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Plant Cell Suspension Cultures as Models for Abiotic Stress Research

Damilola Olofintuyi[1]; Oluwaseun Shodipo[2]; Maksuda Akter[3]; Abdollah Monfared[4]

[1,2,3,4]Department of Biological Sciences, Texas Tech. University, USA

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Abstract

Plant cell suspension cultures have emerged as powerful experimental systems for investigating plant responses to abiotic stress conditions. These homogeneous, rapidly dividing cell populations offer significant advantages over whole-plant systems, including controlled growth conditions, reduced experimental complexity, and enhanced reproducibility. This review examines the application of plant cell suspension cultures in abiotic stress research, with particular emphasis on drought, salinity, and temperature stress. We discuss the molecular, proteomic, and metabolomic responses observed in various species, including *Arabidopsis thaliana*, rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*). The integration of multi-omics approaches has revealed complex regulatory networks governing stress adaptation, including alterations in primary and secondary metabolism, protein secretion pathways, and epigenetic modifications. Plant cell suspension cultures provide unique opportunities to dissect stress signaling pathways, identify novel stress-responsive genes, and evaluate the efficacy of stress-protective compounds. Despite certain limitations, such as the dedifferentiated nature of cultured cells and potential loss of tissue-specific responses, suspension cultures remain invaluable tools for fundamental research and biotechnological applications. This review synthesizes current knowledge on the utility of plant cell suspension cultures in abiotic stress research and highlights future directions for advancing our understanding of plant stress biology.

Keywords: *Plant Cell Suspension Culture,Abiotic Stress, Drought Stress, Salt Stress, Proteomics, Metabolomics, Stress Adaptation, Arabidopsis Thaliana.*

I. INTRODUCTION

Climate change and environmental degradation pose unprecedented challenges to global food security, necessitating a comprehensive understanding of plant responses to abiotic stress conditions (Lambers et al., 2008; Taiz & Zeiger, 2010). Abiotic stresses, including drought, salinity, extreme temperatures, heavy metal toxicity, and nutrient deficiency, significantly limit crop productivity worldwide, with economic losses estimated in billions of dollars annually (Davies & Zhang, 1991; Zhu, 2002). Understanding the molecular mechanisms underlying plant stress responses is essential for developing stress-tolerant crop varieties capable of sustaining agricultural productivity under increasingly adverse environmental conditions (Yamaguchi-Shinozaki & Shinozaki, 2006; Cutler et al., 2010).

Plant cell suspension cultures represent a powerful experimental platform for investigating fundamental aspects of plant biology, including cellular responses to environmental stress (Lambers et al., 2008). These cultures consist of dedifferentiated cells growing in liquid medium under controlled conditions, providing a homogeneous cell population that facilitates precise experimental manipulation and reproducible results. The use of suspension cultures in plant stress research dates back several decades, but recent advances in genomics, proteomics, and metabolomics have revolutionized their application, enabling comprehensive systems-level analyses of stress responses (Kiani et al., 2007; Marondedze et al., 2016).

The advantages of plant cell suspension cultures for abiotic stress research are manifold. First, the uniform cell population eliminates the confounding effects of cellular heterogeneity present in intact plant tissues, allowing researchers to attribute observed responses directly to the applied stress treatment (Krizek, 1985; Strauss & Agenbag, 1998). Second, the controlled growth

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environment enables precise manipulation of stress conditions, including the timing, intensity, and duration of stress exposure (Gray et al., 2011). Third, suspension cultures facilitate rapid growth and high biomass production, providing sufficient material for multi-omics analyses (Marchler-Bauer et al., 2015, 2017). Fourth, the simplified system allows for the study of direct cellular responses without the complex interactions between different tissue types and organs present in whole plants (Davies & Zhang, 1991).

However, it is important to acknowledge the limitations of suspension cultures as model systems. The dedifferentiated nature of cultured cells means they may lack certain tissue-specific responses and specialized metabolic pathways present in intact plants (Taiz & Zeiger, 2010). Additionally, the absence of organ-level organization and long-distance signaling pathways limits the extrapolation of findings to whole-plant responses (Davies & Zhang, 1991; Cutler et al., 2010). Nevertheless, when used judiciously and complemented with whole-plant studies, suspension cultures provide invaluable insights into the fundamental cellular mechanisms of stress perception, signal transduction, and adaptive responses (Yamaguchi-Shinozaki & Shinozaki, 2006; Zhu, 2002).

This review examines the application of plant cell suspension cultures in abiotic stress research, with a focus on recent advances in understanding cellular responses to drought, salinity, and other environmental stresses (Figueiredo et al., 2008; Zhu, 2002). We discuss findings from proteomic, metabolomic, and transcriptomic studies conducted in various plant species, highlighting common stress response mechanisms and species-specific adaptations (Marondedze et al., 2016). Furthermore, we explore the potential of suspension cultures for biotechnological applications, including the identification of stress-protective compounds and the characterization of stress-responsive promoters for genetic engineering applications (Shannon et al., 2003; de Castro et al., 2006; Altschul et al., 1990).

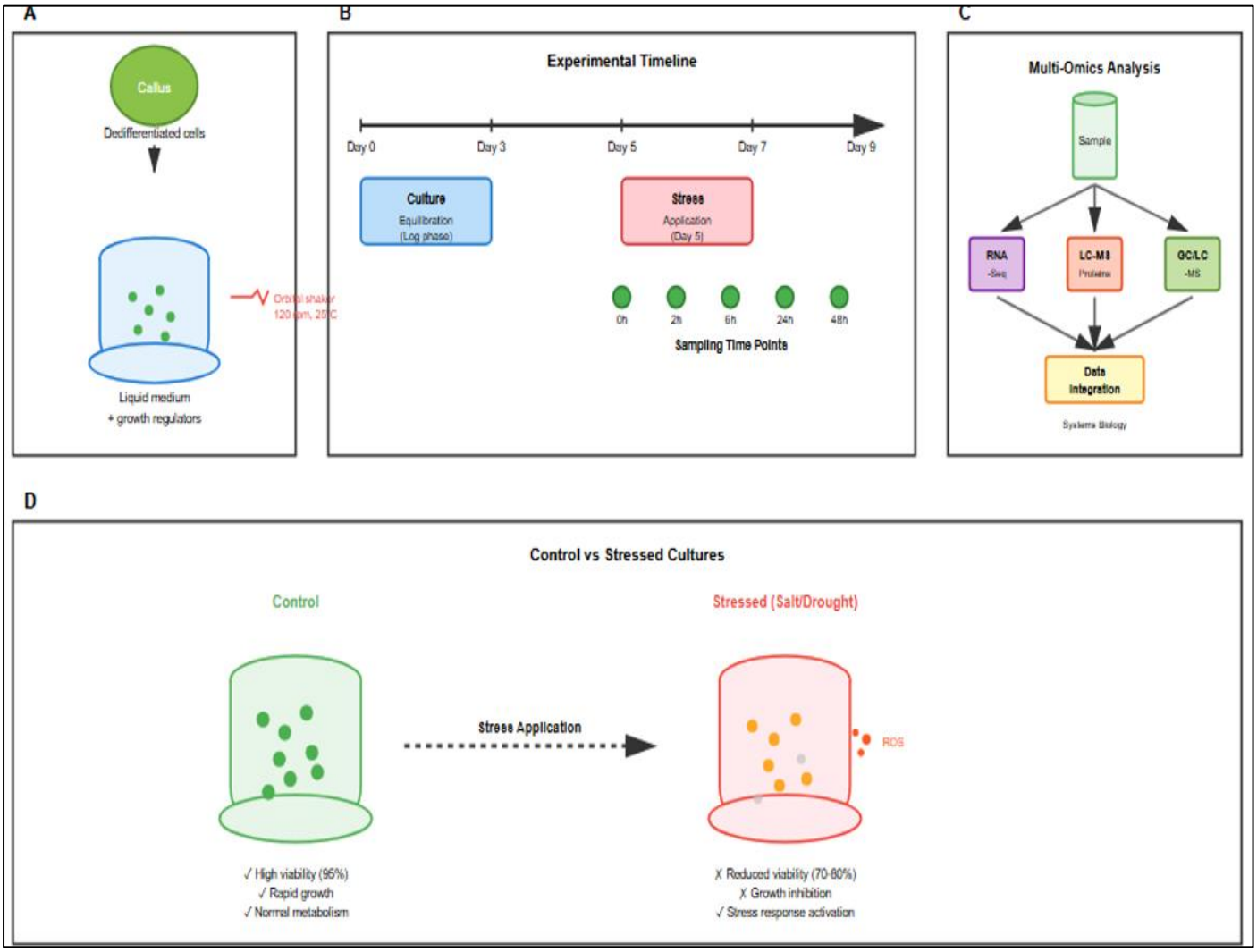

Fig 1 Schematic Overview of Plant Cell Suspension Culture System and Stress Application

- *Panel A:*

Illustration of the establishment of plant cell suspension cultures from callus tissue, showing flask setup with liquid medium and orbital shaker.

- *Panel B:*

Timeline of typical stress treatment protocol, indicating pre-treatment culture period, stress application, and sampling time points.

- *Panel C:*

Flowchart showing downstream applications including proteomic, metabolomic, and transcriptomic analyses.

- *Panel D:*

Comparison between stressed and control suspension cultures, showing typical morphological differences

✓ *Figure Caption:*

Overview of plant cell suspension culture methodology for abiotic stress research. (A) Establishment of suspension cultures from dedifferentiated callus tissue maintained in liquid medium under controlled conditions. (B) Experimental timeline showing critical phases of stress treatment experiments, including equilibration period, stress application, and temporal sampling for omics analyses. (C) Integration of multi-omics approaches for comprehensive characterization of stress responses. (D) Morphological and physiological differences between control and stress-treated suspension cultures, including cell viability, growth rate, and cellular architecture changes.

II. HISTORICAL PERSPECTIVE AND DEVELOPMENT OF PLANT CELL SUSPENSION CULTURES

Plant cell suspension cultures have been utilized in plant biology research since the mid-20th century, following the pioneering work on plant tissue culture by Haberlandt, Gautheret, and others (Lambers et al., 2008). The establishment of the first successful plant cell suspension cultures marked a significant milestone in plant biotechnology, enabling researchers to study plant cells in isolation from the complex organization of whole plants. Early applications focused primarily on fundamental aspects of plant cell biology, including cell division, differentiation, and secondary metabolite production (Taiz & Zeiger, 2010).

The development of standardized culture conditions and defined media formulations facilitated the reproducible establishment of suspension cultures from various plant species (Lambers et al., 2008). Arabidopsis thaliana emerged as a model organism for molecular plant biology research, and the establishment of Arabidopsis cell suspension cultures provided a tractable system for investigating cellular processes at the molecular level. Similarly, suspension cultures from crop species such as rice, maize, and tobacco have been extensively utilized for both basic research and biotechnological applications (Kiani et al., 2007).

➢ *Drought Stress Responses in Plant Cell Suspension Cultures*

Drought stress represents one of the most significant environmental challenges affecting plant growth and productivity (Davies & Zhang, 1991; Zhu, 2002). Plant cells perceive water deficit through multiple sensing mechanisms, triggering complex signaling cascades that lead to adaptive responses (Yamaguchi-Shinozaki & Shinozaki, 2006; Cutler et al., 2010). Studies using plant cell suspension cultures have provided crucial insights into the early molecular events following drought stress perception (Figueiredo et al., 2008).

Alqurashi et al. (2018) conducted a comprehensive proteomic analysis of early responses to severe drought stress in Arabidopsis thaliana cell suspension cultures. Their study revealed significant alterations in protein abundance within hours of stress imposition, affecting multiple cellular processes including photosynthesis-related proteins, stress response proteins, and metabolic enzymes. Notably, the rapid accumulation of late embryogenesis abundant (LEA) proteins and other protective proteins highlighted the activation of cellular defense mechanisms (Yamaguchi-Shinozaki & Shinozaki, 2006). The study also identified changes in proteins involved in protein folding, reactive oxygen species (ROS) scavenging, and osmotic adjustment, underscoring the multifaceted nature of drought stress responses (Zhu, 2002).

The proteomic changes observed in drought-stressed suspension cultures reflect a coordinated cellular response aimed at maintaining cellular homeostasis under water-limited conditions (Davies & Zhang, 1991). Downregulation of energy-intensive processes such as protein synthesis and cell division, coupled with the upregulation of protective mechanisms, represents a strategic reallocation of cellular resources to enhance survival (Cutler et al., 2010). These findings from suspension culture studies have been largely corroborated in whole-plant systems, validating the utility of this model for drought stress research (Taiz & Zeiger, 2010).

➢ *Salt Stress Adaptation and Metabolic Reprogramming*

Soil salinity affects vast areas of agricultural land worldwide, imposing both osmotic and ionic stress on plants (Zhu, 2002). Plant cell suspension cultures have proven particularly valuable for dissecting the mechanisms of salt stress tolerance, as they allow researchers to distinguish between direct cellular effects and systemic responses mediated through root-shoot communication (Davies & Zhang, 1991).

Shinde et al. (2021) investigated metabolic adjustment in Arabidopsis root suspension cells during adaptation to salt stress, revealing profound changes in both primary and secondary metabolism. Their study demonstrated that salt-adapted cells undergo extensive metabolic reprogramming, with alterations in amino acid metabolism, carbohydrate metabolism, and the biosynthesis of compatible solutes (Kiani et al., 2007). Importantly, the research revealed the existence of "mitotic stress memory," whereby cells retained metabolic signatures of previous stress exposure even after several cell divisions.

The accumulation of compatible solutes, including proline, glycine betaine, and sugars, represents a well-characterized adaptive response to salt stress (Zhu, 2002). These compounds serve multiple functions, including

osmotic adjustment, protection of cellular structures, and scavenging of reactive oxygen species (Yamaguchi-Shinozaki & Shinozaki, 2006). Metabolomic analyses of salt-stressed suspension cultures have revealed the dynamic regulation of biosynthetic pathways leading to compatible solute accumulation, providing targets for genetic engineering approaches to enhance salt tolerance (Kiani et al., 2007).

Nam et al. (2013) employed a combined proteomic and metabolomic approach to evaluate rice suspension cultured cells as a model system for studying salt-responsive networks. Their comprehensive analysis revealed coordinated changes in protein abundance and metabolite levels, identifying key regulatory nodes in the salt stress response network.

- *Hormonal Regulation and Protein Secretion Under Abiotic Stress*

Phytohormones play central roles in coordinating plant responses to abiotic stress, with abscisic acid (ABA) serving as a master regulator of stress-induced gene expression (Cutler et al., 2010). The application of exogenous ABA to plant cell suspension cultures has provided insights into ABA-mediated signaling pathways and their downstream effects on cellular physiology (Yamaguchi-Shinozaki & Shinozaki, 2006).

Mock et al. (2023) investigated the effects of exogenous ABA treatment on protein secretion in sorghum cell suspension cultures. Their proteomic analysis revealed that ABA treatment significantly altered the secretome, affecting the abundance of numerous extracellular proteins. This finding highlights the importance of protein secretion in stress responses and suggests that extracellular proteins may play previously underappreciated roles in stress adaptation (Cutler et al., 2010).

- *Multi-Omics Integration in Stress Response Studies*

The integration of multiple omics technologies has revolutionized our understanding of plant stress responses, enabling systems-level analyses that capture the complexity of cellular adaptation (Marondedze et al., 2016). Transcriptomics, proteomics, and metabolomics provide complementary information about stress-induced changes at different levels of biological organization. When applied to plant cell suspension cultures, these approaches yield comprehensive datasets that facilitate the reconstruction of regulatory networks and metabolic pathways involved in stress responses (Shannon et al., 2003).

Several studies have demonstrated the power of integrated multi-omics approaches for elucidating stress response mechanisms. The correlation between transcript abundance, protein levels, and metabolite concentrations provides insights into regulatory control points and reveals post-transcriptional and post-translational regulatory mechanisms (de Castro et al., 2006; Altschul et al.).

- *Advantages and Limitations of Suspension Cultures as Model Systems*

Plant cell suspension cultures offer numerous advantages for abiotic stress research (Lambers et al., 2008). The homogeneous cell population facilitates quantitative analyses and reduces experimental variability. The controlled culture environment enables precise manipulation of stress conditions, including the application of defined stress intensities and durations (Gray et al., 2011). The rapid growth rate and high biomass production of suspension cultures provide sufficient material for comprehensive molecular analyses (Marchler-Bauer et al., 2015; Marchler-Bauer et al., 2017).

However, several limitations must be considered when interpreting results from suspension culture studies (Taiz & Zeiger, 2010). The dedifferentiated nature of cultured cells means they may lack certain specializedstructures, and metabolic pathways present in differentiated plant tissues. The absence of tissue organization and organ-level processes limits the investigation of systemic responses and long-distance signaling (Davies & Zhang, 1991). Despite these limitations, suspension cultures remain invaluable tools when used appropriately and complemented with whole-plant validation studies (Lambers et al., 2008).

Collectively, evidence from physiological, molecular, and systems biology studies underscores the continued relevance of plant cell suspension cultures as robust platforms for abiotic stress research. The integration of physiological ecology principles (Lambers et al., 2008) with high-throughput bioinformatics resources, including sequence alignment, domain annotation, and network visualization tools (Altschul et al., 1990; Shannon et al., 2003; Marchler-Bauer et al., 2015), enables deeper characterization of stress-responsive pathways. Such integrative approaches strengthen the translational potential of suspension culture studies for crop improvement and stress resilience research.

III. MOLECULAR AND CELLULAR RESPONSES TO ABIOTIC STRESS IN SUSPENSION CULTURES

Table 1 Common Abiotic Stress Treatments Applied to Plant Cell Suspension Cultures

Stress Type	Common Treatments	Typical Concentrations/Conditions	Key Responses Observed	References
Drought/Osmotic	Polyethylene glycol (PEG), mannitol, sorbitol	PEG: 10–30% (w/v); Mannitol: 200–400 mM	Osmolyte accumulation, LEA protein induction, ROS scavenging	Alqurashi et al. (2018)
Salt stress	NaCl, Na_2SO_4	50–200 mM NaCl	Compatible solute production, ion homeostasis, metabolic reprogramming	Nam et al. (2013); Shinde et al. (2021)
Heat stress	Elevated temperature	35–45°C for 1–24 hours	Heat shock protein induction, membrane stabilization	Multiple studies
Cold stress	Reduced temperature	4–10°C for hours to days	Cold-responsive gene expression, membrane modifications	Multiple studies
Oxidative stress	H_2O_2, paraquat, methyl viologen	H_2O_2: 1–10 mM; Paraquat: 1–50 μM	Antioxidant enzyme activation, ROS scavenging	Multiple studies
Heavy metal	$CdCl_2$, $CuSO_4$, $ZnSO_4$	50–500 μM depending on metal	Chelation, compartmentalization, antioxidant responses	Multiple studies
Nutrient deficiency	Modified media lacking specific nutrients	Complete omission of target nutrient	Metabolic adjustment, nutrient remobilization	Multiple studies
Hormonal	ABA, salicylic acid, jasmonic acid	ABA: 1–100 μM; SA: 0.1–1 mM	Hormone-responsive gene expression, crosstalk	Mock et al. (2023)

➢ *Early Stress Perception and Signal Transduction*

The initial perception of abiotic stress by plant cells triggers rapid signaling cascades that ultimately lead to adaptive responses. Plant cells employ multiple sensory mechanisms to detect environmental changes, including membrane-localized receptors, ion channels, and osmosensors. In suspension cultures, the absence of complex tissue architecture allows for the direct study of these cellular sensing mechanisms without interference from systemic signals.

Early signaling events following stress perception include changes in cytosolic calcium concentrations, production of reactive oxygen species (ROS), and activation of mitogen-activated protein kinase (MAPK) cascades. These signaling components function as molecular switches that transduce stress signals to downstream effectors, including transcription factors that regulate stress-responsive gene expression. Studies using suspension cultures have elucidated the kinetics of these early signaling events, revealing that significant changes occur within minutes to hours of stress imposition.

The generation of ROS under stress conditions serves both as a signal and as a potential source of cellular damage. Plant cells must carefully balance ROS production and scavenging to harness their signaling function while preventing oxidative damage. Suspension culture studies have revealed the dynamic regulation of antioxidant systems, including superoxide dismutase, catalase, and ascorbate peroxidase, during stress adaptation.

Table 2 Advantages and Limitations of Plant Cell Suspension Cultures for Abiotic Stress Research

Advantages	Limitations
Homogeneous cell population reduces experimental variability	Dedifferentiated cells may lack tissue-specific responses
Precise control of stress intensity, duration, and timing	Absence of tissue organization and organ-level processes
Rapid growth enables high biomass production for omics analyses	Cannot study long-distance signaling and systemic responses
Simplified system for studying direct cellular responses	Potential genetic and epigenetic instability during long-term culture
Efficient transformation for gene function studies	Lack of specialized structures (stomata, trichomes, etc.)
Amenable to high-throughput screening applications	May not fully represent whole-plant stress responses
Reduced complexity facilitates mechanistic studies	Absence of root-shoot interactions and coordination
Cost-effective compared to whole-plant experiments	Potential loss of species-specific adaptations
Reproducible results across independent experiments	Limited applicability for studying developmental responses
Suitable for multi-omics integration approaches	Findings require validation in whole-plant systems

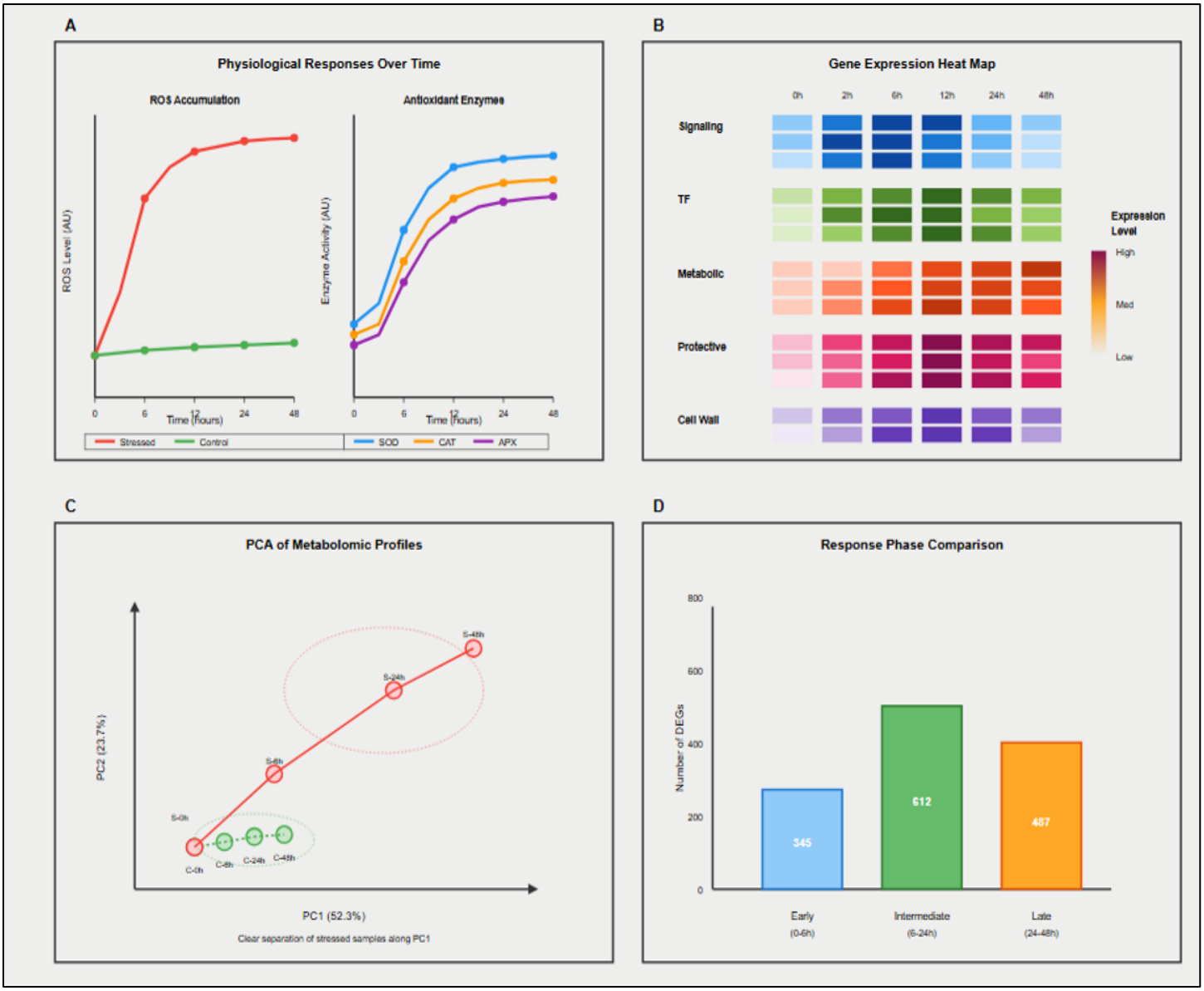

Fig 2 Temporal Dynamics of Cellular Responses to Abiotic Stress

- *Panel A:*

Line graphs showing time-course changes in ROS levels, antioxidant enzyme activities, and cell viability following stress application (0–48 hours)

- *Panel B:*

Heat map showing temporal expression patterns of stress-responsive genes grouped by function (signaling, transcription factors, metabolic enzymes, protective proteins)

- *Panel C:*

Principal component analysis (PCA) plot showing the trajectory of metabolomic profiles over time in stressed versus control cultures

- *Panel D:*

Bar graphs comparing early (1–6 h), intermediate (6–24 h), and late (24–48 h) responses

✓ *Figure Caption:*

Temporal progression of cellular responses to abiotic stress in plant suspension cultures. (A) Time-dependent changes in reactive oxygen species accumulation, antioxidant enzyme activities (SOD, CAT, APX), and cell viability in response to salt stress (150 mM NaCl). (B) Heat map representation of transcriptional responses showing distinct temporal clusters of stress-responsive genes. Early-responsive genes (0–2 h) primarily encode signaling components, while late-responsive genes (12–48 h) include metabolic enzymes and protective proteins. (C) PCA analysis of metabolomic data showing clear separation of stressed and control samples, with temporal progression indicated by connected points. (D) Quantitative comparison of proteomic changes across early, intermediate, and late response phases, highlighting the dynamic nature of stress adaptation. Data represent means ± SE from three independent experiments.

➢ *Transcriptional Regulation of Stress-Responsive Genes*

Transcription factors play pivotal roles in orchestrating stress responses by regulating the expression of hundreds to thousands of downstream target genes. Several transcription factor families, including AP2/ERF, bZIP, MYB, NAC, and WRKY, have been implicated in abiotic stress responses. Studies using suspension cultures

have facilitated the identification and functional characterization of numerous stress-responsive transcription factors.

The application of transcriptomic approaches to stress-treated suspension cultures has revealed complex regulatory networks involving multiple transcription factor families. These analyses have identified both positive and negative regulators of stress responses, highlighting the fine-tuned nature of stress-responsive gene expression. Furthermore, comparative transcriptomic studies across different stress conditions have revealed both stress-specific and common regulatory mechanisms, providing insights into crosstalk between different stress response pathways.

➢ *Proteomic Changes Under Abiotic Stress*

Proteomic analyses provide direct information about the functional molecules that execute cellular responses to stress. Changes in protein abundance reflect the combined effects of transcriptional regulation, protein synthesis, and protein degradation. Furthermore, post-translational modifications, including phosphorylation, acetylation, and ubiquitination, add additional layers of regulatory complexity that can only be captured through proteomic approaches.

Studies employing quantitative proteomics have revealed that abiotic stress induces substantial changes in the abundance of proteins involved in diverse cellular processes. Commonly affected protein categories include stress response proteins (e.g., heat shock proteins, LEA proteins), antioxidant enzymes, metabolic enzymes, and proteins involved in protein folding and quality control. The temporal dynamics of protein abundance changes provide insights into the sequential activation of different protective mechanisms during stress adaptation.

Table 3 Proteomic Changes in Plant Cell Suspension Cultures Under Abiotic Stress

Functional Category	Representative Proteins	Typical Response to Stress	Biological Significance
Stress response proteins	Heat shock proteins (HSPs), Late embryogenesis abundant (LEA) proteins	Upregulated	Protein protection, chaperoning, membrane stabilization
Antioxidant enzymes	Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Glutathione S-transferase (GST)	Upregulated	ROS detoxification, oxidative stress mitigation
Photosynthesis-related	Ribulose bisphosphate carboxylase (RuBisCO), Light-harvesting complex proteins	Downregulated under severe stress	Energy conservation, reduced photodamage
Protein synthesis	Ribosomal proteins, Translation factors	Downregulated	Energy conservation, growth reduction
Metabolic enzymes	Glycolysis enzymes, TCA cycle enzymes, Amino acid biosynthesis enzymes	Variable (context-dependent)	Metabolic reprogramming, energy metabolism adjustment
Osmolyte biosynthesis	Δ1-pyrroline-5-carboxylate synthetase (P5CS), Betaine aldehyde dehydrogenase (BADH)	Upregulated	Compatible solute production, osmotic adjustment
Cell wall modification	Expansins, Xyloglucan endotransglucosylase/hydrolase (XTH), Peroxidases	Variable	Cell wall remodeling, turgor maintenance
Signal transduction	Protein kinases, Phosphatases, 14-3-3 proteins, Calmodulins	Upregulated	Stress signal perception and transduction
Protein degradation	Ubiquitin, Proteasome subunits, Proteases	Upregulated	Protein quality control, damaged protein removal
Redox regulation	Thioredoxins, Peroxiredoxins, Glutaredoxins	Upregulated	Redox homeostasis, protein regulation

Subcellular proteomic approaches, focusing on specific organelles or cellular compartments, have further refined our understanding of stress responses. For example, analyses of the chloroplast proteome have revealed stress-induced changes in photosynthetic apparatus and chloroplast antioxidant systems. Similarly, studies of the mitochondrial proteome have highlighted the importance of mitochondrial metabolism and respiration in stress adaptation.

➢ *Metabolic Reprogramming and Compatible Solute Accumulation*

Metabolic reprogramming represents a fundamental aspect of cellular adaptation to abiotic stress. Plants adjust their metabolism to produce protective compounds, maintain energy homeostasis, and support the biosynthesis of stress-protective molecules. Metabolomic analyses of stressed suspension cultures have revealed extensive changes in both primary and secondary metabolism.

Table 4 Metabolic Changes in Plant Cell Suspension Cultures under Salt and Drought Stress

Metabolite Class	Specific Metabolites	Change Under Stress	Functional Roles	Species Studied
Amino acids	Proline, GABA, Glutamate, Aspartate	Increased	Osmotic adjustment, ROS scavenging, signaling, nitrogen storage	*Arabidopsis*, Rice
Organic acids	Citrate, Malate, Succinate, Fumarate	Variable	TCA cycle intermediates, pH regulation, chelation	*Arabidopsis*, Rice, Sorghum
Sugars	Glucose, Fructose, Sucrose, Trehalose	Increased	Osmotic adjustment, energy provision, signaling, protein stabilization	*Arabidopsis*, Rice
Sugar alcohols	Sorbitol, Mannitol, Inositol	Increased	Osmotic adjustment, ROS scavenging	Multiple species
Quaternary ammonium compounds	Glycine betaine, Choline	Increased	Osmotic adjustment, membrane protection, enzyme stabilization	Multiple species
Polyamines	Putrescine, Spermidine, Spermine	Increased	Membrane stabilization, ROS scavenging, signaling	*Arabidopsis*, Rice
Phenylpropanoids	Flavonoids, Anthocyanins, Lignin precursors	Increased	Antioxidant activity, UV protection, cell wall strengthening	Multiple species
Lipids	Phospholipids, Galactolipids, Free fatty acids	Altered composition	Membrane remodeling, signaling	*Arabidopsis*
Nucleotides	ATP, ADP, AMP	Decreased ATP/ADP ratio	Energy status indicator	Multiple species
Antioxidants	Ascorbate, Glutathione, Tocopherols	Increased or maintained	ROS scavenging, redox buffering	Multiple species

The accumulation of compatible solutes, including proline, glycine betaine, trehalose, and polyamines, represents a conserved stress response mechanism across plant species. These compounds function as osmolytes, protein stabilizers, and ROS scavengers. Studies using suspension cultures have elucidated the biosynthetic pathways and regulatory mechanisms controlling compatible solute accumulation, identifying key enzymes and regulatory points that could be targeted for enhancing stress tolerance.

Changes in carbohydrate metabolism under stress conditions reflect the need to balance energy production with the biosynthesis of protective compounds. Many studies have reported increases in soluble sugars, particularly sucrose and glucose, under stress conditions. These sugars serve multiple functions, including osmotic adjustment, energy provision, and signaling roles. The dynamic regulation of sugar metabolism highlights the central importance of carbohydrate homeostasis in stress adaptation.

- *Cell Wall Modifications and Structural Adaptations*

The plant cell wall plays crucial roles in maintaining cell shape, providing mechanical support, and mediating interactions with the environment. Abiotic stress conditions induce modifications to cell wall composition and architecture that contribute to stress adaptation. Studies using suspension cultures have revealed stress-induced changes in cell wall polysaccharides, lignin content, and the expression of cell wall-modifying enzymes.

Under osmotic stress conditions, cells typically exhibit reduced turgor pressure, necessitating adjustments to cell wall mechanics to prevent collapse. Modifications to cell wall extensibility and rigidity, mediated by enzymes such as expansins, xyloglucan endotransglucosylase/hydrolases (XTHs), and peroxidases, contribute to cellular adaptation. The balance between cell wall loosening and strengthening determines cellular responses to osmotic stress and influences cell growth under stress conditions.

IV. APPLICATIONS OF PLANT CELL SUSPENSION CULTURES IN STRESS BIOLOGY RESEARCH

Fig 3 Application of Suspension Cultures for Biotechnological Applications

- *Panel A:*
  High-throughput screening workflow, showing 96-well plates, automated imaging, and dose-response curves for stress-protective compounds.

- *Panel B:*
  Reporter gene assay results showing stress-responsive promoter activity under various treatments.

- *Panel C:*
  Results from overexpression and knockout studies, comparing stress tolerance phenotypes.

- *Panel D:*
  Bioreactor system for large-scale production of stress-induced secondary metabolites, with graphs showing optimized production parameters.

✓ *Figure Caption:*
Biotechnological applications of plant cell suspension cultures in stress research and compound production.

- High-throughput screening pipeline for identifying stress-protective compounds. Top: 96-well plate format enabling parallel testing of compounds at various concentrations. Middle: Automated fluorescence microscopy for cell viability assessment using fluorescent dyes. Bottom: Dose-response curves showing enhanced salt tolerance in cells treated with candidate osmoprotectants.
- Characterization of stress-responsive promoters using GUS reporter assays. Bar graphs show promoter activity (GUS activity normalized to protein content) under control conditions and various stress treatments, enabling identification of stress-specific promoter elements.
- Functional characterization of stress-responsive genes through overexpression (OE) and CRISPR knockout (KO) approaches. Cell viability, growth rate, and stress tolerance phenotypes demonstrate gene function. *P5CS1-OE* lines show enhanced proline accumulation and improved stress tolerance, while *P5CS1-KO* lines exhibit hypersensitivity.
- Bioreactor system for commercial production of stress-induced secondary metabolites. Left: Schematic of

bioreactor setup with controlled environmental parameters. Right: Optimization curves showing effects of elicitor concentration and treatment duration on secondary metabolite yield. Data represent means ± SE, with asterisks indicating significant differences (*p < 0.05, **p < 0.01, ***p < 0.001).

- *High-Throughput Screening of Stress-Protective Compounds*

Plant cell suspension cultures provide an excellent platform for high-throughput screening of compounds that enhance stress tolerance. The uniform cell population and controlled growth conditions enable reproducible dose-response studies and facilitate the identification of effective concentrations of test compounds. This approach has been used to identify numerous natural and synthetic compounds with stress-protective properties.

Screening programs using suspension cultures have identified various classes of stress-protective compounds, including osmoprotectants, antioxidants, phytohormones, and signaling molecules. Compounds showing promise in suspension culture assays can subsequently be tested in whole-plant systems to evaluate their efficacy under realistic agricultural conditions. This stepwise approach accelerates the discovery of novel stress-protective agents for agricultural applications.

- *Characterisation of Stress-Responsive Promoters*

The identification and characterisation of stress-responsive promoters represent important goals for developing stress-tolerant transgenic crops. Promoters that are specifically activated under stress conditions enable the targeted expression of protective genes when needed, avoiding potential negative effects of constitutive overexpression. Plant cell suspension cultures provide a convenient system for testing promoter activity under various stress conditions.

Reporter gene assays, typically using β-glucuronidase (GUS) or fluorescent proteins as reporters, enable quantitative assessment of promoter activity in suspension cultures. Time-course analyses reveal the kinetics of promoter activation, while dose-response studies determine the stress intensity required for promoter induction. The characterisation of promoter elements responsible for stress responsiveness informs the design of synthetic promoters with optimised properties for biotechnological applications.

- *Functional Characterisation of Stress-Responsive Genes*

Plant cell suspension cultures facilitate the rapid functional characterisation of stress-responsive genes through overexpression or gene silencing approaches. The transformation of suspension cultures is generally more efficient than whole-plant transformation, enabling faster evaluation of candidate genes. Furthermore, the controlled growth conditions and uniform cell population facilitate quantitative assessments of gene function.

Overexpression studies have validated the roles of numerous genes in stress tolerance, including transcription factors, metabolic enzymes, and stress-protective proteins. Conversely, gene silencing approaches, including RNA interference (RNAi) and CRISPR/Cas9-mediated knockout, have revealed the functions of genes involved in stress sensing, signal transduction, and stress responses. The combination of gain-of-function and loss-of-function studies provides comprehensive insights into gene function.

- *Investigation of Stress Memory and Priming Responses*

Recent research has revealed that plants can "remember" previous stress exposure, exhibiting enhanced tolerance to subsequent stress events—a phenomenon termed stress priming or stress memory. Plant cell suspension cultures provide an ideal system for investigating the molecular basis of stress memory, as they allow precise control over the timing and intensity of stress treatments.

Studies using suspension cultures have demonstrated that stress-primed cells exhibit altered responses to subsequent stress exposure, including faster and stronger activation of protective mechanisms. The molecular basis of stress memory involves chromatin modifications, such as histone modifications and DNA methylation, that persist through cell divisions and influence gene expression patterns. Suspension culture systems enable the dissection of these epigenetic mechanisms and their roles in stress memory.

- *Production of Stress-Induced Secondary Metabolites*

Many valuable secondary metabolites, including pharmaceuticals, nutraceuticals, and industrial compounds, accumulate in response to stress conditions. Plant cell suspension cultures offer potential for the commercial production of these compounds under controlled conditions. Elicitation strategies, including the application of stress treatments or signalling molecules, can enhance secondary metabolite production in suspension cultures.

Optimisation of culture conditions, including media composition, elicitor concentrations, and treatment timing, can maximise secondary metabolite yields. In some cases, suspension culture systems achieve higher productivity than whole plants, making them economically viable for commercial production. The use of bioreactors for large-scale cultivation further enhances the feasibility of this approach.

V. COMPARATIVE ANALYSIS ACROSS PLANT SPECIES

- *Model Species Versus Crop Species*

While model species such as *Arabidopsis thaliana* provide powerful systems for fundamental research, the translation of findings to crop species requires validation in relevant agricultural systems. Comparative studies using suspension cultures from both model and crop species have revealed both conserved and species-specific stress response mechanisms.

Arabidopsis suspension cultures offer numerous advantages, including well-characterised genetics, extensive genomic resources, and efficient transformation protocols. However, important crops such as rice, wheat, and maize exhibit distinct physiological and metabolic characteristics that influence their stress responses. Studies using crop suspension cultures complement findings from model systems and provide directly relevant information for crop improvement.

Fig 4 Comparative Analysis of Stress Responses Across Plant Species

- *Panel A:*

Venn diagram showing overlap and species-specific stress-responsive genes among *Arabidopsis*, rice, and sorghum

- *Panel B:*

Heat map comparing proteomic changes across three species under equivalent stress conditions

- *Panel C:*

Bar graphs comparing accumulation of major compatible solutes (proline, glycine betaine, sugars) in different species.

- *Panel D:*

Phylogenetic tree of key stress-responsive genes showing evolutionary conservation.

✓ *Figure Caption:*

- Venn diagram showing the overlap of stress-responsive genes (≥2-fold change, FDR < 0.05) identified in *Arabidopsis*, rice, and sorghum suspension cultures subjected to salt stress (150 mM NaCl for 24 h). The core set of 234 genes represents conserved stress responses across species, while species-specific responses reflect evolutionary adaptations.
- Comparative proteomic heat map showing relative abundance changes of orthologous proteins in the three species. Clustering reveals both conserved response patterns and species-specific protein regulation.
- Quantitative comparison of compatible solute accumulation showing species-specific preferences: *Arabidopsis* and rice accumulate high proline levels, while sorghum shows enhanced glycine betaine production.
- Phylogenetic analysis of LEA protein families showing evolutionary conservation and species-specific expansions. Data represent means ± SE from three biological replicates per species.

➢ *Species-Specific Adaptations to Abiotic Stress*

Different plant species have evolved distinct strategies for coping with environmental stress, reflecting their evolutionary histories and ecological niches. Comparative analyses of stress responses in suspension cultures from diverse species have revealed both common mechanisms and species-specific adaptations.

For example, halophytic species that naturally tolerate high salinity exhibit constitutively active stress tolerance mechanisms that are stress-inducible in glycophytic species. Similarly, species adapted to arid environments show enhanced capacity for osmotic adjustment and antioxidant protection. Understanding these species-specific adaptations provides insights into the genetic and physiological basis of stress tolerance and identifies target traits for crop improvement.

➢ *C3 versus C4 Photosynthesis Types*

The photosynthetic pathway employed by different plant species influences their responses to abiotic stress, particularly drought and high temperature. C4 plants, which employ a CO_2-concentrating mechanism, exhibit inherently higher water-use efficiency than C3 plants. Comparative studies using suspension cultures from C3 and C4 species have revealed differences in metabolic responses to stress.

While suspension cultures lack intact photosynthetic capacity, they retain many components of photosynthetic metabolism and show differential regulation of these pathways under stress. Comparative metabolomic studies have highlighted differences in organic acid metabolism, amino acid profiles, and carbohydrate partitioning between C3 and C4 suspension cultures under stress conditions.

VI. INTEGRATION OF MULTI-OMICS DATA

➢ *Systems Biology Approaches to Stress Response Analysis*

The integration of multi-omics datasets through systems biology approaches provides comprehensive insights into the complexity of stress responses. Network analysis methods reconstruct regulatory and metabolic networks, identifying hub genes and metabolites that play central roles in stress adaptation. These systems-level analyses reveal emergent properties of stress responses that are not apparent from individual omics datasets.

Constraint-based metabolic modelling, integrated with transcriptomic and proteomic data, enables prediction of metabolic fluxes under stress conditions. These models provide insights into metabolic bottlenecks and identify potential targets for metabolic engineering to enhance stress tolerance. The validation of model predictions through targeted metabolomic analyses further refines our understanding of stress metabolism.

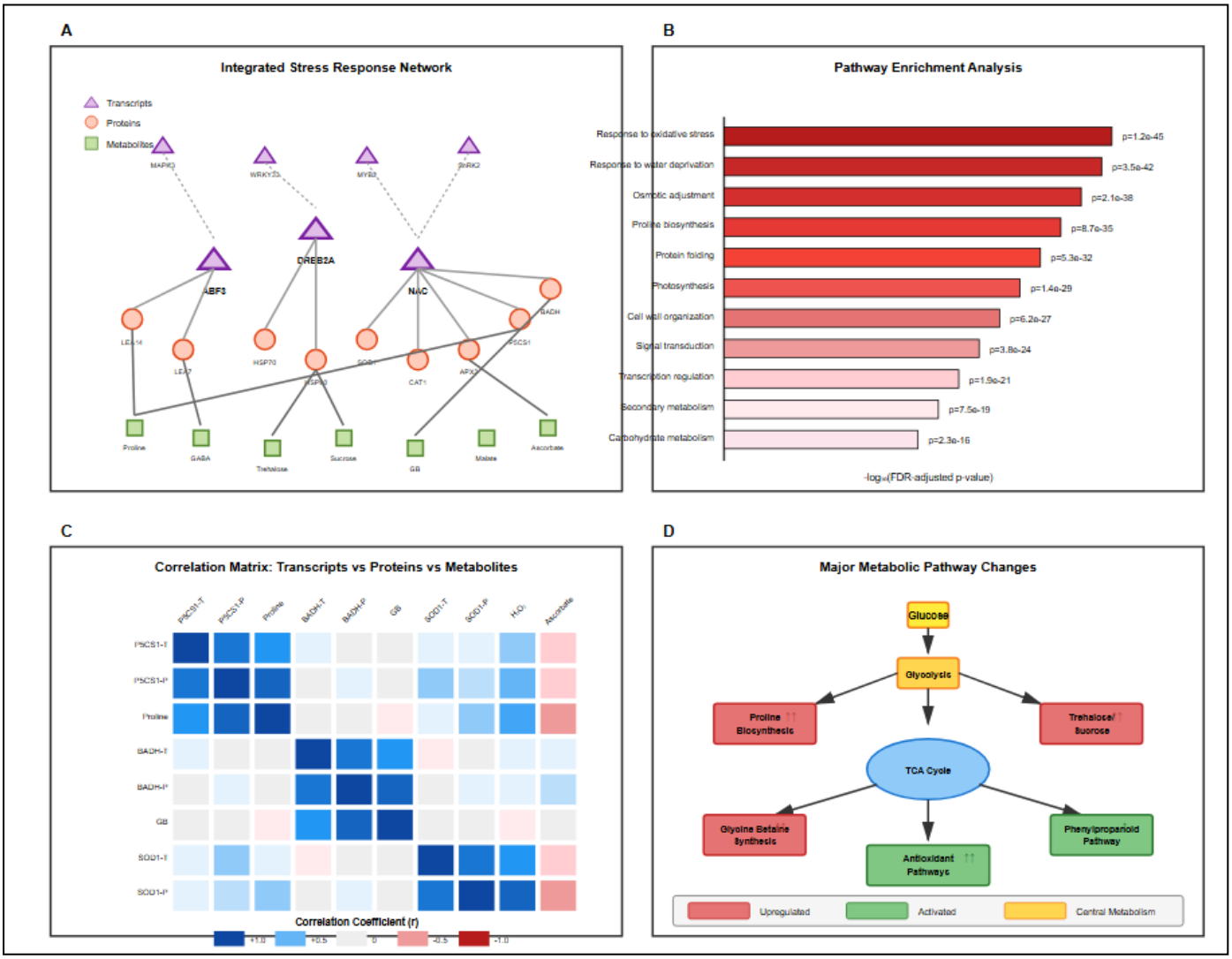

Fig 5 Multi-Omics Integration Reveals Coordinated Stress Response Networks

➢ *Panel A:*

Network diagram integrating transcriptomic, proteomic, and metabolomic data, with nodes representing genes/proteins/metabolites and edges representing correlations or regulatory relationships.

➢ *Panel B:*

Pathway enrichment analysis showing significantly affected biological processes ranked by p-value.

➢ *Panel C:*

Correlation matrix showing relationships between transcript levels, protein abundance, and metabolite concentrations for key stress-responsive components.

➢ *Panel D:*

Schematic representation of major metabolic pathways affected by stress, with colour-coded changes in metabolite levels.

✓ *Figure Caption:*

Systems-level integration of multi-omics data reveals coordinated molecular responses to abiotic stress.

- Integrated regulatory network constructed from transcriptomic, proteomic, and metabolomic datasets. Nodes represent molecular entities (triangles: transcripts; circles: proteins; squares: metabolites), with node size indicating degree of change under stress. Edge thickness represents correlation strength. Hub nodes (highly connected) represent key regulatory points in the stress response network.
- Gene Ontology and pathway enrichment analysis identifying biological processes significantly affected by stress (FDR-corrected $p < 0.05$).
- Correlation heat map showing relationships between molecular levels for 50 selected stress-responsive components. Strong correlations ($r > 0.7$) indicate coordinated regulation, while weak correlations suggest post-transcriptional or post-translational control.
- Schematic metabolic map showing stress-induced changes in central carbon metabolism, amino acid biosynthesis, and osmolyte production pathways. Upregulated pathways are shown in red, downregulated in blue. Data derived from *Arabidopsis* suspension cultures exposed to 20% PEG-induced osmotic stress for 24 hours.

➢ *Machine Learning and Predictive Modelling*

The application of machine learning algorithms to multi-omics datasets has emerged as a powerful approach for identifying biomarkers of stress tolerance and predicting cellular responses to stress. Supervised learning methods can classify cells based on their stress tolerance capacity, while unsupervised methods reveal hidden patterns in complex datasets.

Deep learning approaches, including neural networks, show promise for integrating diverse omics datasets and predicting gene function. These computational methods complement traditional hypothesis-driven research and accelerate the discovery of novel stress response mechanisms. The development of user-friendly bioinformatics tools facilitates the application of these advanced analytical methods by experimental biologists.

➢ *Data Integration Challenges and Solutions*

Despite the potential of multi-omics integration, several challenges must be addressed. Different omics platforms generate data with varying scales, distributions, and levels of noise, complicating direct comparisons. Furthermore, the relationship between transcript abundance, protein levels, and metabolite concentrations is complex, involving multiple regulatory mechanisms.

Normalization methods and statistical approaches have been developed to address these challenges. Pathway-based analysis methods that focus on functional units rather than individual molecules provide more robust insights. Additionally, time-series analyses that account for temporal dynamics reveal regulatory relationships between different molecular levels. The continued development of integrative analytical methods will further enhance the utility of multi-omics approaches.

VII. LIMITATIONS AND CONSIDERATIONS

➢ *Dedifferentiation and Loss of Tissue-Specific Responses*

The dedifferentiated nature of suspension culture cells represents both an advantage and a limitation. While cellular homogeneity facilitates quantitative analyses, the absence of tissue-specific characteristics limits the study of responses that depend on cellular differentiation. Certain stress-responsive pathways may be absent or altered in dedifferentiated cells compared to intact plant tissues.

The lack of organized tissue structure in suspension cultures precludes the investigation of tissue-specific stress responses and organ-level adaptations. For example, specialized structures such as stomata, which play crucial roles in whole-plant drought responses, are absent in suspension cultures. Therefore, findings from suspension culture studies should be validated in intact plants to ensure their physiological relevance.

➢ *Long-Distance Signaling and Systemic Responses*

Whole-plant stress responses involve complex long-distance signaling mechanisms that coordinate responses across different organs. Root-to-shoot and shoot-to-root signaling, mediated by hormones, hydraulic signals, and electrical signals, cannot be studied in suspension cultures. Systemic acquired responses, such as systemic acquired acclimation, require intact plant systems for investigation.

Despite this limitation, suspension cultures provide valuable insights into the local cellular responses that form the basis of systemic responses. Understanding the fundamental cellular mechanisms prepares the ground for

investigating how these local responses are coordinated at the whole-plant level.

➢ *Genetic and Epigenetic Instability*

Prolonged maintenance of cell suspension cultures may lead to genetic and epigenetic changes that alter cellular characteristics. Somaclonal variation, arising from genetic mutations or chromosomal rearrangements, can introduce heterogeneity into supposedly clonal cell lines. Epigenetic drift, involving changes in DNA methylation and histone modifications, may alter gene expression patterns over time.

Regular monitoring of culture characteristics and periodic re-establishment of cultures from fresh plant material can minimise these problems. Additionally, using cultures at consistent passage numbers across experiments improves reproducibility. Researchers should remain aware of these potential sources of variability when designing experiments and interpreting results.

VIII. FUTURE PERSPECTIVES AND EMERGING TECHNOLOGIES

Table 5 Emerging Technologies and Future Applications of Plant Cell Suspension Cultures in Stress Research

Technology/Application	Description	Advantages	Current Status	Potential Impact
Single-cell omics	Transcriptomic, proteomic, metabolomic analysis of individual cells	Reveals cellular heterogeneity, identifies rare cell types	Early development, growing applications	Understanding cell-to-cell variation in stress responses
CRISPR/Cas9 gene editing	Precise genome modification in suspension cultures	Rapid functional characterization, multiplexing capability	Widely adopted	Accelerated gene function studies, synthetic biology
Synthetic biology circuits	Engineered genetic circuits for enhanced stress tolerance	Precise control of gene expression, novel functions	Proof-of-concept stage	Rational design of stress-tolerant cells
Microfluidics	Miniaturized culture systems with precise environmental control	High-throughput, reduced reagent use, automation	Growing applications	Large-scale screening, dynamic stress treatments
AI and machine learning	Computational analysis of multi-omics data, predictive modelling	Pattern recognition, hypothesis generation	Increasing adoption	Accelerated discovery, optimized experimental design
3D culture systems	Three-dimensional culture mimicking tissue organization	Bridges gap between 2D cultures and whole plants	Early development	More physiologically relevant models
Bioreactor systems	Large-scale automated culture systems	Commercial production, consistent conditions	Established for secondary metabolites	Scalable production of stress-protective compounds
Real-time monitoring	Continuous measurement of cellular responses using biosensors	Dynamic response profiles, early detection	Growing applications	Understanding temporal dynamics of stress responses
Spatial metabolomics	Spatially resolved metabolite profiling in cell aggregates	Links metabolism to cellular position	Emerging technology	Understanding metabolic gradients and cell interactions
Optogenetics	Light-controlled manipulation of cellular processes	Precise temporal control, non-invasive	Early plant applications	Dissecting signaling pathways with high precision

➢ *Single-Cell Omics Approaches*

Recent advances in single-cell genomics, transcriptomics, and metabolomics are revolutionizing our understanding of cellular heterogeneity and individual cell responses. Application of these technologies to plant cell suspension cultures could reveal subtle differences in stress responses among individual cells within a culture, identifying subpopulations with distinct characteristics.

Single-cell RNA sequencing has already been applied to plant tissues, revealing unexpected cellular heterogeneity. Extending these approaches to stress-treated suspension cultures could identify rare cell types with enhanced stress tolerance or reveal stochastic variation in stress responses. These insights could inform strategies for selecting elite cells for crop improvement programs.

➢ *CRISPR/Cas9 Gene Editing in Suspension Cultures*

The CRISPR/Cas9 system has transformed plant genetic engineering, enabling precise genome editing with unprecedented efficiency. The application of CRISPR/Cas9 to suspension cultures facilitates rapid functional characterization of stress-responsive genes. Multiplexed editing approaches enable simultaneous

modification of multiple genes, accelerating the evaluation of combinatorial gene effects.

Gene editing in suspension cultures can generate edited cell lines for fundamental research or provide a stepping stone for whole-plant transformation. The rapid generation time of suspension cultures enables faster evaluation of editing efficiency and phenotypic consequences. Furthermore, the use of transient editing approaches in suspension cultures allows functional characterization without stable transformation.

- *Synthetic Biology Approaches*

Synthetic biology aims to design and construct novel biological systems with desired properties. Plant cell suspension cultures provide ideal platforms for testing synthetic genetic circuits and engineered metabolic pathways. The rapid growth and ease of transformation make suspension cultures attractive for prototyping synthetic biology constructs before transferring them to whole plants.

Engineered stress-responsive circuits could enhance stress tolerance or enable novel sensing and reporting capabilities. For example, synthetic promoters designed to respond specifically to certain stress combinations could drive protective gene expression with greater precision than natural promoters. The iterative design-build-test cycle facilitated by suspension cultures accelerates the development of effective synthetic biology solutions.

- *Integration with Artificial Intelligence*

Artificial intelligence (AI) and machine learning are increasingly being applied to plant biology research. In the context of suspension culture studies, AI algorithms can analyze complex multi-omics datasets, identify patterns, and generate testable hypotheses. Image analysis using computer vision can assess cellular morphology and viability in high-throughput screens.

AI-driven experimental design can optimize culture conditions and stress treatments to achieve desired outcomes. Reinforcement learning algorithms could autonomously adjust culture parameters in real-time based on continuous monitoring of cellular responses. These advanced computational approaches promise to accelerate discovery and enable more sophisticated experiments.

- *Development of Standardized Protocols and Resources*

The establishment of standardized protocols for suspension culture establishment, maintenance, and stress treatment would enhance reproducibility and facilitate comparison across studies. Community efforts to develop such standards, like those in microbiology and mammalian cell culture, would benefit the field.

Additionally, the creation of publicly available repositories of characterized suspension culture lines, similar to stock centers for seeds and microorganisms, would facilitate research. These resources, combined with detailed phenotypic and genomic characterization, would enable researchers worldwide to access validated experimental systems.

IX. CONCLUSIONS

Plant cell suspension cultures have proven to be invaluable tools for investigating cellular responses to abiotic stress. The controlled experimental conditions, cellular homogeneity, and amenability to molecular analyses make suspension cultures powerful systems for dissecting complex stress response mechanisms. Studies employing proteomic, metabolomic, and transcriptomic approaches have revealed the multifaceted nature of stress adaptation, encompassing changes in gene expression, protein abundance, metabolite profiles, and cellular architecture.

Key findings from suspension culture studies include the identification of rapid early signaling events following stress perception, the characterization of stress-responsive gene regulatory networks, the elucidation of metabolic reprogramming strategies, and the discovery of stress memory mechanisms. These insights have advanced our fundamental understanding of plant stress biology and provided targets for biotechnological applications aimed at enhancing crop stress tolerance.

While suspension cultures have certain limitations, including the absence of tissue organization and systemic responses, they complement whole-plant studies and provide unique experimental advantages. When used appropriately within a comprehensive research strategy, suspension cultures continue to yield important discoveries about plant responses to environmental stress.

Looking forward, emerging technologies including single-cell omics, CRISPR/Cas9 gene editing, synthetic biology, and artificial intelligence promise to further enhance the utility of plant cell suspension cultures. The integration of these advanced approaches with traditional experimental methods will deepen our understanding of plant stress biology and accelerate the development of stress-tolerant crops needed to ensure global food security in the face of climate change.

The continued use of plant cell suspension cultures, combined with advances in analytical technologies and computational methods, will undoubtedly yield further insights into the fundamental mechanisms by which plants perceive, respond to, and adapt to environmental stress. These discoveries will inform rational strategies for engineering stress-tolerant crops and contribute to sustainable agricultural practices in an era of increasing environmental challenges.

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