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RESEARCH ARTICLE
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Strigolactone and Abscisic Acid Positively Regulate Morphophysiological Traits and Gene Expression in Locally Grown *Sorghum bicolor* L under Drought Conditions

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ABSTRACT

Drought stress markedly constrains plant growth and productivity by disrupting physiological and biochemical homeostasis. *Sorghum bicolor* L., a drought-tolerant C4 crop, is an appropriate model for investigating hormone-mediated stress responses. This study evaluated the morphological, physiological, biochemical, and gene expression responses of local *S. bicolor* genotypes under polyethylene glycol (PEG)-induced drought stress, combined with exogenous abscisic acid (ABA) and strigolactone (SL) treatments, including a post-stress recovery phase. PEG stress significantly reduced shoot and root length and root number (up to 48%), particularly in plants without hormonal application. In contrast, SL substantially improved shoot and root growth during both stress and recovery. The chlorophyll ratio decreased by 72% under PEG stress but was restored by SL and ABA+SL treatments, indicating improved photosynthetic resilience. PEG stress increased proline (45%), malondialdehyde (MDA; 55%), and hydrogen peroxide (H_2O_2; 10%) levels, consistent with drought stress and oxidative damage. Exogenous ABA and SL reduced lipid peroxidation and reactive oxygen species (ROS) accumulation, with SL showing greater efficacy in the local *S. bicolor* genotypes. Antioxidant enzyme activities (CAT, POD, and APX) increased under stress, with relatively higher activities observed under ABA treatment. Gene expression analyses revealed differential regulation of *SbCAT*, *SbAPX*, and *SbSLAC1*. Both ABA and SL strongly induced *SbCAT* and *SbAPX*, whereas *SbSLAC1* was primarily upregulated under PEG stress combined with ABA. Overall, ABA and SL enhance drought tolerance in sorghum through coordinated activation of antioxidant defenses, drought adjustment, and stress-responsive gene expression, with SL playing a prominent role in promoting morphological recovery and maintaining redox balance during post-stress adaptation.

Keywords: *Sorghum bicolor*, Drought stress, Gene expression, Strigolactone, Abscisic acid.

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INTRODUCTION

Sorghum is a versatile crop that can be cultivated in arid and semi-arid regions where many other crops cannot be produced economically. It is used as a grain crop and as a source of forage, fiber, fuel, and other value-added products. Globally, sorghum ranks as the fifth most important cereal crop and is considered a strategic commodity for food security and livestock feed, particularly in Africa, Asia, South America, and the United States. Sorghum genotypes exhibit substantial diversity, providing broad potential for multiple ends uses (Tonapi et al., 2020; Zarei et al., 2022). The genus sorghum comprises 24 species, only a few of which are cultivated; among them, *Sorghum bicolor* L. is the most widely grown and is used primarily for animal feed and fodder, as well as for biofuel production from lignocellulosic biomass (Stamenkovic et al., 2020). *S. bicolor* is also recognized for drought tolerance and resilience to climate variability, as its C_4 photosynthetic pathway supports high water - use

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efficiency and biomass productivity (Wirawan et al., 2024). Importantly, sorghum is a staple crop for more than half a billion people in developing countries, particularly in arid and semi-arid regions where drought stress remains a major constraint on yield (Ge et al., 2023; Wang et al., 2025).

Drought is among the most severe abiotic stresses affecting plant growth and development and can lead to substantial yield losses (Arum et al., 2019; Siswoyo et al., 2021; Prasad et al., 2021; Hussaini et al., 2025). Water deficit impairs key metabolic processes, including leaf gas exchange, and promotes oxidative injury, ultimately reducing productivity. As a multidimensional stress, drought affects plants at molecular, morphological, physiological, and biochemical levels (Chadalavada et al., 2022; Zafar et al., 2023; Chang, 2025). Severe dehydration can suppress photosynthesis, disrupt metabolism, and, in extreme cases, it can lead to plant death. Regulation of leaf photosynthesis is therefore central to controlling water loss under drought. In response to limited water availability, plants typically downregulate photosynthetic activity due to increased transpirational demand, disrupted cellular function, reduced stomatal conductance, and impaired CO_2 diffusion and assimilation (Luqman et al., 2023; Luo et al., 2024; Imtaiz, 2025).

Because plants are sessile, they must continually adjust growth and development to fluctuating enviro nmental conditions. Phytohormones-including ABA, auxin, cytokinins, gibberellins, ethylene, brassinosteroids, jasmonic acid, salicylic acid, and strigolactones-play central roles in orchestrating these adaptive responses. Importantly, these hormones do not act independently; instead, they interact through extensive crosstalk that integrates biosynthesis, catabolism, transport, and signaling pathways. Abscisic acid is a well-characterized phytohormone involved in the regulation of developmental processes, including seed maturation and dormancy, and plays a central role in abiotic stress adaptation (Aslam et al., 2022; Ashraf & Khalid, 2025). During drought, ABA typically accumulates in roots and is transported to shoots via the xylem, where it promotes stomatal closure to reduce water loss and modulates ROS homeostasis (Sharma et al., 2023). ABA is synthesized from C_{40} carotenoid precursors produced in plastids. The first committed and rate-limiting step is the oxidative cleavage of 9-cis-violaxanthin or 9′-cis-neoxanthin to xanthoxin, catalyzed by 9-cis-epoxycarotenoid dioxygenases. Xanthoxin is then converted in the cytosol to abscisic aldehyde by a short-chain dehydrogenase/reductase and subsequently oxidized to ABA by abscisic aldehyde oxidase (Nakajima et al., 2024).

Strigolactones were first identified in root exudates as germination stimulants for parasitic weeds in the Orobanchaceae (e.g., *Striga*, *Phelipanche*, and *Orobanche*) (Jia et al., 2017; Özbilen et al., 2024). They are now recognized as multifunctional metabolites that act both as rhizosphere signals and as a distinct class of endogenous hormones regulating plant growth and development (Yoneyama & Philip, 2021). The earliest described hormonal function of SLs was the inhibition of shoot branching. In the rhizosphere, SLs function as signaling molecules that promote symbiosis with arbuscular mycorrhizal fungi by inducing hyphal branching, a response observed near host roots (Bhoi et al., 2021; Li et al., 2022). Conversely, parasitic weeds exploit SLs as host-location cues; their seeds germinate only after perceiving SL signals released by host roots (Wang et al., 2024; Khan et al., 2024). Genetic and biochemical studies have identified key enzymes involved in SL biosynthesis, including carotenoid isomerase, carotenoid cleavage dioxygenases, and cytochrome P450 monooxygenases. SL biosynthesis begins with all-trans-β-carotene, which is isomerized to 9-cis-β-carotene by D27 (Zhang et al., 2025). Sequential reactions catalysed by CCD7 and CCD8 then convert 9-cis-β-carotene to carlactone, which contains the characteristic A- and D-ring scaffold (Fathi et al., 2025).

Despite increasing studies on hormonal crosstalk under drought stress, limited attention has been given to locally adapted sorghum genotypes grown under specific agroecological conditions. Local genotypes may possess unique physiological and hormonal response patterns shaped by long-term enviro nmental adaptation. Therefore, investigating ABA SL interactions under PEG-induced drought stress in locally cultivated sorghum provides novel insights into genotype specific drought adaptation mechanisms and supports regionally relevant breeding strategies. This study aimed to elucidate the interaction between ABA and SL under PEG-induced drought stress in locally *Sorghum bicolor* L., with emphasis on their regulatory roles in morphological traits, photosynthetic pigments, stress-related metabolites, antioxidant enzyme activities, and stress-responsive gene expression.

MATERIALS & METHODS

Plant Materials, Growth Conditions and Stress Treatments

Mature *Sorghum bicolor* L. seeds (black-grained genotype) were collected from Jember, Indonesia. The experiment was conducted in the Nutraceutical and Pharmaceutical Laboratory, University of Jember, Indonesia (8°11′12.2″S, 113°38′08.6″E) (Fig. 1). Seeds were surface-sterilized by immersion in 70% (v/v) ethanol for 10 minutes, followed by 2% (v/v) sodium hypochlorite for 10 minutes. The seeds were then rinsed five times with sterile distilled water. Sterilized seeds were placed on sterile filter paper and transferred onto Murashige and Skoog (MS) medium for germination (Assem et al., 2023). Seeds were germinated for 7 days on solid MS medium containing 4.43 $g\ L^{-1}$ MS basal salts, 30 $g.L^{-1}$ sucrose, and 8 $g.L^{-1}$ agar. Seven-day-old seedlings were then subcultured for 7 days on treatment media. A total of 12 media (treatments) were prepared, comprising three experimental groups: non-stress (−PEG), drought stress (+PEG), and recovery after drought (+PEG recovery), each with four hormonal conditions (no hormone, ABA, SL, and ABA+SL).

For the non-stress (−PEG) group, seedlings were transferred to MS0 medium supplemented with hormones according to treatment: 0.1mM ABA, 0.01 mM SL, or a combination of ABA + SL. For the drought stress (+PEG) group, seedlings were transferred to MS0 medium containing 6% (w/v) polyethylene glycol (PEG 6000) to induce drought stress, either without hormones or

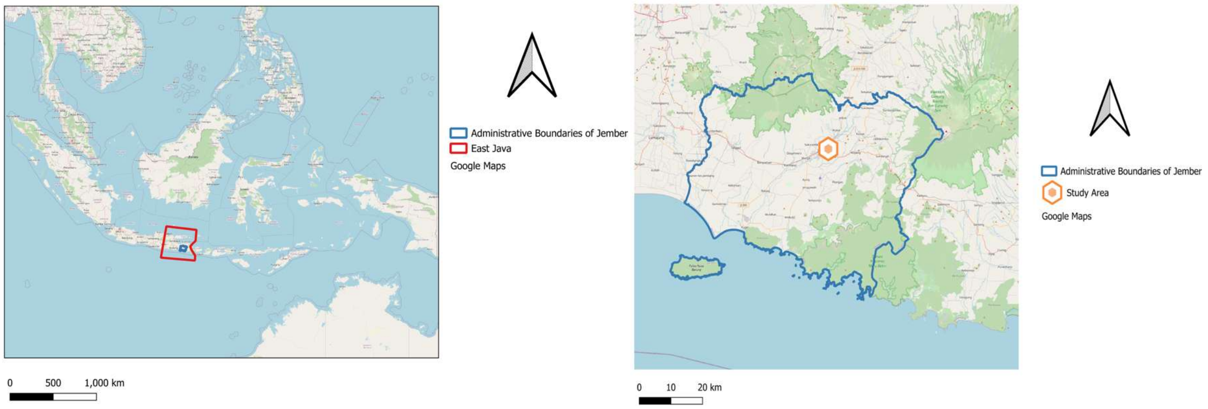

Fig. 1: Location of the study area in Jember, East Java, Indonesia. The Map shows the geographical position of the experimental site within Indonesia and East Java Province. The orange marker indicates the exact location of the study area.

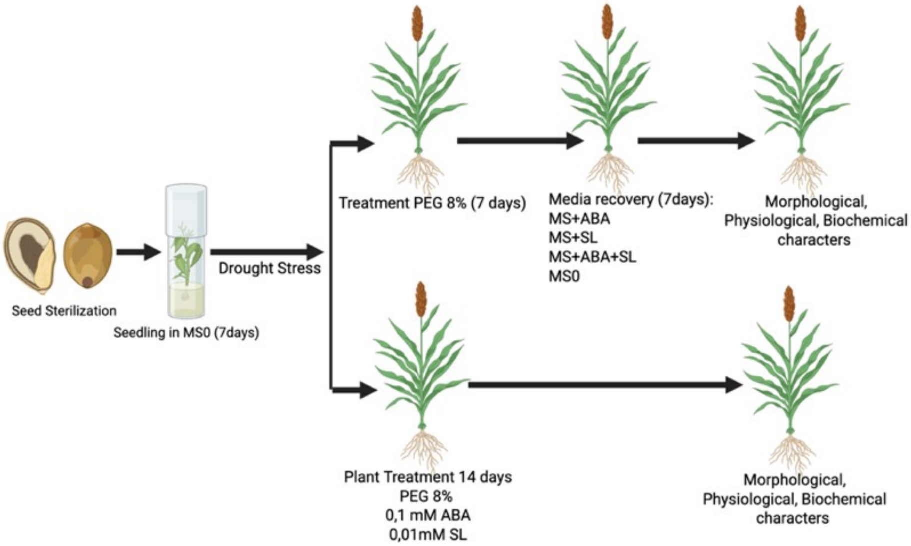

Fig. 2: Experimental design scheme for drought stress and hormone treatments in sorghum *in vitro*. Seven-day-old sorghum seedlings germinated on MS0 medium were exposed to 8% PEG to induce drought stress, followed by a recovery phase with hormone treatments (ABA and SL). Subsequently, morphophysiological, biochemical, and molecular parameters were assessed to evaluate plant responses to drought stress.

supplemented with ABA, SL, or ABA+SL (Korek & Marzec, 2023; Luqman et al., 2023). Stress treatments were applied for 7 days. For the recovery (+PEG recovery) group, seedlings were first exposed to PEG stress for 7 days and then transferred to PEG-free MS0 recovery medium supplemented with ABA, SL, or ABA + SL for an additional 7 days.

Each treatment was replicated three times, with five plants per replicate. Plants were maintained at 25°C during the recovery phase under the same cultivation conditions as in the preceding phase. The overall experimental duration was 21 days (7 days germination + 7 days treatment + 7 days recovery). At the end of the recovery phase, plants were harvested for morphological, physiological, and biochemical analyses. A schematic overview of the experimental design is shown in Fig. 2.

Growth and Morphological Parameters

Morphological traits, including shoot length, root length, and root number, were measured to evaluate the growth responses of locally grown white- and black-grained sorghum under drought stress and during the recovery phase. Measurements were recorded at 7 days intervals, beginning at the onset of stress exposure and continuing through the recovery phase.

Chlorophyll and Carotenoid Contents

Total chlorophyll content was determined from leaf samples collected 7 days after treatment using a spectrophotometric method (Lichtenthaler & Babani, 2022). Leaf tissue was extracted in 80% (v/v) acetone, and absorbance was measured at 645 and 663 nm for chlorophyll and at 470 nm for carotenoids. Chlorophyll a (Chl. a), chlorophyll b (Chl. b), and carotenoid concentrations were calculated using Arnon's equations as follows: Chl.a = 11.75 A_{662} – 2.35 A_{645}; Chl.b = 18.61 A_{645} – 3.96 $A_{662,}$ and Car. = 1000 A_{470} – 2.27 Chl.a – 81.4 Chl.b/ 227. Pigment contents were expressed as mg g^{-1} fresh weight (FW).

Determination of Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA) in Sorghum Leaves

Hydrogen peroxide and MDA levels were quantified as indicators of oxidative stress and lipid peroxidation following Velikova et al., (2000). Fresh leaf tissue (200 mg) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 rpm for 15 minutes. For H_2O_2 determination, 0.5 mL of supernatant was mixed with 0.5 mL of 10mM potassium phosphate buffer (pH 7.0) and 1.0 mL of 1M potassium iodide, then incubated for 15 minutes at room temperature (28°C). Absorbance was measured at 390 nm, and H_2O_2 concentration was calculated using an H_2O_2 standard curve. For MDA determination, 1.0 mL of supernatant was added to 4.0 mL of 20% (w/v) TCA containing 0.5% (w/v) thiobarbituric acid. The mixture was incubated at 95°C for 30 minutes and immediately cooled in an ice bath. Absorbance was read at 532 and 600 nm, and MDA concentration was calculated using an extinction coefficient of $155mM^{-1}cm^{-1}$. Results were expressed as $\mu mol.g^{-1}FW$.

Determination of Proline Content

Proline content was measured as described by Wang et al., (2022) with minor modifications. Fresh leaf tissue (50 mg) was homogenized in 1 mL of 60% (v/v) ethanol and incubated overnight at 4°C. The extract was centrifuged at 12,000 rpm for 5 minutes, and the supernatant was used for analysis (the extraction step could be repeated on the pellet if required). The reaction mixture (final volume 1 mL) consisted of 0.80 mL of ninhydrin reagent 1% (w/v) ninhydrin prepared in 60% (v/v) acetic acid and 20% (v/v) ethanol, and 100 µL of sample extract. After mixing, the samples were incubated at 95°C for 20 minutes, then cooled to room temperature until color stabilization, and the absorbance was measured at 520 nm. Proline concentration was expressed as $\mu mol\ g^{-1}.FW$.

Protein Extraction and Antioxidant Enzyme Activities

Leaf samples from each seedling were homogenized on ice using a chilled mortar and pestle in 5 mL extraction buffer containing 50mM Tris–HCl (pH 7.4), 1mM EDTA, 5mM $mgCl_2$, and 1mM β-mercaptoethanol. Homogenates were centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was used to determine total soluble protein content and antioxidant enzyme activities, which were measured using a UV–Vis spectrophotometer.

Catalase activity (CAT) was determined according to Rudiyanto et al., (2025). The reaction mixture contained 1.0 mL of freshly prepared 20mM H_2O_2 in 50mM sodium–potassium phosphate buffer (pH 7.4) and 100µL enzyme extract. After incubation at 37°C for 3 minutes, the reaction was stopped by adding ammonium molybdate. Absorbance was measured at 374 nm against an appropriate blank.

Peroxidase activity (POD) was assayed following Rudiyanto et al., (2025). The assay mixture consisted of 2.0 mL phenol–buffer solution, 1.0 mL substrate solution, and 0.1 mL aminoantipyrine solution. The mixture was incubated at 37°C for 10 minutes, after which 0.1 mL enzyme extract was added and maintained at 37°C. Absorbance was measured at 500 nm at 2 and 5 minutes after enzyme addition. POD activity was calculated according to the formula provided in the referenced method, based on the formation of quinoneimine dye generated by coupling 4-aminoantipyrine and phenol.

Ascorbate Peroxidase activity (APX) was measured by monitoring the decrease in ascorbate absorbance at 290 nm as described by Kumar (2022). The 1.0 mL reaction mixture contained 0.95 mL of 50mM sodium phosphate buffer (pH 7.0), 0.5mM ascorbic acid, 0.2mM EDTA, 0.2mM H_2O_2, and 50µL enzyme extract. APX activity was calculated using an extinction coefficient of $2.8mM^{-1}\ cm^{-1}$ and expressed as μmol ascorbate oxidized $min^{-1}\ mg^{-1}$ protein.

RNA Isolation and Antioxidant-related Gene Expression

Total RNA was extracted from *Sorghum bicolor* leaves using the inclone biotech™ RNA Mini Extraction Kit, following the manufacturer's instructions. Gene expression was assessed by reverse transcription polymerase chain reaction (RT-PCR). Briefly, 1µg of total RNA was reverse-transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad, USA). The reverse transcription reaction contained 1µg RNA, 10µL of iScript master mix, and nuclease-free water treated with 0.1% diethyl pyrocarbonate to a final volume of 20µL. The resulting cDNA was amplified for 12 PCR cycles.

Primers were designed from conserved regions of *Tubulin*, *SbSLAC1*, *SbCAT*, and *SbAPX* to evaluate transcript accumulation in *S. bicolor*. Primer sequences were as follows: *SbCAT* (350bp), forward 5′-GGCAAGTCCCACTACGTCAA-3′ and reverse 5′-AGCTGCTCGTTCTCGTTGAA-3′; *SbAPX* (450bp), forward 5′-AGAGCGGTCTGGTTTTGAGG-3′ and reverse 5′-GAGCTTGAGGTGGGCTTCTT-3′; *SbSLAC1* (450bp), forward 5′-AACATAACTCGATCGGCGCT-3′ and reverse 5′-GAGGGAGTTCTGCTTGCTCA-3′. PCR amplicons were separated on 2% (w/v) agarose gels and visualized under UV illumination using a Major Science UV transilluminator. Relative band intensity was quantified using ImageJ software.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD) from three biological replicates. Statistical differences among treatments were evaluated by one-way analysis of variance (ANOVA) using SPSS Statistics v25.0 (IBM Corp., USA). Mean comparisons were performed using Duncan's multiple range test, with statistical significance set at $P<0.05$.

RESULTS

Impact of ABA and SL Application on Morphological Characteristics of Sorghum

Table 1 summarizes the effects of PEG-induced drought stress and exogenous hormone treatments (ABA, SL, and ABA+SL) on shoot length, root length, and root number in sorghum. Overall, hormone treatments significantly affected morphological traits ($P<0.05$). Strigolactone consistently promoted shoot elongation during both drought stress and recovery. Under stress (+PEG), SL-treated plants exhibited the greatest shoot length (14.63 ± 0.81cm), which was significantly higher than the corresponding value under non-stress conditions (−PEG; 13.80 ± 0.70cm). By contrast, hormone application did not affect root length, as values were comparable across treatments during both stress and recovery. Notably, SL showed the strongest positive effect on post-stress morphological recovery by enhancing shoot and root elongation, as well as increasing root proliferation, particularly during the recovery phase.

Table 1: Effects of ABA and SL on morphological parameters of local *Sorghum bicolor* L. genotypes under PEG-induced drought stress recovery

Treatment		Shoot length (cm)	Root length (cm)	Number of Roots (sheets)
(-) PEG	Control	22.95 ± 1.44^{f}	16.27 ± 2.99^{cd}	4.78 ± 0.51^{bcd}
	ABA	13.80 ± 0.70^{cd}	8.37 ± 1.02^{ab}	3.60 ± 0.40^{ab}
	SL	19.63 ± 1.11^{e}	16.50 ± 0.79^{cd}	5.56 ± 0.20^{d}
	ABA+SL	10.20 ± 1.32^{a}	14.47 ± 2.54^{c}	5.33 ± 0.34^{d}
(+) PEG	Control	10.67 ± 1.15^{ab}	9.40 ± 0.98^{ab}	3.87 ± 0.67^{abc}
	ABA	12.83 ± 0.61^{bcd}	7.60 ± 0.35^{a}	3.73 ± 0.42^{abc}
	SL	14.63 ± 0.81^{d}	9.83 ± 1.04^{ab}	4.97 ± 1.89^{cd}
	ABA+SL	10.97 ± 2.34^{ab}	8.63 ± 2.57^{ab}	3.33 ± 0.67^{a}
Recovery	Control	12.03 ± 1.72^{abc}	10.80 ± 1.78^{b}	4.78 ± 0.51^{bcd}
	ABA	19.78 ± 0.25^{e}	19.11 ± 1.14^{de}	4.89 ± 0.19^{bcd}
	SL	23.33 ± 1.69^{f}	23.39 ± 1.34^{f}	5.56 ± 0.20^{d}
	ABA+SL	19.52 ± 0.48^{e}	19.47 ± 0.55^{e}	5.33 ± 0.34^{d}

Data are presented as mean ± SD (n = 5). Different superscript letters within the same column indicate significant differences (Duncan's multiple range test, $P<0.05$). PEG, polyethylene glycol; ABA, abscisic acid; SL, strigolactone.

Role of Exogenous ABA and SL Application in Regulating Photosynthetic Pigments

Under non-stressed conditions (control), sorghum showed higher baseline chlorophyll a (6.00 ± 0.05 mg.g^{-1}FW) but lower total chlorophyll (7.50 ± 0.18 mg.g^{-1}FW). SL application increased total chlorophyll to 7.79 ± 0.30 mg.g^{-1}FW and carotenoids to 2.89 ± 0.13 mg.g^{-1}FW, while maintaining a moderate chlorophyll a/b ratio (1.62 ± 0.11). PEG stress caused severe pigment depletion, reducing chlorophyll a to 0.35 ± 0.06 mg.g^{-1}FW and total chlorophyll to 2.47 ± 0.18 mg.g^{-1}FW. Under stress, the ABA+SL treatment most effectively restored chlorophyll content, increasing total chlorophyll to 6.80 ± 0.21 mg.g^{-1}FW and producing the highest chlorophyll b level (4.46 ± 0.15 mg.g^{-1}FW). In contrast, SL alone did not recover chlorophyll under stress, with total chlorophyll remaining low (1.33 ± 0.27 mg.g^{-1}FW), although carotenoids remained relatively high (4.51 ± 0.10 mg.g^{-1}FW).

During recovery, pigment restoration differed among treatments. SL again increased total chlorophyll (6.69 ± 0.10 mg.g^{-1}FW), whereas ABA+SL resulted in a moderate recovery (4.69 ± 0.15 mg.g^{-1}FW). Carotenoid concentrations remained elevated across most recovery treatments, particularly under ABA+SL (4.05 ± 0.02 mg.g^{-1}FW), which may reflect sustained photoprotective or antioxidant capacity after stress. Chlorophyll a/b ratios were generally low during recovery (0.52–0.62), indicating a relative shift toward chlorophyll b dominance following stress (Fig. 3).

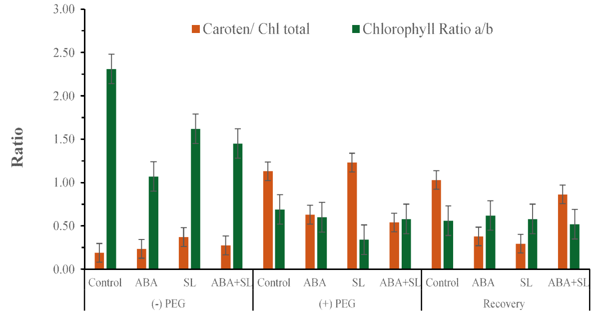

Fig. 3: Carotenoid-to-total chlorophyll ratio and chlorophyll a/b ratio in sorghum under non-stress (−PEG), PEG-induced drought stress (+PEG), and recovery conditions following ABA, SL, or combined ABA+SL treatments.

Effects of Exogenous ABA and SL on Stress-related Metabolites (proline, MDA, and H_2O_2)

Proline accumulation increased markedly under PEG-induced drought stress, consistent with drought adjustment (Table 2). Under non-stress (−PEG), proline levels were low across treatments (0.10 ± 0.01 to 0.90 ± 0.11μmol.g^{-1}FW). Notably, under non-stressed conditions, the ABA+SL combination produced the highest proline level in black sorghum (0.90 ± 0.11μmol.g^{-1}FW), suggesting a potential priming effect of the hormone combination. Under PEG stress, proline increased substantially, reaching 2.42 ± 0.48μmol.g^{-1}FW in sorghum, indicating a strong osmo-protective response.

Table 2: Proline, MDA and H_2O_2 contentss in *Sorghum bicolor* L. under PEG-induced drought stress and recovery with ABA, SL, and ABA+SL treatments

Treatment		Proline (μmol.g^{-1}FW)	MDA (μmol.g^{-1}FW)	H_2O_2 (μmol.g^{-1}FW)
(-) PEG	Control	0.15 ± 0.01^{a}	0.47 ± 0.01^{ab}	0.09 ± 0.00^{ab}
	ABA	0.41 ± 0.03^{a}	0.62 ± 0.03^{abc}	0.20 ± 0.01^{c}
	SL	0.10 ± 0.01^{a}	0.34 ± 0.02^{a}	0.08 ± 0.01^{a}
	ABA+SL	0.90 ± 0.11^{bc}	1.64 ± 0.04^{bcd}	0.11 ± 0.01^{b}
(+) PEG	Control	2.42 ± 0.48^{e}	2.39 ± 0.57^{d}	0.91 ± 0.01^{h}
	ABA	1.20 ± 0.16^{cd}	1.48 ± 0.00^{abcd}	0.92 ± 0.02^{h}
	SL	0.53 ± 0.01^{ab}	1.73 ± 0.02^{cd}	0.95 ± 0.00^{i}
	ABA+SL	1.43 ± 0.19^{d}	1.63 ± 0.01^{bcd}	0.77 ± 0.01^{g}
Recovery	Control	1.19 ± 0.53^{cd}	0.73 ± 0.01^{abc}	0.39 ± 0.02^{e}
	ABA	1.22 ± 0.17^{cd}	1.26 ± 0.01^{abcd}	0.57 ± 0.01^{f}
	SL	1.11 ± 0.02^{cd}	1.29 ± 0.02^{abcd}	0.35 ± 0.01^{d}
	ABA+SL	0.85 ± 0.24^{bc}	1.64 ± 0.02^{bcd}	0.56 ± 0.04^{f}

Data are presented as mean ± SD (n = 5). Different superscript letters within the same column indicate significant differences (Duncan's multiple range test, $P<0.05$). PEG, polyethylene glycol; ABA, abscisic acid; SL, strigolactone; FW, fresh weight.

Hormone application under PEG stress reduced proline accumulation to different extents. ABA resulted in a moderate decrease (1.20 ± 0.16μmol.g^{-1}FW), whereas SL more effectively reduced proline in black sorghum (0.53 ± 0.017μmol.g^{-1}FW), suggesting that SL may alleviate drought stress. During recovery, proline generally declined, consistent with stress relief; however, proline remained relatively high in black sorghum across treatments, indicating a slower recovery response.

Malondialdehyde, an indicator of lipid peroxidation, increased significantly under PEG stress, with the highest value observed in PEG-stressed controls (2.39 ± 0.576μmol.g^{-1}FW), confirming membrane damage under drought. ABA and SL reduced MDA under PEG stress, although MDA remained comparatively high even with hormone treatments (e.g., 1.64 ± 0.01μmol.g^{-1}FW under ABA+SL), suggesting persistent oxidative pressure. During recovery, MDA remained elevated in some treatments, particularly ABA+SL (1.64 ± 0.017μmol.g^{-1}FW), indicating incomplete restoration of membrane integrity.

H_2O_2 levels broadly paralleled MDA. PEG stress increased H_2O_2 to 0.91 ± 0.011 μmol.g^{-1}FW, indicating enhanced oxidative stress. Although hormonal treatments reduced H_2O_2 accumulation under stress, levels remained higher than in the control, with the lowest value observed under ABA+SL (0.77 ± 0.01μmol.g^{-1}FW). During recovery, H_2O_2 declined sharply, and SL markedly limited H_2O_2 accumulation (0.02 ± 0.006μmol.g^{-1}FW).

Impact of ABA and SL on Antioxidant Enzyme Activities under Drought Stress

Table 3 presents CAT, POD, and APX activities in sorghum exposed to PEG-induced drought stress (8%) and subsequent recovery under ABA, SL, and ABA+SL treatments. Under non-stress (−PEG), CAT activity was significantly lower under control conditions than during drought stress and recovery. Hormone application resulted in a modest increase in CAT activity, with the highest value recorded under ABA treatment (3.5U. mg^{-1}), indicating a mild induction of antioxidant defense mechanisms.

Table 3: Activities of CAT, POD and APX in local *Sorghum bicolor* L. under PEG induced drought stress and recovery with ABA, SL and ABA+SL treatments

Treatment		CAT	POD	APX
		(U. mg^{-1}protein)	(U. mg^{-1}protein)	(U. mg^{-1}protein)
(-) PEG	Control	2.25 ± 0.21^{a}	1.14 ± 0.04^{a}	30.10 ± 0.02^{c}
	ABA	3.50 ± 0.08^{c}	1.44 ± 0.13^{abc}	40.49 ± 0.45^{e}
	SL	3.34 ± 0.02^{bc}	1.24 ± 0.06^{ab}	29.47 ± 0.58^{bc}
	ABA+SL	2.90 ± 0.49^{b}	2.05 ± 0.05^{def}	29.02 ± 0.09^{bc}
(+) PEG	Control	5.75 ± 0.04^{f}	2.09 ± 0.31^{def}	40.64 ± 1.03e
	ABA	6.80 ± 0.06^{g}	1.67 ± 0.58^{bcd}	51.90 ± 0.99^{g}
	SL	5.14 ± 0.13^{e}	2.09 ± 0.08^{def}	35.47 ± 0.47^{d}
	ABA+SL	4.42 ± 0.15^{d}	2.20 ± 0.28^{ef}	44.17 ± 1.26^{f}
Recovery	Control	2.90 ± 0.19^{b}	1.06 ± 0.06^{a}	25.26 ± 0.26a
	ABA	4.37 ± 0.58^{d}	2.64 ± 0.11^{g}	44.88 ± 0.21^{f}
	SL	4.33 ± 0.76^{d}	2.42 ± 0.39^{fg}	26.86 ± 0.07^{ab}
	ABA+SL	4.09 ± 0.11^{d}	1.85 ± 0.07^{cde}	33.20 ± 4.64^{d}

Data are presented as mean ± SD (n = 5). Different superscript letters within the same column indicate significant differences (Duncan's multiple range test, $P<0.05$). PEG, polyethylene glycol; ABA, abscisic acid; SL, strigolactone.

Under PEG stress, CAT activity increased markedly, peaking in ABA-treated plants (6.8U. mg^{-1}). SL also enhanced CAT activity (5.14U. mg^{-1}), but to a lesser extent than ABA. Interestingly, ABA+SL-induced lower CAT activity than ABA alone, which may reflect antagonistic effects or regulatory balancing between hormone pathways. In recovery controls (no hormone), CAT activity declined substantially (2.9U. mg^{-1}), indicating post-stress downregulation in the absence of hormonal signaling.

POD showed a similar pattern. Under PEG stress, ABA produced the greatest increase in POD activity (1.67U. mg^{-1}), whereas SL and ABA+SL-induced moderate increases. During recovery, ABA resulted in the highest POD activity (2.64U. mg^{-1}), with SL showing a comparable elevation (2.42U. mg^{-1}).

APX exhibited the strongest hormonal responsiveness, particularly under PEG stress. ABA produced the highest APX activity (51.9U. mg^{-1}), significantly exceeding the other treatments. ABA+SL resulted in intermediate APX activity (44.17U. mg^{-1}), whereas SL alone produced comparatively lower values.

Correlation Analysis of Physiological and Biochemical Parameters under Stress Conditions

A correlation heatmap was used to assess relationships among proline, MDA, and H_2O_2 contents and CAT, POD, and APX activities (Fig. 4). Correlation coefficients (*r*) ranged from weak to strong, indicating variable degrees of association among traits. Proline showed strong positive correlations with MDA (*r* = 0.76), H_2O_2 (*r* = 0.75), and CAT (*r* = 0.68), suggesting tight coupling between drought adjustment, oxidative damage, and catalase-mediated detoxification. MDA also correlated strongly with H_2O_2 (*r* = 0.94) and CAT (*r* = 0.87), supporting an integrated response linking lipid peroxidation, ROS accumulation, and CAT activity.

H_2O_2 exhibited a very strong positive correlation with CAT (*r* = 0.97), consistent with catalase upregulation in response to increased H_2O_2. By comparison, POD showed moderate-to-weak correlations with most variables, with the strongest association observed with CAT (*r* = 0.46). APX generally exhibited weaker correlations across parameters, although it showed a relatively strong association with H_2O_2 (*r* = 0.73), suggesting that APX may be regulated differently or may respond under distinct thresholds of oxidative stress.

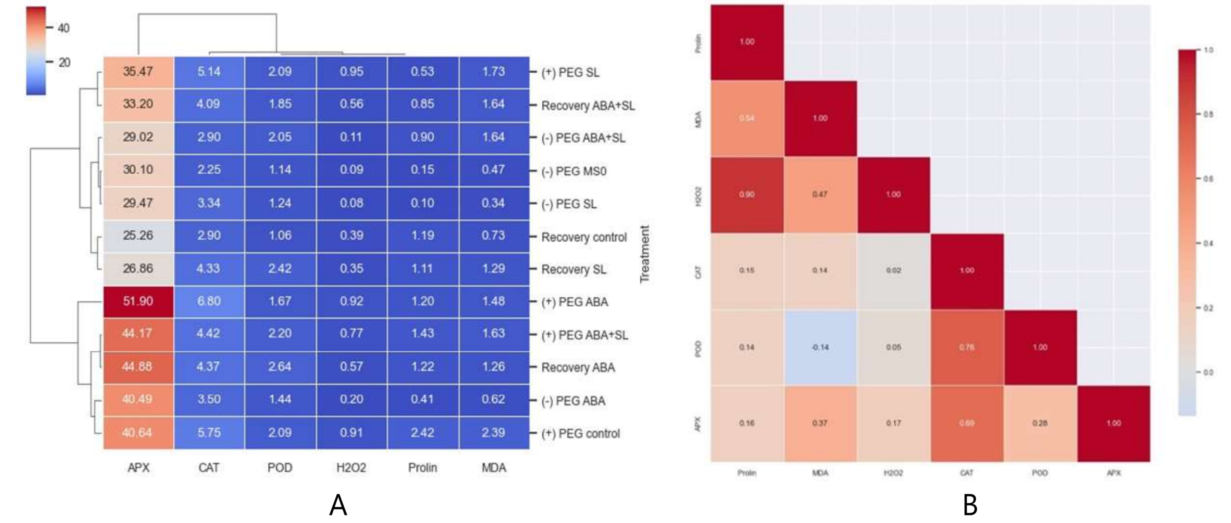

Fig. 4: (A) Hierarchical clustering heatmap illustrating biochemical responses of sorghum across drought stress and recovery treatments. Parameters analyzed were activities of antioxidant enzymes (APX, CAT and POD) and oxidative stress markers (H_2O_2, Proline and MDA). Colors represent normalized mean values (red = higher; blue = lower). (B) Pearson correlation matrix among biochemical traits across treatments. Correlation coefficients (r) are shown within cells, with red indicating positive and blue indicating negative correlation. Values represent means of three biological replicates ($P<0.05$).

In a second correlation set (genotype-specific), several relationships were weaker and included negative associations, indicating divergent physiological behaviour. Proline correlated moderately with MDA (r = 0.54) but only weakly with H_2O_2 (r = 0.15) and CAT (r = 0.14), suggesting a more limited coupling between drought adjustment and antioxidant enzymes in this genotype. The relationship between MDA and H_2O_2 was modest (r = 0.47), and MDA showed negligible association with CAT (r = 0.02). POD displayed a slight negative correlation with MDA (r = −0.14) and weak positive correlations with H_2O_2 (r = 0.05), but a strong positive correlation with CAT (r = 0.76), indicating potential coordination between these enzymes in ROS scavenging. APX remained weakly correlated with most parameters (e.g., H_2O_2, r = 0.37; CAT, r = 0.19), suggesting relatively independent regulation under the conditions tested.

While the biochemical responses provide evidence of stress adaptation at the physiological level, understanding whether these changes are driven by transcriptional regulation is essential. Therefore, we further investigated the expression profile of key genes involved in drought stress signaling and hormone crosstalk.

Gene Expression Analysis

Based on the results described above, gene expression analysis was performed to evaluate transcriptional changes associated with antioxidant defense, focusing on catalase (*SbCAT*), ascorbate peroxidase (*SbAPX*), and Slow Anion Channel 1 (*SbSLAC1*). This analysis aimed to (i) characterize the transcriptional regulation of stress-responsive antioxidant pathways during PEG-induced drought stress and the subsequent recovery phase and (ii) assess the effects of single-hormone (ABA or SL) and combined (ABA+SL) treatments on these responses.

Fig. 5A and 5B show representative electrophoretic profiles and the corresponding relative expression levels of *SbCAT*, *SbAPX*, and *SbSLAC1* across control, stress, and recovery conditions under ABA, SL, and ABA+SL treatments. *Tubulin* was used as the reference gene and exhibited stable expression across all treatments, supporting its suitability as an internal control for normalization.

DISCUSSION

Plant hormonal regulation plays a crucial role in determining photosynthetic resilience under drought stress. Differential hormonal effects can significantly influence pigment stability and recovery capacity following water deficit. ABA was generally less effective than SL in promoting post-stress recovery. The combined ABA+SL treatment produced intermediate responses, suggesting partial redundancy and pathway interaction. Under non-stressed conditions, ABA alone significantly decreased pigment contents, possibly due to suppressed biosynthesis and accelerated degradation. On the other hand, SL consistently increased chlorophyll accumulation and alleviated PEG-induced pigment loss in black sorghum, either by itself or in conjunction with ABA (Fathi et al., 2025). Black sorghum also showed a stronger carotenoid-based response under PEG stress, indicating genotype-specific photoprotection strategies. Although genotype specific responses should be considered in sorghum breeding and agronomic management, these findings collectively support the potential of SL+ABA treatments to mitigate drought induced impairment of the photosynthetic apparatus (Ssebulime et al., 2025; Zhai et al., 2026).

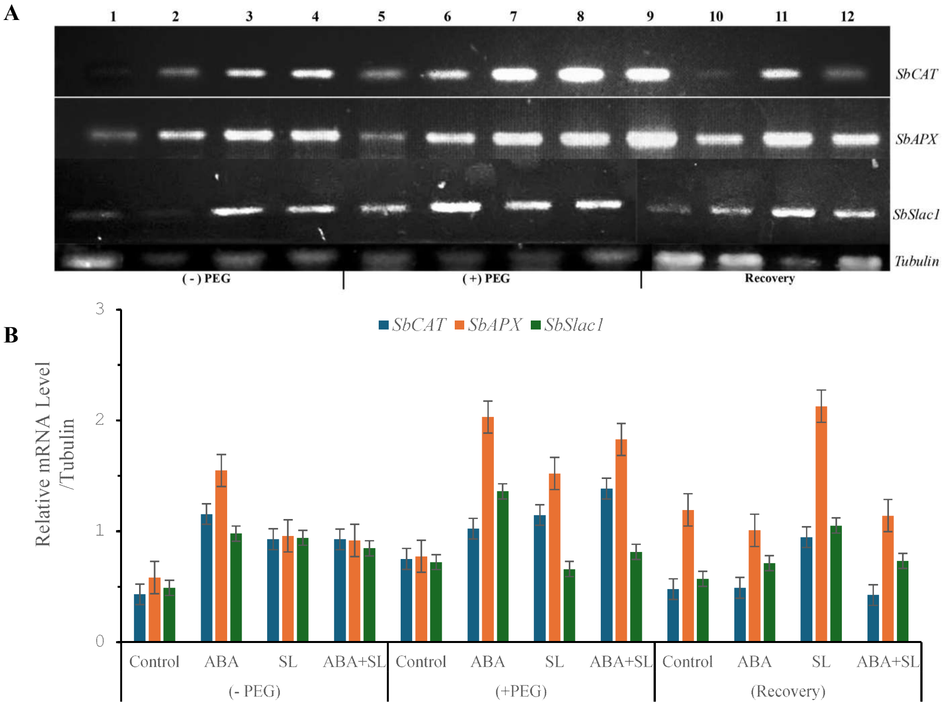

Fig. 5: Molecular analysis. (A) Representative agarose gel electrophoresis image showing expression patterns of *SbCAT* (350bp), *SbAPX* (450bp), and *SbSLAC1* (450bp) under non-stress (−PEG), PEG-induced drought stress (+PEG), and recovery conditions. (B) Relative mRNA abundance quantified using ImageJ.

Proline, MDA and H_2O_2 levels were higher in black sorghum than in white sorghum, indicating stronger metabolic adaptations to drought and a more stress-reactive phenotype. However, black sorghum exhibited slower recovery, particularly in ROS detoxification and membrane integrity. The ABA+SL combination effectively modulated proline and MDA accumulation, while H_2O_2 responses remained inconsistent, suggesting differential hormonal control of ROS production and scavenging. These findings demonstrate that hormone-based strategies, especially SL combination with ABA, increase drought tolerance by regulating key stress metabolites (Bhoi et al., 2021). During drought stress and recovery, ABA most effectively induced antioxidant enzymes, particularly CAT and APX. Although SL improved antioxidant responses, it was generally less successful than ABA. The ABA+SL treatment was not consistently additive, indicating hormonal crosstalk. White sorghum generally showed higher antioxidant enzyme activities, and ABA maintained elevated APX activity during recovery (Kelly et al., 2023). Proline, H_2O_2, and CAT were closely related in the stress response, with correlation analysis indicating an integrated network between osmolyte accumulation and antioxidant defenses. Strong positive correlations suggest coordinated regulation of cellular redox homeostasis during drought stress. In contrast, black sorghum showed weaker correlation, implying more independent or threshold-dependent antioxidant activity. This complexity highlights the need for further investigation of genotype-specific regulatory networks controlling ROS detoxification and osmolyte accumulation in stress-resilient sorghum (Rudiyanto et al., 2025).

At the transcriptional level, *SbCAT,* which encodes catalase, reacted differently to hormone application and drought stress, underscoring its central role in redox regulation during drought adaptation. Catalase decomposes H_2O_2, a major ROS generated under stress conditions, thereby limiting oxidative damage. Induction of *SbCAT* by ABA and SL suggests that both hormones stimulate oxidative stress-responsive genes, probably through partially overlapping but mechanistically distinct signaling pathways. In the absence of stress, *SbCAT* transcript were low in control plants but significantly elevated following ABA or SL treatment. This pattern aligns with previous studies indicating that ABA accumulation triggers the ROS-scavenging system by inducing antioxidant enzymes such as catalase, peroxidases, and superoxide dismutase (Zulfiqar et al., 2024). The SL-dependent induction further supports the role of strigolactones in developmental regulation, symbiotic signaling, and abiotic stress responses. Moreover, SL-mediated upregulation of *SbCAT* suggests interaction with ABA-related transcriptional networks, enhancing redox preparedness before drought onset (Wang et al., 2025). In the recovery phase, *SbCAT* expression declined compared to the stress phase, consistent with reduced ROS production as normal physiological activity resumed. Notably, ABA treatment during recovery brought *SbCAT* expression to near pre-stress levels, indicating its role in redox reprogramming and post-stress homeostasis. In general, these results show that *SbCAT* is a dynamic part of the ROS-detoxification machinery in *S. bicolor*, responsive to both drought stress and hormonal regulation. The non-additive expression pattern under combined ABA-SL treatments suggests complex hormonal crosstalk, enabling effective antioxidant response while reducing the metabolic cost of prolonged stress responses.

In this study, *SbAPX* transcripts were low in control plants but significantly upregulated by ABA and SL treatments, indicating hormonal activation of antioxidant pathways. Under water deficit, ABA upregulates APX and other peroxidase genes through ABA-responsive cis-elements in the promoter regions (Dun et al., 2023). Increasing evidence also links SLs with improved redox regulation, possibly via interactions with ABA and auxin signaling pathways (Sharma et al., 2023; Khan et al., 2024). Thus, SL-induced *SbAPX* expression suggests improved ROS-scavenging capacity and antioxidant priming before stress exposure. After PEG-induced drought stress, *SbAPX* expression increased further, confirming sensitivity to dehydration-induced oxidation stress. The increased induction seen with PEG+ABA and PEG+SL treatments indicates that both hormones amplify stress-responsive transcription. This pattern supports a dual-layer regulation model, in which hormones establish baseline activation and combined stress-hormone signal intensity expression, consistent with synergistic responses reported in rice and maize (Prasad et al., 2021).

Under non-stressed conditions, *SbAPX* expression remained relatively high in the ABA+SL co-treatment, whereas *SbCAT* decreased when both hormones were applied concurrently. This difference suggests that ABA and SL regulate *SbAPX* in a complementary rather than opposing. Promoter regions of APX genes in cereals contain numerous ABA and stress-responsive cis-elements, facilitating the integration of hormonal and enviro nmental signals (Chadalavada et al., 2022). So, *SbAPX* may act as a convergence point where ABA and SL pathways work together to improve ROS detoxification and reduce damage to cells. After PEG stress, *SbAPX* expression slowly went down, but remained higher in ABA or SL treatment plants recovery compared to untreated control. This persistent expression indicates that ongoing hormonal signaling facilitates the restoration of redox homeostasis and repair mechanisms subsequent to stress alleviation (Luo et al., 2024; Tanaka et al., 2025).

The *SbSLACl* gene, which encodes S-type anion channel 1 in *S. bicolor,* showed differential transcription response to drought stress and hormonal treatments (Zhang et al., 2025). In this study, *SbsSLAC1* transcripts were weak or nearly undetectable in control plants and in those treated with ABA or SL without PEG, indicating low basal expression without drought stress. Conversely, PEG-induced drought stress significantly upregulated *SbSLAC1,* indicating its responsiveness to drought-like conditions. This aligns with the recognized role of *SLAC1* orthologs in initiating guard-cell signaling pathways during drought and ABA-mediated response (Zhai et al., 2026). When PEG was combined with ABA, SL, or ABA+SL, *SbSLAC1* expression remained similar or slightly higher than PEG alone, suggesting that both hormones enhance drought signaling. This effect may occur through increased activity of transcription factors regulator the *SbSLAC1* promoter (Brandt et al., 2015). The increased expression in PEG+ABA corroborates the positioning of *SbSLAC1* downstream of ABA signaling in sorghum. Its responsiveness to SL is consistent with documented ABA-SL crosstalk, where SLs improve drought tolerance by modulating stomatal function and ABA sensitivity (Nashar et al., 2025). During recovery, *SbSLAC1* expression remained moderately higher in ABA- or SL-treated plants compared to untreated controls. Keeping *SbSLAC1* expression going during recovery may also help the stomata open slowly while keeping transpiration and secondary oxidative stress from getting too high. The expression profile of *SbSLAC1* shows that it is regulated by stress and hormones (Shimotohno et al., 2025). Unlike *SbCAT* and *SbAPX*, which primarily function in redox detoxification, *SbSLAC1* directly links hormonal signaling and plant water status to stomatal conductance. Its strong induction under PEG highlights a close association with drought perception and ABA signaling, while its responsiveness to SL suggests that strigolactone positively regulates ABA-dependent stomatal closure. Collectively, these findings identify *SbSLAC1* as a key molecular component in drought adaptation of *S. bicolor*, integrating ABA and SL signaling to regulate water loss and maintain physiological homeostasis during stress and recovery.

Conclusion

This study provides integrated evidence of the morphological, physiological, biochemical and molecular bases of drought tolerance in *Sorghum bicolor* under PEG-induced drought stress and hormonal regulation by ABA and SL. Drought stress markedly inhibited plant growth, reduced photosynthetic pigment content, and disrupted cellular redox homeostasis as indicated by decreased chlorophyll levels and increased accumulation of proline, MDA and H_2O_2. Exogenous ABA and SL alleviated these detrimental effects by enhancing antioxidant enzyme activities (CAT, POD and APX) and improving growth and pigment stability, particularly during the recovery phase. At the transcriptional level, *SbCAT* and *SbAPX* were strongly induced by both ABA and SL, underscoring their central roles in enzymatic ROS detoxification. In contrast, *SbSLAC1* responded primarily to PEG stress and ABA treatment, supporting its function in ABA-mediated stomatal regulation and water conservation. The combined effect of ABA and SL indicates complex hormonal crosstalk that fine tunes the balance between oxidative defence and water-use efficiency. Collectively, these findings show that coordinated hormonal regulation drives multi-level adaptive responses in sorghum and suggest practical avenues to improve drought resilience through hormonal priming and molecular breeding strategies targeting the *SbCAT*, *SbAPX* and *SbSLAC1* pathways.

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Data Availability: All the data is available in the article.

Ethics Statement: Not applicable

Author's Contribution: HM and TAS conceived and designed the study. HM and DS performed the experiments and collected the data. HM conducted laboratory analyses and processed the data. TAS, WIDF, and HSA supervised the project. HM and DS performed the statistical analyses. HM and TAS drafted the manuscript. All authors critically revised the manuscript and approved the final version.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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