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Original Article

Synergistic hormonal regulation of drought stress mitigation and recovery in *Oryza sativa* var. glutinous through abscisic acid and strigolactone

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Abstract

Oryza sativa var. glutinous, a native rice variety, has the potential to serve as a source of value-added nutritious processed foods, but it has very limited cultivation due to limited tolerance to abiotic stresses, especially drought, which restricts its growth and development. This study investigated the interactive roles of abscisic acid (ABA) and strigolactone (SL) to alleviate drought stress and promote recovery (R) in *Oryza sativa* var. glutinous. Drought stress and recovery treatments consisted of ABA, SL, and a combination of both, which had previously been subjected to drought stress induced by PEG-6000. Observational traits involved morphological (shoot height, leaf number, root length, and root number). Biochemical and physiological assessments included chlorophyll *a* and *b*, total chlorophyll, carotenoids, proline, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2). Then, changes in gene expression and enzyme activity of catalases (CAT), peroxidase (POD) and ascorbate peroxidases (APX) were assessed. The results showed that drought stress caused a decrease in total chlorophyll (4.46%) and an increase in proline (21.05%). The significant impact of oxidative stress was demonstrated by an increase in MDA (89%) and H_2O_2 (91%), as well as CAT, APX, and POD activity. During the recovery phase, the combination of SL and ABA was able to suppress the accumulation of MDA (44.64%) and H_2O_2 (20%), indicating a reduction in oxidative stress and restoration of membrane integrity. These results highlight the likely existence of an interaction between ABA and SL, which consequently affects not only the response to drought stress but also the recovery pathways.

Keywords: Abscisic acid, Strigolactone, Drought stress, *Oryza sativa* var. glutinous, Plant recovery, Resilience

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Introduction

Black glutinous rice (*Oryza sativa* var. glutinosus) is a less preferred abiotic stress-prone glutinous rice by farmers for cultivation and is highly prone to abiotic stress such as drought. Drought stress is one of the most serious environmental factors and affects the entire growth stages of rice plants (Hou et al., 2024). It causes multiple physiological changes in plants, such as reducing the rate of photosynthesis, transpiration, stomatal conductance, the stability of pigments, and relative water content. Furthermore, under drought stress, overproduction of reactive oxygen species (ROS), such as H_2O_2, induces damage to membranes and degradation of photosynthetic pigments (Siswoyo et al., 2021; Jira-anunkul and Pattanagul, 2021; Quintao et al., 2023; Buqori et al., 2025; Rudiyanto et al., 2025). In addition, drought stress induces stomatal closure through lesser turgor pressure, causing morphological, physiological, and biochemical changes that ultimately affect plant growth and productivity (Mahatthanaphatcharakun and Taratima, 2025). To avoid damage from drought stress, plants exhibit a number of strategies, including the use of stress-responsive hormones that regulate the processes involved in resiliency. These strategies represent an attractive option for producing environmentally stress-tolerant plants that could maintain productivity under conditions of stress (Yao et al., 2020; Mubarik et al., 2021; Cristina et al., 2024). Therefore, increasing drought tolerance is a crucial strategy in supporting food security.

Abscisic acid (ABA) has been well-characterized as a stress hormone that plays an important role in regulating stomatal closure, osmoregulation, and inducing defence related gene expressions during extreme environmental conditions, including drought (Fujita et al., 2011; Ali et al., 2020; Aslam et al., 2022; Nashar et al., 2025). In addition to this, multiple studies have shown that combining ABA with other hormones or compounds will bolster plant resilience against abiotic stresses (Zhou et al., 2022; Ali et al., 2025). For example, Chi et al. (2021) showed that tomato plants receiving ABA in combination with strigolactones (SLs) had a significant improvement in drought tolerance. Previous studies also showed that the ABA and SLs interaction is able to not only regulate cellular redox status and increase antioxidant enzyme activity, but also facilitate physiological recovery from drought stress (Bhoi et al., 2021; Alvi et al., 2022; Vaseva et al., 2024).

SLs were originally identified as stimulators for seed germination in parasitic plants but were recently recognized for regulating root architecture, stem growth, and plant responses to abiotic stresses, including drought stress (Ullah et al., 2018). Studies have suggested a synergism between ABA and SLs, which improves plant adaptations to water-deficit conditions. However, there are few studies on the synergistic roles of ABA and SLs in black glutinous rice, especially in the recovery phase after drought stress. The recovery phase is a critical period during which plants must quickly restore metabolic functions, repair oxidative damage, and regain photosynthetic capacity to grow optimally (Ke et al., 2025). Understanding hormonal mechanisms during the recovery phase is crucial for planning more effective mitigation strategies.

Therefore, this study is designed to determine the effects of ABA and SLs synergism on polyethylene glycol (PEG)-induced drought stress, and during recovery after experiencing stress during the vegetative phase of black glutinous rice. This study looks at changes in morphological features, photosynthetic pigments, stress markers (proline, MDA, and H_2O_2), antioxidant enzyme activities, and the expression of related genes (CAT, APX, and POD) to evaluate a hormonal approach for improving drought tolerance in black glutinous rice.

Material and Methods

Plant materials and experimental treatments

Black glutinous rice (*Oryza sativa* var. glutinous) used in this experimental study was obtained from the collection of the Center for Excellence in Science and Technology of Industrial Plant Biotechnology (PUI-PT BioTIn), University of Jember, Indonesia (8°11'12.2"S 113°38'08.6"E). The seeds were surface-sterilized by soaking in 70% ethanol for 30 s, then soaking in 2% sodium hypochlorite (v/v) for 30 min. The seeds were rinsed 5 times with sterile distilled water. Sterile seeds were placed on filter paper and then laid onto Murashige and Skoog medium, where they were germinated for 7 days (Gupta et al., 2020). The plants (7 days old) were then given three treatment groups, namely: 1. Non-stress (-PEG), the plants were transferred to control medium enriched with hormones according to the treatment, consisting of 0.1 mM abscisic acid (ABA), 0.01 mM strigolactone (SL), and a combination of ABA + SL. 2. Drought stress (+PEG), the plants were transferred to the control

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medium induced with 8% (w/v) polyethylene glycol (PEG-6000) as a drought stress agent, and combined with hormones: ABA, SL, ABA + SL. The stress treatment on the plants was conducted over a period of 7 days. 3. Recovery after drought (+PEG Recovery), after experiencing PEG stress for 7 days, the plants were transferred to a recovery medium (control) consisting of ABA, SL, ABA + SL. The recovery phase lasted for 7 days. Each treatment was repeated 3 times, and each repetition consisted of 5 plants. During the recovery phase, the plants were cultivated at a temperature of 25°C, with the same intensity as the previous phase. The total duration of the experiment was 21 days (7 days of germination + 7 days of treatment + 7 days of recovery). After the recovery phase, the plants were harvested for physiological and chemical analysis. A flow diagram of the experimental framework is presented in Figure 1.

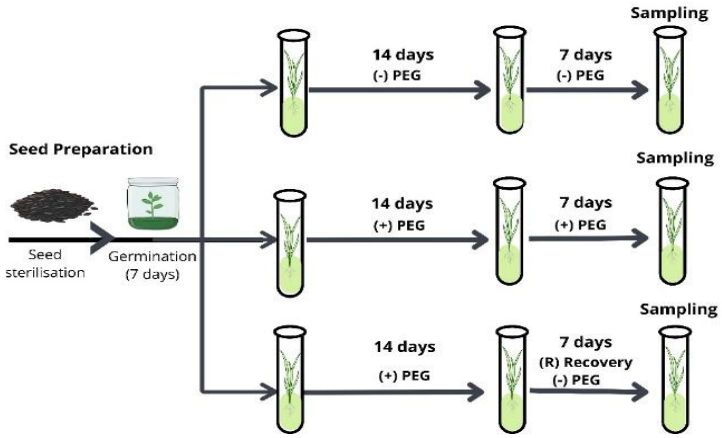

Figure-1. The research workflow illustrates the experimental design, including drought stress induction with 8% PEG-6000, hormone treatments (ABA, SL, and ABA+SL), and the vegetative phase to assess the morphological, biochemical, physiological, and molecular responses of black glutinous rice.

Morphological parameters

Morphological observations under PEG-6000 treatment were conducted 14 days after planting, while observations for the recovery treatments were performed 21 days after planting during the sampling stage. Morphological parameters, including plant height, root length, number of leaves, and number of roots, were measured non-destructively at the end of the drought stress period and following the recovery phase. Measurements were carried out using a digital ruler to ensure accuracy and consistency across replicates. Morphological data were statistically analyzed to evaluate the plant responses to hormonal treatments during post-drought recovery (Begum et al., 2022).

Photosynthetic pigment content in leaves

Total chlorophyll, chlorophyll *a*, chlorophyll *b,* and carotenoid content were measured using spectrophotometry following the method of Lichtenthaler and Babani (2022). A quantity of 0.5 g of fresh rice leaves was accurately weighed and then processed into fine powder in a mortar. The sample was extracted with 1.5 mL of analytical grade methanol (p.a.) and stirred until the pigments were fully released. The homogenate was placed into a 1.5 mL microtube and subjected to centrifugation at 9,600×*g* for 10 min, allowing for the separation of the supernatant from the pellet. The supernatant was collected, placed in a cuvette, and analyzed using a spectrophotometer at wavelengths of 470, 652, and 665 nm to determine the photosynthetic pigment content (mg. g^{-1} FW).

Analysis of proline, MDA and H_2O_2 contents

Proline contents were analyzed using spectrophotometric analysis according to the method of Kamruzzaman et al. (2022). Absorbance for the chromophore was measured at 520 nm. The proline concentration was calculated according to a standard curve as microgram per gram of fresh weight (µg. g^{-1} FW). MDA contents were determined following

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Begum et al. (2022). Absorbance was recorded at 532 and 600 nm, and the MDA concentration was determined based on an extinction coefficient of 155 mM^{-1} cm^{-1}. Results were expressed as μmol. g^{-1} FW. Hydrogen peroxide contents (H_2O_2) were evaluated following Junglee et al. (2014) using 0.5 g of fresh leaf tissue homogenized in 5 mL of 0.1% TCA. Mixtures were then centrifuged at 13,800×*g* for 15 min before measuring absorbance at 390 nm. H_2O_2 concentration was expressed as μmol. g^{-1} FW.

Antioxidant enzyme activities

Enzymes were extracted as previously described by grinding 0.1 g of fresh leaf material in liquid nitrogen and homogenizing in an extraction buffer. The extraction was centrifuged at 6,100×*g* for 15 min at 4 °C. The supernatant was then used for activity assays of catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). CAT activity was measured as described by Hadwan and Abed (2016). The method is based on the reaction of unoxidized H_2O_2 with ammonium molybdate to produce a yellow complex and measured absorbance at 374 nm. CAT activity was calculated using the equation described by Hadwan and Abed (2016). The POD activity was measured as described by Ahsan et al. (2023). The increase in optical density (OD) due to the formation of tetra-guaiacol was measured at 470 nm every 20 s for 3 min by a spectrophotometer. The activity of POD was calculated from the change in OD over time. We assayed APX activity as described by Nakano and Asada (1987). The decrease in absorbance value at 290 nm was tracked.

Expression analysis of *SoCAT*, *SoAPX*, and *SoPOD*

Leaf samples weighing 0.1 g were pulverized in liquid nitrogen to create a fine powder and subsequently homogenized in 1 mL of AccuZol™ total RNA isolation reagent. Following this, 200 μL of chloroform was introduced, and the mixture was subjected to vigorous agitation for 15 s before being cooled on ice for 5 min. The solution was processed through centrifugation at 13,800×*g* for 15 min at 4 °C. An equal volume of isopropyl alcohol was combined with the supernatant and centrifuged again at 13,800×g for 10 min at 4 °C. The resultant pellet was washed with 80% ethanol and centrifuged once more at 13,800×g for 5 min at 4 °C. Finally, the pellet was air-dried and reconstituted in RNase-free water. The concentration and purity of RNA were assessed using spectrophotometric methods by measuring the absorbance at wavelengths of 260 and 280 nm. For the synthesis of first-strand cDNA, approximately 1 μg of total RNA was utilized with the AccuPower® CycleScript™ RT Premix (dT20) kit. A total reaction volume of 20 μL was achieved by adding DEPC-treated water. The cDNA synthesis process is carried out in a thermal cycle according to the conditions stated in the company protocol. The amplification of cDNA through PCR was conducted under the specified thermal cycling parameters: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 48.9-52.5 °C for 30 s, and extension at 72 °C for 1 min; followed by a final elongation at 72 °C for 5 min. Primers for antioxidant-related genes were adopted from Rudiyanto et al. (2025). The analysis of PCR products was conducted on an agarose gel (1.5%), undergoing electrophoresis at 80 V for a duration of 50 min. The Promega 100 bp DNA ladder served as the molecular size marker, with the application of 3 μL. Visualization of the gels was achieved through a gel documentation system, and the intensities of the bands were measured with ImageJ software to assess relative gene expression levels.

Statistical analysis

The research was performed using a completely randomized design with three replicates, each consisting of five plants. Before conducting the analysis of variance (ANOVA), the data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using the Levene test. ANOVA and the least significant difference (LSD) post-hoc test at a significance level of $p<0.05$ were performed using SPSS Statistics version 22 software. Visualization of the data, which encompasses heatmaps and bar graphs, was achieved using Python 3.10 with the Matplotlib, Seaborn, and Pandas libraries.

Results

Morphological responses to treatments

The effects of ABA, SL, and their combined application on the morphological characteristics of *Oryza sativa* var. glutinous plants under normal conditions (−PEG), drought stress (+PEG), and the recovery (R) phase are presented in Table 1. Hormone

treatments had a significant influence on morphological parameters (p<0.05). The application of SL consistently promoted plant height during drought stress and recovery phases. Under stress conditions (+PEG), SL treatment produced the tallest plants (35.90 ± 0.95 cm), which were significantly higher compared with the control under normal conditions (−PEG, 32.10 ± 1.99 cm). In contrast, hormone application had no significant influence on the quantity of leaves, as all treatments yielded similar results in drought stress and recovery phases.

Table-1. Morphological parameters of *Oryza sativa* var. glutinous in response to drought stress triggered by PEG-6000 and during the recovery phase, treated with ABA, SL, and their combination.

Condition	Treatment	Plant height (cm)	Number of Leaves (sheets)	Root length (cm)	Number of Roots (sheets)
(-) PEG	Control	32.10 ± 1.99^{d}	2.00 ± 0.00^{a}	8.90 ± 0.43^{f}	5.00 ± 0.00^{e}
	ABA	14.17 ± 0.84^{a}	2.00 ± 0.00^{a}	1.80 ± 0.36^{a}	3.00 ± 0.00^{ab}
	SL	36.87 ± 0.61^{e}	2.00 ± 0.00^{a}	4.27 ± 0.47^{cde}	3.00 ± 0.00^{ab}
	ABA+SL	14.10 ± 0.75^{a}	2.00 ± 0.00^{a}	5.13 ± 0.31^{e}	3.00 ± 0.00^{ab}
(+) PEG	Control	24.10 ± 0.66^{b}	3.00 ± 1.00^{b}	3.63 ± 0.51^{bcd}	5.00 ± 0.00^{e}
	ABA	14.20 ± 0.46^{a}	2.00 ± 0.00^{a}	1.80 ± 0.56^{a}	3.00 ± 0.00^{ab}
	SL	35.90 ± 0.95^{e}	2.00 ± 0.00^{a}	4.23 ± 0.55^{cde}	3.00 ± 0.00^{ab}
	ABA+SL	10.80 ± 0.60^{a}	2.00 ± 0.00^{a}	2.67 ± 0.74^{ab}	3.00 ± 0.00^{ab}
(+) PEG Recovery (R)	Control	26.67 ± 2.08^{e}	3.67 ± 0.57^{b}	4.83 ± 0.76^{de}	4.33 ± 1.16^{de}
	ABA	17.66 ± 4.04^{c}	3.00 ± 0.00^{b}	3.00 ± 1.00^{bcde}	3.00 ± 0.00^{ab}
	SL	36.67 ± 1.52^{e}	3.30 ± 0.57^{b}	3.83 ± 0.76^{ab}	4.00 ± 1.00^{cd}
	ABA+SL	23.33 ± 4.16^{de}	3.00 ± 1.00^{b}	2.66 ± 0.28^{ab}	2.33 ± 0.57^{a}

Data are represented as mean ± SE (n=3). Different letters indicate significant differences at p<0.05, according to the LSD post-hoc test. Parameters include Plant height (cm), number of leaves, root length (cm), and number of roots (sheets).

For root length, SL treatment under normal conditions (−PEG) produced roots up to 4.27 ± 0.47 cm, while under stress conditions (+PEG), root length reached 4.23 ± 0.55 cm. Both values were significantly greater than those observed with ABA treatment under drought stress (1.80 ± 0.36 cm). Interestingly, in the recovery phase, the control treatment exhibited the longest roots (4.83 ± 0.76 cm), exceeding those of all hormone-treated plants. The number of roots in the control treatment remained constant (5.00 ± 0.00) under normal and drought stress conditions. However, the ABA+SL combination did not significantly increase plant height or root number. Under drought stress (+PEG), this treatment resulted in markedly smaller plants (10.80 ± 0.60 cm) compared to the control (14.10 ± 0.75 cm) and other treatments. A similar pattern was observed during the recovery phase, where ABA+SL treatment did not promote plant height compared with single-hormone applications. These results indicate that while single hormone application, particularly SL, enhances plant morphological responses, the combination treatment may exert antagonistic effects on these parameters. Both single and combined hormone treatments slightly reduced root number; however, these differences were not statistically significant under drought stress or recovery conditions.

Photosynthetic pigment activity in leaves

Single and combined hormone treatments also influenced photosynthetic pigment activity in leaves during drought stress and recovery. Photosynthetic pigments, including carotenoids and chlorophylls (*a* and *b*), serve as indicators of assimilation capacity and are sensitive markers of metabolic disturbances under abiotic stress. Total chlorophyll and carotenoid contents declined sharply following single ABA application during both drought stress and recovery phases. The single SL application showed a similar decreasing trend, although the reduction was less pronounced compared with ABA. The combination of ABA and SL also led to reduced chlorophyll content, though not to the extent observed with ABA alone. Carotenoids were significantly reduced by hormone

treatments; however, ABA+SL maintained values closer to the control compared with single-hormone applications (Table 2). This suggests that combined hormone application may partially mitigate the decline in photosynthetic pigments under drought stress**.**

Table-2. Photosynthetic pigment contents in *Oryza sativa* var. glutinous leave*s* under drought stress and recovery phases, with ABA, SL, and combined treatments (ABA+SL).

Condition	Treatment	Chlorophyll *a* (mg.g^{-1} FW)	Chlorophyll *b* (mg.g^{-1} FW)	Chlorophyll total (mg.g^{-1} FW)	Carotenoid (mg.g^{-1} FW)
(-) PEG	Control	11.56 ± 0.40^{g}	7.47 ± 0.56^{d}	19.03 ± 0.80^{g}	5.56 ± 0.23^{g}
	ABA	4.93 ± 0.10^{b}	6.44 ± 0.02^{bc}	11.37 ± 0.12^{b}	0.22 ± 0.15^{a}
	SL	6.81 ± 0.13^{ef}	5.62 ± 0.09^{a}	12.43 ± 0.21^{c}	1.19 ± 0.60^{b}
	ABA+SL	5.30 ± 0.46^{bc}	5.34 ± 0.26^{a}	10.64 ± 0.41^{b}	1.46 ± 0.36^{bc}
(+) PEG	Control	6.60 ± 0.10^{e}	11.58 ± 0.02^{f}	18.18 ± 0.12^{g}	9.50 ± 0.53^{j}
	ABA	2.71 ± 0.18^{a}	6.51 ± 0.40^{c}	9.22 ± 0.09^{a}	2.14 ± 0.28^{d}
	SL	6.45 ± 0.75^{de}	10.46 ± 1.18^{e}	16.91 ± 1.16^{f}	7.74 ± 0.65^{i}
	ABA+SL	8.02 ± 0.75^{fg}	9.55 ± 0.67^{d}	17.57 ± 1.42^{e}	7.07 ± 0.32^{h}
(+) PEG Recovery (R)	Control	6.72 ± 0.30^{ef}	7.82 ± 0.10^{d}	14.54 ± 0.26^{e}	6.11 ± 0.03^{g}
	ABA	5.91 ± 0.25^{cd}	7.92 ± 0.40^{d}	13.83 ± 0.53^{de}	4.71 ± 0.02^{f}
	SL	6.74 ± 0.05^{ef}	6.60 ± 0.13^{c}	13.34 ± 0.09^{cd}	2.95 ± 0.02^{e}
	ABA+SL	7.68 ± 0.03^{f}	5.71 ± 0.08^{ab}	13.39 ± 0.05^{cd}	1.92 ± 0.03^{cd}

Data values are presented as mean ± SE (n=3). Different letters reflect significant differences at $p<0.05$ (ANOVA followed by LSD test) within each pigment parameter (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids) as indicated by different letters.

Proline, MDA, and H_2O_2 content

Evaluating biochemical responses such as proline, MDA, and H_2O_2 levels is essential for understanding oxidative damage and the activation of adaptive defense mechanisms under stress conditions. These parameters provide insights into the ability of hormonal treatments to regulate ROS levels and enhance physiological stability in *Oryza sativa* var. glutinous during drought stress and recovery phases. As shown in **Table 3**, drought stress induced by (+) PEG application significantly increased the accumulation of proline, MDA, and H_2O_2 compared to normal conditions (-) PEG. Proline levels rose markedly under stress, particularly in the control (0.23 ± 0.09 μmol·g^{-1} FW) and ABA (0.22 ± 0.08 μmol·g^{-1} FW) treatments. However, during the recovery phase, proline content dropped significantly in all treatments, with the lowest observed in the control (0.01 ± 0.01 μmol·g^{-1} FW), followed by SL (0.04 ± 0.01 μmol·g^{-1} FW), ABA (0.05 ± 0.02 μmol·g^{-1} FW), and ABA+SL (0.09 ± 0.05 μmol.g^{-1} FW).

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Table-3. Proline, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) content in *Oryza sativa* var. glutinous under drought stress, recovery phase and hormonal treatments.

Condition	Treatment	Proline (µmol.g^{-1} FW)	MDA (µmol.g^{-1} FW)	H_2O_2 (µmol.g^{-1} FW)
(-) PEG	Control	0.19 ± 0.00^{de}	0.42 ± 0.10^{c}	0.02 ± 0.00^{b}
	ABA	0.20 ± 0.07^{e}	0.72 ± 0.07^{b}	0.02 ± 0.00^{b}
	SL	0.03 ± 0.01^{ab}	0.37 ± 0.04^{a}	0.02 ± 0.00^{b}
	ABA+SL	0.15 ± 0.05^{bcde}	0.40 ± 0.03^{a}	0.04 ± 0.00^{d}
(+) PEG	Control	0.23 ± 0.09^{e}	4.14 ± 0.22^{e}	0.24 ± 0.00^{f}
	ABA	0.22 ± 0.08^{e}	3.70 ± 0.05^{d}	0.05 ± 0.00^{e}
	SL	0.15 ± 0.05^{cde}	2.30 ± 0.03^{c}	0.04 ± 0.00^{c}
	ABA+SL	0.21 ± 0.08^{e}	1.42 ± 0.04^{b}	0.06 ± 0.00^{e}
(+) PEG Recovery (R)	Control	0.01 ± 0.01^{a}	1.12 ± 0.08^{b}	0.05 ± 0.00^{f}
	ABA	0.05 ± 0.02^{abc}	0.56 ± 0.05^{ab}	0.06 ± 0.00^{a}
	SL	0.04 ± 0.01^{ab}	0.53 ± 0.18^{ab}	0.05 ± 0.00^{a}
	ABA+SL	0.09 ± 0.05^{abcd}	0.62 ± 0.05^{ab}	0.04 ± 0.00^{cd}

Data are presented as mean ± SE (n=3). The same letters in the columns indicate no significant differences, while different letters indicate significant differences between treatments at a confidence level of $p<0.05$ (ANOVA followed by LSD post-hoc test). MDA: Malondialdehyde, PEG: Polyethylene glycol, ABA: Abscisic acid, SL: Strigolactone.

Lipid peroxidation, indicated by MDA content, also increased sharply under drought stress. The control (+PEG) showed the highest MDA accumulation (4.14 ± 0.22 µmol·g^{-1} FW), reflecting severe cellular damage. In contrast, the ABA+SL combination effectively reduced MDA levels to 1.42 ± 0.04 µmol·g^{-1} FW, suggesting stronger membrane protection. Upon recovery, MDA content decreased in all treatments, with SL showing the lowest value (0.53 ± 0.18 µmol·g^{-1} FW). Hydrogen peroxide, a key ROS generated during stress-induced metabolic disruption, also accumulated significantly under drought stress. The control exhibited the highest H_2O_2 level (0.24 ± 0.00 µmol·g^{-1} FW), while treatments with ABA, SL, or their combination markedly reduced it to 0.06 ± 0.00 µmol·g^{-1} FW. In the recovery phase, all treatments maintained significantly lower H_2O_2 levels, indicating the restoration of the antioxidant defense system.

Determination of CAT, APX, and POD activities

Under conditions of drought stress (+ PEG), there were notable increases in the activities of antioxidant enzymes (CAT, POD, and APX) in plants when compared with normal conditions (- PEG) (Table 4). The ABA+SL treatment showed the highest CAT activity (0.70 ± 0.01 U.mg^{-1} protein), while POD activity peaked in the control (1.33 ± 0.32 U.mg^{-1} protein), suggesting oxidative damage without hormonal protection. In contrast, POD activity was markedly reduced in hormone-treated plants, with the lowest value in the ABA+SL group (0.51 ± 0.04 U.mg^{-1} protein), indicating enhanced membrane protection. APX activity was strongly enhanced by ABA (51.53 ± 4.40 U.mg^{-1} protein), reaching levels equivalent to the control under drought stress. However, SL and ABA+SL showed moderate APX activities, suggesting differences in how single versus combined hormone treatments regulate ascorbate peroxidase responses. During recovery, CAT activity decreased across treatments, with the control maintaining the highest residual activity (0.26 ± 0.05 U.mg^{-1} protein). POD activity was highest in the ABA treatment (1.22 ± 0.02 U.mg^{-1} protein), reflecting the persistence of ABA-mediated stress signaling. Similarly, APX activity during recovery was maximized in the ABA treatment (43.30 ± 0.00 U.mg^{-1} protein), indicating that ABA continues to drive a strong antioxidant response even after stress relief.

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Table-4. The activities of antioxidant enzymes of CAT, POD, and APX in Oryza sativa var. glutinous under drought stress and recovery stages, treated with ABA, SL, and their combination.

Condition	Treatment	CAT (U.mg^{-1} protein)	POD (U.mg^{-1} protein)	APX (U.mg^{-1} protein)
(-) PEG	Control	0.25 ± 0.00^{bc}	0.29 ± 0.12^{abc}	35.96 ± 3.34^{cd}
	ABA	0.24 ± 0.00^{b}	1.08 ± 0.04^{h}	12.66 ± 1.67^{a}
	SL	0.44 ± 0.00^{e}	0.38 ± 0.32^{abc}	29.16 ± 7.70^{ab}
	ABA+SL	0.46 ± 0.00^{f}	0.86 ± 0.05^{def}	41.76 ± 3.34^{d}
(+) PEG	Control	0.53 ± 0.01^{g}	1.33 ± 0.32^{gh}	51.53 ± 4.40^{e}
	ABA	0.38 ± 0.00^{d}	0.68 ± 0.05^{cde}	51.53 ± 4.40^{e}
	SL	0.53 ± 0.00^{g}	0.92 ± 0.58^{ef}	26.26 ± 5.08^{b}
	ABA+SL	0.70 ± 0.01^{h}	0.51 ± 0.04^{bcd}	31.13 ± 8.37^{bc}
(+) PEG Recovery (R)	Control	0.26 ± 0.05^{a}	0.06 ± 0.05^{a}	26.56 ± 0.51^{b}
	ABA	0.21 ± 0.00^{a}	1.22 ± 0.02^{fg}	43.30 ± 0.00^{d}
	SL	0.21 ± 0.00^{a}	0.22 ± 0.32^{ab}	26.00 ± 0.00^{b}
	ABA+SL	0.24 ± 0.00^{b}	0.18 ± 0.05^{ab}	28.32 ± 0.00^{ab}

Data are presented as mean ± SE (n=3). The same letters in the columns indicate no significant differences, while different letters indicate significant differences between treatments at a confidence level of $p<0.05$ (ANOVA followed by LSD post-hoc test). Enzyme activities are expressed as U.mg^{-1} protein.

Figure 2A (heatmap, Z-score clustering) illustrates the integrated biochemical responses (proline, MDA, H_2O_2, CAT, APX, POD) across the three phases: (-) PEG, (+) PEG, and recovery. The (+) PEG group displayed strong positive Z-scores for MDA, H_2O_2, and antioxidant enzyme activities, reflected by high colour intensity (red), confirming an activated stress response. Importantly, ABA+SL treatments showed negative Z-scores for MDA and H_2O_2 under both (-) PEG and (+) PEG, indicating effective mitigation of oxidative damage. In the recovery phase, cluster separation and reduced colour intensity indicated detoxification and normalization of stress indicators, supporting the role of hormonal treatments in accelerating recovery. Figure 2B illustrates the Pearson correlation matrix among biochemical parameters under drought stress. The analysis revealed significant correlations ($p<0.05$), where correlation coefficient (r) values approaching +1 illustrate a robust positive correlation, while values close to -1 represent a strong negative correlation. APX activity showed a robust positive correlation with CAT ($r = 0.73$; $p<0.01$) and proline ($r = 0.65$), suggesting that APX activity is tightly linked with both osmotic adjustment and enzymatic antioxidant defense. Conversely, CAT activity demonstrated a highly significant negative correlation with H_2O_2 ($r = -0.75$; $p<0.01$), a trend also observed in APX ($r = -0.65$; $p<0.05$), indicating that higher antioxidant enzyme activity effectively reduces H_2O_2 accumulation, a key oxidative stress marker. Moreover, MDA exhibited a strong positive correlation with H_2O_2 ($r = 0.79$; $p<0.01$), reinforcing the notion that enhanced ROS levels directly contribute to lipid peroxidation and cellular damage.

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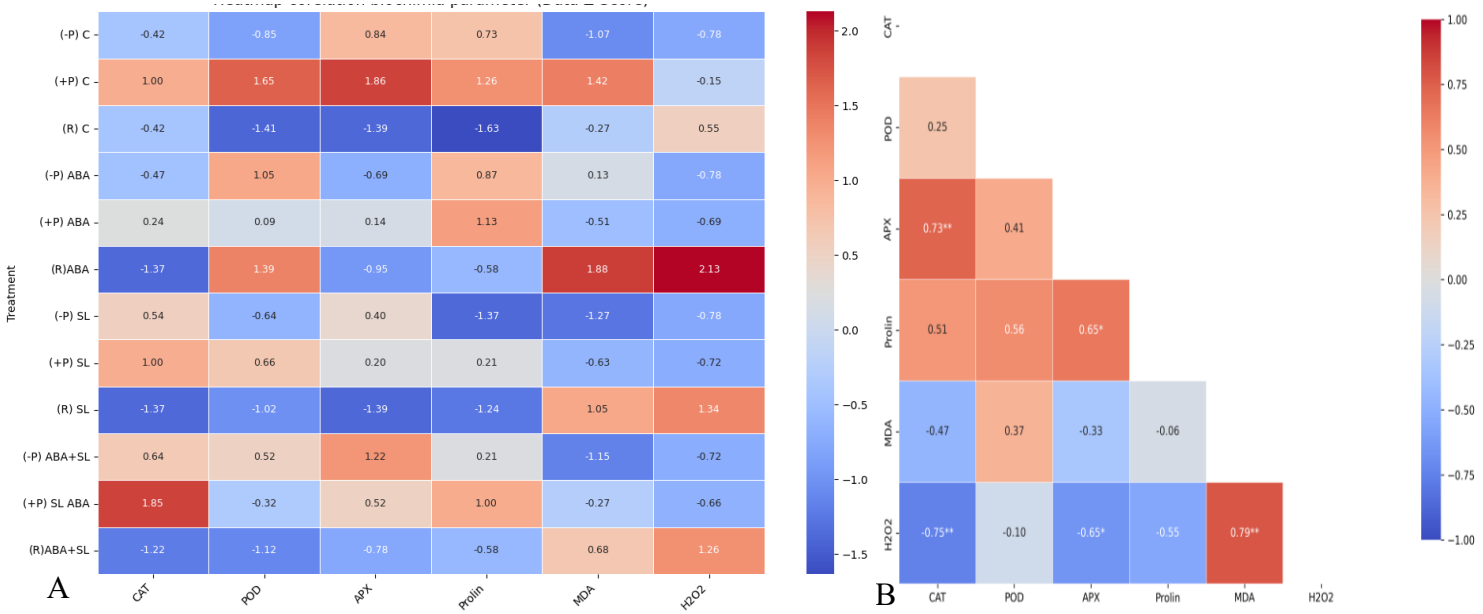
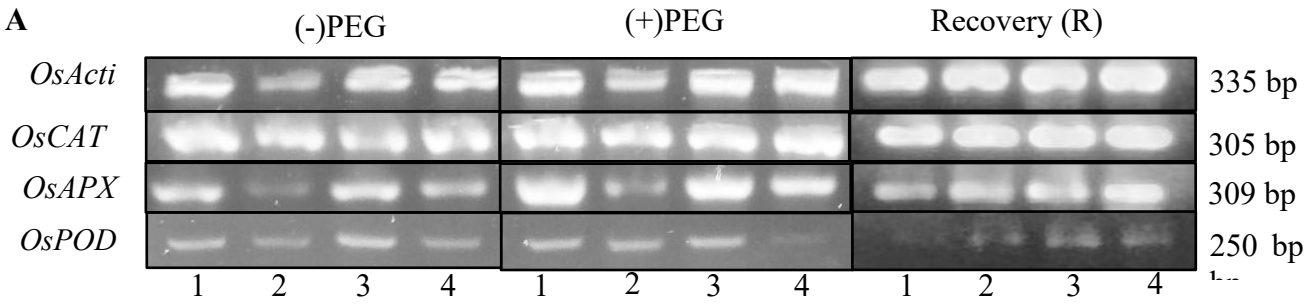


Figure-2. (A) Clustered heat map of the activities of antioxidant enzymes (CAT, POD, and APX) and oxidative stress markers (MDA, H_2O_2, and Proline) across drought stress and recovery treatments. High intensity (red) indicates elevated biochemical activity. (B) Pearson correlation heatmap among biochemical parameters under stress conditions. Red and blue represent significant positive and negative correlations ($p<0.05$). Note p=significant: **=p< 0.01; *= p<0.05.

Gene expression of *OsCAT*, *OsAPX*, and *OsPOD*

Based on the aforementioned results, gene expression analysis was conducted to assess the activity of antioxidant enzymes, specifically catalase (*OsCAT*), ascorbate peroxidase (*OsAPX*), and peroxidase (*OsPOD*). This analysis aimed to investigate the transcriptional regulation of the plant's antioxidant defense system during drought stress and the subsequent recovery phase, as well as to evaluate the effectiveness of single hormone (ABA, SL) and combined (ABA+SL) treatments. Figure 3A and B display the electrophoretic profiles and relative expression levels of *OsCAT*, *OsAPX*, and *OsPOD* genes under control, stress, and recovery conditions, following treatment with single hormones (ABA, SL) and their combination (ABA+SL). *OsActin* was used as a reference gene, exhibiting consistent expression across all treatments, thereby serving as a reliable internal control.



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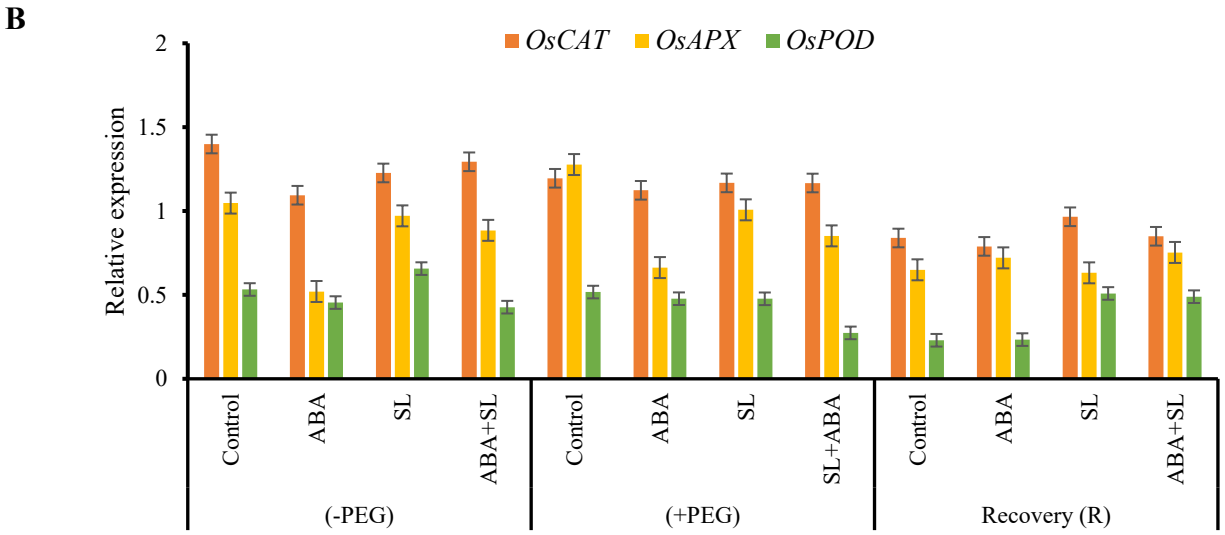

Figure-3. (A) Electrophoretic band profiles of CAT, APX, and POD gene expression under control conditions (-)PEG, drought stress (+)PEG, and recovery phase (R). (B) Relative expression levels of CAT, APX, and POD genes under the same treatment conditions. 1, Control; 2, Abscisic acid (ABA); 3, Strigolactone (SL) and 4, ABA+SL.

All antioxidant genes displayed stable baseline expression when exposed to the control conditions (-PEG). Under drought stress (+PEG), *OsCAT* expression was reduced in all cases, including for each of the hormones applied both singly and in combination, as shown by the fainter bands. This pattern of decreasing levels of expression under stress and a rebound in expression following wetting continues, which likely indicates that *OsCAT* is neither transcriptionally activated by drought stress nor responsive to the hormone treatments that were applied. A similar pattern was observed for the *OsAPX* gene expression that fell under all treatments of stress; however, a large increase was observed in *OsAPX* during the recovery period after applying the ABA and SL, as shown from the thicker bands and relative levels of expression greater than 1. *OsPOD* expression increased considerably during the recovery of ABA and SL but otherwise exhibited a pattern of reduced expression under stress for all cases. The ABA potency of *OsPOD* relative to SL was shown to not increase to a greater degree than pre-stress levels following wetting, following both hormones given concurrently, as evidenced by the thinner bands with relative expression values for this treatment under recovery being less than unit 1.

Discussion

PEG-induced drought stress affects the morpho-physiology of black glutinous rice plants, characterized by a decrease in Plant height and root length, which is reduced under PEG-induced drought stress, reflecting physiological disturbances caused by cellular dehydration and restricted tissue expansion. This observation aligns with the findings of Mahatthanaphatcharakun and Taratima (2025), who reported that water stress directly suppresses cellulose biosynthesis and cell elongation. The application of SL can significantly maintain plant height, even approaching the control value (-PEG). This finding is in line with the results reported by Jarin et al. (2024), that SL enhances auxin accumulation in the root elongation zone, thereby promoting both root growth and shoot development under optimal conditions. In contrast, when administered alone, ABA application led to a reduction in plant height and root length. As a well-known stress hormone, ABA induces excessive stomatal closure, disrupting water and nutrient transport, and resulting in growth inhibition (Mostofa et al., 2021). The simultaneous use of ABA and SL did not significantly improve growth parameters under stress conditions. However, during the recovery phase, this combination notably enhanced plant height, suggesting that the synergistic effect of these

hormones is more pronounced during post-stress recovery. The number of leaves and roots did not exhibit significant changes during either the stress or recovery phases. This suggests that these morphological traits are relatively conservative and do not respond rapidly to hormonal treatments in the short term, as previously noted by Aslam et al. (2022).

Under drought conditions, chlorophyll *a* and *b* contents typically decline due to oxidative stress and reduced photosynthetic efficiency (Zhuang et al., 2020). In the present study, PEG-induced stress led to a reduction in chlorophyll *a*, chlorophyll *b*, and total chlorophyll across all treatments, with the most pronounced decrease observed in the untreated control. This decline is indicative of chloroplast dysfunction and a compromised photosystem II (PSII), largely attributed to ROS accumulation, as described by Faizan et al. (2020).

The application of ABA, SL, and a combination of both hormones during the recovery phase showed an increase in total chlorophyll values. It can be said that plant physiological recovery occurred due to the administration of these hormones, in line with what was stated Jarin et al. (2024) that physiological recovery related to chloroplast structure regeneration and antioxidant pathway activation supports plant chlorophyll recovery after experiencing drought stress.

Drought stress not only affects plant morphology and photosynthesis, but also affects proline, MDA, and H_2O_2. As shown in Table 3, proline, MDA, and H_2O_2 increased significantly during the drought stress phase across all treatments, with the highest accumulation observed in the control group compared to both the pre-stress phase (-PEG) and the recovery phase (R). When plants experience water deficit, there is an increase in proline as a protective osmopolitan, an increase in ROS (H_2O_2), and the accumulation of MDA as an indicator of lipid peroxidation. This suggests that drought stress induces proline accumulation as an adaptive physiological response. Proline functions as an osmoprotectant, stabilizer of protein structures, ROS scavenger, and activator of antioxidant signaling pathways (Nazir et al., 2020). During the recovery phase, plants treated with hormones, particularly the ABA+SL combination, exhibited greater proline accumulation than both the control and the single hormone treatments. This reduction indicates a chloroplast malfunction and alteration in photosystem II (PSII), primarily caused by ROS accumulation, as stated by Faizan et al. (2020).

The MDA and H_2O_2 levels rose sharply during drought stress, particularly within the control group, highlighting the excessive lipid peroxidation and membrane damage that was occurring. This is in stark contrast to results from plants treated with either ABA or SL, as both variables reduced MDA and H_2O_2 build up, where the ABA + SL combination was clearly the treatment with the largest reduction in MDA and H_2O_2 concentration levels. This can help hypothesize that ABA and SL applications can help promote the cellular defence mechanisms in plants by supporting enzymatic and non-enzymatic antioxidant upgrades (Delaix et al., 2024).

At the recovery stage, the hormone-treated plants had a greater proline capacity than both the control and individual hormone treatments, especially the treatment with the combined ABA+SL. This implies that the concurrent application of hormones helps post-stress synthesis of proline, which is an important contributor to osmotic adjustment, metabolic recovery, increased growth and improvement of physiological properties (Hasanuzzaman et al., 2020). In addition to proline, MDA, and H_2O_2, experienced a lower capacity reduction during the recovery phase, indicating that all potential protective systems responsible for membrane repair, lipid peroxidation inhibition and the restoration of redox balance and membrane integrity post-stress period are activated (Liu et al., 2022).

Successful ROS management during abiotic stress is crucial for plant survival and recovery. It is also associated with enzymatic detoxification pathways involving CAT, APX, and POD, as Zhuang et al. (2020) described. In the control group, CAT activity was markedly elevated under PEG stress, which highlights the importance of CAT in detoxifying H_2O_2 (Waadt et al., 2022). CAT activity dropped in response to ABA and SL, and even greater declines in the ABA+SL combination, indicating that hormone treatments influence antioxidant enzyme prioritization. During the recovery phase, APX also showed a downward trend in the control group as well as SL and ABA+SL plants, however, the ABA treatment increased APX, which could indicate a delayed recovery from oxidative damage or perhaps hormonal regulation of antioxidant responses. Overall, these results support the work of Korek and Marzec (2023), who put forth that SL would improve redox signalling through enhancing POD while that

downregulated CAT responses. Furthermore, Chi et al. (2021) proposed that ABA+SL treatment results in silenced CAT expression, likely due to the modulation of all other pathways through POD or APX for dealing with oxidative stress shutdowns. In the recovery phase, a reduction in APX activity was detected in the control, SL-treated, and ABA+SL-treated groups, indicating a reduction of ROS levels after equilibrium was re-established in cellular metabolism. However, ABA-treated leaves maintained elevated APX activity during the recovery phase. This observation may indicate a sustained, prolonged response initiated by ABA as an antioxidant response to manage residual oxidative damage or to prepare for subsequent stress responses (Ma et al., 2024). This finding, along with the results from the previous experimental section, warranted further analysis using a Z-score heatmap to evaluate the effects of individual and combined hormone treatments on antioxidant enzyme activities across all three experimental phases, showing that the hormone treatments were able to elevate antioxidant enzyme activity levels across the transition from non-stress to drought stress experimental phases. Increased MDA and H_2O_2 levels, along with elevated antioxidant enzyme activity, occur in response to oxidative stress. These findings are in agreement with Zhou et al. (2021), demonstrating that drought stress elicits excessive accumulation of ROS, leading to activation of plant enzymatic antioxidant defences. However, H_2O_2 levels remained elevated compared to the non-stressed baseline, indicating that although detoxification mechanisms were active, residual ROS may still accumulate at basal levels to support signalling processes essential for recovery and cellular reprogramming (Xu et al., 2024). A notable inverse relationship was identified between H_2O_2 levels and the functions of CAT and APX, reinforcing their roles in ROS detoxification. Additionally, a positive correlation between APX activity and proline accumulation was identified, suggesting a cooperative interaction between enzymatic and non-enzymatic antioxidant systems. Proline, acting as an osmolyte, also contributes to ROS scavenging and stress signal modulation, as previously reported by Kim et al. (2022). These bolster the hypothesis that the simultaneous use of ABA and SL exerts systemic regulatory effects—effectively suppressing ROS accumulation while balancing enzymatic antioxidant activity and osmolyte levels more efficiently than single hormone treatments.

Under drought stress, *OsCAT* and *OsAPX* expression increased sharply under both ABA and SL single treatments during stress treatments. It indicates the involvement of ABA in activating ROS detoxification pathways. This is consistent with previous studies by Wei et al. (2022), which demonstrated that ABA induces catalase gene expression to neutralize excess H_2O_2. Zhang et al. (2021) also reported that SL modulates *OsAPX* activity through transcriptional regulation. Notably, the ABA+SL combination induced even greater *OsAPX* expression, indicating that this hormonal interaction enhances the plant's oxidative stress defense more effectively than single treatments. The expression pattern of *OsPOD* was more distinct. The highest expression was observed in the ABA-only treatment under stress conditions (+PEG), corroborating findings by Zhou et al. (2024), which identified ABA-induced *OsPOD* expression via the *OsWRKY30* transcriptional pathway.

Conversely, SL treatment suppressed *OsPOD* expression, suggesting SL may rely more on *OsCAT* and *OsAPX* pathways for ROS detoxification. The ABA+SL combination showed reduced *OsPOD* expression compared to ABA alone, indicating a modulatory effect of SL on ABA-driven peroxidase gene regulation. During the recovery phase, expression of all antioxidant genes (*OsCAT*, *OsAPX*, and *OsPOD*) declined across treatments but remained elevated compared to the non-stress control (-PEG). This supports the concept of a 'stress memory' mechanism, where plants retain elevated antioxidant potential to prepare for future stress episodes (Kambona et al., 2023). SL treatment promoted faster recovery of gene expression, reinforcing its role in enhancing long-term plant resilience. However, when ABA and SL were applied individually, the expression of *OsPOD* increased. This indicates that the upregulation of POD during the recovery phase contributes to eliminating residual ROS, particularly H_2O_2, generated from post-stress metabolism, as also reported by Ge et al. (2024). Overall, the results of this study indicate that the administration of a combination of ABA and SL works complementarily in regulating morpho-physiology, strengthening the antioxidant system, accelerating membrane structure recovery, and coordinating the expression of defence genes, thereby providing a more effective adaptive response compared to single administration when administered to *Oryza sativa* var. glutinous plants during and after the recovery phase. The combined application of ABA and SL can enhance drought resistance and

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physiological recovery in black glutinous rice. The resistance and recovery were indicated by the stabilization of photosynthetic pigments, a reduction in MDA and H_2O_2 concentrations, and fortification of the antioxidant defense at both the enzymatic and molecular levels.

Conclusion

The results provided evidence that ABA and SL act synergistically to reduce the negative impact of drought stress induced by PEG-6000. The combination of ABA and SL can restore and maintain morpho-physiological and biochemical balance. The combination of ABA and SL is more effective than a single hormone in stabilizing growth, maintaining and restoring photosynthetic pigments, suppressing the accumulation of proline, MDA, and H_2O_2, and strengthening the antioxidant enzyme system (CAT, POD, APX). Therefore, it is appreciated that interactions between hormones/initiators are considered during ROS detoxification and the maintenance of metabolic integrity under drought stress. Overall, results confirmed that identified applications of combined ABA and SL can be improved to increase plant tolerance and to promote recovery of local varieties, such as *Oryza sativa* var. glutinous. This study also has contributed to our understanding of hormonal crosstalk under drought stress, as well as the potential of combining hormonal approaches to enhance drought tolerance in local rice accessions under climate change.

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Contribution of Authors

Sudiarti D & Siswoyo TA: Studied conception and design.

Sudiarti D, Murtianingsih H, Rizkiantoro R & Agustin DN: Performed experimental execution and collected data.

Sudiarti D & Agustin DN: Performed laboratory analyses, data processing and statistical analysis.

Siswoyo TA: Supervised and coordinated the project.

Sudiarti D, Siswoyo TA, Nugraha AS & Fanata WID: Helped in manuscript write up and review.

All authors critically revised and approved the final manuscript.

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