

**STRESS PROTEOMICS ON THE APPLICATION OF DEFENSE
HORMONES IN *SWIETENIA MACROPHYLLA* KING SEEDLINGS**

By

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled “**STRESS PROTEOMICS ON THE APPLICATION OF DEFENSE HORMONES IN *SWIETENIA MACROPHYLLA* KING SEEDLINGS**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, for any other University or Society.

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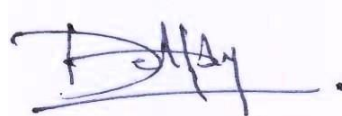
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LIST OF ABBREVIATIONS

%	Percentage
μmol	Micromole
ANOVA	Analysis of variance
Chl	Chlorophyll
cm	Centimeter
E	Transpiration rate
g	Gram
Gs	Stomatal conductance
HCA	Hierarchical cluster analysis
HSP	Heat shock proteins
JA	Jasmonic acid
LT	Leaf temperature
m	Meter
MALDI TOF	Matrix-assisted laser desorption ionization - Time of flight
mM	Millimolar
MS	Mass spectrometry
PCA	Principal component analysis
PMF	Peptide mass fingerprinting
Pn	Photosynthetic rate

RL	Root length
RSR	Root-to-shoot ratio
RWC	Relative water content
SA	Salicylic acid
SDS PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
SQ	Sturdiness quotient
SQI	Seedling quality index
TCA	Trichloroacetic acid
TEMED	Tetramethylethylenediamine

1. INTRODUCTION

In the context of climate change, the increasing frequency and intensity of abiotic stresses such as drought, heat, cold, salinity, radiation, and pollution have posed significant threats to plant health, productivity, and ecological stability, particularly in tropical forestry species (Eckardt *et al.*, 2022). Trees, however, have shown the ability to adapt to a broad range of climatic events by altering their intrinsic physiological and biochemical pathways. Understanding these responses is crucial for tree improvement and breeding efforts to enhance resilience to changing environmental conditions (Tanaka *et al.*, 2023).

Both biotic and abiotic stresses exert detrimental effects on tree growth and productivity, hindering the successful establishment of tree seedlings and imposing substantial losses in overall growth and yield, necessitating protective mechanisms (Saharan *et al.*, 2022). For instance, during drought and salt stress, plants employ a multifaceted strategy encompassing stomatal closure, leaf area reduction, accumulation of osmoregulatory molecules, and alterations in cell wall elasticity. These changes help preserve cell turgor, maintain ion balance, and regulate metabolic processes (Zhang *et al.*, 2023; Muhammad *et al.*, 2023). The duration and intensity of stress significantly influence plant growth and development. In response, plants deploy inherent defense mechanisms such as modulation of defense hormones, accumulating secondary metabolites, increased enzymatic activity, and altered protein expression to avert potential damage (Rehman *et al.*, 2023).

The application of defense hormones, Salicylic acid (SA) and Jasmonic acid (JA), has emerged promising strategies to modulate plant stress responses. These defense hormones are pivotal in activating defense pathways and orchestrating stress signalling networks, enabling plants to acclimate to unfavourable environmental conditions (Khan *et al.*, 2015).

Swietenia macrophylla King, commonly referred to as Indian Mahogany, is one of the most commercially important timber tree species in neo-tropical forests, belonging to the family Meliaceae. Renowned for its rapid growth rate, mahogany has gained significant popularity as a cultivated plantation tree. Its suitability for large-scale timber

production is attributed to its excellent timber quality, distinctive reddish wood color, exceptional workability, and high durability (Anoop *et al.*, 2014). However, climate change scenarios, characterized by frequent drought, elevated temperatures, and salinity stress, present formidable challenges that can result in plantation failures and impede productivity.

Plant stress proteomics, a dynamic discipline, focuses on examining the plant proteome and the biological functions of proteins in response to stress. The role of proteins in plant stress responses is critical, as they directly influence the development of novel phenotypes by modulating physiological traits to adapt to changing environmental conditions (Ahmad *et al.*, 2016). Understanding these intrinsic pathways is crucial for developing strategies to enhance tree stress tolerance.

Research focusing on the physiological, biochemical, and molecular mechanisms of forest trees, particularly concerning protein expression, cellular homeostasis, and metabolic processes, remains critical for advancing tree breeding initiatives (Dey *et al.*, 2022). Gaining deeper insights into these mechanisms will facilitate the development of more resilient and productive tree species, supporting sustainable forestry practices and adaptation to climate change. In this context, the present study aims to achieve the following objectives:

1. Evaluating plant response to defense hormones in *Swietenia macrophylla* seedlings.
2. Understanding the stress proteomics on the application of defense hormones.

2. REVIEW OF LITERATURE

Climate change induces abiotic stresses such as heat, drought, and salinity, which negatively impact plant physiological processes. These stresses result in increased tree mortality and reduced tree growth hindering the ability of forests to regenerate (Kanitkar *et al.*, 2024). Understanding the mechanisms behind stress tolerance and the interactions between defense hormones in regulating plant responses to abiotic stresses is essential for addressing issues related to seedlings growth (Bari *et al.*, 2008). The hormone-mediated stress responses play crucial role in plant adaptation to drought, salinity, temperature fluctuations, and heavy metal contamination, with significant implications for crop yield (Ahmad *et al.*, 2016).

Stress proteomics is a dynamic research area focusing on the the plant proteome and the biological functions of proteins in plants, exposed to various stress conditions (Kosova *et al.*, 2018). Proteomic analyses have provided insights into how these stress responses are mediated at the protein level, revealing complex networks of signalling pathways (Liu *et al.*, 2019). Plant defense hormones such as Salicylic Acid (SA), Jasmonic Acid (JA), and Ethylene (ET), brassinosteroids play crucial roles in regulating developmental processes and defense responses to both biotic and abiotic stresses (Rasool, 2022).

2.1 *SWIETENIA MACROPHYLLA*- SPECIES DESCRIPTION

Indian Mahogany (*Swietenia macrophylla* King) is one of the most commercially valuable timber tree species in neo-tropical forests, belonging to the Meliaceae family. Its natural distribution spans from Mexico to Bolivia and central Brazil (Lamb, 1966). Due to its rapid growth, high-quality timber mahogany has become a popular plantation tree, ideal for large-scale production (Anoop *et al.*, 2014). In India, it is cultivated in Tamil Nadu and Kerala and is appreciated for its good wood quality, reddish colour, workability, and durability (Durai *et al.*, 2017).

2.1.1 Botany of mahogany

Mahogany is a large deciduous tree with an umbrella-shaped crown, reaching heights over 30m and diameters at breast height (DBH) exceeding 1.5m. Historically, it could reach 40m-60m in height and 2.5m-3.5m in diameter before extensive logging

(Lamb, 1966). The trunk is straight, cylindrical, slightly grooved, and has well-developed spurs. Young trees have narrow crowns, while older trees have broad, dense, and heavily branched crowns. The rounded crown features broad, ascending branches and lush foliage. The bark of older trees is scaly, hairy, heavily wrinkled longitudinally, and brownish-grey to reddish-brown, with the inner bark being red-brown to pinkish-red. The leaves are typically paripinnate but can also be imparipinnate, measuring 12cm-45cm in length with 3-6 pairs of lanceolate or ovate leaflets, each 5cm-12cm long and 2cm-5cm wide (Soerianegara and Lemmens, 1993; Schmidt *et al.*, 2000).

The unisexual flowers, 0.5cm-1.0 cm long, are produced in large, branched inflorescences containing both male and female flowers. The capsular fruits are oblong or ovoid, 11.6 cm-38.7 cm long and 6.7 cm-12.0 cm wide, light grey to brown, with 4-5 valves, each containing 22-71 mature seeds. The seeds are samaroid, thick at the base, 7cm -12cm long, and 2 cm-2.5 cm wide, including the wings (Soerianegara and Lemmens, 1993; Schmidt *et al.*, 2000).

2.1.2 Distribution of species- Mahogany

Mahogany occurs naturally in Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru, and Venezuela. Its survival is in peril in Ecuador, Colombia, Panama, and Costa Rica, with Bolivia tackling commercial extinction. Mexico, Belize, and Brazil have also seen significant drops, with Guatemala, Peru, Nicaragua, and Honduras witnessing severe population reductions (Lugo *et al.*, 1999). Extensive planting initiatives have been implemented in Southern Asia and the Pacific, including India, Indonesia, the Philippines, and Sri Lanka, as well as in West Africa (Soerianegara and Lemmens, 1993).

In India, mahogany was first introduced as an ornamental tree in the Botanical Garden of Kolkata in 1795. It is cultivated as a timber species in Kerala, Tamil Nadu, Karnataka, Maharashtra, and West Bengal. Kerala has the greatest mahogany plantation area in India. Because of its high demand and rapid development, it has recently gained appeal among farmers and forest departments, resulting in extensive cultivation throughout Tamil Nadu and Kerala (Durai *et al.*, 2017).

2.1.3 Ecological range of mahogany

Mahogany is remarkably adaptable, thriving in a wide range of soil and environmental conditions, including alluvial soils, volcanic terrains, heavy clays, and lateritic substrates, as well as soils derived from limestone, granite, sedimentary, igneous, or metamorphic rocks (Whitmore *et al.*, 1992). It is a pioneer species in America's tropical settings, restoring degraded agricultural fields (Mayhew and Newton, 1998). Notably, the Philippines is highly resistant to cyclones. In Java, these trees grow well even in low soils, but they thrive in deep, fertile, well-drained soils with a pH of 6.5-7.5. Mahogany grows in wet and dry forest habitats, favoring yearly rainfall between 1000 mm and 2500 mm and dry intervals lasting up to four months (Lamb, 1966).

Mahogany likes ample light and is susceptible to frost but is resistant to fire. It thrives in well-drained soils, avoids waterlogging, and has moderate coppicing habits. When grown in black soil, it shows increased tree height, girth at breast height, and total tree volume per hectare (Akhilraj *et al.*, 2023). This species thrives in tropical dry forests with annual precipitation ranging from 1000 mm to 2000 mm, an average annual temperature of roughly 24 °C, and a potential evapotranspiration ratio of 1 to 2 (Lamb, 1966). Mahogany flourishes in India at elevations ranging from sea level to 1500 meters, with mean annual temperatures ranging from 20 °C to 28 °C (Akhilraj *et al.*, 2023).

2.1.4 Wood characteristic of mahogany

Mahogany is a relatively soft, medium-weight timber with pleasing properties. The heartwood has reddish or pinkish tints that intensify to a rich crimson or brown as it ages, but the sapwood is typically yellowish. It is valued for its attractive look, convenience of use with hand tools, superior finishing qualities, and dimensional stability (Krisnawati *et al.*, 2011). Its ability to polish well without cracking or warping makes it an excellent choice for high-quality furniture, mainly because of its coloration and versatility.

The wood density ranges from 485 kg/m³ to 850 kg/m³ at 15% moisture content, with interlocking or straight grain and a fine to moderately coarse texture (Soerianegara and Lemmens, 1993). Its surface has a glossy sheen, typically emphasized by the irregular grain, resulting in aesthetically pleasing figure patterns.

2.1.5 Economic uses of mahogany:

Mahogany is highly prized for its many uses across a range of industries. It is highly valued in furniture and cabinetry due to its lovely appearance, convenience, and superior finishing qualities. The wood is perfect for creating fine furniture and cabinetry since it polishes well and resists warping and cracking (Krisnawati *et al.*, 2011). It is also appropriate for producing veneer and plywood due to its delicate texture and interlocked grain. Mahogany products are highly valued due to their durability and aesthetic appeal. They are frequently utilized in high-end building and interior decorating (Soerianegara and Lemmens, 1993).

Furthermore, due to its resilience, workability, and sonorous properties, mahogany is preferred for musical instruments like pianos and guitars. Instrument makers value its capacity to generate a warm, rich tone (Lamb, 1966). Wood is also a popular material for building boats because of its strength and capacity to withstand rot. It can create upscale and small vessels and combine aesthetic and practical qualities (Mayhew *et al.*, 1998).

Mahogany is perfect for complex woodwork, such as beautiful carvings and turned objects like bowls and vases, because of its smooth finish and ease of carving and turning (Soerianegara and Lemmens, 1993). Despite being less prevalent because of its high value in other applications, it is occasionally utilized in buildings for flooring, panelling, and structural elements, particularly in high-end applications where appearance and durability are critical (Krisnawati *et al.*, 2011).

2.2 ROLE OF DEFENSE HORMONES IN ALLEVIATING THE ABIOTIC STRESS

Like animals, plants possess intricate defense mechanisms to combat various stressors, including pathogens, herbivores, and environmental challenges. Key to these defense mechanisms is plant defense hormones, which orchestrate a myriad of physiological and biochemical responses to ensure plant survival and fitness. Understanding these hormones is particularly critical in forestry, where maintaining the health and resilience of forest ecosystems is paramount. Plant defense hormones are synthesized during different stress conditions, allowing plants to tailor their defense response accordingly (Rasool, 2022).

2.2.1 Role of SA in plant defense mechanisms

Salicylic acid is a phenolic compound historically recognized for its medicinal properties. The active ingredient was first purified from willow bark in the early 19th century, with Johann Buchner, Henri Leroux, and Raffaele Piria isolating salicin, a precursor to SA (Klessig *et al.*, 2018). Its role in plant defense mechanisms became increasingly evident in the 20th century. In 1961, Frank Ross's experiments with tobacco plants revealed the phenomenon of Systemic Acquired Resistance (SAR), showing that plants develop heightened resistance to reinfection after an initial pathogen exposure (Khan *et al.*, 2015). By the 1990s, studies firmly established SA's critical function in initiating and propagating SAR, as elevated levels of SA were detected in both local and systemic tissues following pathogen infection (Klessig *et al.*, 2018).

SA is crucial for the plant immune response, particularly against biotrophic pathogens that rely on living host tissue. Upon pathogen detection, SA accumulates, and triggers localized cell death, known as the hypersensitive response, to limit pathogen spread (An *et al.*, 2011; Klessig *et al.*, 2018). Additionally, SA is crucial in establishing Systemic Acquired Resistance (SAR), a durable defense mechanism that protects uninfected tissues from future attacks (Klessig *et al.*, 2018).

SA is synthesized *via* two pathways, the phenylpropanoid pathway in the cytoplasm, starting from phenylalanine, and the isochorismate pathway in the chloroplast. Most SA in plants is either glucosylated or methylated, forming derivatives like salicylic acid 2-O- β -D-glucoside (SAG) for storage and methyl salicylate (MeSA) for signaling (Rivas-San Vicente & Plasencia, 2011). SA mediates its signalling functions by regulating the expression of pathogenesis-related (PR) proteins, critical plant defense markers (An *et al.*, 2011; Klessig *et al.*, 2018). These proteins are produced both in infected tissues and systemically, reflecting the broad defensive response initiated by SA. Additionally, SA influences the plant's redox state by interacting with redox-sensitive proteins and adjusting cellular oxidative status to enhance defense signalling (Spoel *et al.*, 2024).

SA perception and signalling involve a complex network of genes and proteins, with NPR1 (Non-expression of Pathogenesis-Related Genes 1) being a key regulator (Backer *et al.*, 2019). When SA accumulates, NPR1 is activated and translocate to the nucleus to modulate PR gene expression. Understanding these pathways is essential for

enhancing disease resistance in forest trees. SA is also recognized for improving abiotic stress tolerance in plants by enhancing the antioxidant defense system and regulating physiological responses. It acts as a signalling molecule, triggering reactive oxygen species (ROS) production, influencing stress-responsive gene expression, and interacting with other hormones and proteins involved in stress tolerance (Yang *et al.*, 2023). SA application boosts antioxidant enzyme activities, helping scavenge ROS and reducing oxidative damage. Additionally, it activates protein kinases that phosphorylate transcription factors involved in stress responses, emphasizing its multifaceted role in stress tolerance (Yang *et al.*, 2023).

SA affects plant functions in a dose-dependent manner, with low concentrations promoting growth and higher concentrations inhibiting it. For instance, foliar application of 50 μ M SA promoted growth in *Matricaria chamomilla*, while 250 μ M inhibited it. Similarly, in *Vigna radiata*, 0.1 mM and 0.5 mM SA enhanced photosynthesis and growth, but 1.0 mM SA caused growth inhibition (Yang *et al.*, 2023). SA has been shown to alleviate various abiotic stresses in different plant species. SA improved growth and photosynthesis in *Oryza sativa* (rice) under lead (Pb) stress, *Zea mays* (maize) under cadmium (Cd) stress, and *Phaseolus vulgaris* (common bean) under copper (Cu) stress (Khan *et al.*, 2015). In *Triticum aestivum*, SA enhanced salt tolerance by boosting antioxidant enzyme activity and regulating stress-responsive gene expression.

SA also strengthens salinity and osmotic stress tolerance by increasing antioxidant enzyme activity, which reduces oxidative damage caused by high salt levels. It improves membrane potential and prevents salt-induced potassium loss, promoting better growth and stress resilience under saline conditions (Khan *et al.*, 2015).

2.2.2 Role of JA in plant defense mechanisms:

Jasmonic acid was later identified as a critical component in plant defense, isolated initially from jasmine. This sesquiterpene lactone plays diverse roles in plant growth, development, and stress responses. Unlike SA, which primarily defends against biotrophic pathogens, JA is crucial for resistance to necrotrophic pathogens and herbivorous insects, making jasmonates essential in plant defense strategies (Lace and Prandi, 2016).

JA and its derivatives defend plants against necrotrophic pathogens and herbivores. JA biosynthesis is triggered by various biotic and abiotic stresses, activating defense genes that bolster the plant's resistance. JA signaling involves the SCF (Skp1-Cullin-F-box) complex, where the ubiquitin-proteasome pathway degrades JAZ repressors, allowing MYC transcription factors to induce JA-responsive gene expression (Rehman *et al.*, 2023; Carvalhais *et al.*, 2013).

Environmental stressors trigger JA biosynthesis. The lipid precursor α -linolenic acid is converted through the octadecanoid pathway, forming 12-oxo-phytodienoic acid, which is then reduced and β -oxidized to produce JA (Li *et al.*, 2022). The active form, jasmonate-L-isoleucine (JA-Ile), binds to the SCF(COI1) complex, leading to the ubiquitination and degradation of JAZ repressors, allowing MYC transcription factors to activate stress-responsive genes (Li *et al.*, 2022).

JA also produces secondary metabolites and proteins, such as proteinase inhibitors, which reduce herbivores digestive efficiency (Dabrowska and Anna, 2009). Additionally, JA signaling interacts with other hormone pathways, including SA, to fine-tune plant defenses, leading to either antagonistic or synergistic effects depending on the stress type (Monte, 2023; Wang *et al.*, 2021).

The concentration of JA significantly affects plant responses, with different levels producing varying effects on growth and defense mechanisms. The optimal JA concentration for enhancing growth and stress tolerance is species-specific and depends on the type and intensity of the stress. Research shows that JA levels increase in plant tissues in response to abiotic stresses by activating JA biosynthetic genes, underscoring its role as a vital hormone in stress responses. However, excessively high JA concentrations can hinder growth by suppressing essential physiological processes (Song *et al.*, 2021). JA enhances drought tolerance by regulating stomatal closure, reducing water loss, and activating drought-responsive genes.

Elevated JA levels boost antioxidant enzyme activity, helping to mitigate oxidative damage from drought-induced reactive oxygen species (ROS) (Ali and Baek, 2020). Additionally, JA improves salinity tolerance by enhancing antioxidant defenses, maintaining ion homeostasis, and regulating osmoprotectant synthesis (Aslam *et al.*, 2021). It contributes to membrane stability, reduces Na⁺ uptake, and promotes K⁺

retention, alleviating salt-induced cytotoxicity. Proper JA concentrations can mitigate salt stress effects more effectively than without JA application (Siddiqi *et al.*, 2019).

In rice, JA treatment enhances drought and salinity tolerance by modulating stomatal conductance and increasing antioxidant enzyme activities. Similar effects were observed in *Arabidopsis thaliana*, where JA application improved tolerance to cold and salinity stress by activating stress-responsive pathways (Rehman *et al.*, 2023). Overall, JA boosts the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which are essential for scavenging reactive oxygen species (ROS) generated under stress (Wang *et al.*, 2020).

2.2.3 Hormonal crosstalk in plant defense responses:

Hormonal crosstalk refers to the interaction between various hormonal signaling pathways in plants, essential for regulating defense responses against pathogens and herbivores. Key hormones involved in these defense mechanisms include JA, SA, ethylene (ET), and abscisic acid (ABA) (Vos *et al.*, 2015). These hormones coordinate complex signalling networks that allow plants to fine-tune their defense strategies based on the type of stress encountered.

The SA and JA signalling pathways often interact antagonistically, activation of one can suppress the other (Vos *et al.*, 2015). This antagonism is a cost-saving strategy, enabling plants to allocate resources efficiently by prioritizing the most effective defense response depending on the attacker. For instance, research on *Arabidopsis thaliana* has demonstrated that inducing SA-dependent defenses can suppress JA-dependent defenses, increasing susceptibility to necrotrophic pathogens when SA pathways are activated. Conversely, activating JA responses can inhibit SA signalling, impacting resistance against biotrophic pathogens (Vos *et al.*, 2015).

Ethylene is a critical hormone in plant defense that often works synergistically with JA to enhance resistance against necrotrophic pathogens and herbivores (Vos *et al.*, 2015). ET stabilizes and activates transcription factors like ERF1, improving necrotrophic pathogen resistance. However, it can also interact antagonistically with salicylic acid (SA), adding complexity to the plant immune network (Vos *et al.*, 2015).

Abscisic acid, primarily known for its role in abiotic stress responses, also significantly impacts biotic stress. It can interact antagonistically with SA and JA

pathways, often suppressing immune responses to prioritize abiotic stress tolerance. For example, ABA signaling can inhibit the SA pathway, reducing resistance to biotrophic pathogens. Additionally, ABA enhances defenses against herbivores by synergizing with JA to promote the MYC branch of JA signalling (Vos *et al.*, 2015).

2.3 ABIOTIC STRESS AND DEFENSE HORMONE-MEDIATED RESPONSE IN PLANTS

Abiotic stress pertains to the deleterious effects of non-living environmental factors on living organisms within a specific ecosystem (Ghosh, S and Majee, M, 2023). These adverse conditions profoundly influence global plant growth, development, and productivity (Mittler, 2002). Various abiotic stressors significantly impact plant development and agricultural yield, including drought, elevated temperatures, salinity, air and soil pollution, and mineral deficiencies (Khokhar *et al.*, 2024).

The primary abiotic stressors impacting plants encompass drought, thermal extremes, salinity, cold stress, heat stress, and heavy metal toxicity (Praveen *et al.*, 2023). These stressors can be systematically categorized into physical and chemical stresses. Physical stresses encompass hydric challenges (drought and flooding), temperature extremes (both high and low), light variations (in terms of quantity and quality), and mechanical disturbances (such as ice, snow, and wind). Conversely, chemical stresses encompass nutrient imbalances (excessive or deficient), salinity, soil characteristics (including pH and composition), air pollution (including CO₂, O₂, NO₂, and SO₂), and the application of pesticides (including herbicides, insecticides, and fungicides) (Khokhar *et al.*, 2024).

2.3.1 Methods of inducing abiotic stress in plants:

Inducing abiotic stress in controlled environments, particularly drought and salinity, is imperative for elucidating plant responses and enhancing stress resilience. One of the most straight-forward and widely employed methods for inducing drought stress involves withholding water from the soil, effectively mimicking natural drought conditions (Krizek, 1985). This approach closely approximates the gradual water loss observed in authentic drought scenarios, although its reproducibility may be influenced by various factors, including soil composition and prevailing environmental conditions (Wang *et al.*, 2003).

Another prevalent technique employs chemical agents such as Polyethylene Glycol (PEG) and mannitol to induce osmotic stress, lowering water potential and simulating drought conditions. For instance, PEG 6000 is frequently used in experimental settings due to its substantial molecular size, which restricts its diffusion through cell walls, thus allowing precise control of water potential and reflecting plant conditions under specified osmotic potentials (Wang *et al.*, 2003).

To induce salinity stress, specific salts such as NaCl, CaCl₂, MgCl₂, and K₂SO₄ are introduced into the growing medium, facilitating the creation of varying salinity levels to study their impact on physiological and biochemical responses in plants. By adjusting the concentrations of these salts, researchers can replicate different severities of natural salinity stress (Atta *et al.*, 2023; Ma *et al.*, 2020).

Recent innovations also encompass the hydroponic drought method and the low-water agar assay. The hydroponic system induces drought stress by decreasing water levels in nutrient solutions, thereby subjecting plant roots to controlled water deficit conditions (Wang *et al.*, 2024). Conversely, the low-water agar assay modifies water content within agar media, creating a more challenging substrate for water absorption and closely simulating real-world drought responses at the molecular level (Wang *et al.*, 2024).

2.3.2 Physiological and biochemical responses of plants to abiotic stress:

Plants exhibit multifaceted responses to abiotic stresses through intricate mechanisms integrated at multiple levels: whole plant, cellular, and molecular. These responses encompass alterations in gene expression, modifications in cellular metabolism, and changes in growth rates and crop yields (Dey *et al.*, 2022). For example, under osmotic conditions such as high salinity and drought, plants initiate immediate responses, including stomatal closure, to conserve water while also enacting longer-term adaptations by regulating stress-responsive genes (Verma *et al.*, 2016).

Physiologically, abiotic stress triggers a cascade of morphological, physiological, biochemical, and molecular changes that detrimentally impact plant growth and productivity (Mittler, 2002). Common physiological adaptations include reduced leaf size and modified root architecture to enhance water uptake, stomatal closure to minimize water loss, and decreased photosynthetic rates due to restricted CO₂ entry. Additionally,

abiotic stress can affect leaf photochemistry and mineral nutrition and activate antioxidant systems to counteract oxidative stress (Dey *et al.*, 2022).

Plants deploy various adaptive strategies at the biochemical level to cope with abiotic stresses. These include the accumulation of osmolytes such as proline and glycine betaine to maintain cellular osmotic balance, increased production of reactive oxygen species (ROS), and activating antioxidant enzymes to mitigate oxidative damage. Furthermore, salinity-tolerant plants maintain a favorable K^+/Na^+ ratio and sequester Na^+ into vacuoles to manage ionic stress effectively (Ben and Lavi, 2012; Khokhar *et al.*, 2024).

2.3.3 Synergistic and antagonistic effects of SA and JA during stress conditions:

SA and JA are pivotal phytohormones that orchestrate plant responses to abiotic stresses. They exhibit synergistic and antagonistic interactions, essential for modulating plant defense mechanisms. The dynamics between SA and JA can profoundly influence plant responses to stress, with SA generally associated with resistance to biotrophic pathogens, while JA is predominantly involved in responses to necrotrophic pathogens and herbivorous threats (Myers *et al.*, 2022; Sultana *et al.*, 2024; Mishra *et al.*, 2024).

The synergistic interactions of SA and JA can enhance stress tolerance by activating overlapping and specific signalling pathways. For instance, co-application of these hormones under certain conditions can upregulate a shared set of genes, thereby providing a more robust defense response (Wang *et al.*, 2020). Research has demonstrated that JA and SA can regulate systemic signalling and may coordinate the balance between plant growth and defense, allowing for reallocating resources as needed (Myers *et al.*, 2022; Sultana *et al.*, 2024).

Conversely, these hormones can exhibit antagonistic effects. For example, JA can suppress SA accumulation by modulating specific transcription factors such as NAC TFs, MAPK, and MYC2, which are crucial for stress signal crosstalk (Mishra *et al.*, 2024; Yang *et al.*, 2019). This intricate balance between SA and JA signalling enables plants to prioritize their responses to multiple stresses effectively, optimizing their survival and growth under adverse conditions (Yang *et al.*, 2019).

2.4 PROTEOMIC ANALYSIS OF PLANT STRESS RESPONSES

Proteomics represents a dynamic and potent approach for investigating the alterations in protein expression in plants subjected to stress, offering critical insights into their responses to various abiotic and biotic stressors (Shoaib *et al.*, 2024). This technique facilitates functional genomic analysis, enabling researchers to comprehend how plants adapt and counteract the adverse impacts of stressors by identifying and quantifying protein expression levels in stressful conditions (Roveda *et al.*, 2011; Ahmad *et al.*, 2016).

Recent advancements in proteomics have significantly enhanced our genetic and molecular understanding of plant responses to abiotic stress, promoting the identification of specific proteins and pathways instrumental in conferring stress tolerance (Ahmad *et al.*, 2016). By elucidating the proteomic landscape, scientists can uncover the intricate mechanisms underlying plant resilience, ultimately contributing to developing strategies to improve stress tolerance in crops and enhance overall agricultural sustainability.

2.4.1 Techniques for proteomic analysis:

Proteomic methodologies employed in investigating plant stress responses are diverse and sophisticated, concentrating on a comprehensive analysis of proteins to elucidate how plants react to various stress conditions, including both biotic and abiotic stresses (Liu *et al.*, 2019). Key techniques encompass mass spectrometry, gel electrophoresis, and advanced chromatographic methods. Among these, liquid chromatography coupled with mass spectrometry (LC-MS) is utilized for spatial proteomics and the analysis of post-translational modifications (PTMs), which are pivotal in mediating plant stress responses (Ruan *et al.*, 2008).

Gel-based techniques such as two-dimensional electrophoresis (2-DE), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and gel-free mass spectrometry-based quantitative proteomic approaches are commonly employed to dissect plant proteomes (Hu *et al.*, 2015). These methodologies facilitate an in-depth examination of protein extraction, separation, and analysis, enabling researchers to unravel the intricate protein networks present in plants (Skultety *et al.*, 2009). Recent technological advancements have also fostered the development of combined subcellular and organ-specific proteomics, enhancing our understanding of the cellular mechanisms that govern stress responses (Ahmad *et al.*, 2016).

Mass spectrometry (MS) is indispensable for proteomic analysis, providing high sensitivity and specificity for identifying and quantifying proteins and their modifications. LC-MS is particularly valuable for separating and identifying peptides from complex mixtures, establishing itself as a cornerstone technique for spatial and PTM proteomics (Liu *et al.*, 2019). Matrix-assisted laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) facilitates rapid and high-throughput protein identification, delivering accurate mass measurements and proving advantageous for analyzing larger biomolecules (Patel *et al.*, 2015). The Orbitrap mass spectrometer, especially models like the Q-Exactive and Fusion Tribrid, offers high resolution and mass accuracy, which are critical for identifying low-abundance proteins and conducting detailed proteomic studies. Additionally, Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) is emerging as a prominent method due to its enhanced dynamic range and throughput, which significantly improve quantitation precision and facilitate the detection of various protein conformations (Perchepped *et al.*, 2023).

2.4.2 Proteomic insights into defense hormone-induced stress responses:

Research focusing on proteomic responses to defense hormone treatments, specifically JA and SA, has yielded significant insights into the types and abundances of proteins involved in stress responses. The signaling pathways associated with JA and SA, integral to plant defense mechanisms, progress through various stages, including hormone biosynthesis, modification, and both early and late responses (Stroud *et al.*, 2022). These pathways encompass distinct genetic components and regulatory mechanisms that critically influence the types and quantities of proteins synthesized during stress responses.

In *Arabidopsis thaliana*, the SA defense network exhibits rapid and dynamic transcriptional reprogramming following hormone treatment, with notable alterations in protein expression levels observed within the first 0 to 12 hours post-treatment (hpt) (Stroud *et al.*, 2022). Similarly, JA treatment triggers a swift and sustained expression profile of stress-responsive proteins, extending the response duration to 24 hpt (Stroud *et al.*, 2022).

In rice proteomic analyses of SA-treated leaves have identified 36 differentially expressed proteins, indicating a significant reconfiguration of the rice proteome in

response to SA application (Li *et al.*, 2012). Subcellular proteomic investigations have revealed that SA affects protein localization and interactions within various cellular compartments. For instance, proteins involved in SA biosynthesis and signalling have accumulated in chloroplasts, underscoring the critical role of this organelle in SA-mediated defense responses (Li *et al.*, 2012; Zhou *et al.*, 2022).

JA treatment in rice similarly induces rapid and extended-expression profiles of stress-responsive proteins. A study investigating rice leaf blades treated with 100 μ M JA over two days found 12 upregulated proteins and two downregulated proteins (Li *et al.*, 2012). The differentially expressed proteins are primarily associated with defense mechanisms against necrotrophic pathogens, herbivores, and metabolic adjustments necessary to endure stress. In maize, both JA and methyl jasmonate (MeJA) play crucial roles in regulating plant development, stress resistance, and responses to insect attacks by inducing specific gene expressions (Zhang *et al.*, 2023). The proteins identified in this study serve vital biological functions, comprising photosynthesis and energy-related proteins (38.4%), proteins involved in folding, degradation, and regulation (15.0%), stress and defense-related proteins (11.7%), and redox-responsive proteins (8.3%) (Zhang *et al.*, 2023).

2.4.3 Subcellular localization of proteins and protein-protein interactions:

Proteomics, especially when integrated with advanced mass spectrometry techniques, has emerged as a crucial methodology for elucidating the subcellular localization of proteins during stress responses. Techniques such as Free Flow Electrophoresis (FFE), Flow Field-Flow Fractionation (FIFFF), and Laser Capture Microdissection (LCM) enable the precise separation of proteins into distinct subcellular components, facilitating accurate localization and functional analysis (Li *et al.*, 2012).

Numerous studies have demonstrated that treatments with defense hormones significantly affect protein localization. For instance, SA and JA modulate the spatial distribution of proteins associated with stress responses across various organelles. In particular, hormone-responsive proteins, including Receptor-Like Kinases (RLKs) and Receptor-Like Proteins (RLPs), are found to accumulate at the plasma membrane, underscoring their critical roles in signal perception and transduction during stress conditions (Li *et al.*, 2012).

In chloroplasts, proteins responsible for the biosynthesis of defense-related phytohormones, such as SA and JA, are localized within these organelles. Chloroplast-localized proteins are vital for mediating stress responses and coordinating the functions of nuclear-encoded plastid-localized proteins (Li *et al.*, 2012). Additionally, both mitochondria and chloroplasts are implicated in generating reactive oxygen species (ROS) that act as signalling molecules. Research has identified mitochondrial proteins that participate in Programmed Cell Death (PCD) and stress responses, further elucidating the interconnectedness of these organelles in stress signalling (Li *et al.*, 2012).

Protein-protein interaction assays further illuminate the complexity of hormone-induced signalling networks. For example, interactions between JA-responsive proteins and transcription factors such as JASMONATE INSENSITIVE 1/MYC2 (JIN1/MYC2) highlight these proteins' regulatory roles in activating downstream stress responses (Bar *et al.*, 2020). This intricate interplay of protein localization and interactions is essential for understanding the multifaceted mechanisms underlying plant stress responses.

2.4.4 Differential protein expression during stress conditions:

Proteomic studies have demonstrated that abiotic stresses significantly influence plant proteomes, leading to changes in protein relative abundance, cellular localization, and post-translational modifications (Kosová *et al.*, 2018). Protein expression profiling has played a crucial role in elucidating the molecular responses of plants to various abiotic stressors, such as drought, salinity, cold, and flooding. These studies offer valuable insights into the adaptive mechanism's plants employ to withstand environmental challenges (Kitavi *et al.*, 2023; Srivastava *et al.*, 2024).

Research has identified specific proteins differentially expressed in response to drought stress. The protein Glycine-rich RNA binding proteins (GR-RBPs) have shown differential expression during drought stress, suggesting their potential role in enhancing stress tolerance (Singh *et al.*, 2022). Additionally, genes encoding Late Embryogenesis Abundant (LEA) proteins and Heat Shock Proteins (HSPs) have been utilized to improve drought tolerance in transgenic plants (Shinozaki *et al.*, 2007). Studies across model and crop species consistently identify common differentially expressed genes related to

drought stress, indicating evolutionary conservation in drought response mechanisms (Devitt *et al.*, 2022).

In response to salinity stress, numerous proteins are differentially expressed, aiding plant adaptation. Proteins such as ascorbate peroxidase, chaperonins, and osmotin play significant roles in mitigating the adverse effects of high salinity (Dzinyela *et al.*, 2023). Furthermore, seagrasses accumulate compatible solutes like proline, carbohydrates, and LEA proteins to cope with salt stress (Athar *et al.*, 2022). Heat shock proteins (HSPs) are highly responsive to heat stress and play a critical role in protecting plants by facilitating proper protein folding and preventing aggregation (Timperio *et al.*, 2008). These proteins are universally induced across different plant species under heat stress conditions, emphasizing their importance in heat tolerance (Shinozaki *et al.*, 2022).

Transcription factors (TFs) are essential in regulating gene expression in response to abiotic stress, functioning as molecular switches that control the activation or repression of stress-responsive genes (Wani *et al.*, 2020). Six major TF families—ABF, DREB, bZIP, WRKY, MYB, and NAC—are primarily involved in abiotic stress tolerance. Specific transcription factors like DREB (Dehydration-Responsive Element Binding) TFs are crucial for drought and cold stress responses by regulating the expression of various stress-responsive genes (Shinozaki *et al.*, 2022). Other TFs, such as NAC, modulate responses to multiple stresses, including drought and salinity, by regulating downstream genes involved in stress adaptation (Nakashima *et al.*, 2012). The WRKY TF family responds to various abiotic stresses by regulating genes associated with pathogen defense, oxidative stress, and senescence (Wani *et al.*, 2020).

Functional annotation methods, including gene ontology enrichment analysis, are frequently employed to categorize genes based on their biological functions and identify pathways involved in the stress response (Warsi *et al.*, 2023). This method is essential for functional annotation, providing insights into the roles of differentially expressed proteins in stress adaptation (Haak *et al.*, 2017). Additionally, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis enhances the understanding of biochemical pathways activated or suppressed under stress conditions. This technique has been applied to validate findings from transcriptome analyses, revealing specific plant responses to abiotic stresses like drought and salinity (Jan *et al.*, 2022).

3. MATERIALS AND METHODS

The current study, "Stress proteomics on the application of defense hormones in *Swietenia macrophylla* King seedlings," is conducted to evaluate the response of Mahogany seedlings to defense hormones Salicylic Acid (SA) and Jasmonic Acid (JA), and evaluate their effectiveness under abiotic stress conditions. The research aimed to gain insights into stress-related proteomic changes triggered by the application of these hormones. This chapter provides a detailed account of the experimental setup, including the materials used and the methodologies employed in the investigation.

3.1 STUDY LOCATION:

The study is conducted at the College of Forestry, Kerala Agricultural University, Kerala, India (13°32'N, 76°26'E) from July 2023 to September 2024. Different experiments were conducted at the Department of Forest Biology and Tree Improvement, College of Forestry, Vellanikkara of Kerala Agricultural University. The location experiences a warm, humid climate with an average annual rainfall of about 2100mm to 2500mm. The average temperature ranges from 24.4°C to 42.8°C, with a relative humidity of 80 percent to 100 percent.

3.2 FOLIAR APPLICATION OF DEFENSE HORMONES TO MAHOGANY SEEDLINGS:

Six-month-old mahogany seedlings were treated with varying concentrations of the defense hormones SA and JA to evaluate their effects on growth and development. The objective is to determine the optimal concentration of each hormone that promotes enhanced growth in the seedlings.

3.2.1 Experimental material:

Uniform mahogany seedlings were obtained from the Tree and High-Tech nursery unit at the College of Forestry, Kerala Agricultural University, Thrissur. The experiment was conducted in the nursery shade house at the College of Forestry, Vellanikkara.

3.2.2 Experimental design:

The experiment followed a Completely Randomized Design (CRD) with six treatments of SA and JA at different concentrations (Table 01). Each treatment is

replicated three times with five plants per replication. Defense hormone solutions were applied to the leaves of mahogany seedlings using the foliar spray method. The sprays were administered twice, with a two-day interval between applications, between 9:00 AM and 11:00 AM to optimize absorption and reduce evaporation. A control group was used to establish a baseline for comparing the effects of the hormone treatments.

A 1 mM stock solution of JA was prepared by dissolving 0.04206 g of JA in ethanol and adjusting the volume to 200 mL with distilled water to achieve the desired concentration. Working solutions were subsequently prepared by diluting this stock solution. Similarly, a 1 mM stock solution of SA was prepared by dissolving 0.02762 g of SA in ethanol and bringing the volume to 200 mL with distilled water. This stock solution was also diluted with distilled water to obtain the required working concentrations for experimental use.

3.2.3 Observation

Morphological and physiological parameters of the seedlings are recorded five days after hormone application and subsequently at 30-day intervals over 150 days. During this time, the concentration of each hormone was evaluated and standardized based on the morphological and physiological observations.

3.2.3.1 Morphological parameters

The morphological parameters, including seedling height (cm), collar diameter (cm), number of leaves, leaf area (cm²), and root-to-shoot ratio, were measured and recorded.

3.2.3.1.1 Volume Increment (cm³):

The volume index is a parameter used to quantify the overall growth of each seedling by combining its height (h) and collar diameter (d). It was determined both at the initial stage, prior to hormone application, and after 150 days of observation, using the formula:

$$\text{Volume index} = d^2h$$

Table 01. Concentrations of defense hormones applied in the study:

Treatments	JA (mM)	SA (mM)
T1	Control	Control
T2	0.2	0.2
T3	0.4	0.4
T4	0.6	0.6
T5	0.8	0.8
T6	1.0	1.0

Table 02. Standardized defense hormone concentrations for drought and salinity stress conditions:

Stress	Treatment
Drought Stress	Control
	SA (0.8mM)
	JA (0.6mM)
Salinity stress	Control
	SA (0.8mM)
	JA (0.6mM)

Table 03. Samples for proteomic analysis under drought and salinity stress conditions

Stress	Samples
Salinity stress	Normal condition
	Salinity stress condition
	Recovery without treatment
	Recovery with SA
	Recovery with JA
Drought stress	Normal condition
	Drought Stress Condition
	Recovery without treatment
	Recovery with SA
	Recovery with JA

3.2.3.1.2 Sturdiness Quotient:

The sturdiness quotient is a key indicator of the resilience of seedlings to adverse climatic conditions. It reflects the balance between height and collar diameter, where a lower quotient indicates sturdier seedlings. The formula, was proposed by Ritchie *et al.*, 1984.

$$\text{Sturdiness Quotient} = \frac{\text{Shoot length (cm)}}{\text{Collar diameter (cm)}}$$

3.2.3.1.3 Seedling quality index:

The seedling quality index (SQI), introduced by Dickson *et al.* (1960), is a comprehensive measure of seedling vigour and overall health, incorporating morphological traits and biomass distribution. It was used to evaluate the quality of seedlings by considering height, collar diameter, and the ratio of shoot-to-root dry weights.

$$\text{Seedling Quality Index} = \frac{\text{Total seedling dry weight (g)}}{\left(\frac{\text{Plant height (cm)}}{\text{Collar diameter (cm)}} \right) + \left(\frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}} \right)}$$

3.2.3.2 Physiological parameters

Physiological parameters such as photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), and leaf temperature ($^{\circ}\text{C}$) were measured on the third leaf from the tip using an IRGA (LI6400 portable photosynthetic system). Total chlorophyll content ($\text{mg g}^{-1} \text{ FW}$) was estimated using the DMSO (Dimethyl Sulphoxide) method developed by Hiscox and Israelstam (1979). The relative water content (%) of the leaves was determined using Barrs (1968) method.

3.2.4 Statistical analysis:

3.2.4.1 Analysis of Variance:

The data collected on morphological and physiological factors were analyzed using analysis of variance (ANOVA) through the general linear model procedure in R software (version 4.3.1) to determine the significance of various parameters. Least Square Difference (LSD) test was performed to assess differences among treatment means, evaluating the effects of defense hormones (JA and SA) on growth enhancement.



Plate 1. Study location of the experiment

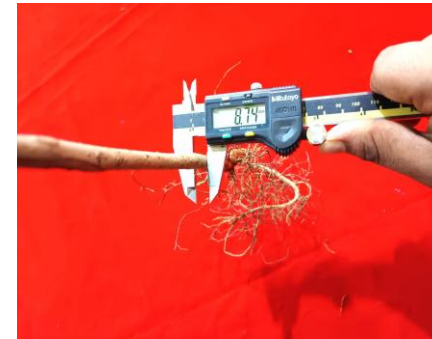


Plate 2. Measurements of morphological and physiological parameters

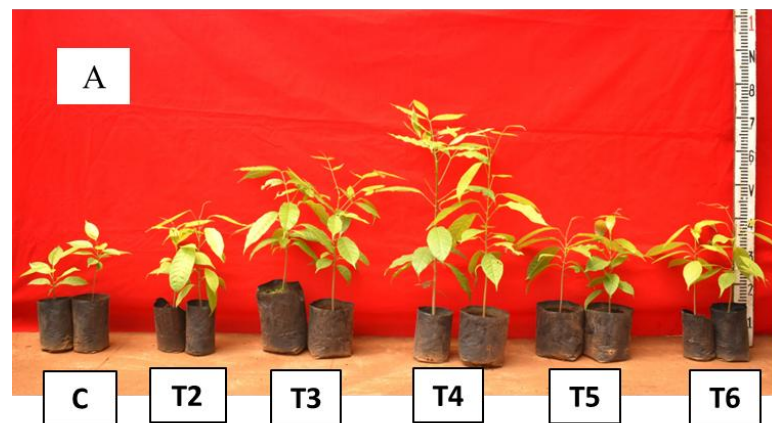


Plate 3. Height increment(cm) under different treatments of defense hormones; A. Height(cm) increase after 45 days of JA treatment, B. Height increase after 150 days of JA treatment, C. Height increase after 150 days of SA treatment

Each growth stage was analyzed independently using R software. Duncan's Multiple Range Test (DMRT) was then applied to compare the means and identify significant variations between treatments.

3.2.4.2 Cluster analysis

Using morphological and physiological parameters, different hormonal treatments undergo hierarchical cluster analysis employing Average Linkage (between groups) with Euclidean Distance as the measurement method. A dendrogram was created to illustrate the degree of similarity among the treatments, utilizing the 'agricolae' package. Additionally, the interclass distances were calculated and visually represented.

3.2.4.3 Principal component analysis of treatment of treatment response

The collected data on morphological and physiological traits were subjected to Principal Component Analysis (PCA) separately using R software. Principal components were computed and visualized using the 'prcomp' function in R version 4.3.1, along with the 'factoextra' and 'agricolae' packages. The selection index was constructed using 'factoextra,' with final scoring based on the principal components, achieving a cumulative variance of 0.75. An index model was applied to compare the different defense hormone treatments. A repeated PCA for the calculated physiological and morphological scores was used to construct the final index.

3.3 EFFECT OF DEFENSE HORMONES ON ALLEVIATING ABIOTIC STRESS IN MAHOGANY SEEDLINGS

The experiment examined the effects of standardized exogenous applications of the defense hormones JA and SA on mahogany seedlings exposed to abiotic stress conditions, specifically drought and salinity stress.

3.3.1 Experimental Material

Eight-month-old mahogany seedlings was collected from the forest nursery at the College of Forestry in Vellanikkara were utilised for the experiment.

3.3.2 Experimental design:

The experiment consists of six treatment groups, with two standardized concentrations of JA and SA applied for each stress type and a control group for each

condition without hormone application (Table 2). A CRD was used, with five replications per treatment and five seedlings per replication, totalling 25 seedlings per treatment group. Before stress screening, the seedlings were irrigated to field capacity, considered the "normal condition."

Moisture stress is imposed by withholding irrigation until the plants showed signs of wilting, marking this phase as the "drought condition." After wilting, the plants were treated with standardized concentrations of defense hormones (JA and SA), referred to as the "regain condition." Morphological, physiological, and biochemical parameters were monitored under all three water stress conditions, with the experiment repeated twice for confirmation.

Salinity stress was induced by applying a 100 mM sodium chloride (NaCl) solution to the root zone of the seedlings, raising the soil's electrical conductivity (EC) to around 4 dS/m. The seedlings were closely observed for stress indicators such as leaf browning, chlorosis, and stunted growth, confirming the onset of the "salinity stress condition." After successful stress induction, the seedlings were treated with standardized concentrations of defense hormones, initiating the "regain condition."

3.3.3 Observations

Morphological, physiological, and biochemical observations are taken throughout all the three conditions: normal, stress, and regain.

3.3.3.1 Morphological parameters

The number of leaves at all three conditions was recorded, and the percentage of leaves retained, along with the number of epicormic shoots that emerged during the recovery phase, was calculated using the following formula:

$$\text{Percentage of Leaves Retained (\%)} = \left(\frac{\text{Number of Leaves Retained}}{\text{Total Number of Leaves}} \right) \times 100$$

3.3.3.2 Physiological parameters

The physiological traits of the plants, such as photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration rate ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), leaf temperature ($^{\circ}\text{C}$), relative water content (%), chlorophyll content

(mg g⁻¹), and chlorophyll stability index (%), were recorded during all three conditions. The effects of defense hormones on these traits were then assessed.

3.3.3.2.1 Photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (E)

The photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and transpiration rate ($\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were measured for the third leaf from the tip using an IRGA (LI6400 portable photosynthetic system) between 9:00 AM and 11:00 AM providing a photosynthetic photon flux density of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The leaf chamber air humidity is maintained at 60% RH, $370\text{--}380 \mu\text{mol mol}^{-1} \text{ CO}_2$ concentration, $26\text{--}28^\circ\text{C}$ air temperature, and $500 \mu\text{mol s}^{-1}$ flow rate.

3.3.3.2.2 Leaf temperature (°C)

Leaf Temperature was measured for the third leaf from the tip using an IRGA (LI6400 portable photosynthetic system) between 9:00 AM and 11:00 AM, providing a photosynthetic photon flux density of $2,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

3.3.3.2.3 Relative water content (%)

The relative water content (RWC) of the leaves was determined following Barrs's method (1968). RWC measures the difference between the current water content of leaf tissue and its maximum capacity at full turgidity. Fresh leaf samples are collected and immediately taken to the laboratory, where they are cut into small discs with a one-centimeter diameter, and the fresh weight (FW) is recorded. The discs were soaked in distilled water for six hours to reach full saturation. Afterward, excess water was removed, and the turgid weight (TW) was recorded. The discs were wrapped in aluminium foil and oven-dried for 24 hours at $70\pm5^\circ\text{C}$. Once completely dried, the dry weight (DW) was recorded. The RWC was then calculated using the following formula:

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

3.3.3.2.4 Chlorophyll content (mg g⁻¹)

The photosynthetic pigments and chlorophyll content were estimated by Hiscox and Israelstam (1979), which involves DMSO (Dimethyl sulphoxide). Fresh leaf samples weighing 0.1 g (100 mg) were cut into small pieces and placed in a test tube containing 7 ml of DMSO. The samples were incubated in the dark for 24 hours. After incubation, the extract was collected and mixed with 3 ml of DMSO to achieve a final volume of 10 ml. The absorbance of the final extract was measured at 645 nm (A₆₄₅), 663 nm (A₆₆₃), and 652 nm (A₆₅₂) using a visible spectrophotometer (Thermo Orion AquaMate 7000 Vis). Chlorophyll a (mg g⁻¹), Chlorophyll b (mg g⁻¹), and total chlorophyll (mg g⁻¹) were calculated using the following formulas:

$$\text{Chlorophyll a (mg g}^{-1}\text{)} = [12.7 (A_{663}) - 2.69 (A_{645})] \times V / 1000 \times W$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)} = [22.9 (A_{645}) - 4.68 (A_{663})] \times V / 1000 \times W$$

$$\text{Carotenoid (mg g}^{-1}\text{)} = [7.6 (A_{480}) - 1.49 (A_{510})] \times V / 1000 \times W$$

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = [A_{652} \times 1000 \times V] / 34.5 \times 1000 \times W$$

where V = volume of the extract (mL) and W = weight of the fresh leaf (g).

3.3.3.2.5 Chlorophyll Stability Index (CSI) (%)

Fresh leaves were collected, and two replicates weighing 0.1 g (100 mg) were cut into pieces and placed in test tubes containing 20 ml of distilled water. One test-tube remains untreated as a control, while the other is incubated at 55°C for 30 minutes in a hot water bath as the treatment group. After cooling, the leaf residues were transferred to fresh tubes, and the distilled water was discarded. Then, 7 ml of dimethyl sulfoxide (DMSO) was added to the control and treatment tubes and left at room temperature overnight. The final volume was adjusted to 10 ml by adding the DMSO solution to the necessary amount of DMSO. The absorbance of the final extract (V) is measured at 652 nm (A₆₅₂) using a visible spectrophotometer (Thermo Orion AquaMate 7000 Vis). The chlorophyll stability index (CSI) was calculated as the percentage of chlorophyll content in the treatment compared to that of the control (Murthy, 1962).

$$\text{CSI (\%)} = \left(\frac{\text{Total Chlorophyll of Treatment}}{\text{Total Chlorophyll of Control}} \right) \times 100$$



Plate 4. Measuring the physiological and biochemical parameters during the drought and salinity stress condition in Mahogany seedlings



Plate 5. Stress symptoms and Recovery stage for drought and Salinity stress in mahogany seedlings; a. Wilting of leaves as a symptom of drought stress, b. Accumulation of salts and browning of lower leaves as a symptom of salinity stress, c. Recovery of drought after application of defense hormones, d. Recovery of salinity stress after application of defense hormones

3.3.3.2.6 Membrane Stability Index (MSI) (%)

The membrane stability index (MSI) was determined following the standard protocol established by Sairam (1994). Two test tubes were filled with 100 mg of fresh leaf samples and 10 ml of distilled water each. One set of test tubes was incubated in a boiling water bath at 35 °C for 30 minutes (control), while the second set was maintained at 100 °C for 15 minutes (treatment) before being allowed to cool to room temperature.

After the leaf discs were removed, the electrical conductivity (µS/cm) of both the control (C1) and treatment (C2) solutions was measured using a conductivity bridge. Finally, the MSI was calculated using the formula provided below:

$$\text{MSI (\%)} \text{ or Electrolyte Leakage} = \left(1 - \frac{C1}{C2}\right) \times 100$$

3.3.3.3 Biochemical parameters

The biochemical parameters were evaluated under all three conditions. Healthy third leaves from the top were collected in each stress condition and transported in an ice box to the lab. The leaves were subsequently wrapped in aluminium foil and stored at -20°C for further analysis. Key biochemical parameters related to osmoregulation and reactive oxygen species, particularly proline (µg g⁻¹), were analysed to understand how defense hormones respond to abiotic stress conditions.

3.3.3.3.1 Proline content

Proline was determined using the acid ninhydrin reagent (Bates *et al.*, 1973).

Reagents -

- Sulphosalicylic acid (3%): 3 g in 100 ml distilled water.
- Acid ninhydrin: 1.25 g ninhydrin in 30 ml glacial acetic acid. This solution was then warmed for complete solubility until the color became greenish blue, and then 20 ml of 6M phosphoric acid was added, and the solution changed to yellow. The mixture was used within 24 hours of preparation.
- Phosphoric acid (6M): Mixing 41.176 ml of Ortho-phosphoric acid in 58.824 ml of distilled water.

A leaf sample of 0.2 g was ground with 2 ml of 3% sulphosalicylic acid and then centrifuged at 10,000 rpm for 10 minutes. The filtrate was collected in a test tube, ensuring the supernatant did not exceed 2 ml, and mixed with 2 ml each of glacial acetic acid and 2 ml of acid ninhydrin. The test tubes were incubated in water bath at 100°C for one hour. After incubation, the test tubes were placed on ice for 10-15 minutes to cool and stop the reaction. Next, 4 ml of toluene was added, and the mixture was vortexed for 15-20 seconds before bringing it to room temperature.

Two separate layers were formed, and the top layer containing toluene was removed. The intensity of the color was measured at 520 nm using a spectrophotometer, with toluene as blank. A proline standard curve was prepared using L-proline at known concentrations, and the proline content was calculated on a fresh-weight basis as follows:

$$\text{Proline Content } (\mu\text{mol/g tissue}) = \frac{(\mu\text{g Proline/mL}) \times \text{Volume of Toluene (mL)}}{115.5 \times 5 \times \text{Weight of Sample (g)}}$$

3.3.4 Statistical Analysis:

3.3.4.1 Analysis of Variance:

The data collected on morphological, physiological, and biochemical parameters were analysed using analysis of variance (ANOVA) by linear model procedure in R software (version 4.3.1) to determine the significance of various parameters. LSD test independently assesses differences among treatment means, drought, salinity stress stages, and defense hormone treatment groups using R software. DMRT was then applied to compare the means and identify significant variations between treatments. Pearson correlation analysis assesses the correlation among the morphological, physiological, and biochemical parameters.

3.4 PROTEOMIC ANALYSIS OF STRESS RESPONSE MECHANISMS INDUCED BY DEFENSE HORMONES.

A comparative proteomic analysis examined the differential protein expression between stress-induced mahogany seedlings treated with defense hormones and untreated control seedlings across three conditions: Normal, Stress, and Regain (Table 03). Proteins were initially extracted from seedlings under normal irrigated conditions, referred to as the "normal condition." After inducing drought and salinity stress, proteins

were extracted from leaf samples labelled "drought" and "salinity stress." Following successful stress induction, defense hormones (SA and JA) were applied separately, and proteins were extracted again after 48 hours, marking as "regain condition."

3.4.1 Extraction and visualization of proteins:

3.4.1.1 Protein extraction using TCA-acetone method:

Proteins were extracted using the TCA-acetone method (Niu *et al.*, 2018). The frozen leaf samples (2g) were homogenized with polyvinyl polypyrrolidone (10% of sample weight) in liquid nitrogen (Fig. 1). The resulting powder was transferred to a 50 ml tube containing 25 ml of a 10% trichloroacetic acid (TCA)-acetone mixture. The samples were then centrifuged at 5000 rpm for 20 minutes at 4°C. After discarding the supernatant, the pellet was washed with 80% methanol 5 ml containing 0.1 M ammonium acetate to remove residual TCA. This mixture was centrifuged at 5000 rpm for 20 minutes at 4°C, and the supernatant was discarded.

The pellet was resuspended and vortexed in 80% acetone (5 ml) and centrifuged at 5000 rpm for 20 minutes at 4°C. The resulting pellets were air-dried to remove residual acetone and re-suspended in a 1:1 phenol (pH 7.9)/SDS buffer (30% sucrose, 2% SDS, 0.1 M Tris-HCl, 5% beta-mercaptoethanol, 1 mM PMSF, pH 8.0) at a volume of 0.6 ml per 0.1 g of starting material. This mixture was incubated at room temperature for 5 minutes and then centrifuged at 5000 rpm for 20 minutes.

The upper phenol phase was transferred to a new 50 ml tube, and protein precipitation was induced by adding five volumes of methanol containing 0.1 M ammonium acetate. The mixture was left to precipitate overnight at -20°C. After centrifugation, the resulting pellet was washed once with 100% methanol and twice with 80% acetone. The dried pellets were dissolved in the re-suspension buffer. In cases where white pellets were visible, the mixture was centrifuged at 5000 rpm for 20 minutes at 4°C, and the supernatant was discarded. If no phase separation occurred, an additional 100 µl of phenol was added, followed by mixing and centrifugation. The final pellet was washed once with 100% methanol (3 ml) and twice with 80% acetone (3 ml).

3.4.1.2 Protein estimation by Bradford's assay:

After protein extraction, the proteins were estimated for different samples using the Bradford assay. The Bradford reagent was prepared by adding 50 mg of Coomassie Brilliant Blue G-250 in 50 ml of methanol. Next, 100 ml of 85% phosphoric acid (H_3PO_4) was added to the methanol solution. This mixture was then thoroughly mixed with 500 ml of distilled water. The solution was filtered to remove any precipitates. Finally, 350 ml of distilled water was added to bring the total volume to approximately 1 liter. The prepared reagent was stored at 4°C for future use. The principle of assay worked by using this reagent, where the absorbance maximum of Coomassie Brilliant Blue G-250 shifted from 465 nm to 595 nm upon binding to proteins. The sample was mixed with the Bradford reagent and incubated for 5 minutes at room temperature, followed by measuring the OD at 595 nm to quantify the protein concentration.

3.4.2 SDS-PAGE analysis of leaf protein extracts:

After protein extraction and estimation, the proteins are separated using SDS-PAGE based on their molecular weight. This analysis uses the discontinuous buffer system on mini gels (10 cm x 8 cm).

Reagents-

- Acrylamide-N, N'-methylene bis(acrylamide) (30%)
- Tetramethylethylenediamine (TEMED)
- Ammonium persulphate (APS) (10%)
- Sodium dodecyl sulfate (SDS) (10%)
- Resolving gel (12%) and stacking gel (4%)
- Running buffer (pH 8.3)
- Pre-stained molecular weight marker (Precision Plus Protein Dual Xtra Standards, Bio-Rad)

Protein extracts were mixed with SDS sample buffer, which contained SDS to denature the proteins by disrupting their secondary and tertiary structures and coating them with a negative charge. A reducing agent (DTT) was added to break disulfide bonds, glycerol was added to increase sample density, and a tracking dye was used for electrophoresis monitoring. The protein-buffer mixture was prepared at a 1X working

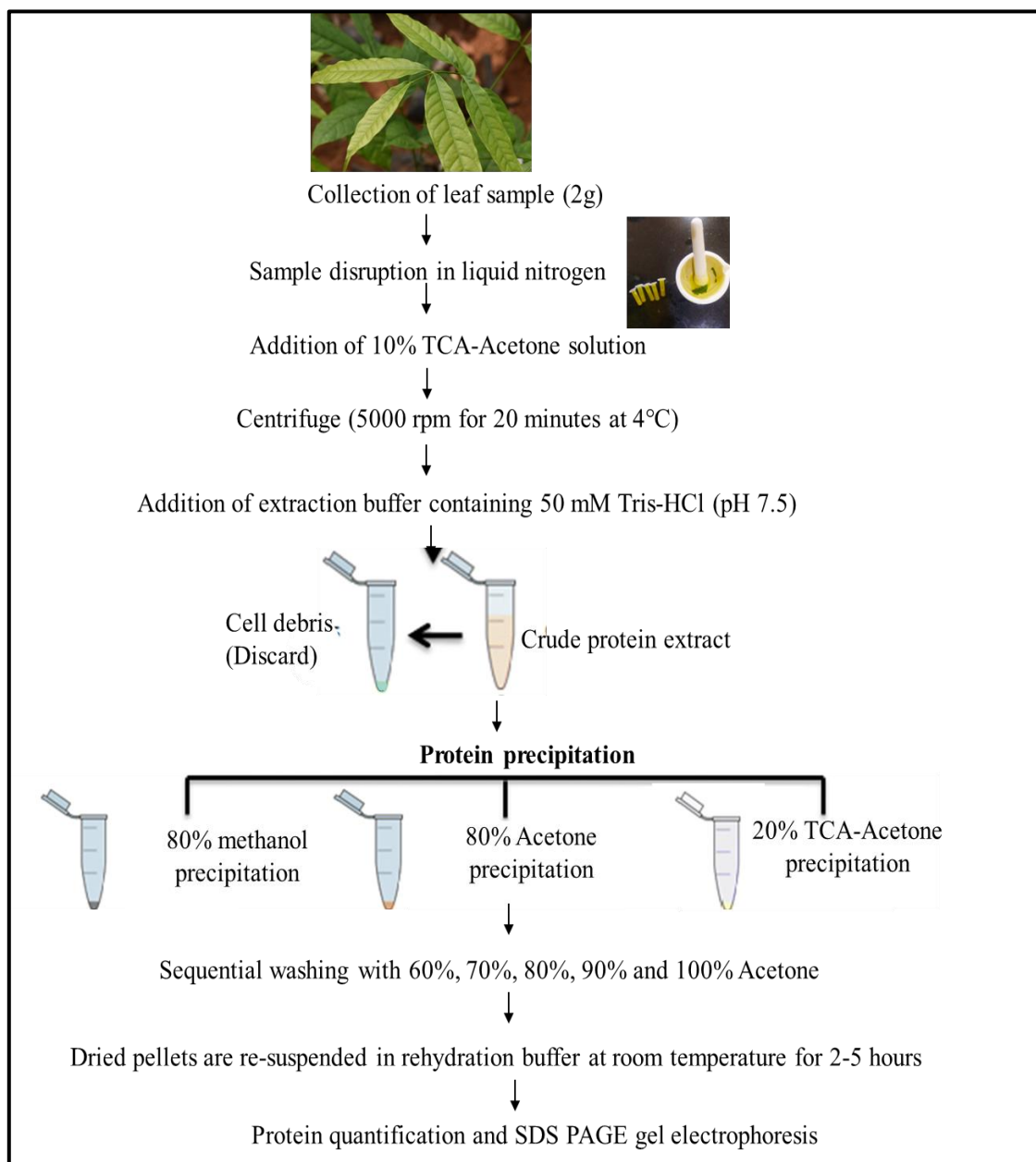


Fig. 01 Protein extraction protocol from mahogany leaves using the TCA-Acetone method

concentration and then heated in a boiling water bath for 5 minutes to ensure complete denaturation. After cooling to room temperature, the samples were centrifuged for 10 minutes to remove any insoluble debris, leaving only soluble denatured proteins for accurate loading onto the gel.

The gel apparatus was assembled with glass plates and spacers, and the resolving gel was poured between the plates, followed by the stacking gel. Once polymerized, the stacking gel's wells were cleaned, and 30 μ l of each sample and 15 μ l of a molecular weight marker were loaded into the wells. Electrophoresis was initiated at 75V through the stacking gel and increased to 95V once the samples reached the resolving gel. After electrophoresis, the gel was removed, washed with distilled water, and treated with a fixing solution. Protein bands were visualized by staining with Coomassie Brilliant Blue R250. Excess stain was removed by washing the gel with distilled water, followed by destaining for 2-3 hours with periodic solution changes. The protein bands were then visualized using the Bio-Rad Chemi-Doc imaging system.

3.4.3 In-gel digestion protocol for Mass Spectrometry

After SDS-PAGE, the protein bands were excised and digested using the protease trypsin. Two sets of 500 μ l snap cap microcentrifuge tubes were washed before use with 2X 500 μ l methanol and a 500 μ l Milli-Q water rinse. The protein band was cut as small as possible using a razor and transferred into the microcentrifuge tube.

Reagents

- Stained polyacrylamide gel containing expressed proteins
- 50 mM ammonium bicarbonate (NH_4HCO_3 ; 3.96 mg/mL)/50% (v/v) acetonitrile
- 10 mM dithiothreitol (DTT, 1.54 mg/mL) in 50 mM NH_4HCO_3
- 55 mM iodoacetamide (10.2 mg/mL) in 50 mM NH_4HCO_3
- 0.1% TFA
- 50 mM NH_4HCO_3 , pH 8
- HPLC grade acetonitrile
- 0.5 mL non-sticky tubes

3.4.3.1 Reduction/alkylation:

The gel slices were rinsed with 25 mM ammonium bicarbonate to begin the reduction process. The gel slice was dehydrated by adding 50 µl of solution A, a 2:1 mixture of acetonitrile (ACN) and 50 mM ammonium bicarbonate, for 5 minutes. After removing the supernatant, 50 µl of 25 mM ammonium bicarbonate was added for 2 minutes. The dehydration step was repeated with 50 µl of solution A for another 5 minutes, and the supernatant was removed again to add 50 µl of 25 mM ammonium bicarbonate for 2 minutes. Finally, the gel slice was dehydrated with 50 µl of solution A for 5 minutes.

3.4.3.2 Trypsin digestion

Following the dehydration and rehydration cycles, the gel slice was rehydrated in 20-50 µl of 10-20 mM dithiothreitol (DTT) and placed at 60 °C for 1 hour. After incubation, the DTT was removed, and the gel slice was rinsed with 50 µl of 25 mM ammonium bicarbonate. Next, 20-50 µl of 50-100 mM iodoacetamide was added, and the gel slice was incubated at room temperature in the dark for 20 minutes. After this incubation, the iodoacetamide was removed, and the gel slice was rinsed with 50 µl of ammonium bicarbonate. The gel slice was then dehydrated with 50 µl of solution A for 5 minutes, after which the supernatant was removed and 50 µl of 25 mM ammonium bicarbonate was added for 2 minutes. Optionally, the dehydration and rehydration process were repeated twice. After removing the final supernatant, the gel spots were dried in a speed vac for approximately 5-15 minutes.

For digestion, the trypsin stock was prepared by adding 1 ml of ice-cold 1 mM HCl to a standard 20 µg trypsin(5X) vial (Sigma/Promega MS grade trypsin) stored on ice. Aliquots of 5 µl were made for each gel band, and 95 µl of 25 mM ammonium bicarbonate was added to achieve a concentration of 100 ng. The gel slice was rehydrated with 100 µl of chilled trypsin solution incubated in ice for 20-30 minutes until the trypsin was absorbed. After rehydration, 25 mM ammonium bicarbonate was added to cover the gel slice in the tube, and the gel slice was incubated at 37 °C for 16 hours. Periodically, it was checked to ensure the gel slice remained covered with 25 mM ammonium bicarbonate.

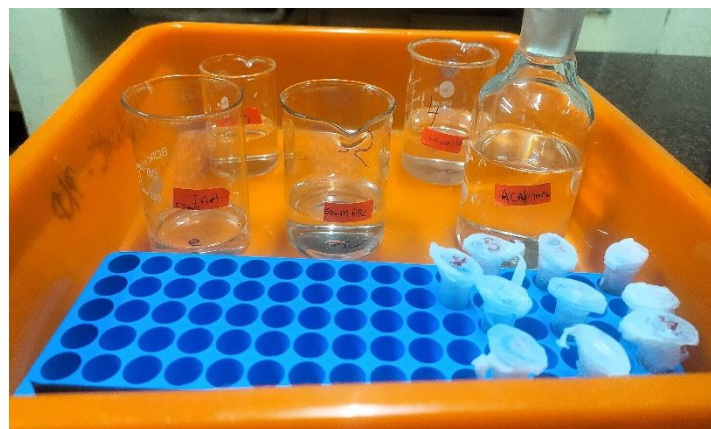
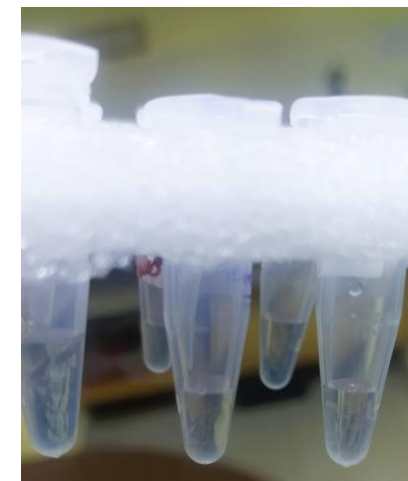


Plate 6. Extraction and visualisation of proteins for different conditions

3.4.3.3 Peptide extraction

For peptide extraction, 5 μL of 1% aqueous TFA was added to halt digestion, followed by 15 minutes of shaking and brief centrifugation. The supernatant containing tryptic peptides was transferred to a clean tube and kept on ice. The gel pieces were extracted twice with 50 μL and 25–50 μL of extraction solution (60% acetonitrile, 1% TFA) by gentle vortexing. The pooled supernatants were dried to near dryness via centrifugal evaporation and resuspended in 15 μL of 0.1% TFA for mass spectrometry analysis.

3.4.3 MALDI-TOF MS analysis

3.4.4.1 Sample preparation:

After digestion of proteins into peptides, an aliquot of 1.5 μL peptide digest was mixed with an equal volume of saturated α -Cyano-4 hydroxycinnamic acid (HCCA) matrix solution (Bruker Daltonics) and spotted onto an MTP 384 ground steel target plate (Bruker Daltonics), then dried in laminar airflow chamber. Mass spectra were obtained on an Autoflex II MALDI TOF/TOF (Bruker Daltonics, Germany) mass spectrometer equipped with a pulsed N₂ laser ($\lambda=337\text{nm}$, 50Hz).

3.4.4.2 MS/MS analysis and database search:

Peptide monoisotopic signals were analyzed in the FlexAnalysis software (version 2.4, Bruker Daltonics). The processed peaks were transferred through the MS BioTools™ (version 3.0) program as inputs to MASCOT search engine version 2.2 for protein identification. The following specified parameters were applied for database search: database (MSDB/NCBItr/ Swissport); taxonomy (green plants, Viridiplantae); proteolytic enzyme (Trypsin); peptide mass tolerance (≤ 100 ppm); global modification (Carbamidomethyl, Cys); variable modification (Oxidation, Met); peptide charge state (1+) and maximum missed cleavage of 1. The significance threshold is set to a minimum of 95% ($p \leq 0.05$).

The criteria used to accept protein identification were based on the molecular weight search (MOWSE) score, the percentage of the sequence coverage, and a minimum of five matched peptides. MS/MS is performed to confirm the identification with

matched peptides, selected based on their suitability for fragmentation (signal strength and relative isolation). The parent and daughter ions were analyzed using the SNAP algorithm using the Flex Analysis software (version 2.4, Bruker Daltonics).

For MS/MS, signal/noise ratio peaks > 15 were transferred through the MS Biotoools™ program as inputs to MASCOT for peptide identification. In order to determine the false discovery rate (FDR) of peptide identification, an automatic decoy database search is performed by choosing the Decoy checkbox on the search form in the Mascot search engine. When the search was complete, the statistics for matches to the random sequences, which were effectively sequenced from a decoy database, are reported in the result. The results were accepted the result if the false discovery rate is 0%. All collected MS/MS data were run in combined mode (combined TOF and TOF/TOF) with MS data in the Mascot search engine for final protein identification.

3.4.4 Data analysis and functional annotation:

The analysis of MALDI-TOF results provided valuable insights into the proteomic response of mahogany seedlings under stress-induced defense hormone-treated plants.

3.4.4.1 Correlated heat maps:

Heat maps provide distinct clustering patterns among differentially expressed proteins, highlighting groups with similar expression profiles. These visualizations indicated potential co-regulation and involvement in related biological pathways, distinguishing upregulated and downregulated proteins between control and hormone-treated seedlings.

3.4.4.2 Fold change analysis and functional annotation:

Fold changes were compared across the treatment groups based on protein concentration (mg/ml). The fold change analysis identified key proteins with significant variations in expression. Proteins with a fold change greater than 0 were upregulated, suggesting a role in stress response, while those with a fold change less than 0 were downregulated, potentially indicating suppressed pathways. Proteomic analysis categorized differentially expressed proteins into relevant biological processes,

molecular functions, and cellular components, with significant involvement in stress response and signal transduction, further elucidating the roles of these identified proteins in stress-related mechanisms.

4. RESULTS

The present study, titled “Stress proteomics on the application of defense hormones in *Swietenia macrophylla* King seedlings,” aimed to explore the effects of defense hormones in alleviating abiotic stress and to identify the proteins involved in stress tolerance. This chapter provides a comprehensive presentation of the findings, organized into the following sections:

4.1 APPLICATION OF DEFENSE HORMONES TO MAHOGANY SEEDLINGS

4.1.1 Effect of defense hormones on morphological parameters:

The morphological parameters, including plant height, collar diameter, root architecture, leaf area, and leaf count, were systematically evaluated over 150 days with 30-day intervals to assess the effects of varying concentrations of JA and SA on the growth dynamics and development of mahogany seedlings. The temporal progression of these traits offered critical insights into the optimal hormone concentrations for promoting growth vigour and enhancing stress resilience in the seedlings.

The exogenous application of defense hormones significantly influenced the height of mahogany seedlings as outlined in Table 04. Among the treatment groups, JA showed the most significant effect at a concentration of 0.6 mM (T4), resulting in a 158.87% increase in height, reaching an average of 106.42 cm. Similarly, SA at a concentration of 0.8 mM (T5) led to a 139.62% increase, with an average height of 100.64 cm. In contrast, the control groups for both JA and SA exhibited the lowest growth rates, with height increases of 74.13% and 69.38% increases, respectively (Table 04). However, height growth was inhibited at higher concentrations (1.0 mM) of both hormones, indicating that concentrations beyond a certain threshold can adversely impact growth.

The collar diameter of mahogany seedlings was also significantly affected by hormone application, as shown in Table 05. JA at 0.6 mM (T4) had the greatest impact, resulting in a 180.53% increase and reaching a mean collar diameter of 11.53 mm. SA showed the most pronounced effect at a concentration of 0.8 mM (T5), resulting in a 146.93% increase with a mean diameter of 10.63 mm (Table 02). Similar to height, the

Table 04 Effect of exogenous application of JA and SA on the height increment (cm) in six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D	Percentage Increase
Jasmonic Acid							
T1 (control)	41.78	47.16 ^c	53.11 ^d	59.64 ^d	67.87 ^d	72.75 ^d	74.13%
T2 (0.2 mM)	42.33	52.87 ^b	62.33 ^{bc}	67.00 ^{cd}	75.04 ^{bcd}	81.71 ^{bc}	93.03%
T3 (0.4 mM)	42.00	56.13 ^b	68.28 ^b	77.51 ^b	81.92 ^b	86.28 ^b	105.43%
T4 (0.6 mM)	41.11	69.04 ^a	86.15 ^a	94.29 ^a	97.07 ^a	106.42 ^a	158.87%
T5 (0.8 mM)	42.89	54.12 ^b	64.02 ^{bc}	73.08 ^{bc}	78.92 ^{bc}	81.43 ^{bc}	89.86%
T6 (1.0 mM)	40.22	50.91 ^{bc}	57.41 ^{cd}	64.15 ^{cd}	71.88 ^{cd}	77.69 ^{cd}	93.16%
CD	NS	5.681	6.987	8.777	8.648	6.541	
P-value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	
Salicylic Acid							
T1 (control)	41.77	47.15 ^c	53.11 ^c	59.64 ^c	65.47 ^d	70.75 ^d	69.38%
T2 (0.2 mM)	42.66	48.33 ^{bc}	54.40 ^c	61.62 ^{de}	67.87 ^{cd}	75.28 ^{cd}	76.47%
T3 (0.4 mM)	46.11	52.70 ^b	59.02 ^{bc}	65.66 ^{cd}	69.12 ^{cd}	79.44 ^{bcd}	72.28%
T4 (0.6 mM)	41.11	48.92 ^{bc}	54.79 ^{bc}	68.49 ^c	74.65 ^c	83.22 ^{bc}	102.43%
T5 (0.8 mM)	42.00	58.17 ^a	76.39 ^a	85.78 ^a	93.39 ^a	100.64 ^a	139.62%
T6 (1.0 mM)	44.55	57.92 ^a	65.59 ^b	75.74 ^b	82.55 ^b	87.42 ^b	96.23%
CD	NS	4.244	5.573	5.633	6.952	8.675	
P-value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	

Significant at 0.05 levels; NS - non significant at 0.05 levels

Table 05 Effect of exogenous application of JA and SA on the collar diameter increment (cm) of six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D	Percentage Increase
Jasmonic Acid							
T1 (control)	4.20	4.83	5.64	6.84 ^b	7.04 ^c	7.36 ^c	75.24%
T2 (0.2 mM)	4.33	4.98	5.73	7.16 ^b	8.28 ^b	8.40 ^{ab}	94.15%
T3 (0.4 mM)	4.39	5.05	5.92	7.27 ^{ab}	8.40 ^{ab}	9.42 ^b	114.58%
T4 (0.6 mM)	4.11	4.72	6.32	8.39 ^a	9.69 ^a	11.53 ^a	180.53%
T5 (0.8 mM)	4.11	4.71	5.89	6.77 ^b	7.83 ^b	8.04 ^{ab}	95.56%
T6 (1.0 mM)	4.13	4.75	5.68	6.95 ^b	8.04 ^b	8.12 ^{ab}	96.49%
CD	NS	NS	NS	1.165	1.347	1.452	
P-value	>0.05	>0.05	>0.05	<0.05	<0.05	<0.05	
Salicylic Acid							
T1 (control)	4.25	4.48	4.80 ^c	5.23 ^c	5.65 ^c	7.59 ^c	74.02%
T2 (0.2 mM)	4.39	4.43	4.99 ^{bc}	5.77 ^{abc}	6.14 ^{bc}	7.89 ^{bc}	79.73%
T3 (0.4 mM)	4.26	4.59	5.30 ^{abc}	5.85 ^{bc}	6.63 ^{bc}	8.02 ^b	88.35%
T4 (0.6 mM)	4.34	4.95	5.35 ^{abc}	6.20 ^{abc}	6.89 ^{bc}	8.48 ^b	95.18%
T5 (0.8 mM)	4.30	5.12	6.01 ^a	7.25 ^a	8.48 ^a	10.63 ^a	146.93%
T6 (1.0 mM)	4.20	5.13	5.70 ^{ab}	6.60 ^{ab}	7.46 ^{ab}	9.82 ^{ab}	133.81%
CD	NS	NS	0.712	0.828	0.854	0.892	
P-value	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	

Significant at 0.05 levels; NS - non-significant at 0.05 levels

control groups for both JA and SA displayed the lowest collar diameter growth, with increases of 75.24% and 74.02%, respectively.

Height and collar diameter, critical indicators of volume increment, play essential role in assessing overall growth and biomass accumulation in mahogany seedlings. The final volume measurements at the last observation (150 days) demonstrated optimal growth at specific concentrations of JA and SA, as shown in Table 06. The application of JA at 0.6 mM (T4) resulted in the highest volume, reaching approximately 155.48 cm³ (Fig 03). Similarly, SA at 0.8 mM (T5) produced a peak volume of around 111.17 cm³ (Fig 04). In contrast, the control groups and higher concentrations (1.0 mM) of both SA and JA showed the least growth, suggesting that elevated hormone levels might have inhibited seedling development.

Both JA and SA treatments significantly influenced the leaf production in mahogany seedlings, reflecting improved overall plant vigor (Table 07). JA treatments showed the highest increase at 0.4 mM, with a 221.70% rise, reaching 39 leaves, while SA peaked at 0.8 mM, resulting in a 210.69% increase to 41 leaves. In contrast, the control groups exhibited the lowest leaf increase, with JA at 116.96% and SA at 107.07%, highlighting the superior efficacy of intermediate hormone concentrations in promoting leaf growth. Higher concentrations (1.0 mM) of SA and JA showed an inhibitory effect.

The application of JA and SA significantly influenced leaf area expansion in mahogany seedlings (Table 08). The most significant increase in leaf area was observed with 0.6 mM JA, which showed a 206.57% rise, while 0.8 mM SA resulted in a 180.39% increase. Among JA treatments, the largest leaf areas were recorded at 0.6 mM (43.38 cm²) and 0.4 mM (38.96 cm²). However, higher JA concentrations (1.0 mM and 0.8 mM) showed a reduction in leaf area (Table 05). Similarly, SA at 0.8 mM produced the largest leaf area (40.74 cm²), followed by 1.0 mM SA (35.77 cm²). Lower concentrations in the control groups resulted in smaller leaf areas, understanding the importance of optimizing hormone dosages to 22 maximum leaf growth.

The application of JA and SA significantly influenced shoot length in mahogany seedlings, exhibiting a concentration-dependent response (Table 09). After 150 days of observation, the highest shoot length for JA was recorded with 0.6 mM (T4), reaching

Table 06 Effect of exogenous application of JA and SA on the seedling characteristics in six-month-old mahogany seedlings

Treatments	Initial Volume (cm ³)	Final Volume (cm ³)	Sturdiness Quotient	Seedling Quality Index	Initial Volume Index (cm ³)	Final Volume Index (cm ³)	Sturdiness Quotient	Seedling Quality Index
	JA				SA			
T1 (control)	7.44	49.83 ^b	6.13 ^c	1.08 ^c	8.23	43.59 ^c	8.36 ^a	1.37 ^d
T2 (0.2 mM)	8.02	59.70 ^b	7.58 ^b	1.67 ^b	8.36	55.50 ^c	6.92 ^{bc}	1.58 ^{cd}
T3 (0.4 mM)	8.69	68.06 ^b	5.84 ^c	1.77 ^b	7.77	58.09 ^c	7.38 ^b	1.84 ^{bc}
T4 (0.6 Mm)	7.11	155.48 ^a	10.35 ^a	2.32 ^a	7.79	54.50 ^c	6.04 ^c	1.58 ^{cd}
T5 (0.8 mM)	7.43	54.46 ^b	5.82 ^d	1.99 ^{ab}	7.82	111.17 ^a	7.41 ^b	2.29 ^a
T6 (1.0 mM)	7.13	58.11 ^c	6.82 ^{bc}	1.67 ^b	7.88	83.55 ^b	6.02 ^c	2.01 ^{ab}
CD	NS	3.10	1.199	0.450	NS	1.6	0.900	0.333
P- Value	>0.05	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05

Significant at 0.05 levels; NS - non significant at 0.05 levels

Table 07 Effect of exogenous application of JA and SA on the number of leaves in six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D	Percentage Increase
Jasmonic Acid							
T1 (control)	13.67	15.53 ^c	21.66 ^c	25.20 ^c	27.24 ^b	29.65 ^c	116.96%
T2 (0.2 mM)	13.22	16.26 ^{bc}	22.69 ^{bc}	28.50 ^b	32.60 ^b	35.62 ^{bc}	169.39%
T3 (0.4 mM)	12.11	16.83 ^{bc}	23.48 ^{bc}	29.50 ^{bc}	36.13 ^{ab}	38.96 ^{ab}	221.70%
T4 (0.6 Mm)	14.33	21.36 ^a	29.79 ^a	37.42 ^a	40.13 ^a	43.38 ^a	202.65%
T5 (0.8 mM)	14.78	20.39 ^{ab}	28.45 ^{ab}	35.73 ^{ab}	34.70 ^{ab}	37.28 ^{bc}	152.27%
T6 (1.0 mM)	14.67	19.36 ^{abc}	27.01 ^{abc}	33.92 ^{abc}	34.85 ^{ab}	38.34 ^{ab}	161.42%
CD	NS	4.139	5.774	7.253	5.535	5.226	
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	
Salicylic Acid							
T1 (control)	14.33	17.47	23.76 ^{abc}	25.77 ^c	28.00 ^b	29.68 ^b	107.07%
T2 (0.2 mM)	13.11	15.42	20.97 ^{bc}	28.51 ^{bc}	32.08 ^{ab}	32.90 ^b	150.93%
T3 (0.4 mM)	13.44	14.96	23.93 ^{abc}	29.31 ^{abc}	32.10 ^{ab}	34.10 ^b	153.61%
T4 (0.6 Mm)	13.00	17.59	20.34 ^c	27.66 ^{bc}	31.12 ^{ab}	31.92 ^b	145.54%
T5 (0.8 mM)	13.11	18.82	25.59 ^{ab}	34.80 ^a	31.00 ^{ab}	40.74 ^a	210.69%
T6 (1.0 mM)	13.00	19.15	26.05 ^a	32.27 ^{ab}	34.35 ^a	35.77 ^{ab}	175.15%
CD	NS	NS	5.23	5.51	NS	6.16	
P- Value	>0.05	>0.05	<0.05	<0.05	>0.05	<0.05	

Significant at 0.05 levels; NS - non significant at 0.05 levels

Table 08 Effect of exogenous application of JA and SA on the leaf area (cm²) in six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D	Percentage Increase
		Jasmonic Acid					
T1 (control)	26.22	29.52	33.15 ^d	40.23 ^{cd}	41.09 ^{cd}	50.28 ^b	91.76%
T2 (0.2 mM)	27.72	37.84	50.33 ^{bc}	56.52 ^{bc}	57.82 ^{bc}	64.76 ^b	133.59%
T3 (0.4 mM)	27.96	39.01	54.46 ^b	61.16 ^b	62.56 ^b	70.07 ^b	150.57%
T4 (0.6 mM)	28.94	46.01	70.50 ^a	79.17 ^a	81.00 ^a	88.72 ^a	206.57%
T5 (0.8 mM)	25.49	33.78	43.99 ^{bcd}	49.40 ^{bcd}	50.54 ^{bcd}	56.60 ^b	122.02%
T6 (1.0 mM)	27.22	30.93	36.59 ^{cd}	41.09 ^{cd}	42.03 ^{cd}	57.16 ^b	109.97%
CD	NS	NS	15.19	17.05	17.45	18.32	
P- Value	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	
		Salicylic Acid					
T1 (control)	25.25	28.06	32.36 ^{bc}	34.94 ^b	40.50 ^b	48.20 ^b	90.86%
T2 (0.2 mM)	27.72	33.54	34.87 ^{bc}	41.49 ^{ab}	51.08 ^{ab}	60.78 ^{ab}	119.26%
T3 (0.4 mM)	25.49	33.65	35.33 ^{bc}	42.05 ^{ab}	57.18 ^{ab}	62.05 ^{ab}	143.39%
T4 (0.6 mM)	27.22	36.21	39.20 ^{abc}	46.65 ^{ab}	63.44 ^a	65.49 ^{ab}	140.58%
T5 (0.8 mM)	28.94	41.09	47.75 ^a	56.82 ^a	68.18 ^a	81.14 ^a	180.39%
T6 (1.0 mM)	27.96	38.87	43.69 ^{ab}	52.00 ^a	61.36 ^a	70.01 ^a	150.37%
CD	NS	NS	11.88	14.13	17.80	21.19	
P- Value	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	

Significant at 0.05 levels; NS - non-significant at 0.05 level

81.00 cm, followed by 0.4 mM (T3) with 68.00 cm. The shortest shoot length for JA was observed with 1.0 mM (T6) with 56.11 cm, while the control group (T1) had the lowest at 51.44 cm. For SA treatments, the highest shoot length was observed at 0.8 mM (T5) reaching 71.11 cm, followed by 1.0 mM (T6) at 63.56 cm. Lower concentrations showed a decline in shoot length, with the shortest recorded for the control group (T1) at 50.11 cm.

The sturdiness quotient, a vital measure of seedling stability and resilience, showed significant variation with the application of JA and SA after 150 days of observation (Table 06). A lower sturdiness quotient indicates greater stability and resilience. The application of JA at 0.6 mM (T4) resulted in the lowest sturdiness quotient, indicating enhanced structural integrity. Lower concentrations (0.4 mM and 0.2 mM) also promoted sturdiness, albeit to a lesser extent. The control groups exhibited the highest sturdiness quotient, indicating reduced stability. Similarly, for SA treatments, 1 mM (T6) showed the lowest sturdiness quotient, followed by 0.8 mM (T5), reflecting improved seedling stability. Lower concentrations (0.6 mM and 0.4 mM) had moderate effects, with the control group showing the highest sturdiness quotient.

The seedling quality index, a vital measure of plant vigour and survival potential, is crucial in determining the success of early plant development and long-term growth. Both JA and SA treatments significantly enhanced this index, with optimal concentrations of 0.6 mM for JA and 0.8 mM for SA producing the highest quality seedlings (Table 06). Excessive concentrations slightly diminished seedling quality, indicating a threshold for effective hormone application.

The exogenous application of JA and SA significantly influenced shoot dry weight, a crucial indicator of biomass allocation and overall plant growth (Table 09). In JA treatments, the highest shoot dry weight of 30.16 g was observed at 0.6 mM, with notable reductions at higher and lower concentrations. Similarly, SA treatments peaked at 0.8 mM, yielding a weight of 24.17 g. Both hormones demonstrated that specific concentrations optimize shoot growth, while excess or insufficient doses hinder biomass accumulation.

The exogenous application of JA and SA notably influenced root length, the number of roots, and root dry weight, which are essential indicators of nutrient acquisition

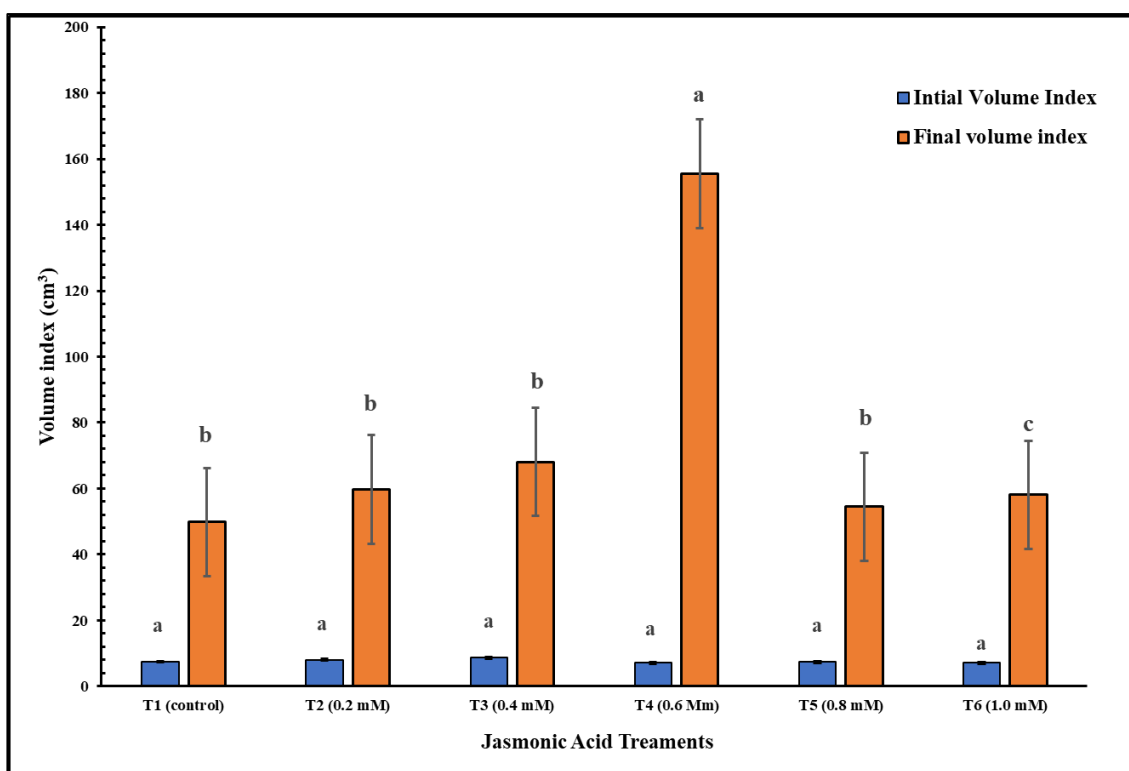


Fig 2. Variation in volume increment (cm³) with JA application in six-month-old mahogany seedlings

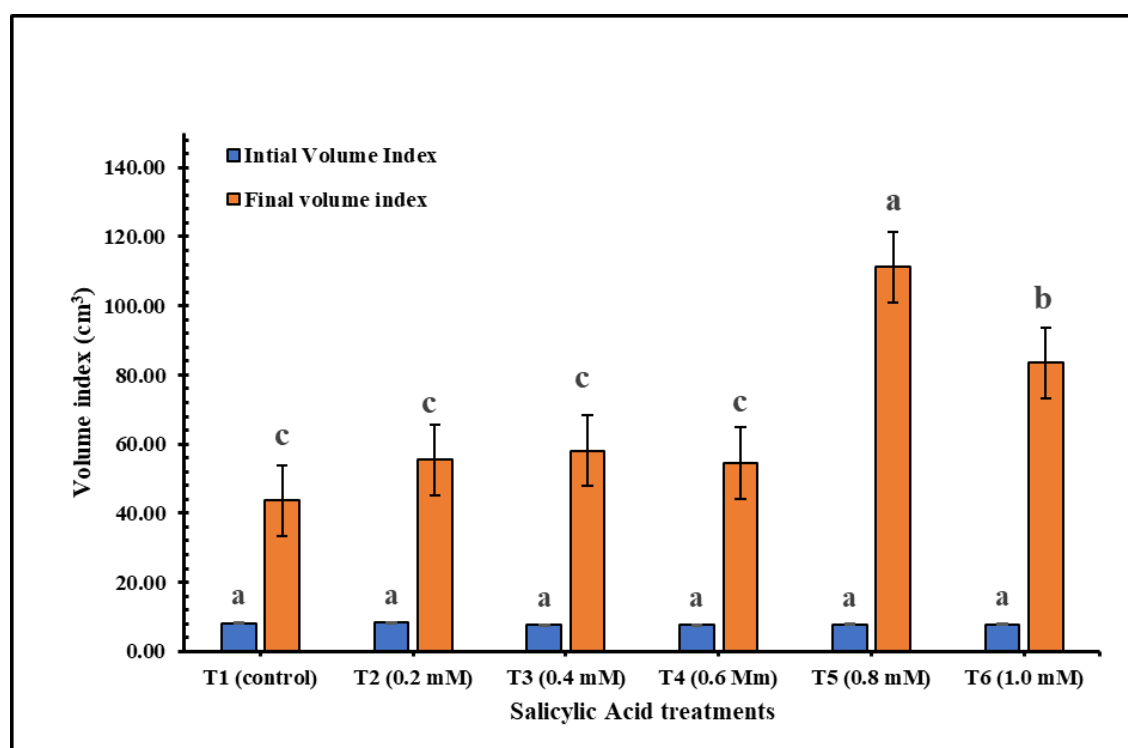


Fig 3. Variation in volume increment (cm³) with SA application in six-month-old mahogany seedlings

Table 09 Effect of exogenous application of JA and SA on the root and shoot parameters in six-month-old mahogany seedlings.

Days/ Treatments	Shoot Length (cm)	Root Length (cm)	Number of Roots	Shoot Dry Weight (g)	Root Dry Weight (g)	Root-Shoot Ratio
Jasmonic Acid						
T1 (control)	51.44 ^e	15.00	46.89	14.91 ^c	5.33 ^{ab}	0.385
T2 (0.2 mM)	62.11 ^c	17.44	43.33	22.43 ^b	6.12 ^{ab}	0.309
T3 (0.4 mM)	68.00 ^b	19.33	46.67	19.77 ^{bc}	5.30 ^{ab}	0.273
T4 (0.6 Mm)	81.00 ^a	23.11	43.78	30.16 ^a	4.14 ^b	0.143
T5 (0.8 mM)	60.11 ^{cd}	17.00	32.78	19.12 ^{bc}	6.88 ^a	0.384
T6 (1.0 mM)	56.11 ^d	17.33	45.44	17.69 ^{bc}	5.94 ^{ab}	0.399
CD	4.306	NS	NS	6.583	2.110	NS
P- Value	<0.05	>0.05	>0.05	<0.05	<0.05	>0.05
Salicylic Acid						
T1 (control)	50.11 ^d	15.22	45.11 ^{ab}	12.60 ^c	5.28 ^{bc}	0.597
T2 (0.2 mM)	57.00 ^c	16.89	44.44 ^{ab}	16.52 ^{bc}	5.24 ^{bc}	0.342
T3 (0.4 mM)	59.11 ^c	15.33	43.00 ^{abc}	21.13 ^{ab}	6.85 ^{ab}	0.395
T4 (0.6 Mm)	62.89 ^b	20.56	32.00 ^c	15.87 ^{bc}	5.42 ^{bc}	0.370
T5 (0.8 mM)	71.11 ^a	23.56	37.89 ^{bc}	24.17 ^a	5.82 ^b	0.208
T6 (1.0 mM)	63.56 ^b	18.00	49.78 ^a	19.87 ^{ab}	7.06 ^a	0.376
CD	2.188	NS	10.503	5.335	2.663	NS
P- Value	<0.05	>0.05	<0.05	<0.05	<0.05	>0.05

Significant at 0.05 levels; NS - non-significant at 0.05 levels

and overall plant health, measured after 150 days (150D). JA treatments achieved the longest root length of 23.11 cm at 0.6 mM, while SA treatments peaked at 0.8 mM with a length of 23.56 cm. However, no statistically significant differences were observed between treatments. In terms of the number of roots, the control group (T1) in the JA-treated groups had the highest number of roots (46.89), closely followed by T3 (0.4 mM), whereas the lowest count was recorded at 0.8 mM (T5), with a significant drop to 33.

In the SA treatments, the highest number of roots of 50 occurred at 0.6 mM (T4), but this declined to 32.00 at 1.0 mM (T6), suggesting that higher concentrations may inhibit root development (Table 09). Additionally, JA significantly affected root dry weight, with the highest value of 6.88 g at 0.8 mM (T5) and relatively high root dry weight (6.12 g) at the lower concentration of 0.2 mM (T2). Meanwhile, the control group (T1) and other treatments exhibited moderate values. In contrast, SA treatments demonstrated no significant differences in root biomass distribution.

The application of JA and SA influenced the root-to-shoot ratio, although statistically insignificant differences were observed across treatments (Table 06). In JA-treated plants, the highest root-to-shoot ratio of 0.399 was recorded at 1.0 mM (T6). In comparison, the lowest ratio of 0.143 was observed at 0.6 mM (T4), indicating a greater biomass allocation to shoots. Similarly, in SA treatments, the control group (T1) exhibited the highest root-to-shoot ratio at 0.597. In contrast, the lowest ratio of 0.208 was observed at 0.8 mM (T5), suggesting a trend towards increased biomass distribution favoring shoot growth (Table 09).

4.1.2 Effect of defense hormones on physiological parameters

The physiological parameters of mahogany seedlings, such as photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), leaf temperature ($^{\circ}\text{C}$), total chlorophyll content (mg m^{-2}), and relative water content (%), were measured over 150 days, with 30 days interval. This assessment elucidated the impact of varying concentrations of JA and SA on the physiological responses of seedlings.

4.1.2.1 Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^2/\text{s}$)

JA and SA significantly influenced the photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^2/\text{s}$) in mahogany seedlings over 150 days (Table 10). JA at 0.6 mM (T4) recorded the peak rates

(T2, T6) improved rates but did not surpass T4. Meanwhile, SA at 1.0 mM (T6) produced a maximum rate of $4.50 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$ on day 30. Lower SA concentrations (T4, T3) showed moderate enhancements in photosynthetic efficiency. These results highlight the importance of optimizing hormone concentrations to enhance photosynthesis under stress conditions. of $3.78 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$ by day 120th, while the control group (T1) showed the lowest rates, declining from 2.55 to $2.14 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$ (Table 10). Intermediate JA concentrations (T2, T6) improved rates but did not surpass T4. Meanwhile, SA at 1.0 mM (T6) produced a maximum rate of $4.50 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$ on day 30. Lower SA concentrations (T4, T3) showed moderate enhancements in photosynthetic efficiency. These results highlight the importance of optimizing hormone concentrations to enhance photosynthesis under stress conditions.

4.1.2.2 Transpiration rate ($\text{mmol H}_2\text{O m}^2\text{s}^{-1}$)

JA and SA significantly influenced the transpiration rate of mahogany seedlings. JA at 0.6 mM (T4) recorded the highest transpiration rate of $0.178 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$ at 30 days and $0.145 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$ at 120 days, enhancing gas exchange compared to the control group (T1), which decreased from 0.086 to $0.078 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$ over 150 days which is summarised in Table 11. Intermediate JA concentrations (0.2 mM, T2, and 0.8 mM, T5) showed moderate increases but did not surpass T4. For SA, 0.8 mM (T5) yielded peak transpiration rates of $0.177 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$ at 30 days and $0.142 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$ at 120 days, with the control showing the lowest rates. Intermediate SA treatments (0.4 mM and 1.0 mM) resulted in modest improvements. Overall, optimal hormone concentrations effectively enhanced transpiration, benefiting water regulation in the seedlings.

4.1.2.3 Stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$)

Stomatal conductance is crucial for gas exchange and overall plant metabolism, and the exogenous application of defense hormones, particularly JA and SA, significantly influenced this parameter in mahogany seedlings (Table 12). For JA, the highest stomatal conductance was recorded in T5 (0.8 mM), peaking to $0.0758 \text{ mol m}^{-2} \text{ s}^{-1}$ at 30 days and declining to $0.0265 \text{ mol m}^{-2} \text{ s}^{-1}$ by 150 days. In contrast, the control group (T1) exhibited the lowest conductance, starting from $0.0023 \text{ mol m}^{-2} \text{ s}^{-1}$ and increasing to $0.0120 \text{ mol m}^{-2} \text{ s}^{-1}$. Intermediate JA concentrations (0.6 mM, T4, and 1.0 mM, T6) significantly

Table 10 Effect of exogenous application of JA and SA on the Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$) of six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D
Jasmonic Acid						
T1 (control)	2.55	2.99 ^c	2.72 ^c	2.78 ^c	2.87 ^c	2.14 ^{bc}
T2 (0.2 mM)	2.72	3.30 ^{ab}	3.07 ^{ab}	3.14 ^b	3.24 ^b	2.01 ^c
T3 (0.4 mM)	2.69	3.11 ^{bc}	2.76 ^c	3.10 ^b	3.20 ^b	2.24 ^b
T4 (0.6 Mm)	2.46	3.43 ^a	3.23 ^a	3.67 ^a	3.78 ^a	2.65 ^a
T5 (0.8 mM)	2.85	3.29 ^{ab}	2.89 ^{bc}	2.96 ^{bc}	3.06 ^{bc}	2.27 ^b
T6 (1.0 mM)	2.74	3.37 ^{ab}	2.96 ^{bc}	3.07 ^b	3.17 ^b	2.22 ^b
CD	NS	0.273	0.247	0.262	0.271	0.189
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Salicylic Acid						
T1 (control)	2.54	3.13 ^c	2.30 ^c	2.50 ^c	2.66 ^c	2.53 ^c
T2 (0.2 mM)	2.39	3.41 ^{bc}	2.51 ^{bc}	2.73 ^{bc}	2.90 ^{bc}	2.75 ^{bc}
T3 (0.4 mM)	2.44	3.18 ^c	2.83 ^b	2.54 ^c	2.63 ^c	2.50 ^c
T4 (0.6 Mm)	2.19	3.10 ^c	2.28 ^c	2.48 ^c	2.70 ^c	2.57 ^c
T5 (0.8 mM)	2.27	3.84 ^b	3.31 ^a	3.07 ^b	3.27 ^b	3.64 ^a
T6 (1.0 mM)	2.42	4.50 ^a	2.34 ^c	3.60 ^a	3.83 ^a	3.10 ^b
CD	NS	0.616	0.453	0.492	0.524	0.497
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Significant at 0.05 levels; NS - non significant at 0.05 levels

Table 11 Effect of exogenous application of JA and SA on the Transpiration rate (mmol H₂O m²s⁻¹) of six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D
Jasmonic Acid						
T1 (control)	0.086	0.122 ^d	0.111 ^d	0.116 ^d	0.099 ^d	0.078
T2 (0.2 mM)	0.091	0.148 ^{bc}	0.135 ^{bc}	0.141 ^{bc}	0.120 ^{bc}	0.073
T3 (0.4 mM)	0.087	0.143 ^c	0.131 ^c	0.136 ^c	0.117 ^c	0.071
T4 (0.6 Mm)	0.094	0.178 ^a	0.163 ^a	0.170 ^a	0.145 ^a	0.076
T5 (0.8 mM)	0.084	0.160 ^b	0.146 ^b	0.152 ^b	0.130 ^b	0.079
T6 (1.0 mM)	0.086	0.153 ^{bc}	0.140 ^{bc}	0.146 ^{bc}	0.124 ^{bc}	0.078
CD	NS	0.011	0.011	0.011	0.009	NS
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	>0.05
Salicylic Acid						
T1 (control)	0.091	0.113 ^d	0.095 ^b	0.110 ^c	0.119 ^d	0.101 ^d
T2 (0.2 mM)	0.097	0.124 ^{cd}	0.096 ^b	0.111 ^c	0.130 ^c	0.110 ^{cd}
T3 (0.4 mM)	0.093	0.126 ^c	0.093 ^b	0.115 ^c	0.171 ^a	0.112 ^c
T4 (0.6 Mm)	0.091	0.123 ^{cd}	0.095 ^b	0.128 ^b	0.133 ^{bc}	0.110 ^{cd}
T5 (0.8 mM)	0.092	0.177 ^a	0.122 ^a	0.142 ^a	0.142 ^b	0.158 ^a
T6 (1.0 mM)	0.095	0.154 ^b	0.114 ^a	0.132 ^{ab}	0.137 ^{bc}	0.137 ^b
CD	NS	0.012	0.009	0.010	0.011	0.011
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Significant at 0.05 levels; NS - non significant at 0.05 levels

Table 12 Effect of exogenous application of JA and SA on the stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) of mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D
Jasmonic Acid						
T1 (control)	0.0023	0.0152 ^d	0.0147 ^{abc}	0.0030 ^c	0.0025 ^b	0.0120
T2 (0.2 mM)	0.0030	0.0194 ^{cd}	0.0088 ^{abc}	0.0062 ^c	0.0028 ^b	0.0053
T3 (0.4 mM)	0.0026	0.0335 ^c	0.0017 ^{bc}	0.0075 ^c	0.0026 ^b	0.0034
T4 (0.6 mM)	0.0045	0.0559 ^b	0.0168 ^{ab}	0.0031 ^c	0.0326 ^a	0.0168
T5 (0.8 mM)	0.0021	0.0758 ^a	0.0211 ^a	0.0271 ^b	0.0212 ^a	0.0265
T6 (1.0 mM)	0.0036	0.0051 ^d	0.0002 ^c	0.0626 ^a	0.0231 ^a	0.0084
CD	NS	0.017	NS	0.015	0.013	NS
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	>0.05
Salicylic Acid						
T1 (control)	0.0026	0.0057 ^c	0.0145 ^c	0.0253 ^c	0.0025 ^b	0.0028
T2 (0.2 mM)	0.0030	0.0055 ^c	0.0071 ^c	0.0027 ^d	0.0026 ^b	0.0037
T3 (0.4 mM)	0.0023	0.0052 ^c	0.0058 ^c	0.0027 ^d	0.0028 ^b	0.0041
T4 (0.6 mM)	0.0045	0.0277 ^b	0.0335 ^b	0.0335 ^c	0.0326 ^a	0.0037
T5 (0.8 mM)	0.0021	0.0787 ^a	0.0677 ^a	0.0758 ^a	0.0231 ^a	0.0040
T6 (1.0 mM)	0.0036	0.0264 ^b	0.0224 ^{bc}	0.0559 ^b	0.0212 ^a	0.0038
CD	NS	0.018	0.016	0.016	0.013	NS
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	>0.05

Significant at 0.05 levels; NS - non significant at 0.05 levels

improved. Similarly, for SA, T5 (0.8 mM) demonstrated the highest conductance of $0.0787 \text{ mol m}^{-2} \text{ s}^{-1}$ at 30 days and decreased to $0.0231 \text{ mol m}^{-2} \text{ s}^{-1}$ at 120th days. The control group showed the lowest values, while other SA treatments yielded intermediate results. Overall, both JA and SA at optimal concentrations effectively enhanced stomatal conductance, thereby improving gas exchange and metabolic efficiency in the seedlings.

4.1.2.4 Leaf temperature ($^{\circ}\text{C}$):

Leaf temperature reflects the adaptive responses of plants to environmental conditions. There were no significant differences among the treatments for the leaf temperature of mahogany seedlings over most of the entire evaluation period (Table 13).

4.1.2.5 Relative Water Content (%):

Relative Water Content (RWC) was significantly influenced by defense hormones JA and SA, indicating variations in plant hydration and health. High RWC is crucial for cell turgor, photosynthesis, and overall growth. In JA treatments, T4 (0.6 mM) recorded the highest RWC at 80.62% at 30 days, while the control (T1) declined to 60.23% by 150 days (Table 14). Other JA treatments varied, T2 (0.2 mM) peaked at 74.50%, T3 (0.4 mM) reached 79.21%, T5 (0.8 mM) peaked at 73.94%, and T6 (1.0 mM) peaked at 75.77%. For SA treatments, T5 (0.8 mM) achieved the highest RWC at 86.68% at 30 days, maintaining 65.55% by 150 days, whereas T1 reached to 56.05%. Other SA treatments included T2 (0.2 mM) at 72.12%, T3 (0.4 mM) at 74.10%, T4 (0.6 mM) at 73.23%, and T6 (1.0 mM) at 78.06%. These results highlight SA's effectiveness in enhancing plant hydration.

4.1.2.5 Total chlorophyll content (mg g^{-1}):

Total chlorophyll content is a vital indicator of plant health and photosynthetic efficiency. Significant variations in chlorophyll levels were observed in response to defense hormones (Table 15). In JA treatments, T4 (0.6 mM) had the highest chlorophyll content, peaking at 4.448 mg g^{-1} at 90 days, while T3 (0.4 mM) had the lowest at 2.576 mg g^{-1} by 150 days. The control (T1) showed 3.746 mg g^{-1} at 90 but declined to 2.809 mg g^{-1} by 150 days. T5 (0.8 mM) showed to 4.070 mg g^{-1} by 90, ending at 2.775 mg g^{-1} , and T6 (1.0 mM) at 3.897 mg g^{-1} , dropping to 2.768 mg g^{-1} . For SA treatments, T1 showed at 3.181 mg g^{-1} on 30 days but decreased to 2.556 mg g^{-1} by 150 days. T5 (0.8 mM) showed the highest chlorophyll content of 3.704 mg g^{-1} at 30 days, ending at 3.159

Table 13 Effect of exogenous application of JA and SA on the leaf temperature ($^{\circ}\text{C}$) of six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D
Jasmonic Acid						
T1 (control)	35.29	33.99 ^c	35.79	37.37	36.24	33.99 ^c
T2 (0.2 mM)	36.07	35.00 ^{bc}	36.57	38.15	37.02	35.00 ^{bc}
T3 (0.4 mM)	36.64	36.60 ^a	36.64	38.22	37.09	36.60 ^a
T4 (0.6 Mm)	36.61	35.81 ^{ab}	36.61	38.19	37.06	35.81 ^{ab}
T5 (0.8 mM)	37.13	36.23 ^{ab}	37.13	38.71	37.58	36.23 ^{ab}
T6 (1.0 mM)	37.00	36.89 ^a	37.00	38.58	37.45	36.89 ^a
CD	NS	1.242	NS	NS	NS	1.242
P- Value	>0.05	<0.05	>0.05	>0.05	>0.05	<0.05
Salicylic Acid						
T1 (control)	38.13	38.13 ^a	37.38	36.58	37.03	38.39
T2 (0.2 mM)	38.02	38.03 ^a	36.90	36.10	36.55	37.91
T3 (0.4 mM)	37.75	37.20 ^{ab}	36.81	36.01	36.46	37.82
T4 (0.6 Mm)	37.71	37.04 ^{abc}	36.77	35.97	36.42	37.78
T5 (0.8 mM)	37.67	36.19 ^{bc}	36.70	35.90	36.35	37.71
T6 (1.0 mM)	37.20	35.95 ^c	36.48	35.68	36.13	37.49
CD	NS	1.11	NS	NS	NS	NS
P- Value	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05

Significant at 0.05 levels; NS - non-significant at 0.05 levels

Table 14 Effect of exogenous application of JA and SA on the relative water content (%) in six-month-old mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D
		Jasmonic Acid				
T1 (control)	54.56	63.96 ^b	58.30 ^c	62.44 ^b	64.77 ^c	60.23 ^d
T2 (0.2 mM)	52.89	74.50 ^a	64.52 ^{ab}	73.20 ^a	69.76 ^{bc}	65.25 ^{bcd}
T3 (0.4 mM)	57.44	79.21 ^a	66.46 ^a	70.23 ^a	72.28 ^{ab}	66.85 ^{abc}
T4 (0.6 Mm)	58.89	80.62 ^a	67.78 ^a	73.14 ^a	76.74 ^a	72.07 ^a
T5 (0.8 mM)	57.11	73.94 ^a	60.23 ^{bc}	67.61 ^a	72.71 ^{ab}	69.17 ^{ab}
T6 (1.0 mM)	57.00	75.77 ^a	66.69 ^a	68.22 ^a	69.97 ^{bc}	63.65 ^{cd}
CD	NS	7.401	4.857	5.636	5.726	4.969
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Salicylic Acid				
T1 (control)	62.90	69.38 ^c	58.97 ^d	63.51 ^c	56.46 ^b	56.05 ^b
T2 (0.2 mM)	64.18	72.12 ^{bc}	61.30 ^{cd}	64.87 ^c	57.67 ^b	55.36 ^b
T3 (0.4 mM)	67.00	74.10 ^{bc}	67.91 ^{bc}	74.70 ^b	66.41 ^a	63.75 ^a
T4 (0.6 Mm)	65.21	73.23 ^{bc}	64.01 ^{bcd}	65.67 ^c	58.38 ^b	54.20 ^b
T5 (0.8 mM)	69.69	86.68 ^a	78.01 ^a	83.27 ^a	68.28 ^a	65.55 ^a
T6 (1.0 mM)	69.69	78.06 ^b	68.84 ^b	75.72 ^b	67.31 ^a	64.62 ^a
CD	NS	7.502	6.615	7.175	6.333	6.080
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Significant at 0.05 levels; NS - non-significant at 0.05 levels

Table 15 Effect of exogenous application of JA and SA on the total chlorophyll content (mg/g) of mahogany seedlings

Days/ Treatments	Initial	30 D	60 D	90 D	120 D	150 D
Jasmonic Acid						
T1 (control)	3.121	3.434 ^{bcd}	2.747 ^c	3.478 ^{cd}	3.746	2.809
T2 (0.2 mM)	2.941	3.294 ^{cd}	2.635 ^{cd}	3.626 ^{bcd}	3.529	2.647
T3 (0.4 mM)	2.863	3.206 ^d	2.565 ^d	3.386 ^d	3.435	2.576
T4 (0.6 Mm)	3.191	4.212 ^a	3.370 ^a	4.448 ^a	3.830	2.872
T5 (0.8 mM)	3.084	3.855 ^{ab}	3.084 ^{ab}	4.070 ^{ab}	3.700	2.775
T6 (1.0 mM)	3.075	3.690 ^{bc}	2.952 ^{bc}	3.897 ^{bc}	3.690	2.768
CD	NS	0.426	0.341	0.450	NS	NS
P- Value	>0.05	<0.05	<0.05	<0.05	>0.05	>0.05
Salicylic Acid						
T1 (control)	2.840	3.181 ^{bc}	2.668 ^c	3.171 ^{bc}	3.025	2.556 ^c
T2 (0.2 mM)	2.676	2.997 ^c	2.831 ^{bc}	2.988 ^c	2.850	2.713 ^{bc}
T3 (0.4 mM)	2.605	3.152 ^{bc}	2.805 ^{bc}	3.142 ^{bc}	2.774	2.688 ^{bc}
T4 (0.6 Mm)	2.904	3.427 ^{ab}	3.050 ^{ab}	3.416 ^{ab}	3.093	2.923 ^{ab}
T5 (0.8 mM)	2.806	3.704 ^a	3.297 ^a	3.692 ^a	2.988	3.159 ^a
T6 (1.0 mM)	2.798	3.414 ^{ab}	3.038 ^{ab}	3.403 ^{ab}	2.980	2.912 ^{ab}
CD	NS	0.338	0.300	0.337	0.287	0.288
P- Value	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Significant at 0.05 levels; NS - non-significant at 0.05 levels

mg g⁻¹. Overall, SA T5 (0.8 mM) enhanced and maintained chlorophyll content most effectively, followed by T4 and T6.

4.1.3 Hierarchical Clustering of SA and JA treatment groups:

The hierarchical clustering performed under the given treatment groups, and organized based on their similarities or dissimilarities, with interclass distances used to indicate the degree of relatedness between treatments. Smaller distances suggest that the treatments within those clusters are more closely related, while larger distances reflect greater dissimilarity.

Under the SA and JA treatments, hierarchical clustering revealed three distinct clusters. JA treatment T4 (0.6 mM) formed an individual cluster with a considerable interclass distance, indicating a significantly different response from the other treatments. Similarly, the control treatment T1 was placed in a separate cluster (Cluster 2) but with a smaller interclass distance than T4, suggesting a moderate similarity with the remaining treatments. Cluster 3 included T2 (0.2 mM), T3 (0.4 mM), and T6 (1.0 mM), indicating that these treatments produced more similar responses. Treatment T5 (0.8 mM) formed a distinct cluster with a larger interclass distance, highlighting a unique response compared to other treatments (Fig 04).

In the second dendrogram for SA (Fig.5), the control treatment T1 formed a separate cluster (Cluster 1), with a noticeable interclass distance distinguishing it from the other treatments. T5 (0.8 mM) formed Cluster 2, indicating its response remained distinct under this condition. Treatments T2 (0.2 mM), T3 (0.4 mM), T4 (0.6 mM), and T6 (1.0 mM) were grouped in Cluster 3, with smaller interclass distances between them, suggesting more similar responses compared to T1 and T5. Overall, the interclass distances in both dendrograms highlighted that some treatments, such as T2, T3, T4, and T6, exhibited similar response patterns. In contrast, T1 and T5, showed distinct reactions, reflecting varying degrees of dissimilarity among the treatments.

4.1.4 PCA and Selection Index:

PCA was conducted to develop a selection index and score plant performance under different treatments. Individual PCA was performed for physiological and morphological parameters separately, with the scoring based on maintaining control conditions as a baseline for the index. Following this, a combined PCA was performed

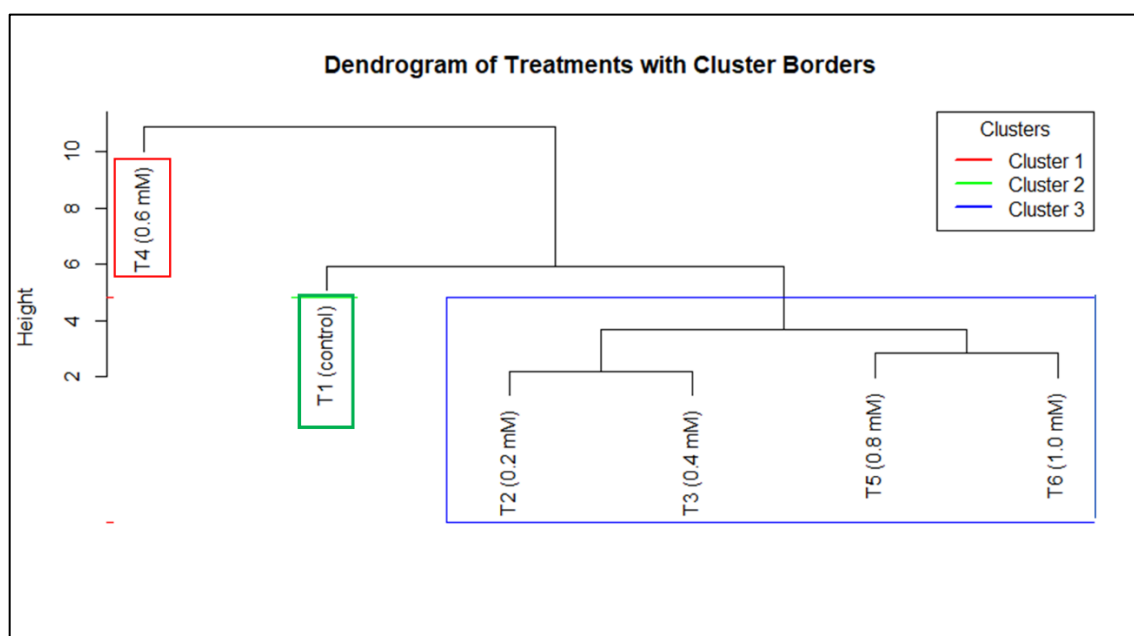


Fig 4. Dendrogram of hierarchical clustering based on morphological and physiological parameters in JA treatment groups

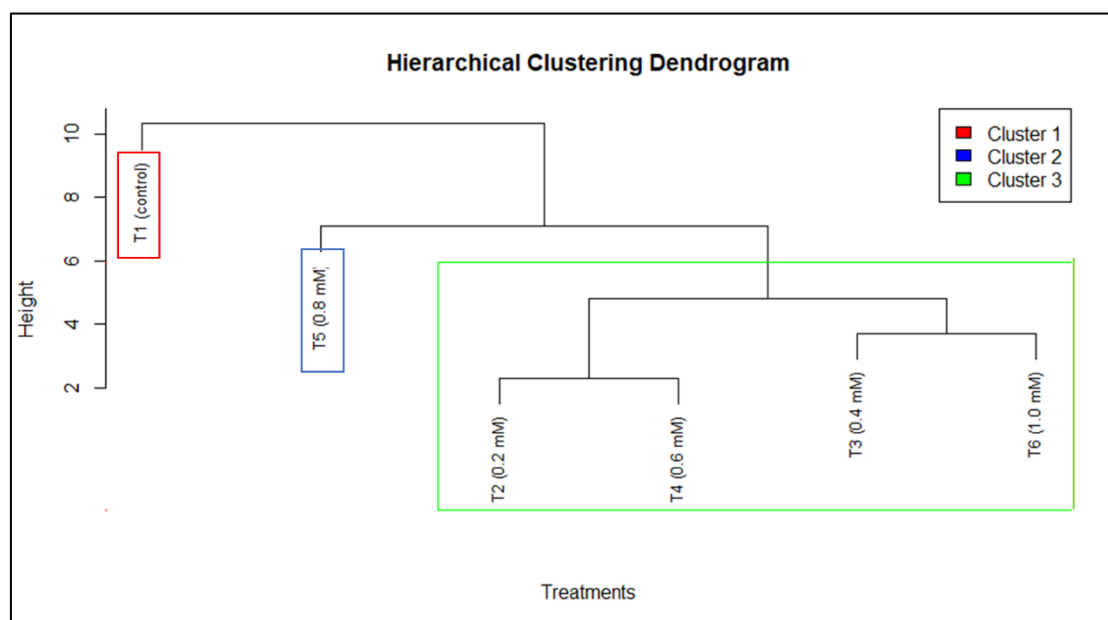


Fig 5. Dendrogram of hierarchical clustering based on morphological and physiological parameters in SA treatment groups

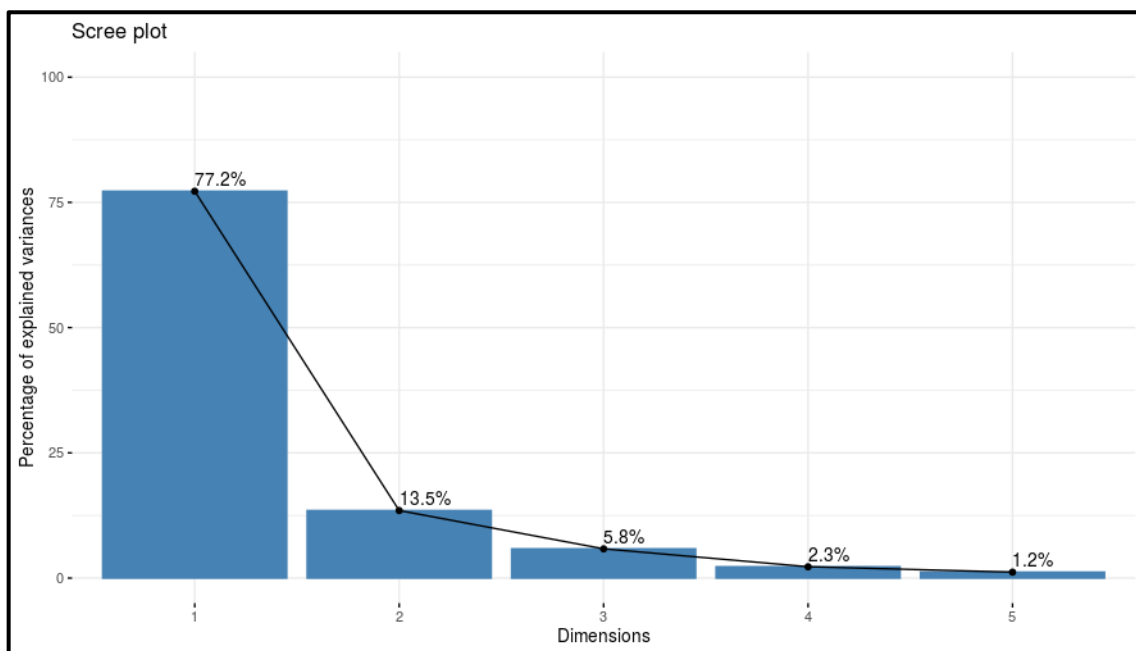


Fig 6. Scree plot depicting the variance explained by each principal component for morpho-physiological parameters under JA treatment

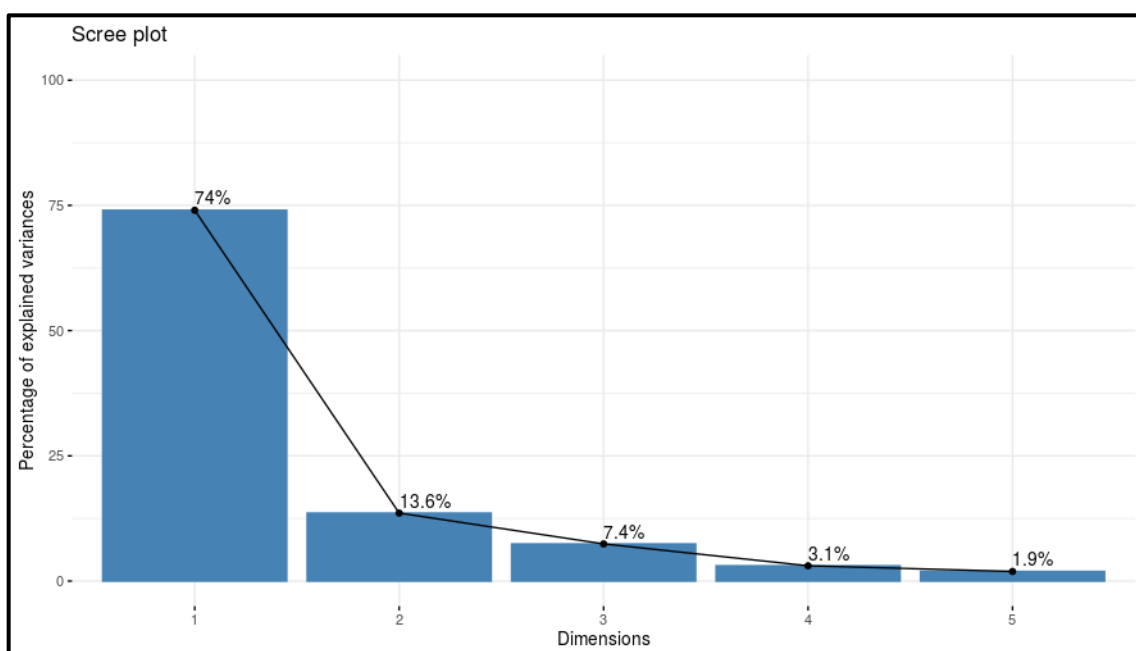


Fig 7. Scree plot depicting the variance explained by each principal component for morpho-physiological parameters under SA treatment

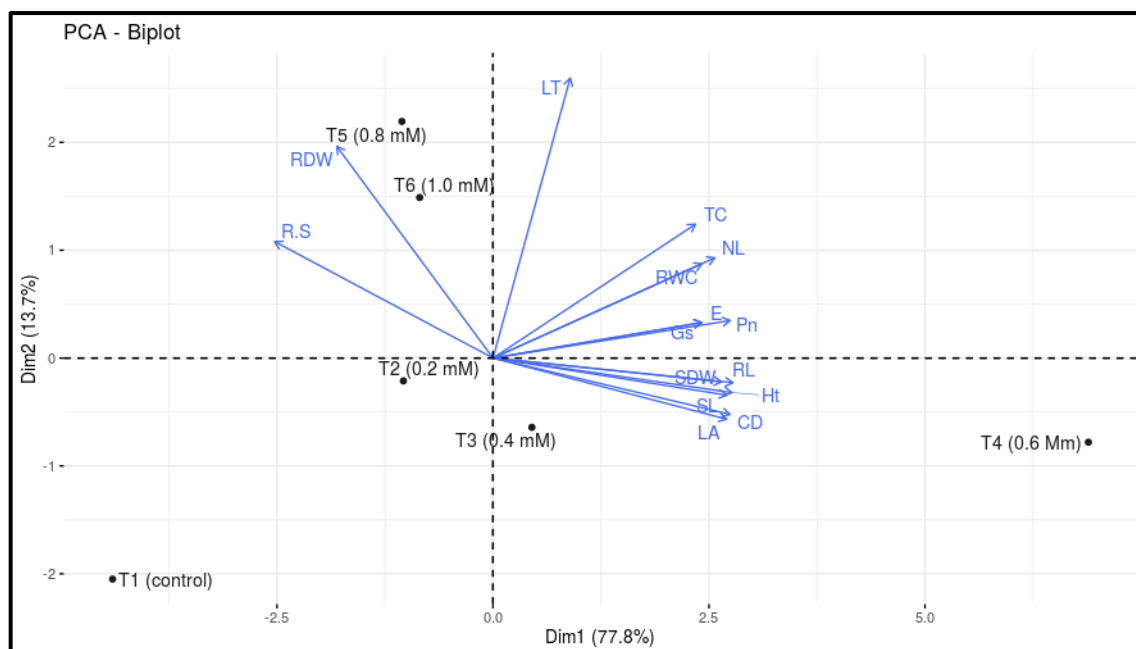


Fig 8. Biplot depicting the distribution of different JA treatments derived from PCA

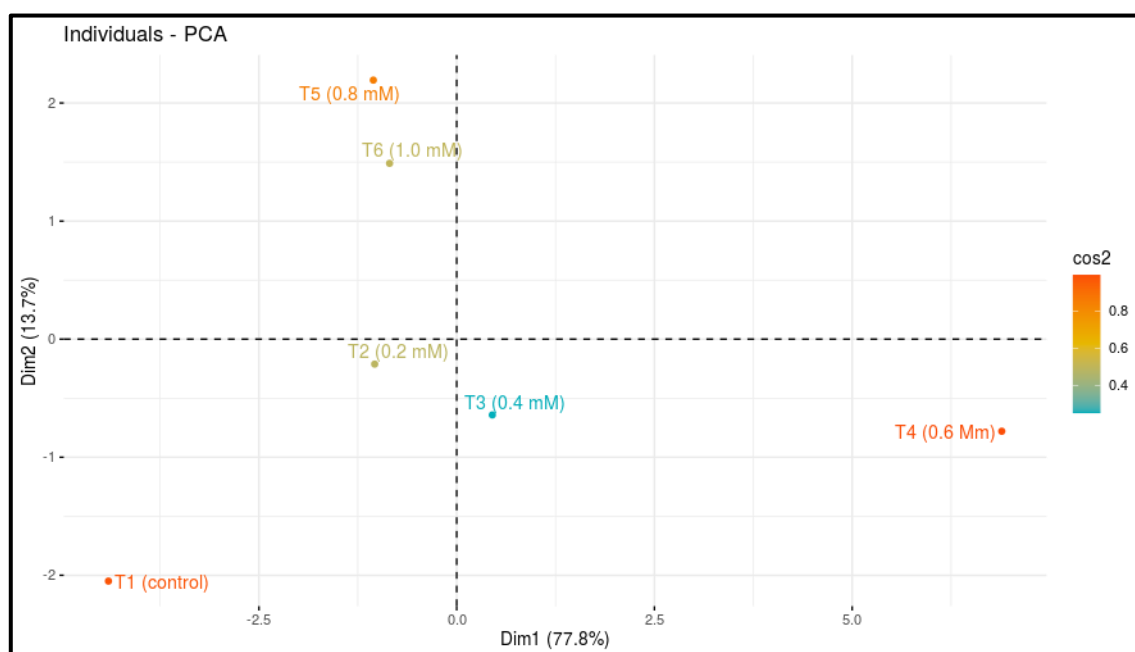


Fig 9. Individual PCA of different JA treatment groups in mahogany seedlings

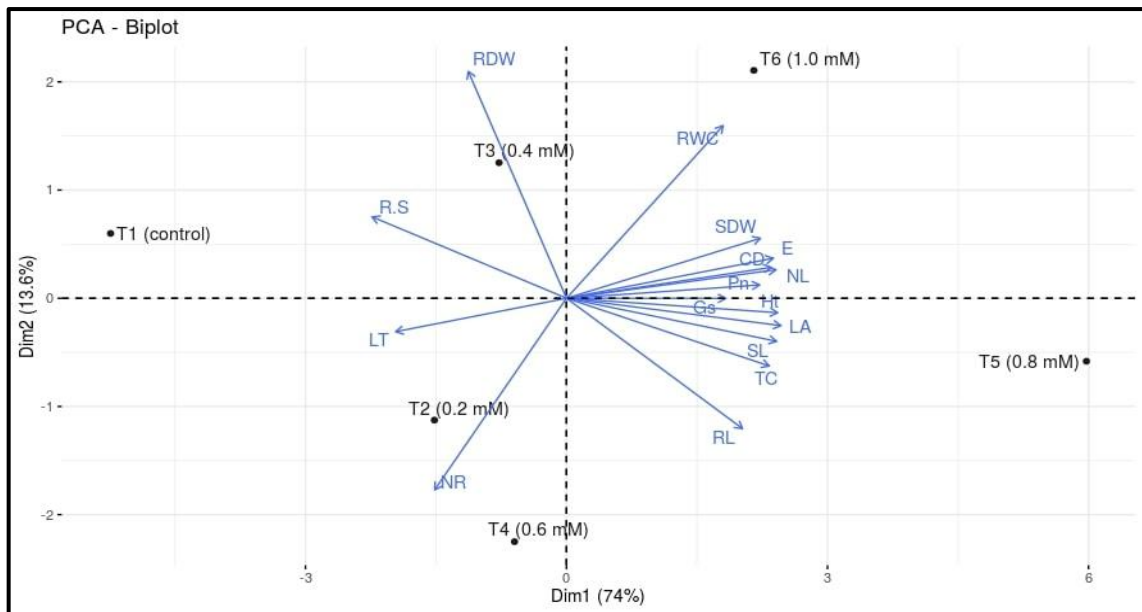


Fig 10. Biplot depicting the distribution of different SA treatments derived from PCA

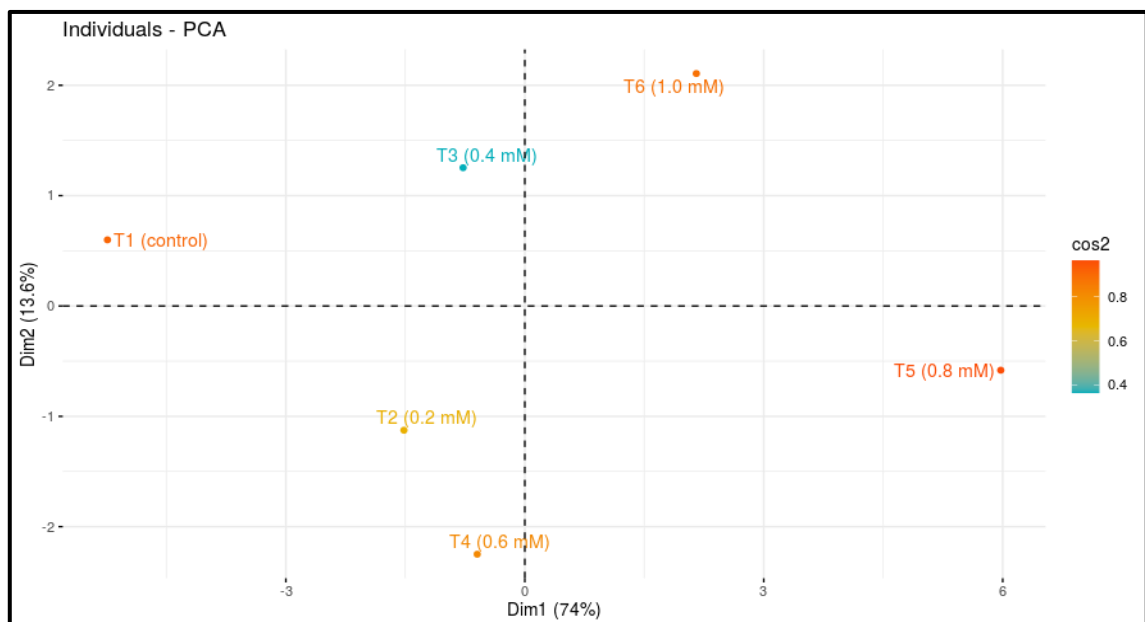


Fig 11. Individual PCA of different SA treatment groups in mahogany seedlings

using the scores from both analyses, and final scoring was applied to rank plant performance under JA and SA treatments.

Among JA treatment groups, six principal components were formed to explain the variation of the physiological parameters, as presented. Based on the cumulative variance, two principal components (PC1 and PC2) were selected for further analysis. PC1 explained 77.76% of the total variation, while PC2 explained 13.66%, resulting in a cumulative variance of 91.42% (Fig 6). The loading values of variables, which reflect their contribution to each principal component, varied across components. For instance, photosynthetic rate (Pn) had loading values of 0.286 for PC1 and -0.087 for PC2, while stomatal conductance (Gs) had values of 0.252 for PC1 and -0.082 for PC2. In terms of variable contributions, root length (RL), height (Ht), shoot dry weight (SDW), and photosynthetic rate (Pn) contributed most to PC1, indicating that these parameters were key drivers of plant response to JA. Meanwhile, leaf temperature (LT) and root dry weight (RDW) were more influential in PC2, reflecting the plant's thermal regulation and biomass allocation under JA treatment. Considering the cumulative variance for physiological and morphological parameters, PC1 and PC2 were selected to score plant performance under various JA treatments. This final scoring system ranked the treatments based on their effects on significant growth and physiological traits, clearly understanding plant response to JA.

The physiological parameters of the SA treatment groups and six principal components were generated to explain the variation. Based on the cumulative proportion of variance, PC1 and PC2 were selected for further analysis. PC1 explained 74.02% of the total variation, and PC2 explained an additional 13.57%, resulting in a cumulative variance of 87.60% (Fig 7). The loadings of each component variable varied, reflecting their contribution to the variance. The photosynthetic rate (Pn) had loading values of 0.259 for PC1 and 0.034 for PC2, while stomatal conductance (Gs) had 0.214 for PC1 and 0 for PC2. In terms of variable contribution, leaf area (LA), height (Ht), shoot length (SL), and number of leaves (NL) contributed most to PC1, indicating that SA heavily influenced these traits. On the other hand, root dry weight (RDW) and relative water content (RWC) had the highest contributions to PC2, highlighting the impact of SA on root biomass and water management. Based on the cumulative variance from physiological and morphological parameters, PC1 and PC2 were used to score plant performance under SA treatments. This scoring method allowed for ranking plant

responses, revealing that key physiological traits such as water retention, biomass allocation, and growth were central to the plant's overall response to SA application.

4.2 ABIOTIC STRESS ADAPTATION MECHANISM IN MAHOGANY SEEDLINGS

4.2.1 Morphological adaptations of mahogany seedlings to abiotic stress:

The morphological parameters like leaf retention and the number of epicormic shoot production during the stress and recovery phase were explored, and results are presented in Table. 16.

As summarized in the Table 16, leaf retention exhibited significant variation across treatments under normal irrigation, drought, salinity stress (100 mM), and recovery conditions. Under normal irrigation, the control group maintained the highest leaf retention with an average of 37 leaves. However, leaf retention dropped significantly during drought stress to an average of 20 leaves, while salinity-stressed plants retained 28 leaves. In the recovery phase, plants without defense hormones showed the lowest leaf retention, with drought-stressed plants retaining only 17 leaves (45.92% of the initial count) and salinity-stressed plants retaining 14 leaves (37.99%) shown in Fig. 12.

The application of defense hormones, Jasmonic Acid (JA) and Salicylic Acid (SA), significantly improved leaf retention. After drought stress, plants treated with 0.8 mM SA retained 20 leaves (59.35%), while those treated with 0.6 mM JA exhibited even better recovery, retaining 23 leaves (66.09%). In salinity-stressed conditions, SA treatment led to a retention of 19 leaves (54.73%), while JA treatment resulted in 22 leaves (64.61%).

Moreover, the production of epicormic shoots increased significantly during the recovery phase following stress (Table 16). Under drought stress, plants produced an average of 2 shoots, while salinity stress slightly increased to 1.67 shoots. During recovery, plants without defense hormones showed the lowest shoot production, with drought-stressed plants averaging 1.78 shoots and salinity-stressed plants producing 1.67 shoots. Notably, the application of SA significantly enhanced shoot production during the recovery phase, with drought-stressed plants treated with 0.8 mM SA producing 3 shoots. Plants treated with 0.6 mM JA showed remarkable recovery, producing 7 shoots. In salinity-stressed conditions, SA treatment produced 3 shoots, whereas JA treatment resulted in 6 shoots shown in Fig. 13.

4.2.2 Physiological and biochemical adaptations of mahogany seedlings to abiotic stress condition:

The physiological parameters measured under abiotic stress conditions demonstrated significant differences across treatments in recovery phases (Table 17). Under normal irrigation conditions, the photosynthetic rate was recorded at $3.574 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$, but this rate significantly decreased during drought stress ($2.016 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$) and further dropped under salinity stress ($1.406 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$). Following drought recovery, the application of defense hormones led to improved photosynthetic rates, with 0.6 mM (JA) increasing the rate to $2.985 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$, while 0.8 mM Salicylic Acid (SA) resulted in a moderate increase to $2.686 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$. In salinity-stressed plants, JA also enhanced the photosynthetic rate to $2.748 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$ during recovery, shown in Fig.14.

Stomatal conductance remained low under stress treatments, measuring $0.007 \text{ mol m}^{-2} \text{ s}^{-1}$ for drought and $0.006 \text{ mol m}^{-2} \text{ s}^{-1}$ for salinity stress. However, during recovery, stomatal conductance reached $0.121 \text{ mol m}^{-2} \text{ s}^{-1}$, particularly in the absence of defense hormones. Notably, recovery treatments with SA showed higher stomatal conductance ($0.091 \text{ mol m}^{-2} \text{ s}^{-1}$) than JA ($0.006 \text{ mol m}^{-2} \text{ s}^{-1}$), indicating a more favourable response with SA.

The transpiration rate followed a similar trend, starting at $1.063 \text{ mmol H}_2\text{O m}^2/\text{s}$ under normal conditions but decreasing significantly to $0.540 \text{ mmol H}_2\text{O m}^2/\text{s}$ under drought stress. After recovery, JA treatment during salinity stress resulted in the highest transpiration rate of $1.323 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$, while SA treatment during drought recovery also increased transpiration to $1.206 \text{ mmol H}_2\text{O m}^2/\text{s}$.

Relative water content (RWC) exhibited notable variation across stress treatments and recovery phases. Under normal irrigation, RWC was 74.77% , decreasing to 52.25% during drought stress. After drought recovery, the control group showed an RWC of 56.08% . The 0.8 mM SA treatment recorded RWC values of 76.44% (normal), 56.99% (stress), and 62.67% (recovery), while the 0.6 mM JA treatment had the highest RWC, with values of 78.84% (normal), 53.67% (stress), and 70.14% (recovery). Under salinity stress, the control group displayed an RWC of 73.66% (normal), 56.70% (stress), and 61.90% (recovery). For SA, RWC values were 72.90% (normal), 52.19% (stress), and 65.23% (recovery), while the JA treatment showed 75.48% (normal), 54.89% (stress),

Table 16 Effect of exogenous application of JA and SA on morphological parameter during drought and salinity stress conditions

Treatments	No of leaves			Percentage leaf retained (%)	Number of shoots produced	No of leaves			Percentage leaf retained (%)	Number of shoots produced
Drought stress						Salt stress				
	Normal	Stress	Regain		Regain	Normal	Stress	Regain		Regain
Control	36.46	20.41	12.74 ^b	34.94	1.78 ^b	36.46	27.71	13.85 ^c	37.99	1.67 ^b
SA(0.8mM)	34.00	19.04	16.18 ^a	47.59	2.89 ^b	33.90	25.76	18.55 ^b	54.73	3.44 ^b
JA(0.6mM)	35.11	19.66	19.20 ^a	54.69	6.89 ^a	34.49	26.21	22.28 ^a	64.61	6.22 ^a
P-value	NS	NS	0.00		0.00	NS	NS	0.00		0.00

Significant at 0.05 levels; NS - non-significant at 0.05 levels

Table 17 Effect of exogenous application of JA and SA on physiological parameters during drought and salinity stress conditions

Treatments	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$)			Stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$)			Transpiration rate ($\text{mmol H}_2\text{O m}^2\text{s}^{-1}$)			Leaf Temp ($^{\circ}\text{C}$)		
Drought stress												
	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain
Control	3.574	1.992	2.037 ^c	0.0335	0.0071 ^b	0.1211 ^a	0.540	1.034 ^a	0.206 ^b	33.52	37.12	33.61
SA(0.8mM)	3.443	2.016	2.686 ^b	0.0377	0.0079 ^{ab}	0.0908 ^a	0.607	0.964 ^{ab}	0.047 ^b	32.95	37.18	32.48
JA(0.6mM)	3.512	2.041	2.985 ^a	0.0224	0.0203 ^a	0.0060 ^b	0.565	0.864 ^b	0.712 ^a	33.47	37.57	32.96
P-value	NS	NS	0.000	NS	0.049	0.007	NS	0.042	0.000	NS	NS	NS
Salt stress												
Treatments	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain
Control	3.421	1.542	0.569 ^c	0.0315	0.0056	0.1211 ^a	0.034	0.005	0.012 ^b	34.42	35.12	34.91
SA(0.8mM)	3.430	1.406	2.164 ^b	0.0327	0.0058	0.0122 ^b	0.038	0.006	0.016 ^b	33.53	34.55	35.07
JA(0.6mM)	3.471	1.543	2.748 ^a	0.0324	0.0057	0.0165 ^b	0.022	0.005	0.121 ^a	33.82	35.07	34.81
P-value	NS	NS	0.000	NS	NS	0.000	NS	NS	0.000	NS	NS	NS

Significant at 0.05 levels; NS - non-significant at 0.05 levels

and 70.45% (recovery) as shown in Fig.17. Both SA and JA treatments significantly improved RWC, with JA demonstrating superior recovery under drought and salinity stress.

Leaf temperature remained relatively stable across treatments, ranging from 32.17°C to 33.53°C, with no significant differences observed. Overall, JA treatment consistently resulted in better recovery in photosynthetic rate, transpiration, and relative water content, indicating a more substantial response compared to SA. These results highlight the potential of JA to mitigate stress effects and enhance physiological recovery under abiotic stress conditions. Transpiration and relative water content indicate a more substantial response than SA, especially impact and enhance physiological stress recovery conditions. These results highlight the potential of JA to mitigate stress (Table 17).

The physiological parameters related to photosynthetic pigments and biochemical stress indicators exhibited significant variations across treatments and stress conditions (Table 18). Under normal irrigation, chlorophyll-a (Chl-a) content was recorded at 1.846 mg/g. In comparison, chlorophyll-b (Chl-b) was higher at 1.401 mg/g, resulting in a total chlorophyll content of 3.672 mg/g. During drought stress, Chl-a decreased to 1.263 mg/g, Chl-b dropped to 0.873 mg/g, and total chlorophyll fell to 2.872 mg/g. Further reductions were noted under salinity stress, with Chl-a at 0.942 mg/g, Chl-b at 0.990 mg/g, and total chlorophyll at 2.332 mg/g. Upon recovery, the application of defense hormones significantly enhanced chlorophyll content. After drought recovery, treatment with 0.6 mM JA restored Chl-a to 1.879 mg/g and Chl-b to 1.235 mg/g, resulting in a total chlorophyll content of 3.091 mg/g. Similarly, 0.8 mM SA treatment under drought recovery led to Chl-a levels of 1.549 mg/g and Chl-b levels of 1.100 mg/g, with total chlorophyll reaching 3.200 mg/g. For salinity stress recovery, JA treatment increased Chl-a to 1.378 mg/g and Chl-b to 1.391 mg/g, achieving a total chlorophyll content of 3.242 mg/g (Fig. 15).

Carotenoid content mirrored these trends, starting at 1.257 mg/g under normal conditions and declining to 0.795 mg/g under drought stress and 0.921 mg/g under salinity stress. During recovery, SA treatment under salinity regains yielded the highest carotenoid content (1.320 mg/g), while JA showed 1.080 mg/g during drought regain.

Table 18 Effect of exogenous application of defense hormones on photosynthetic pigment parameters during drought and salinity stress conditions in mahogany seedlings

Treatments	Chl-a (mg/g)			Chl-b (mg/g)			Carotenoids (mg/g)			Total Chl (mg/g)		
Drought stress												
	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain
Control	1.772	1.400	1.484 ^b	1.343	0.873	0.961 ^b	1.257	0.804	0.977	3.672	2.512 ^b	2.829 ^b
SA(0.8mM)	1.749	1.382	1.879 ^a	1.343	0.873	1.100 ^{ab}	1.274	0.892	1.177	3.460	2.641 ^b	3.200 ^a
JA(0.6mM)	1.694	1.338	1.686 ^{ab}	1.484	0.965	1.235 ^a	1.197	0.766	1.080	3.368	2.872 ^a	3.091 ^{ab}
P-value	NS	NS	0.023	NS	NS	0.018	NS	NS	NS	NS	0.002	NS
Salt stress												
Treatments	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain
Control	1.846	0.941	0.947 ^b	1.356	0.990	1.138 ^b	1.346	0.941	0.947 ^{ab}	3.754	2.153	2.476 ^c
SA(0.8mM)	1.764	0.900	1.134 ^{ab}	1.385	1.011	1.280 ^{ab}	1.264	0.900	1.234 ^a	3.628	2.190	2.847 ^b
JA(0.6mM)	1.822	0.929	1.264 ^a	1.401	1.023	1.391 ^a	1.222	0.929	1.264 ^a	3.618	2.332	3.242 ^a
P-value	NS	NS	0.007	NS	NS	0.002	NS	NS	0.002	NS	NS	0.000

Significant at 0.05 levels; NS - non-significant at 0.05 levels

Table 19 Effect of exogenous application of JA and SA on physiological parameters during drought and salinity stress conditions in mahogany seedlings

Treatments	RWC (%)			CSI (%)			MSI (%)			Proline (µg/g)		
Drought stress												
	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain
Control	74.77	52.25	56.08 ^b	64.80	54.43	54.88 ^b	65.78	52.22	58.11 ^b	1.878	3.857 ^a	2.454 ^a
SA(0.8mM)	76.44	56.99	62.67 ^b	67.27	56.51	67.58 ^a	66.89	52.56	63.44 ^b	1.820	3.450 ^b	1.502 ^b
JA(0.6mM)	78.84	53.67	70.14 ^a	65.28	54.84	65.04 ^a	66.67	54.67	66.11 ^a	1.971	3.648 ^{ab}	1.180 ^c
P-value	NS	NS	0.000	NS	NS	0.010	NS	NS	0.000	NS	0.003	0.000
Salt stress												
Treatments	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain	Normal	Stress	Regain
Control	73.66	56.70	61.90 ^b	67.50	52.44	59.00	61.78	52.22	58.11 ^b	2.067 ^{ab}	4.391	3.293 ^a
SA(0.8mM)	72.90	52.19	65.23 ^{ab}	70.08	53.56	60.25	63.89	52.56	63.44 ^a	1.872 ^b	4.268	2.774 ^b
JA(0.6mM)	75.48	54.89	70.45 ^a	68.00	55.22	62.13	63.67	54.67	66.11 ^a	2.303 ^a	4.176	2.297 ^c
P-value	NS	NS	0.013	NS	NS	NS	NS	NS	0.000	0.094	NS	0.000

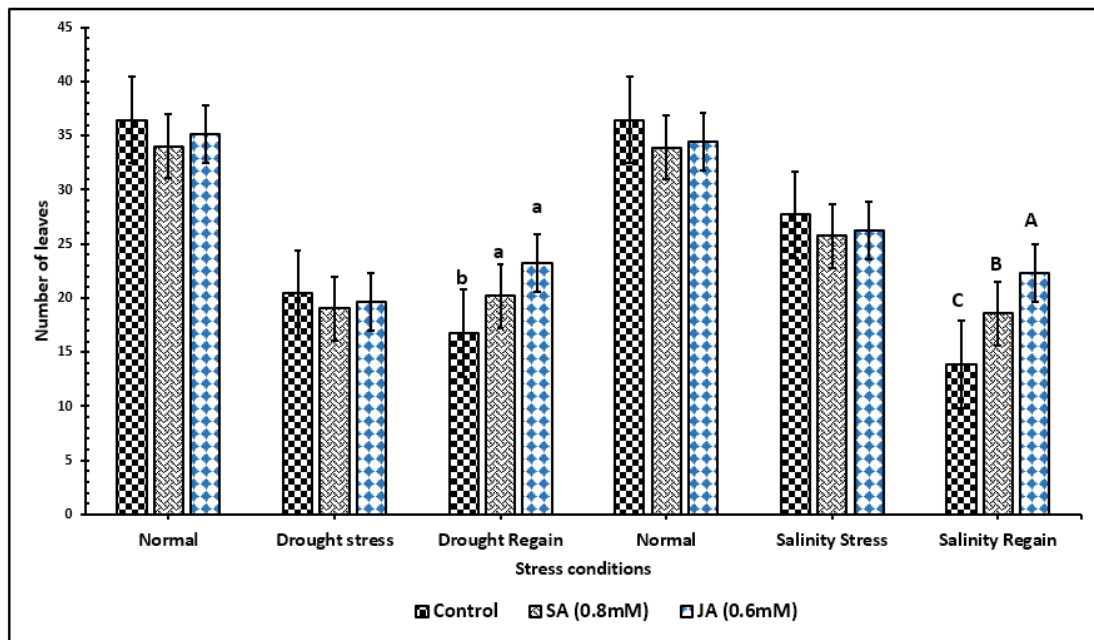


Fig 12. Variation in leaf retention across different treatments under drought and salinity stress conditions in mahogany seedlings

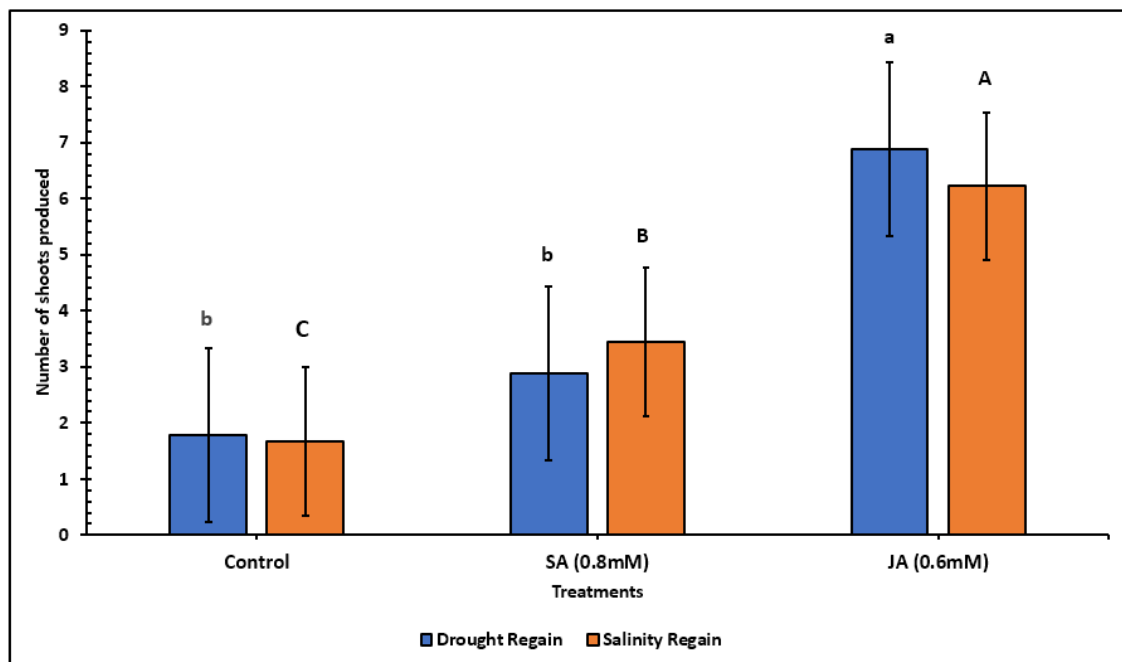


Fig 13. Variation in the number of shoots produced during regain condition in mahogany seedlings

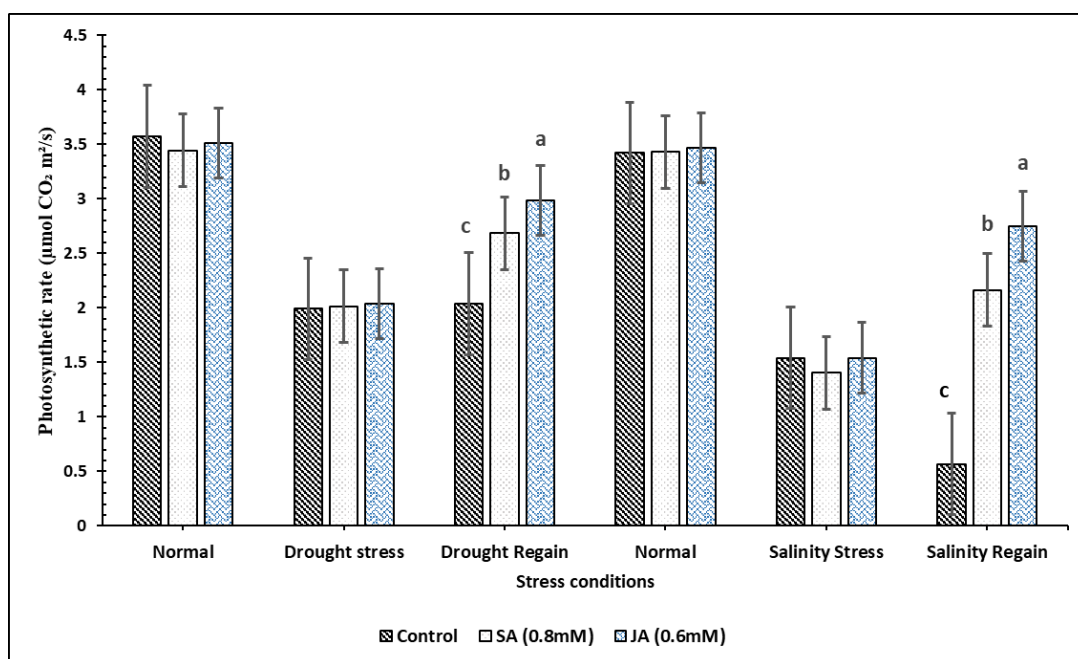


Fig 14. Variation in photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) across different treatments under drought and salinity stress conditions in mahogany seedlings

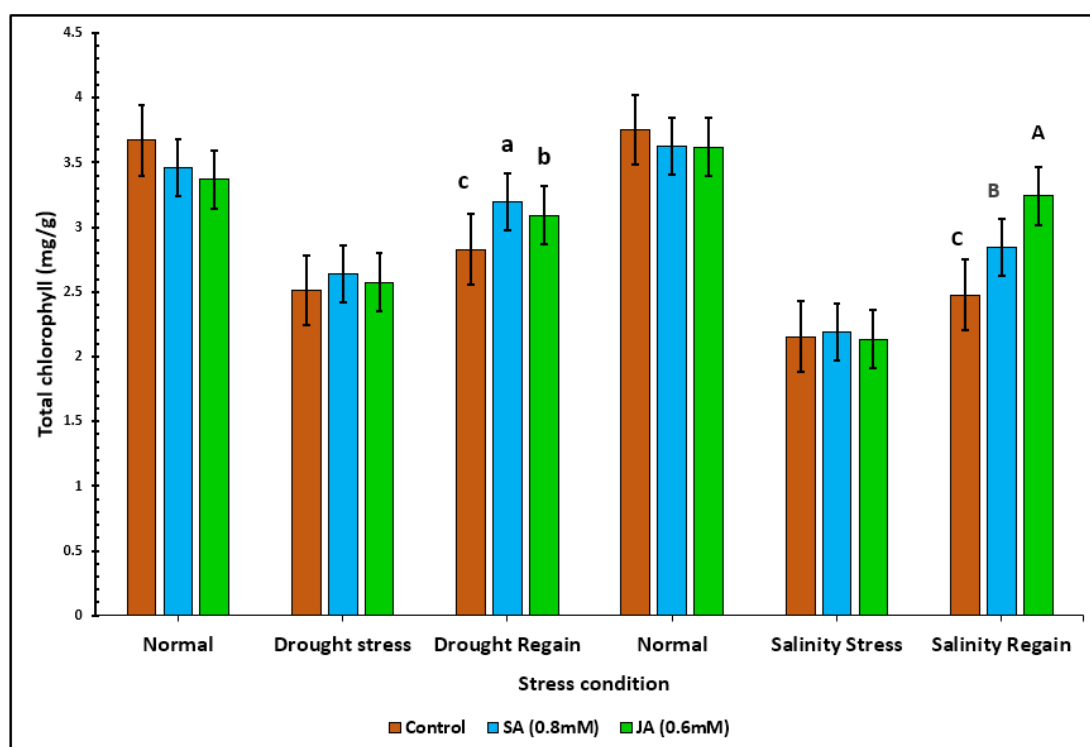


Fig 15. Variation in total chlorophyll content (mg/g) across different treatments under drought and salinity stress conditions in mahogany seedlings

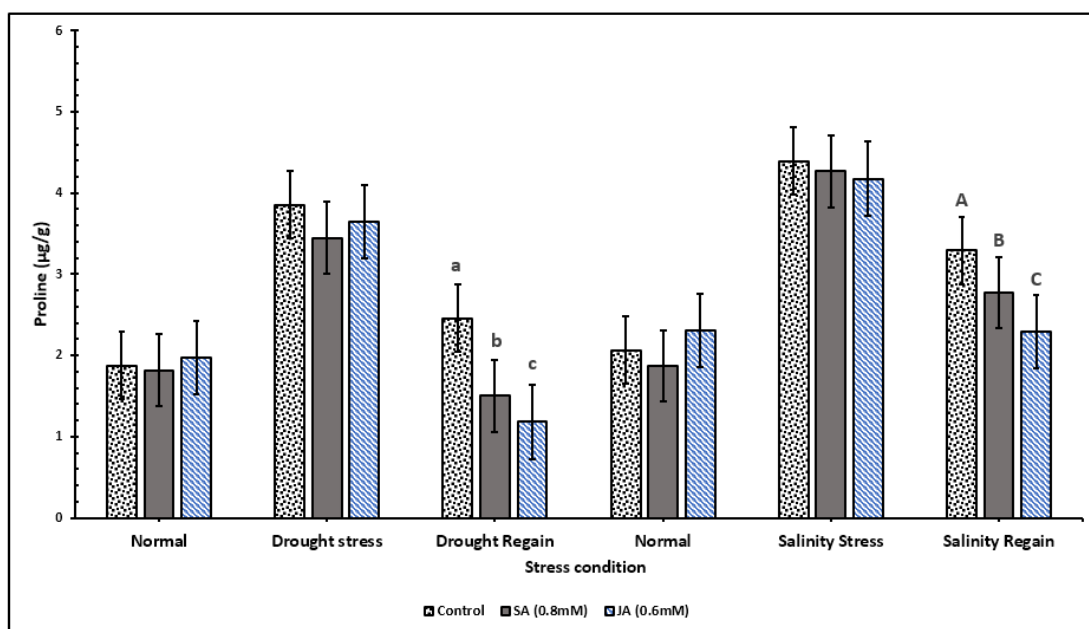


Fig 16. Variation in proline content ($\mu\text{g/g}$) across different treatments under drought and salinity stress conditions in mahogany seedlings

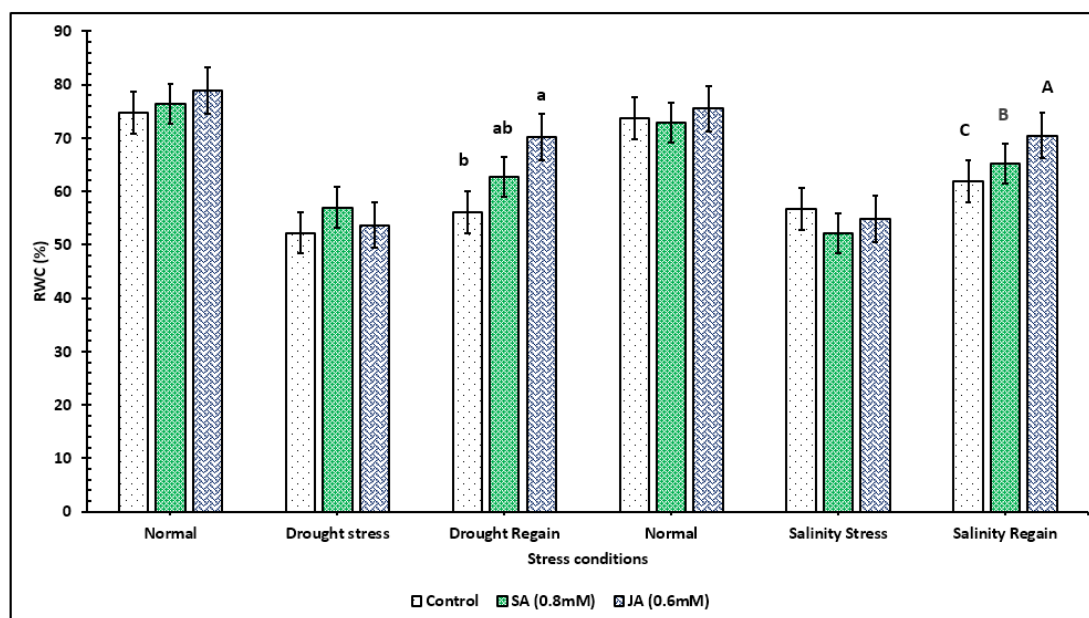


Fig 17. Variation in RWC (%) across different treatments under drought and salinity stress conditions in mahogany seedlings

The chlorophyll stability index (CSI) and membrane stability index (MSI) were also significantly affected. Normal conditions yielded a CSI of 67.27% and an MSI of 63.89%.

These values decreased to 49.25% and 52.22%, respectively, under drought stress. Recovery treatments showed improvements, with SA under drought regain achieving a CSI of 67.58% and an MSI of 63.44%. JA treatment also exhibited a strong response during drought recovery, with a CSI of 65.04% and the highest MSI of 66.11%. Proline accumulation, a key indicator of stress response, increased under stress conditions. Normal conditions recorded proline levels of 1.878 $\mu\text{g/g}$, which rose to 3.857 $\mu\text{g/g}$ during drought stress and peaked at 4.391 $\mu\text{g/g}$ under salinity stress (Fig 16). Recovery treatments indicated significant reductions in proline levels, with JA treatment reducing proline to 1.180 $\mu\text{g/g}$ during drought regain and SA leading to proline levels of 1.903 $\mu\text{g/g}$. These findings demonstrate that JA and SA treatments effectively restored chlorophyll content and stability indices while reducing proline accumulation, with JA exhibiting superior recovery effects, especially under drought conditions (Table 19).

4.2.3 Pearson Correlation during abiotic stress condition:

The Pearson correlation analysis under abiotic stress revealed significant relationships between key physiological and biochemical parameters, highlighting their influence on plant stress responses (Table 20, Fig.18). Chlorophyll a (Chl a) positively correlated with the photosynthetic rate (Pn), total chlorophyll, relative water content (RWC), and membrane stability index (MSI), indicating its vital role in photosynthesis and water balance under stress. In contrast, Chl a showed a strong negative correlation with proline, suggesting that higher proline levels coincide with reduced chlorophyll during stress. Chlorophyll b (Chl b) was positively correlated with carotenoids, RWC, and chlorophyll stability index (CSI), emphasizing its function in water retention and stress protection, while its negative correlation with proline indicates increased proline accumulation when Chl b decreases. Total chlorophyll strongly correlated with RWC and Pn, underscoring the importance of pigment levels in maintaining photosynthetic efficiency during stress. Proline, a key stress marker, is negatively correlated with most photosynthetic traits, suggesting its accumulation is linked to compromised photosynthetic functions. Carotenoids are positively associated with CSI and transpiration (E), indicating their protective role under stress.

Table 20. Pearson correlation coefficient for physiological and biochemical parameters during drought and salinity stress conditions

	Chl a	Chl b	Total chl	Carotenoids	Proline	Pn	GS	E	LT	RWC	CSI	MSI	Leaf retain
Chl a	1												
Chl b	0.46	1											
Total chl	0.86	0.63	1										
Carotenoids	0.36	0.87	0.56	1									
Proline	-0.87	-0.60	-0.75	-0.56	1								
Pn	0.89	0.57	0.93	0.51	-0.75	1							
GS	-0.14	-0.24	-0.15	-0.15	-0.10	-0.37	1						
E	0.52	0.71	0.66	0.74	-0.76	0.68	-0.03	1					
LT	0.17	0.12	0.24	-0.24	-0.21	0.03	0.27	0.02	1				
RWC	0.81	0.83	0.84	0.70	-0.84	0.86	-0.16	0.80	0.09	1			
CSI	0.70	0.77	0.71	0.81	-0.85	0.65	0.09	0.76	-0.06	0.84	1		
MSI	0.85	0.63	0.80	0.63	-0.98	0.76	0.09	0.74	0.20	0.81	0.86	1	
Leaf retain	0.49	0.40	0.54	0.28	-0.15	0.63	-0.51	0.16	-0.28	0.59	0.31	0.14	1

Significant at 0.05 levels;(P-value-<0.05)

Pn-Photosynthetic rate, GS- Stomatal conductance, E – Transpiration rate, LT- Leaf temperature, RWC- Relative water content, CSI- Chlorophyll stability index, MSI- Membrane stability index

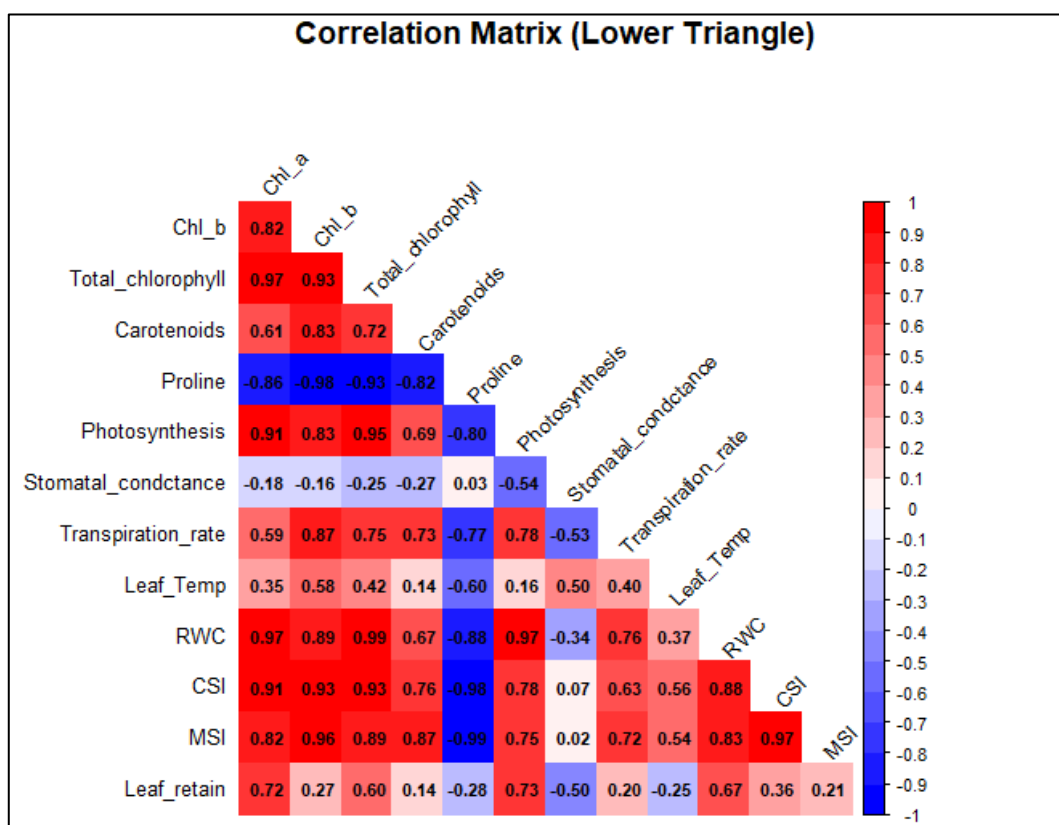


Fig 18. Correlation among morphological, physiological, and biochemical parameters under drought and salinity stress conditions in mahogany seedlings

Stomatal conductance (Gs) showed weaker correlations overall but had a notable negative relationship with leaf retention, emphasizing its role in maintaining leaf stability during stress. Transpiration (E) positively correlated with RWC and CSI, highlighting its role in water regulation and stress adaptation. In contrast, leaf temperature (LT) positively correlated with Gs, indicating its influence on stomatal regulation. Overall, parameters such as chlorophyll content, proline accumulation, RWC, and CSI emerged as key indicators of plant stress tolerance, providing insight into the mechanisms underlying plant responses to abiotic stress, particularly the importance of photosynthetic efficiency, water retention, and biochemical adjustments in stress adaptation.

4.3 PROTEOMIC ANALYSIS OF STRESS RESPONSE MECHANISMS INDUCED BY DEFENSE HORMONES:

A comparative proteomic analysis was conducted under normal, stress, and recovery conditions. Stress-induced seedlings treated with SA and JA were analyzed 48 hours after the application of defense hormones. Proteins were extracted using the TCA-acetone method and visualized by SDS-PAGE, revealed distinct protein expression patterns. These proteins were further analyzed using Peptide Mass Fingerprinting (PMF), and protein identification was carried out through MALDI-TOF MS. The functional annotation of the identified proteins offered valuable insights into the stress response mechanisms activated by defense hormones.

4.3.1 Visualization of proteins by SDS-PAGE:

SDS-PAGE analysis revealed distinct changes in protein expression across different treatments, highlighting the effects of stress and defense hormone application in mahogany seedlings (Fig.19,20). Under normal condition, the protein profile exhibited balanced expression patterns, with prominent bands around 50 kDa, 37 kDa, and 25 kDa representing the baseline protein profile. However, under stress conditions (salt and drought), protein bands, particularly low molecular weight proteins below 37 kDa, showed a marked reduction, indicating stress-induced suppression of specific proteins. In untreated seedlings during recovery, protein levels are partially restored, with fainter bands reappearing, reflecting incomplete recovery from stress. In contrast, SA treatment significantly enhanced protein expression, especially at 50 and 37 kDa protein bands, suggesting its role in stimulating defense-related proteins. JA treatment resulted in the most pronounced effect for stress recovery, increasing the expression of high molecular weight proteins, with intense bands around 50 kDa, implying activation of structural and defense-related proteins.

4.3.2 Peptide Mass Fingerprinting (PMF) and protein identification:

Proteins of interest were excised from SDS-PAGE gels, digested with trypsin, and analyzed using MALDI-TOF MS to determine the mass-to-charge (m/z) ratio of the resulting peptides. The generated spectrum displayed distinct peaks, each corresponding to a specific peptide. These m/z values were then matched against protein databases for identification using Mascot software from the NCBI database (Table 21). The mass

spectra facilitated precise protein identification, with detailed m/z values for each protein being summarised (Fig 21A,B).

4.3.3 Differential Protein Expression of mahogany seedlings to abiotic stress condition:

Under normal conditions, the protein profile exhibited balanced expression patterns, with prominent bands representing mid-molecular weight (35 kDa to 60 kDa) proteins constituting the baseline protein profile. Ten significant protein spots were identified, including D-3-phosphoglycerate dehydrogenase 1 (PGDH1) (0.219 mg/mL), which was the most abundant. Guanine nucleotide-binding protein alpha-1 (0.197 mg/mL) closely followed. Other proteins, such as ribulose biphosphate carboxylase (RuBISCO) (0.076 mg/mL), chlorophyll a-b binding protein (0.007 mg/mL), and pyruvate decarboxylase (0.001 mg/mL), exhibited normal expression levels. The total protein concentration was recorded at 0.631 mg/mL (Table 22), highlighting the critical role of these proteins in maintaining cellular homeostasis. A varied expression pattern was observed under stress conditions (drought and salt), marked by a significant reduction in lower molecular weight proteins, indicating membrane breakdown and a decline in photosynthesis and metabolic activity.

During drought stress, sixteen protein spots were identified for defense, metabolism, and photosynthesis, predominantly localized in the cytosol, plastids, and cell membranes. Heat shock protein 90 (HSP 90) (0.013 mg/mL), heat stress transcription factor A-4a (0.015 mg/mL), and sulfiredoxin (0.002 mg/mL) primarily enhance defense mechanisms during drought stress. Proteins such as DAHP synthase (0.108 mg/mL), D-3-phosphoglycerate dehydrogenase (0.045 mg/mL), and guanine nucleotide-binding protein alpha-1 (0.302 mg/mL) support metabolism and photorespiration. Photosynthesis and metabolism are maintained by ribulose biphosphate carboxylase (RuBISCO) (0.061 mg/mL), allene oxide synthase (0.002 mg/mL), pyruvate decarboxylase 3 (PDC3) (0.000 mg/mL), and chlorophyll a-b binding protein (0.003 mg/mL). Additionally, proteins like MEI2-like 7 protein (0.017 mg/mL) regulate gene expression and defense responses.

Similarly, during salt stress, eight proteins were identified (Table 23), with guanine nucleotide-binding protein alpha-1 being the most abundant at 0.302 mg/mL, followed by ribulose biphosphate carboxylase (RuBISCO) (0.137 mg/mL) and DAHP synthase (0.092 mg/mL). The total protein concentration decreased to 0.594 mg/mL,

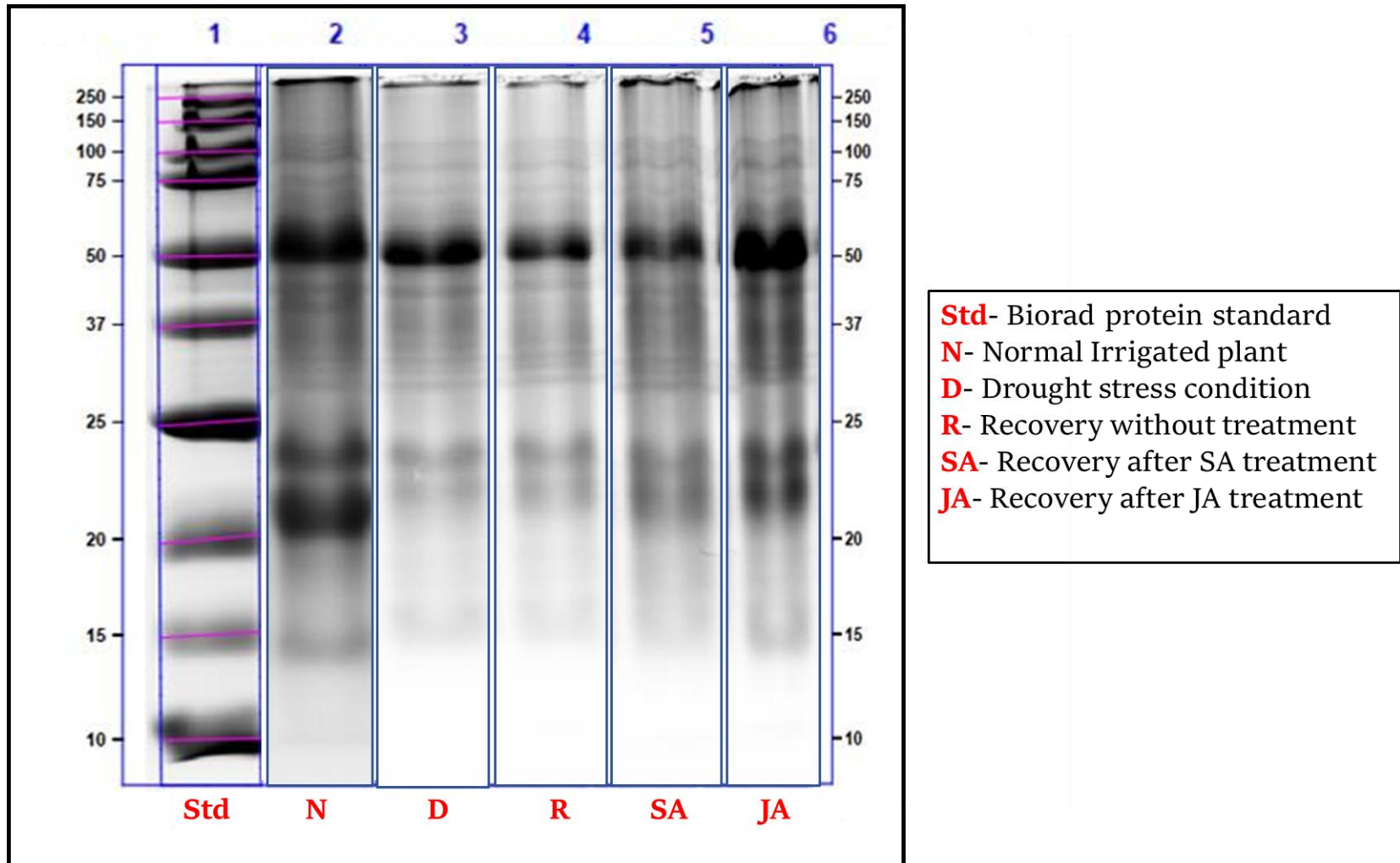


Fig 19. SDS-PAGE protein bands for different conditions under drought stress

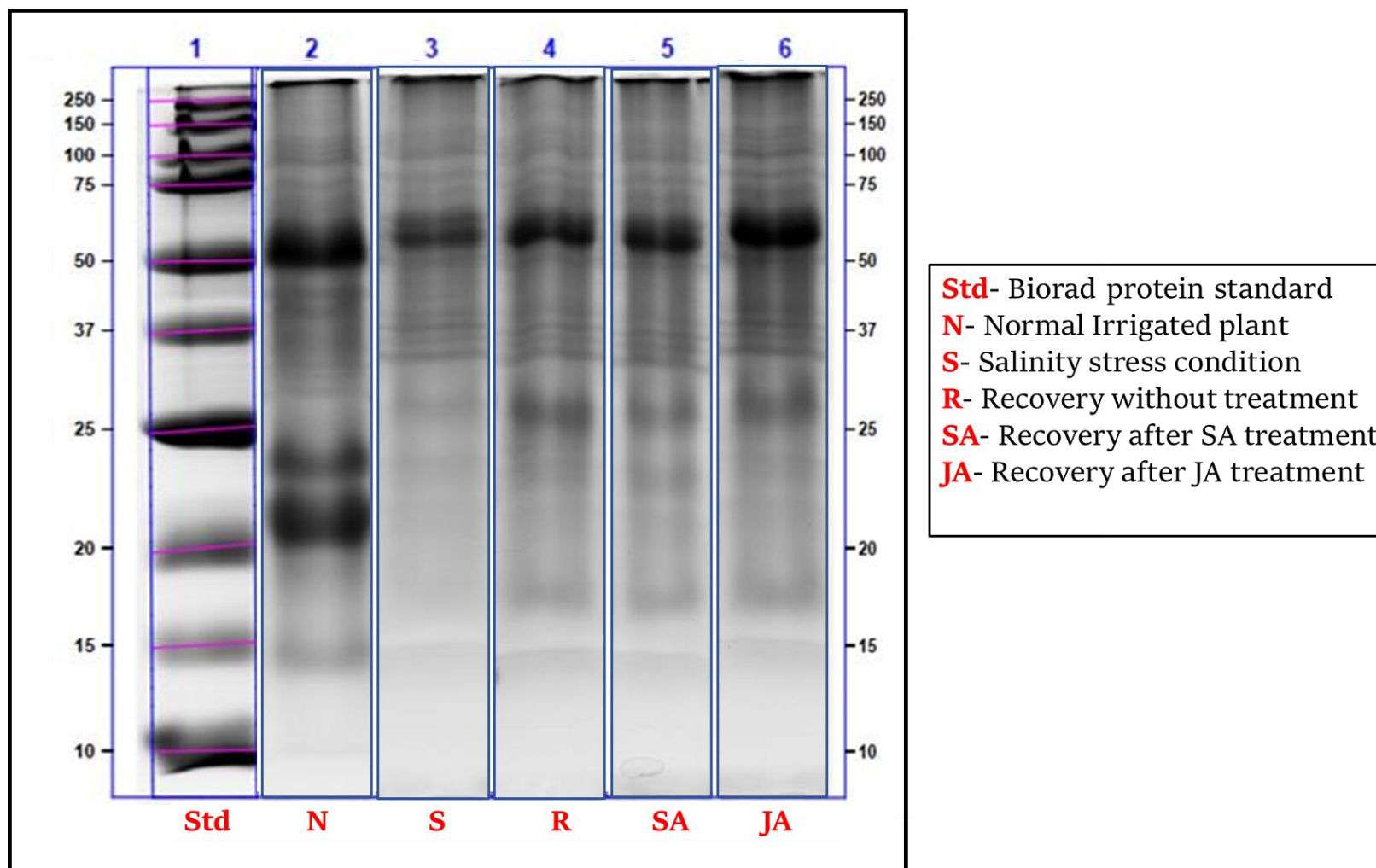


Fig 20. SDS-PAGE protein bands for different conditions under salinity stress

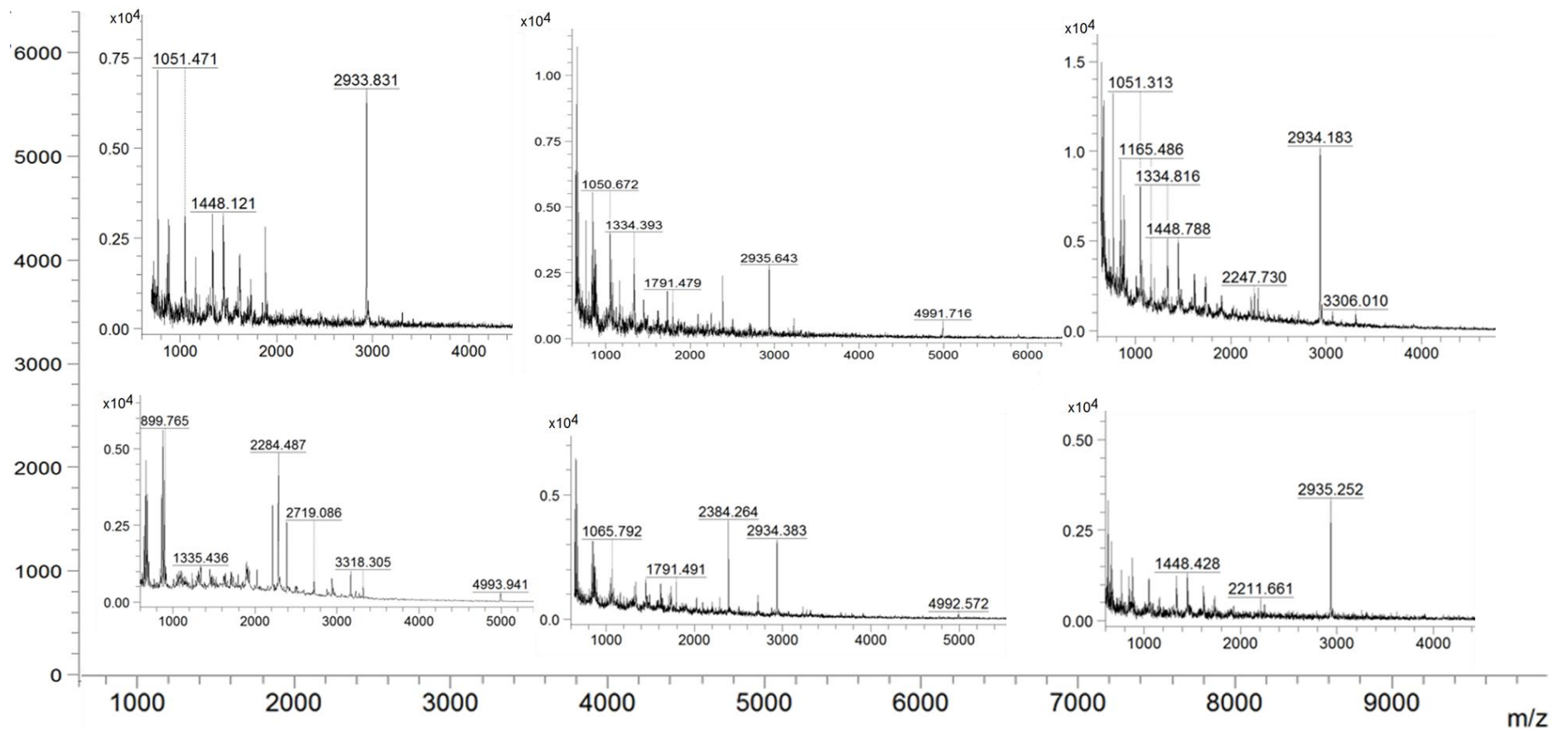


Fig 21.A Peptide mass fingerprint for unique proteins using MALDI TOF MS

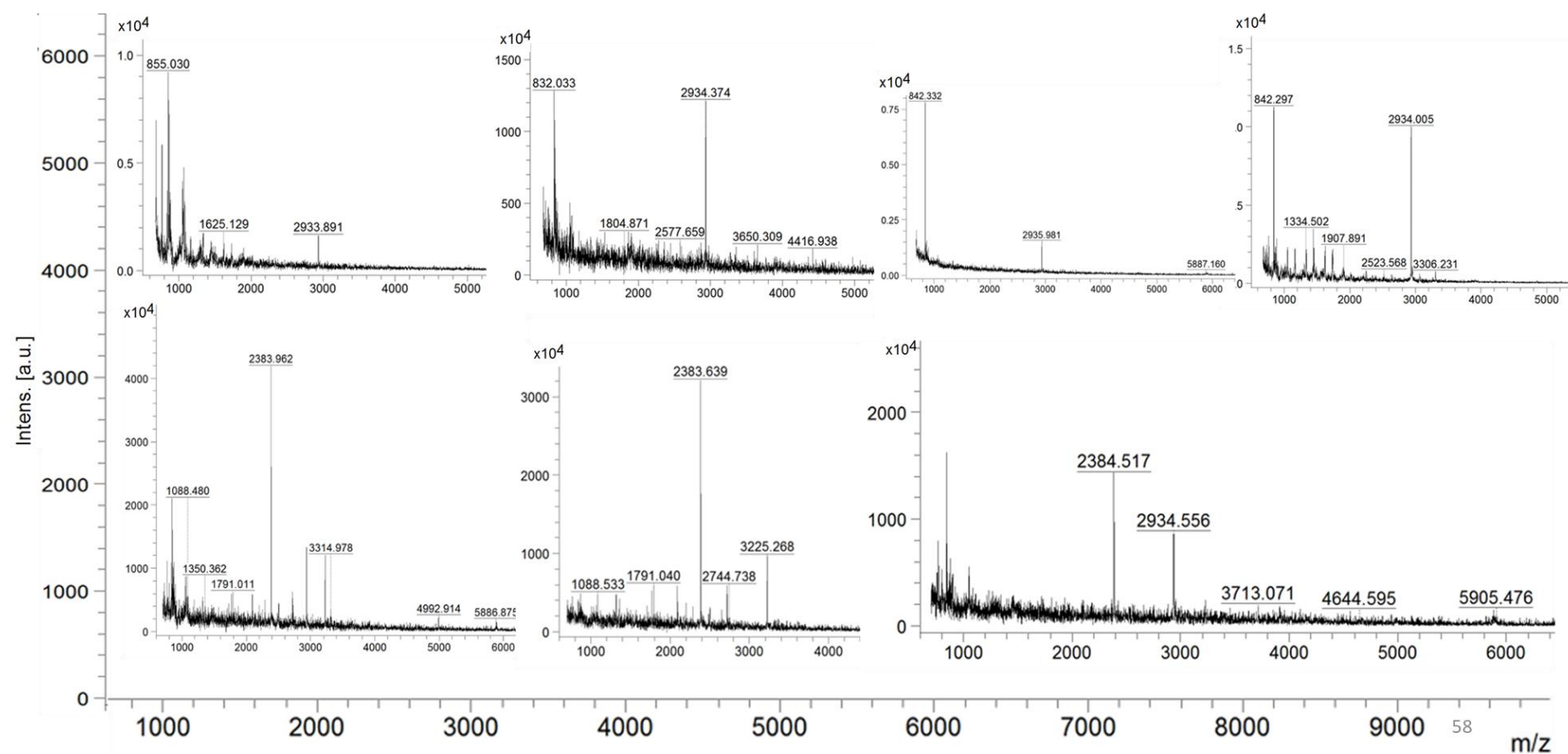


Fig 21B. Peptide mass fingerprint for unique proteins using MALDI TOF MS

Table 21 Proteins identified by MALDI TOF Mass Spectrometry

Sl.no	Mwt (kDa)	PI	Protein	Role	No. of AA	Accession no.	Protein ID	Mascot score
1	15	6.44	Phospho-2-dehydro-3-deoxyheptonate aldolase (DAHP synthase)	Defense	493	A0MH68	AROF_CATRO	65
2	20	6.16	D-3-phosphoglycerate dehydrogenase 1 (PGDH1)	Metabolism and photorespiration	603	Q8LGJ6	SERA1_ARATH	53
3	23	6.44	Ribulose biphosphate carboxylase large chain (RUBP)	Photosynthesis and metabolism	455	P69573 P52781	RBL_LUPAB	44
4	28	6.52	Allene oxide synthase (AOS3)	Defense (Jasmonate activity)	500	Q6Z6L1	C74A3_ORYSJ	44
5	32	6.16	Cation/H(+) antiporter (CA)	Defense (Ion homeostasis)	783	Q9FYB9	CHX11_ARATH	43
6	34	5.00	Heat Shock Proteins (HSP)	Defense	701	P51819	HSP83_IPONI	52
7	36	6.09	Pyruvate decarboxylase 3 (PDC3)	Metabolic plasticity (Hypoxia and drought stress)	592	Q9M039	PDC3_ARATH	48
8	44	10.17	Sulfiredoxin (Srx)	Defense	125	Q8GY89	SRX_ARATH	48
9	53	5.57	Guanine nucleotide-binding protein alpha-1 (GNP)	Metabolism	384	Q40224	GPA1_LUPLU	54
10	60	5.77	Calcium-dependent protein kinase 14 (CDP)	Defense	522	B9FKW9	CDPKE_ORYSJ	42
11	70	5.36	Salicylic acid-binding protein (SAP)	Defense and gene expression	260	Q6RYA0	SABP2_TOBAC	37
12	75	5.14	Chlorophyll a-b binding protein 1 (ChlaP)	Photosynthesis	262	P12329	CB21_MAIZE	45
13	80	9.30	Guanylate kinase 2 (GKP)	Defense and metabolism	285	Q10M74	GMK2_ORYSJ	62

14	95	9.18	Polyadenylate-binding protein 7	Gene Expression	609	Q9ZQA8	PABP7_ARATH	50
15	100	10.70	Protein MEI2-like 7	Gene Expression and Defense	389	Q6ET49	OML7_ORYSJ	47
16	118	8.50	Zinc finger CCCH domain-containing protein	Gene Expression	82	Q3ECU8	C3H13_ARATH	62
17	127	5.33	Heat stress transcription factor A-4a	Defense	401	O49403	HFA4A_ARATH	62
18	114	6.68	Formate dehydrogenase 1	Respiration and Defense	376	Q9SXP2	FDH1_ORYSJ	46
19	135	8.63	Cytochrome b6-f complex iron-sulfur subunit,	Photosynthesis	230	P26291	UCRIA_PEA	50
20	168	9.42	Zinc finger A20 and AN1 domain-containing stress-associated protein 3	Stress gene regulation	355	Q5JN07	SAP3_ORYSJ	49

indicating adaptive protein shifts to cope with salinity stress. Sulfiredoxin also increased under salt stress, with 0.032 mg/mL concentration.

During drought recovery without defense hormones, guanine nucleotide-binding protein alpha-1 remained prominent at 0.375 mg/mL, playing a crucial role in metabolic signaling. The ribulose biphosphate carboxylase large chain contributed 0.104 mg/mL, restoring photosynthesis. The total protein concentration during recovery was measured at 0.615 mg/mL, reflecting significant restoration of protein functions. In contrast, six proteins were identified during salt stress recovery without defense hormones, with guanine nucleotide-binding protein alpha-1 constituting 0.375 mg/mL. Sulfiredoxin followed at 0.047 mg/mL, and allene oxide synthase 3 contributed 0.024 mg/mL. The total protein concentration was recorded at 0.481 mg/mL, indicating partial recovery from salt stress.

The application of the defense hormone SA significantly enhanced the defense response under both stress conditions. During drought recovery with SA, guanine nucleotide-binding protein alpha-1 exhibited the highest expression at 0.291 mg/mL, followed by D-3-phosphoglycerate dehydrogenase 1 at 0.081 mg/mL. Ribulose biphosphate carboxylase (0.116 mg/mL) contributed significantly to photosynthesis. Other proteins included chlorophyll a-b binding protein (0.004 mg/mL), guanylate kinase (0.010 mg/mL), formate dehydrogenase (0.046 mg/mL), and cytochrome b6-f complex iron-sulfur subunit (0.026 mg/mL), all involved in restoring cellular activity and metabolism. The total protein concentration during recovery was measured at 0.691 mg/mL, underscoring the enhancement of metabolic and defense-related proteins. In salinity stress (100 mM) recovery with SA treatment, protein expression was further increased, with guanine nucleotide-binding protein alpha-1 contributing at 0.291 mg/mL, DAHP synthase at 0.107 mg/mL, and ribulose biphosphate carboxylase at 0.081 mg/mL. Additionally, the cation/H(+) antiporter (0.006 mg/mL) and MEI2-like 7 protein (0.022 mg/mL) maintained cellular ion homeostasis. The total protein concentration reached 0.540 mg/mL, reflecting an active defense response triggered by SA.

During drought recovery with JA, guanine nucleotide-binding protein alpha-1 dominated at 0.282 mg/mL, emphasizing its role in JA signaling. DAHP synthase contributed 0.136 mg/mL, and D-3-phosphoglycerate dehydrogenase 1 accounted for 0.089 mg/mL. Other proteins included ribulose biphosphate carboxylase (0.047

mg/mL), allene oxide synthase 3 (0.030 mg/mL), and sulfiredoxin (0.008 mg/mL). The total protein concentration was measured at 0.637 mg/mL, reflecting the interplay between stress response and primary metabolic proteins, underscoring JA's role in plant defense and adaptation (Table 22). During salinity stress recovery with JA treatments, nine proteins were identified, with guanine nucleotide-binding protein alpha-1 accounting for 0.257 mg/mL and DAHP synthase at 0.137 mg/mL. Ribulose biphosphate carboxylase was also significant, contributing 0.116 mg/mL. Other proteins included MEI2-like 7 protein (0.022 mg/mL), chlorophyll a-b binding protein (0.006 mg/mL), calcium-dependent protein kinase (0.011 mg/mL), sulfiredoxin (0.027 mg/mL), cation/H(+) antiporter (0.006 mg/mL), and allene oxide synthase 3 (0.004 mg/mL). The total protein concentration increased to 0.580 mg/mL, indicating robust protein expression involved in stress recovery facilitated by JA (Table 23).

Table 22 Differential protein expression (mg/ml) during drought stress conditions

Sl no	Protein	Mol wt. (kDa)	Normal	Droght Stress	Recovery without DH	Recovery with SA	Recovery with JA
1	DAHP synthase	15	0.108	0.141	0.064	0.177	0.136
2	D-3-phosphoglycerate dehydrogenase	22	0.219	0.045	Nil	Nil	0.089
3	RUBP carboxylase large chain	23	0.076	0.084	0.104	0.061	00.047
4	Allene oxide synthase	28	0.002	0.009	0.031	Nil	0.030
5	Heat shock protein	32	Nil	0.013	0.025	0.005	Nil
6	Pyruvate decarboxylase	36	0.001	Nil	Nil	Nil	Nil
7	Salicylic acid-binding protein	35	Nil	Nil	Nil	0.009	Nil
8	Sulfiredoxin	44	0.002	0.014	0.014	0.007	0.008
9	Guanine nucleotide-binding protein	53	0.197	0.326	0.347	0.346	0.282
10	Chlorophyll a-b binding protein	75	0.007	0.002	Nil	0.004	0.004
11	Guanylate kinase	80	Nil	Nil	0.003	0.010	Nil
12	Protein MEI2-like	100	0.017	0.015	0.004	Nil	Nil
13	Zinc finger CCCH domain-containing protein	118	Nil	Nil	Nil	Nil	0.014
14	Heat stress transcription factor A-4a	127	Nil	0.015	0.024	Nil	0.007
15	Formate dehydrogenase 1	114	Nil	Nil	Nil	0.046	0.006
16	Cytochrome b6-f complex iron-sulfur subunit	135	Nil	Nil	Nil	0.026	0.015
Total protein (mg/mL)			0.631	0.665	0.615	0.691	0.637

Table 23 Differential protein expression (mg/ml) during salinity stress conditions

Sl no	Protein	Mol wt. (kDa)	Normal	Salinity Stress	Recovery without DH	Recovery with SA	Recovery with JA
1	DAHP synthase	15	0.108	0.092	Nil	0.107	0.137
2	D-3-phosphoglycerate dehydrogenase	20	0.219	Nil	Nil	0.081	Nil
3	Ribulose biphosphate carboxylase	23	0.076	0.137	Nil	Nil	0.116
4	Allene oxide synthase	28	0.002	Nil	0.024	0.014	0.004
5	Cation/H (+) antiporter	32	0.003	0.014	0.021	0.005	0.006
6	Pyruvate decarboxylase	36	0.001	0.006	Nil	Nil	Nil
7	Sulfiredoxin	44	0.002	0.032	0.047	0.018	0.027
8	Guanine nucleotide-binding protein	53	0.197	0.302	0.375	0.291	0.257
9	Calcium-dependent protein kinase	60	Nil	Nil	Nil	Nil	0.011
10	Chlorophyll a-b binding protein	75	0.007	0.003	0.006	0.003	0.006
11	Protein MEI2	100	0.017	Nil	0.009	0.021	0.022
12	Zinc finger CCCH domain-containing protein	118	Nil	0.007	Nil	Nil	Nil
Total Protein concentration (mg/ml)			0.631	0.594	0.481	0.540	0.580

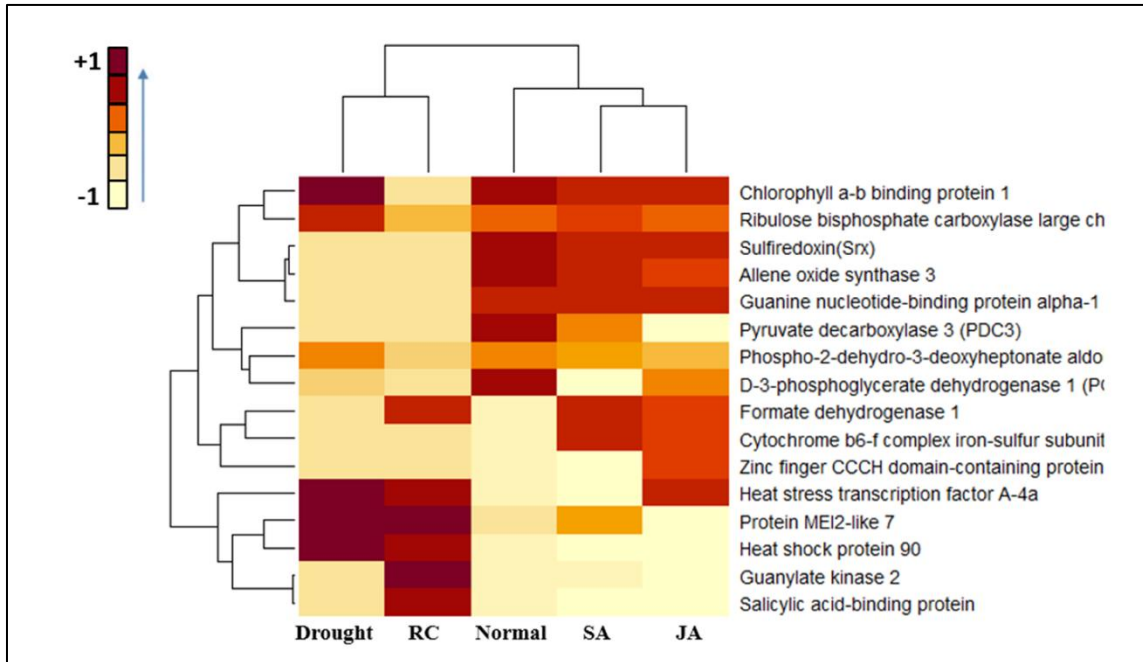


Fig 22. Clustered heatmap for differential expression of proteins for different conditions under drought stress

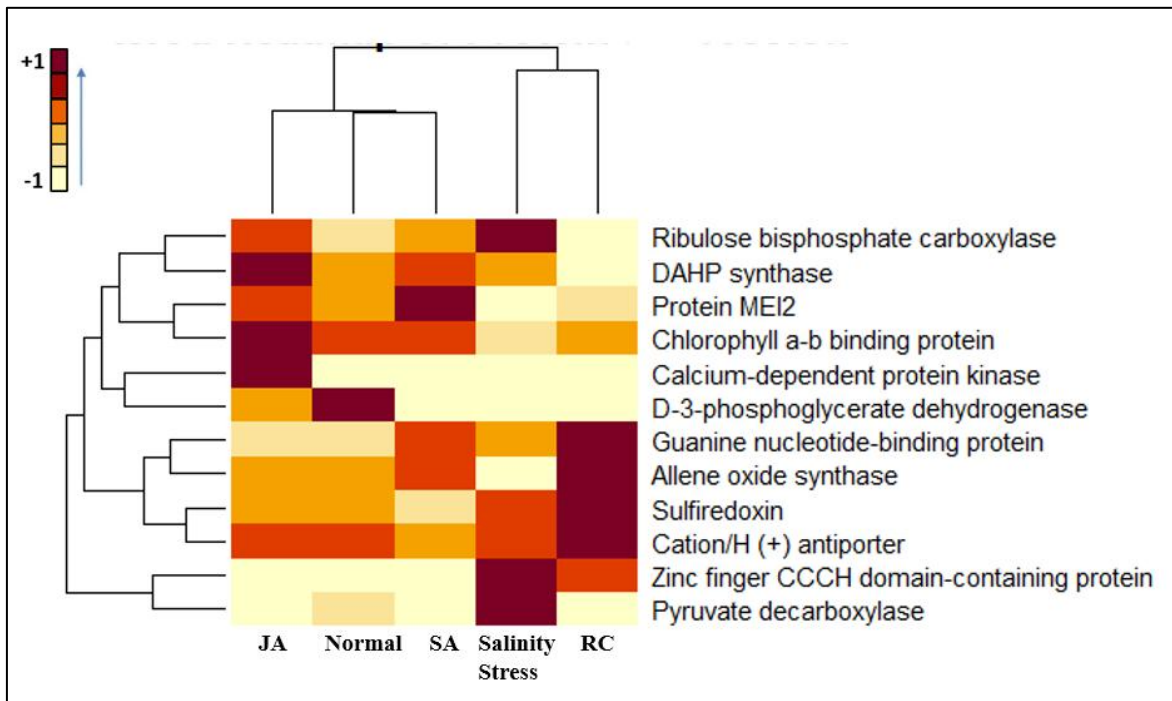


Fig 23. Clustered heatmap for differential expression of proteins for different conditions under salinity stress

4.3.4 Clustered heat map of differentially expressed proteins:

The clustered heatmap presents the differential expression of proteins across different conditions, highlighting distinct up- or down-regulation in response to stress, recovery, and hormone treatments (SA and JA). The colour intensity reflects protein abundance, with brighter colors representing higher expression levels.

During drought stress treatment groups (Fig.22), Chlorophyll a-b binding protein one and Ribulose biphosphate carboxylase exhibit varied expression patterns, indicating their involvement in photosynthesis is affected by stress. Elevated Sulfiredoxin (Srx) levels during drought and RC highlight its role in oxidative stress response, while Allene oxide synthase 3 peaks under RC, underscoring its significance in JA biosynthesis. Guanine nucleotide-binding protein alpha-1 is markedly upregulated across all conditions, suggesting its pivotal role in cellular signaling. Hormonal treatments with SA and JA promote the expression of proteins linked to stress adaptation, while D-3-phosphoglycerate dehydrogenase 1 (PGDH1) showed differential expression across treatments.

During salt stress treatment groups (Fig.23), proteins such as Allene oxide synthase, D-3-phosphoglycerate dehydrogenase, Sulfiredoxin, and Meiotic protein 2 (MEI2) were slightly down-regulated, while Ribulose biphosphate carboxylase (RUBP), Guanine nucleotide-binding protein, and Zinc Finger proteins are up-regulated, reflecting their roles in growth and development in normal condition. In salt stress conditions, proteins like cation/H⁺ antiporter (C/H⁺), DAHP synthase, and AOS3 are down-regulated, while RUBP, Guanine, and Zinc Finger proteins are up-regulated, indicating the activation of defense mechanisms. During the recovery phase, C/H⁺ and DAHP synthase show moderate up-regulation, while RUBP, Guanine, and Zinc Finger proteins continue to exhibit strong up-regulation, underscoring their role in recovery processes. SA treatment results in the up-regulation of C/H⁺, DAHP synthase, PGDH1, and pyruvate decarboxylase 3 (PDC3), while AOS3, Srx, and MEI2 like 7 are down-regulated, indicating diverse responses to SA. In contrast, JA treatment leads to significant up-regulation of C/H⁺, DAHP synthase, and PDC3, with PGDH1, Srx, and MEI2-like 7 showing down-regulation. Essential proteins such as C/H⁺, DAHP synthase, RUBP, and Guanine nucleotide-binding proteins play significant roles in stress response

and recovery, exhibiting a distinct regulatory pattern under SA and JA treatments. AOS3 and PGDH1 demonstrate a heightened sensitivity to stress, showing sustained down-regulation.

4.3.5 Fold change analysis:

Fold change analysis is crucial for understanding the relative abundance of proteins in response to different treatment and stress conditions (Fig 26 and 27). It allows for identifying significantly upregulated or downregulated proteins, providing insights into their functional roles in plant defense, metabolism, and stress response mechanisms. The fold change values for the drought and salt stress are detailed in Tables (24 and 25.)

The functional annotation of proteins under drought and salinity stress highlights critical shifts in protein roles as plants adapt to these abiotic stresses. In both conditions, proteins show a marked shift towards defense and ion/metabolic balance, reflecting the plant's prioritization of survival mechanisms. Under drought stress, proteins are predominantly associated with defense (38%) and metabolism (25%), while photosynthesis and gene expression are downregulated. Similarly, in salinity stress, the focus shifts to defense (29%) and ion balance (29%), with other processes reduced.

Recovery patterns differ depending on the treatment applied. Without treatment, plants rely heavily on defense and metabolism for recovery, as seen in both stress conditions. However, when treated with salicylic acid (SA), proteins show enhanced roles in photosynthesis (28%) and metabolism (27%), suggesting a restoration of growth processes alongside defense (18%). On the other hand, treatment with JA results in a balanced enhancement of photosynthesis (22%), metabolism (22%), and secondary metabolite production (21%), alongside contributions from defense and hormonal signaling, indicating a comprehensive recovery mechanism.

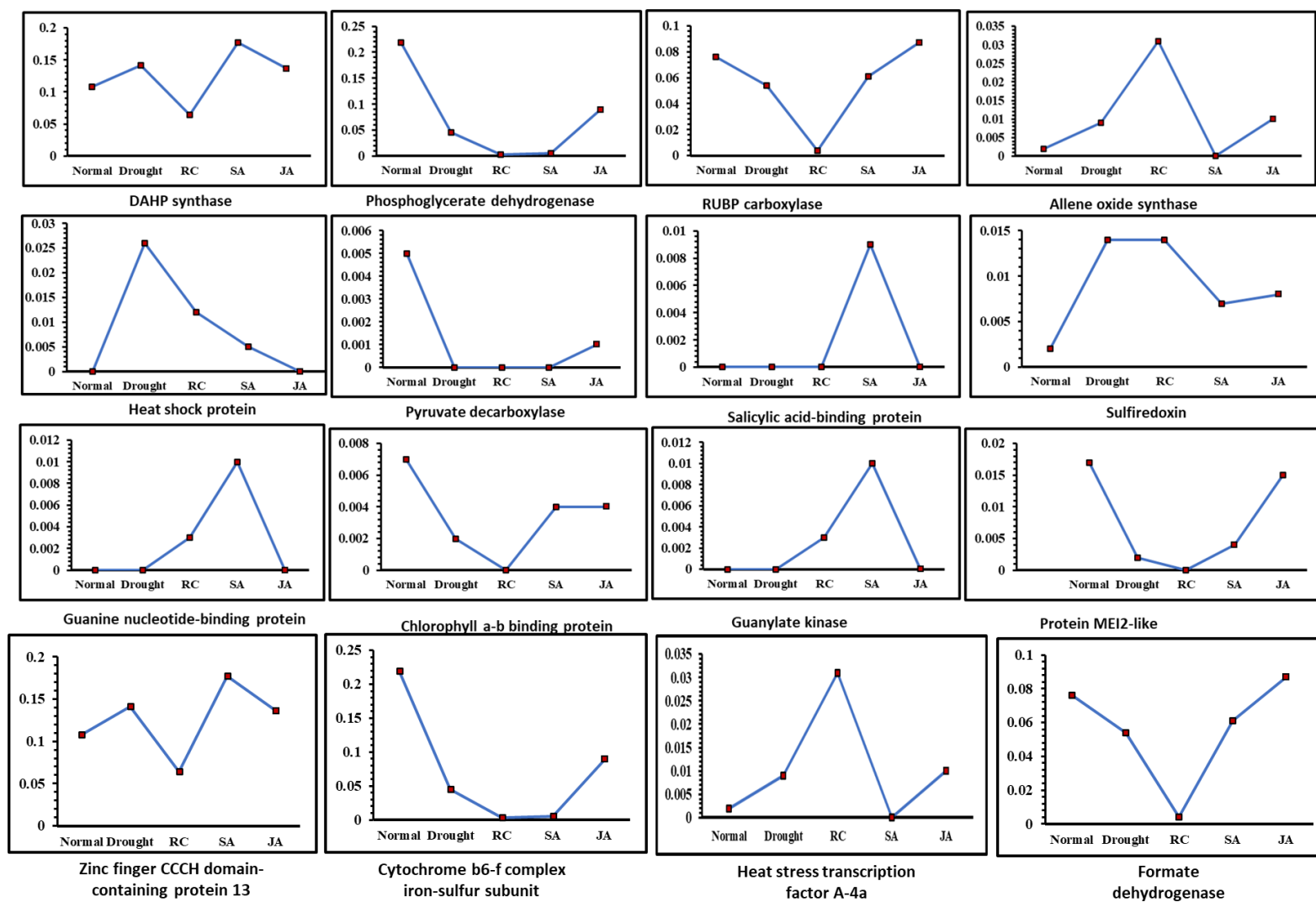


Fig 24. Fold change (Conc. v/s Treatment) of differentially expressed proteins for different conditions under drought stress

Table 24 Functional annotation of identified proteins during drought condition

Sl. No	Protein name	Function	Sub-cellular localization	Role of protein
1	DAHP synthase	Plant defense through secondary metabolite productions (flavonoids, lignin, and phenolic acids)	Chloroplast	Defense (Oxidative and dehydration stress)
2	D-3-phosphoglycerate dehydrogenase	Redox homeostasis and protect plants from oxidative damage.	Plastids (chloroplasts)	Metabolism and photorespiration
3	Ribulose biphosphate carboxylase large chain	Regulation of photosynthetic electron transport, Calvin cycle, and modulation of stomatal aperture and gas exchange	Stroma (chloroplasts)	Photosynthesis
4	Allene oxide synthase	AOS3 is involved in responses to drought, salt, and temperature stress, regulating stress-related genes and pathways.	The endoplasmic reticulum, Plastids.	Defense (Regulates Jasmonate activity)
5	Heat shock protein	Stress response, apoptosis, and cell survival	Cytoplasm and nucleus	Defense
6	Pyruvate decarboxylase 3	Involved in plant signaling and developmental processes (root growth and differentiation).	Mitochondria, Vesicles and Vacuoles	Metabolic plasticity (Hypoxia and drought stress)
7	Salicylic acid-binding protein	Defense gene regulation, hormone signaling	Cytoplasm	Stress signaling and defense
8	Sulfiredoxin	Maintains cellular redox balance, protecting against oxidative stress and regulating stress	Peroxisome, Chloroplasts	Defense
9	Guanine nucleotide-binding protein	Regulate plant growth, development, and stress responses.	Plasma membrane and cytosol	Metabolism

10	Chlorophyll a-b binding protein 1	Light-harvesting complex, ensuring efficient photosynthetic energy conversion and plant survival.	Thylakoid membranes Chloroplasts	Photosynthesis
11	Guanylate kinase 2	Integrating meiotic, chromatin, and hormonal regulation.	Cytosol	Gene Expression and Defense
12	Protein MEI2-like 7	Integrating meiotic, chromatin, and hormonal regulation.	Nucleus	Gene Expression and Defense
13	Zinc finger CCCH domain-containing protein 13	Post-transcriptional gene regulation and organogenesis	ER and nucleus	Gene Expression
14	Heat stress transcription factor A-4a	Regulator of the heat stress response.	Cytoplasm and Nucleolus	Defense
15	Formate dehydrogenase 1	FDH1 is a key enzyme in carbon metabolism, energy production, and stress responses	Mitochondria	Respiration and Defense
16	Cytochrome b6-f complex iron-sulfur subunit,	Essential for photosynthetic energy conversion and plant survival	Thylakoid membranes Chloroplasts Lamellae	Photosynthesis

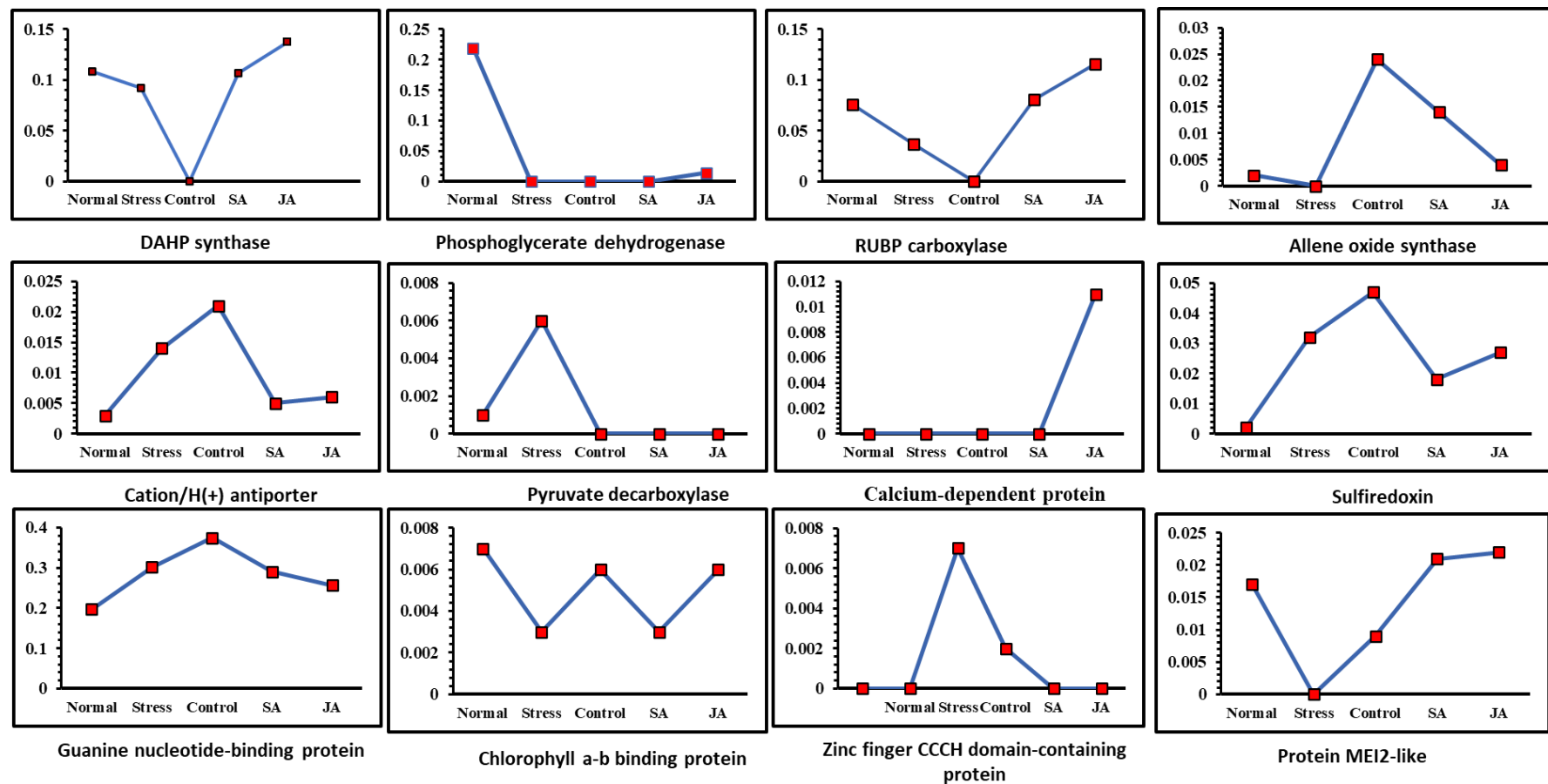


Fig 25. Fold change (Conc. v/s Treatment) of differentially expressed proteins for different conditions under salinity stress

Table 25 Functional annotation of identified proteins during salinity stress condition

Sl. No	Protein name	Function	Sub-cellular localization	Role of protein
1	DAHP synthase	Plant defense through secondary metabolite productions	Chloroplast	Defense (Oxidative and dehydration stress)
2	D-3-phosphoglycerate dehydrogenase	Redox homeostasis and protect plants from oxidative damage.	Plastids (chloroplasts)	Metabolism and photorespiration
3	Ribulose biphosphate carboxylase large chain	Regulation of photosynthetic electron transport, Calvin cycle, and modulation of stomatal aperture and gas exchange	Stroma (chloroplasts)	Photosynthesis
4	Allene oxide synthase 3	AOS3 is involved in responses to drought, salt, and temperature stress, regulating stress-related genes and pathways.	The endoplasmic reticulum, Plastids.	Defense (Regulates jasmonate activity)
5	Cation/H(+) antiporter	Involved in salt, drought, and metal stresses by regulating ion fluxes and maintaining cellular ion balance.	Tonoplast and Polarized cell compartments:	Defense (Ion homeostasis)
6	Pyruvate decarboxylase 3	Involved in plant signaling and developmental processes (root growth and differentiation).	Mitochondria, Vesicles	Metabolic plasticity (Hypoxia and drought stress)
7	Sulfiredoxin	Maintains cellular redox balance, protecting against oxidative stress and regulating stress	Peroxisome, Chloroplasts	Defense
8	Guanine nucleotide-binding protein alpha-1	Regulate plant growth, development, and stress responses.	Plasma membrane and cytosol	Metabolism
9	Calcium-dependent protein kinase 14	Integrating calcium signals and hormonal signals to regulate plant growth, development, and stress responses.	Plasma membrane, Chloroplast	Defense

10	Chlorophyll a-b binding protein 1	Light-harvesting complex, ensuring efficient photosynthetic energy conversion and plant survival.	Thylakoid membranes Chloroplasts	Photosynthesis
11	Protein MEI2-like 7	Integrating meiotic, chromatin, and hormonal regulation.	Nucleus Meiotic structures	Gene Expression and Defense
12	Zinc finger CCCH domain-containing protein 13	Post-transcriptional gene regulation and organogenesis	ER and nucleus	Gene Expression

4.3.6 Functional annotation of identified proteins during abiotic stress condition:

During drought stress, functional annotations reveal varied responses across different conditions. Under normal conditions, the highest functional allocation is toward photosynthesis (28%) and metabolism (27%), with substantial resources directed toward defense (18%) and secondary metabolite production (9%), indicating a balanced physiological state. In drought conditions, there's a shift, with defense mechanisms predominating (38%) and a reduction in photosynthesis (25%) and metabolism (12%), showing a prioritization of protective functions. Upon recovery without treatment, the focus slightly shifts back to photosynthesis (29%) and metabolism (27%), though defense (14%) and secondary metabolites (14%) remain elevated, reflecting an adaptive response. When SA is applied during recovery, photosynthesis remains the highest priority (28%), with defense and metabolism each at 18%, highlighting a stable recuperation pattern that incorporates hormonal signaling (9%). With JA treatment, photosynthesis (22%) and metabolism (22%) are nearly equal in priority, alongside defense (21%) and secondary metabolites (14%), showing a more balanced response, with a unique emphasis on hormonal signaling (7%), indicating JA's role in modulating multiple functional pathways (Fig 24).

Similarly, for salinity stress conditions, during normal conditions, the plant primarily focuses on photosynthesis, which constitutes 28% of its functional allocation, closely followed by metabolism at 27%, reflecting a priority on growth and energy production in the absence of stress (Fig 25). Defense mechanisms contribute 18%, indicating a baseline level of protection, while secondary metabolite production and ion balance each account for 9%, supporting regulatory functions and maintaining stability. When exposed to salinity stress, the plant shifts resources significantly towards defense, which takes up 35% of its functional allocation, demonstrating a strategic response to mitigate the negative effects of salt stress. Photosynthesis and metabolism decrease to 20% and 15%, respectively, as the plant conserves energy under stress, while ion balance rises to 18%, indicating efforts to manage salt levels and uphold cellular stability. In the recovery phase without any treatment, the plant begins to restore its focus on growth processes, with photosynthesis and metabolism occupying 26% and 24%, respectively, suggesting a return to energy production and metabolic activity. Defense remains substantial at 22%, as the plant continues protecting itself from residual stress effects,

while ion balance and secondary metabolite production each contribute 12%, highlighting ongoing attempts to re-establish homeostasis. During recovery with salicylic acid (SA) treatment, photosynthesis is prioritized at 28%, followed by metabolism at 20% and defense at 18%, which underscores SA's role in supporting energy production while still maintaining protective functions. Secondary metabolite production and ion balance each make up 12%, and hormonal signaling, introduced at 10%, suggests that SA facilitates recovery by balancing growth and stress adaptation through signaling pathways. In recovery with jasmonic acid (JA) treatment, photosynthesis and defense are nearly equally prioritized, accounting for 25% and 22% of functional allocation, respectively, indicating JA's effect in enhancing both growth and stress responses. Metabolism remains at 18%, secondary metabolite production at 15%, and ion balance at 10%, suggesting JA's role in managing stress while regulating nutrient stability. Hormonal signaling, also at 10%, reflects JA's ability to modulate growth and recovery pathways effectively.

These findings illustrate that salinity stress prompts a strong defensive response and enhanced ion regulation, while recovery treatments with SA and JA help rebalance growth and adaptation. SA enables a faster return to growth functions, whereas JA promotes a more balanced recovery, aiding resilience across various physiological processes.

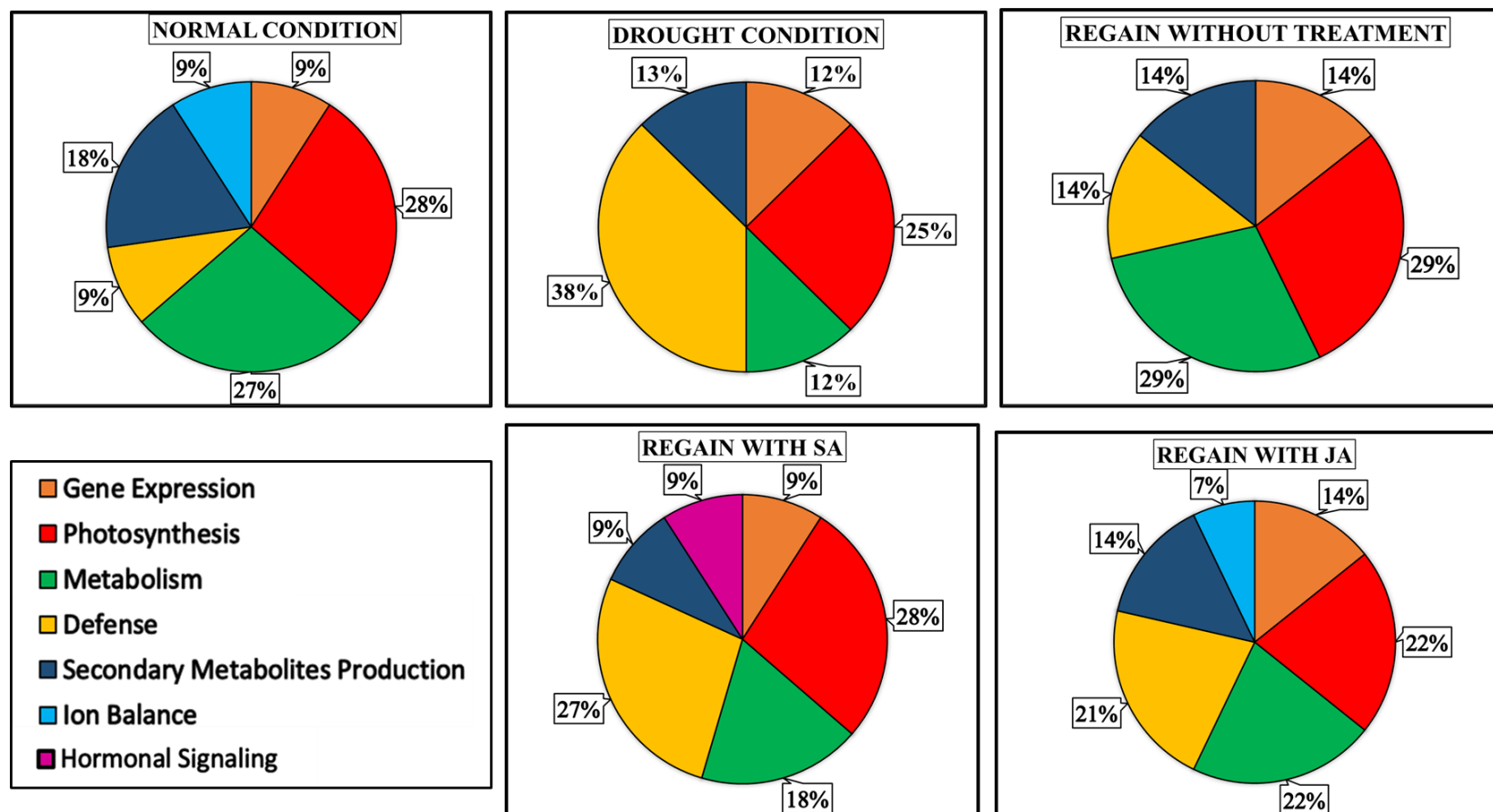


Fig 26. Functional annotation of differentially expressed proteins for different conditions under drought stress

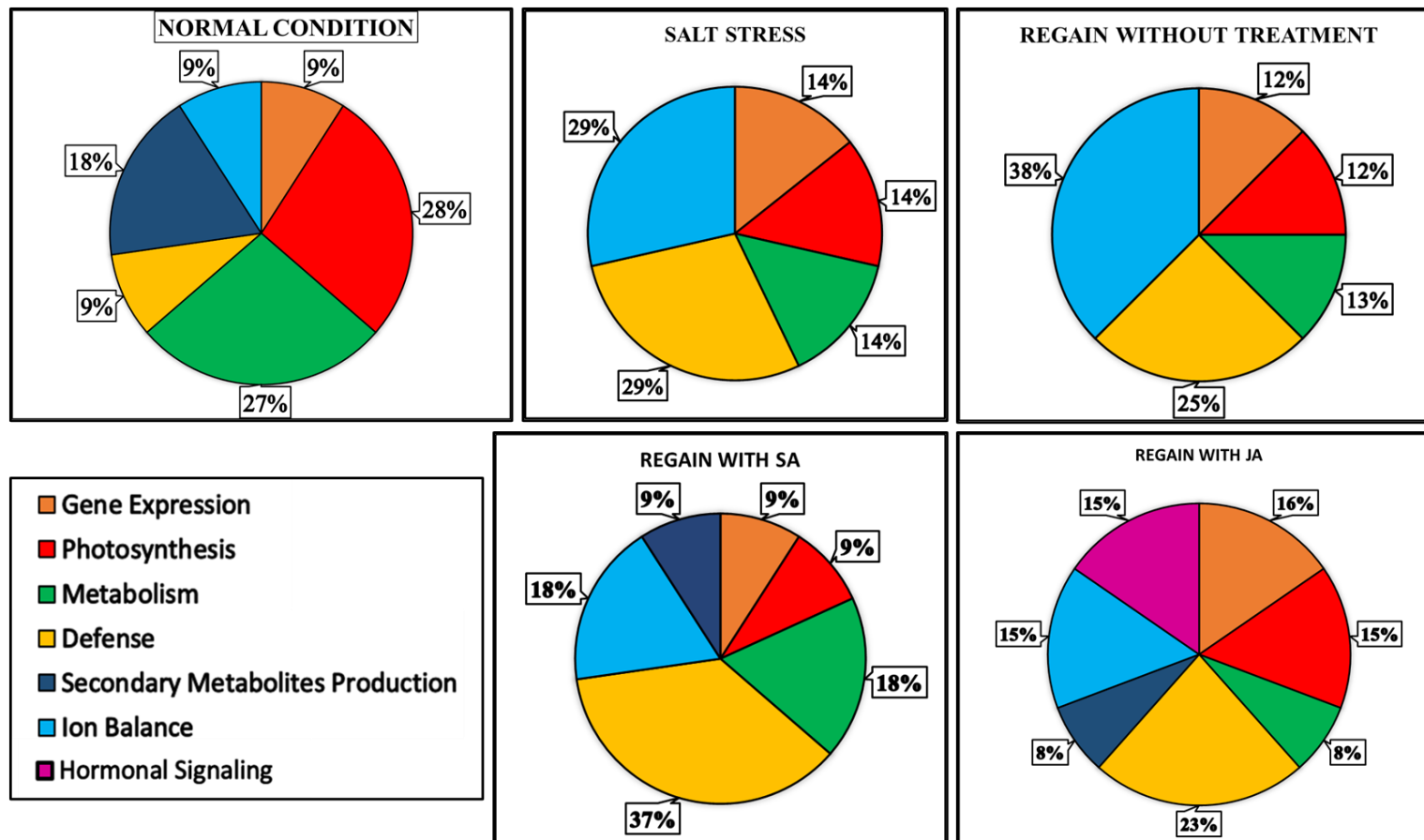


Fig 27. Functional annotation of differentially expressed proteins for different conditions under salinity stress

5. DISCUSSION

This chapter presents a detailed discussion of the findings from the investigation, “Stress proteomics on the application of defense hormones in *Swietenia macrophylla* King seedlings” under the following subheads:

5.1 DEFENSE HORMONES APPLICATION TO MAHOGANY SEEDLINGS:

Evaluating morphological and physiological parameters in mahogany seedlings over 150 days provided valuable insight into how different concentrations of JA and SA affect growth dynamics. Exogenous application of these defense hormones has enhanced plant growth by modulating key morphological and physiological traits. Specifically, the application of optimal concentrations of JA and SA resulted in significant improvements in seedling height, collar diameter, photosynthetic efficiency, and chlorophyll content, thereby fostering greater overall vigor and stress tolerance. These findings are consistent with previous research indicating that SA and JA enhance plant growth by stimulating nutrient uptake, optimizing photosynthesis, and activating stress response pathways (Rivas-San Vicente & Plasencia, 2011; Monte, 2023).

5.1.1 Effect of defense hormones on morphological parameters:

The exogenous application of JA and SA significantly impacted the height of mahogany seedlings. Optimal concentrations of JA (0.6 mM) and SA (0.8 mM) to marked growth increases, with JA-treated seedlings exhibiting a 158.87% increase and SA-treated seedlings showing a 139.62% increase in height. These results align with earlier studies highlighting the roles of JA and SA in regulating plant growth through mechanisms such as cell elongation, division, and nutrient allocation (Rohwer & Erwin, 2008). However, as Wang *et al.* (2020) reported, higher concentrations (1.0 mM) of both hormones inhibited growth, suggesting a threshold beyond which hormonal imbalances disrupt normal metabolic processes. This highlights the importance of precise hormone application, as excessive levels can suppress growth. Control groups with minimal height increases further underscore the efficacy of optimal JA and SA concentrations in promoting plant growth and resilience.

JA and SA significantly influenced collar diameter growth in mahogany seedlings. JA at 0.6 mM produced the most significant increase (180.53%, reaching 11.53 mm), while SA at 0.8 mM led to a 146.93% rise (10.63 mm). These results highlight the

positive effects of optimal hormone concentrations on structural development. In contrast, control groups showed much lower growth (75.24% for JA, 74.02% for SA), reinforcing the hormones impact. Higher (1.0 mM) and lower concentrations inhibited growth, indicating a narrow optimal range. The growth effects of JA and SA are linked to their roles in cell division, cell wall expansion, and lignification. JA promotes vascular development and cell wall strengthening (Rivas-San Vicente & Plasencia, 2011), while SA enhances structural growth through reactive oxygen species (ROS) signaling. Excessive ROS at higher concentrations can damage cells, inhibiting growth, as reported by Wang *et al.* (2020), while suboptimal levels fail to stimulate sufficient growth. This concentration-dependent response aligns with research emphasizing the importance of precise hormonal regulation for optimal structural development (Zheng *et al.*, 2023).

Height and collar diameter are key indicators of volume increment, crucial for assessing growth and biomass accumulation in mahogany seedlings. After 150 days, optimal growth was observed with specific JA and SA concentrations. JA at 0.6 mM (T4) resulted in the highest volume at 155.48 cm³, while SA at 0.8 mM (T5) yielded 111.17 cm³. Control and higher hormone concentrations (1.0 mM) showed minimal growth, indicating that excessive hormone levels may hinder development. JA and SA promote cell division and elongation, especially in the apical meristem and cambium, facilitating vertical growth and collar expansion through enhanced auxin transport (Pauwels & Goossens, 2011).

JA and SA treatments significantly influenced the number of leaves in mahogany seedlings, with intermediate concentrations promoting the higher leaf development and overall plant vigor. The greatest leaf increase was observed at 0.4 mM JA (221.70%) with 39 leaves and 0.8 mM SA (210.69%) with 41 leaves. This increase in leaf number is attributed to the crosstalk between defense hormones and auxin, which is crucial for cell division and leaf formation. According to Pauwels & Goossens (2011), defense hormones modulate auxin distribution, stimulating meristematic activity, which leads to the proliferation of leaf primordia and a subsequent increase in leaf numbers. The enhanced photosynthetic performance further supports this growth by providing the necessary energy and resources (Khan *et al.*, 2015).

The application of JA and SA significantly enhanced leaf area expansion in mahogany seedlings, highlighting the necessity of optimizing hormone concentrations

for effective leaf area growth. The most significant increase in leaf area was observed at 0.6 mM JA, resulting in a 206.57% increase, while 0.8 mM SA yielded a 180.39% increase. Maximum leaf areas were recorded at 0.6 mM JA (43.38 cm²) and 0.4 mM JA (38.96 cm²), indicating that intermediate hormone concentrations are most effective for promoting leaf development. SA promotes cell expansion and division, increasing leaf area (Singh & Usha, 2003). By enhancing overall plant vigor, both SA and JA play critical roles in increasing leaf area in treated seedlings (Majeed *et al.*, 2024).

The application of JA and SA significantly influenced both shoot length and shoot dry weight in mahogany seedlings, demonstrating a concentration-dependent effect on growth and biomass accumulation. After 150 days of observation, the optimal concentration of JA was 0.6 mM (T4), resulting in a maximum shoot length of 81.00 cm, followed by 0.4 mM (T3) at 68.00 cm. Regarding shoot dry weight, JA treatments at 0.6 mM yielded the highest biomass, with a shoot dry weight of 30.16 g, while higher and lower concentrations resulted in significant reductions. Likewise, SA treatments showed higher biomass yield for 0.8 mM, achieving a shoot dry weight of 24.17 g. Najafi *et al.*, 2019 reported that specific concentrations of both JA and SA optimize shoot growth and biomass accumulation, whereas excessive or insufficient hormone levels diminish their effectiveness.

The exogenous application of SA and JA significantly influenced both shoot dry weight and shoot length in mahogany seedlings. In JA treatments, the highest shoot dry weight of 30.16 g was observed at 0.6 mM, with reductions at higher and lower concentrations. Similarly, the SA treatments peaked at 0.8 mM, yielding a shoot dry weight of 24.17 g, highlighting that specific concentrations of JA and SA optimize biomass allocation. In terms of shoot length, the seedlings exhibited a clear concentration-dependent response. After 150 days, the maximum shoot length for JA was 81.00 cm at 0.6 mM (T4), while the shortest was 56.11 cm at 1.0 mM (T6). The highest shoot length for SA was 71.11 cm at 0.8 mM, followed by 63.56 cm at 1.0 mM. The control group consistently had the lowest values for both parameters. The significant increases are attributed to photosynthetic performance and nutrient uptake enhancement. By improving chlorophyll content, stomatal conductance and promoting nutrient transporter genes, JA and SA boosted photosynthetic efficiency and nutrient availability, leading to greater biomass accumulation (Keya *et al.*, 2023).

The sturdiness quotient, an important indicator of seedling stability and resilience, reflects the plant's ability to withstand stress. According to Wasternack & Hause (2013), JA enhances sturdiness by promoting lignin biosynthesis and cell wall fortification, with the highest quotient observed at 0.6 mM due to optimal structural reinforcement. Lower concentrations were less effective, while higher levels (1.0 mM) overstimulated stress pathways, reducing sturdiness. Similarly, SA strengthens cell wall integrity, with the highest sturdiness at 0.8 mM, where growth and defense responses were balanced (Vlot *et al.*, 2009). Excessive SA (1.0 mM) showed diminishing returns, likely due to SA toxicity.

The seedling quality index, a vital measure of plant vigor and survival potential, reflects the overall health and long-term growth prospects of seedlings. According to Pauwels & Goossens (2011), JA enhances seedling quality by improving nutrient uptake and photosynthetic efficiency, with the highest index observed at 0.6 mM, where growth and stress responses were optimally balanced. Lower concentrations were less effective, while higher levels (1.0 mM) triggered stress responses that reduced seedling quality. Similarly, SA improved seedling vigor, with the highest quality index at 0.8 mM (Khan *et al.*, 2015). Excessive SA (1.0 mM) showed diminishing returns, likely due to stress-related growth inhibition.

The exogenous application of JA and SA significantly influenced root length, number of roots, root dry weight, key indicators of nutrient acquisition and overall plant health. After 150 days of observation, JA achieved a maximum root length of 23.11 cm at 0.6 mM, and SA peaked at 23.56 cm at 0.8 mM, though no statistically significant differences were found between treatments. Regarding the number of roots, the JA control group had the highest number (46.89), significantly dropping to 33 at 0.8 mM, while SA treatments showed the highest root number (50) at 0.6 mM, declining to 32 at 1.0 mM. JA also significantly affected root dry weight, peaking at 6.88 g at 0.8 mM, whereas SA showed no significant differences in biomass. Both hormones influenced the root-to-shoot ratio, with the highest JA ratio of 0.399 at 1.0 mM and the lowest of 0.143 at 0.6 mM. For SA, the control group had the highest ratio at 0.597 and the lowest at 0.208 at 0.8 mM, indicating a trend toward increased biomass allocation to shoots. These effects can be attributed to JA and SA's roles in promoting root elongation through enhanced gene expression related to cell division (Pauwels & Goossens, 2011), improving root architecture for better nutrient uptake (Zhang *et al.*, 2015), which triggers

physiological adaptations to stress (Wasternack & Hause, 2013), and demonstrating effectiveness within specific concentration ranges, as excessive levels may lead to inhibitory effects (Khan *et al.*, 2015).

5.1.2 Effect of defense hormones on physiological parameters

The evaluation of physiological parameters in mahogany seedlings demonstrated that the exogenous application JA and SA significantly improved the photosynthetic rate, transpiration rate, stomatal conductance, leaf temperature, total chlorophyll content, and relative water content, thereby promoting overall growth and metabolic activity.

Photosynthesis is the process by which plants convert carbon dioxide into sugars and oxygen by capturing light energy (Gest H, 2002). In mahogany seedlings, the application of JA and SA significantly enhanced photosynthetic rates. JA at 0.6 mM (T4) reached peak rates of 3.78 $\mu\text{mol CO}_2 \text{ m}^2/\text{s}$ by day 120, while the control group (T1) declined from 2.55 to 2.14 $\mu\text{mol CO}_2 \text{ m}^2/\text{s}$. Although intermediate JA concentrations (T2 and T6) improved photosynthesis, they did not exceed the rates observed at T4. Furthermore, SA at 1.0 mM (T6) attained a maximum rate of 4.50 $\mu\text{mol CO}_2 \text{ m}^2/\text{s}$ on day 30, with lower concentrations (T4 and T3) showing moderate enhancements. The increase in photosynthetic rates with JA and SA application can be attributed to their roles in enhancing chlorophyll content and improving stomatal conductance, which are critical for effective gas exchange and light absorption (Keya *et al.*, 2023). The decrease in photosynthetic rates toward the end of the evaluation period can be attributed to excessive heat waves in the study location and a potential reduction in the effects of JA and SA after 120 days post-application.

JA and SA significantly enhanced the transpiration rate of mahogany seedlings, which is crucial for maintaining water balance and facilitating gas exchange. JA at 0.6 mM (T4) achieved peak transpiration rates of 0.178 $\text{mmol H}_2\text{O m}^2/\text{s}$ at 30 days and 0.145 $\text{mmol H}_2\text{O m}^2/\text{s}$ at 120 days, while the control group (T1) decreased from 0.086 to 0.078 $\text{mmol H}_2\text{O m}^2/\text{s}$ (Table 08). This improvement can be attributed to increased stomatal conductance, enhancing water vapor release and CO_2 uptake (Zhang *et al.*, 2015). Similarly, SA at 0.8 mM (T5) recorded peak rates of 0.177 $\text{mmol H}_2\text{O m}^2/\text{s}$ at 30 days and 0.142 $\text{mmol H}_2\text{O m}^2/\text{s}$ at 120 days. JA and SA promote stomatal opening, facilitating gas exchange and optimizing transpiration processes. Additionally, they modulate the expression of aquaporins, proteins that enhance water transport across cell membranes,

leading to more efficient water uptake (Curticăpean, 2020). Overall, the application of JA and SA effectively enhances transpiration, contributing to improved water regulation, growth performance, and stress resilience in mahogany seedlings.

The exogenous application of JA and SA significantly enhanced stomatal conductance in mahogany seedlings, which is crucial for gas exchange and plant metabolism (Table 09). JA at 0.8 mM (T5) achieved the highest stomatal conductance of $0.0758 \text{ mol m}^{-2} \text{ s}^{-1}$ at 30 days, declining to $0.0265 \text{ mol m}^{-2} \text{ s}^{-1}$ by 150 days, while the control group (T1) increased from 0.0023 to $0.0120 \text{ mol m}^{-2} \text{ s}^{-1}$. Similarly, SA at 0.8 mM (T5) demonstrated peak conductance of $0.0787 \text{ mol m}^{-2} \text{ s}^{-1}$ at 30 days, decreasing to $0.0231 \text{ mol m}^{-2} \text{ s}^{-1}$ by day 120. Both JA and SA significantly improved stomatal conductance compared to control treatments. This increase in stomatal conductance is attributed to the ability of JA and SA to promote stomatal opening, enhancing gas exchange for CO₂ uptake and water vapor loss (Zamora *et al.*, 2021). Additionally, these hormones may regulate genes associated with stomatal development, improving seedlings' metabolic efficiency (Bouvier *et al.*, 2024). Thus, JA and SA at optimal concentrations effectively enhance stomatal conductance, improving gas exchange and metabolic performance in mahogany seedlings (Abebe *et al.*, 2009).

The analysis of leaf temperature in mahogany seedlings revealed no significant differences across the various treatments throughout the evaluation period. This finding suggests that the exogenous applications of JA and SA did not have a discernible effect on leaf temperature regulation under the given experimental conditions.

The application of JA and SA significantly influenced the Relative Water Content (RWC) of mahogany seedlings, reflecting variations in plant hydration and overall health. In JA treatments, the highest RWC was observed in T4 (0.6 mM) at 80.62% on day 30, while the control group (T1) declined to 60.23% by day 150. For SA treatments, T5 (0.8 mM) achieved the highest RWC of 86.68% at day 30, decreasing to 65.55% by day 150, compared to T1, which reached only 56.05%. These results underscore the effectiveness of SA in enhancing plant hydration. The significant improvement in Relative Water Content (RWC) with JA and SA treatments is attributed to their enhanced physiological processes promoting water retention and cell turgor. Wang *et al.* (2020) found that these hormones increase aquaporin expression, improving water transport across cell membranes. Additionally, SA enhances stomatal conductance, facilitating better gas

exchange and water regulation (Khoshbakht, 2015). These hormonal applications effectively support plant hydration, which is essential for maintaining turgor pressure and optimizing growth.

Total chlorophyll content, an essential indicator of plant health and photosynthetic efficiency, varied significantly with defense hormone treatments. In JA treatments, T4 (0.6 mM) reached the highest chlorophyll content of 4.448 mg g⁻¹ at 90 days, while T3 (0.4 mM) had the lowest at 2.576 mg g⁻¹ by 150 days. The control group (T1) decreased from 3.746 mg g⁻¹ at 90 days to 2.809 mg g⁻¹ at 150 days. For salicylic acid (SA) treatments, T5 (0.8 mM) recorded the highest content of 3.704 mg g⁻¹ at 30 days, maintaining 3.159 mg g⁻¹ by 150 days. Overall, SA T5 (0.8 mM) most effectively enhanced and maintained chlorophyll content, followed by JA T4 and T6. The increase in total chlorophyll content due to Jasmonic Acid (JA) and Salicylic Acid (SA) can be attributed to photosynthetic pigment-enhancing proteins, upregulation of chlorophyll biosynthesis, inhibition of chlorophyll degradation, and improved photosynthetic efficiency. These hormones enable plants to maintain chlorophyll levels under stress, promoting better photosynthesis and growth (Rehman, 2023; Farhangi-Abriz *et al.*, 2018).

5.1.3 Hierarchical clustering of SA and JA treatment groups

The hierarchical clustering results provide valuable insights into the variability of plant responses to different concentrations of Jasmonic Acid (JA) and Salicylic Acid (SA). By examining the interclass distances, we can infer the degree of similarity or dissimilarity between treatments, shedding light on how the plants react to each treatment under stress conditions. Hierarchical clustering under JA treatments revealed three distinct clusters, reflecting varying plant responses to different hormone concentrations. T4 (0.6 mM) formed an isolated cluster with the largest interclass distance, indicating a unique response likely due to its optimal concentration for stress adaptation. The control group (T1) also formed a separate cluster with a smaller interclass distance, showing moderate similarity to the JA treatments. Cluster 3, grouping T2 (0.2 mM), T3 (0.4 mM), and T6 (1.0 mM), showed more similar responses, likely due to comparable effects on key physiological traits like photosynthesis and stomatal conductance. T5 (0.8 mM) formed a distinct cluster, possibly indicating an intermediate concentration that induced a different stress response.

Similarly, SA treatments showed distinct patterns. The control (T1) formed a separate cluster, highlighting its physiological responses without SA. T5 (0.8 mM) also formed a distinct cluster, indicating its unique impact on plant physiology. T2, T3, T4, and T6 grouped together with smaller interclass distances, suggesting more uniform responses related to growth and stress adaptation, particularly in water management and biomass allocation.

5.1.4 PCA and selection index of treatment responses

PCA provided key insights into the physiological and morphological traits driving plant responses under JA and SA treatments. For JA, PC1 accounted for 77.76% of the variation, with root length, shoot dry weight, and photosynthetic rate contributing most, highlighting their role in stress response. PC2 emphasized leaf temperature and root dry weight, suggesting JA's influence on thermal regulation and biomass allocation under stress. For SA, PC1 explained 74.02% of the variation, with leaf area, height, shoot length, and number of leaves as major contributors, indicating SA's role in promoting growth under stress. PC2 highlighted root dry weight and water retention, showing SA's impact on water management and root biomass during stress. The final PCA-based scoring ranked plant performance, emphasizing the importance of traits like photosynthetic rate, biomass allocation, and water retention in stress tolerance. Hierarchical clustering and PCA revealed distinct response patterns, with certain JA (T4 0.6mM) and SA (T5 0.8mM) treatments inducing unique growth dynamics (Fig 9, 11). These findings provide a framework for optimizing hormone treatments to improve plant stress tolerance (Maizel *et al.*, 2020).

5.2 ABIOTIC STRESS ADAPTATION MECHANISM IN MAHOGANY SEEDLINGS:

Mahogany seedlings exhibit a wide range of morphological, physiological, and biochemical adaptations to cope with abiotic stresses such as drought and salinity. Under drought conditions, these seedlings undergo significant changes, including reduced leaf area, lower stomatal conductance, and diminished transpiration rates, improving water use efficiency. These changes help minimize water loss, allowing the plant to conserve vital resources (Khatun *et al.*, 2021; Rahman *et al.*, 2013). In response to salinity stress, physiological adjustments include the regulation of ion homeostasis, where seedlings balance sodium (Na⁺) and potassium (K⁺) ions to prevent cellular toxicity. Salinity-induced stress also reduces photosynthetic efficiency and stomatal conductance, limiting

carbon fixation, further aggravated by osmotic stress and oxidative damage (Rahman *et al.*, 2013).

The application of defense hormones, such as JA and SA, plays a pivotal role in modulating these stress responses. SA has been shown to improve drought tolerance by regulating stomatal closure and enhancing antioxidant activity, thereby mitigating oxidative damage (Khan *et al.*, 2015). In contrast, JA promotes the expression of stress-responsive proteins and secondary metabolites, which aid in maintaining cellular homeostasis and provide structural protection against the harmful effects of drought and salinity (Isah and Tasiu, 2019). These hormonal treatments enhance the resilience of mahogany seedlings to abiotic stresses by boosting their defense mechanisms and overall physiological stability.

5.2.1 Morphological adaptations in response to drought and salinity stress

Leaf retention is crucial in plant stress responses, particularly under drought and salinity stress. Plants often shed leaves as a survival strategy to reduce water loss and conserve resources during unfavorable conditions, a process known as stress-induced leaf senescence involving various physiological and molecular changes (Rehman *et al.*, 2023). In mahogany seedlings, abiotic stress significantly reduced leaf retention. Under normal irrigation, plants maintained 36 leaves, whereas drought stress reduced this number to 20 leaves, and salinity stress to 28 leaves. Stress alters leaf water potential, affecting the plant's ability to retain water in leaves. Reduced leaf water potential under drought and salinity stress can lead to wilting, decreased photosynthetic activity, and eventual leaf abscission if stress persists (Ma *et al.*, 2020; Ahluwalia *et al.*, 2021).

Leaf retention varied significantly across treatments during drought and salinity stress recovery, highlighting the impact of defense hormones. In drought-stressed control plants, only 17 leaves (45.92% retention) remained, reflecting resource reallocation to critical functions like root growth. Similarly, salinity stress reduced leaf retention to 14 leaves (37.99%) due to osmotic stress. However, JA and SA significantly improved leaf retention. SA (0.8mM) treated plants retained 21 leaves (59.35%) under drought and 19 leaves (54.73%) under salinity stress, likely due to SA's regulation of stomatal conductance, ion balance, and antioxidant activity (Seleiman *et al.*, 2023). JA-treated plants (0.4mM) showed even greater retention, with 23 leaves (66.09%) under drought and 22 leaves (64.61%) under salinity, as JA induces stress-responsive proteins and

secondary metabolites, strengthening cellular structures (Isah & Tasiu, 2019). These findings suggest that JA provides stronger protection against drought and salinity stress than SA.

5.2.2 Physiological and biochemical adaptations in response to drought and salinity stress

The physiological parameters measured under abiotic stress conditions showed significant differences in the recovery phase across the treatments. Under normal irrigation, the photosynthetic rate was $3.574 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$, significantly decreasing under drought ($2.016 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$) and salinity stress ($1.406 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$). Reduced photosynthesis during drought is common, as stomatal closure limits CO_2 absorption, thereby reducing photosynthesis (Niinemets, 2016). However, defense hormone applications improved recovery. Treatment with 0.6 mM JA during drought recovery increased the photosynthetic rate to $2.985 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$, while 0.8 mM SA resulted in a moderate increase to $2.686 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$. Similarly, JA (0.4 mM) during salinity stress recovery boosted the photosynthetic rate to $2.748 \mu\text{mol CO}_2 \text{ m}^2/\text{s}$. Jasmonic acid's ability to induce stress-responsive proteins and regulate metabolic activities contributes to maintaining higher photosynthesis during recovery (Isah & Tasiu, 2019).

Stomatal conductance followed a similar pattern, low under stress conditions, with $0.007 \text{ mol m}^{-2} \text{ s}^{-1}$ under drought and $0.006 \text{ mol m}^{-2} \text{ s}^{-1}$ under salinity. Defense hormone treatments, particularly SA, increased stomatal conductance during recovery, with SA-treated plants reaching $0.091 \text{ mol m}^{-2} \text{ s}^{-1}$. Stomatal conductance plays a vital role in gas exchange, and its improvement in recovery enhances photosynthesis (Borah *et al.*, 2019). JA, however, showed a lower stomatal conductance in recovery ($0.006 \text{ mol m}^{-2} \text{ s}^{-1}$), despite its superior effects on other parameters like photosynthetic rate.

The transpiration rate also decreased significantly under drought ($0.540 \text{ mmol H}_2\text{O m}^2/\text{s}$), but increased during recovery, particularly with JA treatment under salinity ($1.323 \text{ mmol H}_2\text{O m}^2/\text{s}$) and SA under drought ($1.206 \text{ mmol H}_2\text{O m}^2/\text{s}$). Transpiration is essential for cooling and nutrient transport, and its reduction under stress helps conserve water (Niinemets, 2016). Increased transpiration in recovery helps to re-establish these processes, allowing plants to regain physiological stability (Liu *et al.*, 2010).

Relative water content (RWC), which reflects plant hydration status, dropped from 76.44% under normal conditions to 52.25% during drought and 56.70% under

salinity. Recovery treatments significantly improved RWC, with JA under drought achieving 70.45% and under salinity 70.14%, indicating better water retention during stress recovery (Galeano *et al.*, 2019). This suggests that JA helps maintain cellular water content more effectively than SA.

Leaf temperature remained stable across treatments, ranging from 32.17°C to 33.53°C. Under drought conditions, increased leaf temperatures, as high as 35.81°C, were observed due to reduced transpiration and cooling (Clausnitzer *et al.*, 2011). Maintaining optimal leaf temperatures is critical to prevent heat-induced damage during stress (Gonzalez-Dugo *et al.*, 2012).

Photosynthetic pigments, particularly chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), and total chlorophyll, declined under stress but recovered after hormone treatments. Under normal conditions, total chlorophyll was 3.672 mg/g, which dropped to 2.872 mg/g during drought. JA application during recovery restored Chl-a and Chl-b to 1.879 mg/g and 1.235 mg/g, respectively, increasing total chlorophyll to 3.091 mg/g, while SA showed a moderate increase to 3.200 mg/g. This recovery highlights the role of defense hormones in regulating chlorophyll synthesis and degradation (Rehman *et al.*, 2023).

Carotenoid content, essential for photoprotection, followed a similar trend, with normal levels at 1.257 mg/g, decreasing under drought and salinity stress but recovering with hormone applications. JA improved carotenoid content to 1.080 mg/g under drought recovery, while SA under salinity recovery increased it to 1.32 mg/g. Enhanced carotenoid levels help mitigate photo-oxidative damage during stress recovery (Taiz *et al.*, 2015).

The chlorophyll stability index (CSI) and membrane stability index (MSI), indicators of cellular integrity, were also affected by stress. Normal conditions had a CSI of 67.27% and MSI of 63.89%, which decreased under drought to 49.25% and 52.22%, respectively. SA treatment during drought recovery improved CSI (67.58%) and MSI (63.44%), while JA showed slightly lower CSI but the highest MSI (66.11%). Improved stability indices suggest better protection of cellular structures during stress recovery (Rahman *et al.*, 2013).

Proline accumulation, a marker of stress response, increased significantly under stress, from 1.878 µg/g under normal conditions to 3.857 µg/g during drought. Hormone treatments reduced proline levels, with JA showing stronger recovery by lowering proline

to 1.180 µg/g, compared to SA's 1.903 µg/g. Lower proline levels indicate reduced stress, highlighting JA's superior role in promoting recovery (Martyn *et al.*, 2022). In summary, JA consistently showed better photosynthetic rate, RWC, and pigment content recovery, indicating a more robust response than SA. This suggests that JA is more effective in mitigating the impacts of drought and salinity stress on physiological parameters.

5.3.3 Correlation analysis for different conditions of drought and salinity stress

The Pearson correlation analysis and hierarchical clustering highlight the interconnectedness of physiological and biochemical parameters in plant responses to abiotic stress. Chlorophyll a (Chl a) is central to photosynthesis, positively correlating with the photosynthetic rate (Pn), total chlorophyll, relative water content (RWC), and membrane stability index (MSI), while negatively correlating with proline, indicating that higher proline levels can reduce chlorophyll content and photosynthetic efficiency during stress.

Chlorophyll b (Chl b) supports water retention and stress protection, positively correlating with carotenoids, RWC, and chlorophyll stability index (CSI), but also negatively correlates with proline, suggesting that proline accumulation is linked to decreased Chl b levels. Proline is a stress indicator, showing negative correlations with most photosynthetic traits, thus reflecting reduced photosynthetic capacity under stress. Carotenoids enhance photoprotection, positively correlating with CSI and transpiration (E), which aids in oxidative damage mitigation and water movement. Stomatal conductance (Gs) has weaker correlations but negatively correlates with leaf retention, indicating its role in maintaining leaf stability under stress. Transpiration (E) positively correlates with RWC and CSI, signifying its importance in water regulation, while leaf temperature (LT) positively correlates with Gs, reflecting its influence on stomatal behaviour (Sun *et al.*, 2020).

5.3 PROTEOMIC ANALYSIS OF STRESS RESPONSE MECHANISMS INDUCED BY DEFENSE HORMONES:

This experiment investigated the proteomic response of *Swietenia macrophylla* seedlings to abiotic stress and subsequent treatment with defense hormones JA and SA using SDS-PAGE and Peptide Mass Fingerprinting (PMF). The findings revealed notable shifts in protein expression patterns, shedding light on how defense hormones mediate stress responses and facilitate recovery.

5.3.1 Visualization of proteins by SDS-PAGE:

SDS-PAGE analysis demonstrated distinct protein expression profiles under normal, stress, and recovery conditions, highlighting the dynamic responses of mahogany seedlings to abiotic stress and hormonal treatment. Under normal conditions, key protein bands around 50 kDa, 37 kDa, and 25 kDa reflected baseline metabolic and structural proteins, supporting findings in other studies where similar molecular weights were linked to primary physiological functions in plants (Sakamoto & Murata, 2002). However, under salt and drought stress, a reduction in low molecular weight proteins below 37 kDa was observed, suggesting suppression of stress-sensitive proteins, which aligns with previous reports on the impact of osmotic stress on protein expression (Mittler, 2006; Kannaujia *et al.*, 2019). These findings imply that specific proteins are downregulated in a stress-induced response, likely to conserve energy for essential survival mechanisms.

During recovery, untreated seedlings partially restored protein expression, evidenced by the reappearance of fainter bands. This indicates a gradual and incomplete recovery, possibly due to the residual effects of stress (Redestig *et al.*, 2011). However, SA treatment significantly enhanced the expression of proteins around 50 and 37 kDa, indicating its role in upregulating defense-related proteins. SA is known to activate pathogenesis-related proteins and other components of the systemic acquired resistance (SAR) pathway (Durrant & Dong, 2004), which could explain the enhanced protein expression observed in this study. Furthermore, JA treatment induced the most pronounced recovery response, with intense bands around 50 kDa, pointing to the activation of high molecular weight proteins associated with structural integrity and stress resilience. Studies have shown that JA effectively promotes protein expression related to cell wall strengthening and defense against abiotic stress (Wasternack & Song, 2017), supporting our findings that JA may be particularly influential in post-stress recovery.

5.3.2 Peptide mass fingerprinting (PMF) and protein identification:

To delve deeper into the identity and functions of the proteins involved, PMF analysis was conducted on proteins excised from SDS-PAGE gels. The MALDI-TOF MS analysis provided peptide mass fingerprints that facilitated the identification of proteins through Mascot software, matched with entries in the NCBI database. This method, widely used

in plant proteomics, allowed for precisely identifying stress-responsive proteins (Gygi *et al.*, 1999). Among the identified proteins, many are implicated in stress tolerance mechanisms, including enzymes involved in antioxidant defense, metabolic regulation, and cellular repair (Kosová *et al.*, 2011).

The identified proteins' functional annotation revealed that, under stress conditions, proteins associated with ROS scavenging, metabolic adjustments, and cellular repair were predominant. This aligns with findings from prior studies on proteomic responses to drought and salinity stress, where increased expression of antioxidant enzymes and molecular chaperones was reported as a means to mitigate oxidative damage (Wang *et al.*, 2003; Jorrín-Novo *et al.*, 2015). In seedlings treated with SA and JA, proteins linked to defense and cellular signalling were highly expressed, particularly in the JA-treated group, which underscores JA's role in upregulating structural proteins and signaling molecules that fortify stress resistance (Ali *et al.*, 2007; Wasternack, 2014).

Overall, the proteomic analysis provided critical insights into the stress response mechanisms activated by SA and JA. These hormones helped restore protein expression disrupted by salt and drought stress and induced specific proteins linked to defense and structural integrity. The increased expression of high molecular weight proteins in JA-treated seedlings, in particular, suggests that JA may be more effective in bolstering stress resilience through structural reinforcement. At the same time, SA appears to target defense-related pathways more prominently. These observations align with existing literature on the differential roles of SA and JA in plant defense signaling (Pieterse *et al.*, 2012; Zhang *et al.*, 2013).

5.3.3 Differential protein expression of mahogany seedlings under drought and salinity stress conditions

Under normal conditions, mahogany seedlings exhibited a balanced protein expression profile, with mid-molecular weight proteins (ranging from 35 kDa to 60 kDa) dominating the baseline profile. Ten significant protein spots were identified, with D-3-phosphoglycerate dehydrogenase 1 (PGDH1) being the most abundant at a concentration of 0.219 mg/mL, followed closely by guanine nucleotide-binding protein alpha-1 at 0.197 mg/mL. Additionally, ribulose biphosphate carboxylase (RuBISCO) was detected at 0.076 mg/mL, along with chlorophyll a-b binding protein at 0.007 mg/mL and pyruvate

decarboxylase at 0.001 mg/mL, all contributing to normal cellular functions. The total protein concentration was recorded at 0.631 mg/mL (Table 1), underscoring the critical role of these proteins in maintaining cellular homeostasis under optimal conditions. These findings align with prior studies suggesting that these mid-molecular weight proteins are crucial in supporting metabolic processes and photosynthesis under non-stressful conditions (Jorrín-Novo *et al.*, 2015; Zhang *et al.*, 2018).

Under stress conditions, including drought and salt stress, a varied expression pattern was observed. A marked reduction in low molecular weight proteins indicated potential membrane breakdown and reduced metabolic and photosynthetic activity, signaling a shift towards stress response mechanisms (Kramer *et al.*, 2016). During drought stress, sixteen protein spots were identified, associated with defense, metabolism, and photosynthesis, primarily localized in the cytosol, plastids, and cell membranes. Heat shock protein 90 (HSP 90) at 0.013 mg/mL, heat stress transcription factor A-4a at 0.015 mg/mL, and sulfiredoxin at 0.002 mg/mL were upregulated, supporting enhanced defense mechanisms to counteract drought stress. This observation aligns with findings from other plant species, where heat shock proteins and stress-related transcription factors play a critical role in stabilizing proteins and protecting cells under stress (Kosová *et al.*, 2011; Yadav, 2010).

Metabolism and photorespiration were supported by DAHP synthase (0.108 mg/mL), D-3-phosphoglycerate dehydrogenase (0.045 mg/mL), and guanine nucleotide-binding protein alpha-1 (0.302 mg/mL), suggesting that certain metabolic pathways remain active under drought conditions. Ribulose biphosphate carboxylase (RuBISCO) at 0.061 mg/mL, allene oxide synthase at 0.002 mg/mL, pyruvate decarboxylase 3 (PDC3) at 0.000 mg/mL, and chlorophyll a-b binding protein at 0.003 mg/mL were also maintained, albeit at reduced levels, contributing to limited photosynthesis and metabolism. MEI2-like 7 proteins at 0.017 mg/mL were detected as well, which is known to regulate gene expression and bolster defense responses during stress (Wang *et al.*, 2003; Liao *et al.*, 2020). These proteins indicate a shift towards energy conservation and survival mechanisms, as reported in drought-stressed plants of other species (Mittler, 2006).

Similarly, under salt stress conditions, eight proteins were identified, with guanine nucleotide-binding protein alpha-1 as the most abundant at 0.302 mg/mL,

followed by ribulose biphosphate carboxylase (RuBISCO) at 0.137 mg/mL and DAHP synthase, further supporting the maintenance of metabolic activities during salt stress. These observations are consistent with proteomic studies in other plants, where salt stress often triggers the accumulation of stress-responsive proteins and enzymes related to osmotic balance and ion transport (Kannaujia *et al.*, 2019; Munns & Tester, 2008). Under salt stress, the protein profile demonstrated a decrease in the concentration of certain proteins, with a total protein concentration of 0.594 mg/mL (Table 23), down by 0.092 mg/mL from normal conditions, indicating adaptive shifts to cope with salinity stress. Sulfiredoxin also increased, reaching 0.032 mg/mL, contributing to stress defense (Xiao *et al.*, 2009).

During drought recovery without defense hormones, guanine nucleotide-binding protein alpha-1 remained prominent at 0.375 mg/mL, playing a key role in metabolic signaling restoration. Ribulose biphosphate carboxylase large chain was recorded at 0.104 mg/mL, restoring photosynthetic activity. The total protein concentration during recovery was measured at 0.615 mg/mL (Table 22), suggesting a significant recovery of protein functionality. In contrast, six proteins were identified during salt stress recovery without defense hormones, with guanine nucleotide-binding protein alpha-1 again the most abundant at 0.375 mg/mL. Sulfiredoxin followed with 0.047 mg/mL, and allene oxide synthase 3 contributed 0.024 mg/mL. The total protein concentration was recorded at 0.481 mg/mL, reflecting partial recovery from salt stress (Wang *et al.*, 2003; Liao *et al.*, 2020).

The application of the defense hormone SA notably enhanced the defense response under both stress conditions. During drought recovery with SA, guanine nucleotide-binding protein alpha-1 exhibited the highest expression at 0.291 mg/mL, followed by D-3-phosphoglycerate dehydrogenase 1 at 0.081 mg/mL. Ribulose biphosphate carboxylase (0.116 mg/mL) contributed significantly to photosynthetic recovery. Other proteins such as chlorophyll a-b binding protein (0.004 mg/mL), guanylate kinase (0.010 mg/mL), formate dehydrogenase (0.046 mg/mL), and cytochrome b6-f complex iron-sulfur subunit (0.026 mg/mL) were also involved, aiding in the restoration of cellular activity and metabolism. The total protein concentration during recovery with SA treatment reached 0.691 mg/mL, underscoring the enhancement of metabolic and defense-related proteins. In salinity stress recovery (100 mM) with SA, protein expression was further elevated, with guanine nucleotide-binding protein alpha-

1 contributing 0.291 mg/mL, DAHP synthase 0.107 mg/mL, and ribulose biphosphate carboxylase 0.081 mg/mL. Additionally, proteins like the cation/H(+) antiporter (0.006 mg/mL) and MEI2-like 7 protein (0.022 mg/mL) played a role in maintaining cellular ion homeostasis, bringing the total protein concentration to 0.540 mg/mL, reflecting an active defense response induced by SA (Kannaujia *et al.*, 2019).

In drought recovery with JA treatment, guanine nucleotide-binding protein alpha-1 was prominent at 0.282 mg/mL, emphasizing its role in JA signaling pathways. DAHP synthase contributed 0.136 mg/mL, while D-3-phosphoglycerate dehydrogenase 1 accounted for 0.089 mg/mL. Other proteins, including ribulose biphosphate carboxylase (0.047 mg/mL), allene oxide synthase 3 (0.030 mg/mL), and sulfiredoxin (0.008 mg/mL), also contributed to the total protein concentration, which was recorded at 0.637 mg/mL. This profile suggests an interplay between stress response and primary metabolic proteins, highlighting JA's role in plant defense and adaptation (Nicolas *et al.*, 2016). During salinity stress recovery with JA, nine proteins were identified, with guanine nucleotide-binding protein alpha-1 accounting for 0.257 mg/mL and DAHP synthase at 0.137 mg/mL. Ribulose biphosphate carboxylase was also significant, contributing 0.116 mg/mL. Other proteins identified included MEI2-like 7 protein (0.022 mg/mL), chlorophyll a-b binding protein (0.006 mg/mL), calcium-dependent protein kinase (0.011 mg/mL), sulfiredoxin (0.027 mg/mL), cation/H(+) antiporter (0.006 mg/mL), and allene oxide synthase 3 (0.004 mg/mL). The total protein concentration increased to 0.580 mg/mL, indicating robust protein expression linked to stress recovery facilitated by JA (Wang *et al.*, 2003; Liao *et al.*, 2020).

These findings illustrate the complex adaptive responses of mahogany seedlings to abiotic stress and recovery phases, with significant variations in protein expression driven by specific defense hormones. The enhanced protein concentrations during recovery phases, especially with SA and JA treatments, underscore the critical role of these hormones in bolstering metabolic and defense pathways under stress conditions (Sharma *et al.*, 2016; Wang *et al.*, 2020).

5.3.4 Clustered heat map of differentially expressed proteins

The clustered heat map illustrates the differential expression of proteins under various conditions, highlighting the distinct up- or down-regulation in response to stress, recovery, and hormone treatments (SA and JA). The color intensity reflects protein

abundance, with brighter colors representing higher expression levels (Mund *et al.*, 2022).

During drought stress, proteins such as Chlorophyll a-b binding protein 1 and Ribulose biphosphate carboxylase exhibit varied expression patterns, indicating that stress significantly affects their involvement in photosynthesis (Dai *et al.*, 2023). Elevated levels of Sulfiredoxin (Srx) during drought and recovery conditions highlight its role in oxidative stress response (Miller *et al.*, 2010), while Allene oxide synthase 3 peaks during recovery, underscoring its importance in JA biosynthesis (Nishimura *et al.*, 2014). Notably, Guanine nucleotide-binding protein alpha-1 is markedly upregulated across all conditions, suggesting its pivotal role in cellular signaling (Chen *et al.*, 2023). Hormonal treatments with SA and JA promote the expression of proteins associated with stress adaptation, while D-3-phosphoglycerate dehydrogenase 1 (PGDH1) displayed differential expression across treatments, indicating a complex regulatory network (Zhang *et al.*, 2016).

In the context of salt stress, proteins such as Allene oxide synthase, D-3-phosphoglycerate dehydrogenase, Sulfiredoxin, and Meiotic protein 2 (MEI2) were slightly down-regulated, while Ribulose biphosphate carboxylase (RuBP), Guanine nucleotide-binding protein, and Zinc Finger proteins were upregulated, reflecting their roles in growth and development under normal conditions (Gonzalez *et al.*, 2019). Under salt stress, proteins like the cation/H⁺ antiporter (C/H⁺), DAHP synthase, and AOS3 showed down-regulation. In contrast, RuBP, Guanine, and Zinc Finger proteins were upregulated, indicating an activation of defense mechanisms (Kumari *et al.*, 2015).

During the recovery phase, C/H⁺ and DAHP synthase exhibited moderate up-regulation, while RuBP, Guanine, and Zinc Finger proteins continued to show strong up-regulation, underscoring their roles in recovery processes (Munns & Tester, 2008). Treatment with SA resulted in the up-regulation of C/H⁺, DAHP synthase, PGDH1, and pyruvate decarboxylase 3 (PDC3), while AOS3, Srx, and MEI2-like 7 were down-regulated, indicating diverse responses to SA (Xu *et al.*, 2012). In contrast, JA treatment led to significant up-regulation of C/H⁺, DAHP synthase, and PDC3, with PGDH1, Srx, and MEI2-like 7 exhibiting down-regulation. Essential proteins such as C/H⁺, DAHP synthase, RuBP, and Guanine nucleotide-binding protein play significant roles in stress response and recovery, demonstrating a distinct regulatory pattern under SA and JA

treatments (Wang *et al.*, 2015). AOS3 and PGDH1 showed heightened sensitivity to stress, indicating sustained down-regulation.

5.3.5 Fold change analysis of identified proteins:

Fold change analysis is crucial for understanding the relative abundance of proteins in response to different treatment and stress conditions. It facilitates the identification of significantly upregulated or downregulated proteins, providing insights into their functional roles in plant defense, metabolism, and stress response mechanisms (Kumar *et al.*, 2021). The fold change values for drought and salt stress are detailed in Tables 16 and 17.

Functional annotation of proteins under drought and salinity stress highlights critical shifts in their roles as plants adapt to these abiotic stresses. In both conditions, proteins exhibited a marked shift toward defense and ion/metabolic balance, reflecting the plant's prioritization of survival mechanisms (Zhang *et al.*, 2016). Under drought stress, proteins are predominantly associated with defense (38%) and metabolism (25%), while photosynthesis and gene expression are downregulated. Similarly, in salinity stress, the focus shifts to defense (29%) and ion balance (29%), with other processes reduced (Munns & Tester, 2008).

Recovery patterns differ depending on the treatment applied. Without treatment, plants rely heavily on defense and metabolism for recovery, as observed in both stress conditions (Li *et al.*, 2019). However, when treated with salicylic acid (SA), proteins show enhanced roles in photosynthesis (28%) and metabolism (27%), suggesting a restoration of growth processes alongside defense (18%) (Yin *et al.*, 2015). Conversely, treatment with JA results in a balanced enhancement of photosynthesis (22%), metabolism (22%), and secondary metabolite production (21%), alongside contributions from defense and hormonal signaling, indicating a comprehensive recovery mechanism (Sharma *et al.*, 2017).

These findings collectively highlight the dynamic interplay between stress response, recovery, and hormonal modulation, reinforcing the necessity of targeted treatments to enhance stress resilience in mahogany seedlings.

5.3.6 Functional annotation of identified proteins during drought and salinity stress

Functional annotations reveal varied responses of proteins to abiotic stress, particularly during drought and salinity conditions. Under normal conditions, the highest functional allocation is directed toward photosynthesis (28%) and metabolism (27%), with considerable resources allocated to defense (18%) and secondary metabolite production (9%), indicating a balanced physiological state conducive to optimal growth and development (Gonzalez *et al.*, 2019).

In drought conditions, there is a notable shift in functional allocation, with defense mechanisms predominating (38%), accompanied by a reduction in photosynthesis (25%) and metabolism (12%). This shift reflects prioritizing protective functions in response to stress (Munns & Tester, 2008). Upon recovery without treatment, the focus slightly shifts back to photosynthesis (29%) and metabolism (27%). However, defense (14%) and secondary metabolites (14%) remain elevated, indicating an adaptive response aimed at stabilizing physiological functions (Li *et al.*, 2024).

When salicylic acid (SA) is applied during recovery, photosynthesis continues to be the highest priority (28%), with both defense and metabolism at 18%. This highlights a stable recuperation pattern that incorporates hormonal signaling (9%), demonstrating SA's critical role in facilitating recovery (Yin *et al.*, 2015). In contrast, jasmonic acid (JA) treatment yields a nearly equal emphasis on photosynthesis (22%) and metabolism (22%), alongside defense (21%) and secondary metabolites (14%), showcasing a more balanced response that uniquely emphasizes hormonal signaling (7%). This indicates JA's role in modulating multiple functional pathways to enhance plant resilience (Sharma *et al.*, 2017).

Similarly, during normal conditions for salinity stress, the plant primarily focuses on photosynthesis, constituting 28% of its functional allocation, closely followed by metabolism at 27%. This reflects a priority on growth and energy production in the absence of stress, with defense mechanisms contributing 18%, indicating a baseline level of protection (Kumari *et al.*, 2015). Secondary metabolite production and ion balance each account for 9%, supporting regulatory functions to maintain stability. When exposed to salinity stress, the plant significantly reallocates resources toward defense, which constitutes 35% of its functional allocation, demonstrating a strategic response to mitigate the negative effects of salt stress (Munns, 2002). Photosynthesis and metabolism

decrease to 20% and 15%, respectively, as the plant conserves energy under stress, while ion balance rises to 18%, indicating active management of salt levels and cellular stability (Zhang *et al.*, 2016).

In the recovery phase without treatment, the plant begins to restore its focus on growth processes, with photosynthesis and metabolism occupying 26% and 24%, respectively. This suggests a return to energy production and metabolic activity, while defense remains substantial at 22%, allowing the plant to continue protecting itself from residual stress effects (Miller *et al.*, 2010). Ion balance and secondary metabolite production each contribute 12%, highlighting ongoing efforts to re-establish homeostasis.

During recovery with SA treatment, photosynthesis is prioritized at 28%, followed by metabolism at 20% and defense at 18%. This underscores SA's role in supporting energy production while still maintaining protective functions, with secondary metabolite production and ion balance each making up 12% and hormonal signaling introduced at 10%. This suggests that SA facilitates recovery by balancing growth and stress adaptation through signaling pathways (Xu *et al.*, 2012). In recovery with JA treatment, photosynthesis and defense are nearly equally prioritized, accounting for 25% and 22% of functional allocation, respectively. This indicates JA's effect in enhancing both growth and stress responses (Wang *et al.*, 2015). Metabolism remains at 18%, secondary metabolite production at 15%, and ion balance at 10%, suggesting JA's role in managing stress while regulating nutrient stability. Hormonal signaling at 10% reflects JA's ability to effectively modulate growth and recovery pathways (Swami *et al.*, 2011).

These findings illustrate that salinity stress prompts a robust defensive response and enhanced ion regulation, while recovery treatments with SA and JA help rebalance growth and adaptation. SA enables a faster return to growth functions, whereas JA promotes a more balanced recovery, aiding resilience across various physiological processes (Chin *et al.*, 2023; Nishimura *et al.*, 2014).

6. SUMMARY

This study, titled "Stress Proteomics on the Application of Defense Hormones in *Swietenia macrophylla* King Seedlings," explores how mahogany seedlings respond to defense hormones, specifically JA and SA, in terms of their physiological and morphological characteristics. The main objectives are to assess the effects of these hormones on various morphological and physiological parameters. Additionally, the research involves subjecting the seedlings to abiotic stress conditions, such as drought and salinity, followed by hormone treatments to evaluate their recovery patterns. Through proteomic analyses, the study identifies differentially expressed proteins, their functional roles, and the biological pathways involved in stress and recovery phases. This comprehensive assessment reveals significant morphological, physiological, and biochemical changes in response to hormone treatments under drought and salinity stress. Furthermore, the functional annotation of differentially expressed proteins indicates a shift towards enhanced defense and metabolic functions during stress, providing valuable insights into how the application of defense hormones may bolster resilience in mahogany, thereby advancing our understanding of stress proteomics in tropical forestry species.

Experiment 01. Optimization of JA and SA concentrations for enhanced growth and development in mahogany seedlings

This experiment investigated the effects of JA and SA on the growth of mahogany seedlings, aiming to identify optimal hormone concentrations for enhanced development. The results demonstrated that JA significantly improved seedling growth, with the best performance observed at a concentration of 0.6 mM, however, both lower and higher concentrations were found to have detrimental effects. Similarly, SA treatment exhibited the highest activity at a concentration of 0.8 mM. Notably, JA application was associated with uneven leaf expansion and malformations, indicating potential physiological stress. This study underscores the importance of optimizing hormone concentrations to maximize growth benefits while minimizing adverse effects in mahogany seedlings.

Major findings includes:

- For seedling height, JA at 0.6 mM (T4) increased growth by 158.87% to 106.42 cm, while SA at 0.8 mM (T5) enhanced height by 139.62% to 100.64 cm. Control groups demonstrated lower growth rates, with increases of 74.13% for JA and 69.38% for SA.
- For collar diameter, JA at 0.6 mM (T4) resulted in a 180.53% increase, achieving a mean diameter of 11.53 mm, while SA at 0.8 mM (T5) led to a 146.93% increase, reaching 10.63 mm. Control groups showed lower growth, with increases of 75.24% for JA and 74.02% for SA.
- For the number of leaves, JA at 0.6 mM (T4) resulted in a 145.38% increase, averaging 25.62 leaves per seedling, while SA at 0.8 mM (T5) led to a 125.00% increase, with an average of 23.15 leaves. Control groups exhibited lower growth, with increases of 63.18% for JA and 61.74% for SA.
- For volume increment, JA at 0.6 mM (T4) achieved a 158.74% increase, reaching approximately 155.48 cm³, while SA at 0.8 mM (T5) resulted in a 137.75% increase, averaging around 111.17 cm³. Control groups displayed lower volume growth, with increases of 63.25% for JA and 58.19% for SA.
- For leaf area, JA at 0.6 mM (T4) resulted in a 206.57% increase, averaging 43.38 cm² per seedling, while SA at 0.8 mM (T5) led to a 180.39% increase, with an average of 40.74 cm². Control groups exhibited lower leaf area growth, with increases of 89.32% for JA and 75.21% for SA.
- For shoot length, JA at 0.6 mM (T4) resulted in a significant increase of 57.73%, reaching an average of 81.00 cm, while SA at 0.8 mM (T5) led to a 53.18% increase, averaging 71.11 cm. Control groups displayed lower growth, with increases of 21.20% for JA and 17.98% for SA.
- JA at 0.6 mM (T4) yielded the highest sturdiness quotient, indicating enhanced stability and for SA, 0.8 mM (T5) resulted in the highest sturdiness, followed by 1.0 mM (T6), while lower concentrations exhibited moderate effects.

- Both JA and SA treatments significantly improved the seedling quality index, with optimal results at 0.6 mM for JA and 0.8 mM for SA.
- JA treatments showed the longest root length at 0.6 mM (23.11 cm), while SA peaked at 0.8 mM (23.56 cm), although no significant differences were found. The number of roots was highest in the control group (T1) for JA (46.89), with SA reaching 50 at 0.6 mM (T4), but declined at 1.0 mM (T6). JA had a maximum root dry weight of 6.88 g at 0.8 mM (T5), while SA showed no significant differences across treatments.
- For Photosynthetic Rate ($\mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1}$), JA at 0.6 mM (T4) achieved peak rates of $3.78 \mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1}$ on day 120, while the control (T1) decreased from 2.55 to $2.14 \mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1}$. SA at 1.0 mM (T6) peaked at $4.50 \mu\text{mol CO}_2 \text{ m}^2 \text{ s}^{-1}$ on day 30, indicating the importance of optimizing hormone concentrations for enhanced photosynthesis.
- For transpiration rate ($\text{mmol H}_2\text{O m}^2 \text{ s}^{-1}$), JA at 0.6 mM (T4) recorded the highest rates of $0.178 \text{ mmol H}_2\text{O m}^2 \text{ s}^{-1}$ at 30 days and 0.145 at 120 days. The control (T1) declined from 0.086 to $0.078 \text{ mmol H}_2\text{O m}^2 \text{ s}^{-1}$, while SA at 0.8 mM (T5) peaked at $0.177 \text{ mmol H}_2\text{O m}^2 \text{ s}^{-1}$ at 30 days and $0.142 \text{ mmol H}_2\text{O m}^2 \text{ s}^{-1}$ at 120 days, emphasizing the role of hormones in improving water regulation.
- For stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$), the highest conductance for JA was in T5 (0.8 mM), reaching $0.0758 \text{ mol m}^{-2} \text{ s}^{-1}$ at 30 days. The control (T1) had the lowest values, highlighting the effectiveness of optimal hormone concentrations in enhancing gas exchange.
- Leaf Temperature ($^{\circ}\text{C}$) has no significant differences in leaf temperature were observed across treatments, indicating consistent plant responses to environmental conditions.
- For relative water content (RWC), JA at 0.6 mM (T4) recorded the highest RWC at 80.62% at 30 days, while the control declined to 60.23% by 150 days. SA at

0.8 mM (T5) achieved 86.68% at 30 days, showing SA's effectiveness in enhancing plant hydration.

- Total Chlorophyll Content (mg g^{-1}) for JA at 0.6 mM (T4) peaked at 4.448 mg g^{-1} at 90 days, while the control declined from 3.746 mg g^{-1} to 2.809 mg g^{-1} by 150 days. SA T5 (0.8 mM) effectively maintained chlorophyll content at 3.704 mg g^{-1} at 30 days, indicating its positive impact on photosynthetic efficiency.

Experiment 02. Evaluating the role of JA and SA in alleviating drought and salinity stress in mahogany seedlings

This experiment assessed the efficacy of JA and SA in enhancing the tolerance of mahogany seedlings to drought and salinity stress. The findings revealed that both hormones significantly improved stress resilience, with JA (at 0.6 mM) demonstrating high effectiveness in alleviating salt and drought stress conditions. Conversely, SA (at 0.8 mM) was particularly beneficial for mitigating drought stress. The study highlighted the critical roles of both JA and SA in modulating morphological, physiological, and biochemical adaptations, showcasing their potential as effective agents for improving stress tolerance in mahogany seedlings.

Major findings include:

- During drought stress and salinity stress the JA (0.6 mM) significantly increased the number of leaves during recovery (19.20), (22.28) compared to control (12.74), (13.85) respectively.
- During drought stress and salinity stress, the JA (0.6 mM) significantly increased the number of shoots produced during recovery (6.89) and (6.22) compared to control (1.78) and (1.67), respectively.
- During drought stress and salinity stress, JA (0.6 mM) significantly increased the photosynthetic rate during recovery ($2.985 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$) and ($2.748 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$) compared to the control ($2.037 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$) and ($0.569 \mu\text{mol CO}_2 \text{ m}^2\text{s}^{-1}$), respectively.

- JA (0.6 mM) significantly improved stomatal conductance during stress and recovery phases ($0.0203 \text{ mol m}^{-2} \text{ s}^{-1}$) and ($0.0060 \text{ mol m}^{-2} \text{ s}^{-1}$) compared to control ($0.0071 \text{ mol m}^{-2} \text{ s}^{-1}$) and ($0.1211 \text{ mol m}^{-2} \text{ s}^{-1}$), respectively, under drought stress, while SA (0.8 mM) showed moderate improvements during recovery ($0.0122 \text{ mol m}^{-2} \text{ s}^{-1}$) compared to control ($0.1211 \text{ mol m}^{-2} \text{ s}^{-1}$) under salinity stress.
- Under drought stress, the JA (0.6 mM) treatment significantly enhanced the transpiration rate during recovery ($0.712 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$) compared to the control ($0.206 \text{ mmol H}_2\text{O m}^2\text{s}^{-1}$), while SA (0.8 mM) showed modest improvements in the recovery phase (0.016) compared to the control (0.012 $\text{mmol H}_2\text{O m}^2\text{s}^{-1}$).
- Under drought stress, the SA (0.8 mM) treatment significantly enhanced Chl-a during recovery (1.879) compared to the control (1.484).
- JA (0.6 mM) significantly increased Chl-b during recovery under drought stress (1.235) compared to the control (0.961), while there were no significant differences during salt stress.
- JA (0.6 mM) significantly increased total chlorophyll during recovery under salt stress (3.242 mg g^{-1}) compared to the control (2.476 mg g^{-1}), highlighting the effectiveness of JA in enhancing overall chlorophyll content during stress recovery.
- For drought, JA (0.6 mM) significantly improved RWC during recovery (70.14%) compared to control (56.08%). Similarly, JA (0.6 mM) significantly increased RWC during recovery (70.45%) compared to control (61.90%).
- The control showed significantly higher proline accumulation during drought stress ($3.857 \text{ }\mu\text{g/g}$) compared to JA (0.6 mM) ($1.180 \text{ }\mu\text{g/g}$). Additionally, the for salinity stress control had significantly elevated proline levels under normal conditions ($2.067 \text{ }\mu\text{g/g}$) compared to JA (0.6 mM) ($2.303 \text{ }\mu\text{g/g}$).

Experiment 03. Comparative proteomic analysis of stress response mechanisms induced by JA and SA in mahogany Seedlings

This experiment conducted a comparative proteomic analysis to explore the stress response mechanisms induced by JA and SA in mahogany seedlings. The findings indicated that JA (at 0.6 mM) significantly modulated protein expression, mirroring patterns observed under normal conditions, and was particularly effective in addressing salt and drought stress. In contrast, SA (at 0.8 mM) showed strong efficacy in modulating protein expression under drought conditions. Notably, distinct protein expression profiles emerged in response to each stress condition, underscoring the targeted and hormone-specific responses of mahogany seedlings to abiotic stress. This analysis provides valuable insights into the differential expression of proteins and their roles in enhancing stress resilience.

- The study investigates the differential protein expression in response to drought and salinity stress, recovery phases, and hormonal treatments (SA and JA), revealing distinct shifts in functional allocation that highlight the roles of key proteins in stress adaptation and recovery mechanisms.
- During drought and salinity stress, plants demonstrate a significant shift in functional allocation towards defense mechanisms and ion regulation, prioritizing protective responses over growth. At the same time, recovery treatments with JA and SA facilitate a gradual return to metabolic functions, highlighting their roles in resilience and adaptation.

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**STRESS PROTEOMICS ON THE APPLICATION OF DEFENSE
HORMONES IN *SWIETENIA MACROPHYLLA* KING
SEEDLINGS**

By

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ABSTRACT OF THE THESIS

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8. ABSTRACT

Climate change poses severe challenges to ecosystems, agriculture, and forestry by intensifying extreme weather events like droughts, floods, and heat waves, compromising plant health, productivity, and ecosystem stability. Additionally, climate-induced salinity stress, driven by rising sea levels and increased irrigation, disrupts plant water and nutrient absorption, stunts root growth, and causes ion toxicity, reducing forest productivity, especially in tropical and subtropical regions. In response to these stresses, plants activate intricate defense mechanisms that mitigate damage and facilitate adaptation to adverse conditions. The defense hormones like Jasmonic Acid (JA) and Salicylic Acid (SA) play a vital role in enhancing plant resilience by regulating the expression of stress-related genes, promoting the production of protective proteins, and initiating physiological adjustments that optimize water-use efficiency and sustain cellular balance.

This study investigated the effects of defense hormones, JA and SA, on mahogany seedlings, a species valued for its rapid growth, high-quality wood, workability, and durability. The research aimed to understand the role of JA and SA in alleviating drought and salinity stress and explore the associated stress proteomics, offering valuable insights into adaptive responses and resilience of mahogany seedlings to abiotic stress.

The effects of various concentrations of JA and SA on the growth and development of six-month-old mahogany seedlings were investigated. Seedlings were treated with foliar applications of JA and SA at six different concentrations (0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1 mM and control), with measurements taken every 30 days over a 150-day period. The results indicated that JA at 0.6 mM (T4) and SA at 0.8 mM (T5) were the most effective treatments, leading to optimal growth performance. These treatments significantly enhanced overall plant vigor, promoting stability as indicated by the sturdiness quotient and improving seedling quality. The findings demonstrate the potential of JA and SA in enhancing mahogany growth, particularly under stress conditions, highlighting their promising applications in forestry practices.

The effectiveness of JA and SA in alleviating drought and salinity stress in mahogany seedlings was thoroughly examined. Drought stress was induced by withholding irrigation until wilting, while salinity stress was applied using a 100 mM NaCl solution (EC = 6 dS/m) over four days. Following stress induction, JA and SA were applied to leaves to enhance acclimatization and recovery, with measurements taken under normal, stressed, and 8-day post-hormone application conditions. Results indicated that JA at 0.6 mM significantly enhanced leaf retention and shoot production under drought and salinity stress conditions. It also improved photosynthetic rates and chlorophyll pigments during recovery compared to control seedlings. In contrast, control seedlings demonstrated higher proline accumulation during recovery from both stress types than those treated with SA and JA. The defense hormone JA at 0.6 mM is highly effective in alleviating drought and salinity stress, while SA (0.8 mM) was particularly effective against drought stress. Both hormones significantly influenced morphological, physiological, and biochemical adaptations in response to stress.

A comparative proteomic analysis of mahogany seedlings was performed under normal, stress, and recovery conditions (48 hours post-hormone application). Results indicated that JA (0.6 mM) significantly influenced protein expression, effectively mitigating drought and salinity stress, while SA (0.8 mM) was particularly effective against drought stress. Each stress condition produced unique protein expression profiles, reflecting hormone-specific responses. Key proteins identified included Heat Shock Proteins and Heat Stress Transcription Factor 4a for drought stress, and Cation/H⁺ Antiporter and Guanine Nucleotide-Binding Protein for salinity stress, emphasizing their roles in enhancing stress resilience and recovery.