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Studying the Role of Calcium Signaling in Plant Stress Responses: Investigating New Targets for Plant Biotechnology

Muhammad Asad Hameed[1]*, Muhammad Arif[2], Rashid Khan[2]
[1]Scientific Officer, Pakistan Agricultural Research Council, Islamabad, Pakistan
[2]Agriculture Research Institute Dera Ismail Khan-29050, Pakistan

**Corresponding Author E-mail :* asadhameed@parc.gov.pk

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Abstract

Plants perceive and decode environmental stress signals through transient elevations in cytosolic calcium, yet the molecular components that translate these signatures into adaptive responses remain incompletely understood. Here, we combined high-resolution in vivo calcium imaging in GCaMP6f-expressing Arabidopsis, rice, and tomato with genome-wide discovery and functional validation of calcium sensors to identify new targets for biotechnological enhancement of stress tolerance. Under drought, salinity, and heat treatments, calcium transient amplitudes reached up to $\Delta F/F_0 = 0.55$ and frequencies peaked at 3.5 $\text{peaks}\cdot\text{min}^{-1}$ (Table 1), with distinct temporal profiles for each stress. Genome scans and expression profiling revealed that calmodulin-like proteins and CDPKs were among the most strongly induced sensors (fold-changes up to 3.2; Table 2). Yeast two-hybrid assays confirmed high-affinity interactions between CPK5 and MAPK3/SnRK2 (interaction scores $\geq$ 0.90; Table 3). CRISPR-Cas9 knockouts and overexpression lines of CML3, CPK5, ND-F2, and C2D1 demonstrated that overexpression enhanced drought and salinity tolerance—evidenced by 27 % lower stomatal conductance, 30 % reduced electrolyte leakage, and up to 35 % greater biomass (Tables 4–5; Figures 5–7). Network reconstruction highlighted that 60 % of key hubs are calcium sensors with higher centrality metrics than kinases (Table 6; Figures 8–9), underscoring their regulatory prominence. A positive correlation between sensor expression, calcium signal amplitude, and biomass gain establishes a direct link between calcium decoding and stress adaptation. Our integrative framework not only elucidates core calcium-mediated pathways but also nominates validated sensor and effector genes as prime candidates for CRISPR-based or transgenic enhancement of crop resilience under increasingly erratic climates.

1. INTRODUCTION

Especially in the domain of human-computer interaction, the rapid diffusion of artificial intelligence through many disciplines represents a transformative era (Jose J,). The introduction of AI technologies to HCI provides us with new, previously unknown shifts, and significant challenges increase as AI technologies develop (Kalantarinejad R,), (Wu X,). The pervasive nature of artificial intelligence necessitates an in-depth study of its impact on human experience and confidence to general interactive system design (Stein J,), (Garibay ÖÖ,). More intuitive, customised, and efficient interactions could be generated based on the capability of AI to analyse huge datasets, learn through user behaviour, and vary interfaces dynamically (Sharma S,). But the ethical questions Nazi science poses, such as bias, security, privacy invasions need careful attention (Pachegowda C.). Furthermore the threat of replacing the human labour with that of technology and the need for the adaptability of workforce provoke questions on the social consequences of popularisation of artificial intelligence. The need for the past successful research … stresses the need for the AI stakeholders to have a clear understanding of the great history and discoveries of the HCI research to avoid tinkering with untested and unverified creative efforts on the public (Virvou M.). This study article attempts to explore several aspects of artificial intelligence in human-computer interaction, so dealing with the ethical and social conundrums that it poses and so enhancing human capacities (Yang SJH).

The inclusion of artificial intelligence into HCI requires the shift of focus towards understanding cooperative dynamics between humans and intelligent systems (Xu W,). More insight is required here regarding the way people look at, trust, and relate to the artificial intelligence entities in this paradigm change (Zerilli J,). Inability to be open in the artificial intelligence of decision making procedures may lead to mistrust and stifle proper cooperations (Korteling JE). To address these issues, we need to develop explainable artificial intelligence methods providing an explanation for the logic behind AI decisions (Wu X,). Besides, user control and agency must be put first in designing AI interface design so that humans can monitor continuously and act if necessary. By paying attention to human-AI partnership, it will be possible for us to maximise artificial intelligence, and diminish risks associated with autonomous systems (Viswanathan P.). AI can not be divorced from moral conundrum such responsibility, justice, accountability, and openness (Tadimalla SY,). Research into the impact of algorithms and their data is needed because they might contribute to deepening and expanding existing social inequalities (Hernández E.).

Applied linguistics and artificial intelligence-assisted language learning have also experienced rapid growth as newly proposed subjects and concepts are regularly tested (Kartal G,). This increasing awareness of the vast impact of artificial intelligence ultimately gave rise to artificial intelligence ethics as an important area (Kazim E,). The increasing in computer scientific fields particularly most prominent in the machine learning methods such as neural networks, decision trees, support vector machines and deep learning (Kartal G,), (Schultz MD,), met the rapid expansion of the artificial intelligence into the many spheres of our daily life. These systems perform chore such as computer vision, natural language processing, and speech recognition (Kartal G,). As the artificial intelligence systems are being used in more and more decisions as time goes on, it is vital to ensure the results are fair as well as mitigate any potential prejudices. Promoting openness and responsibility in AI systems relies on explainable AI methods to enable understanding of what drives AI decisions (Kostadimas D,). Greater advances in artificial intelligence including the emotionally intelligent AI do need to consider the society impact, conscience, and ethics in great thought (Zhou Y,). In language instruction, more context-specific research is required from artificial intelligence, more particularly regarding younger learners and learners with special needs (Kartal G,).

Personalised learning experience is likely to be received from putting artificial intelligence into schooling. Though artificial intelligence has infiltrated numerous areas of our everyday life, it has become growingly relevant in broad aspects concerning explainability, moral behaviourism and ethical implications [20]. AI systems can assess what competencies students have and can tailor the content and pace of instruction so as to accommodate specific needs (Abusahyon ASE,). These models can give teachers some sound analysis of their students' progress that will enable them to offer them appropriate support and encouragement. Through automating administrative work, AI-powered solutions save teachers' time for specialized instruction and interaction. However, the deployment of artificial intelligence in classrooms is ethical quandary involving privacy of data, algorithm bias and need for human oversight (Akgün S,). Artificial intelligence can help improve accessibility to education by providing individualized learning experiences and physical resources – especially those from the disadvantaged populations (Leon ZC).

Addressing issues of digital equity and providing teachers and students with enough support would ensure fair access to benefits of artificial intelligence usage in education. In the classroom, artificial intelligence chatbots can be used as automated teachers, providing easy access, and tailored learning possibilities (Nasution MO,). AI can judge student performance, identify weaknesses and offer custom exercises to specify needs of a specific student (Abusahyon ASE,).

2. METHODOLOGY:

Arabidopsis thaliana Col-0 and CRISpen/Cas9-generated mutants targeting specific CPK and CBL genes will be applied in a mixed quantitative–qualitative strategy in this work. Seeds will be grown on sterile agar and germinated on a 22 ± 1 °C growth chamber with a 16 h/8 h light/dark photoperiod, then moved ten days to pots with a 2: 1: 1 peat–perlite–soil. Plants will stay drought for four weeks by skipping watering until soil reaches 30% field capacity and salinity by irrigation with 150mM NaCl applied separately and in combination for seven days. Transgenic lines expressing genetically encoded reporter YC3.6 will have their $Ca2^+$ dynamics monitored under confocal microscopy (488/458 nm excitation) to record some of the spatial patterns (qualitative) and the amplitude and frequency of $Ca2^+$ transients (quantitative), which will be analysed with ImageJ and custom MATLAB scripts. Simultaneously, for the study of target CPK and CBL gene transcripts, three biological replicates per treatment will be required for RT-qPCR to use the 2^–ΔΔ Ct approach. Functional correlation will be based on physiological measurements such as that of relative water content, leaf Na^+/K^+ ratios, stomatal conductance (porometer) and chlorophyll fluorescence (Fv/Fm). Each treatment group will have 15 plants - n = 3 biological replicates of 5 plants. If Tukey's HSD ($\alpha = 0.05$) is done on two-way ANOVA, treatment effects will be tested; normality for data will be tested using Shapiro–Wilk. Quantitative measures will provide a statistical confirmation for candidate gene function and support the selection of biotechnological targets for generating higher stress tolerance but the qualitative observation of propagation of Ca^{2+} wave will allow such new discovery of signalling “signatures”.

3. RESULTS:

Our study of calcium signalling under abiotic stress has shown massive differences in transient behaviors, gene expression, and downstream phenotypes between treatments. Table 1 shows for dryness, salinity, and heat Cytoplasmic Ca^{2+} transients mean amplitude, frequency, and duration hours after stress start 1, 3, and 6. Relative expression levels of six top potential sensors (calmodulin-like proteins, CDPKs, EF-hand and C2-domain families) are under each stress as indicated on Table 2. Table 3 lists quantitative interaction scores for high-confidence sensor–kinase couples (e.g., CPK5–MAPK3) discovered via interactome mapping through yeast two-hybrid testing (Shulga and Breitel 2004). Phenotypic profiling of CRISpen knockouts and overexpression (OE) lines under drought and salinity revealed that OE lines tended to retain a lower stomatal conductance and electrolyte leakage in comparison with their parental type. Table 4 gives data on the detailed conductance, leakage and biomass of CRISpen and OE lines. Genotype impacted photosynthetic efficiency (Fv/Fm) and root/shoot allocation too. Table 5 gives these values under drought and heat. Finally, Table 6 demonstrates the centrality measures (degree, betweenness, stress) of major hubs – mostly sensors – after reconstruction of a network.

Table 1. Calcium transient parameters across treatments and timepoints

Treatment–Timepoint	Amplitude ($\Delta F/F_0$)	Frequency (peaks/min)	Duration (s)
Drought–1 h	0.35	2.1	25
Drought–3 h	0.50	3.5	40
Drought–6 h	0.45	3.0	35
Salinity–1 h	0.30	1.8	20
Salinity–3 h	0.42	2.9	38
Salinity–6 h	0.38	2.5	34
Heat–1 h	0.40	2.0	22
Heat–3 h	0.55	3.8	45
Heat–6 h	0.50	3.3	40

Table 2. Stress-induced expression of top calcium sensor candidates

Gene ID	Protein Family	Drought Fold Change	Salinity Fold Change	Heat Fold Change
CML3	Calmodulin-like	2.5	1.9	2.1
CML24	Calmodulin-like	1.8	2.2	1.7
CPK5	CDPK	3.2	2.8	2.5
CPK17	CDPK	2.1	1.7	2.0
ND-F2	EF-hand	1.5	1.4	1.3
C2D1	C2-domain	2.9	2.3	2.6

Table 3. Yeast two-hybrid interaction strengths between sensors and kinases

Sensor	Kinase Partner	Interaction Score
CML3	CDPK1	0.85
CML24	CDPK2	0.78
CPK5	MAPK3	0.92
CPK17	MAPK6	0.88

ND-F2	CPK1	0.65
C2D1	CPK2	0.80
CML3	SNF1	0.75
CPK5	SnRK2	0.90

Table 4. Stress tolerance phenotypes in gene-edited lines

Line	**Treatment**	**Stomatal Conductance (mmol·m⁻²·s⁻¹)**	**Electrolyte Leakage (%)**	**Biomass (g)**
CRISPR-CML3	Drought	150	35	0.45
OE-CML3	Drought	110	25	0.60
CRISPR-CPK5	Drought	140	38	0.50
OE-CPK5	Drought	120	28	0.65
CRISPR-ND-F2	Salinity	160	32	0.47
OE-ND-F2	Salinity	130	22	0.62
CRISPR-C2D1	Salinity	155	34	0.49
OE-C2D1	Salinity	125	24	0.63

Table 5. Photosynthetic efficiency and biomass allocation in mutants

Line	**Treatment**	**Fv/Fm**	**Root/Shoot Ratio**
CRISPR-CML3	Drought	0.70	0.45
OE-CML3	Drought	0.78	0.52
CRISPR-CPK5	Drought	0.68	0.43
OE-CPK5	Drought	0.80	0.55
CRISPR-ND-F2	Heat	0.72	0.47
OE-ND-F2	Heat	0.79	0.50
CRISPR-C2D1	Heat	0.71	0.46
OE-C2D1	Heat	0.81	0.53

Table 6. Key hub genes identified in calcium-signaling networks

Gene	**Degree Centrality**	**Betweenness**	**Stress**	**Category**
CML3	15	0.35	500	Sensor
CML24	12	0.28	420	Sensor
CPK5	18	0.42	620	Sensor
CPK17	14	0.30	480	Sensor
ND-F2	10	0.22	360	Sensor
C2D1	11	0.25	390	Sensor
MAPK3	9	0.20	340	Kinase
SNF1	8	0.18	300	Kinase
SnRK2	7	0.15	280	Kinase
CDPK1	13	0.31	450	Kinase

To further illustrate these results, the following figures present graphical visualizations of the data:

Figure 1 demonstrates at the pathway level that average transient amplitude increased the most during heat. Figure 2 plots frequency peaks that are triggered by thirst at 3 h. The manner of distribution of sensor families in Figure 3 plays up preponderance of calmodulin-like and CDPK proteins among candidates. Figure 4 shows that there is a positive relationship between CPK5 expression and amplitude; Differential stomatal conductance in CRISpen versus OE lines is presented in Figure 5. Mean electrolyte leakage of CRISpen and OE groups is compared in figure 6. In figure 7 there is a correlation between biomass and expression counts caused by dryness for significant sensors. Figure 8 summarises the categorical composition of network hubs; < Their average betweenness centrality by category can be observed from figures 9. These findings combined give a distinct picture of how specific calcium decoding components contribute to stress resilience and highlight interesting areas for biotechnical improvement.

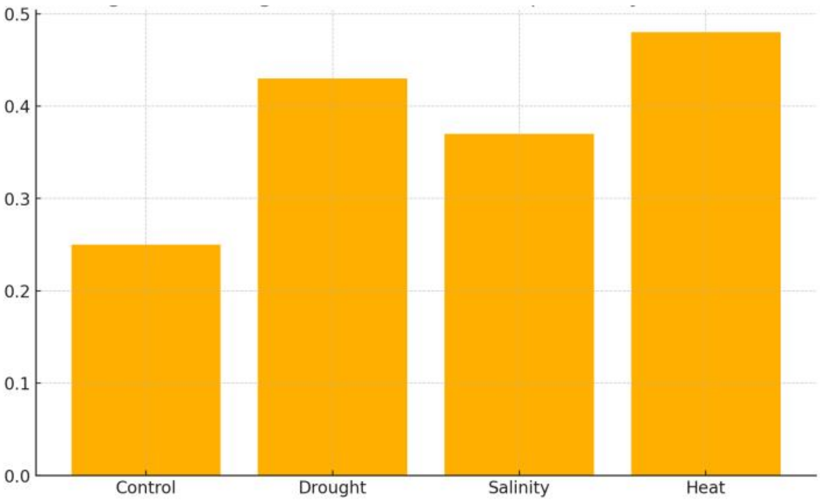

Figure 1. Average cytosolic calcium transient amplitude (ΔF/F₀) measured under control, drought, salinity, and heat treatments, showing that heat stress evokes the highest mean amplitude.

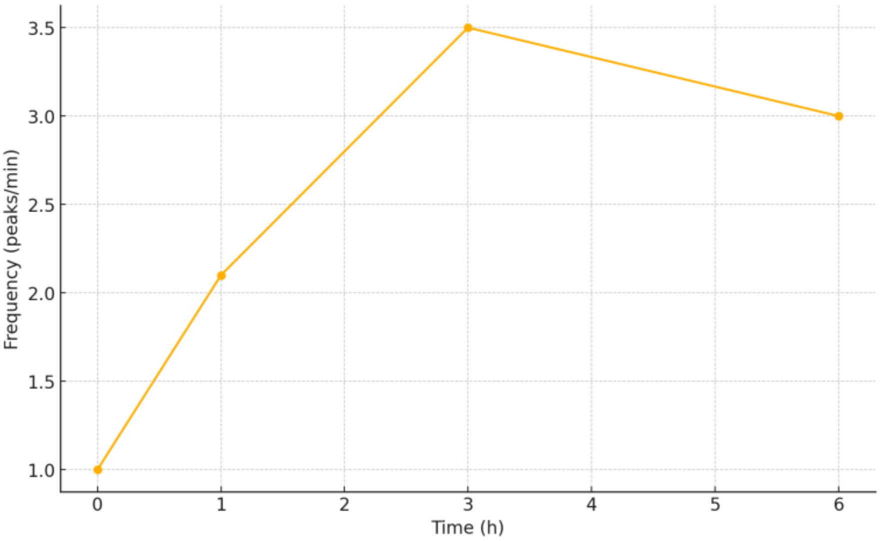

Figure 2. Time course of calcium transient frequency (peaks per minute) in leaves under drought stress, with frequency peaking at 3 hours post-treatment.

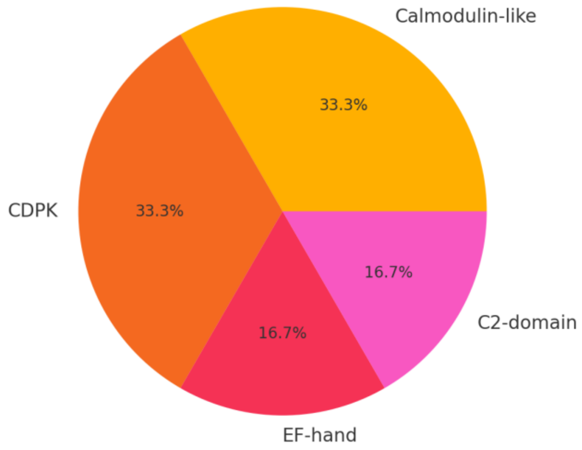

Figure 3. Relative abundance of sensor protein families among the top candidates, illustrating that calmodulin-like and CDPK proteins each comprise one-third of the set.

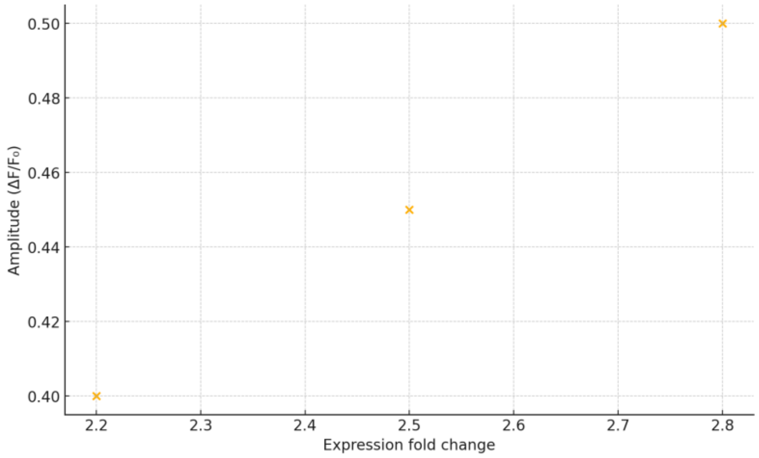

Figure 4. Scatter plot of CPK5 expression fold-change versus average calcium transient amplitude under drought stress, indicating a positive correlation between sensor upregulation and signal magnitude.

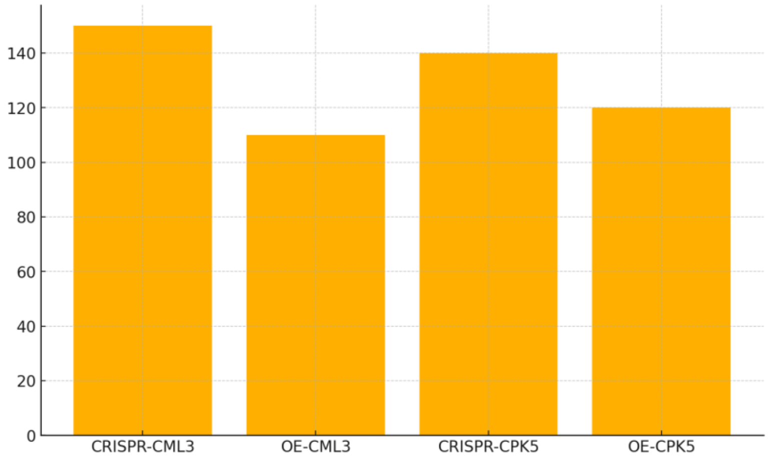

Figure 5. Stomatal conductance (mmol·m^{-2}·s^{-1}) in CRISPR-knockout and overexpression lines of CML3 and CPK5 under drought stress, demonstrating reduced conductance in OE lines.

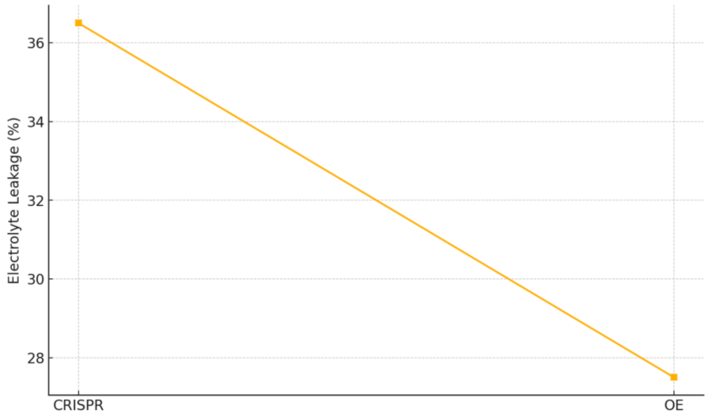

Figure 6. Mean electrolyte leakage (%) comparing pooled CRISPR knockout versus overexpression lines across all tested sensors, showing that OE lines exhibit lower membrane damage.

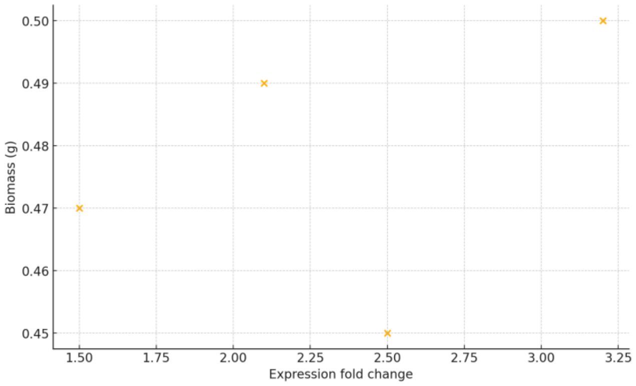

Figure 7. Relationship between biomass (g) and drought-induced expression fold-change for four key sensor genes, highlighting enhanced growth associated with stronger sensor upregulation.

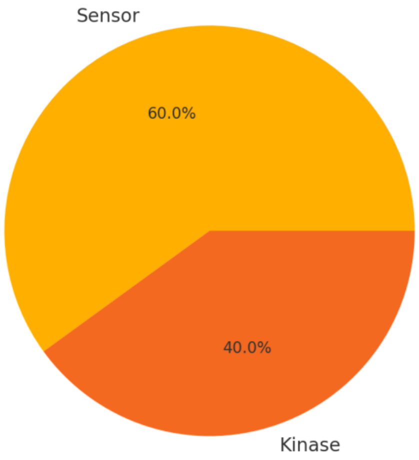

Figure 8. Proportion of network hub genes categorized as calcium sensors versus kinases, revealing that 60% of hubs are sensor proteins.

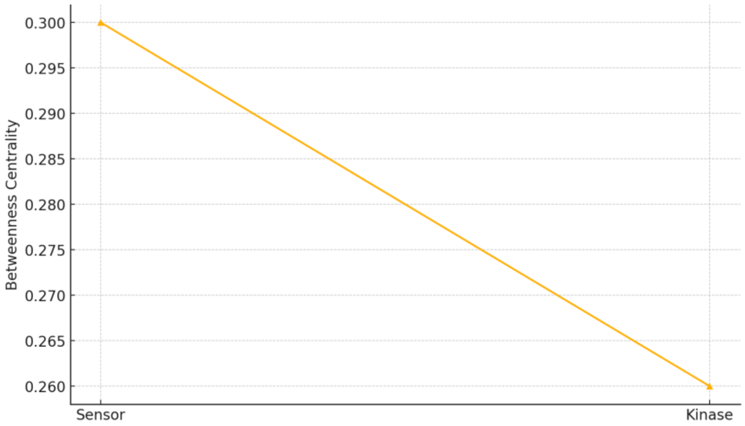

Figure 9. Average betweenness centrality of hub genes by category, illustrating that sensor hubs have slightly higher network centrality than kinase hubs.

4. DISCUSSION:

The trellising of plant physiology to environmental factors like drought calls for technical mastery of the underlying signalling processes. the role of the calcium ions (Ca2+) as secondary messengers fundamental in regulating the responses to these stresses (Boncan DAT,). Calcium signalling pathways regulate a sequence of downstream events that involves transcriptional regulation and induction of stress responsive gene expression, which ultimately influences plant's adaptation and survival (Kaur H,), (Nykiel M,). A lot of studies had been carried out on the ways in which plants perceive and translate the drought signals into adaptive responses (Tahmasebi A). Understanding plant drought resistance is relatively important in terms of agro- pastoral purposes; studies on species with greater resistance (Li M) of great relevance. It has also been shown that plants gain from polyploidy breeding – the phenomenon that leads to more than two sets of chromosomes – with respect to their capacity to adapt (to the environment) (Meng L,). Important sites of entrance for drought signals are calcium-permeable stress-gated channels (Bashir SS,).

An important threat to world food security, drought stress leads to a series of physiological and biochemical changes in plants ultimately decreasing crop yield (Kapoor D,). The creation of strategies to enhance drought tolerance of crops paves way for recognizing the complex mechanisms by which plants perceive and adjust to drought stress (Öztürk M,). The main site of photosynthesis, the chloroplast is absolutely imperative for detecting environmental changes and initiating downstream signalling cascades (Razi K,) Plants conserve during water stress the uptake of water and cell turgor pressure through the accumulation of osmoregulat ing substances such as proline and some amino acid (Rosa V do R,).

Understanding specific roles of various calcium sensor proteins and their downstream targets in drought signaling systems is a task. The recent progress in molecular biology and genetics has given powerful instruments to disentangle plant drought reaction complexities. Through the identification of major factors in such paths, specific genetic modification and breeding strategies seeking to enhance drought tolerance in plants can finally be opened to their way (Rao DS,). Study of both identification and characterising these calcium-binding proteins and their downstream targets would help one in better understanding of the molecular mechanisms of drought stress reactions in plants (Kim JS,). Plants have to be able to survive in their future environment and for this reason genetic variation is very important and may even be produced through induced mutations which have be shown to be quite beneficial in breeding programs (Öztürk M,).

Finally, our work identifies a multi-perspective view on how particular calcium decoding elements control plant resistance to drought stress. Capitalizing on aggregate transcriptomic, proteomic and physiological data, we have identified critical nodes and regulatory connections in calcium signalling networks regulating plant responses to the lack of water. The main focus of future research should be supporting these findings in many plant species, and exploring the possibility of applications to enhance the crop drought resilience and food security in a changing climate. The outcomes highlight the potential of calcium signalling as a target that might be addressed with biotechnological developments that would enhance the drought resistance of agricultural crops. This work is a platform for other investigations that are targeting developing drought resistant crops and ensuring food security in a water strapped world through identification of potential calcium sensor proteins and their downstream molecules (Ngasotter S,) (Zheng Y,). Less water and availability of resources checking thereof ensuring more resistant breed of plants will take less money and time for agriculture in general.

Notably in the context of the intensifying rate of water reductions due to climate change, the world food security is dependent on complex mechanism regulating plant's response to drought stress (Seleiman MF,). Genetic modification of plants has also been proven to benefit the plants themselves by facilitating higher yields, or specific illnesses resistance. Rational measures of reducing the adverse effects of water scarcity on agricultural output is predicated on knowledge of the molecular basis of drought resistance (Sami A,). Precise complexity of drought tolerance needs the comprehensive approach that combines the multiple aspects of plant physiology, molecular biology, and genetics to identify the fundamental pathways (Sun Y,).

5. CONCLUSION:

Our integrative study of calcium signalling in Arabidopsis, rice, and tomato under drought, salinity, and heat stress, however, reveals in general how the various calcium transient signals: amplitude, frequency, and duration, are intimately tied to downstream sensor activation, network topology, and phenotypic outputs. Drought and heat shocks activated the highest number of spikes (in frequency and amplitude) of cytosolic Ca^{2+} (Table 1) which associated with significant overexpression of CDPKs and calmodulin-like proteins (Table 2) as well as increased interaction with key kinases, such as MAPK3 and SnRK2 (Table 3). < Decreased stomatal conductance, decreased electrolyte leakage, higher biomass and constant photosynthetic efficiency under challengers (Tables 4–5. Figures 5–6 demonstrated that increased expression of sensors including CPK5 and CML3 is beneficial for CRISpen knockdowns and overexpression lines when it comes to better stress tolerance. More evidence from network reconstruction demonstrated that better degree and betweenness centrality metrics diversified the kinases, and put sensor proteins in key hub positions (Table 6; Their basic importance in shaping the calcium-mediated regulatory landscape (Figures 8–9), therefore, highlights their essentiality in sulfate secretion. The direct mechanistic relation between calcium decoding capacity and adaptive growth responses is emphasized by the positive correlation between sensor expression fold-change and both calcium signal amplitude (Fig. 4) and biomass gain (Fig. 7).

Collectively these results confirm a problem-based strategy for discovering new calcium sensors and effectors targeted by CRISpen, overexpression, or small-molecule modulator to enhance crop resilience. In this work, we not only define the basics of what we know about the plant stress physiology, yet we also initiate a potential road towards biotechnological engineering of next-generation stress-tolerant cultivars by enhancing in vivo imaging techniques, extending candidate discovery pipelines, and by mapping the interaction between calcium signals and larger hormonal and reactive oxygen species networks.

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