relative electrolyte conductivity by 2.88-fold. Relative to the control group, drought-stressed leaves on day 30 showed significantly higher $H_2O_2$ content (+ 74.75%), $O_2^{-}\cdot$ production rate (+ 4.92-fold), MDA content (+ 4.96-fold), and relative electrolyte conductivity (+ 1.87-fold) (Fig. 3).

In summary, drought stress caused significant oxidative damage in grapevine leaves, as indicated by membrane lipid peroxidation and substantial increases in ROS-related physiological indicators.

- *3.3.2 Correlation analysis between ultraweak luminescence and reactive oxygen species in grapevine leaves under drought stress*

As drought stress progressed, all ROS-related indicators in grapevine leaves increased substantially, while UWL intensity showed a pronounced decline. To further investigate this relationship, a correlation analysis was conducted between UWL intensity and ROS-related parameters under drought conditions (Table 1). The results indicated that UWL intensity was significantly and negatively correlated with $H_2O_2$ content, $O_2^{-}\cdot$ production rate, MDA content, and relative electrolyte conductivity (Table 1).

In summary, as ROS accumulated under drought stress, UWL intensity declined correspondingly, suggesting that ROS is not a direct source of UWL in grapevine leaves.

Table 1
Correlation analysis between UWL intensity and ROS levels in grapevine leaves under drought stress

| Reactive oxygen index | UWL regression equatioin | Correlation coefficient $R^2$ |
|---|---|---|
| $H_2O_2$ | Y=-10.543x + 24.254 | -0.8668** |
| Production rate of $O_2^{-}\cdot$ | Y=-0.4536x + 19.812 | -0.8749** |
| MDA | Y=-82.291x + 19.666 | -0.9397** |
| Relative conductivity | Y=-0.2039x + 20.812 | -0.9689** |

In the regression equation, X refers to the reactive oxygen index, and Y refers to UWL intensity. The asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$).

# 3.4. Relationship between ultraweak luminescence and photosynthetic characteristics in grapevine leaves under drought stress

## 3.4.1. Changes in chlorophyll content in grapevine leaves under drought stress

Changes in chlorophyll content in grapevine leaves under drought stress are shown in Fig. 4. As the duration of the treatment increased, the levels of chlorophyll *a*, chlorophyll *b*, and total chlorophyll (*a + b*) in the control group remained relatively stable, exhibiting only minor fluctuations. In contrast, leaves subjected to drought stress showed a continuous and pronounced decline in all three chlorophyll components. By day 30, compared to day 0 (pre-treatment), the contents of chlorophyll *a*, chlorophyll *b*, and total chlorophyll (*a + b*) in drought-stressed leaves had decreased by 70.60%, 51.05%, and 66.38%, respectively. Relative to the control, these values were reduced by 64.26%, 60.11%, and 63.05%, respectively, at the end of the drought period (Fig. 4).

In summary, chlorophyll biosynthesis in grapevine leaves was markedly inhibited under drought stress, as indicated by significant reductions in chlorophyll content.

## 3.4.2. Changes in photosynthetic parameters in grapevine leaves under drought stress

Changes in photosynthetic parameters in grapevine leaves under drought stress are shown in Fig. 5. In the control group, Pn, Tr, Ci, and Gs remained relatively stable, exhibiting only minor fluctuations throughout the treatment period. In contrast, drought-stressed leaves showed an initial increase followed by a sustained decline in Pn, Tr, and Gs. By day 30, these parameters had decreased significantly by 58.73%, 57.58%, and 61.26%, respectively, compared to the control (Fig. 5A, 5B, 5D). For Ci, drought stress induced an initial decrease followed by a subsequent increase. On day 30, Ci in drought-stressed leaves was 27.79% higher than that of the control (Fig. 5C).

In summary, drought stress markedly reduced photosynthetic performance in grapevine leaves, reflecting a strong inhibition of photosynthetic activity and a decline in overall carbon assimilation capacity.

## 3.4.3. Changes in chlorophyll fluorescence parameters in grapevine leaves under drought stress

Changes in chlorophyll fluorescence parameters in grapevine leaves under drought stress are shown in Fig. 6. The Fv/Fm, which reflects the efficiency of light energy conversion in PSII reaction centers, remained stable at approximately 0.75 in the well-watered control throughout the experiment. In contrast, drought-stressed leaves exhibited a progressive decline in Fv/Fm, with values decreasing by 39.23% relative to day 0 and by 35.28% compared to the control on day 30 (Fig. 6A). The Yield, measured without prior dark adaptation, represents the actual quantum efficiency of PSII and indicates the rate of photosynthetic electron transport. In control plants, Yield remained steady at around 0.73. However, in

drought-stressed plants, Yield declined continuously with increasing stress duration. By day 30, it had decreased by 52.90% relative to day 0 and by 52.67% compared to the control (Fig. 6B). The qP, which reflects the proportion of open PSII reaction centers, remained relatively constant in the control group. Under drought conditions, however, qP began to decline from day 10, reaching a 30.78% reduction relative to pre-treatment levels and a 31.28% reduction compared to the control by day 30 (Fig. 6C). The qN, a regulatory mechanism that protects the photosynthetic apparatus under stress, remained stable at approximately 0.2 in the control group. In drought-stressed plants, qN increased markedly from day 10 onward, reaching a 1.99-fold increase compared to day 0 and a 2.22-fold increase compared to the control by day 30 (Fig. 6D).

In summary, drought stress significantly disrupted the photosynthetic light reactions in grapevine leaves. The reductions in Fv/Fm, Yield, and qP indicate damage to PSII and impaired photochemical efficiency, while the sharp rise in qN suggests the activation of photoprotective mechanisms under sustained stress conditions.

## 3.4.4. Correlation analysis between ultraweak luminescence and photosynthetic characteristics in grapevine leaves under drought stress

As drought stress progressed, the photosynthetic capacity of grapevine leaves declined, as indicated by reductions in chlorophyll content, photosynthetic parameters, and chlorophyll fluorescence parameters. Simultaneously, UWL intensity also decreased. To further examine this relationship, a correlation analysis was performed between UWL intensity and various photosynthetic traits under drought conditions (Table 2).

The results showed that UWL intensity was strongly and positively correlated with chlorophyll content, including chlorophyll *a*, chlorophyll *b*, and total chlorophyll (*a* + *b*). It was moderately and positively correlated with photosynthetic parameters such as Pn, Tr, and Gs, and moderately and negatively correlated with Ci. UWL intensity was also strongly and positively correlated with chlorophyll fluorescence parameters including Fv/Fm, Yield, and qP, and strongly negatively correlated with qN (Table 2). These findings suggest that under drought stress, UWL intensity declines in parallel with decreases in chlorophyll content, Pn, Tr, Gs, Fv/Fm, Yield, and qP, and increases in Ci and qN. Collectively, the data indicate that UWL emission is closely linked to photosynthetic function: enhanced photosynthetic activity promotes UWL generation, whereas impaired photosynthesis under drought conditions suppresses it.

Table 2
Correlation analysis between UWL intensity and photosynthetic characteristics in grapevine leaves under drought stress

| Photosynthetic characteristic index | | UWL regression equation | Correlation coefficient $R^2$ |
|---|---|---|---|
| Chlorophyll | Chlorophyll *a* | Y = 9.5731X + 4.7496 | 0.9603** |
| | Chlorophyll *b* | Y = 48.058X + 0.4952 | 0.9715** |
| | Chlorophyll *a* + *b* | Y = 8.022x + 3.9946 | 0.9669** |
| Photosynthetic parameters | Pn | Y = 6.9317X + 6.9647 | 0.5054 |
| | Tr | Y = 10.547X + 7.8831 | 0.5504 |
| | Gs | Y = 0.3483X + 7.828 | 0.4353 |
| | Ci | Y=-0.0208X + 24.472 | -0.5573 |
| Chlorophyll fluorescence parameters | Fv/Fm | Y = 27.102X-4.5296 | 0.8660** |
| | Yield | Y = 22.352X + 0.6233 | 0.9332** |
| | qP | Y = 27.207X-9.689 | 0.8934** |
| | qN | Y=-22.355X + 20.149 | -0.8766** |

In the regression equation, X refers to the photosynthetic characteristic index, and Y refers to UWL intensity. The asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$).

# 3.5. Relationship between ultraweak luminescence and energy status in grapevine leaves under drought stress

# 3.5.1 Changes in energy status in grapevine leaves under drought stress

Changes in energy-related metabolites in grapevine leaves under drought stress are shown in Fig. 7. In the control group, levels of ATP, ADP, AMP, and energy charge remained relatively stable throughout the treatment period. In contrast, drought-stressed leaves exhibited a gradual decline in both ATP content and energy charge, which were significantly lower than those in the control. Meanwhile, levels of ADP and AMP increased progressively under drought conditions and were significantly higher than in the control group. Compared to pre-treatment levels, by day 30 of drought stress, ATP content had decreased by 67.48%, while ADP and AMP contents had increased by 42.27% and 58.90%, respectively. Energy charge declined by 26.66%. Relative to the control group, drought-stressed leaves on day 30 showed a 65.74% reduction in ATP, a 41.30% increase in ADP, a 61.48% increase in AMP, and a 26.10% decrease in energy charge (Fig. 7).

In summary, drought stress markedly impaired energy metabolism in grapevine leaves, resulting in reduced ATP synthesis, elevated ADP and AMP accumulation, and a substantial decline in cellular energy charge.

## 3.5.2. Correlation analysis between ultraweak luminescence and energy status in grapevine leaves under drought stress

The correlation between UWL intensity and energy-related parameters in grapevine leaves under drought stress is shown in Table 3. Drought stress led to a pronounced reduction in ATP levels and energy charge (EC), along with a progressive accumulation of ADP and AMP, accompanied by a significant decline in UWL intensity. Correlation analysis revealed that UWL intensity was strongly and positively correlated with both ATP content and EC, while exhibiting strong negative correlations with ADP and AMP levels. These results indicate that UWL emission is closely linked to energy metabolism under drought conditions.

In particular, the reduction in ATP and EC coincided with a decrease in UWL intensity, whereas the rise in ADP and AMP was associated with diminished UWL emission. These findings suggest that drought stress impairs energy synthesis, thereby constraining UWL generation. Furthermore, the data imply that ATP may constitute one of the metabolic sources of UWL: when ATP levels are maintained, UWL emission is enhanced; conversely, when ATP synthesis is suppressed, UWL intensity declines accordingly.

Table 3
Correlation analysis between UWL intensity and energy status in grapevine leaves under drought stress

| energy-related parameters | UWL regression equatioin | Correlation coefficient $R^2$ |
|---|---|---|
| ATP | Y = 16.41x + 4.4775 | 0.9372** |
| ADP | Y=-74.405x + 39.914 | -0.9423** |
| AMP | Y=-240.51x + 32.998 | -0.9629** |
| EC | Y = 40.499x-15.973 | 0.9203** |

In the regression equation, X refers to the energy-related parameters, and Y refers to UWL intensity. The asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$).

# 4. Discussion

UWL in plants is closely associated with growth, metabolic activity, and physiological responses. Due to its high sensitivity to environmental stimuli, even subtle changes in external conditions can readily alter

UWL emission. Consequently, investigating how environmental factors influence UWL intensity—and how UWL reflects underlying physiological dynamics—has significant implications for understanding plant stress responses. Previous studies have shown that unfavorable environmental conditions frequently result in reduced UWL intensity [20, 21]. For instance, Wang et al. [22] reported that UWL emitted from plant roots decreased progressively with prolonged soil drought. In the present study, grapevine leaves exhibited a consistent decline in UWL intensity throughout the drought treatment, with levels significantly lower than those in the control group. This reduction coincided with decreases in relative soil water content, leaf relative water content, free water content, and the free-to-bound water ratio—indicating that drought stress markedly impaired water metabolism. These results suggest that as drought severity increased, the metabolic activity and physiological vigor of grapevines declined accordingly, leading to suppressed UWL emission. Therefore, UWL intensity may serve as a sensitive indicator of drought-induced physiological stress in plants. This observation is consistent with the findings of Zhang [23], who reported a decline in UWL intensity in apple seedlings under water stress and emphasized the close association between UWL and metabolic processes. Collectively, these findings support the hypothesis that UWL is closely coupled to physiological function and may act as a photon-based signal of drought stress, reflecting the internal physiological state of the plant in response to environmental changes.

Under normal conditions, the amount of ROS generated by plant cellular metabolism is minimal and can be effectively scavenged by the plant's own antioxidant defense systems, thus causing no harm. However, under drought stress, this balance is disrupted—ROS production increases, while the plant's scavenging capacity declines. Consequently, antioxidants are degraded, leading to the accumulation of ROS, which in turn causes oxidative damage to the plant [16, 24]. Among the current theories explaining the mechanism of biological UWL, the most representative is the biochemical perspective, which posits that UWL is closely associated with ROS. During a series of metabolic reactions in biological systems, ROS generated as by-products may serve as one of the sources of UWL [25, 26]. In the present study, drought stress led to increased $H_2O_2$ content, $O_2^{-}\cdot$ production rate, MDA content, and relative electrical conductivity in the leaves, all of which were higher than those in the control. Meanwhile, UWL intensity exhibited a decreasing trend and was significantly lower than in the control. Correlation analysis revealed a highly significant negative relationship between UWL intensity and hydrogen peroxide content, superoxide anion production rate, MDA content, and relative electrical conductivity. In other words, UWL intensity decreased as ROS levels and membrane damage increased. These results are consistent with previous findings. For example, Jie et al. [21] found that UWL intensity in *Malus hupehensis* seedlings decreased with increasing ROS accumulation under drought stress. Wen [27] also observed that in *Sedum* species under heat stress, the production rate of superoxide anions, MDA content, and relative conductivity increased, while UWL intensity declined. Similarly, Zhang [23] reported that under water deficit conditions, apple seedlings accumulated ROS while exhibiting reduced UWL, suggesting that ROS is not the direct source of UWL. The present study supports the view that UWL intensity is negatively associated with ROS accumulation. Although ROS is involved, it may not be the direct source of UWL. The observed decline in UWL may be attributed to severe ROS accumulation under drought stress, which induces or aggravates membrane lipid peroxidation and MDA production. This damages the cell

membrane system, increases membrane permeability, and severely disrupts plant metabolism and tissue structure, thereby weakening UWL [28].

Chlorophyll, the central pigment in chloroplasts, plays a pivotal role in photosynthesis. It comprises both light-harvesting pigments and reaction center pigments, which function to absorb and transfer light energy, ultimately converting it into chemical energy through photochemical reactions. This process constitutes the initial and essential step in photosynthetic energy conversion [29]. In this study, chlorophyll content in grapevine leaves decreased significantly under drought stress. As stress severity increased, the reduction became more pronounced. These findings suggest that drought stress suppresses chlorophyll biosynthesis, thereby reducing photosynthetic efficiency. Photosynthesis is a fundamental process that sustains plant life by driving both material and energy metabolism. Its efficiency directly influences plant growth, development, and resistance to environmental stress. The impact of drought on photosynthesis is typically attributed to both stomatal and non-stomatal limitations [30]. In this study, as drought stress intensified, chlorophyll content in grapevine leaves progressively declined. Photosynthetic parameters—including Pn, Gs, and Tr—initially increased and then declined, while Ci exhibited the opposite pattern, first decreasing and then increasing. This trend suggests that in the early stages of drought, the water deficit was not yet sufficient to substantially impair photosynthesis. However, under prolonged drought stress, reductions in Pn, Gs, and Tr, alongside elevated Ci, indicate that the decline in photosynthetic capacity was primarily driven by non-stomatal factors. These non-stomatal limitations are likely related to chlorophyll degradation, decreased photosystem efficiency, reduced activity of key enzymes involved in carbon fixation, and lower mesophyll conductance. Changes in chlorophyll fluorescence parameters are sensitive indicators of photosynthetic performance and stress response, often described as an “intrinsic probe” for examining the interaction between photosynthesis and environmental stress [31]. In this study, as drought stress intensified, Fv/Fm, Yield, and qP in grapevine leaves gradually declined, while qN increased. These trends indicate that increasing drought severity impaired the photochemical efficiency of PSII and reduced the openness of its reaction centers, thereby suppressing the rate of photosynthetic electron transport. As a result, the proportion of light energy harvested by antenna pigments available for photochemistry decreased, leading to diminished photosynthetic activity. The excess excitation energy accumulating in PSII was dissipated via enhanced qN, reflecting the activation of protective mechanisms to prevent photooxidative damage to the photosynthetic apparatus. An integrated analysis of chlorophyll content, photosynthetic parameters, and chlorophyll fluorescence parameters reveals a pronounced decline in photosynthetic capacity in grapevine leaves under drought stress. Drought markedly inhibited photosynthetic performance, as indicated by reductions in chlorophyll concentration, net photosynthetic rate, and photochemical efficiency. Subsequent correlation analysis demonstrated a strong association between declining UWL intensity and photosynthetic inhibition. UWL intensity exhibited highly significant positive correlations with chlorophyll content and fluorescence parameters (Fv/Fm, Yield, and qP), suggesting that UWL generation is tightly linked to photosynthetic system activity. When photosynthesis is active, electron transport rates are elevated and sufficient excitation energy is available—conditions that favor UWL emission. Conversely, the decline in photosynthetic function reduces excitation energy,

thereby diminishing UWL intensity. Several studies have underscored the close association between UWL and photosynthetic activity. Li et al. [32, 33] reported that UWL in mung bean and peanut, as well as in peanut seedlings exposed to different light spectra, was strongly correlated with chloroplast development and photosynthetic performance. Wen [27] further proposed that plant vigor and photosynthetic efficiency may be key contributors to UWL emission. Similarly, Zhu et al. [34], in a study examining UWL dynamics during strawberry fruit development, also suggested a strong linkage between UWL and photosynthesis. The present findings align with these prior observations, providing additional evidence that UWL emission is closely coupled to the intensity of photosynthetic activity. The strong correlation between UWL and chlorophyll content further implies that UWL may be influenced not only by energy transfer processes, but also by chlorophyll biosynthesis and metabolic dynamics [35]. Nevertheless, the underlying regulatory mechanisms remain unclear and merit further elucidation using molecular and metabolomic approaches.

ATP is a central molecule supporting plant physiological activity, and its abundance serves as a key indicator of cellular energy status. Emerging evidence suggests that any reaction involving the synthesis or consumption of ATP—or other photosynthetically derived metabolites—can result in photon emission as a byproduct of metabolic transitions [36]. Accordingly, UWL is increasingly recognized as being closely coupled to intracellular energy dynamics. Dong et al. [12] reported that UWL intensity in sugarcane leaves reflects not only the plant's metabolic state but also its capacity for energy conversion. In the present study, drought stress induced a progressive decline in ATP content and energy charge in grapevine leaves, accompanied by a corresponding accumulation of ADP and AMP. These observations suggest compromised ATP biosynthesis and disrupted energy homeostasis under stress conditions, consistent with the findings of Zhang [16], who reported similar decreases in UWL intensity and physiological function in water-stressed apple seedlings. Notably, the reduction in ATP levels closely mirrored the decline in UWL intensity throughout the drought period. Correlation analysis revealed a highly significant positive relationship between UWL intensity and both ATP content and energy charge, implying that UWL emission is energetically constrained. This notion is further supported by studies on strawberry fruit senescence [37] and apricot flower development [38], where UWL intensity decreased in parallel with ATP depletion. Collectively, these findings indicate that UWL in grapevine leaves is intimately linked to cellular energy status under drought stress. ATP may act not only as a metabolic energy currency but also as a potential source of luminescent emission. These results provide novel insight into the physiological basis of UWL and highlight its potential as a non-invasive indicator for monitoring plant energy dynamics.

# 5. Conclusion

In conclusion, drought stress significantly reduced UWL intensity in grapevine leaves by impairing water metabolism, disrupting redox homeostasis, and suppressing photosynthetic performance and energy synthesis. Throughout the stress period, key physiological parameters—including chlorophyll content, Pn, Gs, Tr, chlorophyll fluorescence parameters (Fv/Fm, Yield, qP), ATP concent and energy charge—declined progressively and exhibited strong positive correlations with UWL intensity. Conversely, drought led to

marked increases in reactive oxygen species (ROS)-associated indicators, such as $H_2O_2$, $O_2^{-}\cdot$ production rate, MDA content, and relative electrolyte conductivity. These parameters were strongly and negatively correlated with UWL intensity. Together, these results suggest that UWL emission is tightly coupled to the functional integrity of the photosynthetic machinery and intracellular energy state. The decline in UWL under drought conditions appears to result primarily from metabolic dysregulation induced by stress, rather than from ROS acting as direct luminescent sources. Elevated ROS levels likely inhibit UWL generation indirectly by driving lipid peroxidation, disrupting membrane structure, and impairing downstream metabolic activity required for UWL excitation.

Therefore, UWL holds promise as a non-invasive, integrative indicator of plant responses to drought stress, capturing changes in water metabolism, photosynthetic efficiency, energy status, and oxidative imbalance. This underscores both the theoretical importance and practical utility of UWL in plant physiological research and stress diagnostics. Future studies should focus on elucidating the regulatory mechanisms governing UWL generation through integrative approaches such as molecular biology and metabolomics. In particular, uncovering the signaling functions of UWL in plant stress physiology may pave the way for its broader application in assessing crop stress tolerance and monitoring environmental challenges in agricultural systems.

# Declarations

### CRediT authorship contribution statement

Li Zhang: Methodology, Resources, Formal analysis, Visualization, Data curation, Writing—original draft, Writing—review and editing. Yafen Meng: Methodology, Formal analysis, Investigation, Resources, Data curation. Jinli Guo: Conceptualization, Writing—review and editing, Supervision, Project administration, Funding acquisition.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Ethics approval and Consent to participate

Not applicable.

**Consent for publication**

Not applicable.

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# Figures

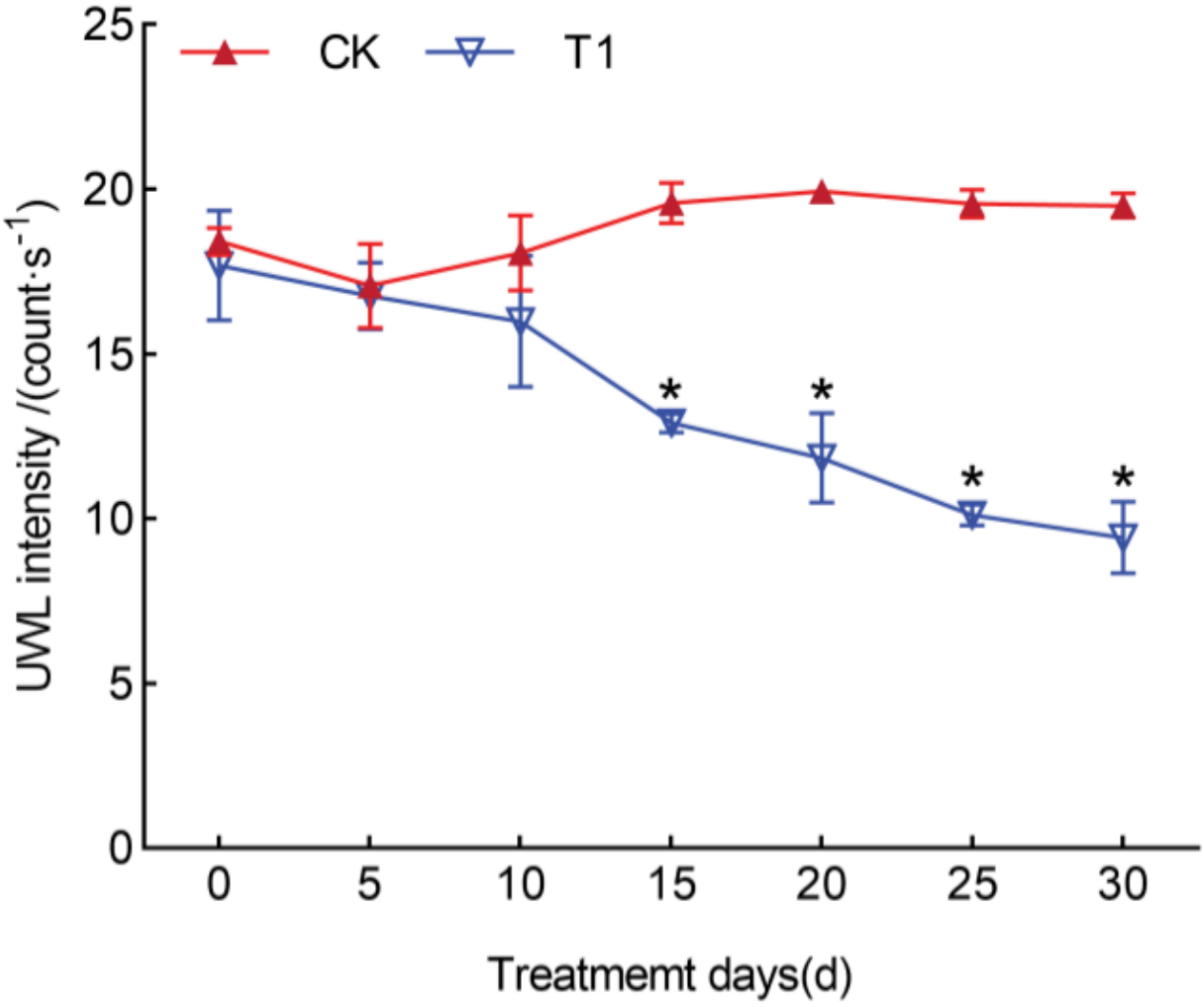
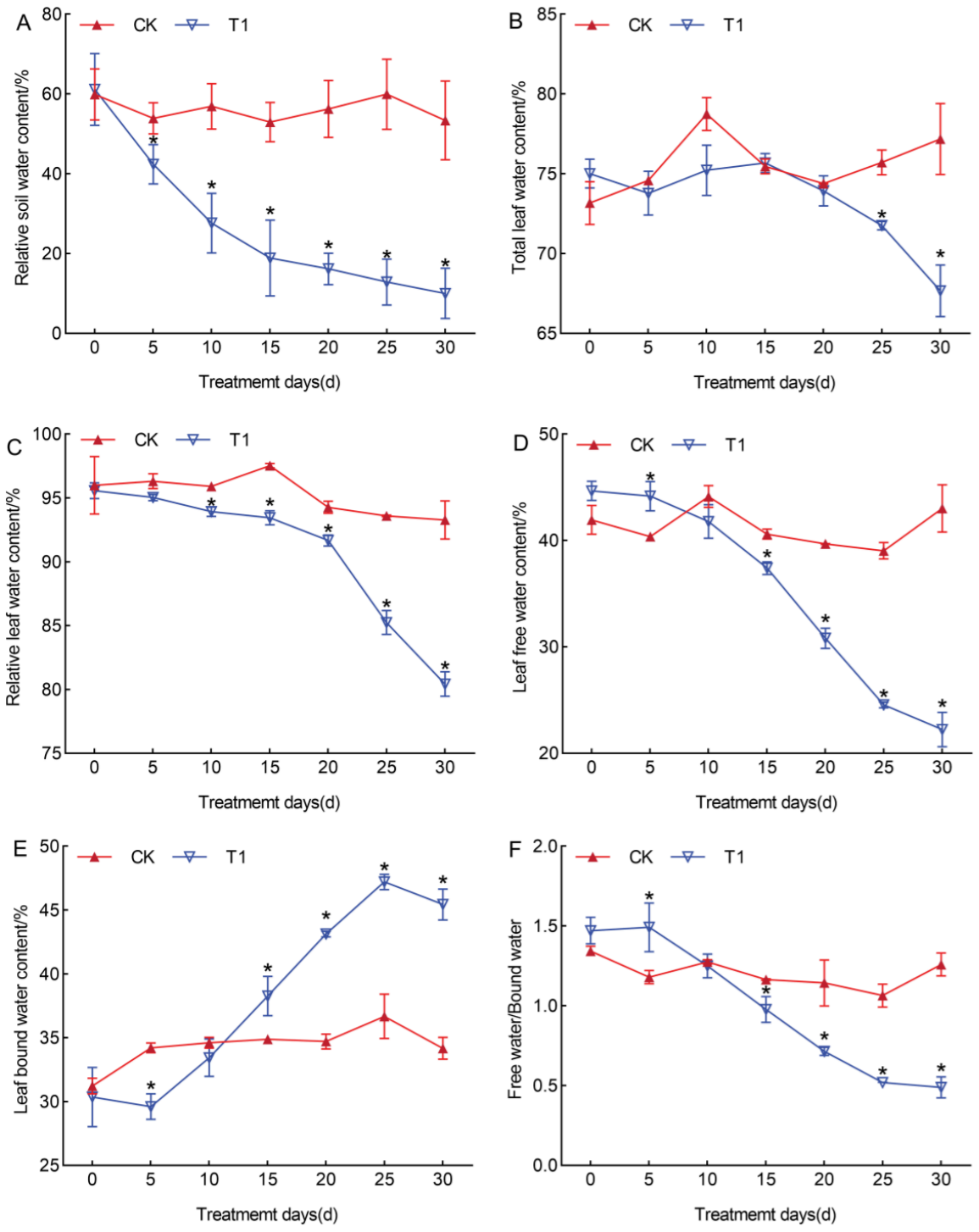



**Figure 1**

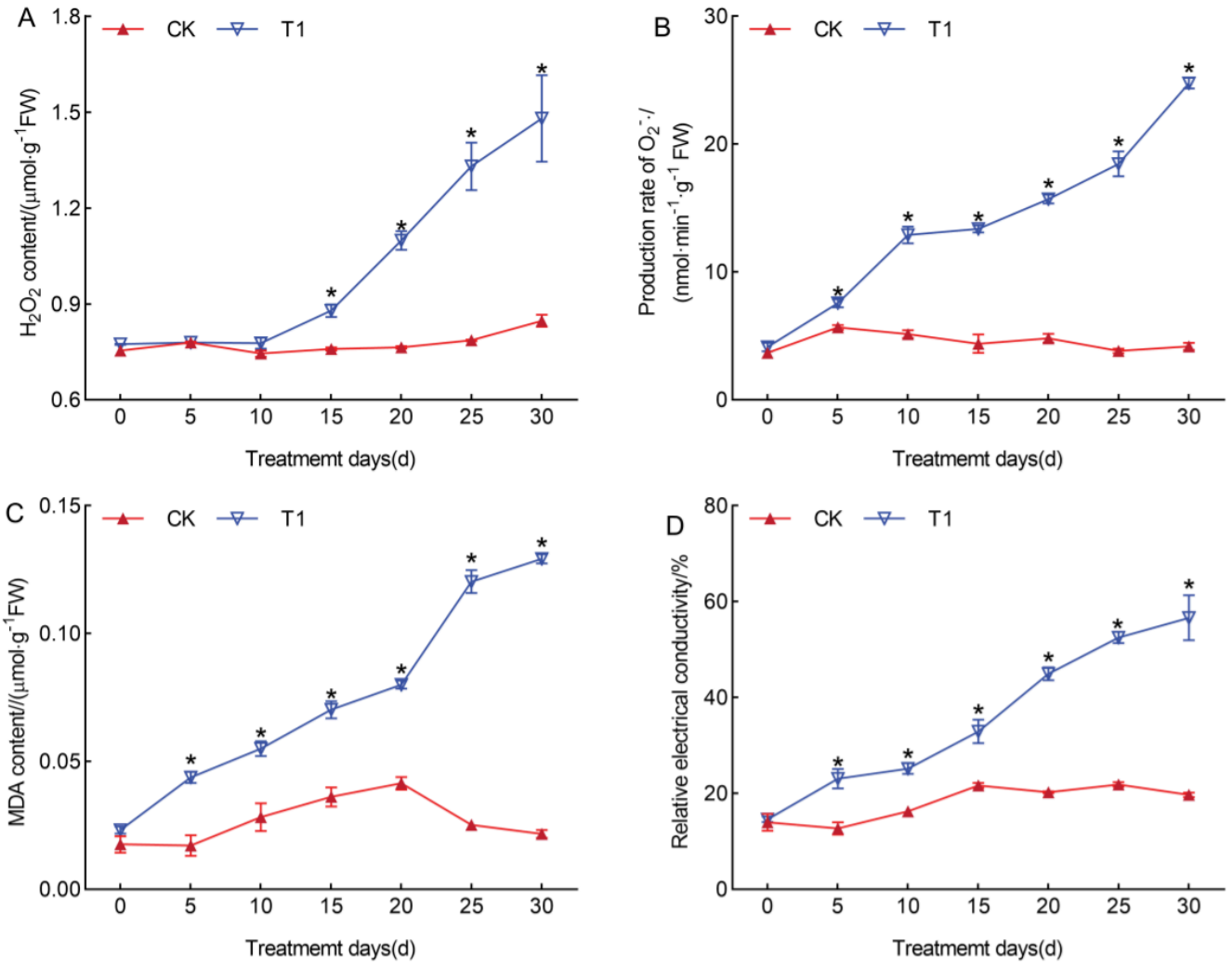
Changes in UWL intensity in the leaves of *Cabernet Sauvignon* grapevine under drought stress. CK: *Cabernet Sauvignon*, control; T1: *Cabernet Sauvignon*, drought stress. * indicates a statistically significant difference ($p < 0.05$) between CK and T1 in grapevine leaves at the same time point based on LSD test. The error bars represent mean±standard error (SE) ($n = 3$).




## Figure 2

Changes in water metabolism parameters in *Cabernet Sauvignon* grapevine under drought stress. (A) Relative soil water content; (B) Total leaf water content; (C) Relative leaf water content; (D) leaf free water content; (E) leaf bound water content; (F) ratio of free water to bound water. CK: *Cabernet Sauvignon*, control; T1: *Cabernet Sauvignon*, drought stress. * indicates a statistically significant

difference ($p$< 0.05) between CK and T1 in grapevine leaves at the same time point based on LSD test. The error bars represent mean±standard error (SE) ($n$ = 3).




## Figure 3

Changes in ROS accumulation in the leaves of *Cabernet Sauvignon* grapevine under drought stress. (A) $H_2O_2$ content; (B) production rate of $O_2^{\cdot-}$; (C) MDA content; (D) relative electrical conductivity. CK: *Cabernet Sauvignon*, control; T1: *Cabernet Sauvignon*, drought stress. * indicates a statistically significant difference ($p$ < 0.05) between CK and T1 in grapevine leaves at the same time point based on LSD test. The error bars represent mean±standard error (SE) ($n$ = 3).

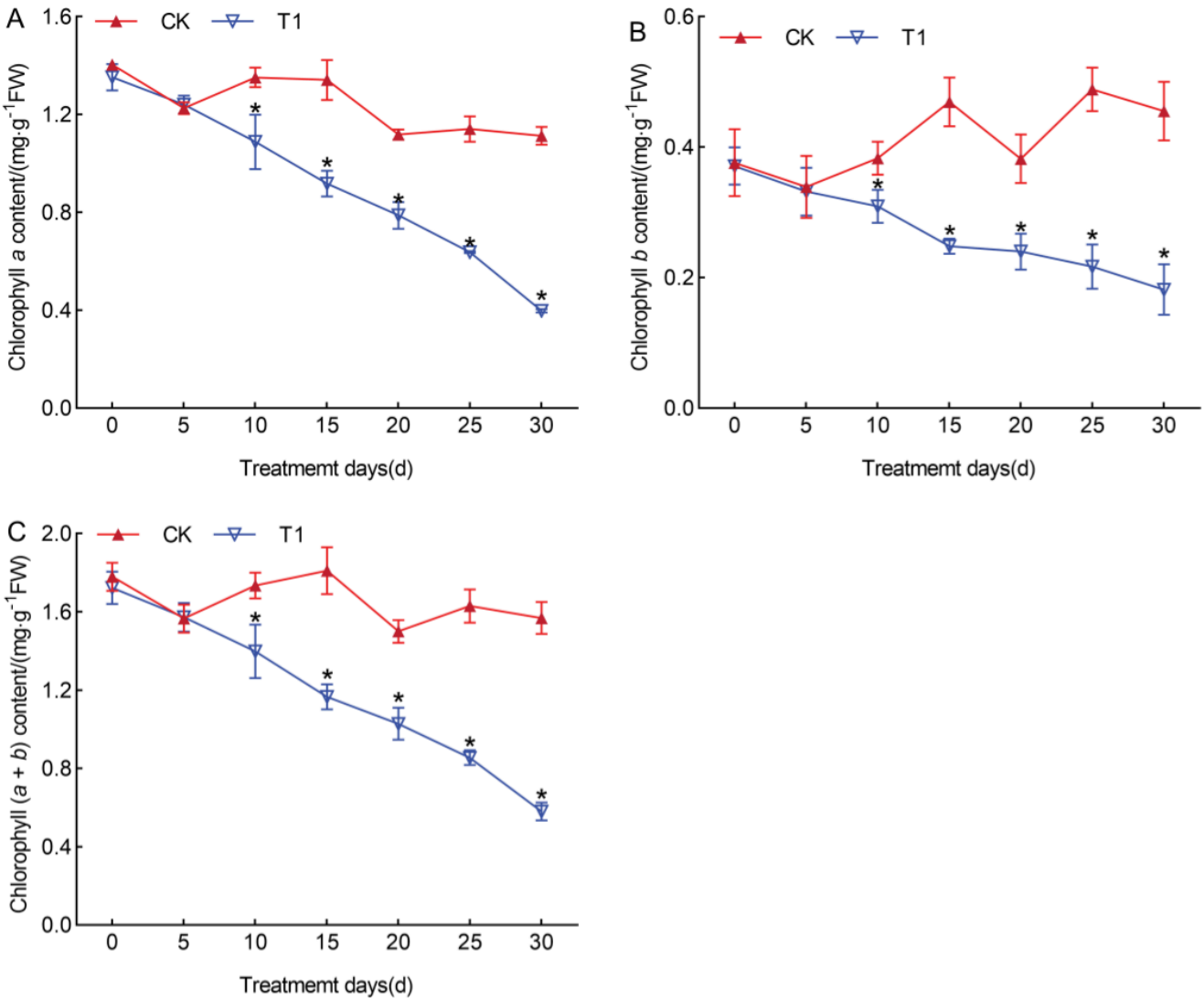
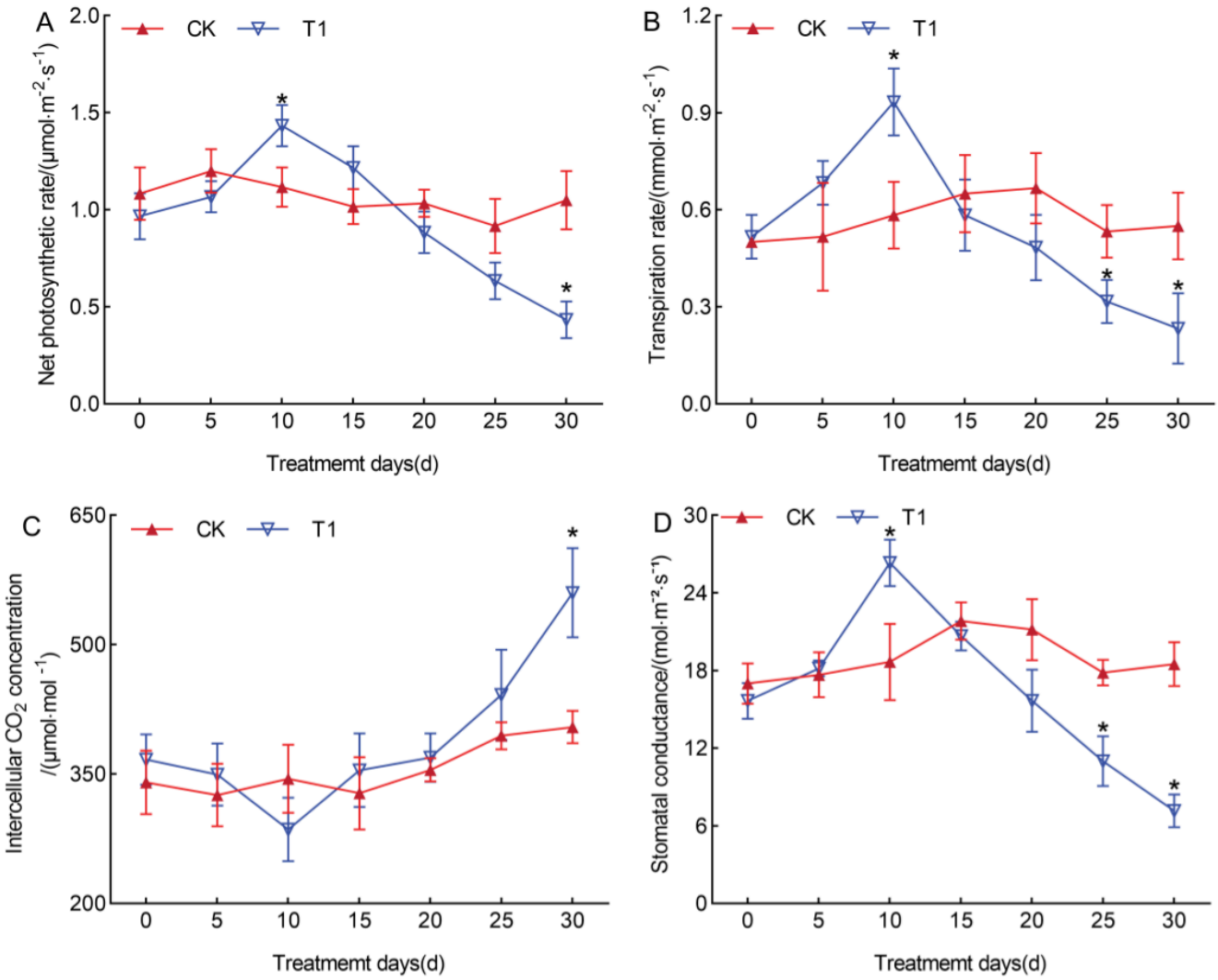



**Figure 4**

Changes in chlorophyll content in the leaves of *Cabernet Sauvignon* grapevine under drought stress. (A) chlorophyll *a*content; (B) chlorophyll *b* content; (C) chlorophyll (*a+b*) content. CK: *Cabernet Sauvignon*, control; T1: *Cabernet Sauvignon*, drought stress. * indicates a statistically significant difference ($p< 0.05$) between CK and T1 in grapevine leaves at the same time point based on LSD test. The error bars represent mean±standard error (SE) ($n = 3$).




## Figure 5

Changes in photosynthetic parameters in the leaves of *Cabernet Sauvignon* grapevine under drought stress. (A) net photosynthetic rate (Pn); (B) transpiration rate (Tr); (C) intercellular $CO_2$ concentration (Ci); (D) stomatal conductance (Gs). CK: *Cabernet Sauvignon*, control; T1: *Cabernet Sauvignon*, drought stress. * indicates a statistically significant difference ($p < 0.05$) between CK and T1 in grapevine leaves at the same time point based on LSD test. The error bars represent mean±standard error (SE) ($n = 3$).

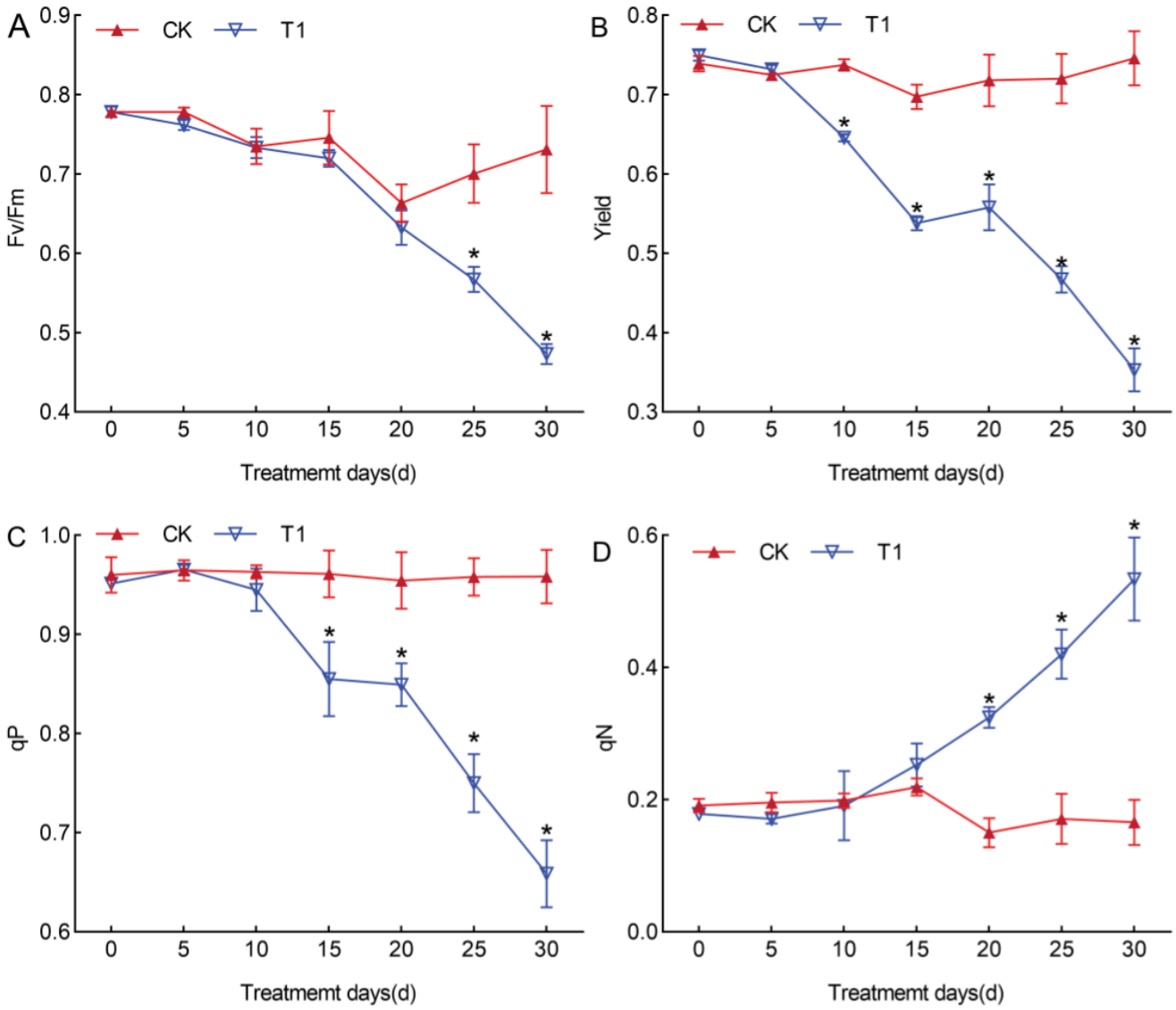



**Figure 6**

Changes in chlorophyll fluorescence parameters in the leaves of *Cabernet Sauvignon* grapevine under drought stress. (A) Fv/Fm; (B) Yield; (C) qP; (D) qN. CK: *Cabernet Sauvignon*, control; T1: *Cabernet Sauvignon*, drought stress. * indicates a statistically significant difference ($p < 0.05$) between CK and T1 in grapevine leaves at the same time point based on LSD test. The error bars represent mean±standard error (SE) ($n = 3$).

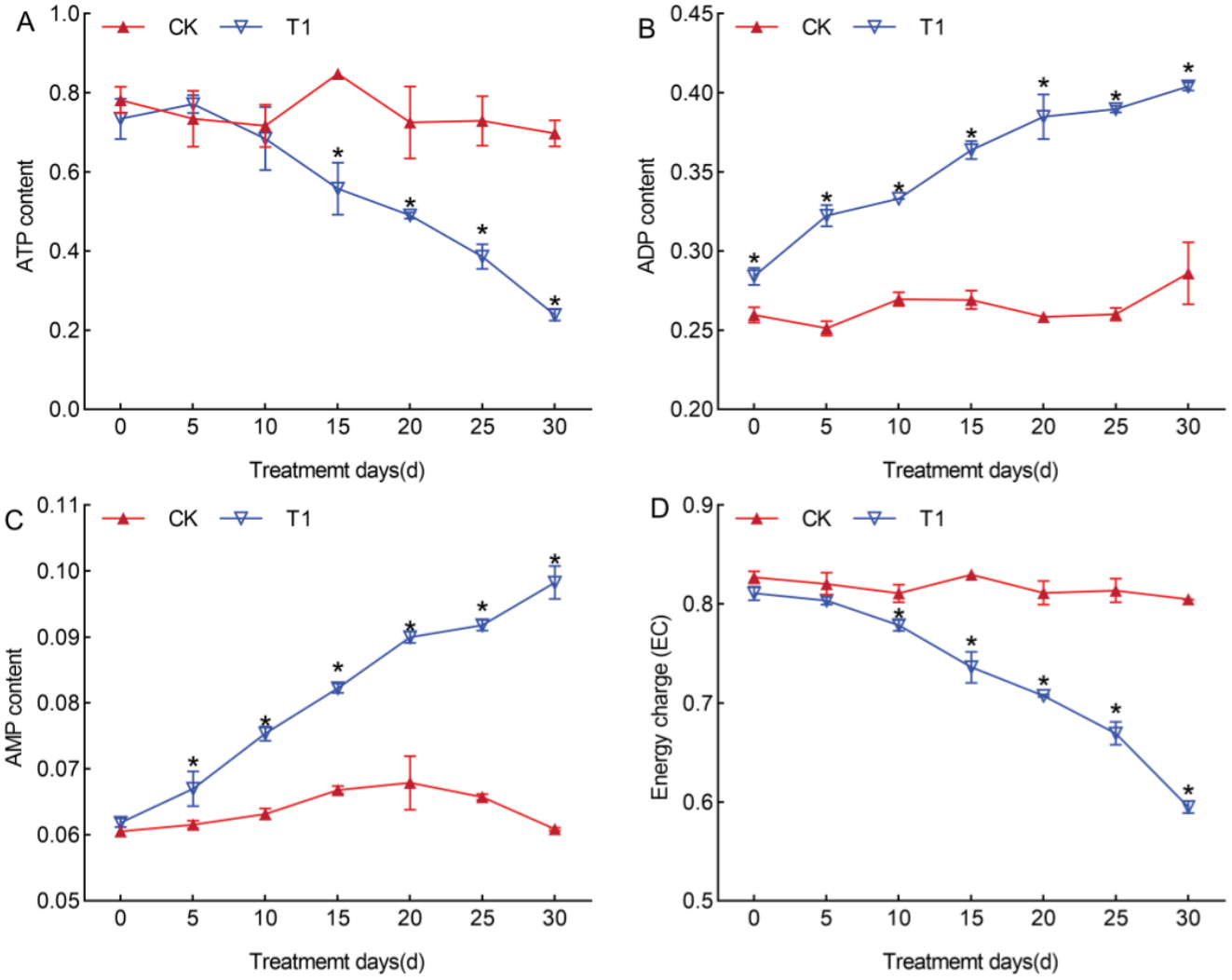



**Figure 7**

Changes in energy status in the leaves of *Cabernet Sauvignon* grapevine under drought stress. (A) ATP content; (B) ADP content; (C) AMP content; (D) Energy charge (EC). CK: *Cabernet Sauvignon*, control; T1: *Cabernet Sauvignon*, drought stress. * indicates a statistically significant difference ($p < 0.05$) between CK and T1 in grapevine leaves at the same time point based on LSD test. The error bars represent mean±standard error (SE) ($n = 3$).