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MELEK EKİNCİ

ERTAN YILDIRIM


SELDA ÖRS

METİN TURAN

DURAN ŞİMŞEK

See next page for additional authors

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MELEK EKİNCİ, ERTAN YILDIRIM, SELDA ÖRS, METİN TURAN, DURAN ŞİMŞEK, and YÜSRA İNCİ

Change in plant growth and some bioactive components of the tomato under water deficit with exogenous chrysin treatment

Melek EKİNCİ^{1*}, Ertan YILDIRIM¹, Selda ÖRS², Metin TURAN³, Duran ŞİMŞEK⁴, Yüstra İNCİ⁵

¹Department of Horticulture, Agriculture Faculty, Atatürk University, Erzurum, Türkiye

²Department of Agricultural Structures and Irrigation, Agriculture Faculty, Atatürk University, Erzurum, Türkiye

³Department of Agricultural Trade and Management, Faculty of Economy and Administrative Sciences, Yeditepe University, İstanbul, Türkiye

⁴Areo Seed Company, Akdeniz University, Konyaalti, Antalya, Türkiye

⁵Organized Industrial Zone Vocational School, Harran University, Şanlıurfa, Türkiye

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Abstract: Drought is an important factor causing decreased productivity and quality in plant cultivation. Stress tolerance in plants can be increased, or damage can be reduced through various exogenous applications. One of them is flavonoids, which play an essential role in responding to stress through changes occurring at the endogenous level in plants due to drought stress. The effects of an exogenous treatment of chrysin, a member of the flavonoid group, on tomato seedlings grown under water deficit were examined in this study. Different doses (C0: control with no treatment; C1: 0.1 mM; C2: 0.5 mM; and C3: 1.0 mM) of chrysin solutions were applied to plant leaves under full irrigation (100% field capacity (FC)) and water deficit (60% FC). The treatment effects were investigated in terms of numerous biochemical, physiological, and morphological properties, as well as their bioactive components. It was determined that the adverse effects of drought stress on plants were alleviated by chrysin, and positive results were obtained, especially with applications at 0.5 and 1.0 mM doses. With drought, plant fresh weight, plant dry weight, root fresh weight, root dry weight, plant height, stem diameter, and leaf relative water content decreased by 35%, 56%, 23%, 20%, 25%, 12%, 24%, and 10%, respectively; however, the same reductions under drought with chrysin treatment were lower. While the abscisic acid content increased with water stress, the various treatments suppressed this increase. The effect of these applications is also thought to occur through plant antioxidant activity, phenolic compounds, and osmoprotectants such as amino acids, sugar, and organic acids. The changes in bioactive components due to the chrysin treatment decreased the rate of damage from stress. This study is one of the first to examine chrysin applications to the tomato; however, it would be useful to detail the research at the molecular level in future studies to understand the effectiveness of this treatment in all its aspects.

Key words: Water deficit, antioxidant enzyme, osmoprotectant, phenolic compound

1. Introduction

Drought, among the major constraints on plant growth and development in agricultural production worldwide, disrupts normal plant growth and water relations, reduces stem elongation, leaf size, and root development, as well as water use efficiency (Farooq et al., 2009; Farooq et al., 2012). Various metabolic, physiological, biochemical, and molecular events occurring within plants play a role in the drought stress reaction. During drought, the synthesis of reactive oxygen species (ROS), production of stress hormones, and changes in plant morphology occur in plants, forming mechanisms that resist stress (Ahluwalia et al., 2021). Plant continue their normal growth in the event of short-term stress, with reactions such as decreased growth, decrease in carbon assimilation, closure

of stomata, osmotic adaptation, and hydraulic changes in case of stress. However, prolonged stress decreases plant growth and metabolic functions, resulting in aging and death (Ahluwalia et al., 2021; Razi and Muneer, 2021). Photoassimilation, carbon fixation, and photosynthesis decrease under drought with a change in leaf structure and stomata closure (Ghadirnezhad Shiade et al., 2023). Osmolytes such as amino acids, organic acids, and polyols, which have low molecular weight, effectively maintain cellular functions under drought. Plant growth agents (auxins, gibberellins, cytokinins, salicylic acid, and abscisic acid) regulate a plant's response to drought. Polyamines and various enzymes reduce the adverse effects of water deficiency due to their antioxidant properties (Farooq et al., 2009; Farooq et al., 2012).

* Correspondence: ekincim@atauni.edu.tr

Plants have various mechanisms that provide resistance to adverse environments and protect development. Tolerance to various stresses is sometimes attributed to flavonoids or other phenolic compounds according to their functions in the cell wall and membrane (Treutter, 2006). Flavonoids are low molecular weight polyphenolic secondary metabolic compounds that play a role in plant growth, development, reproduction, and stress defense (Ma et al., 2014; Shah and Smith, 2020). Flavonoids are important in response mechanisms to many abiotic stress factors in higher plants. They protect plants by acting like UV filters, acting as allelopathic compounds, signaling molecules, phytoalexins, antimicrobial defense compounds, and detoxifying agents (Samanta et al., 2011). Flavonoids also play an important role in stressed plants by inhibiting or reducing the formation of ROS (Di Ferdinando et al., 2012). The basic structure of flavonoids is a three-phenolic ring structure consisting of A (6-carbon) and B (6-carbon) attached to the central C (3-carbon) ring (Aherne and O'Brien, 2002). Flavonoids are subdivided into flavones, flavonols, flavanones, flavanols, flavanonols, isoflavonoids, anthocyanins, and chalcones. In addition, according to the connection of the B ring to the C ring, flavonoids are divided into three main subgroups: flavonoids, isoflavonoids, and neoflavonoids (Samanta et al., 2011). Flavones, a flavonoid subgroup, are found in many vegetables and fruits. Chrysin (5,7-OH), apigenin (5,7,4'-OH), rutin (glycoside), and luteolin (5,7,3',4'-OH) are important flavones (Heim et al., 2002; Shah and Smith, 2020). Chrysin (5,7-dihydroxyflavone) belongs to the flavone class of polyphenolic compounds in the 15-carbon skeleton; passion fruit (*Passiflora* sp.), honey, propolis, mushrooms such as *Pleurotus ostreatus*, and other fruits and vegetables are the main natural sources of chrysin (Mani and Natesan, 2018; Naz et al., 2019). In the synthesis of chrysin, which is converted from the amino acid phenylalanine, phenylalanine is first converted to cinnamic acid by the phenylalanine ammonia-lyase enzyme, and the synthesis of chrysin occurs as a result of a series of enzymatic reactions (Mani and Natesan, 2018). Chrysin has estrogenic, antiinflammatory, antibacterial, antidiabetic, and antitumor effects in humans and plays many essential functions in protecting humans from various diseases. However, its effects on plants are not clearly understood (Naz et al., 2019; Ozcan et al., 2020).

Many strategies, such as selection, breeding, and exogenous application of various hormones, osmoprotectants, and biostimulants against plant drought stress, have been introduced in scientific studies (Yildirim et al., 2019; Ekinci et al., 2020; Yildirim et al., 2021; Turan et al., 2023). One of these biostimulants is the flavonoid, which plays a vital role in the stress response, with changes occurring in the plant's endogenous level with drought

stress. Flavonoids are effective in scavenging oxygen free radicals and alleviating stress-induced oxidative damage. In a study on maize, the authors found that flavonoids can provide drought tolerance by regulating stomatal movement and reducing oxidative damage caused by drought (Li et al., 2021). Gao et al. (2020) stated that drought stress promotes the accumulation of secondary metabolites such as flavonoids and supports drought resistance in plants by reducing H_2O_2 content. Flavonols are also involved in H_2O_2 accumulation and stomatal movement in guard cells under stress conditions in plants (Brunetti et al., 2019). However, it is unclear how the exogenous application of flavonoids affects plants. Although many studies exist on how flavonoids affect humans and animals, no studies have been conducted on their effect on plants, especially tomatoes. For this reason, this study aims to specify the effect of applying chrysin to tomato seedlings against drought, which has caused some of the most significant problems in agricultural production in recent years—and to determine the mechanism of increasing tolerance. The current study was planned with the hypothesis that the exogenous application of chrysin can improve drought tolerance in tomato seedlings.

2. Materials and method

2.1. Materials and experiment design

The study was carried out as a pot study in the controlled greenhouses of Atatürk University's Plant Production Application and Research Center. Tomato (*Solanum lycopersicon* L.) seedlings were initially cultivated in trays filled with peat in a perlite mixture (2:1, v:v). Once the seedlings reached the 2–3 leaf growth stage, they were transplanted into 2.5-liter pots. These pots were filled with a mixture of soil, peat, and sand (2:1:1; by volume). The growth conditions in the greenhouse were carefully controlled: humidity was maintained at $50\% \pm 5\%$, and the average temperature was $25\text{ }^\circ\text{C}$ during the day and $18\text{ }^\circ\text{C}$ at night, with a variation of $\pm 2\text{ }^\circ\text{C}$.

Shortly after transplanting the seedlings into pots, they were subjected to leaf spraying with three distinct concentrations of (C0: control with no treatment; C1: 0.1 mM; C2: 0.5 mM; and C3: 1.0 mM) (GR24-Chrysin synthetic analogue) ($\text{Chr-C}_{15}\text{H}_{10}\text{O}_4$, Thermo Fisher Scientific Chemicals, Waltham, MA, USA). This treatment regimen was administered thrice, each time at weekly intervals.

Irrigation water amounts were calculated using a soil moisture meter (WET Sensor, Delta-T Devices, Cambridge, UK). The soil moisture levels were checked before each irrigation, and the necessary amount of water was calculated to bring the available pot moisture up to field capacity (full irrigation; 100%) for control treatment. For water deficit treatments, the water amount was adjusted according to 60% of complete irrigation.

Water deficit treatment occurred one day after the chrysin application. Irrigation was performed every 2–3 days according to the measured soil moisture values. The study concluded 50 days after the commencement of the water treatments.

2.2. Measurements and analysis

At the end of the experiment, measurements were taken for plant fresh weight (PFW), plant dry weight (PDW), root fresh weight (RFW), root dry weight (RDW), plant height (PH), and stem diameter (SD). The material was dried at 67 °C for 48 h to determine dry weights. The electrolyte leakage (EL) of the plants was calculated as a percentage, as determined by Yildirim et al. (2021)

For leaf relative water content (LRWC) analysis, leaf discs were first weighed to obtain the fresh weight (FW). The discs were then placed in distilled water to determine their turgor weights (TW). The samples were then oven-dried and weighed to obtain the dry weight (DW) (Yildirim et al., 2021). The LRWC was calculated using the following equation: $LRWC (\%) = [(FW - DW)/(TW - DW)] \times 100$. The total leaf area (LA) of the plants was measured using a CI-202 Portable Laser Leaf Area Meter (CID Bio-Science, Camas, WA, USA).

Lipid peroxidation was assessed by measuring the content of malondialdehyde (MDA). The absorbance of the obtained supernatant was measured at 400, 500, and 600 nm, and the MDA concentration was determined from these absorbance values with an extinction coefficient of $155 \text{ mmol l}^{-1} \text{ cm}^{-1}$ (Shams et al., 2019).

The hydrogen peroxide (H_2O_2) content was measured following the method described by Velikova et al. (2000). The absorbance of the supernatant was read at 390 nm, and the H_2O_2 concentration was determined by referencing a standard calibration curve prepared with known H_2O_2 concentrations. The proline concentration was quantified using a spectrophotometer at 520 nm, according to Bates et al.'s method (1973).

For antioxidant enzyme activities, fresh leaf samples were homogenized in the extraction solution following the protocols outlined by Angelini et al. (1990) and Angelini and Federico (1989). The resulting supernatant was used to assess enzyme activities. Superoxide dismutase (SOD) activity was measured at 560 nm, catalase (CAT) at 240 nm, and peroxidase (POD) at 436 nm using spectrophotometric methods detailed by Abedi and Pakniyat (2010) and Angelini et al. (1990). Ascorbate peroxidase (APX) activity was assessed using techniques outlined by Mittler and Zilinskas (1993). Glutathione reductase (GR) and glutathione S-transferase (GST) activities were determined following protocols described by Chikezie et al. (2009) and Minucci et al. (2009).

The abscisic acid (ABA) content was analyzed following the methods described by Battal and Tileklioglu (2001) and

Kuraishi et al. (1991). ABA quantification was conducted using high-performance liquid chromatography (HPLC) with a Zorbax Eclipse-AAA C-18 column on an Agilent 1200 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA), and detection was performed at 265 nm using a UV detector, as detailed by Ekinici et al. (2014). Sugar extraction followed the procedure outlined by Nikolidaki et al. (2017). Subsequently, sugar analysis was performed using an Agilent HPLC system (HP1100, Agilent Technologies Inc., Santa Clara, CA, USA).

Amino acid analysis was conducted using an Agilent HPLC system (HP1100, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a diode array detector. A Zorbax Eclipse AAA analytical column (Agilent Technologies Inc., Santa Clara, CA, USA) was employed for amino acid determination, following the methodology described by Henderson et al. (1999).

Phenolic compounds were quantified using reversed-phase high-performance liquid chromatography (HPLC) equipment (Shimadzu Scientific Instruments, Tokyo, Japan). The phenolic profiles of the extracts were determined using an adapted protocol derived from Schulz et al. (2015). The concentration of each phenolic compound was calculated in micrograms per gram of extract. Organic acid analysis was done with the HPLC equipped with a pump system and a UV/Vis detector (SPD-20A) monitored at 210 nm (Ekinici et al., 2014).

This study, conducted in a greenhouse environment, employed a factorial experimental design and a completely randomized trial setup. Statistical analysis was performed using ANOVA in SPSS (IBM Corps., Chicago, IL, USA), and differences between means were assessed using Duncan's multiple comparison test. Pearson correlation analysis (PCA) was employed to examine relationships between variables and explore interrelationships among variables.

3. Results and discussion

The effect of chrysin treatment on tomato seedlings under drought stress revealed important effects on plant growth and development parameters and some bioactive components.

Tomato seedlings are sensitive to drought, and it was determined that stress caused a decline in the growth and development of the tomato seedlings in this study. Previous research has shown that drought causes a decrease in plant growth in tomatoes (Pervez et al., 2009; Liang et al., 2020; Ors et al., 2021). With drought, PFW, PDW, RFW, and RDW decreased by 35%, 56%, 23%, and 20%, respectively, in this study (Figure 1). The decrease in water content under drought stress causes a decline in leaf water potential, loss of turgor, closure of stomata, and a decrease in cell expansion and growth, thus causing adverse effects on plants (Jaleel et al., 2009; Yang et al.,

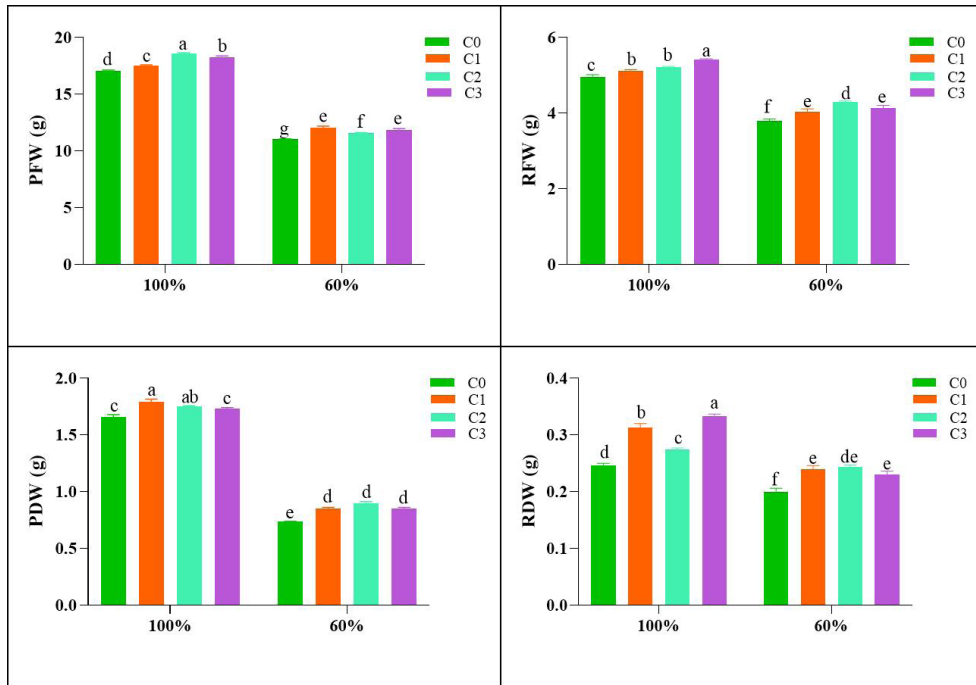


Figure 1. Effects of chrysin on plant fresh weight (PFW), plant dry weight (PDW), root fresh weight (RFW), and root dry weight (RDW) of tomato seedlings under different irrigation levels. No statistical differences exist between the means shown with the same letter in the bars ($p < 0.001$).

2021). Developed roots help the plant to take up and use water in the soil; therefore, it is effective in the survival of plants during periods of drought (Yang et al., 2021). However, these reductions under drought with exogenous chrysin treatments (C1, C2, and C3) were lower. With the applications, lower rates of reductions occurred at 29%–32%, 46%–49%, 13%–19%, and 4%–8% in PFW, PDW, RFW, and RDW, respectively, compared to C0 (control) (Figure 1).

PH, SD, LA, and LRWC with no treatments (C0) decreased by 25%, 12%, 24%, and 10%, respectively, while EL increased by 84% under drought (Figures 2 and 3). Plant height affected by drought is associated with cell growth and leaf senescence, and the causes of plant height reduction are a decrease in cell expansion under drought, an increase in leaf shedding, and disruption in mitosis (Yang et al., 2021). Leaves, important for plant assimilation and transpiration, usually have greater leaf thickness, a smaller leaf area, and higher leaf tissue density to acclimate to drought (Werner et al., 1999; Yang et al., 2021). Water stress causes low turgor pressure and prevents cell expansion and growth in plants (Jaleel et al., 2009). Decreased soil water potential under drought causes a decrease in the number of leaves, leaf size, and leaf lifespan. This decrease in leaf area results from a suppression of leaf expansion by drought and a decrease in photosynthesis (Anjum et al., 2011). With water

deficiency, the flow of water from the xylem to the nearby elongating cells in plants is interrupted, preventing cell elongation; in addition, the decrease in cell expansion and elongation causes a decrease in growth and productivity characteristics (Nonami, 1998; Hussain et al., 2008; Anjum et al., 2011). With chrysin treatment, the decrease in these parameters was less significant in plants under drought conditions. PH, SD, and LA decreased by 11%–14%, 6%–9%, and 7%–14%, respectively, with chrysin treatment (Figure 2). The change in LRWC and EL was less significant with chrysin treatment than in the control group (Figure 3). In general, it can be said that a 0.5 mM dose (C2) is the correct application for the parameters mentioned above. Similarly, luteolin, a flavone-like chrysin, increased germination and seedling growth in corn under normal conditions and mitigated salt stress damage (El-Shafey and AbdElgawad, 2012).

ABA plays an important role in plant reactions to stress; it accumulates in response to stress and mediates stress responses that help plant survival (Zhang et al., 2006). With decreased leaf water potential or turgor parallel to increasing water deficiency, ABA biosynthesis in leaves and ABA concentration in all leaf tissues, including guard cells, increase (Saradadevi et al., 2017). ABA regulates stomatal closure, reduces water transpiration, and improves drought resistance; a high ABA content in tolerant plants can achieve this process (Zhang et al.,

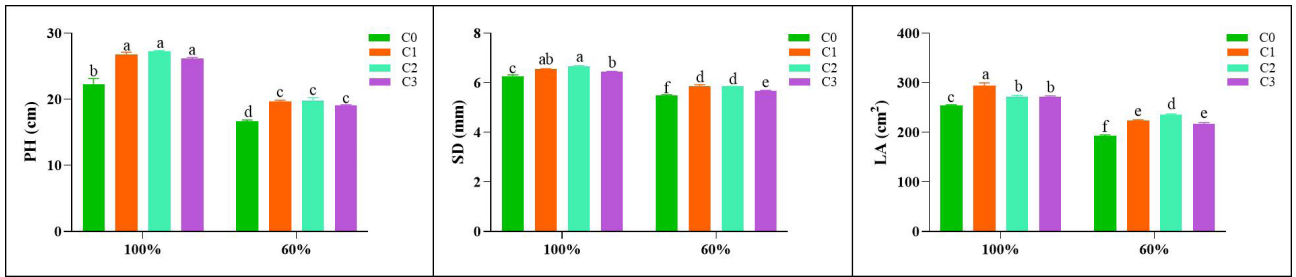


Figure 2. Effects of chrysin on plant height (PH), stem diameter (SD), and leaf area (LA) of tomato seedlings under different irrigation levels. No statistical differences exist between the means shown with the same letter in the bars ($p < 0.001$).

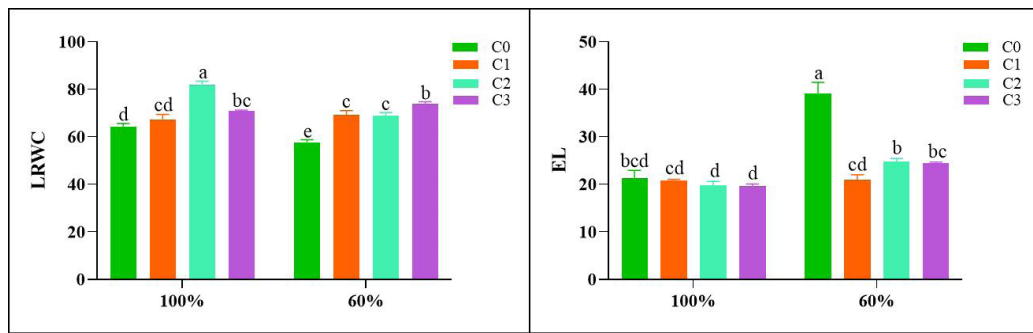


Figure 3. Effects of chrysin on the leaf relative water content (LRWC) and electrical leakage (EL) of tomato seedlings under different irrigation levels. No statistical differences exist between the means shown with the same letter in the bars ($p < 0.001$).

2020). However, the ABA content, which increases with drought, has an important impact on plants like soybeans and tomatoes (Dong et al., 2019; Liu et al., 2022; Turan et al., 2023). When the results regarding ABA are evaluated, while the ABA content also increased with water stress, this increase was lower during the chrysin treatment in this study (Figure 4). The reason may be that chrysin application reduces the effect of stress on tomatoes.

Under stress, the production of ROS such as singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals is accelerated. Excessive production of these ROS with stress is due to the disruption of electron transport processes in mitochondria and chloroplasts. Therefore, plants respond to stress by strengthening various endogenous mechanisms and these defense mechanisms to adapt to ROS production (Hasanuzzaman et al., 2013). A study on sugar beet showed a significant increase in leaf proline, MDA, H_2O_2 , APX, CAT, and POD enzymatic activities with drought stress (Ghaffari et al., 2019). There was an increase in H_2O_2 and MDA under water deficit (75% and 69%, respectively). However, the increase in H_2O_2 and MDA content under stress conditions was at the lowest level at the 1.0 mM dose (C3) (Figure 4). Flavonoids can scavenge active oxygen species, and the superoxide anion radical does not easily diffuse into the vacuoles where flavonoids are localized from the chloroplast (Samanta et al., 2011).

Researchers have also noted that the flavonoid peroxidase reaction functions as an H_2O_2 scavenging mechanism, and flavonoids act as detoxifying agents (Samanta et al., 2011).

Increasing resistance to drought is a strategy that increases the functions of antioxidant components and reduces or prevents oxidative damage (Hasanuzzaman et al., 2013). When plants are exposed to stress, SOD enables the removal of superoxide by converting it to O_2 and H_2O_2 ; on the other hand, CAT converts H_2O_2 into water and oxygen, and POD functions in the extracellular space and clears H_2O_2 . GR catalyzes the decrease of oxidized glutathione to reduced glutathione, while APX scavenges H_2O_2 into water using ascorbate (Rajput et al., 2021). In several other studies, the antioxidant enzymes activity increased under stress, the activity of CAT, SOD, and GPX enzymes in chickpeas was significantly affected (Mohammadi et al., 2011), and the activity of CAT, APX, and GR in melons changed with stress (Kavas et al., 2013). Drought-tolerant plants have higher levels of antioxidants, which reprogram their metabolism and increase their antioxidant capacity under stress conditions; sensitive species also activate their antioxidant systems because an antioxidant capacity serves an essential function in drought tolerance and response to drought (Laxa et al., 2019).

Similarly, there was an increase in antioxidant enzyme activity (except for POD) with drought in this study.

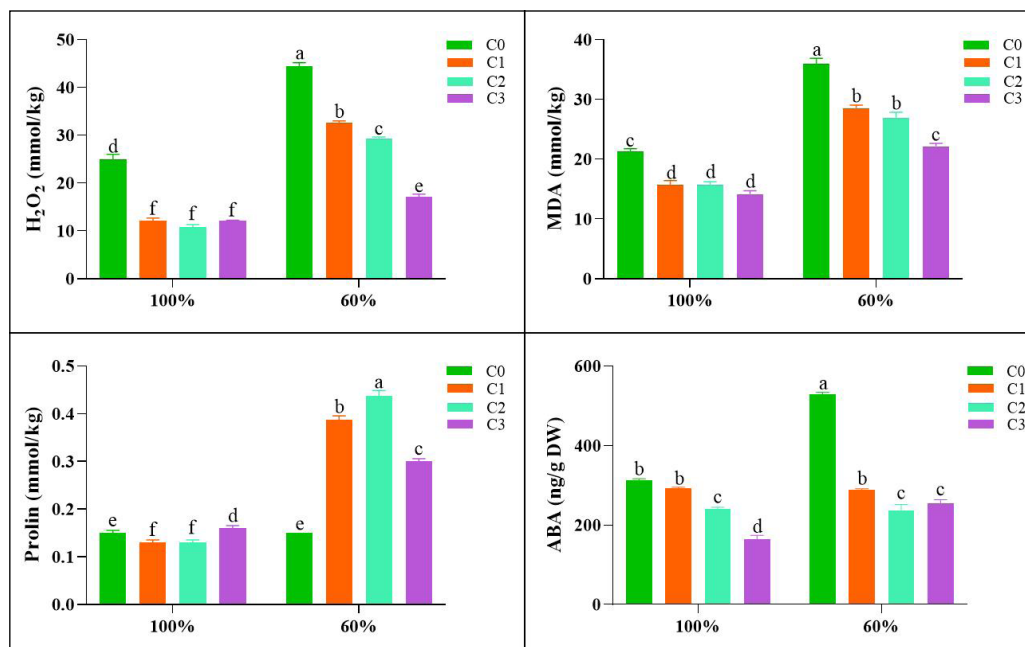


Figure 4. Effects of chrysin on the H₂O₂, MDA, proline, and ABA content of tomato seedlings under different irrigation levels. No statistical differences exist between the means shown with the same letter in the bars ($p < 0.001$).

However, the chrysin treatment caused changes in enzyme activity under both normal and drought conditions. Under drought stress, antioxidant enzyme activity with chrysin treatment was lower than in the control treatment, in which the plants did not go through chrysin application. Under stress conditions, the CAT, SOD, and APX contents were lower with the 1.0 mM treatment, while GR with the 0.5 mM treatment and GST with the 0.1 mM treatment were at lower levels (Figure 5). Flavonoids scavenge ROS in plants under biotic and abiotic stresses with their antioxidant properties (Baskar et al., 2018). Flavonols act as ROS scavengers and inhibitors of auxin transport in various cell and tissue types to regulate plant development and responses to stress (Daryanavard et al., 2023). Flavonols have a strong antioxidant capacity due to their ability to neutralize singlet oxygen and hydrogen peroxide (Csepregi and Hideg, 2018). Flavonols can affect growth and development by inhibiting auxin transport and regulating the distribution of this hormone. In addition, due to their antioxidant capacity, flavonols play a protective role in plant reaction to abiotic stresses such as drought (Daryanavard et al., 2023).

A PCA analysis was performed considering the effects of the applications on several morphological, physiological, and biochemical variables during the deficit period. The PCA biplot of the first two components (PC1 and PC2) explained 87.09% of the variance for the tested variables (Figure 6, Table 1). Specifically, a negative

correlation occurred in the EL, proline, H₂O₂, MDA, POD, CAT, SOD, GR, GST, and APX parameters (PC1). The control (C0) treatment under drought was more affected by these parameters. All applications under full irrigation treatments significantly affected PFW, RFW, PDW, RDW, PH, SD, and LA. While different doses of chrysin treatment under drought stress were included in the same category, the control treatment occurred in a separate place.

Various morphological and structural changes occur in plants that alleviate drought stress. In addition, various drought response mechanisms are activated, such as gene expression, hormone synthesis, and the synthesis of osmotic regulatory substances (Yang et al., 2021). In plants, low water potential produces osmoregulation, which is formed by the synthesis and accumulation of various osmoprotectants or osmolytes (such as sugars, soluble proteins, ammonium compounds, and amino acids such as proline) (Öztürk et al., 2021). These osmoprotectants and osmolytes are small molecules that protect membranes and proteins under abiotic stress conditions (Munns, 2002).

In this study, there was an increase in phenolic compounds (except ferulic acid and quercetin) in the plants under drought stress. Treatments of chrysin increased phenolic compounds in both unstressed and stressed plants. While the 1.0 mM chrysin treatment produced the highest values in gallic acid, vanillic acid, transcafeic acid, trans-p-cumaric acid, kaftaric acid, catechin, epicatechin, rutin, myricetin, and mlvidin-3-o-glucoside contents, the

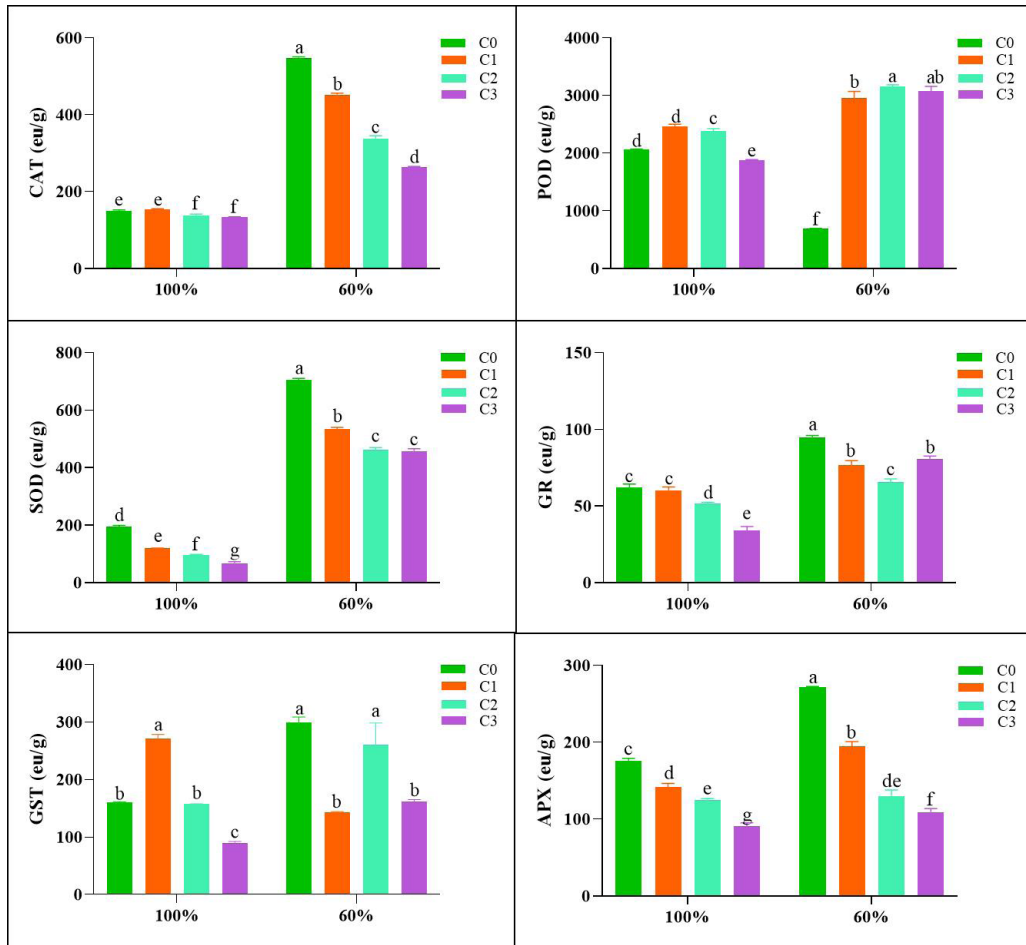


Figure 5. Effects of chrysin on the CAT, POD, SOD, GR, GST, and APX activity of tomato seedlings under different irrigation levels. No statistical differences exist between the means shown with the same letter in the bars ($p < 0.001$).

0.1 mM treatment resulted in the highest values in terms of tyrosol. While ferulic acid and quercetin decreased with water stress, the 0.1 mM chrysin treatment was the highest (Figure 7). Many phenols play a role in plant growth by helping develop cell walls. Hydroxycinnamic acids, such as p-coumaric and ferulic acid, are found as esters or insoluble in the cell wall fraction. Phenolic acids, which have large molecular numbers, play a role in functions that cause changes in cell wall structure, turgor pressure, water flow, and growth (Pratyusha, 2022). During drought stress, plants produce polyphenols to control water ion flow and cope with stress conditions (González-Chavira et al., 2018). Increases in plant phenol content during drought stress prevent further water loss through the closure of stomata, maintaining redox homeostasis and creating a tolerance mechanism to improve plant health and prevent oxidative damage (Pratyusha, 2022). During drought, phenylpropanoid pathway enzymes regulate the biosynthesis and accumulation of phenolic compounds. Changes in the transcription level of genes

encoding phenylalanine ammonia-lyase and chalcone synthase are effective in regulating the activity of these enzymes and in the synthesis of phenolics as a response to various stresses (Chowdhary et al., 2021). According to a study conducted on grapevines, the content of caffeic acid in roots and leaves increased, and the content of ferulic acid and phenolic acids decreased under drought stress (Król et al., 2014). Flavonoids and polyphenols are natural compounds that enable ROS scavenging in plants under drought stress. Plant phenolic compounds and flavonoids are important in the stress tolerance mechanism. It has been determined that some leaf amaranth vegetables with drought tolerance contain significant amounts of phenols and flavonoids (such as caffeic acid, catechin, ferulic acid, gallic acid, myricetin, quercetin, p-coumaric acid, rutin, trans-cinnamic acid, vanillic acid, etc.) (Sarker and Oba, 2020).

During drought stress, there is an increase in the levels of various compounds, such as sugars, that have osmoprotective functions in plants, preventing the

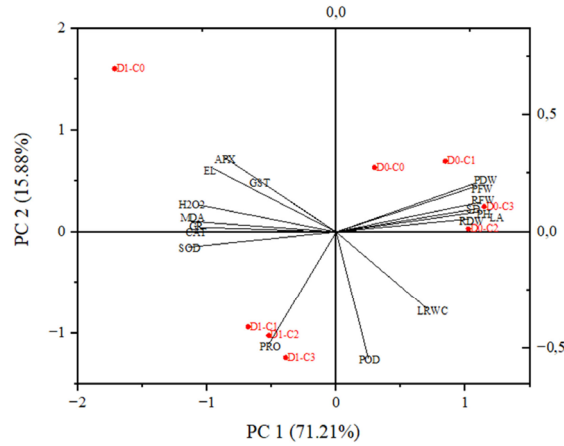


Figure 6. Principle component analysis (PCA) of some morphological, physiological, and biochemical parameters of tomato seedlings under different irrigation levels. PFW: plant fresh weight; RFW: root fresh weight; PDW: plant dry weight; RDW: root dry weight; PH: plant height; SD: stem diameter; LA: leaf area; LRWC: leaf relative water content; EL: electrolyte leakage; H₂O₂: hydrogen peroxide; MDA: malondialdehyde; PRO: proline; CAT: catalase; POD: peroxidase; SOD: superoxide dismutase; GR: glutathione reductase; GST: glutathione-S-transferase; APX: ascorbate peroxidase.

Table 1. Coefficients for principal components.

Variable	PC 1	PC 2
Proportion of variance	71.21%	15.88%
Eigen value	12.82	2.86
PFW	0.26	0.19
RFW	0.27	0.13
PDW	0.26	0.21
RDW	0.24	0.06
PH	0.27	0.08
SD	0.27	0.10
LA	0.26	0.07
LRWC	0.17	-0.33
EL	-0.23	0.27
H ₂ O ₂	-0.26	0.12
MDA	-0.27	0.05
PRO	-0.13	-0.48
CAT	-0.26	0.01
POD	0.06	-0.54
SOD	-0.28	-0.07
GR	-0.26	0.02
GST	-0.14	0.22
APX	-0.21	0.32

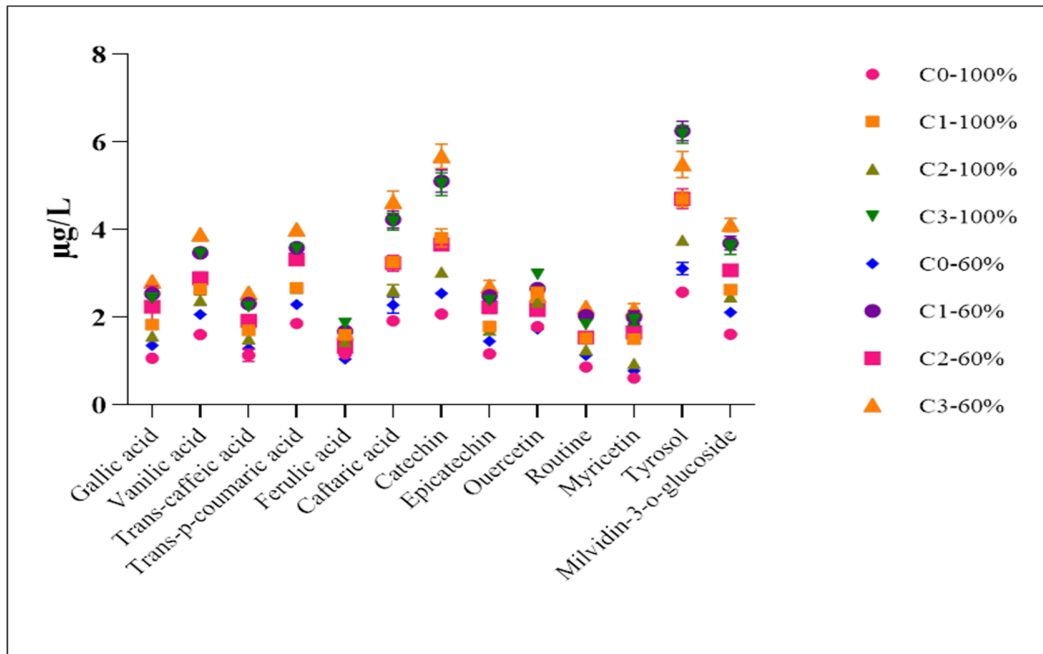


Figure 7. Effects of chrysin on the phenolic compounds of tomato seedlings under different irrigation levels.

degradation of enzymes and reducing the amount of ROS (Poonam et al., 2016; Ozturk et al., 2021). ROS are produced in plants as the end compounds of sugars and photosynthesis, which are necessary for normal plant metabolism and growth; these increase under stress as various sugar molecules ensure osmoregulation (Poonam et al., 2016). Under drought stress, the concentration of reduced form sugars (fructose, glucose, sucrose, and fructans) and sugar alcohols—also called polyols (such as myoinositol, pinitol, sorbitol, mannitol, xylitol, and ribitol)—is high, and these types of sugars function as osmoprotectants (Singh et al., 2015). Similarly, in our study, an increase was observed in the sugar content of tomatoes under drought stress. In the stressed tomato plants, there was a 25%, 22%, 39%, 25%, 34%, 44%, and 22% increase in arabinose, fructose, galactose, glucose, rhamnose, sucrose, and xylose contents, respectively. Chrysin treatments increased the sugar content in both the unstressed and stressed plants. The highest content of arabinose, fructose, galactose, glucose, rhamnose, and sucrose was obtained with an application of 1.0 mM chrysin; the highest xylose content was determined from an 0.5-mM chrysin treatment (Figure 8) under drought. In another study, the authors found that luteolin flavone stimulates α -amylase activity, which increases the accumulation of soluble sugars and starch mobilization and strengthens antioxidative defense (El-Shafey and AbdElgawad, 2012).

Chrysin effectively increases the response of organic acids (especially succinic, malic, and galacturonic acids) in plant metabolism to long-term drought (Khan et al.,

2020). It plays a role in the stress response mechanism in plants through changes in the organic acid content with stress, and this has been previously determined in wheat (Khosravi-Nejade et al., 2022), chickpeas (Khan et al. 2019), and pepper (Sariyer and Kuzucu, 2019). The organic acid contents (except maleic acid) of the tomato seedlings decreased with water deficit in this study. Oxalic, propionic, tartaric, butyric, malonic, malic, lactic, citric, fumaric, and succinic acids contents with water deficit decreased by 21%, 39%, 25%, 23%, 43%, 37%, 33%, 35%, 51%, and 32%, respectively. However, chrysin application increased the organic acid content in the plants under both stress and normal conditions. While the organic acid content, especially 0.5 mM chrysin, was at the highest level in the unstressed environment, almost all chrysin doses positively affected the stressed plants; however, the results were higher with the 0.1 mM and 1.0 mM chrysin doses (Tables 2 and 3). After organic acids are synthesized in the cell by a photosynthetic (by fixing carbon) or nonphotosynthetic (using phosphoenolpyruvate carboxylase) pathway, they circulate among different plant parts through various transporters for functions such as xylem loading of minerals, maintenance of cell pH, redox balance, and drought tolerance (Panchal et al., 2021). In our study, we observed that plants can tolerate drought stress owing to increased organic acid content with its applications.

Amino acids serve as regulatory and signaling molecules. They are effective as precursors of other organic compounds, such as nucleic acids and proteins, that play a

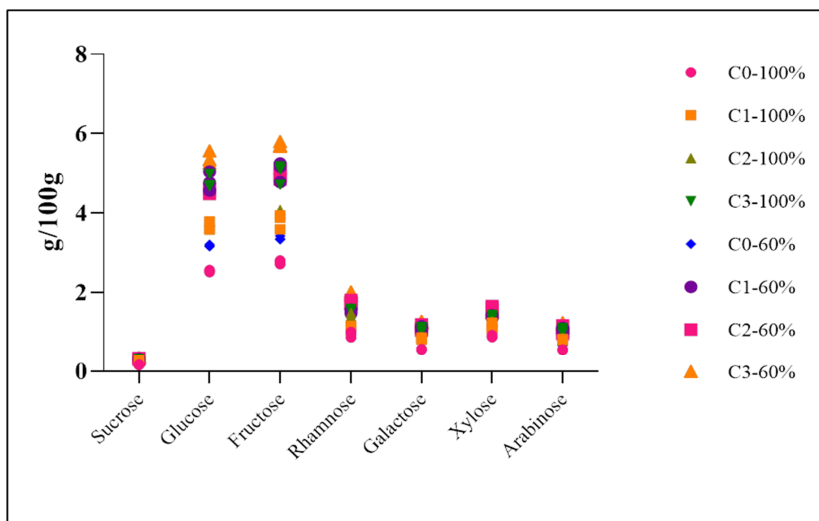


Figure 8. Effects of chrysin on the sugar content of tomato seedlings under different irrigation levels.

Table 2. Effects of chrysin on the organic acid content of tomato seedlings under different irrigation levels (mg/μg).

Irrigation (%FC)	Chr (mM)	Oxalic acid	Propionic acid	Tartaric acid	Butyric acid	Malonic acid	Malic acid
100	0	5.71 c	13.12 c	22.20 c	64.47 c	66.89 bc	25.70 b
	0.1	7.34 b	13.70 c	25.02 c	66.92 c	63.11 c	23.72 bc
	0.5	8.87 a	19.42 a	32.13 a	92.10 a	93.66 a	39.12 a
	1.0	7.43 b	15.61 b	28.69 b	76.89 b	68.75 b	26.10 b
60	0	4.53 d	8.06 d	16.64 d	49.53 d	38.41 e	16.23 ef
	0.1	6.06 c	13.80 c	22.84 c	61.76 c	62.80 c	22.06 cd
	0.5	5.65 c	12.41 c	24.46 c	65.97 c	54.89 d	15.19 f
	1.0	6.04 c	12.09 c	22.28 c	68.90 c	50.85 d	19.27 de

No statistical difference exists between the means shown with the same letter in the same column ($p < 0.001$).

Table 3. Effects of chrysin on the organic acid content of tomato seedlings under different irrigation levels (mg/μg)

Irrigation (%FC)	Chr (mM)	Lactic acid	Citric acid	Maleic acid	Fumaric acid	Succinic acid
100	0	88.18 cd	86.46 bc	19.23 c	31.93 b	93.35 cd
	0.1	99.48 bc	78.53 c	38.74 a	26.27 cd	101.33 bc
	0.5	129.28 a	122.06 a	28.86 b	44.19 a	130.51 a
	1.0	112.59 b	90.29 bc	42.08 a	22.77 cd	110.90 b
60	0	59.05 e	56.28 d	21.75 c	15.77 f	63.00 f
	0.1	87.97 cd	91.71 b	20.27 c	27.95 bc	75.38 e
	0.5	80.44 d	89.80 bc	16.91 c	17.30 ef	102.67 bc
	1.0	91.09 cd	87.77 bc	29.87 b	21.55 de	83.40 de

No statistical difference exists between the means shown with the same letter in the same column ($p < 0.001$).

functional role in plant reactions under stress conditions. Amino acid accumulation significantly affects stress tolerance in terms of ROS detoxification, pH regulation, and contribution to osmotic adjustment (Khan et al., 2020). In our study, with water stress, the aspartate, serine,

glutamate, glutamine, threonine, histidine, glycine, arginine, tyrosine, alanine, cystine, valine, isoleucine, methionine, tryptophan, leucine, phenylalanine, lysine, hydroxyproline, proline, and sarcosine contents decreased at rates of 38%, 86%, 83%, 48%, 34%, 88%,

91%, 49%, 40%, 36%, 44%, 61%, 33%, 48%, 48%, 22%, 45%, 64%, 66%, 59%, and 54% compared to the control, respectively. However, chrysin treatments increased amino acids in both the unstressed and stressed plants, and significant results were obtained, especially with a 0.5 mM chrysin application (Tables 4, 5, and 6). Amino acids are included in pH adjustment and the detoxification of ROS by acting as compatible osmolytes; they also have a functional impact on plant

stress tolerance by acting as a carbon and nitrogen supply during the synthesis of certain enzymes and their precursors. In addition, the protective roles they play by acting as regulatory and signaling molecules show a correlation between the level of amino acid and stress tolerance (Ali et al., 2019).

Changes in the amino acid content under drought stress have also been observed in maize, wheat, and cabbage in previous research (Ranieri et al., 1989;

Table 4. Effects of chrysin on the amino acid content of tomato seedlings under different irrigation levels (pmol/ μ L).

Irrigation (%FC)	Chr (mM)	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Glycine
100	0	68.01 d	179.78 c	374.75 e	353.04 c	204.87 c	91.77 b	101.12 a
	0.1	75.20 c	229.26 b	372.20 e	358.44 c	265.92 b	87.81 b	77.32 c
	0.5	100.77 a	255.43 a	555.24 d	518.68 a	289.37 a	133.20 a	94.47 a
	1.0	79.36 b	236.31 b	387.25 e	384.98 b	301.02 a	93.50 b	84.62 b
60	0	41.99 f	93.38 f	591.60 d	48.05 f	35.02 e	11.05 c	9.31 f
	0.1	57.73 e	109.69 ef	1110.01 b	77.36 e	41.75 e	16.76 c	17.84 e
	0.5	74.43 c	130.41 d	1543.77 a	106.68 d	64.86 d	17.53 c	28.44 d
	1.0	57.65 e	117.81 de	812.15 c	63.61 ef	46.36 de	15.03 c	12.32 ef

No statistical difference exists between the means shown with the same letter in the same column ($p < 0.001$).

Table 5. Effects of chrysin on the amino acid content of tomato seedlings under different irrigation levels (pmol/ μ L).

Irrigation (%FC)	Chr (mM)	Theonine	Arginine	Alanine	Tyrocine	Cystine	Valin	Methionine
100	0	208.29 cd	362.48 b	214.72 c	231.60 c	35.40 c	26.06 b	71.20 bc
	0.1	203.45 cd	289.27 c	232.56 bc	230.40 c	26.72 d	14.81 cd	62.49 c
	0.5	293.29 a	464.67 a	306.77 a	330.88 a	50.58 b	37.24 a	104.54 a
	1.0	222.65 bc	301.79 c	254.52 b	255.48 bc	29.24 d	16.21 c	66.55 bc
60	0	138.05 e	185.53 e	137.48 e	138.68 e	19.88 e	10.20 d	36.99 e
	0.1	201.54 cd	289.73 c	181.53 d	204.32 d	35.21 c	27.76 b	65.47 c
	0.5	246.64 b	361.45 b	247.94 b	263.95 b	66.33 a	37.42 a	75.47 b
	1.0	182.75 d	238.81 d	184.25 d	183.59 d	26.31 d	13.50 cd	50.32 d

No statistical difference exists between the means shown with the same letter in the same column ($p < 0.001$).

Table 6. Effects of chrysin on the amino acid content of tomato seedlings under different irrigation levels (pmol/ μ L).

Irrigation (%FC)	Chr (mM)	Tryptophan	Phenylalanine	Isoluecine	Leucine	Lysine	Hydroxyproline	Sarcosine	Proline
100	0	45.45 b	66.09 c	46.78 bc	102.21 b	127.37 b	84.58 b	164.03 c	12.66 b
	0.1	35.83 d	85.96 b	48.31 bc	69.18 cd	63.69 cd	33.71 de	108.26 d	8.03 cd
	0.5	64.93 a	96.80 a	71.60 a	68.70 cd	77.21 c	33.44 de	224.83 a	18.09 a
	1.0	39.21 cd	94.07 ab	52.87 b	73.66 c	69.70 cd	36.89 de	118.48 d	8.79 c
60	0	23.44 f	51.75 d	31.14 d	55.81 d	45.43 e	29.14 e	77.52 e	5.24 e
	0.1	43.57 bc	63.12 c	49.99 b	113.30 ab	129.68 b	101.62 a	157.32 c	12.11 b
	0.5	65.95 a	94.01 ab	66.11 a	125.55 a	185.34 a	64.63 c	204.74 b	11.16 b
	1.0	31.03 e	68.51 c	41.22 c	75.94 c	60.14 d	38.58 d	102.63 d	6.94 d

No statistical difference exists between the means shown with the same letter in the same column ($p < 0.001$).

Shawon et al., 2018; Yadav et al., 2019; Haghghi et al., 2020); in these studies, the authors stated that amino acids were involved in the tolerance mechanism under stress conditions. In the current study, both drought stress and applications caused changes in the amino acid content, and the chrysin application was especially important in terms of its effect on the amino acid content in increasing drought stress tolerance.

4. Conclusion

This study determined that an exogenous chrysin treatment could alleviate the damage induced by drought stress in tomato seedlings. A chrysin treatment changes many vital functional properties of plants. Exogenous applications can also achieve the effect of flavonoids on plants' internal mechanisms under stress conditions; this effect may vary depending on the properties of the applied flavonoid and dose. In this sense, the chrysin

flavone used in this study may have provided stress tolerance, but more research needs to be conducted to fully understand its mechanism of action. Therefore, collecting more information on its application would be beneficial to better understand how the mechanism functions and its effectiveness.

Author contributions

All authors contributed to the designing, conducting, analyzing, and writing of the manuscript.

Declaration of competing interest

The authors declare that they have no competing financial interests or conflict of interest.

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