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Comparative analysis of peg-induced *in-vitro* drought stress responses in strawberry (*Fragaria* × *ananassa* Duch.) cultivars

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Abstract: This study investigated the morphological, physiological, and biochemical responses of three commercially important strawberry cultivars ('Camarosa', 'Albion', and 'Sweet Charlie') to *in-vitro* drought stress induced by polyethylene glycol (PEG). Increasing osmotic stress resulted in clear cultivar-specific response patterns. 'Albion' exhibited the highest drought tolerance, characterised by sustained vegetative growth, higher chlorophyll content, improved relative water content, and enhanced antioxidant enzyme activities under moderate and severe stress conditions. 'Camarosa' showed moderate resilience, particularly through stable root development and maintenance of physiological traits under osmotic stress. In contrast, 'Sweet Charlie' was highly sensitive to PEG-induced drought stress, displaying pronounced reductions in growth, impaired water status, and elevated oxidative stress indicators. Multivariate analysis further confirmed the distinct adaptive strategies of the cultivars by clearly separating tolerant and sensitive genotypes based on their physiological and biochemical traits. These findings confirm the suitability of PEG-induced *in-vitro* screening as a rapid and controlled tool for drought tolerance evaluation, with 'Albion' emerging as a strong candidate for breeding programs targeting water-deficit conditions.

Keywords: osmotic adjustment; antioxidant defence system; relative water content; proline metabolism; root development; total phenolic content

The increasing frequency and severity of drought events due to climate change have become major environmental stressors threatening agricultural productivity and plant biomass yield worldwide (Dönmez, Yıldız 2024). Drought stress adversely affects essential physiological processes, including photosynthesis, respiration, growth, and cellular metabolism, by limiting the plant's capacity to absorb water, ultimately resulting in reduced yield and diminished crop quality (Aytaç et al. 2024; Nyaupane et al. 2024). In fruit crops, evaluating the adaptive responses of young plants to drought stress is critical for developing effective mitigation strategies. Strawberry (*Fragaria* × *ananassa* Duch.) ranks among the most widely consumed fruits globally, renowned for its high nutritional value and antioxidant properties. However, its shallow rooting system renders it highly susceptible to water deficit conditions (Aykoç et al. 2025). Türkiye is one of the leading strawberry producers in Europe, and the identification of drought-tolerant cultivars is essential for sustainable production under increasingly frequent climate-induced drought episodes (Muradoğlu et al. 2020; Tekin, Ünal 2024).

Plants employ diverse morphological, physiological, and biochemical mechanisms to cope

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with drought stress. Morphologically, drought often results in reduced shoot growth, enhanced root development, and decreased biomass accumulation (Ali et al. 2025). Physiologically, the accumulation of osmolytes such as proline, the generation of reactive oxygen species (ROS), and the activation of antioxidant defence systems, including superoxide dismutase (SOD) and catalase (CAT), play pivotal roles in stress alleviation (Sachdev et al. 2021). Furthermore, chlorophyll content and cell membrane integrity, frequently assessed via malondialdehyde (MDA) levels, serve as important indicators of drought-induced physiological damage. Biochemically, elevated phenolic compound synthesis and enhanced antioxidant capacity under drought conditions reflect plants' efforts to mitigate oxidative stress (Ünal et al. 2024; Öztürk et al. 2025). Under controlled laboratory conditions, drought stress is commonly simulated using polyethylene glycol (PEG), a high-molecular-weight polymer that imposes osmotic stress without ionic toxicity. PEG reduces water availability at the cellular level, effectively mimicking drought conditions (Kylyshbayeva et al. 2024). Therefore, PEG-induced *in-vitro* drought models are reliable tools for screening genotypic variation in stress tolerance.

In this study, three commercially important strawberry cultivars widely cultivated in Türkiye 'Camarosa', 'Albion', and 'Sweet Charlie' were evaluated under PEG-induced *in-vitro* drought stress. The objective was to assess their morphological, physiological, and biochemical adaptations and determine their relative tolerance levels. The results are expected to provide valuable scientific and practical insights for selecting drought-resistant cultivars and developing effective stress adaptation strategies in future strawberry production systems.

MATERIAL AND METHOD

Plant material. This study involved three commercially cultivated strawberry (*Fragaria* × *ananassa* Duch.) cultivars: 'Camarosa', 'Albion', and 'Sweet Charlie'. Plant materials were obtained from commercial strawberry plants harvested during the 2024 growing season. Each cultivar was introduced into *in-vitro* culture under aseptic conditions for micropropagation. Explants were prepared from the sub-leaf nodal regions of the plants, measuring approximately 1.0–1.5 cm in length. These segments were selected for their high totipotency and suitability for shoot induction. After sterilisation, explants were transferred to appropriate culture media under aseptic conditions.

Surface sterilisation procedure. To minimise contamination while preserving explant viability, sub-leaf nodal segments measuring approximately 1.0–1.5 cm were initially rinsed under running tap water for 10 min to remove surface debris. Subsequently, explants were immersed in sterile distilled water containing 2% (v/v) Tween-20 for 10 min with gentle agitation. Surface sterilisation was then performed under a laminar airflow cabinet. Explants were treated with 70% (v/v) ethanol for 30 s, followed by immersion in 1.5% (v/v) sodium hypochlorite (NaOCl) solution supplemented with a few drops of Tween-20 for 12 minutes. Finally, explants were rinsed three times with sterile distilled water to remove any residual sterilants and aseptically transferred to culture media for shoot induction.

Culture medium and drought stress treatment. Murashige and Skoog (MS, 1962) full-strength nutrient medium served as the basal medium for both propagation and drought stress treatments. The medium was supplemented with 30 g/L sucrose and solidified with 7 g/L agar. During the in vitro establishment phase, the MS medium was additionally supplemented with 1.0 mg/L 6-benzylaminopurine (BAP) for shoot proliferation and 0.5 mg/L indole-3-butyric acid (IBA) to promote uniform root development prior to PEG treatments. The pH was adjusted to 5.8 prior to autoclaving at 121 °C for 20 minutes. After sterilisation, the medium was dispensed into 100 mL culture jars with 25 mL per jar and allowed to solidify. Explants were aseptically inoculated into these jars. To simulate drought stress, polyethylene glycol (PEG-6000) was applied at 0% (control), 5% (moderate drought stress), and 10% (severe drought stress) concentrations. PEG solutions were prepared in sterilised medium at room temperature and added aseptically without re-autoclaving. Each treatment included three replicates per cultivar, with 10 explants per replicate. Cultures were incubated in a growth chamber under a 16/8 h light/dark photoperiod at 25 ± 2 °C and approximately 3 000 lux light intensity for 21 days. At the end of the incubation, morphological, physiological, and biochemical parameters were evaluated.

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Measured parameters and analytical procedures. Morphological traits were assessed via direct counting and precise measurements: shoot and leaf numbers were recorded; shoot and leaf lengths were measured with a digital calliper. Rooting percentage was calculated as the ratio of rooted explants to total explants. Root number and length were individually recorded for each explant. Fresh biomass was weighed immediately after harvest, followed by oven drying at 70 °C for 72 h to determine dry biomass.

Physiological analyses focused on water status, membrane integrity, and oxidative stress markers. Chlorophyll content was measured using a SPAD 502 Plus portable chlorophyll meter (Konica Minolta, Osaka, Japan) at three points per leaf, averaging the results. Relative water content (RWC) was calculated using the formula:

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100 \quad (1)$$

where: FW – fresh weight; TW – turgid weight; DW – dry weight.

Proline content was quantified following Bates et al. (1973) using ninhydrin reagent and spectrophotometric absorbance at 520 nm. MDA levels, indicative of lipid peroxidation, were measured by the thiobarbituric acid (TBA) method as described by Heath and Packer (1968), with absorbance readings at 532 and 600 nm. SOD activity was determined by inhibition of nitro blue tetrazolium (NBT) reduction according to Giannopolitis and Ries (1977). CAT activity was assayed by monitoring the decomposition of hydrogen peroxide at 240 nm following Aebi (1984).

Biochemical analyses assessed antioxidant defence capacity. Total phenolic content was determined via the Folin-Ciocalteu method (Singleton, Rossi 1965) and expressed as mg gallic acid equivalents (GAE) per g fresh weight. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Brand-Williams et al. 1995) with absorbance measured at 517 nm to calculate percentage inhibition. Glutathione reductase (GR) activity was measured by monitoring nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of oxidised glutathione (GSSG) at 340 nm, according to Foyer and Halliwell (1976).

Statistical analysis. All statistical analyses were performed using IBM SPSS Statistics 25.0. The effects of cultivar, PEG concentration, and their interaction on the measured morphological, physiological, and biochemical parameters were evaluated using two-way analysis of variance (ANOVA). Mean comparisons were conducted using the least significant difference (LSD) test at the $P < 0.05$ significance level. To enable comparative visualisation across variables with different measurement units, physiological and biochemical parameters were subjected to min–max normalisation (0–10 scaling) prior to radar chart construction and multivariate analysis. Principal component analysis (PCA) was performed on standardised (*z*-score transformed) data to identify dominant stress-responsive traits and to evaluate genotype-specific clustering patterns. PCA biplots were generated using Python (Matplotlib and Seaborn) to illustrate the relationships among measured variables and the distribution of samples under different PEG concentrations.

RESULTS AND DISCUSSION

In this study, the morphological, physiological, and biochemical responses of three strawberry cultivars ('Camarosa', 'Albion', and 'Sweet Charlie') were evaluated under different PEG concentrations (control 0%, 5% and 10% PEG). Morphological traits measured under controlled *in-vitro* conditions were analysed and visualised using radar charts to reveal the variation among cultivars and treatments. The present study revealed statistically significant, genotype-dependent differences in the morphological responses of strawberry cultivars to PEG-induced osmotic stress, based on comprehensive morphometric analyses (Table 1, Figure 1). Under severe osmotic stress (10% PEG), 'Camarosa' maintained the highest shoot number (9.57 ± 0.28; $P < 0.05$) and exhibited robust root system development, as indicated by elevated root number (8.89 ± 0.30; $P < 0.05$) and rooting rate (8.39 ± 0.23) at moderate stress (5% PEG), suggesting superior root plasticity and water uptake capacity. 'Albion' showed vigorous vegetative growth under non-stress and moderate PEG conditions, achieving the highest values for leaf number (9.26 ± 0.19), leaf length (9.10 ± 0.47 cm), fresh biomass (9.46 ± 0.26 g), and dry biomass (7.95 ± 0.12 g) at 5% PEG. However, a marked decline in shoot and root traits was observed in 'Albion' under severe PEG stress ($P < 0.05$), indicating moderate tolerance. In contrast, 'Sweet

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Table 1. Morphological parameters of 'Camarosa', 'Albion', and 'Sweet Charlie' under polyethylene glycol (PEG)-induced osmotic stress

Cultivar	PEG (%)	Shoot No.	Shoot length (cm)	Leaf No.	Leaf length (cm)	Rooting rate (%)	Root No.	Root length (cm)	Fresh biomass (g)	Dry biomass (g)
Camarosa	0	8.52 ± 0.20^{b}	6.49 ± 0.16^{a}	3.61 ± 0.17^{b}	6.76 ± 0.21^{b}	7.11 ± 0.17^{b}	8.22 ± 0.46^{a}	6.02 ± 0.13^{a}	3.89 ± 0.31^{c}	4.99 ± 0.26^{b}
Camarosa	5	9.51 ± 0.23^{a}	6.90 ± 0.15^{a}	7.00 ± 0.13^{a}	4.96 ± 0.50^{c}	8.39 ± 0.23^{a}	4.31 ± 0.42^{b}	5.27 ± 0.20^{b}	5.98 ± 0.37^{b}	6.55 ± 0.40^{a}
Camarosa	10	9.57 ± 0.28^{a}	4.27 ± 0.25^{b}	3.47 ± 0.29^{b}	8.19 ± 0.22^{a}	7.02 ± 0.40^{b}	8.89 ± 0.30^{c}	3.98 ± 0.19^{c}	8.57 ± 0.46^{a}	4.41 ± 0.25^{b}
Albion	0	7.34 ± 0.42^{b}	6.73 ± 0.16^{b}	9.26 ± 0.19^{a}	8.52 ± 0.39^{b}	4.06 ± 0.39^{b}	5.18 ± 0.36^{b}	4.74 ± 0.38^{c}	8.21 ± 0.32^{b}	3.23 ± 0.20^{c}
Albion	5	6.54 ± 0.49^{c}	7.28 ± 0.44^{a}	3.13 ± 0.15^{c}	9.10 ± 0.47^{a}	9.52 ± 0.45^{a}	6.96 ± 0.31^{a}	7.88 ± 0.34^{a}	9.46 ± 0.26^{a}	7.95 ± 0.12^{b}
Albion	10	8.16 ± 0.13^{a}	6.17 ± 0.50^{b}	4.57 ± 0.30^{b}	6.17 ± 0.34^{c}	3.99 ± 0.13^{c}	4.23 ± 0.40^{c}	6.49 ± 0.18^{b}	5.93 ± 0.46^{c}	9.40 ± 0.18^{a}
Sweet Charlie	0	5.81 ± 0.11^{a}	6.92 ± 0.14^{b}	4.09 ± 0.42^{a}	4.27 ± 0.17^{b}	9.03 ± 0.36^{a}	9.62 ± 0.20^{a}	5.61 ± 0.14^{c}	4.90 ± 0.20^{c}	7.51 ± 0.39^{a}
Sweet Charlie	5	4.46 ± 0.41^{b}	3.19 ± 0.16^{c}	4.27 ± 0.45^{a}	7.08 ± 0.29^{a}	5.95 ± 0.46^{b}	9.25 ± 0.42^{a}	8.72 ± 0.27^{a}	5.39 ± 0.11^{b}	4.82 ± 0.21^{b}
Sweet Charlie	10	3.13 ± 0.27^{c}	9.40 ± 0.25^{a}	3.82 ± 0.3^{b}	7.04 ± 0.12^{a}	4.92 ± 0.17^{c}	6.88 ± 0.40^{b}	7.56 ± 0.13^{b}	8.81 ± 0.34^{a}	4.44 ± 0.20^{b}

Mean ± SE (n = 3); different letters indicate significant differences ($P < 0.05$)

Charlie' consistently exhibited the lowest values for all measured morphological indices, with shoot number dropping from (8.05 ± 0.31) at 0% PEG to (6.52 ± 0.19) at 10% PEG, as well as pronounced reductions in root and biomass metrics, reflecting a high sensitivity to water limitation (Table 1, Figure 1).

The radar chart (Figure 1) provides a multidimensional and integrative visualisation of these data, enabling side-by-side evaluation of cultivar performance across water-deficit gradients. 'Camarosa's' radar profile remains broad and stable, especially for root-associated variables, underscoring its superior osmotic stress resilience. In contrast, 'Albion's' radar area contracts sharply as stress intensifies, reflecting limited adaptability. 'Sweet Charlie's' consistently restricted profile further highlights its low plasticity and stress adaptation. Our findings are consistent with those of Raffaelli et al. (2025), who reported that water scarcity in strawberry production imposes dual constraints on both fruit quality and yield. Moderate water deficits can enhance specific fruit quality attributes, but yield stability remains largely genotype-dependent. Notably, certain cultivars ('Lauretta', 'AN15,07,53') maintained high yields (up to 98%) under mild water stress, whereas more severe restriction resulted in substantial and statistically significant yield reductions across genotypes. Together with our observations, these results underscore how precision phenotyping and cultivar-specific irrigation protocols are fundamental for ensuring sustained fruit quality and yield in strawberries exposed to drought. Recent literature further supports the notion that abiotic stresses linked to climate change are major challenges for strawberry cultivation, negatively impacting root development, nutrient uptake, and plant health (Akhtar et al. 2025). Salt accumulation, drought, and temperature extremes disrupt physiological and metabolic processes. Plants respond by producing growth-promoting hormones and relying on beneficial soil microorganisms such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), which, when applied as bioinoculants, improve soil health, fruit quality, and crop yield through root-zone associations. In our experiment, the capacity of 'Camarosa' to sustain root growth and high shoot production under osmotic stress may reflect enhanced water uptake and adaptive advantage under such conditions. The marked

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decrease in vegetative parameters observed in 'Albion' under severe PEG, alongside 'Sweet Charlie's consistently weak performance, underscores the pivotal role of genetic background in determining cultivar stability under abiotic stress conditions. Collectively, these insights underscore the necessity of integrating advanced phenotyping, root trait analysis, and microbial strategies in breeding and management programs to improve drought resilience, resource use efficiency, and sustainability in strawberry cultivation.

Following the morphological comparisons provided by the radar chart, the physiological and biochemical parameters measured under different PEG concentrations are presented in Table 2. PEG-induced osmotic stress resulted in statistically significant and genotype-dependent shifts in water status and oxidative balance. For instance, the SPAD index in 'Sweet Charlie' decreased from 4.62 ± 0.11 at 0% PEG to 4.23 ± 0.37 at 10% PEG, whereas 'Albion' maintained relatively stable SPAD index values (3.89 ± 0.16 at 0% and 4.04 ± 0.31 at 10% PEG). Similarly, the RWC index in 'Camarosa' increased markedly under severe stress (8.72 ± 0.42 at 10% PEG), while fluctuations in 'Sweet Charlie' indicated less consistent water regulation.

The proline index increased progressively with PEG concentration in all cultivars. In 'Sweet Charlie', proline values rose from 7.01 ± 0.37 at 0% PEG to 9.12 ± 0.39 at 10% PEG. However, this osmotic adjustment was accompanied by a sharp increase in the MDA index, reaching 12.74 ± 0.38 under severe stress, indicating intensified lipid peroxidation and membrane damage. Antioxidant enzyme indices displayed distinct genotype-specific patterns. The CAT index in 'Albion' remained high under stress (9.48 ± 0.47 at 5% PEG and 9.73 ± 0.28 at 10% PEG), suggesting an efficient ROS-

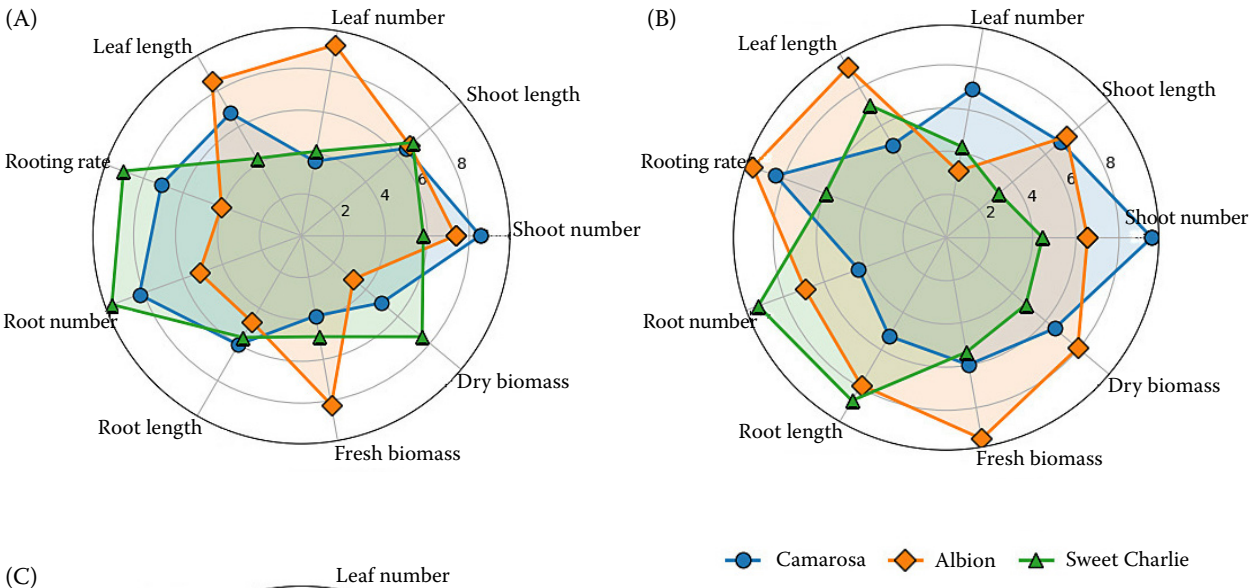

Figure 1. Radar plots illustrating the mean morphological and biomass parameters of three strawberry cultivars under polyethylene glycol (PEG)-induced osmotic stress: (A) 0% PEG (control), (B) 5% PEG, (C) 10% PEG treatments

Nine traits are displayed on the radial axes for cultivars 'Albion', 'Camarosa', and 'Sweet Charlie'; coloured shaded areas indicate the overall performance profiles for each PEG level (blue = 0%, orange = 5%, green = 10%); concentric gridlines (0.0–9.6) represent fixed value increments; values are expressed as arithmetic means of replicate measurements

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Table 2. Min-max normalised (0–10 scaled) physiological and biochemical indices of 'Camarosa', 'Albion', and 'Sweet Charlie' under polyethylene glycol (PEG)-induced osmotic stress

Cultivar	PEG (%)	Total phenolics	Antioxidant activity	GR	Proline	MDA	SOD	CAT	SPAD	RWC
Camarosa	0	5.54 ± 0.49^{a}	7.52 ± 0.14^{a}	7.00 ± 0.26^{b}	5.49 ± 0.49^{b}	9.91 ± 0.45^{a}	7.24 ± 0.43^{a}	4.66 ± 0.20^{c}	3.71 ± 0.17^{c}	4.07 ± 0.37^{b}
Camarosa	5	4.70 ± 0.34^{b}	3.80 ± 0.29^{c}	7.27 ± 0.26^{b}	5.02 ± 0.24^{ab}	7.07 ± 0.47^{b}	4.08 ± 0.43^{c}	6.37 ± 0.49^{b}	6.73 ± 0.15^{a}	3.36 ± 0.39^{c}
Camarosa	10	4.15 ± 0.32^{b}	4.15 ± 0.46^{b}	8.70 ± 0.35^{a}	7.66 ± 0.15^{a}	6.66 ± 0.48^{c}	5.51 ± 0.35^{b}	9.14 ± 0.23^{a}	5.75 ± 0.16^{b}	8.72 ± 0.42^{a}
Albion	0	6.99 ± 0.24^{a}	8.34 ± 0.17^{a}	9.14 ± 0.46^{a}	5.39 ± 0.33^{b}	8.75 ± 0.26^{a}	3.77 ± 0.28^{c}	8.93 ± 0.48^{b}	3.89 ± 0.16^{c}	5.78 ± 0.33^{a}
Albion	5	4.07 ± 0.23^{c}	7.03 ± 0.42^{b}	7.25 ± 0.10^{b}	5.97 ± 0.23^{b}	8.16 ± 0.26^{a}	9.54 ± 0.31^{a}	9.48 ± 0.47^{a}	6.16 ± 0.24^{a}	3.79 ± 0.24^{b}
Albion	10	5.54 ± 0.18^{b}	7.06 ± 0.11^{b}	7.43 ± 0.29^{b}	7.00 ± 0.33^{a}	7.64 ± 0.13^{b}	4.25 ± 0.41^{b}	9.73 ± 0.28^{a}	4.04 ± 0.31^{b}	5.90 ± 0.28^{a}
Sweet Charlie	0	5.86 ± 0.44^{a}	3.18 ± 0.43^{b}	4.09 ± 0.26^{b}	7.01 ± 0.37^{c}	5.87 ± 0.18^{c}	3.19 ± 0.22^{c}	4.55 ± 0.46^{c}	4.62 ± 0.11^{b}	7.70 ± 0.13^{b}
'Sweet Charlie	5	5.66 ± 0.32^{a}	7.13 ± 0.35^{a}	4.88 ± 0.20^{ab}	8.37 ± 0.16^{b}	7.18 ± 0.43^{b}	6.86 ± 0.49^{b}	6.05 ± 0.31^{b}	5.06 ± 0.17^{a}	9.64 ± 0.21^{a}
'Sweet Charlie	10	3.08 ± 0.26^{b}	3.96 ± 0.22^{ab}	9.30 ± 0.24^{a}	9.12 ± 0.39^{a}	12.74 ± 0.38^{a}	7.20 ± 0.33^{a}	7.66 ± 0.29^{a}	4.23 ± 0.37^{b}	9.40 ± 0.47^{a}

GR – glutathione reductase; MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase; SPAD – chlorophyll content; RWC – relative water content; values represent min-max normalised data (0–10 scale); mean ± SE (n = 3); different lowercase letters within the same column indicate significant differences according to LSD test at $P < 0.05$ following two-way ANOVA (cultivar × PEG concentration)

scavenging capacity. In contrast, the simultaneous increase of oxidative damage markers in 'Sweet Charlie' reflects a less coordinated antioxidative defence response. 'Camarosa' exhibited an intermediate biochemical adjustment profile across treatments.

To integrate these interrelated responses and identify dominant drought-associated traits, PCA was conducted. The resulting PCA biplot (Figure 2) provided a multivariate framework clearly discriminating tolerant and sensitive genotypes based on coordinated patterns of SPAD, RWC, antioxidant enzyme activities, proline accumulation, MDA levels, and total phenolics across increasing PEG concentrations (0%, 5%, and 10%).

The distribution of samples in the PCA biplot revealed marked, genotype-dependent clustering that reflects the divergent adaptive strategies of each cultivar. 'Albion' samples, especially under 0% and 5% PEG, consistently clustered in the positive regions of PC1 and PC2, which were closely associated with elevated SPAD, RWC, antioxidant enzyme activity, and total phenolic content. This pattern suggests that 'Albion' possesses a highly coordinated drought defence system, maintaining photosynthetic capacity, cellular hydration, and an effective antioxidant network, hallmarks of superior drought tolerance reported in recent studies (Askari-Khorasgani et al. 2021; Muradoğlu et al. 2025). By contrast, 'Sweet Charlie' shifted strongly toward the negative PC1 axis under 10% PEG, where high MDA and proline accumulation coincided with reduced SPAD and RWC. This profile is characteristic of sensitive genotypes prone to oxidative damage and impaired cellular homeostasis during water stress (Faaek, Pırlak 2021; Ibrahim et al. 2022). 'Camarosa' showed an intermediate profile, maintaining relatively balanced RWC, SPAD, and antioxidant activity, especially under moderate PEG stress, reflecting a partial resilience and capacity for osmotic adjustment.

The orientation and proximity of trait vectors in the PCA biplot further illustrate the relationships among key stress indicators. The alignment of SPAD, RWC, CAT, and SOD vectors toward 'Albion' underscores the synergistic contribution of photosynthetic performance, water status, and antioxidative enzymes to drought resilience. High MDA and proline levels observed in 'Sweet Charlie' reflect a stress response marked by pronounced oxidative damage and osmolyte buildup.

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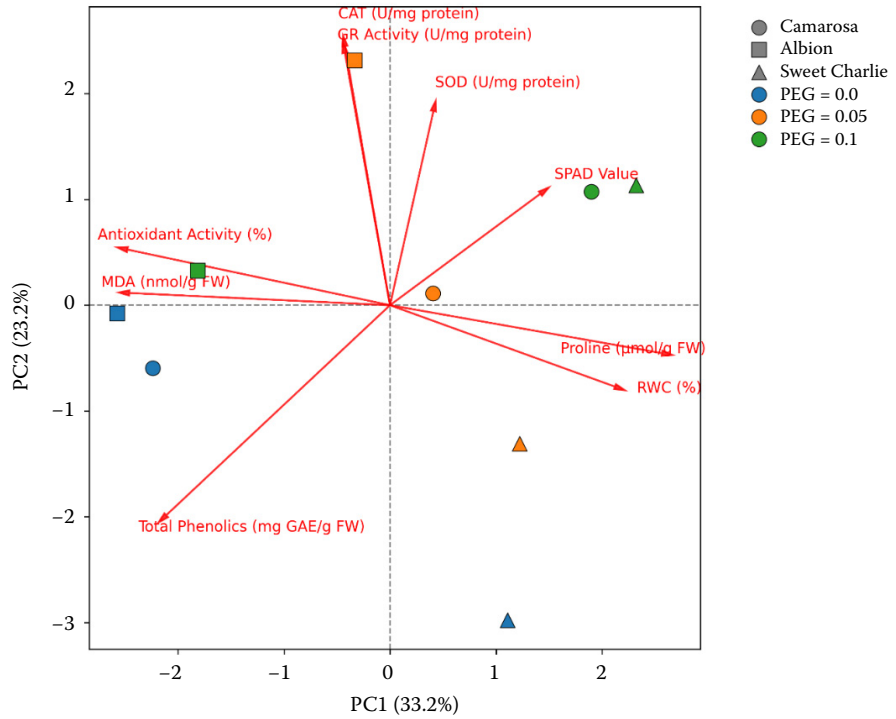

Figure 2. Principal component analysis (PCA) biplot of physiological and biochemical responses of three strawberry cultivars under PEG-induced osmotic stress

Points represent mean scores for each cultivar; PC1 (33.2% of total variance) and PC2 (23.2% of total variance) are shown with dashed reference lines; red vectors indicate scaled loadings of nine normalised indices; arrow length reflects the relative contribution of each variable to the principal components; all data are arithmetic means of three biological replicates; CAT – catalase; GR – glutathione reductase; SOD – superoxide dismutase; SPAD – chlorophyll content; RWC – relative water content MDA – malondialdehyde

These patterns are highly consistent with recent advances in the literature. Qin et al. (2025) highlighted that the enhancement of antioxidant systems and secondary metabolites is fundamental to abiotic stress tolerance in strawberry. Similarly, Mozafari et al. (2019) emphasised that drought-resilient cultivars maintain higher leaf relative water content (LRWC), chlorophyll content, and antioxidant enzyme activity under water deficit, while more susceptible genotypes experience elevated MDA levels and impaired photosynthetic performance. The alignment of our PCA-based findings with those reported by Ariza et al. (2021), who demonstrated a strong association between physiological parameters and yield stability in strawberry under drought, further supports the validity of *in vitro* PEG-based screening as a rapid and effective tool for early identification of drought tolerance in breeding programs.

The integrated perspective afforded by the PCA biplot not only underscores the multidimensional nature of drought tolerance but also highlights the utility of advanced quantitative phenotyping for cultivar selection and breeding. 'Albion' emerges as the most resilient genotype, displaying coordinated physiological and biochemical responses to osmotic stress, while 'Sweet Charlie' is identified as the most sensitive. 'Camarosa' represents an intermediate yet valuable profile for future improvement. Integrating high-throughput phenotyping with detailed physiological and biochemical assessments, alongside advanced visualisation tools, facilitates the targeted selection and breeding of strawberry cultivars optimised for drought resilience and long-term sustainability.

CONCLUSION

This study comprehensively evaluated the morphological, physiological, and biochemical adaptations of three commercially important strawberry

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cultivars, 'Camarosa', 'Albion', and 'Sweet Charlie', under *in-vitro* drought stress simulated by PEG. The results clearly demonstrated that cultivar-specific responses significantly influence drought stress adaptation. Among the cultivars tested, 'Albion' consistently outperformed others across most parameters under both control and drought conditions. Its vigorous vegetative growth, higher chlorophyll content (SPAD), effective osmotic adjustment through proline accumulation, and enhanced antioxidant enzyme activities (SOD, CAT, and GR) underscore its superior drought tolerance. 'Camarosa' exhibited moderate resilience, particularly in root development and antioxidant defence, whereas 'Sweet Charlie' was the most sensitive, showing marked declines in growth, water status, and stress defence traits as PEG concentration increased. These findings validate the efficacy of PEG-induced *in-vitro* models as reliable tools for early-stage drought tolerance screening, enabling rapid and controlled assessment of genotypic variation. Furthermore, the results provide valuable insights into the physiological and biochemical mechanisms underlying cultivar-specific stress responses.

From an applied perspective, 'Albion' emerges as a promising candidate for breeding programs aimed at developing drought-resilient strawberry cultivars. The *in-vitro* platform established herein can serve as an effective preliminary screening tool prior to field evaluations. Future studies should incorporate gene expression and metabolomic analyses to elucidate the molecular mechanisms of drought tolerance and to identify stress-responsive biomarkers that can expedite cultivar selection in strawberry breeding programs.

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