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Research Article

Genus -specific physiological and biochemical adaptations of turfgrasses to drought stress and exogenous calcium

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Abstract

Drought stress is one of the most significant challenges to the sustainability of grasslands in the era of climate change. Given the increasing scarcity of freshwater resources worldwide and the severe impacts of water deficit stress on plant performance, extensive global research efforts are underway to combat this stress and identify plant drought response mechanisms, aiming to enhance plant drought tolerance. This study aimed to investigate the effect of different calcium levels on the physiological and biochemical responses of four common turfgrass genera used in urban green spaces (*Agropyron*, *Festuca*, *Cynodon*, and *Lolium*) under drought stress. Plants were treated in two groups, control (well-watered) and under drought stress, with four different calcium concentrations (0, 5, 10, and 20 mM) in a completely randomized design. Growth, physiological, and biochemical parameters, including root and shoot longitudinal growth, relative water content, leaf ion leakage, changes in membrane lipid peroxidation, photosynthetic pigment content, proline content, and the activity of some antioxidant enzymes, were measured and analyzed. The results indicated that drought stress reduced shoot growth in all genera but led to increased root growth in *Festuca* and *Agropyron*. Calcium application had no significant effect on shoot growth but significantly increased the root growth of *Agropyron* under drought stress compared to the other genera. In the sensitive genera (*Cynodon* and *Lolium*), calcium caused a significant improvement in leaf width, relative water content, chlorophyll a content, and a reduction in ion leakage and membrane lipid peroxidation. In contrast, the resistant genera (*Agropyron* and *Festuca*) showed less response to calcium and primarily relied on intrinsic mechanisms such as high activity of SOD and APX enzymes. Based on the results, it can be concluded that the response to calcium is genus-dependent. Sensitive genera utilize calcium as an external modulator to enhance their defense system, whereas resistant genera, due to possessing more efficient intrinsic defense mechanisms, are less dependent on external calcium. The findings demonstrate that calcium application can be used as a management strategy, particularly in more sensitive turfgrass genus, to improve drought resistance.

Keywords: Ascorbate peroxidase, Catalase, Chlorophyll, Ion leakage, Turfgrass

Introduction

Global climate change and the increasing frequency of prolonged drought events pose serious threats to agricultural productivity and ecosystem sustainability. Grasses are fundamental components of natural grasslands, pastures, turf systems, and urban landscapes,

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contributing to food security, soil conservation, carbon sequestration, and aesthetic value. Given their extensive global distribution, understanding drought tolerance mechanisms in grasses and identifying practical strategies to enhance their resilience to water scarcity have become major priorities in plant physiological and ecological research (Stevens *et al*., 2022).

Plants respond to drought stress through a range of physiological and biochemical mechanisms that are generally classified as drought avoidance and drought tolerance strategies. Avoidance mechanisms include enhanced root development, stomatal regulation, and reduced leaf area, whereas tolerance mechanisms involve osmolyte accumulation, activation of antioxidant defense systems, and maintenance of membrane integrity (Alam *et al*., 2018; Bashir *et al*., 2021; Ranjan *et al*., 2022). The relative contribution of these mechanisms varies widely among species and reflects distinct adaptive strategies.

Among the diverse regulators of plant drought responses, calcium (Ca^{2+}) occupies a central position as both a structural element of cell walls and membranes and a ubiquitous secondary messenger in stress signaling pathways (Ranty *et al*., 2016). Transient increases in cytosolic Ca^{2+} represent one of the earliest cellular responses to abiotic stress and are closely interconnected with reactive oxygen species (ROS) signaling networks. Calcium-ROS cross-talk is mediated through Ca^{2+}-permeable ion channels, respiratory burst oxidase homologs (RBOHs), and Ca^{2+}-sensing proteins such as calmodulin, calcineurin B-like proteins, and Ca^{2+}-dependent protein kinases, which collectively regulate antioxidant defenses, stomatal behavior, gene expression, and metabolic adjustments under drought (Ranty *et al*., 2016; Ravi *et al*., 2023; Xu *et al*., 2022).

A growing body of evidence indicates that exogenous calcium application can alleviate drought-induced damage by preserving ionic balance and membrane integrity, enhancing antioxidant capacity, stabilizing the photosynthetic apparatus, and improving plant water relations. Beneficial effects of calcium supplementation under drought stress have been reported in a range of species, including tobacco, maize, and Datura, where calcium improved relative water content, maintained photosynthetic performance, and reduced oxidative injury (Hu *et al*., 2018; Naeem *et al*., 2017; Qin *et al*., 2019; Feng *et al*., 2023). Calcium is also known to interact with other signaling molecules such as abscisic acid and nitric oxide, forming an integrated regulatory network that fine-tunes drought responses at physiological and molecular levels.

Despite these advances, comparative information on calcium-mediated drought responses in grasses remains limited. Most previous studies have focused on crop or model species, while systematic evaluations across different grass genera are scarce. Moreover, grasses are known to exhibit substantial diversity in drought-adaptation strategies, suggesting that their dependence on calcium-regulated mechanisms may differ considerably.

Therefore, the present study was designed to comparatively investigate the physiological and biochemical responses of four widely used turfgrass genera (*Lolium*, *Agropyron*, *Festuca*, and *Cynodon*) to drought stress under varying levels of exogenous calcium. Specifically, this study aimed to: (i) characterize and compare growth performance, plant water status, photosynthetic pigments, proline accumulation, antioxidant enzyme activities, membrane stability, and lipid peroxidation under drought conditions; (ii) evaluate the effectiveness of calcium application in mitigating drought-induced damage and identify optimal calcium concentrations; and (iii) determine whether calcium-mediated responses differ among genera, thereby providing insight into distinct drought-resistance strategies in grasses.

Materials and methods

Plant material and experimental design: The experiment was conducted during 2020 and 2021 at the Propagation Laboratory of the Kashan Municipality Center. Seeds of four grass species representing four common genera *Cynodon dactylon*, *Agropyron elongatum*, *Festuca arundinacea*, and *Lolium perenne*—were obtained from Pakan Bazr Company (Isfahan, Iran). Seeds were surface-sterilized in 5% (v/v) sodium hypochlorite and rinsed three times with distilled water prior to sowing.

For each species, seeds were planted in a 1:1 mixture of ammonium-sulfate-treated (30 mg N kg^{-1}) cocopeat and peat moss, using individual plastic pots (12 cm diameter × 20 cm height; ~2.26 L volume). Plants were grown in a propagation facility at the Kashan Municipality Center under controlled conditions: 25°C, a photon flux density of 220 µmol m^{-2} s^{-1}, and regular irrigation as needed. The experiment followed a completely randomized design with four calcium treatments (0, 5, 10, and 20 mM $CaCl_2$), three replications per treatment, and six seedlings per replication. Plants were maintained for one month at 25 ± 2°C under fluorescent light with a 16 h light / 8 h dark photoperiod. Irrigation was applied immediately after planting and subsequently once per week using a 50% Hoagland nutrient solution (Hoagland and Arnon, 1950).

Calcium pre-treatment was applied twice at a four-day interval via irrigation water containing $CaCl_2$ at the designated concentrations. Following pre-treatment, drought stress was applied to three replicates per group via complete irrigation withdrawal for nine days, achieving ~30% field capacity. Three additional replicates per group served as well-watered controls. Samples were subsequently taken for analysis.

Morphological measurements: Plant height, root length, and leaf width were measured in control and drought-stressed plants using a digital caliper.

Photosynthetic pigments: Photosynthetic pigments

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were determined according to Lichtenthaler and Wellburn (1983). Fresh leaf tissue (0.2 g) was homogenized in 5 mL of cold 80% acetone and centrifuged at 13,000 rpm for 20 min at 4°C (Eppendorf 5415R). The supernatant volume was adjusted to 5 mL with 80% acetone. Absorbance was measured at 663.2, 646.8, and 470 nm, and total chlorophyll and carotenoid contents were calculated using standard equations.

Electrolyte leakage: Cell membrane stability was assessed by electrolyte leakage following a modified method of Liu *et al.* (2011). Shoot samples of equal weight were rinsed with distilled water, placed in test tubes containing 10 mL of double-distilled water, and incubated in the dark at 25°C for 24 h. Initial electrical conductivity (EC_1) was measured using a conductivity meter (Jenway 4510). Samples were then autoclaved at 121°C for 20 min, cooled to room temperature, and final conductivity (EC_2) was recorded. Electrolyte leakage was calculated as:

$$\mathrm{EL}\ (\%) = \left(\frac{\mathrm{EC_1}}{\mathrm{EC_2}}\right) \times 100$$

Lipid peroxidation: Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content following Heath and Packer (1968). Fresh shoot tissue (0.1 g) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 25 min. One milliliter of the supernatant was mixed with 4 mL of 20% TCA containing 0.5% (w/v) thiobarbituric acid (TBA) and heated at 95°C for 30 min, then rapidly cooled on ice. Absorbance was measured at 532 and 600 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800). MDA concentration was calculated using an extinction coefficient of 155 $mM^{-1}\ cm^{-1}$ and expressed as nmol g^{-1} dry weight (DW).

Relative water content: Relative water content (RWC) was determined according to Ings *et al.* (2013). Fresh weight (FW) of aerial tissues was recorded immediately after sampling. Samples were then immersed in distilled water at 10°C in the dark for 24 h to obtain turgid weight (TW), followed by oven-drying at 70°C for 48 h to determine dry weight (DW). RWC was calculated as:

$$\mathrm{RWC}\ (\%) = \left(\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\right) \times 100$$

Proline content: Proline content was measured following Bates *et al.* (1973). Leaf tissue (0.5 g) was homogenized in 10 mL of 3% sulfosalicylic acid and centrifuged. Two milliliters of the supernatant were reacted with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid and incubated at 100°C for 1 h. After cooling, the reaction mixture was extracted with 4 mL of toluene, and absorbance of the toluene phase was measured at 520 nm. Proline concentration was calculated using an L-proline standard curve and expressed as µmol g^{-1} dry weight.

Enzyme extraction and assay: Fresh leaf samples (0.2 g) were homogenized in 1 mL of 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM α-dithiothreitol (DTT), 2 mM EDTA, and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 3000 × g for 10 min at 4°C, and the resulting supernatant was used as the enzyme extract.

Catalase (CAT) activity: Catalase (CAT) activity was determined by monitoring the decomposition of H_2O_2 through the decrease in absorbance at 240 nm for 2 min (Aebi, 1984). The reaction mixture consisted of 1 mL of 50 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and 50 µL of enzyme extract. One unit of CAT activity was defined as a change in absorbance of 0.01 per minute.

Ascorbate peroxidase (APX) activity: Ascorbate peroxidase (APX) activity was measured by following the decrease in absorbance at 290 nm for 2 min due to ascorbate oxidation ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) (Nakano and Asada, 1981). The total reaction volume (3 mL) contained 50 mM sodium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 0.2 mM H_2O_2, and 50 µL of enzyme extract.

Superoxide dismutase (SOD) activity: Superoxide dismutase (SOD) activity was assayed based on the inhibition of 50% reduction of nitro blue tetrazolium (NBT) to blue formazan at 560 nm (Beauchamp and Fridovich, 1971). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM L-methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA, and 50 µL of enzyme extract. The reaction was initiated by exposing the mixture to fluorescent light (5000 lux) for 15 min. Blank and control samples were prepared without light exposure and without enzyme extract, respectively, and treated similarly.

Statistical analysis: All experiments were conducted with at least three independent replicates. Data were analyzed using GraphPad Prism software (version 10). Two-way analysis of variance (ANOVA) was performed, and mean comparisons were conducted using Duncan’s multiple range test at a significance level of $P \leq 0.05$.

Results

The effects of drought, genus, calcium, and their interactions were significant on shoot and root length. However, the Ca^{+2} × drought interaction was not significant. Data analysis indicated that the highest shoot elongation was observed in the genera *Lolium* and *Agropyron* under non-drought stress conditions. Drought stress significantly reduced shoot growth in *Lolium*, *Cynodon*, and *Festuca*. Moreover, the lowest shoot growth in response to drought stress was recorded in *Cynodon* and *Festuca*, both in the presence and absence of calcium application (Fig. 1a). Overall, calcium application under drought stress did not exert a significant effect on shoot growth (Fig. 1b).

In contrast, root elongation increased in response to drought stress in *Festuca* and *Agropyron* (Fig. 1c). The greatest root length under drought stress in media containing 5, 10, and 20 mM Ca^{+2} was observed in *Agropyron*. For instance, the application of 20 mM Ca^{+2} under drought stress in *Agropyron* resulted in an

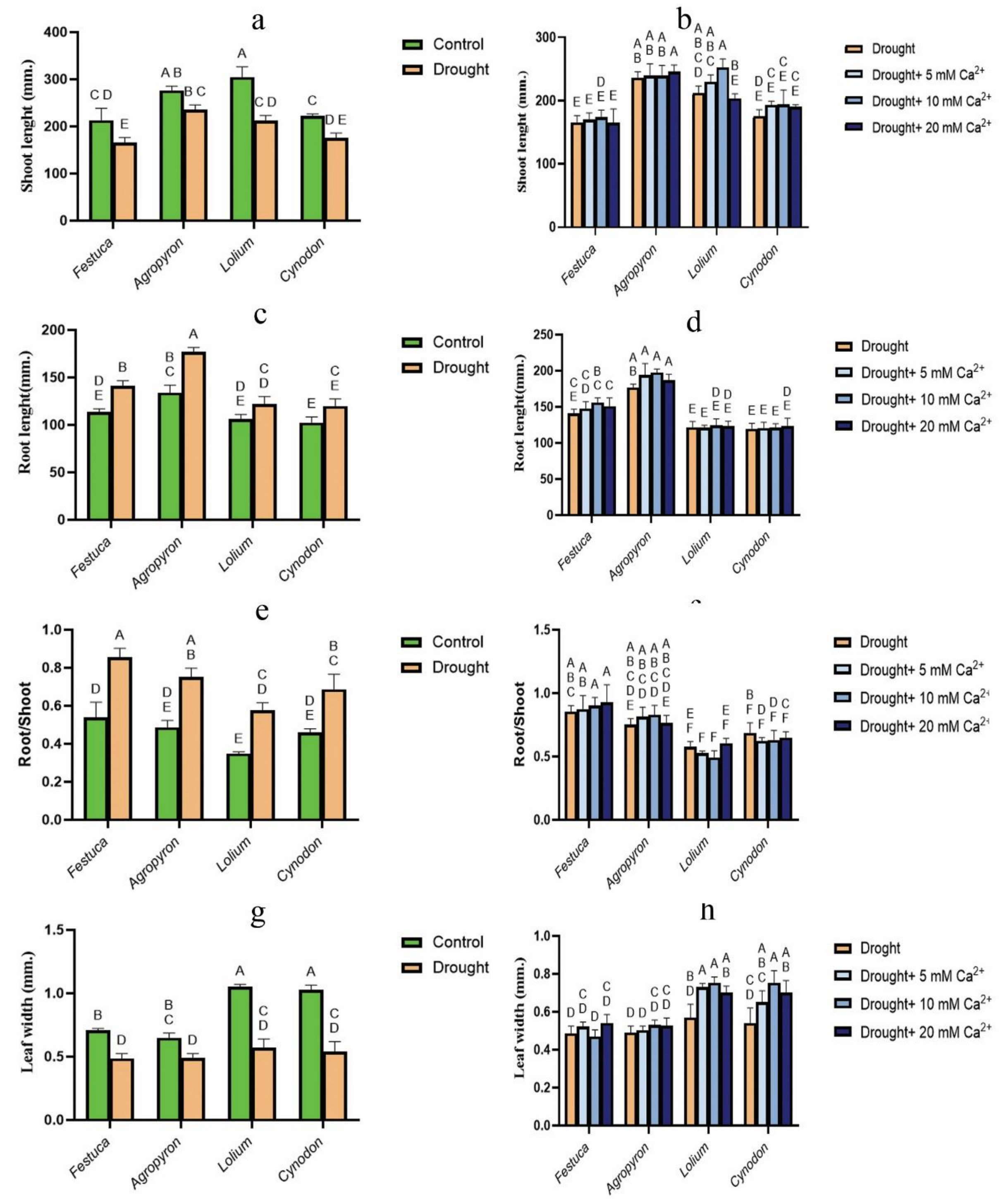

Figure 1. Effects of drought stress and calcium on the morphology of four common turfgrass genera. Drought stress and calcium treatments (5, 10, and 20 mM Ca^{2+}) influenced shoot length (a, b), root length (c, d), root-to-shoot ratio (e, f), and leaf width (g, h) in four turfgrass genera (*Agropyron*, *Festuca*, *Cynodon*, and *Lolium*). Different letters indicate significant differences among treatments at $P < 0.05$ according to Duncan's multiple range test. Values represent the means of at least three independent experiments.

approximately 24%, 52%, and 51% increase in root length compared with *Festuca*, *Lolium*, and *Cynodon* under the same treatment, respectively (Fig. 1d).

Furthermore, drought and genus each had a significant main effect on the root-to-shoot ratio, whereas their interaction was not significant. The root/shoot length ratio increased in all genera in response to drought stress, with the most pronounced increases observed in *Festuca* and *Agropyron* (Fig. 1e). However, calcium application did not have a significant effect on this parameter (Fig. 1f).

Leaf width: Leaf width was significantly influenced by the main effects of drought, genus, and calcium, as well as by their interactions. The greatest leaf width was observed in *Lolium* and *Cynodon* under non-drought stress conditions, being approximately 46% higher than that of *Festuca* and *Agropyron* (Fig. 1g). Drought stress reduced leaf width in all genera. The highest percentage reduction in leaf width in response to drought stress was recorded in *Lolium* and *Cynodon*, with decreases of 47% and 46%, respectively, compared with their controls.

Furthermore, the results indicated that the application of 10 and 20 mM Ca^{+2} improved leaf width in *Lolium* and *Cynodon* under drought stress. For example, treatment with 10 mM Ca^{+2} under drought stress increased leaf width by approximately 32% and 38% in *Lolium* and *Cynodon*, respectively, compared with their drought-stressed controls (Fig. 1h). In contrast, calcium application did not result in significant differences in leaf width in *Festuca* and *Agropyron* under drought stress.

Photosynthetic pigments: The main effects of drought, genus, and calcium, as well as all associated interactions, had a significant effect on chlorophyll *a*, *b*, and total chlorophyll concentrations. In response to drought stress, chlorophyll *a* and total chlorophyll contents decreased in all genera. Under non-drought conditions, the highest chlorophyll *a* content was observed in *Cynodon* and *Agropyron*, while the highest total chlorophyll content was recorded in *Cynodon*. The greatest percentage reduction in chlorophyll *a* under drought stress occurred in *Cynodon* and *Lolium*, with decreases of 46% and 51%, respectively. However, under drought stress, chlorophyll *a* content in *Agropyron* remained higher than in the other genera (Fig. 2a).

Application of calcium under drought stress improved chlorophyll *a* content in *Lolium* and *Cynodon* compared with their respective drought-stressed controls. The results indicated that calcium application at 10 and 20 mM was more effective in increasing chlorophyll *a* concentration in these two genera. In *Cynodon*, chlorophyll *a* content under drought stress increased by approximately 49% at calcium concentrations of 10 and 20 mM compared with the drought control. Similarly, in *Lolium*, application of 10 and 20 mM Ca^{+2} resulted in an approximately 48% increase in chlorophyll *a* relative to the drought control. In contrast, calcium application did not significantly affect chlorophyll *a* content in *Festuca* and *Agropyron* under drought stress (Fig. 2b).

Drought stress significantly reduced chlorophyll *b* content in *Lolium* and *Cynodon*, whereas no statistically significant reduction was observed in *Festuca* and *Agropyron* (Fig. 2c). Nevertheless, calcium application under drought stress markedly enhanced chlorophyll *b* content in *Cynodon*, such that the highest chlorophyll *b* concentrations under drought stress were recorded in *Cynodon* plants treated with 5, 10, and 20 mM Ca^{+2} (Fig. 2d).

Moreover, the greatest percentage reduction in total chlorophyll under drought stress was observed in *Lolium* and *Cynodon*, where total chlorophyll content declined by more than 50% (Fig. 2e). Calcium application under drought stress improved total chlorophyll content in *Lolium* and *Cynodon*, with 10- and 20-mM Ca^{+2} being more effective. The highest total chlorophyll content under drought stress in calcium-containing media was observed in *Cynodon* treated with 5, 10, and 20 mM Ca^{+2}, showing an approximately 45% increase compared with the drought control. Similarly, in *Lolium*, application of 10 and 20 mM Ca^{+2} increased total chlorophyll content by approximately 49% relative to the drought control. In contrast, calcium application had no significant effect on total chlorophyll content in *Festuca* and *Agropyron* (Fig. 2f).

Regarding carotenoids, the results revealed that drought stress did not cause a significant reduction in carotenoid content in any of the genera (Fig. 2g). However, calcium application—particularly at 20 mM—enhanced carotenoid concentration in *Lolium* and *Cynodon*. In these genera, treatment with 20 mM Ca^{+2} increased carotenoid content by approximately 42% and 46%, respectively, compared with their drought-stressed controls (Fig. 2h).

Ion leakage: The main effects of drought, genus, and calcium, as well as all interactions except that between Ca^{+2} × genus, had a significant effect on ion leakage. Data analysis indicated that drought stress increased ion leakage in all genera. The highest ion leakage was observed in *Lolium* and *Cynodon* under drought stress, which were approximately more than 30% higher than *Festuca* and *Agropyron* (Figure 3a). Application of calcium at 10 and 20 mM reduced ion leakage in *Lolium* and *Cynodon* under drought stress. Specifically, supplementation with 10 and 20 mM Ca^{+2} under drought conditions resulted in approximately 16% and 15% reductions in ion leakage in *Lolium* and 12% and 13% reductions in *Cynodon*, respectively, compared with the drought control of each genus (Figure 3b). Although calcium application reduced ion leakage in *Lolium* and *Cynodon*, their ion leakage levels remained higher than those of *Festuca* and *Agropyron* under drought stress, with or without calcium application. In contrast, calcium treatment did not cause a significant reduction in ion leakage in *Festuca* and *Agropyron*.

Lipid peroxidation: The main effects of drought, genus, and calcium, as well as all interactions except that between Ca^{+2} × genus, had a significant effect on lipid peroxidation. The level of lipid peroxidation, assessed by measuring malondialdehyde (MDA) content, increased in response to drought stress in all genera, similar to the pattern observed for ion leakage. However, the highest MDA accumulation under drought stress was detected in *Cynodon*, with values approximately 38%, 36%, and 39% higher than those of *Lolium*, *Agropyron*, and *Festuca*, respectively, under the same conditions (Figure 3c). The results further showed that calcium application under drought stress significantly reduced MDA content only in *Cynodon*, while no significant effects were observed in the other

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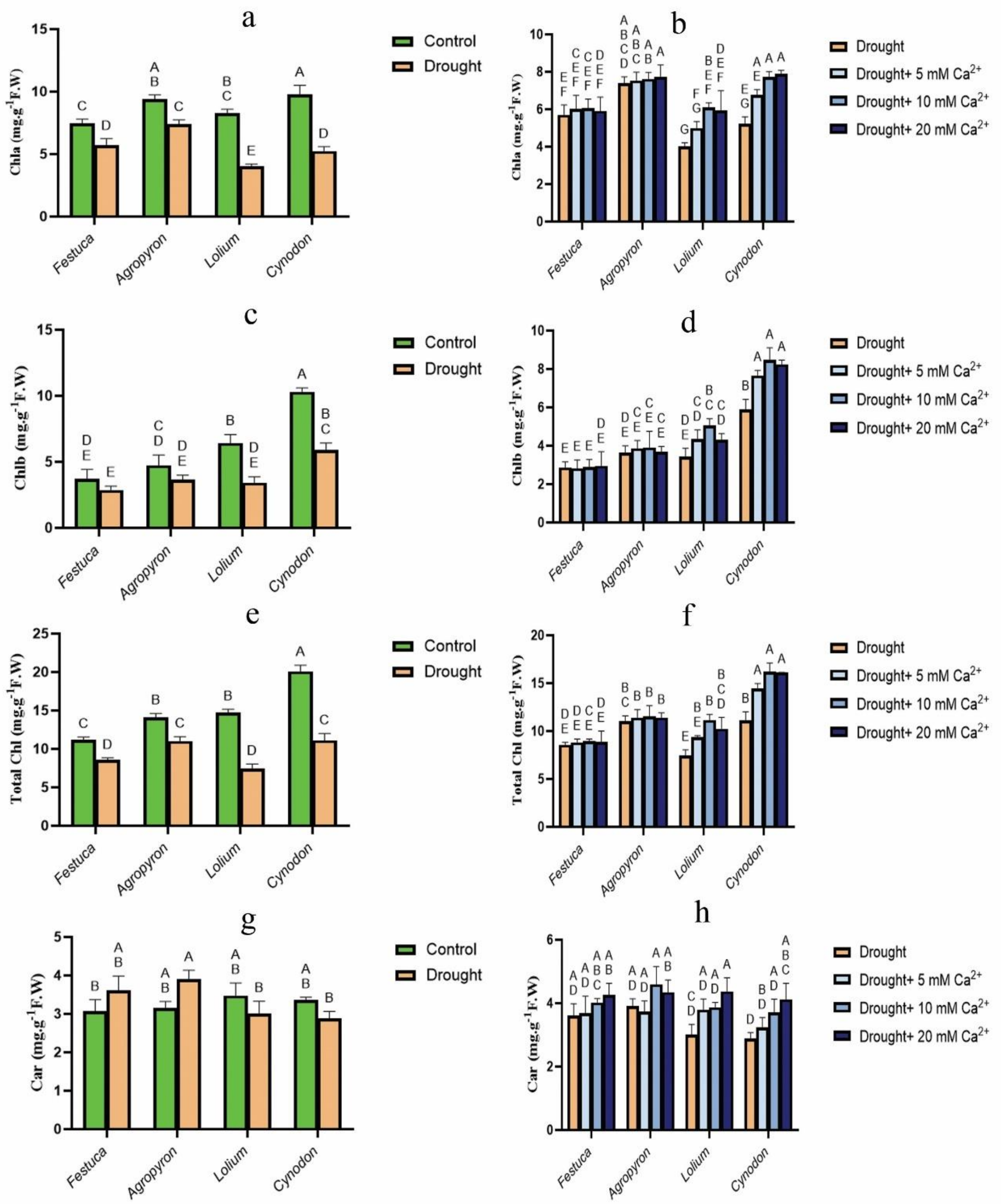

Figure 2. Effects of drought stress and calcium (5, 10, and 20 mM Ca^{+2}) on chlorophyll *a* (a, b), chlorophyll *b* (c, d), total chlorophyll (e, f), and carotenoids (g, h) in four grass genera (*Agropyron*, *Festuca*, *Cynodon*, and *Lolium*). Bars sharing the same letters indicate no significant differences at P < 0.05 according to Duncan's multiple range test. Values represent the means of at least three independent experiments.

genera. In *Cynodon*, supplementation with 5, 10, and 20 mM Ca^{+2} under drought conditions resulted in reductions of approximately 17%, 15%, and 19%, respectively, compared with the drought control (Figure 3d).

Relative water content: The main effects of drought, genus, and calcium, along with all of their interactions, significantly influenced relative water content. Drought stress reduced the relative water content (RWC) in all genera. However, the smallest reduction in RWC was observed in *Agropyron*. Under drought stress, RWC in this genus was approximately 33%, 51%, and 44% higher than that of *Festuca*, *Lolium*, and *Cynodon*, respectively (Figure 3e). In

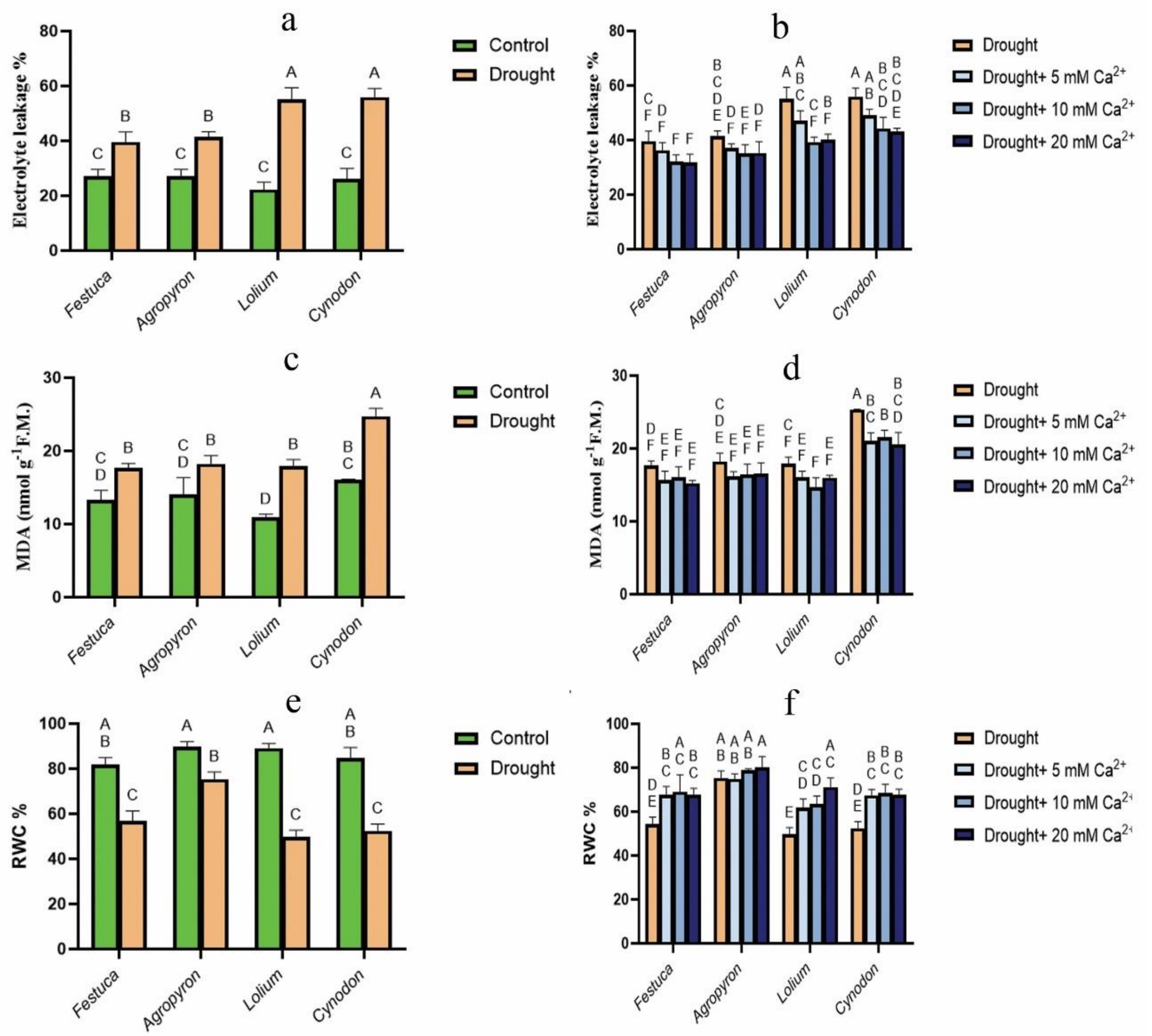

Figure 3. Effects of drought stress and calcium (5, 10, and 20 mM Ca^{+2}) on ion leakage (a, b), lipid peroxidation (c, d), and relative water content (e, f) in four grass genera (*Agropyron, Festuca, Cynodon,* and *Lolium*). Bars sharing the same letters indicate no significant differences at $P < 0.05$ according to Duncan's multiple range test. Values represent the means of at least three independent experiments.

addition, calcium application (5, 10, and 20 mM Ca^{+2}) improved RWC in all genera except *Agropyron*. For instance, supplementation with 20 mM Ca^{+2} under drought stress increased RWC by 15.2% in *Cynodon*, 21.49% in *Lolium*, and 13.3% in *Festuca* compared with the drought control of each genus (Figure 3f).

Proline: Proline content was significantly influenced by drought, genus, and their interaction, but not by calcium or the Ca^{+2} × drought interaction. Data analysis revealed that proline content increased significantly in response to drought stress in all grass genera. The highest proline accumulation under drought stress was observed in *Festuca* (Figure 4a), with levels approximately 42%, 23%, and 48% higher than those in *Agropyron*, *Lolium*, and *Cynodon*, respectively. However, calcium application did not have a significant effect on proline content in any of the grass genera (Figure 4b).

Antioxidant enzymes: Data analysis showed that superoxide dismutase (SOD) activity increased in response to drought stress in *Festuca* and *Agropyron*, whereas no significant changes in SOD activity were observed in *Lolium* and *Cynodon* under drought stress. The highest SOD activity was recorded in *Festuca* and *Agropyron*, which showed increases of more than 60% compared with *Lolium* and more than 90% compared with *Cynodon* under drought conditions (Figure 5a). Calcium supplementation did not alter SOD activity under drought stress in any of the genera (Figure 5b).

Catalase (CAT) activity increased in all grass genera under drought stress. Under these conditions, the lowest CAT activity was observed in *Festuca*. CAT activity in *Lolium* under drought stress was approximately 15% higher than that in *Festuca* (Figure 5c). Moreover, the application of 20 mM Ca^{+2} under drought stress enhanced CAT activity in *Lolium* and *Festuca*. Specifically, supplementation with 20 mM Ca^{+2} resulted in a 21% increase in CAT activity in *Lolium* and a 22%

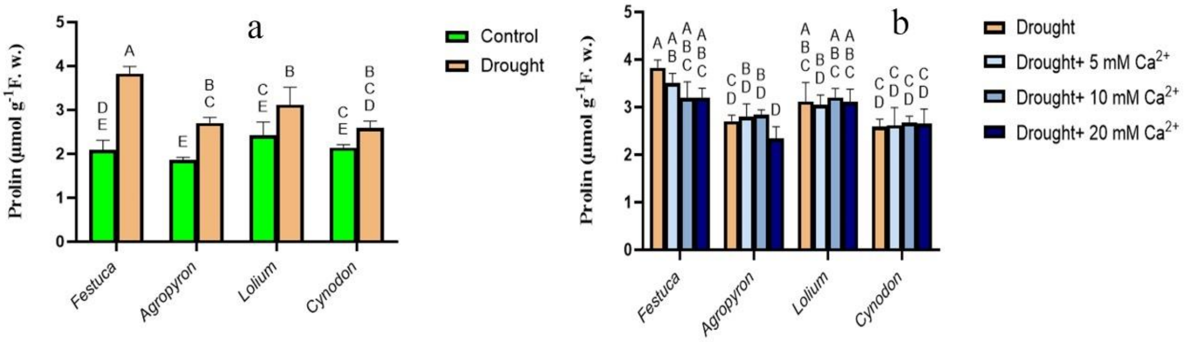

Figure 4. Effects of drought stress and calcium (5, 10, and 20 mM Ca^{+2}) on proline content in four grass genera (*Agropyron*, *Festuca*, *Cynodon*, and *Lolium*). Bars sharing the same letters indicate no significant differences at P < 0.05 according to Duncan's multiple range test. Values represent the means of at least three independent experiments.

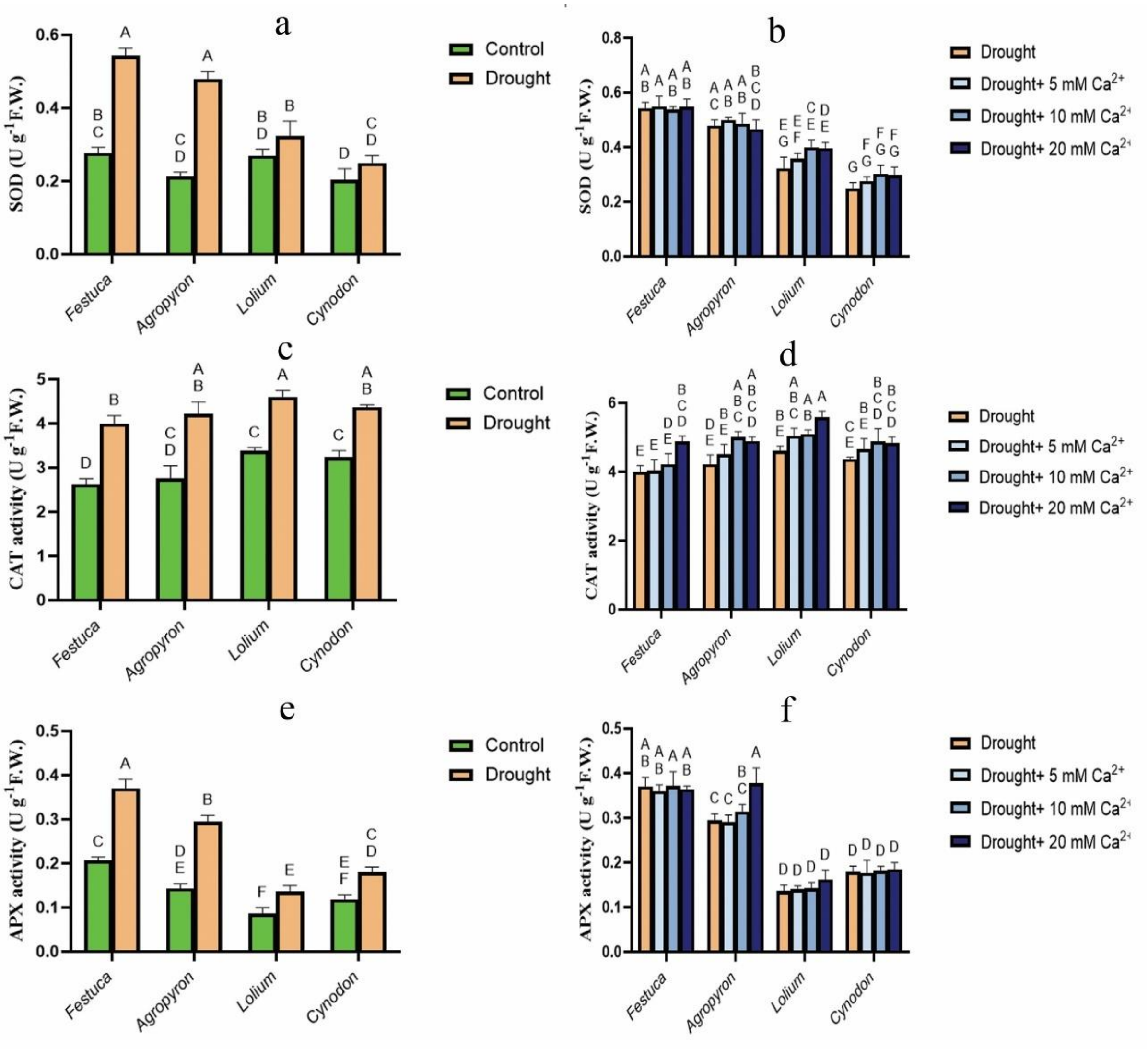

Figure 5. Effects of drought stress and calcium (5, 10, and 20 mM Ca^{+2}) on the activities of superoxide dismutase (a, b), catalase (c, d), and ascorbate peroxidase (e, f) in four grass genera (*Agropyron*, *Festuca*, *Cynodon*, and *Lolium*). Bars sharing the same letters indicate no significant differences at P < 0.05 according to Duncan's multiple range test. Values represent the means of at least three independent experiments.

increase in *Festuca* compared with the drought control of each genus (Figure 5d).

Regarding ascorbate peroxidase (APX) activity, the data indicated that drought stress increased APX activity in all genera. However, the highest APX activity under drought stress was observed in *Festuca*, followed by

Agropyron (Figure 5e), while the lowest APX activity was recorded in *Lolium*. Under drought stress, APX activity in *Festuca* was significantly 27%, 164%, and 105% higher than in *Agropyron*, *Lolium*, and *Cynodon*, respectively. With respect to calcium treatments, only the application of 20 mM Ca^{+2} in *Agropyron* significantly increased APX activity compared with the drought control (Figure 5f).

Discussion

The findings of this study highlighted the complex physiological and biochemical responses to drought stress and the modulatory role of calcium in four grass genera. These responses were discussed within the general framework of drought avoidance (maintenance of plant water status) and drought tolerance (cellular and metabolic adjustments). Previous studies suggested that plant performance under drought often reflected a combination of these strategies (Fang and Xiong, 2015; Ranjan *et al.*, 2022).

In this study, *Agropyron* and *Festuca* exhibited greater root elongation and biomass under drought, indicating a shift toward belowground growth. Such responses were commonly associated with drought avoidance, as deeper or more extensive root systems were reported to enhance access to soil moisture (Khanthavong *et al.*, 2021; Ranjan *et al.*, 2022). The increased root-to-shoot ratio observed in these genera suggested a reallocation of growth under water deficit. Conversely, shoot growth was reduced across all genera, particularly in *Lolium* and *Cynodon*, which was consistent with typical drought responses reported in grasses and may have reflected a strategy to limit transpirational water loss (Bashir *et al.*, 2021; Kane, 2011). Root responses to drought in grasses were species-specific. Our observation of increased root growth in *Festuca* and *Agropyron* was consistent with reports on *Agropyron cristatum*, which showed that this genus responds to drought through resource allocation to roots (Bayat *et al*., 2016). Notably, calcium had no significant effect on shoot growth or root-to-shoot ratio, consistent with findings in *Cynodon dactylon*, where calcium improved physiological parameters without necessarily restoring aboveground biomass (Shi *et al.*, 2013). However, calcium specifically enhanced root growth in *Agropyron* under drought, which suggested that this genus could utilize calcium to reinforce its drought-avoidance strategy via root system development (Ravi *et al.*, 2023; Xu *et al.*, 2022).

At the leaf level, reductions in leaf width and relative water content across all genera were clear indicators of water deficit, a common drought response in grasses, as reported in *Festuca arundinacea* (Manuchehri and Salehi, 2015). Improvements in these parameters in *Lolium* and *Cynodon* following calcium application indicated that calcium had a beneficial effect on plant water status. Although calcium was widely reported to participate in stress signaling and membrane stabilization (Ravi *et al.*, 2023; Xu *et al.*, 2022), the present results demonstrated physiological outcomes only and did not provide direct evidence for specific molecular pathways. This direct improvement in hydration status provided a key drought avoidance mechanism in the sensitive genera. However, calcium had little effect on *Festuca* and *Agropyron*, likely because these genera already possessed more efficient water retention mechanisms and are less dependent on external calcium signaling.

At the cellular level, reductions in photosynthetic pigments in *Lolium* and *Cynodon* indicated damage to the photosynthetic apparatus. The marked improvement of chlorophyll *a*, total chlorophyll, and carotenoids with calcium application highlighted its protective role in stabilizing chloroplast membrane systems and enhancing antioxidant capacity. This was evident in the reductions of lipid peroxidation (MDA) and ion leakage in these two genera. Similar studies had confirmed that calcium prevented chlorophyll degradation by activating PSII repair enzymes and inhibiting reactive oxygen species (ROS) production (Shi *et al.*, 2013). A notable contrast was the lack of calcium effect on MDA in *Festuca* and *Agropyron*, further underscoring their strong intrinsic defense mechanisms.

Calcium acted as a frontline defense against oxidative stress by maintaining membrane stability (Feng *et al.*, 2023). However, enzymatic antioxidant responses varied greatly among genera, which defined distinct drought-resistance strategies. *Festuca* and *Agropyron* strongly activated key enzymes such as SOD and APX, establishing an efficient endogenous defense system. Strong enzymatic responses were considered a key indicator of drought resistance (Ranty *et al.*, 2016; Xu *et al.*, 2022). In contrast, *Lolium* and *Cynodon* lacked such robust enzymatic responses and relied more on non-enzymatic and external protection, such as calcium supplementation.

Finally, proline accumulation was observed in all genera as a universal osmotic response. The lack of calcium effect on proline, as reported in other studies (Ravi *et al.*, 2023), suggested that osmotic regulation under these conditions was likely mediated by alternative signaling pathways, such as ABA-dependent mechanisms.

Conclusion

In summary, *Festuca* and *Agropyron* primarily rely on intrinsic drought-resistance and avoidance mechanisms, including robust root growth and an efficient antioxidant system, and thus exhibited limited responses to exogenous calcium. In contrast, *Lolium* and *Cynodon*, which lack these efficient intrinsic mechanisms, significantly benefited from calcium as an exogenous protector, enhancing membrane integrity, improving water status, and safeguarding the photosynthetic apparatus. This study not only highlights the diverse drought-resistance strategies among grass genera but also provided valuable insights for targeted turfgrass management, where calcium application could serve as

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a practical approach to enhance drought resilience in sensitive cultivars.

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