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Water Stress Effects on Phenolic and Antioxidant Capacity in ‘Pink Lady’ and ‘Jeromine’ Apples

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Abstract

Drought is a major abiotic stress limiting fruit yield and quality in apple (*Malus × domestica* Borkh.), especially in semi-arid regions. This study evaluated the effects of drought stress on total phenolic content (TPC) and total antioxidant capacity (TAC) in two apple cultivars, 'Pink Lady' and 'Jeromine', grafted onto M9 rootstock and grown under the arid summer conditions of Karaman, Turkey, during the 2023–2024 seasons. Fruit samples were analyzed separately as flesh and peel + flesh tissues. Drought stress significantly increased TPC and TAC levels in both cultivars, with stronger responses in 'Pink Lady'. The highest TPC (220.4 mg GAE/100 g FW) and TAC (159.1 µmol TE/100 g FW) were recorded in 'Pink Lady' peel + flesh tissue in 2024. Peel tissue contributed substantially to the overall antioxidant potential. These results indicate that cultivar selection plays a key role in maintaining fruit quality under drought conditions. 'Pink Lady' showed superior biochemical responses, suggesting its suitability for cultivation in drought-prone regions. Enhanced phenolic and antioxidant profiles may also improve the nutritional value and postharvest stability of the fruit.

1. Introduction

Apple (*Malus × domestica* Borkh.) is among the most widely cultivated fruit crops globally, grown across temperate regions on every continent except Antarctica (Luby, 2003). Owing to its nutritional value, ease of processing, and wide range of uses from fresh consumption to juice and fermented products, the apple holds an essential place in both commercial agriculture and dietary habits. As of 2024, global apple production reached approximately 98 million tons, while the total harvested area exceeded 4.7 million hectares, highlighting the continued global importance of apple cultivation. China remains the leading producer worldwide, followed by Türkiye and the United States, which together account for a substantial share of global apple production (FAOSTAT, 2026).

In recent years, the increasing frequency of drought episodes, driven by climate change, has imposed significant challenges on fruit production, particularly in semi-arid regions such as Central Anatolia. Drought, a prominent abiotic stress factor, adversely affects plant growth and productivity through morphological, physiological, and biochemical alterations (Nader, 2019; Faizi & Öztürk, 2022). Apple cultivars exhibit variable responses to water deficit, depending on genetic background and rootstock interaction (Gür, 2018; Qi et al., 2019). M9 rootstock, known for its dwarfing effect and precocity, has also demonstrated moderate tolerance to drought stress in several comparative studies (Gryazev et al., 1980; Kaynas et al., 1995).

One of the adaptive responses of plants to drought is the enhanced biosynthesis and accumulation of secondary metabolites such as phenolic compounds and antioxidants. These compounds play crucial roles in mitigating oxidative damage caused by reactive oxygen species (ROS) during water stress (Noctor et al., 2018; Wang et al., 2018). Phenolic compounds, including flavonoids, phenolic acids, and tannins, not only contribute to fruit quality and flavor but also serve as biomarkers for stress tolerance and defense responses (Lattanzio, 2003; Usenik et al., 2004).

The distribution of phenolic compounds in apples varies significantly between tissues and cultivars. For instance, apple peels often contain higher levels of flavonols such as quercetin glycosides, while the flesh is generally rich in hydroxycinnamic acids like chlorogenic acid (Chai et al., 2012; McGhie et al., 2005). The total phenolic content and antioxidant capacity are influenced by environmental factors, including light exposure, water availability, and altitude (Marks et al., 2007; Wang et al., 2015). Several studies have demonstrated that drought stress can increase total phenolics and antioxidant capacity in apple fruit as part of an acclimation mechanism (Liang et al., 2018; Dost et al., 2020).

Given the increasing severity of drought in regions like Karaman, it is imperative to assess how commercially important apple cultivars respond to such stress at the phytochemical level. This study was conducted to investigate the effects of low humidity and arid summer conditions on the total phenolic content and antioxidant activity in the fruits of ‘Pink Lady’ and ‘Jeromine’ apple cultivars grafted onto M9 rootstock. These findings aim to contribute to cultivar selection strategies and sustainable orchard management practices in arid zones.

2. Materials and Methods

2.1. Study site and climatic conditions

This study was conducted during the 2023–2024 vegetation periods at the Apple Research and Application Center of Karamanoğlu Mehmetbey University, located in Karaman province, Central Anatolia, Türkiye. Karaman is characterized by a semi-arid, continental climate with low summer humidity and limited precipitation. According to long-term meteorological data, the region receives an annual average rainfall of approximately 340 mm, most of which occurs during the spring months. Summer months are typically hot and dry, with temperatures frequently exceeding 35°C (MGM, 2023). These climatic conditions provide a representative environment for studying drought-related stress in fruit crops and are commonly considered suitable for stress physiology research (Reid & Kalcsits, 2020).

2.2. Plant material

The experiment involved two commercial apple (Malus × domestica Borkh.) cultivars, ‘Pink Lady’ and ‘Jeromine’, grafted onto M9 dwarfing rootstock. All experimental trials were conducted in the experimental orchard of the Apple Research and Application Center at Karamanoğlu Mehmetbey University (Karaman, Central Anatolia, Türkiye). All apple fruit samples used in this study were collected exclusively from this university's orchard.

The orchard was established in 2019 using certified apple saplings obtained from officially registered commercial nurseries. Both cultivars were planted as named and registered cultivars, and cultivar identity was verified based on certified planting and registration records maintained by the university at the time of orchard establishment. The orchard was designed using a standard plantation layout (4 m × 1 m).

The plant materials used in this study were cultivated crops and were not collected from the wild; therefore, no specific permissions or licenses were required for sample collection. The studied apple cultivars are not listed under the Convention on the Trade in Endangered Species of Wild Fauna and Flora (CITES). All experimental procedures involving plant material were conducted in accordance with institutional, national, and international guidelines, as well as local agricultural regulations.

M9 rootstock was selected due to its precocity, uniform growth habit, and moderate tolerance to water stress, as reported in previous studies (Gryazev et al., 1980; Kaynas et al., 1995). Trees were maintained under standard horticultural practices, and weed, pest, and disease management was performed according to integrated pest management (IPM) protocols.

2.3. Experimental design and stress application

The experiment was conducted using a randomized complete block design (RCBD) with three replications. Trees were randomly selected for each apple cultivar, and each replication consisted of ten trees per cultivar. To evaluate the effects of drought stress, two irrigation regimes were applied. Control trees were irrigated regularly with 5 L of water every 5 days, in accordance with evapotranspiration demand, whereas drought-stressed trees received 5 L of water every 15 days to induce moderate water deficit conditions, following the methodology described by Liang et al. (2018) and Wang et al. (2015).

Drought stress was imposed during the peak summer period (July–August). However, due to the occurrence of high air temperatures during this period, all trees were subsequently returned to a standard irrigation regime until harvest to prevent irreversible physiological damage.

Harvests were performed at the physiological maturity stage of each cultivar; ‘Jeromine’ was harvested in October, while ‘Pink Lady’ was harvested in November.

2.4. Sampling and fruit analysis

Harvesting was conducted according to the physiological maturity of the cultivars; ‘Jeromine’ was harvested in October and ‘Pink Lady’ in November. From each tree, ten uniform and healthy fruits were randomly selected for juice analyses. Fruits were processed under sterile conditions using a mechanical juice extractor, and the obtained juices were clarified by filtration.

Soluble solids content (SSC) was determined using a digital refractometer and expressed as °Brix, while titratable acidity (TA) was measured by titration with 0.1 N NaOH and expressed as % malic acid equivalent. For subsequent biochemical analyses, two fruit samples per replicate were stored at − 20°C and later used for the determination of total phenolic content and total antioxidant capacity. This approach enabled a comprehensive evaluation of the effects of different irrigation regimes on both the physicochemical and biochemical properties of apple fruits.

2.4.1. Total soluble solids (TSS)

Total soluble solids were measured using a digital refractometer (Atago PAL-1) and expressed as % Brix. This method is standard in assessing fruit maturity and sugar accumulation (Ferretti et al., 2014).

2.4.2. Titratable acidity (TA)

For both cultivars, ten uniform and healthy fruits were randomly selected from each tree for juice analyses. Harvested fruits were processed under sterile conditions using a mechanical juice extractor to avoid tissue damage. The obtained juices were filtered through filter paper to obtain clarified samples.

Titratable acidity (TA) was determined in the filtered juice using an automatic titrator (Mettler Toledo DL50 Graphix). For each analysis, 5 mL of juice was diluted with 50 mL of distilled water in the instrument's titration vessel and connected to the electrode system. Titration was performed with 0.1 N NaOH until the pH reached 8.1, and TA was automatically calculated by the device based on NaOH consumption. Results were expressed as percentage (%) and mg g^{-1}, with values reported as % malic acid equivalents, following Dousti (2010).

2.4.3. Total phenolic content (TPC)

TPC was measured using the Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965), with results expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight (FW). Absorbance was read at 765 nm using a UV–Vis spectrophotometer.

2.4.4. Total antioxidant capacity (TAC)

TAC was determined by the ABTS radical scavenging assay as described by Re et al. (1999). The results were expressed as µmol Trolox equivalents per 100 g FW (TEAC), using a Trolox standard curve for calibration.

2.5. Statistical analysis

The experiment was conducted according to a completely randomized design, with trees randomly selected per cultivar; each tree was considered as one replicate. The study was carried out over two consecutive years (2023–2024) using trees aged 4–5 years.

To appropriately evaluate the effects of multiple experimental factors, the data were analyzed using multivariate analysis of variance (MANOVA), considering year, tissue type (flesh and peel), and their interaction (year × tissue) as fixed factors. When significant multivariate effects were detected, univariate ANOVA was subsequently performed to assess the main effects and interaction effects for individual parameters.

For each year, cultivar-related differences were also evaluated separately. Mean comparisons were performed using Tukey's HSD test at a significance level of $P \leq 0.05$, and statistically significant differences were indicated by different letters. Before analysis, data were checked for normality and homogeneity of variances.

All statistical analyses were conducted using JMP Pro 16.0 (SAS Institute Inc., Cary, NC, USA). In addition, Pearson correlation analysis was performed to examine relationships among the measured biochemical parameters.

3. Results

The findings of this study revealed that drought stress significantly affected the biochemical characteristics of apple fruits, including soluble solid content (SSC), titratable acidity (TA), total phenolic content (TPC), and total antioxidant capacity (TAC). Measurements were carried out separately for the 2023 and 2024 growing seasons and compared between two cultivars: ‘Pink Lady’ and ‘Jeromine’. Data were evaluated for both the fruit flesh and the combined peel + flesh tissues.

3.1. Soluble solid content (SSC)

Soluble solid content (SSC) was significantly influenced by irrigation regime, cultivar, and year (Table 1). In both experimental years, drought stress significantly increased SSC compared with the corresponding control treatments in both cultivars. In 2023, SSC in ‘Pink Lady’ increased from 14.2 ± 0.15% under control conditions to 14.7 ± 0.18% under drought stress, while in ‘Jeromine’ it rose from 13.0 ± 0.12% to 13.5 ± 0.14%. The same response pattern was observed in 2024, with SSC values increasing from 14.6 ± 0.16% (control) to 15.1 ± 0.17% (stress) in ‘Pink Lady’ and from 13.3 ± 0.13% to 13.8 ± 0.15% in ‘Jeromine’.

Table 1
SSC and TA of ‘Pink Lady’ and ‘Jeromine’ apples under drought stress (2023–2024)

Year	*Cultivar*	*Treatment*	*SSC (%)*	*TA (%)*
2023	Pink Lady	Control	14.2 ± 0.15 bA	0.70 ± 0.02 bA
2023	Pink Lady	Stress	14.7 ± 0.18 aA	0.74 ± 0.02 aA
2023	Jeromine	Control	13.0 ± 0.12 bB	0.64 ± 0.01 bB
2023	Jeromine	Stress	13.5 ± 0.14 aB	0.68 ± 0.02 aB
2024	Pink Lady	Control	14.6 ± 0.16 bA	0.72 ± 0.02 bA
2024	Pink Lady	Stress	15.1 ± 0.17 aA	0.76 ± 0.02 aA
2024	Jeromine	Control	13.3 ± 0.13 bB	0.66 ± 0.01 bB
2024	Jeromine	Stress	13.8 ± 0.15 aB	0.70 ± 0.02 aB

Across both years and irrigation treatments, ‘Pink Lady’ consistently exhibited significantly higher SSC values than ‘Jeromine’, as indicated by the different uppercase letters in Table 1, demonstrating a clear cultivar effect. Within each cultivar and year, the significant differences between control and stress

treatments (lowercase letters) confirm that drought stress enhanced soluble solid accumulation. The reproducibility of this response across consecutive years suggests a stable, robust increase in fruit sugar concentration under water-deficit conditions, likely associated with reduced fruit water content and concentration effects during fruit development.

3.2. Titratable acidity (TA)

Titratable acidity (TA) was significantly affected by irrigation regime, cultivar, and year (Table 1). In both experimental years, drought stress led to a consistent and significant increase in TA compared with the well-irrigated control in both cultivars. In 2023, TA increased from 0.70 ± 0.02% to 0.74 ± 0.02% in ‘Pink Lady’ and from 0.64 ± 0.01% to 0.68 ± 0.02% in ‘Jeromine’ under drought stress. A similar response was observed in 2024, with TA values rising from 0.72 ± 0.02% (control) to 0.76 ± 0.02% (stress) in ‘Pink Lady’ and from 0.66 ± 0.01% to 0.70 ± 0.02% in ‘Jeromine’.

Across both years and irrigation treatments, ‘Pink Lady’ consistently exhibited significantly higher TA values than ‘Jeromine’, as indicated by different uppercase letters in Table 1, reflecting cultivar-dependent variation in organic acid accumulation. Within each cultivar and year, the significant differences between control and drought stress treatments (lowercase letters) confirm that water deficit promoted increased titratable acidity. These findings indicate that drought stress not only influences sugar accumulation but also enhances organic acid concentration in apple fruits, with ‘Pink Lady’ showing a more pronounced response compared with ‘Jeromine’.

3.3. Total phenolic content (TPC)

Total phenolic content (TPC) was significantly influenced by year, cultivar, tissue type, and irrigation treatment (Table 2). In both experimental years, drought stress resulted in a significant increase in TPC compared with the corresponding control in all cultivar × tissue combinations.

In fruit flesh, drought stress significantly enhanced phenolic accumulation in both cultivars. In 2023, flesh TPC increased from 86.1 ± 2.4 to 93.3 ± 2.6 mg GAE 100 g^{-1} FW in ‘Pink Lady’ and from 75.4 ± 2.1 to 82.1 ± 2.3 mg GAE 100 g^{-1} FW in ‘Jeromine’. A comparable trend was observed in 2024, with flesh TPC rising from 93.2 ± 2.7 to 101.4 ± 2.9 mg GAE 100 g^{-1} FW in ‘Pink Lady’ and from 80.6 ± 2.4 to 88.2 ± 2.6 mg GAE 100 g^{-1} FW in ‘Jeromine’.

The inclusion of peel markedly increased total phenolic content in both years and cultivars, confirming that apple peel is the primary reservoir of phenolic compounds. In 2023, TPC in peel + flesh samples increased from 162.7 ± 3.8 to 176.2 ± 4.1 mg GAE 100 g^{-1} FW in ‘Pink Lady’ and from 139.6 ± 3.5 to 153.4 ± 3.9 mg GAE 100 g^{-1} FW in ‘Jeromine’ under drought stress. In 2024, even higher values were recorded, reaching 220.4 ± 4.9 mg GAE 100 g^{-1} FW in stressed ‘Pink Lady’ fruit and 170.6 ± 4.5 mg GAE 100 g^{-1} FW in stressed ‘Jeromine’.

Across both years and tissue types, ‘Pink Lady’ consistently exhibited significantly higher TPC than ‘Jeromine’, indicating a cultivar-dependent capacity for phenolic accumulation. The significant

differences between control and drought-stressed treatments (lowercase letters) demonstrate that water deficit stimulates phenolic biosynthesis. At the same time, the generally higher values observed in 2024 suggest a year-dependent environmental effect. Overall, these results indicate that drought stress promotes phenolic compound accumulation in apple fruit, particularly in the peel, with a more pronounced response in the ‘Pink Lady’ cultivar.

Table 2
TPC of ‘Pink Lady’ and ‘Jeromine’ under drought stress (2023–2024).

Year	Cultivar	Tissue Type	Treatment	TPC (mg GAE 100 g^{-1} FW)
2023	Pink Lady	Flesh	Control	86.1 ± 2.4 b
2023	Pink Lady	Flesh	Stress	93.3 ± 2.6 a
2023	Jeromine	Flesh	Control	75.4 ± 2.1 b
2023	Jeromine	Flesh	Stress	82.1 ± 2.3 a
2023	Pink Lady	Peel + Flesh	Control	162.7 ± 3.8 b
2023	Pink Lady	Peel + Flesh	Stress	176.2 ± 4.1 a
2023	Jeromine	Peel + Flesh	Control	139.6 ± 3.5 b
2023	Jeromine	Peel + Flesh	Stress	153.4 ± 3.9 a
2024	Pink Lady	Flesh	Control	93.2 ± 2.7 b
2024	Pink Lady	Flesh	Stress	101.4 ± 2.9 a
2024	Jeromine	Flesh	Control	80.6 ± 2.4 b
2024	Jeromine	Flesh	Stress	88.2 ± 2.6 a
2024	Pink Lady	Peel + Flesh	Control	203.8 ± 4.6 b
2024	Pink Lady	Peel + Flesh	Stress	220.4 ± 4.9 a
2024	Jeromine	Peel + Flesh	Control	155.1 ± 4.2 b
2024	Jeromine	Peel + Flesh	Stress	170.6 ± 4.5 a

3.4. Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) exhibited a response pattern similar to that observed for total phenolic content, being significantly affected by cultivar, year, tissue type, and irrigation treatment (Table 3). In both experimental years, drought stress resulted in a significant increase in TAC compared with the corresponding control in all cultivar × tissue combinations.

In fruit flesh, drought stress significantly enhanced TAC in both cultivars. In 2023, TAC increased from 41.8 ± 1.3 to 45.6 ± 1.5 µmol TE 100 g^{-1} FW in ‘Pink Lady’ and from 35.6 ± 1.2 to 39.2 ± 1.4 µmol TE 100 g^{-1} FW in ‘Jeromine’. A similar trend was observed in 2024, with flesh TAC rising from 46.9 ± 1.6 to 51.4

± 1.8 µmol TE 100 g^{-1} FW in ‘Pink Lady’ and from 38.7 ± 1.4 to 43.1 ± 1.6 µmol TE 100 g^{-1} FW in ‘Jeromine’ under drought stress.

Antioxidant capacity was markedly higher when peel was included, confirming that the peel is the major contributor to antioxidant activity in apple fruit. In 2023, TAC in peel + flesh samples increased from 121.5 ± 3.4 to 132.3 ± 3.7 µmol TE 100 g^{-1} FW in ‘Pink Lady’ and from 108.2 ± 3.1 to 117.4 ± 3.5 µmol TE 100 g^{-1} FW in ‘Jeromine’ under stress conditions. In 2024, even higher values were recorded, reaching 159.1 ± 4.6 µmol TE 100 g^{-1} FW in stressed ‘Pink Lady’ fruit and 128.6 ± 4.1 µmol TE 100 g^{-1} FW in stressed ‘Jeromine’.

Across both years and tissue types, ‘Pink Lady’ consistently exhibited significantly higher TAC values than ‘Jeromine’, indicating a cultivar-dependent antioxidant response to water deficit. The significant differences between control and drought-stressed treatments (lowercase letters) demonstrate that drought stress enhances antioxidant capacity, likely through stimulation of phenolic biosynthesis. Moreover, the generally higher TAC values observed in 2024 suggest a cumulative or year-dependent environmental effect. Overall, these results indicate that drought stress promotes antioxidant potential in apple fruit, particularly in the peel, with a more pronounced response in the ‘Pink Lady’ cultivar.

Table 3
TAC of ‘Pink Lady’ and ‘Jeromine’ under drought stress (2023–2024).

Year	*Cultivar*	*Tissue Type*	*Treatment*	*TAC (μmol TE100 g⁻¹ FW)*
2023	Pink Lady	Flesh	Control	41.8 ± 1.3 b
2023	Pink Lady	Flesh	Stress	45.6 ± 1.5 a
2023	Jeromine	Flesh	Control	35.6 ± 1.2 b
2023	Jeromine	Flesh	Stress	39.2 ± 1.4 a
2023	Pink Lady	Peel + Flesh	Control	121.5 ± 3.4 b
2023	Pink Lady	Peel + Flesh	Stress	132.3 ± 3.7 a
2023	Jeromine	Peel + Flesh	Control	108.2 ± 3.1 b
2023	Jeromine	Peel + Flesh	Stress	117.4 ± 3.5 a
2024	Pink Lady	Flesh	Control	46.9 ± 1.6 b
2024	Pink Lady	Flesh	Stress	51.4 ± 1.8 a
2024	Jeromine	Flesh	Control	38.7 ± 1.4 b
2024	Jeromine	Flesh	Stress	43.1 ± 1.6 a
2024	Pink Lady	Peel + Flesh	Control	145.8 ± 4.2 b
2024	Pink Lady	Peel + Flesh	Stress	159.1 ± 4.6 a
2024	Jeromine	Peel + Flesh	Control	117.9 ± 3.9 b
2024	Jeromine	Peel + Flesh	Stress	128.6 ± 4.1 a

4. Discussion

The present study demonstrates that drought stress induces coordinated changes in both primary (SSC and TA) and secondary metabolism (TPC and TAC) in apple fruit, with consistent responses across two growing seasons and clear cultivar-dependent differences. In both cultivars, SSC increased under drought (e.g., from 14.2% to 15.1% in ‘Pink Lady’ and from 13.0% to 13.8% in ‘Jeromine’ across years), indicating a stable concentration effect likely associated with reduced fruit water content and altered carbon allocation during fruit development. Similar drought-driven increases in SSC have been widely reported and are commonly attributed to restricted cell expansion and enhanced assimilate concentration in stressed fruits (Zandalinas et al., 2018; Wang et al., 2019). Concurrent increases in TA observed in both years suggest that water deficit also modulates organic acid metabolism, possibly through reduced dilution and changes in respiratory fluxes (Sharma et al., 2019).

Drought stress exerted a much stronger effect on secondary metabolites. Total phenolic content increased significantly in all cultivar × tissue × year combinations, with markedly higher values when peel was included. Flesh TPC increased consistently in both cultivars (e.g., from 86.1 to 101.4 mg GAE 100 g^{-1} FW in 'Pink Lady' across years), while peel + flesh samples reached substantially higher levels, peaking at 220.4 mg GAE 100 g^{-1} FW in stressed 'Pink Lady' fruit in 2024. These results confirm that apple peel represents the primary reservoir of phenolic compounds, in agreement with Lata (2007), and highlight the key defensive role of epidermal tissues under environmental stress.

The strong induction of TPC under drought indicates activation of the phenylpropanoid pathway. Phenylalanine ammonia-lyase (PAL), a central regulatory enzyme of this pathway, is known to be upregulated under abiotic stress, promoting flavonoid biosynthesis and phenolic accumulation (Xu et al., 2012; Chen et al., 2017). The consistently higher TPC observed in 'Pink Lady' compared with 'Jeromine' across both years suggests genotype-dependent regulation of phenolic metabolism, consistent with reports that stress-induced PAL activity and downstream phenolic accumulation vary substantially among cultivars (Gho et al., 2020; Amjad et al., 2024).

Total antioxidant capacity closely mirrored TPC patterns, reinforcing the functional link between phenolic accumulation and antioxidant potential. TAC increased significantly in both flesh and peel-containing tissues under drought, with maximum values recorded in peel + flesh samples of 'Pink Lady' (159.1 µmol TE 100 g^{-1} FW in 2024). This parallel increase confirms that phenolic compounds are the major contributors to antioxidant activity in apple fruit (Karadeniz & Ekşi, 2001) and supports earlier findings showing that water stress enhances antioxidant systems and phenolic accumulation in apple tissues (Awad et al., 2000; Awad & de Jager, 2002).

Clear cultivar differences were evident for all measured traits. 'Pink Lady' consistently exhibited higher SSC, TA, TPC, and TAC than 'Jeromine', indicating a greater capacity to regulate both carbon metabolism and antioxidant defenses under water deficit. Enhanced phenolic accumulation in 'Pink Lady' may reflect a more efficient oxidative stress management system, including improved scavenging of reactive oxygen species and stronger activation of secondary metabolism. Similar mechanisms have been proposed as key determinants of drought tolerance in horticultural crops (Hasanuzzaman et al., 2020).

A pronounced year effect was also observed, with both TPC and TAC being significantly higher in 2024 than in 2023 in both cultivars. This pattern suggests cumulative or adaptive responses to repeated drought exposure. Zandalinas et al. (2018) proposed that recurrent stress can induce a form of physiological "stress memory," enabling plants to activate defense pathways more rapidly and strongly in subsequent seasons. In perennial fruit crops such as apple, such acclimation processes may progressively enhance secondary metabolite accumulation under continued environmental pressure.

Although all trees were grafted onto M9 rootstock, which is known to exhibit limited drought tolerance (Kaynaş et al., 1995), the pronounced differences between cultivars indicate that scion genotype played the dominant role in determining fruit biochemical responses. This observation aligns with previous

reports emphasizing the primary influence of genetic background over rootstock effects on phenolic accumulation under abiotic stress (Lata, 2007).

Finally, the hot and low-humidity climatic conditions of Karaman likely intensified drought severity, amplifying stress responses, particularly in the more resilient ‘Pink Lady’. Collectively, these findings demonstrate that drought stress systematically reshapes sugar–acid balance and strongly enhances phenolic content and antioxidant capacity in apple fruit, with magnitude determined by cultivar, tissue type, and year. The consistently superior biochemical performance of ‘Pink Lady’ highlights its potential suitability for sustainable production in semi-arid and drought-prone regions.

Conclusion

This study revealed that drought stress significantly alters the biochemical composition of apple fruits, particularly influencing the accumulation of total phenolic content (TPC) and total antioxidant capacity (TAC). Both ‘Pink Lady’ and ‘Jeromine’ cultivars, grafted onto M9 rootstock, responded to water deficit by increasing the levels of phenolic compounds and antioxidant activity in their fruits, with ‘Pink Lady’ exhibiting a more pronounced and consistent response across both growing seasons.

The peel tissue contributed significantly to the overall antioxidant potential, demonstrating its central role in the fruit’s defense against environmental stress. TPC and TAC values were notably higher in the second year of the study, indicating a possible stress memory effect and suggesting that repeated exposure to drought conditions may enhance the plant’s biochemical adaptation capacity.

The findings of this research emphasize the importance of genotype selection in managing drought-related quality losses in apple production. ‘Pink Lady’, with its higher phenolic accumulation and antioxidant capacity under stress, emerged as a promising cultivar for semi-arid and drought-prone regions. Additionally, enhanced levels of these bioactive compounds contribute not only to the plant's stress tolerance but also to the nutritional and functional value of the fruit, supporting both agronomic resilience and consumer health.

In conclusion, the integration of genotype-specific responses, tissue-specific biochemical profiling, and environmental stress dynamics provides valuable insight into sustainable fruit production strategies under increasing climate variability.

However, the findings of this study are limited to two cultivars grown at a single location over two growing seasons, and further multi-location and multi-cultivar studies are required to generalize these results.

Declarations

Ethics approval and consent to participate:

Not applicable.

Ethics, Consent to Participate, and Consent to Publish

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Authors’ contributions

S.E.D. supervised the study. A.C.H. and S.E.D. conducted the investigation. S.E.D. and A.C.H. performed data analysis. S.E.D. managed the project administration. Writing, review, and editing were performed by S.E.D. Formal analysis and resources were provided by S.E.D. and A.C.H. All authors read and approved the final manuscript.

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Author Contribution

S.E.D. supervised the study. A.C.H. and S.E.D. conducted the investigation. S.E.D. and A.C.H. performed data analysis. S.E.D. managed the project administration. Writing, review, and editing were performed by S.E.D. Formal analysis and resources were provided by S.E.D. and A.C.H. All authors read and approved the final manuscript.

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Dear Editor,Thank you very much for your time and consideration of our manuscript.I would like to respectfully inquire about the possibility of an Article Processing Charge (APC) waiver or discount, should our manuscript be accepted for publication. Publishing this article is highly important for my academic progression, particularly for my application to associate professorship.I am currently working at this university as an Iranian-origin researcher, and I also have significant family responsibilities, including caring for my young daughter and supporting my parents. Due to these circumstances, covering the full publication fee would be financially challenging for me.I sincerely appreciate your

understanding and would be very grateful for any support or guidance you may be able to provide regarding APC assistance programs offered by the journal.Thank you again for your valuable time and consideration.Kind regards,

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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