

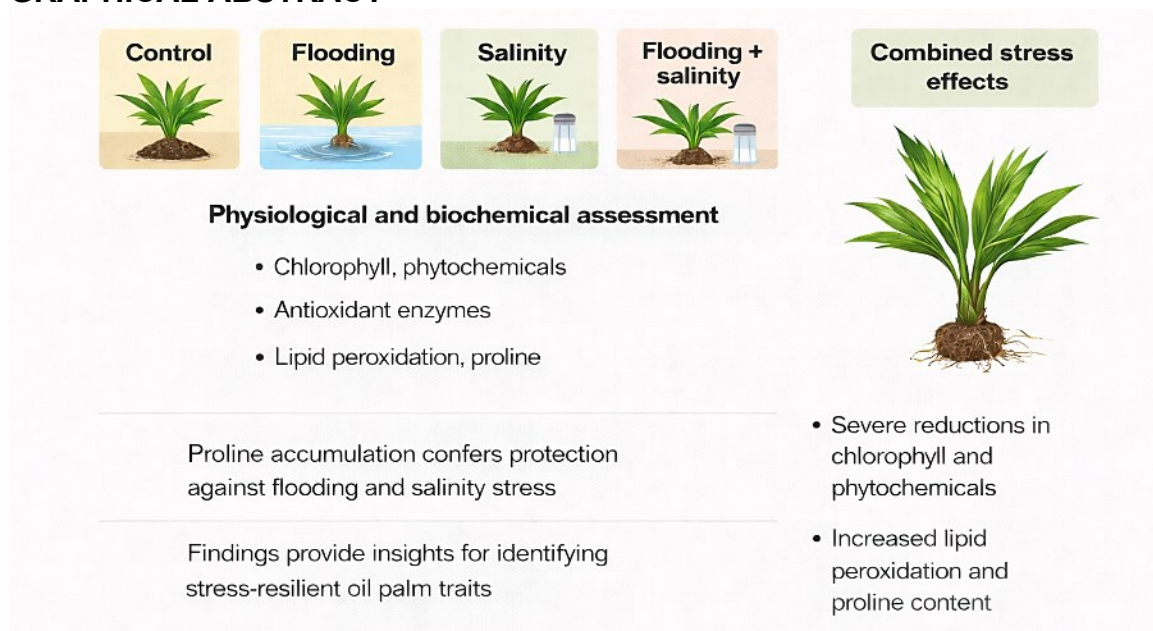
Differential Physiological and Biochemical Responses in Oil Palm Seedlings Exposed to Single and Combined Flooding–Salinity Stress

Munirah Adibah Kamarul Zaman ^a, Kua Jay Mie ^b, Muhammad Amirul Jemuladin@Jamaludin ^a, Noor Azmi Shaharuddin ^a, Mohamad Zulfazli Mohd Sobri ^c, Siti Nor Akmar Abdullah ^d, Adibah Mohd Amin ^e, Potjamarn Suraninpong ^f, and Azzreena Mohamad Azzeme ^{a,*}

* Corresponding author: azzreena@upm.edu.my

DOI: 10.15376/biores.21.3.5931-5953

GRAPHICAL ABSTRACT



Differential Physiological and Biochemical Responses in Oil Palm Seedlings Exposed to Single and Combined Flooding–Salinity Stress

Munirah Adibah Kamarul Zaman ^a, Kua Jay Mie ^b, Muhammad Amirul Jemuladin@Jamaludin ^a, Noor Azmi Shaharuddin ^a, Mohamad Zulfazli Mohd Sobri ^c, Siti Nor Akmar Abdullah ^d, Adibah Mohd Amin ^e, Potjamarn Suraninpong ^f, and Azzreena Mohamad Azzeme ^{a,*}

Increases in temperature, rainfall, and sea level associated with climate change have intensified flooding and saltwater intrusion, thereby posing a growing threat to oil palm cultivation. To evaluate the effects of these environmental stressors, 12-month-old oil palm seedlings were subjected to control, flooding, salinity, and combined flooding and salinity treatments. Physiological and biochemical parameters were measured, including total chlorophyll, antioxidant enzyme activities, lipid peroxidation, and proline content. Phytochemical profiles, such as total phenolics, flavonoids, alkaloids, terpenoids, and antioxidant activity, were also assessed. Combined stress caused the most severe reductions in chlorophyll and phytochemicals, with increased lipid peroxidation and visible symptoms such as leaf yellowing and adventitious root formation. Proline accumulation was markedly higher under combined stress, suggesting a protective role. In contrast, individual stress treatments induced antioxidant enzyme activity, contributing to reactive oxygen species mitigation. These findings highlight the distinct and synergistic impacts of flooding and salinity on oil palm seedling health. The results offer a foundation for identifying stress-resilient traits, which can support future breeding or management strategies to sustain oil palm productivity under changing climate conditions.

DOI: 10.15376/biores.21.3.5931-5953

Keywords: Oil palm; Physiological and biochemical responses; Combined stresses; Single stress; Flooding; Salinity

Contact information: a: Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; b: Department of Botany, Faculty of Science, The University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada; c: Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; d: Institute of Plantation Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; e: Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia; f: School of Agricultural Technology and Food Industry, Walailak University, Nakhon Si Thammarat 80161, Thailand; * Corresponding author: azzreena@upm.edu.my

INTRODUCTION

Oil palm plays a crucial role in ensuring food security. Global demand is rising due to its widespread use in the food industry and household cooking. Among oilseed crops,

palm oil stands out for its high oil yield per unit area. According to Voora *et al.* (2023), palm oil is one of the leading sources of edible oils worldwide. The United States Department of Agriculture (USDA) reported that global palm oil consumption increased from 71 million metric tons in 2021/2022 to approximately 76 million metric tons in 2022/2023. In Malaysia, oil palm cultivation contributes significantly to the agricultural gross domestic product (GDP), accounting for RM 48.31 billion, or 56.8% of the total agricultural GDP in 2020. In the first quarter of 2022 alone, the sector contributed RM 9.5 billion, representing 51.8% of the agrocommodity sector (Siti-Dina *et al.* 2023). As of the third quarter of 2023, the total planted area reached 5.65 million hectares, contributing 2.85% to Malaysia's national GDP (Ministry of Plantation and Commodities). The success of the Malaysian oil palm industry has been attributed to coordinated efforts among the government, industry stakeholders, and relevant agencies (Mohd Hanafiah *et al.* 2022).

However, climate change poses a significant threat to oil palm productivity. Fluctuations in global temperature and rainfall patterns are likely to disrupt oil palm production (Abdullah *et al.* 2017). Projected economic losses by 2099 are estimated to reach RM 341.29, RM 127.43, and RM 51.80 per hectare for Peninsular Malaysia, Sabah, and Sarawak, respectively (Abubakar and Ishak 2022). Malaysia's equatorial climate, characterized by consistently high temperatures and rainfall, generally favors oil palm cultivation. However, climate-induced flooding events have become increasingly frequent, affecting both coastal and inland areas. Expansion of oil palm cultivation into coastal regions has contributed to mangrove deforestation, which further intensifies seawater intrusion (Bhowmik *et al.* 2022). Prolonged flooding under such conditions can expose oil palm to the combined stresses of waterlogging and salinity, severely hindering growth and reducing yield.

Combined salinity and flooding stress has been considered as a way to simulate conditions arising from typhoon- or monsoon-associated storm surges and seawater intrusion, in which saline water inundates plantations, leading to simultaneous root-zone hypoxia and ionic/osmotic stress. Such events have been reported in coastal oil palm-growing regions and are expected to increase with sea-level rise and climate change (Panggabean *et al.* 2025). In contrast, flooding without salinity represents inland or poorly drained plantations subjected to prolonged or intense monsoonal rainfall, river overflow, or high water tables (Asalam *et al.* 2023). By distinguishing among salinity, flooding and combined salinity–flooding treatments, this distinction enhances the ecological relevance of the experimental design and supports interpretation of oil palm stress regulation under field conditions.

Salinity stress adversely affects plant development by disrupting osmotic and ionic balance. It impedes water uptake by roots, leading to stomatal closure and inhibition of leaf expansion (Azzeme and Abdullah 2019; Ji *et al.* 2022). Ion toxicity resulting from salt accumulation further restricts nutrient absorption and induces oxidative damage. Excess sodium (Na^+) interferes with the uptake and transport of essential ions, particularly potassium (K^+), leading to impaired growth (Alharbi *et al.* 2022). Physiological symptoms, such as wilting, leaf senescence, and chlorophyll degradation are commonly observed. Similar physiological responses are also triggered by drought stress, which induces comparable metabolic and developmental changes (Azzeme *et al.* 2016, 2017; Suraninpong *et al.* 2023). Salt stress also leads to oxidative stress, disrupting metabolic activity and compromising membrane integrity (Alharbi *et al.* 2022).

In contrast, flooding induces hypoxia by restricting oxygen availability to the root system, thereby impairing overall plant growth and development. Flooding often results in

reduced roots and shoots elongation, enhanced aerenchyma formation, and altered leaf morphology (Mozo *et al.* 2021). The shift towards anaerobic respiration under flooded conditions promotes the generation of reactive oxygen species (ROS) and inhibits photosynthesis by reducing stomatal conductance and carbon dioxide (CO₂) diffusion (Aslam *et al.* 2023; Jensen *et al.* 2024). Therefore, when flooding and salinity occur simultaneously, the stress effects intensify due to elevated accumulation Na⁺ and chloride (Cl⁻) disrupts nutrient uptake and exacerbates inhibition of K⁺ transport (Duan *et al.* 2018; Renziehausen *et al.* 2024).

The excessive accumulation of ROS under abiotic stress leads to lipid peroxidation, membrane damage, enzyme inhibition, and DNA disruption (Park and Lee 2019). Consequently, photosynthesis efficiency and plant growth decline, ultimately reducing crop productivity (Godoy *et al.* 2021). To counteract oxidative stress, plants activate antioxidant defense systems, including enzymatic antioxidants and the synthesis of protective compounds such as proline (Hasanuzzaman *et al.* 2020; Nadarajah 2020).

Proline is a key osmolyte that plays a vital role under numerous stress conditions. As an amino acid, proline functions in osmotic adjustment, stabilization of membrane and protein structure, as well as scavenging of free radicals (El-Beltagi *et al.* 2020). Stress-signaling molecules, particularly ABA, have been shown to stimulate the expression of the pyrroline-5-carboxylate synthase (*P5CS*) gene, thereby enhancing proline biosynthesis under salinity stress (Singh *et al.* 2022). Conversely, increased expression of proline dehydrogenase (*PRODH*) promotes proline catabolism in mitochondria, supporting respiration and energy production, hence contributing to plant growth maintenance under low water potential conditions (Launay *et al.* 2019). Therefore, proline accumulation is critical for mitigating ROS-induced damage under salinity and drought stresses. Proline is transported and accumulated in the cytoplasm, where it lowers osmotic potential, enhances water uptake, and maintains cellular osmotic balance (Yang *et al.* 2021). In addition, proline enhances antioxidant capacity by supporting the activities of key antioxidant enzymes, including peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), and other ROS-scavenging enzymes (Yang *et al.* 2021). This enhancement may be explained by the ability of proline to stabilize protein structures and maintain cellular redox balance under stress conditions. By preserving enzyme conformation and preventing oxidative damage to proteins, proline helps sustain antioxidant enzyme functionality during salinity and flooding stress. Supporting this observation, Rho *et al.* (2025) reported that proline accumulation increased significantly under flooding stress across five *Capsicum* species during both summer and winter seasons.

While salinity and flooding often co-occur in coastal ecosystems, evaluating their impacts independently remains a significant experimental challenge. To address this issue, the present study employed a controlled hydroponic approach using a standardized Hoagland nutrient solution (Hoagland and Arnon 1950). This methodology allows precise decoupling of stressors by maintaining a constant and optimal nutrient baseline while independently manipulating NaCl concentration. Such an experimental framework ensures that the individual and combined effects of salinity and flooding can be monitored accurately. Similar approaches using Hoagland nutrient solution have been reported in studies on barley (Zeng *et al.* 2013), ryegrass (Yin *et al.* 2017), and ryegrass and alkaligrass (Isweiri *et al.* 2022).

In this study, it was hypothesized that combined flooding and salinity stresses would exert more detrimental effects on oil palm than single flooding or salinity stress. This hypothesis was based on the expectation that the synergistic interaction of flooding

and salinity would impair plant health and development, which could be observed morphologically and physiologically. In particular, combined stress was anticipated to induce greater oxidative stress, thereby increasing reliance on osmoprotectants such as proline and antioxidant defense systems. To evaluate these responses, biochemical and antioxidant analyses were conducted to assess the effects of individual and combined stress treatments. Therefore, this study aimed to investigate the physiological and biochemical responses of oil palm seedlings to flooding and salinity, with particular emphasis on the differential responses of leaves and roots. The findings provide insights into key stress-responsive mechanisms and potential genetic targets for developing stress-resilient oil palm varieties.

EXPERIMENTAL

Plant Materials

Twelve-month-old Calix-600 oil palm seedlings were obtained from SD Guthrie Seeds and Agricultural Services, Banting, Selangor, Malaysia. The seedlings were subsequently acclimatized in the greenhouse at the Institute of Plantation Studies, Universiti Putra Malaysia, Selangor. Both the acclimatization process and the application of abiotic stress treatments were conducted under ambient temperature conditions.

Salinity, Flooding, and Combined Stress Treatments

Four different treatments were applied to the 12-month-old seedlings: control, salinity, flooding, and combined flooding and salinity. The control group was maintained under normal conditions in Hoagland solution without the addition of sodium chloride (NaCl) or submergence. For the salinity treatment, seedlings were irrigated with Hoagland solution supplemented with NaCl. The salt concentration was gradually increased (50, 100, 150, 250, and 300 mM) every other day to avoid osmotic shock (Akbudak and Filiz 2018). Flooding stress was induced by submerging the seedlings in Hoagland solution (without added NaCl) until the aerial parts were fully covered (Chen *et al.* 2016). In the combined treatment, seedlings were submerged in Hoagland solution containing NaCl, with the salt concentration increased gradually as in the salinity treatment. All seedlings were maintained under their respective treatments for 12 days before being harvested for further analysis. Hoagland solution was used throughout the experiment to prevent nutrient deficiency in the seedlings.

Estimation of Chlorophyll Content

Chlorophyll content in control and treated oil palm leaves was determined following the method described by Azzeme *et al.* (2016). Approximately 0.5 g of leaf sample was extracted using 10 mL of 80% (v/v) acetone, and 0.5 mg of calcium carbonate (CaCO₃) was added to the mixture. The extract was then filtered using Whatman No. 1 filter paper. Excess sample residue was washed with acetone until it became colourless. All filtrates were combined, and the final volume was adjusted to 20 mL. Absorbance readings were taken at 645 nm (maximum absorption for chlorophyll b in acetone) and 663 nm (maximum absorption for chlorophyll a in acetone). The contents of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (TC) were calculated using Eqs. 1, 2, 3,

$$Chl_a (mg g^{-1}) = \frac{[12.3 (A_{663}) - 0.86 (A_{645})] \times V}{\alpha \times 1000 \times W} \quad (1)$$

$$Chl_b (mg g^{-1}) = \frac{[19.3 (A_{645}) - 3.6 (A_{663})] \times V}{\alpha \times 1000 \times W} \quad (2)$$

$$TC = Chl_a (mg g^{-1}) + Chl_b (mg g^{-1}) \quad (3)$$

where A_{645} and A_{663} are the absorbances at 645 and 663 nm, V is the volume in mL, α is the length of the light path in the cell (1 cm), and W is the fresh weight in g.

Estimation of Proline Content

Proline content was determined according to the method described by Bates *et al.* (1973). Approximately 0.5 g of leaf and root samples were extracted with 10 mL of 3% (w/v) sulfosalicylic acid. The homogenate was centrifuged at $11,200 \times g$ for 30 min. A 2 mL aliquot of the clear supernatant was collected and reacted with 2 mL of ninhydrin solution and 2 mL of glacial acetic acid. The mixture was incubated at 100 °C for 1 h, after which the reaction was terminated by placing the tubes on ice for 5 min. Subsequently, 4 mL of toluene was added and mixed vigorously. The chromophore-containing toluene phase was separated from the aqueous phase and allowed to equilibrate at room temperature. Absorbance was measured at 520 nm using toluene as the blank. Proline concentration was determined from a standard curve and expressed on a fresh weight (FW) basis using Eq. 4:

$$\begin{aligned} &\mu\text{moles proline} / gFW \text{ materials} = \\ &[(\frac{\mu g \text{ proline}}{ml} \times ml \text{ toluene}) / 115.5 \mu g / \mu\text{mole}] / [g \text{ sample}] / 5 \end{aligned} \quad (4)$$

Estimation of Catalase (CAT), Guaiacol Peroxidase (POD), and Ascorbate Peroxidase (APX) Assay

The activities of catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), and ascorbate peroxidase (APX; EC 1.11.1.11) were determined following the method described by Azzeme *et al.* (2016). For CAT analysis, crude protein was extracted in 50 mM potassium phosphate buffer (pH 7.5) containing 5 mM dithiothreitol (DTT) at 4 °C. The homogenate was centrifuged at $11,200 \times g$ for 15 min at 4 °C, and the supernatant was collected and centrifuged again under the same conditions. The assay mixture contained 50 μ L of crude extract, 20 mM hydrogen peroxide (H_2O_2 , 30%), and 52 mM potassium phosphate buffer (pH 7.5). The CAT activity was defined as the amount of enzyme catalyzing the decomposition of 1 μ mol of H_2O_2 per minute for each mg of protein ($\mu\text{mol min}^{-1} \text{mg}^{-1}$), with absorbance measured at 240 nm over 3 min.

For POD analysis, 0.5 g of frozen leaves and roots were homogenized separately in liquid nitrogen. The powdered samples were mixed with extraction buffer (0.1 M potassium phosphate buffer, pH 6.5, containing 0.5 M sodium chloride and 2% [w/v] polyvinylpyrrolidone, PVP) at 4 °C. The homogenate was gently mixed and centrifuged at $11,200 \times g$ for 15 min at 4 °C, and the supernatant was collected and centrifuged again under the same conditions. A 50 μ L aliquot of crude extract was added to 3 mL of substrate solution containing 0.1 M potassium phosphate buffer (pH 6.0), 15 mM guaiacol, and 7.5 mM H_2O_2 (30%). One unit of POD activity was defined as the amount of enzyme catalyzing the oxidation of 1 μ mol of guaiacol per minute and for each mg of protein ($\mu\text{mol min}^{-1} \text{mg}^{-1}$), measured at 470 nm over 3 min.

For APX analysis, 0.5 g of leaves or roots was ground in liquid nitrogen. The chilled powder was homogenized in 50 mM phosphate buffer (pH 7.8) containing 5 mM ascorbate,

2% PVP, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM DTT, and 0.1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at $11,200 \times g$ for 15 min at 4 °C, and the supernatant was re-centrifuged under the same conditions. The APX activity was measured in a 3 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 50 μ L crude extract, 0.1 mM H₂O₂, and 0.5 mM ascorbate. One unit of APX activity was defined as the amount of enzyme oxidizing 1 μ mol of ascorbate per minute per mg of protein (μ molmin⁻¹mg⁻¹), with absorbance recorded at 290 nm for 3 min.

Estimation of Lipid Peroxidation Activity

Lipid peroxidation in control and treated oil palm leaves and roots was assessed by measuring malondialdehyde (MDA) content using the thiobarbituric acid (TBA) method, as described by Heath and Packer (1968). Approximately 250 mg of fresh tissue was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at $10,000 \times g$ for 5 min at 4 °C. Subsequently, 1 mL of the supernatant was mixed with 4 mL of 20% TCA containing 0.5% TBA and incubated at 95 °C for 30 min. The reaction mixture was rapidly cooled in crushed ice and centrifuged again at $10,000 \times g$ for 10 min at 4 °C. Absorbance was measured at 532 nm and 600 nm using a spectrophotometer. The MDA concentration was calculated using a standard calibration curve and expressed as mmol MDA per gram fresh weight (mmolMDAg⁻¹FW).

Preparation of Crude Extract for Phytochemical Analysis

Control and treated oil palm leaves and roots were extracted following the method described by Kamarul Zaman *et al.* (2020a). Approximately 1 g of leaf and root tissue was ground into a fine powder and extracted with 100 mL of methanol. The mixture was kept at room temperature under continuous shaking at 150 rpm for 24 h. After incubation, the extract was filtered using Whatman No. 1 filter paper. The resulting supernatant was collected and stored at 4 °C for subsequent phytochemical analysis.

Estimation of Total Phenolic Content

The total phenolic content (TPC) of oil palm leaves and roots was determined using the Folin–Ciocalteu method. Methanolic extracts prepared as described earlier were used. Approximately 1 mL of each extract was transferred into a test tube, followed by the addition of 0.5 mL of 10-fold diluted Folin–Ciocalteu reagent and 7 mL of distilled water. The mixture was incubated for 5 min, after which 1.5 mL of 7.5% (w/v) sodium carbonate (Na₂CO₃) was added and mixed thoroughly. The reaction mixture was then incubated in the dark for 2 h. Absorbance was measured at 765 nm using a spectrophotometer. TPC was expressed as milligrams of gallic acid equivalents per gram of fresh weight (mgGAeg⁻¹FW) (Kamarul Zaman *et al.* 2020b).

Estimation of Total Flavonoid Content

The total flavonoid content (TFC) of oil palm leaves and roots was determined using the aluminum chloride colorimetric assay, as described by Kamarul Zaman *et al.* (2020b). A total of 1 mL of each methanolic extract (prepared as described earlier) was added to a reaction mixture containing 4 mL of distilled water. Then, 0.3 mL of 5% (w/v) sodium nitrite (NaNO₂) was added, and the mixture was allowed to react for 5 min. Subsequently, 0.3 mL of 10% (w/v) aluminum chloride (AlCl₃) and 2 mL of 1 M sodium hydroxide (NaOH) were added. The final volume was adjusted to 10 mL with distilled

water. Absorbance was measured at 510 nm using a spectrophotometer. The TFC was expressed as milligrams of quercetin equivalents per gram of fresh weight (mgQEq⁻¹ FW).

Estimation of Total Terpenoid Content

The total terpenoid content (TTC) of oil palm leaves and roots was determined using a sulfuric acid colorimetric assay. A total of 3 mL of chloroform were added to 1 mL of each methanolic extract. The mixture was vortexed and left to stand for 3 min. Then, 200 µL of concentrated sulfuric acid (H₂SO₄) was added, and the reaction mixture was incubated at room temperature for 1.5 h in the dark until a reddish-brown precipitate formed. The supernatant was carefully decanted without disturbing the precipitate. Subsequently, 3 mL of 95% (v/v) methanol was added and vortexed thoroughly until the precipitate was completely dissolved. Absorbance was measured at 538 nm using a spectrophotometer. The TTC was expressed as milligrams of linalool equivalents per gram of fresh weight (mgLEg⁻¹ FW) (Kamarul Zaman *et al.* 2020b).

Estimation of Total Alkaloid Content

The total alkaloid content (TAC) of oil palm leaves and roots was determined using Dragendorff's precipitation method, as described by Mishra *et al.* (2018). Methanolic extracts were transferred into test tubes, and the pH was adjusted to 2.3 using a few drops of 1 M hydrochloric acid (HCl). Subsequently, 400 µL of Dragendorff's reagent (DR) was added to 1 mL of extract. The mixture was centrifuged at 1,844 × g for 10 min at 24 °C. To confirm complete precipitation, another 400 µL of DR was added to the supernatant. After centrifugation, the supernatant was discarded, and the resulting precipitate was washed twice with 1 mL of methanol. The aqueous phase was removed, and the residue was treated with 400 µL of 1% (w/v) disodium sulfide solution (Na₂S). The brownish-black precipitate was then centrifuged again at 1,844 × g for 10 min at 24 °C. Two drops of 1% (w/v) Na₂S solution were added to confirm the completion of the reaction, indicated by the formation of an intense brownish-black precipitate, followed by re-centrifugation. The final supernatant was discarded, and the pellet was dissolved in 400 µL of concentrated nitric acid (HNO₃) by gentle warming at 25 °C. The resulting solution was then diluted with 1.6 mL of autoclaved distilled water. A total of 1 mL of this solution was further reacted with 5 mL of 3% (w/v) thiourea solution. Absorbance was measured at 430 nm against a blank containing concentrated HNO₃ and 3% (w/v) thiourea. Alkaloid content was expressed as mg per gram of fresh weight (mgg⁻¹ FW) and calculated using Eq. 5:

$$\text{Alkaloid content (mgg}^{-1}\text{FW)} = (\text{Concentration } (\mu\text{g/mL})) / (\text{g FW}) \quad (5)$$

Estimation of Antioxidant Activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay, as described by Kamarul Zaman *et al.* (2020b). The initial absorbance of the DPPH solution (without sample) was measured at 517 nm. Approximately 0.2 mL of each sample extract was mixed with 3 mL of 0.1 mM DPPH solution. The mixture was incubated at room temperature in the dark for 30 min. After incubation, the absorbance was measured again at 517 nm. The antioxidant activity was calculated and expressed as the percentage of DPPH free radical scavenging activity using the following Eq. 6,

$$\text{DPPH free radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (6)$$

where A control is the absorbance of DPPH solution without sample and A sample is the absorbance of sample with DPPH solution.

Statistical Analysis

Abiotic stress treatments were arranged in a completely randomized design (CRD), with seven replicates per treatment. For biochemical analysis, three individual plants were randomly selected from each treatment. Data for all measured parameters were subjected to one-way analysis of variance (ANOVA), and differences between means were determined using Tukey's multiple range test at $p \leq 0.05$. Statistical analyses were performed using SAS 9.4 software (Cary, NC, USA). Results are presented as means $\pm$ standard error (SE) from triplicate determinations. Different letters indicate statistically significant differences among treatments.

RESULTS AND DISCUSSION

Morphological Changes of Oil Palm Seedlings

Oil palm seedlings exhibited distinct morphological responses to individual and combined stresses of flooding and salinity, revealing key aspects of their resilience mechanisms. Control seedlings displayed healthy, verdant foliage (Fig. 1A), whereas those exposed to salinity, flooding, and combined stresses showed evident yellowing symptoms (Fig. 1B, 1C, and 1D), with the most pronounced effects observed under salinity and combined stress conditions. In terms of root response, flooding treatment (Fig. 2C) notably induced the development of numerous adventitious roots with aerenchyma formation, a feature not observed in seedlings from other treatments (Fig. 2A, 2B, and 2D).

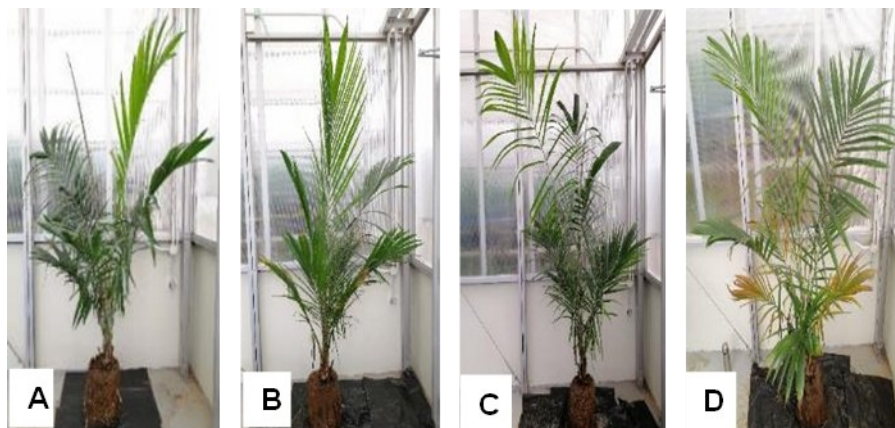


Fig. 1. Morphological changes in oil palm seedlings under (A) control, (B) salinity, (C) flooding, and (D) combined flooding and salinity treatments after twelve days of exposure

Under salinity and combined stresses, notable leaf yellowing indicated the severe impact of high sodium (Na^+) and chloride (Cl^-) accumulation in oil palm leaves. This striking foliage transformation may result from the deleterious effects of Na^+ and Cl^- ions, which trigger the degradation of vital pigments, particularly chlorophyll, and other essential chloroplast components under abiotic stress. Alharbi *et al.* (2022) reported that Na^+ and Cl^- accumulation impairs chlorophyll biosynthesis and disrupts the photosynthetic machinery by inhibiting pigment-producing enzymes, thereby reducing carotenoid and

chlorophyll synthesis and leading to chlorosis. Additionally, salt stress may damage cell wall structure, exacerbate dehydration, and impede overall plant growth and development. Ma *et al.* (2020) and Raju *et al.* (2020) highlighted that salinity-induced dehydration and ionic toxicity promote leaf senescence and reduce photosynthetic activity.

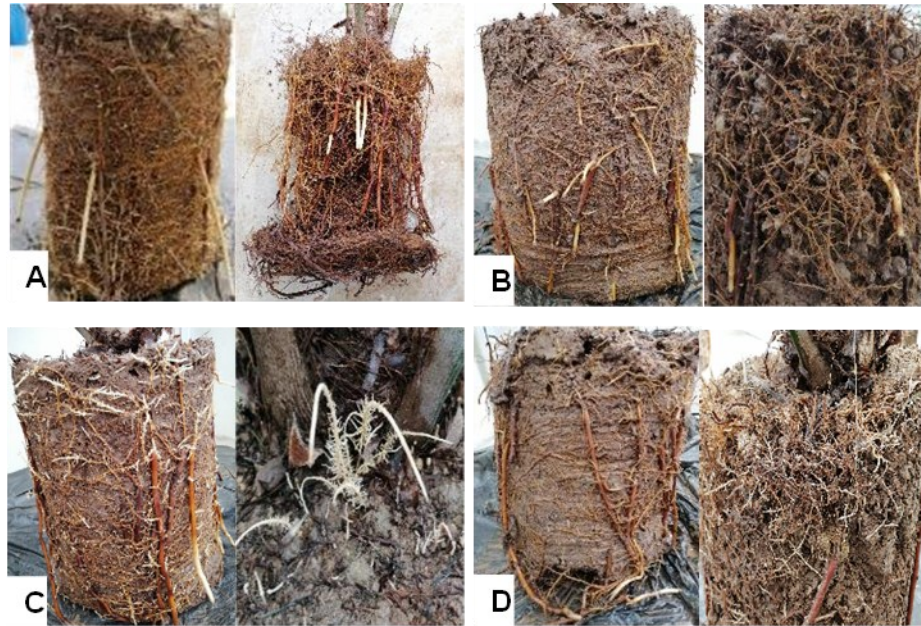


Fig. 2. Morphological changes of oil palm roots under (A) control, (B) salinity, (C) flooding, and (D) combined flooding and salinity stress treatments after twelve days of exposure

Maintaining the negative charge of cell walls is crucial for calcium (Ca^{2+}) binding and pectin cross-linking, which reinforce cell wall integrity. However, excess Na^+ may displace Ca^{2+} , thereby hindering pectin cross-linking and reducing cell elongation (Dabravolski and Isayenkov 2023). The synergy of flooding and salinity may intensify leaf yellowing, potentially due to reduced oxygen (O_2) availability, which promotes toxic Na^+ accumulation in leaves. Oxygen deficiency may impair energy-dependent Na^+ exclusion from the xylem, mediated by high-affinity potassium transporters (HKT) and salt overly sensitive (SOS) transporters, thereby increasing Na^+ levels in mesophyll cells and suppressing photosynthesis (Renziehausen *et al.* 2024).

Roots, being the primary sensors of soil moisture changes, perceive flooding-induced limitations in gas diffusion, leading to elevated ethylene levels and O_2 depletion (Verslues *et al.* 2023). Aerenchyma formation facilitates gas exchange between roots and shoots during submergence, while adventitious roots replace damaged primary roots under O_2 deficiency, ensuring continued oxygen uptake and transport (Aslam *et al.* 2023). Studies by Nuanlaong *et al.* (2021) and Rivera-Mendes *et al.* (2016) reported the development of aerenchyma and pneumatophores in oil palm seedlings under waterlogging stress, demonstrating adaptive strategies to cope with flooding.

In contrast, salinity and combined stress treatments (Fig. 2B and 2D) failed to induce adventitious root formation, potentially due to disrupted Na^+ and Cl^- dynamics that impede root development. Salinity-induced reductions in plant cell water potentially result in dehydration, further aggravated by excess NaCl , which restricts water availability and induces physiological drought (Hannachi *et al.* 2022).

Under combined flooding and salinity stress, low water potential, oxygen deficiency, and ionic toxicity likely compound root growth inhibition. Oxygen deprivation disrupts O₂-dependent ion transporters responsible for Na⁺ exclusion and ion homeostasis. Tahjib-Ul-Arif *et al.* (2023) reported that salinity-induced membrane depolarization, coupled with root O₂ deficiency, diminishes ATP production and impairs SOS1 Na⁺/H⁺ antiporter activity, leading to reduced Na⁺ exclusion and K⁺ retention. As a result, an unfavorable Na⁺/K⁺ ratio impairs plant metabolism and hinders both root and shoot development. In a study by Chakraborty *et al.* (2021), aerenchyma formation in the rice variety Varshadhan was inhibited under saline flooding due to suppression of reactive oxygen species (ROS) signaling by metallothioneins.

Estimation of Chlorophyll Content

A reduction in chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), and total chlorophyll (TC) was observed in treated oil palm leaves compared to the control (Table 1). The combined stress treatment had the most severe effect, with Chl*a*, Chl*b*, and TC levels reduced 5.92-, 5.88-, and 5.93-fold, respectively, relative to the control. However, no significant differences were observed between the effects of single and combined stresses on Chl*a*, Chl*b*, and TC, indicating that salinity, flooding, and their combination elicited comparable impacts on chlorophyll content. Among all treatments, the combined stress resulted in the lowest levels of Chl*a*, Chl*b*, and TC. These reductions are consistent with the yellowing symptoms observed in oil palm leaves under combined stress, as illustrated in Fig. 1D.

Table 1. The Chl*a*, Chl*b*, and TC Contents in Leaf Tissues of Oil Palm Seedlings Subjected to Control, Flooding, Salinity, and Combined Flooding and Salinity Stress Treatments

Treatment	Chl <i>a</i> (mgg ⁻¹ FW)	Chl <i>b</i> (mgg ⁻¹ FW)	TC (mgg ⁻¹ FW)
Control	0.79 ± 0.13 ^a	0.35 ± 0.04 ^a	1.15 ± 0.17 ^a
Salinity	0.16 ± 0.01 ^b	0.06 ± 0.05 ^b	0.22 ± 0.02 ^b
Flooding	0.35 ± 0.12 ^b	0.15 ± 0.05 ^b	0.51 ± 0.16 ^b
Combined	0.13 ± 0.00 ^b	0.06 ± 0.00 ^b	0.19 ± 0.01 ^b

Means with different letters are significantly different at $p \leq 0.05$ Tukey's range test

The effects of combined salinity and flooding stress on oil palm seedlings reveal intricate physiological and biochemical responses, particularly related to oxidative stress and antioxidant defense mechanisms. Unlike individual stresses, combined stress conditions often lead to more complex interactions that can either intensify damage or trigger distinct adaptive responses. A prominent negative effect observed under combined stress was the reduction in chlorophyll content in oil palm leaves. Similar reductions in chlorophyll under combined stress conditions have also been reported in maize, alfalfa, and barley (Zeng *et al.* 2013; Sghaier *et al.* 2020; Mahmood *et al.* 2021).

This chlorophyll decline is likely associated with ion imbalance, particularly the disruption of sodium (Na⁺) and potassium (K⁺) uptake. Under combined stresses, low oxygen levels caused by flooding impair root respiration and reduce the efficiency of selective ion transporters, leading to excessive Na⁺ accumulation (Tahjib-Ul-Arif *et al.* 2023). This ion toxicity is further exacerbated by competition between Na⁺ and K⁺ for

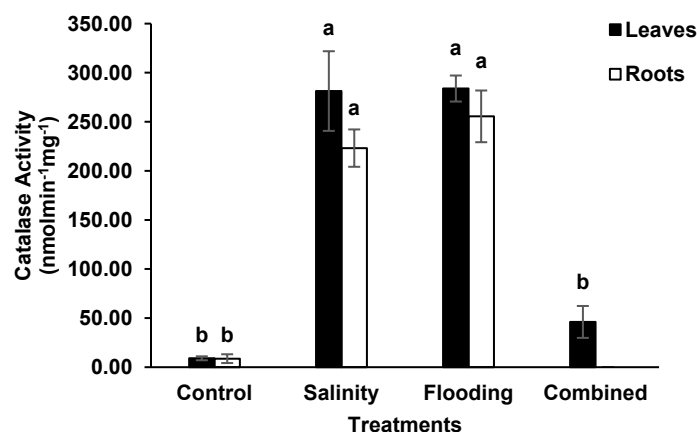
uptake, resulting in K^+ deficiency. Potassium plays a critical role in stomatal regulation, enzyme activation, and photosynthetic function; therefore, its deficiency compromises the structural and functional integrity of the chloroplasts.

The destabilization of chloroplast membranes and disruption of pigment–protein complexes (Renziehausen *et al.* 2024) contribute to accelerated chlorophyll degradation. This process is not only driven by Na^+ toxicity but also by the induction of chlorophyll-degrading enzymes such as chlorophyllase. Activation of chlorophyllase under stress conditions (Guyer *et al.* 2018; Wang *et al.* 2018), along with thylakoid shrinkage within chloroplasts, likely contributes to visible chlorosis, leaf necrosis, and impaired plant development (Mohsin *et al.* 2020; Hannachi *et al.* 2022). The plant's inability to maintain cellular homeostasis under combined salinity and flooding stress ultimately accelerates chlorophyll loss, reducing photosynthetic efficiency and overall growth.

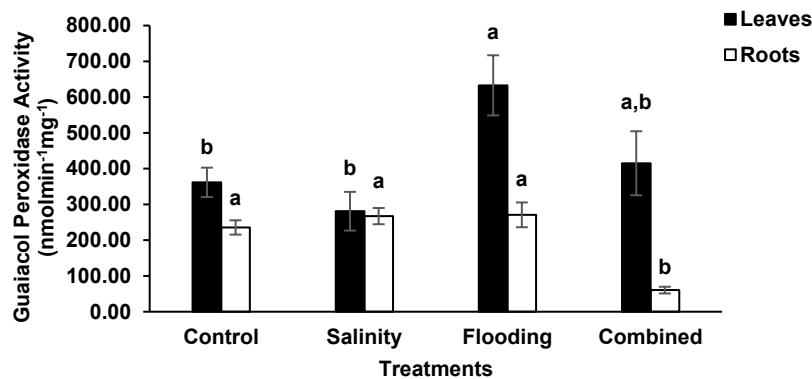
Estimation of Catalase, Guaiacol Peroxidase, and Ascorbate Peroxidase

The present study revealed that the antioxidant enzymes catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) exhibited tissue-specific responses to the imposed stress treatments (Fig. 3). In roots, combined salinity and flooding stress had a markedly negative impact on antioxidant enzyme activities. No CAT (Fig. 3A) or APX (Fig. 3C) activity was detected, while POD activity (Fig. 3B) was significantly reduced by 3.90-fold compared to the control. In contrast, oil palm leaves demonstrated an increase in CAT and POD activities under combined stress, with 5.09-fold and 1.15-fold increases, respectively, compared to the control. However, APX activity in leaves decreased 1.85-fold. When compared to the effects of individual salinity or flooding stress, the combined stress treatment resulted in the lowest CAT, POD, and APX activities in roots, indicating a compounded inhibitory effect. In leaves, only POD and APX showed increased activity under combined stress, suggesting a partial compensatory antioxidant response. These findings indicate that combined salinity and flooding stress exerts a strong negative influence on the antioxidant defense system of oil palm seedlings, particularly in the roots, where enzymatic activity was largely suppressed.

(A)



(B)



(C)

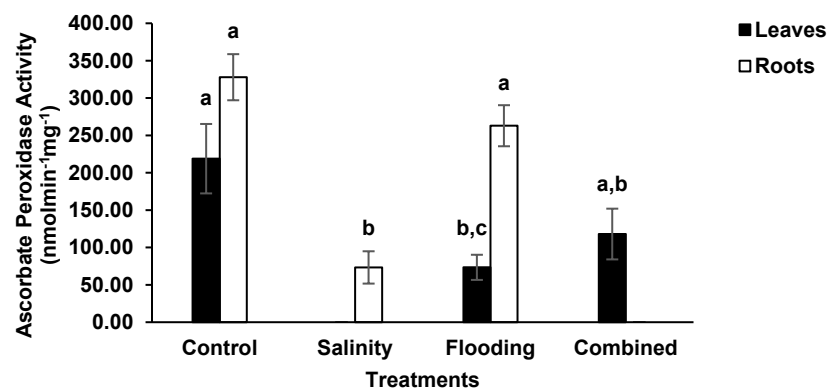


Fig. 3. Effect of single and combined flooding and salinity stresses on the activities of antioxidant enzymes: (a) catalase (CAT), (b) guaiacol peroxidase (POD), and (c) ascorbate peroxidase (APX). Data are presented as mean $\pm$ standard error (SE) of three replicates. Different letters indicate statistically significant differences at $p \leq 0.05$ according to Tukey's multiple range test.

Combined salinity and flooding stresses also trigger oxidative stress, primarily due to the overproduction of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radicals ($\text{OH}^\cdot$). These ROS can damage cellular structures, including membranes, proteins, and nucleic acids, and their accumulation is exacerbated under dual stress conditions due to Na^+ toxicity and low oxygen availability.

In this study, antioxidant enzyme activities showed distinct tissue-specific patterns in response to combined stresses. While catalase (CAT) and guaiacol peroxidase (POD) activities were slightly elevated in leaves, their activities were markedly reduced in roots. The increased CAT and POD activities in leaves suggest that oil palm seedlings retain some capacity to mitigate oxidative damage in aerial tissues. These enzymes function synergistically to decompose H_2O_2 , a major by-product of ROS accumulation, thereby playing a protective role in photosynthetically active tissues (Xiong *et al.* 2020).

In contrast, the decline in antioxidant enzyme activity in roots indicates severe physiological impairment under combined stress. This may be attributed to the dysfunction of key ion transporters, such as salt overly sensitive (SOS) and high-affinity potassium transporter (HKT), which are energy-dependent and adversely affected by low oxygen

levels. Impaired transporter function reduces the exclusion of Na^+ from root cells, leading to its toxic accumulation. Excessive Na^+ and Cl^- disrupt root enzymatic function, destabilize antioxidant proteins, and reduce the plant's ability to detoxify ROS. As suggested by López-Cristoffanini *et al.* (2020), excessive ionic accumulation can overwhelm enzymatic defense systems, resulting in reduced scavenging capacity and heightened oxidative damage.

Estimation of Proline Content

A notable aspect of the response to combined salinity and flooding stresses in oil palm seedlings is the accumulation of proline, a key osmoprotectant and antioxidant molecule. In this study, proline content increased significantly in both leaves and roots under combined stress compared to the control and single stress treatments (Fig. 4). In leaves, proline levels increased 1.74-fold, 1.80-fold, and 2.35-fold relative to the control, salinity, and flooding treatments, respectively. Similarly, in roots, proline content increased 1.46-fold, 1.35-fold, and 1.40-fold compared to the control, salinity, and flooding treatments, respectively.

Interestingly, flooding alone led to a significant reduction in proline content in leaves, with a 1.35-fold decrease compared to the control. In contrast, salinity caused a slight but non-significant increase in proline levels in both leaves and roots, while flooding slightly elevated proline in roots only. However, the changes in proline content under single salinity and flooding stresses were not statistically significant when compared to the control.

Proline serves as a critical component in maintaining cellular homeostasis and mitigating damage caused by reactive oxygen species (ROS). The elevated proline content observed in oil palm seedlings under combined stress conditions suggests that proline plays a compensatory role when enzymatic antioxidant activity is insufficient. As noted by Badruzaman *et al.* (2021), proline may also function as a non-enzymatic antioxidant, protecting cells from oxidative injury associated with excessive ROS accumulation. This supports the hypothesis that non-enzymatic antioxidants, such as proline, become especially important under complex environmental conditions, such as the concurrent occurrence of salinity and flooding.

This finding is consistent with the work of Sghaier *et al.* (2020) and Urmi *et al.* (2023), who reported that proline biosynthesis is a direct response to disrupted cellular homeostasis, aiding in the maintenance of turgor pressure and ROS detoxification. Notably, the current study revealed a reduction in proline content under individual stress treatments (salinity or flooding alone), which coincided with higher enzymatic antioxidant activities, such as those of catalase (CAT) and peroxidase (POD). This suggests a potential inverse relationship between proline accumulation and enzymatic antioxidant activity under single-stress conditions.

The decrease in proline under salinity or flooding alone may be attributed to the plant's capacity to manage oxidative stress primarily through its enzymatic defense systems, reducing the need for non-enzymatic antioxidants. However, under combined stresses, the simultaneous presence of multiple stressors may overwhelm the enzymatic antioxidant system, thereby necessitating greater proline accumulation to compensate for reduced enzymatic efficacy.

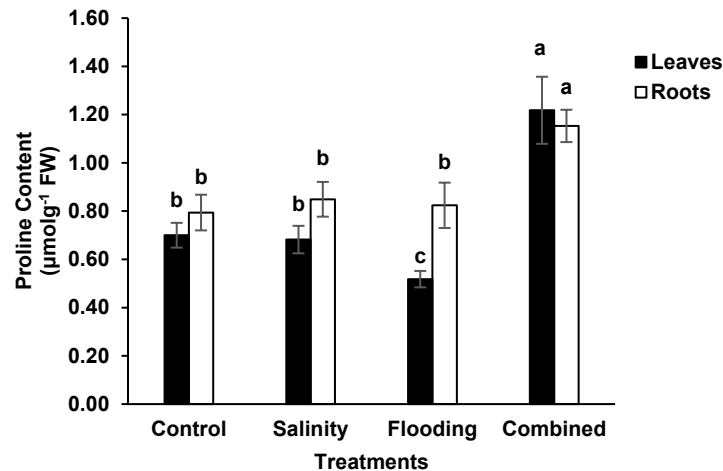


Fig. 4. Proline content in the leaves and roots of oil palm seedlings subjected to single and combined flooding and salinity stress treatments. Data are presented as mean $\pm$ SE of three replicates. Different letters indicate statistically significant differences at $p \leq 0.05$ according to Tukey's multiple range test.

Estimation of Lipid Peroxidation Activity

This study also provides insight into lipid peroxidation activity, assessed through malondialdehyde (MDA) content, a widely recognized indicator of oxidative damage to cellular membranes. The MDA levels were significantly elevated under both single and combined stress treatments compared to the control (Fig. 5). Under combined salinity and flooding stress, oil palm leaves and roots exhibited markedly higher MDA contents, with 2.51-fold and 1.38-fold increases, respectively, relative to the control. Similarly, exposure to either salinity or flooding alone also resulted in significant increases in MDA content in both tissues compared to the control.

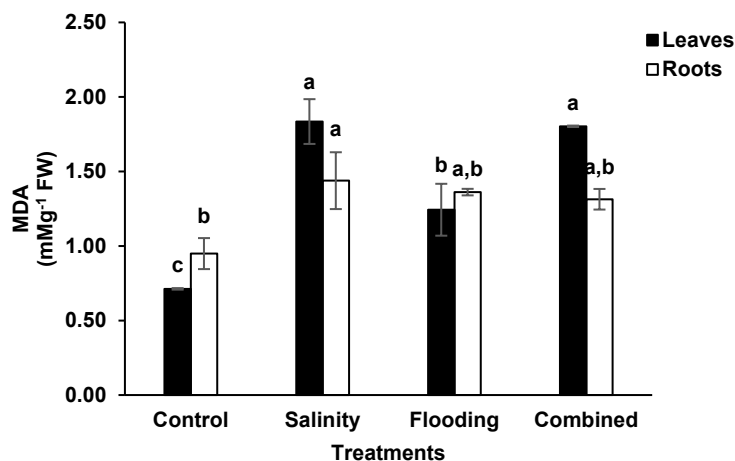


Fig. 5. MDA content in the leaves and roots of oil palm seedlings subjected to control, flooding, salinity, and combined flooding and salinity stress treatments. Data are presented as mean $\pm$ SE of three replicates. Different letters indicate statistically significant differences at $p \leq 0.05$ according to Tukey's multiple range test.

The significant increase in MDA content under combined stress conditions suggests that oil palm seedlings experienced higher levels of oxidative stress compared to single-stress treatments. This observation aligns with the findings of Wang *et al.* (2019), who reported that combined abiotic stresses can exacerbate oxidative damage due to the excessive accumulation of reactive oxygen species (ROS), leading to increased lipid peroxidation. For example, the combination of salinity and waterlogging disrupts ion homeostasis, particularly through elevated Na^+ and Cl^- accumulation, which contributes to both ionic and osmotic stress and subsequently enhances ROS production.

Additionally, oxygen deprivation under waterlogged conditions may impair the plant's capacity to maintain ion selectivity, resulting in increased Na^+ uptake and reduced K^+ transport to the shoots, further aggravating oxidative stress (Duan *et al.* 2019). In contrast, a study by Liu *et al.* (2020) on *Elaeagnus angustifolia* reported lower MDA levels under combined waterlogging and 0.6% NaCl stress, indicating reduced membrane lipid peroxidation, greater membrane stability, and lower relative membrane conductivity. This discrepancy may be attributed to species-specific stress tolerance mechanisms or differences in stress intensity and duration.

In oil palm seedlings, the elevated MDA levels likely reflect more severe oxidative damage, possibly due to prolonged exposure to both salinity and flooding, or a limited capacity to activate sufficient defense mechanisms against the compounded stress effects.

Estimation of Total Phenolic Content, Total Flavonoid Content, Total Terpenoid Content, and Total Alkaloid Content

Total phenolic content (TPC), total flavonoid content (TFC), total terpenoid content (TTC), and total alkaloid content (TAC) were measured to evaluate the effects of single and combined salinity and flooding stresses on non-enzymatic antioxidant activity in oil palm leaves and roots (Fig. 6). Among the four phytochemical classes, TTC (Fig. 6C) exhibited the highest concentrations overall, compared to TPC (Fig. 6A), TFC (Fig. 6B), and TAC (Fig. 6D).

Plants synthesize secondary metabolites, such as phenolics, flavonoids, terpenoids, and alkaloids, as part of their antioxidant defense mechanisms. These compounds play essential roles in neutralizing reactive oxygen species (ROS) and enabling plants to adapt to environmental stressors (Li *et al.* 2020). In the present study, combined salinity and flooding stresses elicited variable effects on the production of these secondary metabolites. Among the metabolites evaluated, terpenoids exhibited the highest accumulation under both single and combined stress conditions. Given their known roles in membrane stabilization and antioxidant activity, the elevated levels of terpenoids suggest a potential protective function in enhancing membrane integrity under abiotic stress.

However, the absence of significant differences in terpenoid content across treatments indicates that their accumulation may not be a primary or specific response to the combined stress of flooding and salinity. In contrast, the reduced accumulation of phenolics, flavonoids, and alkaloids under combined stress conditions suggests that oil palm seedlings may not rely extensively on these compounds for ROS scavenging when facing multiple concurrent stressors. This implies a potential shift in the antioxidant defense strategy under combined stresses, where non-enzymatic antioxidants, such as proline or other physiological adaptations, may play a more dominant role.

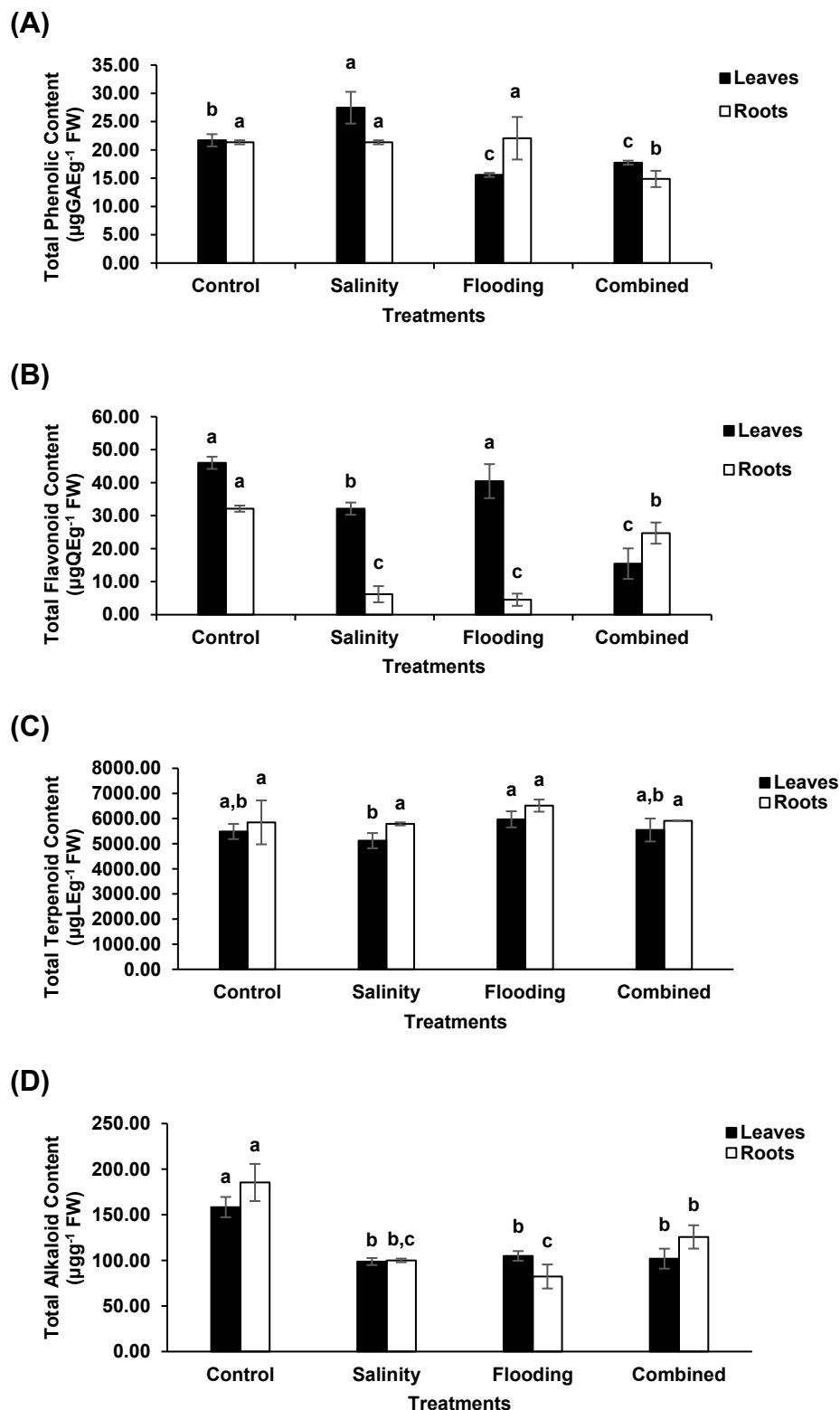


Fig. 6. Effect of single and combined flooding and salinity stresses on (a) total phenolic content (TPC), (b) total flavonoid content (TFC), (c) total terpenoid content (TTC), and (d) total alkaloid content (TAC) in the leaf and root tissues of oil palm seedlings. Data are presented as mean $\pm$ SE of three replicates. Different letters indicate statistically significant differences at $p \leq 0.05$ according to Tukey's multiple range test.

Estimation of Antioxidant Activity

Antioxidant activity in oil palm leaves and roots was assessed using the DPPH free radical scavenging assay. In roots, DPPH scavenging activity increased under single salinity and flooding treatments by 1.48-fold and 1.19-fold, respectively, compared to the control (Fig. 7). However, under combined salinity and flooding stress, a reduction in DPPH activity was observed in both leaves and roots, by 1.70-fold and 1.13-fold, respectively, relative to the control.

A similar decline was also recorded in leaves under individual stress treatments, with a 1.76-fold decrease under salinity and a 1.28-fold decrease under flooding. Among all treatments, the highest DPPH activity in leaves was recorded in the control group ($58.68 \pm 1.08\%$), while in roots, the highest activity was observed under salinity stress ($49.96 \pm 5.21\%$).

The antioxidant capacity of oil palm seedlings, as indicated by DPPH scavenging activity, was highest under flooding in leaves and under salinity in roots. This suggests that different tissues may employ distinct defense strategies depending on the type of stress imposed. The elevated antioxidant activity in roots under salinity stress may be attributed to the enhanced production of phenolics, flavonoids, and alkaloids, which are known to scavenge ROS generated during salt stress.

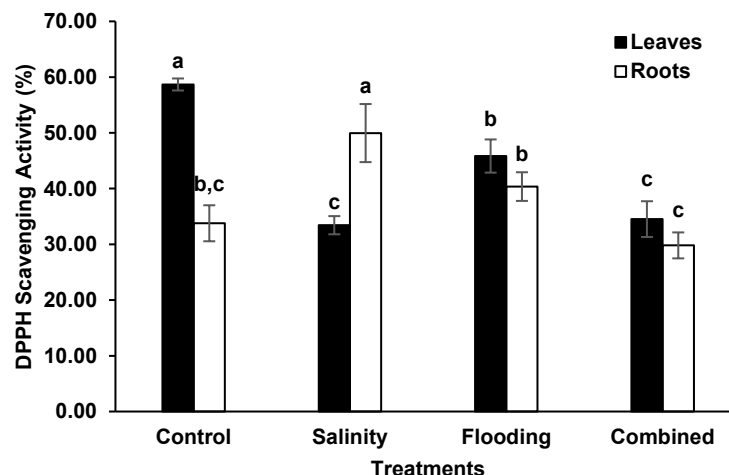


Fig. 7. DPPH free radical scavenging activity in the leaf and root tissues of oil palm seedlings subjected to control, flooding, salinity, and combined flooding and salinity stress treatments. Data are presented as mean $\pm$ SE of three replicates. Different letters indicate statistically significant differences at $p \leq 0.05$ according to Tukey's multiple range test.

In contrast, under combined salinity and flooding stress, the lowest antioxidant activity was recorded in both leaves and roots. This decline may reflect either the depletion of antioxidant compounds or the suppression of their biosynthetic pathways, which is likely due to the severe physiological damage induced by the dual stress. Simultaneous exposure to salinity and flooding may also disrupt the plant's energy balance, thereby limiting its ability to sustain the synthesis of antioxidant metabolites required for effective ROS detoxification.

CONCLUSIONS

1. Oil palm seedlings exhibited distinct physiological and biochemical responses when subjected to single and combined flooding and salinity stress, confirming that stress interactions produce unique effects not predictable from individual stress responses alone.
2. Combined stress led to a more pronounced reduction in chlorophyll content and photosynthetic capacity, as reflected by visible leaf chlorosis and significant decreases in chlorophyll *a*, *b*, and total chlorophyll, compared to single-stress treatments.
3. Lipid peroxidation, indicated by elevated malondialdehyde (MDA) content, was highest under combined stress, signifying enhanced oxidative damage in both leaves and roots due to the overaccumulation of reactive oxygen species (ROS).
4. While catalase (CAT) and peroxidase (POD) activities increased in leaves under combined stress, all antioxidant enzyme activities were significantly suppressed in roots, indicating tissue-specific limitations in enzymatic ROS detoxification.
5. Proline levels increased substantially in both leaves and roots under combined stress, highlighting its role as a key osmoprotectant and non-enzymatic antioxidant when enzymatic defenses are insufficient.
6. The content of secondary metabolites, including phenolics, flavonoids, terpenoids, and alkaloids, generally declined under combined stress, suggesting compromised biosynthetic capacity or diversion of metabolic resources under severe physiological strain.
7. Collectively, these results demonstrate that combined flooding and salinity stress overwhelms the antioxidant defense system in oil palm seedlings, especially in roots, underscoring the need for stress-resilient varieties and adaptive management strategies for areas exposed to multiple abiotic stressors.

ACKNOWLEDGMENTS

This work is supported by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS) (FRGS/1/2019/STG04/UPM/02/1).

REFERENCES CITED

- Abdullah, S. N. A., Azzeme, A. M., Ebrahimi, M., Ariff, E. A. K. E., and Hanifah, F. H. A. (2017). "Transcription factors associated with abiotic stress and fruit development in oil palm," in: *Crop Improvement: Sustainability Through Leading-Edge*, Abdullah, S. N. A. *et al.* (eds.), Springer International Publishing AG, Cham, Switzerland, pp. 71-99. https://doi.org/10.1007/978-3-319-65079-1_4
- Abubakar, A., and Ishak, M. Y. (2022). "An overview of the role of smallholders in oil palm production systems in changing climate," *Nature Environment and Pollution Technology* 21, 2055-2071. <https://doi.org/10.46488/NEPT.2022.v21i05.004>

- Akbudak, M. A., and Filiz, E. (2018). "Genome-wide analyses of ATP sulfurylase (ATPS) genes in higher plants and expression profiles in sorghum (*Sorghum bicolor*) under cadmium and salinity stresses," *Genomics* 111(2), 579-589. <https://doi.org/10.1016/j.ygeno.2018.03>
- Alharbi, K., Al-Osaimi, A. A., and Alghamdi, B. A. (2022). "Sodium chloride (NaCl)-induced physiological alteration and oxidative stress generation in *Pisum sativum* (L.): A toxicity assessment," *ACS Omega* 7(24), 20819-20832. <https://doi.org/10.1021/acsomega.2c01427>
- Aslam, A., Mahmood, A., Ur-Rehman, H., Li, C., Liang, X., Shao, J., Negm, S., Moustafa, M., Aamer, M., and Hassan, M. U. (2023). "Plant adaptation to flooding stress under changing climate conditions: Ongoing breakthroughs and future challenges," *Plants* 12(22), article 3824. <https://doi.org/10.3390/plants12223824>
- Azzeme, A. M., Abdullah, S. N. A., Abd Aziz, M., and Wahab, P. E. M. (2017). "Oil palm drought inducible DREB1 induced expression of DRE/CRT-and non-DRE/CRT-containing genes in lowland transgenic tomato under cold and PEG treatments," *Plant Physiology and Biochemistry* 112, 129-151. <https://doi.org/10.1016/j.plaphy.2016.12>
- Azzeme, A. M., Abdullah, S. N. A., Aziz, M. A., and Wahab, P. E. M. (2016). "Oil palm leaves and roots differ in physiological response, antioxidant enzyme activities and expression of stress-responsive genes upon exposure to drought stress," *Acta Physiologiae Plantarum* 38, article 52. <https://doi.org/10.1007/s11738-016-2073-2>
- Azzeme, A. M., and Abdullah, S. N. A. (2019). "Adaptive mechanisms of plants against salt stress and salt shock," in: *Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches*, M. Akhtar (ed.), Springer Singapore, Singapore, Singapore. https://doi.org/10.1007/978-981-13-8805-7_2
- Badrulzaman, S. Z. S., Ramlan, N. N., Kamarul Zaman, M. A., and Azzeme, A. M. (2021). "Physio-biochemical responses of *in vitro* cooking banana *Musa paradisiaca* cv Lang towards polyethylene glycol (PEG)-induced drought stress," *Malaysian Journal of Fundamental and Applied Sciences* 17(6), 805-817. <https://doi.org/10.11113/mjfas.v17n6.2341>
- Bates, L. S., Waldren, R. P., and Teare, I. D. (1973). "Rapid determination of free proline for water-stress studies," *Plant and Soil* 39, 205-207. DOI: 10.1007/BF00018060
- Bhowmik, A. K., Padmanaban, R., Cabral, P., and Romeiras, M. M. (2022). "Global mangrove deforestation and its interacting social-ecological drivers: A systematic review and synthesis," *Sustainability* 14(8), article 4433. <https://doi.org/10.3390/su14084433>
- Chakraborty, K., Ray, S., Vijayan, J., Molla, K. A., Nagar, R., Jena, P., Mondal, S., Panda, B. B., Shaw, B. P., Swain, P., et al. (2021). "Preformed aerenchyma determines the differential tolerance response under partial submergence imposed by fresh and saline water flooding in rice," *Physiologia Plantarum* 173(4), 1597-1615. <https://doi.org/10.1111/ppl.13536>
- Chen, W., Yao, Q., Patil, G. B., Agarwal, G., Deshmukh, R. K., Lin, L., Wang, B., Wang, Y., Prince, S. J., Song, L., et al. (2016). "Identification and comparative analysis of differential gene expression in soybean leaf tissue under drought and flooding stress revealed by RNA-seq," *Frontiers in Plant Science* 7, article 1044. <https://doi.org/10.3389/fpls.2016.01044>

- Dabravolski, S. A., and Isayenkov, S. V. (2023). "The regulation of plant cell wall organisation under salt stress," *Frontiers in Plant Science* 14, article 1118313. <https://doi.org/10.3389/fpls.2023.1118313>
- Duan, H., Ma, Y., Liu, R., Li, Q., Yang, Y., and Song, J. (2018). "Effect of combined waterlogging and salinity stresses on euhalophyte *Suaeda glauca*," *Plant Physiology and Biochemistry* 127, 231-237. <https://doi.org/10.1016/j.plaphy.2018.03>
- El-Beltagi, H. S., Mohamed, H. I., and Sofy, M. R. (2020). "Role of ascorbic acid, glutathione, and proline applied as singly or in sequence combination in improving chickpea plant through physiological change and antioxidant defense under different levels of irrigation intervals," *Molecules* 25(7), article 1702. <https://doi.org/10.3390/molecules25071702>
- Godoy, F., Olivos-Hernández, K., Stange, C., and Handford, M. (2021). "Abiotic stress in crop species: Improving tolerance by applying plant metabolites," *Plants* 10(2), article 186. <https://doi.org/10.3390/plants10020186>
- Guyer, L., Salinger, K., Krügel, U., and Hörtensteiner, S. (2018). "Catalytic and structural properties of pheophytinase, the phytol esterase involved in chlorophyll breakdown," *Journal of Experimental Botany* 69(4), 879-889. <https://doi.org/10.1093/jxb/erx326>
- Hannachi, S., Steppe, K., Eloudi, M., Mechi, L., Bahrini, I., and Van Labeke, M. C. (2022). "Salt stress induced changes in photosynthesis and metabolic profiles of one tolerant ('Bonica') and one sensitive ('Black beauty') eggplant cultivars (*Solanum melongena* L.)," *Plants* 11(5), article 590. <https://doi.org/10.3390/plants11050590>
- Hasanuzzaman, M., Bhuyan, M. H. M. B., Zulfikar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., Fujita, M., and Fotopoulos, V. (2020). "Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator," *Antioxidants* 9(8), article 681. <https://doi.org/10.3390/antiox9080681>
- Heath, R. L., and Packer, L. (1968). "Photoperoxidation in isolated chloroplasts: I. Kinetics and Stoichiometry of fatty acid peroxidation," *Archives of Biochemistry and Biophysics* 125(1), 189-198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Hoagland, D. R., and Arnon, D. I. (1950). "The water-culture method for growing plants without soil," *Circular & California Agricultural Experiment Station* 347.
- Isweiri, H., Qian, Y., and Davis, J. G. (2022). "Interactive effects of waterlogging and salinity on perennial ryegrass and alkali grass," *International Turfgrass Society Research Journal* 14(1), 266-275. <https://doi.org/10.1002/its2.60>
- Jensen, A. B., Eller, F., and Sorrell, B. K. (2024). "Comparative flooding tolerance of *Typha latifolia* and *Phalaris arundinacea* in wetland restoration: Insights from photosynthetic CO₂ response curves, photobiology and biomass allocation," *Heliyon* 10(1), article e23657. <https://doi.org/10.1016/j.heliyon.2023.e2>
- Ji, X., Tang, J., and Zhang, J. (2022). "Effects of salt stress on the morphology, growth and physiological parameters of *Juglans microcarpa* L. seedlings," *Plants* 11(18), article 2381. <https://doi.org/10.3390/plants11182381>
- Kamarul Zaman, M. A., Azzeme, A. M., Ramle, I. K., Normanshah, N., Ramli, S. N., Shaharuddin, N. A., Ahmad, S., and Abdullah, S. N. A. (2020a). "Induction, multiplication, and evaluation of antioxidant activity of *Polyalthia bullata* callus, a woody medicinal plant," *Plants* 9(12), article 1772. <https://doi.org/10.3390/plants9121772>

- Kamarul Zaman, M. A., Azzeme, A. M., Ramle, I. K., Normanshah, N., Ramli, S. N., Shaharuddin, N. A., Ahmad, S., and Abdullah, S. N. A. (2020b). "Solvent extraction and its effect on phytochemical yield and antioxidant capacity of woody medicinal plant, *Polyalthia bullata*," *BioResources* 15(4), 9555-9568. <https://doi.org/10.15376/biores.15.4.9555-9568>
- Launay, A., Cabassa-Hourton, C., Eubel, H., Maldiney, R., Guivarc'h, A., Crilat, E., Planchais, S., Lacoste, J., Bordenave-Jacquemin, M., Clément, G., Richard, L., Carol, P., Braun, H. P., Lebreton, S., and Savouré, A., (2019). "Proline oxidation fuels mitochondrial respiration during dark-induced leaf senescence in *Arabidopsis thaliana*," *Journal of Experimental Botany* 70(11), 6203-6214. <https://doi.org/10.1093/jxb/erz351>
- Li, Y., Kong, D., Fu, Y., Sussman, M. R., and Wu, H. (2020). "The effect of developmental and environmental factors on secondary metabolites in medicinal plants," *Plant Physiology and Biochemistry* 148, 80-89. <https://doi.org/10.1016/j.plaphy.2020.01>
- Liu, X., Chen, C., Liu, Y., Liu, Y., Zhao, Y., and Chen, M. (2020). "The presence of moderate salt can increase tolerance of *Elaeagnus angustifolia* seedlings to waterlogging stress," *Plant Signaling & Behavior* 15(4), article 1743518. <https://doi.org/10.1080/15592324.2020.1743518>
- López-Cristoffanini, C., Bundó, M., Serrat, X., San Segundo, B., López-Carbonell, M., and Nogués, S. (2020). "A comprehensive study of the proteins involved in salinity stress response in roots and shoots of the FL478 genotype of rice (*Oryza sativa* L. sp. indica)," *The Crop Journal* 9(5), 1154-1168. <https://doi.org/10.1016/j.cj.2020.10.009>
- Ma, Y., Dias, M. C., and Freitas, H. (2020). "Drought and salinity stress responses and microbe-induced tolerance in plants," *Frontiers in Plant Science* 11, article 591911. <https://doi.org/10.3389/fpls.2020.591911>
- Mahmood, U., Hussain, S., Hussain, S., Ali, B., Ashraf, U., Zamir, S., Al-Robai, S. A., Alzahrani, F. O., Hano, C., and El-Esawi, M. A. (2021). "Morpho-physio-biochemical and molecular responses of maize hybrids to salinity and waterlogging during stress and recovery phase," *Plants* 10(7), article 1345. <https://doi.org/10.3390/plants10071345>
- Mishra, M. R., Srivastava, R. K., and Akhtar, N. (2018). "Enhanced alkaloid production from cell culture system of *Catharanthus roseus* in combined effect of nutrient salts, sucrose and plant growth regulators," *Journal of Biotechnology and Biomedical Science* 1(4), 14-34. <https://doi.org/10.14302/issn.2576-6694.jbbs-18-2475>
- Mohd Hanafiah, K., Abd Mutalib, A. H., Miard, P., Goh, C. S., Mohd Sah, S. A., and Ruppert, N. (2022). "Impact of Malaysian palm oil on sustainable development goals: Co-benefits and trade-offs across mitigation strategies," *Sustainability Science* 17, 1639-1661. <https://doi.org/10.1007/s11625-021-01052-4>
- Mohsin, S. M., Hasanuzzaman, M., Parvin, K., and Fujita, M. (2020). "Pretreatment of wheat (*Triticum aestivum* L.) seedlings with 2,4-D improves tolerance to salinity induced oxidative stress and methylglyoxal toxicity by modulating ion homeostasis, antioxidant defenses, and glyoxalase systems," *Plant Physiology and Biochemistry* 152, 221-231. <https://doi.org/10.1016/j.plaphy.2020.04.035>
- Mozo, I., Rodríguez, M. E., Monteoliva, S., and Luquez, V. M. (2021). "Floodwater depth causes different physiological responses during post-flooding in willows," *Frontiers in Plant Science* 12, article 575090. <https://doi.org/10.3389/fpls.2021.575090>

- Nadarajah, K. K. (2020). "ROS homeostasis in abiotic stress tolerance in plants," *International Journal of Molecular Science* 21(5), article 5208. <https://doi.org/10.3390/ijms21155208>
- Nuanlaong, S., Wuthisuthimathavee, S., and Suraninpong, P. (2021). "Lysigenous aerenchyma formation: Responsiveness to waterlogging in oil palm roots," *Biologia Plantarum* 65, 167-176. <https://doi.org/10.32615/bp.2021.002>
- Panggabean, J., Kurnia, J., and Shaumul, T. (2025). "Sea level rise impacts on coastal oil palm plantations," *International Journal of Oil Palm* 8(1), 1-14. <https://doi.org/10.35876/ijop.v8i1.138>
- Park, J. S. and Lee, E. J. (2019). "Waterlogging induced oxidative stress and the mortality of the Antarctic plant, *Deschampsia antarctica*," *Journal of Ecology and Environment* 43, 29. <https://doi.org/10.1186/s41610-019-0127-2>
- Raju, A. D., Parihar, P., Singh, R., Kumar, J., and Prasad, S. M. (2020). "Synergistic action of indole acetic acid with homobrassinolide in easing the NaCl-induced toxicity in *Solanum melongena* L. seedlings," *Acta Physiologiae Plantarum* 42, article 68. <https://doi.org/10.1007/s11738-020-03054-8>
- Renziehausen, T., Frings, S., and Schmidt-Schippers, R. (2024). "Against all floods': Plant adaptation to flooding stress and combined abiotic stresses," *The Plant Journal* 117(6), 1836-1855. <https://doi.org/10.1111/tpj.16614>
- Rho, H., Kang, S. B., Lin, S. W., Lin, T. H., and Barchenger, D. W. (2025). "Physiological characteristics of flooding tolerance in wild and domesticated species of *Capsicum*," *HortScience* 60(10), 1785-1792. <https://doi.org/10.21273/HORTSCI18728-25>
- Rivera-Mendes, Y. D., Cuenca, J. C., and Romero, H. M. (2016). "Physiological responses of oil palm (*Elaeis guineensis* Jacq.) seedlings under different water soil conditions," *Agronomía Colombiana* 34(2), 163-171. <https://doi.org/10.15446/agron.colomb.v34n2.55568>
- Sghaier, H., Aloui, A., Khadri, A., Aschi-Smiti, S., and Brouquisse, R. (2020). "Effects of combined salt and flooding stresses on the growth and physiological behaviour of alfalfa (*Medicago sativa* L.)," *African Journal of Agricultural Research* 16(6), 758-764. <https://doi.org/10.5897/AJAR2020.14831>
- Singh, P., Choudhary, K. K., Chaudhary, N., Gupta, S., Sahu, M., Tejaswini, B., and Sarkar, S. (2022). "Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones," *Frontiers in Plant Science* 13, article 1006617. <https://doi.org/10.3389/fpls.2022.1006617>
- Siti-Dina, R. P., Er, A. C., and Cheah, W. Y. (2023). "Social issues and challenges among oil palm smallholder farmers in Malaysia: Systematic literature review," *Sustainability* 15(4), article 3123. <https://doi.org/10.3390/su15043123>
- Suraninpong, P., Thongkhao, K., Azzeme, A. M., and Suksa-Ard, P. (2023). "Monitoring drought tolerance in oil palm: Choline monooxygenase as a novel molecular marker," *Plants* 12(17), article 3089. <https://doi.org/10.3390/plants12173089>
- Tahjib-Ul-Arif, M., Hasan, M. T., Rahman, M. A., Nuruzzaman, M., Rahman, A. S., Hasanuzzaman, M., Haque, M. R., Hossain, M. A., Latef, A. A. H. A., Muarat, Y., *et al.* (2023). "Plant response to combined salinity and waterlogging stress: Current research progress and future prospects," *Plant Stress* 7, article 100137. <https://doi.org/10.1016/j.stress.2023.10>
- Urmi, T. A., Islam, M. M., Zumur, K. N., Abedin, M. A., Haque, M. M., Siddiqui, M. H., Murata, Y., and Hoque, M. A. (2023). "Combined effect of salicylic acid and proline

- mitigates drought stress in rice (*Oryza sativa* L.) through the modulation of physiological attributes and antioxidant enzymes,” *Antioxidants* 12(7), article 1438. <https://doi.org/10.3390/antiox12071438>
- Verslues, P. E., Bailey-Serres, J., Brodersen, C., Buckley, T. N., Conti, L., Christmann, A., Dinneny, J. R., Grill, E., Hayes, S., Heckman, R. W., *et al.* (2023). “Burning questions for a warming and changing world: 15 unknowns in plant abiotic stress,” *The Plant Cell* 35(1), 67-108. <https://doi.org/10.1093/plcell/koac263>
- Voora, V., Bermúdez, S., Farrell, J. J., Larrea, C., and Luna, E. (2023). “Palm oil prices and sustainability. IISD Market Report,” (<https://www.evidensia.eco/app/uploads/2023/07/2023-global-market-report-palm-oil.pdf>), Accessed 20 Jan 2024.
- Wang, J., Sun, H., Sheng, J., Jin, S., Zhou, F., Hu, Z., and Diao, Y. (2019). “Transcriptome, physiological and biochemical analysis of *Triarrhena sacchariflora* in response to flooding stress,” *BMC Genetics* 20, article 88. <https://doi.org/10.1186/s12863-019-0790-4>
- Wang, Q. L., Chen, J. H., He, N. Y., and Guo, F. Q. (2018). “Metabolic reprogramming in chloroplasts under heat stress in plants,” *International Journal of Molecular Sciences* 19(3), article 849. <https://doi.org/10.3390/ijms19030849>
- Xiong, R., Sang, L., Liu, R., Cheng, R., Li, P., Huang, L., and Cao, G. (2020). “Effects of waterlogging on maize seedling growth during seed germination,” *IOP Conference Series: Earth Environment Science* 598(1), article 012075. <https://doi.org/10.1088/1755-1315/598/1/012075>
- Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., and Chen, S. (2021). “Response mechanism of plants to drought stress,” *Horticulturae* 7(3), article 50. <https://doi.org/10.3390/horticulturae7030050>
- Yin, X., Zhang, C., Song, X., and Jiang, Y. (2017). “Interactive short-term effects of waterlogging and salinity stress on growth and carbohydrate, lipid peroxidation, and nutrients in two perennial ryegrass cultivars,” *Journal of the American Society for Horticultural Science* 142(2), 110-118. <https://doi.org/10.21273/JASHS04023-17>
- Zeng, F., Shabala, L., Zhou, M., Zhang, G., and Shabala, S. (2013). “Barley responses to combined waterlogging and salinity stress: separating effects of oxygen deprivation and elemental toxicity,” *Frontiers in Plant Science* 4, article 313. <https://doi.org/10.3389/fpls.2013.00313>

Article submitted: June 14, 2025; Peer review completed: January 8, 2026; Revised version received: February 28, 2026; Accepted: March 8, 2026; Published: May 15, 2026. DOI: 10.15376/biores.21.3.5931-5953