2025年臺灣國際科學展覽會
優勝作品專輯

作品編號　060016

參展科別　植物學

作品名稱　Decoding Climate Resilience: Functional Profiling of Protein Phosphatase 2C Family Genes for Abiotic Stress Tolerance in Rice

得獎獎項　一等獎

就讀學校　Rastogi Homeschool
作者姓名　Nandini Rastogi

作者照片

Decoding Climate Resilience: Functional Profiling of Protein Phosphatase 2C Family Genes for Abiotic Stress Tolerance in Rice

Nandini Rastogi
United States of America

All charts and images created by student unless otherwise noted. Work performed at an independent laboratory.

Introduction

Problem

- Rice is the primary cereal crop consumed by nearly half the population worldwide
- By 2050, there will be a 50% increase in demand for rice
- The world's poor populations depend more on rice, both for income and consumption, than any other food. Rice is the single-largest source of employment and income for rural people
- Worldwide, 51–82% of agricultural crop yield is lost annually due to abiotic stress due to climate change
- Climate change causes extreme temperatures, erratic rainfall, dangerous droughts, and increased salinity from rising sea levels

Solution

- To adapt to abiotic stress, rice has intricate signaling pathways, particularly those mediated by the phytohormone abscisic acid (ABA), that cause an increase in stress tolerance
- Clade A genes of the Protein Phosphatase 2C (PP2C) gene family are known to be negative regulators of the ABA signaling pathway.
- "Deleting" these genes activates the ABA pathway and increases stress tolerance in rice without inducing stress

CRISPR gene editing technology is the ideal solution

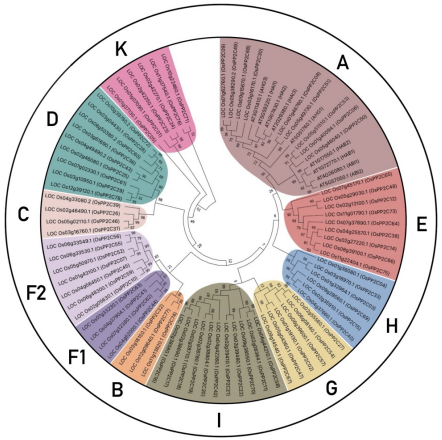
Research Goal

- While the role of PP2C genes in stress response is recognized, there is a gap in understanding the specific genes within this family that contribute significantly to stress signaling. Furthermore, there is a need for a detailed investigation into the effects of targeted CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) genome editing on rice stress response pathways.

Method

Choosing the genes of interest using bioinformatics:

- Created a list of amino acid sequences of all 80 PP2C genes in Oryza sativa and Clade A PP2C genes of Arabidopsis thaliana. Sequences were aligned by ClustalW method.
- Made a neighbor-joining phylogenetic tree with 1000 bootstrap replicates.
- Chose 5 genes of interest from Clade A based on prior research and correlation with Arabidopsis genes: OsPP2C08, OsPP2C09, OsPP2C30, OsPP2C49, and OsPP2C68.
- Software used: Phytozome website, NCBI website, MEGA



Abiotic Stress Treatments:

- Began stress treatments after seeds reached 5cm of growth.
- Conditions: 100 µM ABA (abscisic acid), 100 µM GA3 (gibberellic acid), 20% PEG6000 (polyethylene glycol), 150 mM NaCl, 150mM Mannitol, cold stress at 4 degrees Celsius, and MS media as the control.
- Each stressor was tested for 0, 12, 24, and 48 hours for each of the 5 genes. Tests were done in triplicates
- Samples were sent out for qRT-PCR expression profiling.

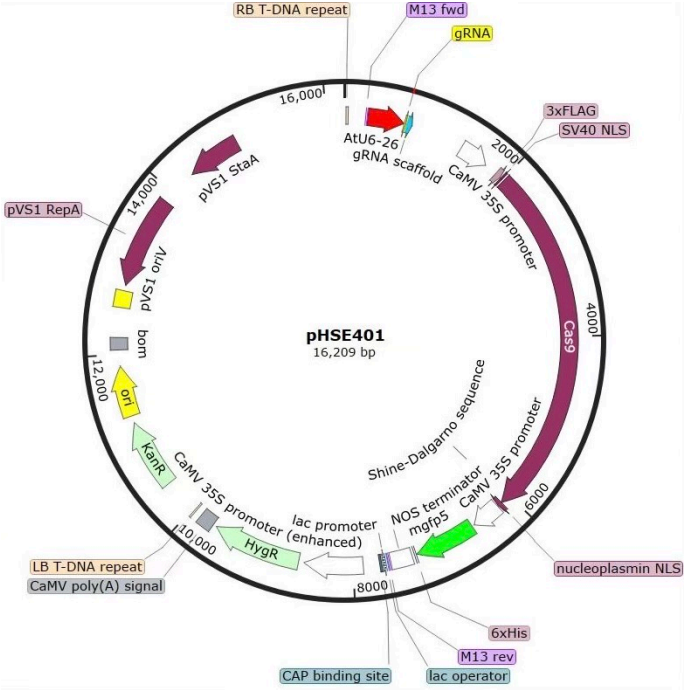
Method

Design and Validation of CRISPR constructs:

- The target sequences for each gene were selected using the Crispr-P website, a site used to identify viable target sequences for CRISPR work. The sequences were chosen based on a set of criteria: 23 bp region ending with NGG (PAM sequence), part of the exon (coding region), high efficiency, 40-60% GC content, and low off-site hits.
- gRNAs were synthesized and cloned under the AtU6-26 promoter in a plant expression vector

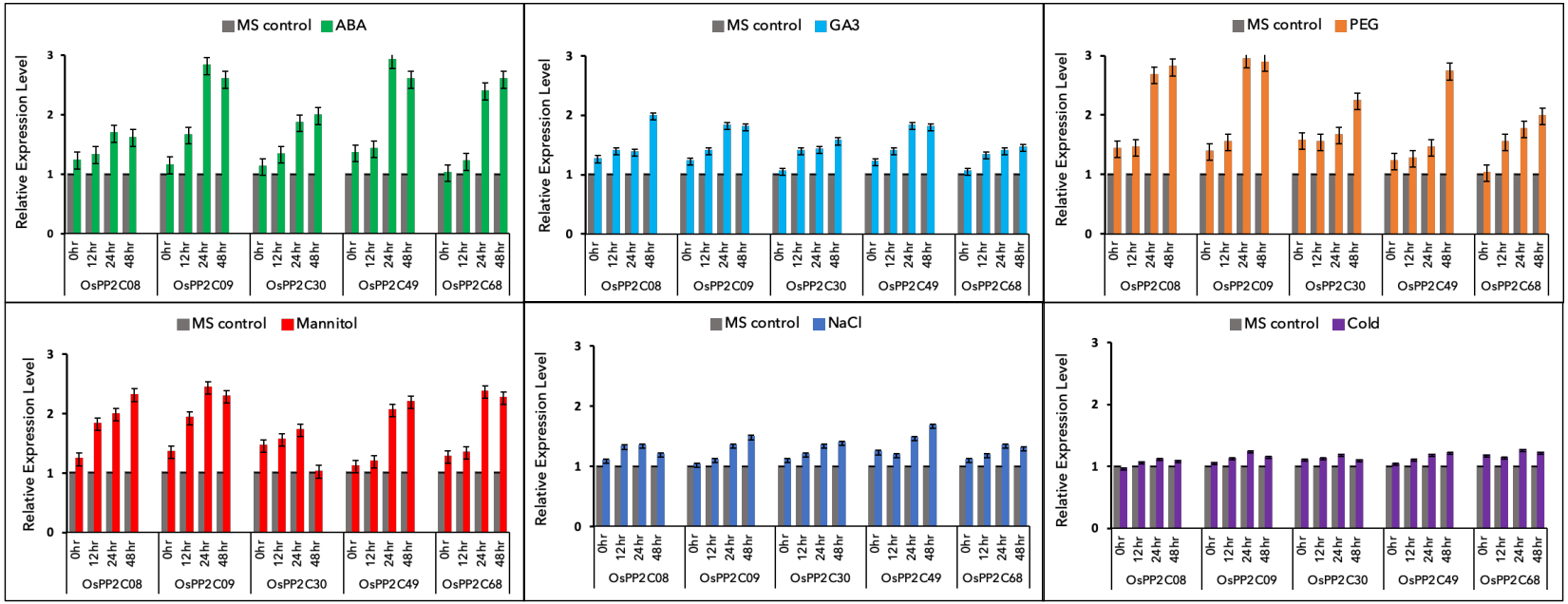
Protoplast Isolation and Transformation:

- Protoplast isolation (removing cell wall) and transformation were completed according to standard protocol.
- Transformation was done by introducing the plasmid to the protoplasts through the use of PEG-CTS.
- Samples were sent for expression profiling and were viewed under regular and confocal microscopes



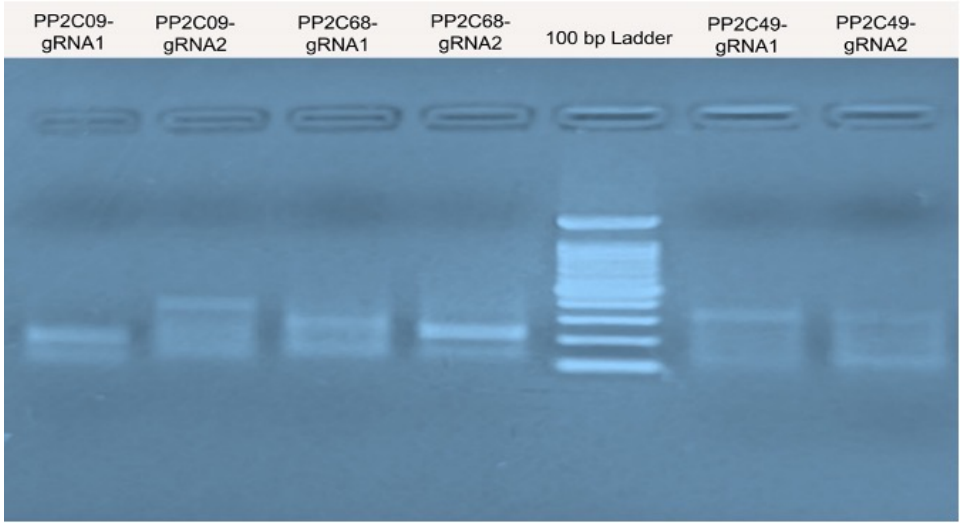
Plasmid map obtained through mentor.

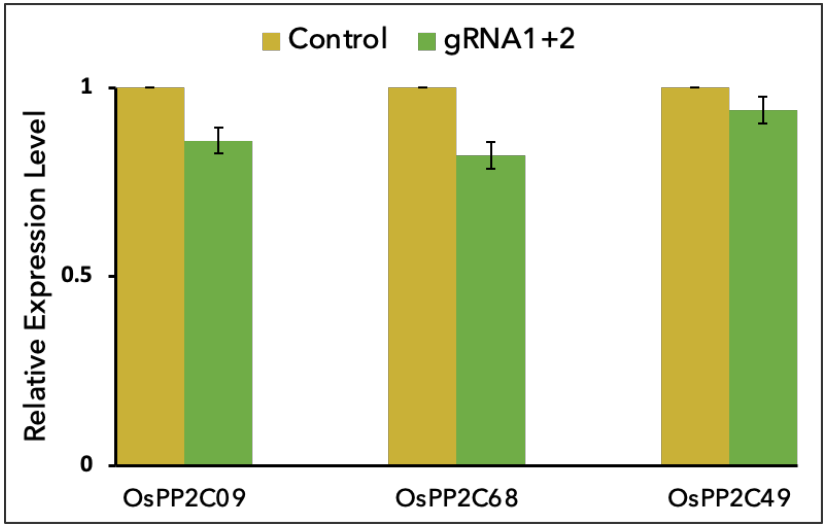
Gene Expression Analysis of PP2C Genes Under Stress



qPCR analysis of selected PP2C genes all stressors shows increased expression of all the selected genes at 24 hr and 48 hr. Further, the expression of PP2C genes was found to be significantly higher under ABA, PEG, and Mannitol stress in comparison to NaCl, GA3, and cold stress. However, upon cold stress treatment, the expression of PP2C genes has not changed much in comparison to control, while NaCl and GA3 still had a moderate increase in PP2C gene expression.

Validation of PP2C Gene Knockouts

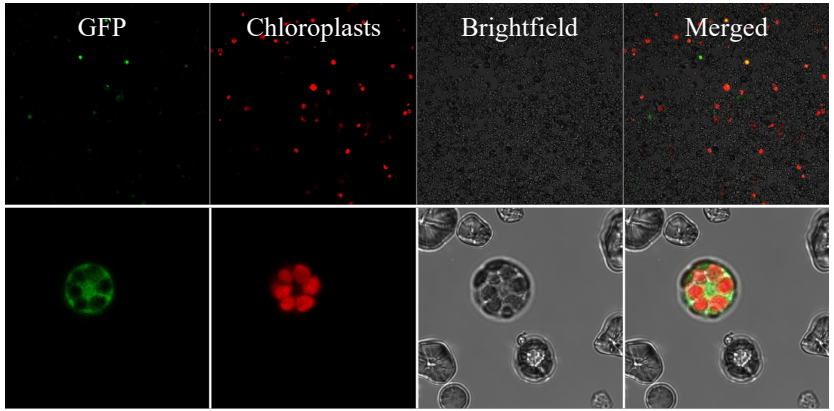




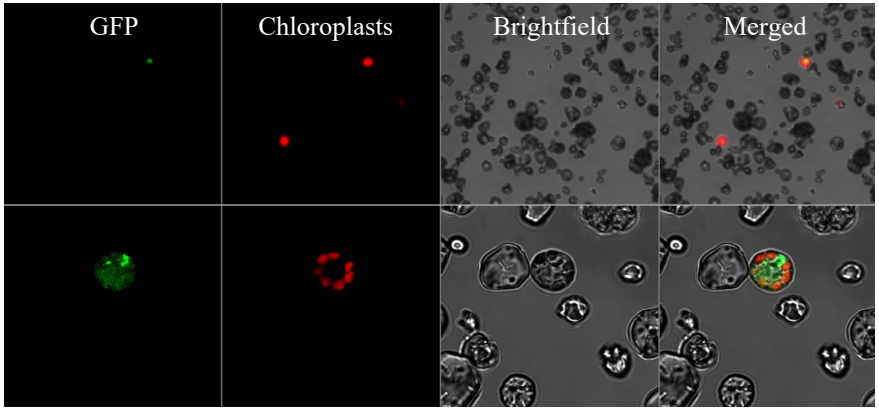
In-vitro cleavage of target amplicon is validated through PCR gel electrophoresis. Total RNA isolated from transformed and control protoplasts were used for cDNA preparation and qRT-PCR analysis, which showed decreased expression of target PP2C genes.

Confocal Microscopy for Knockout Validation

OsPP2C09

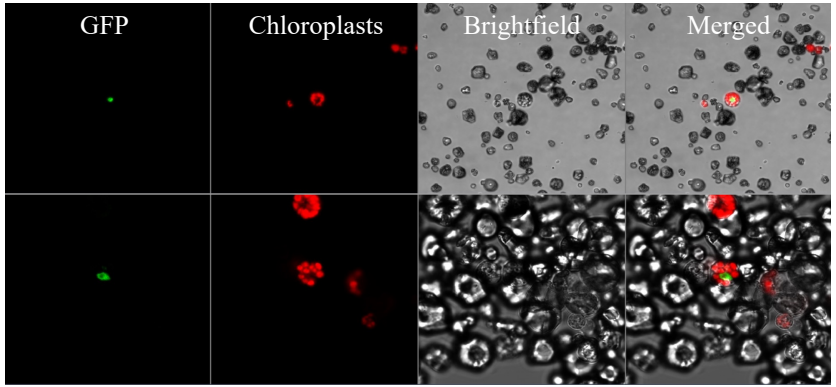


OsPP2C49



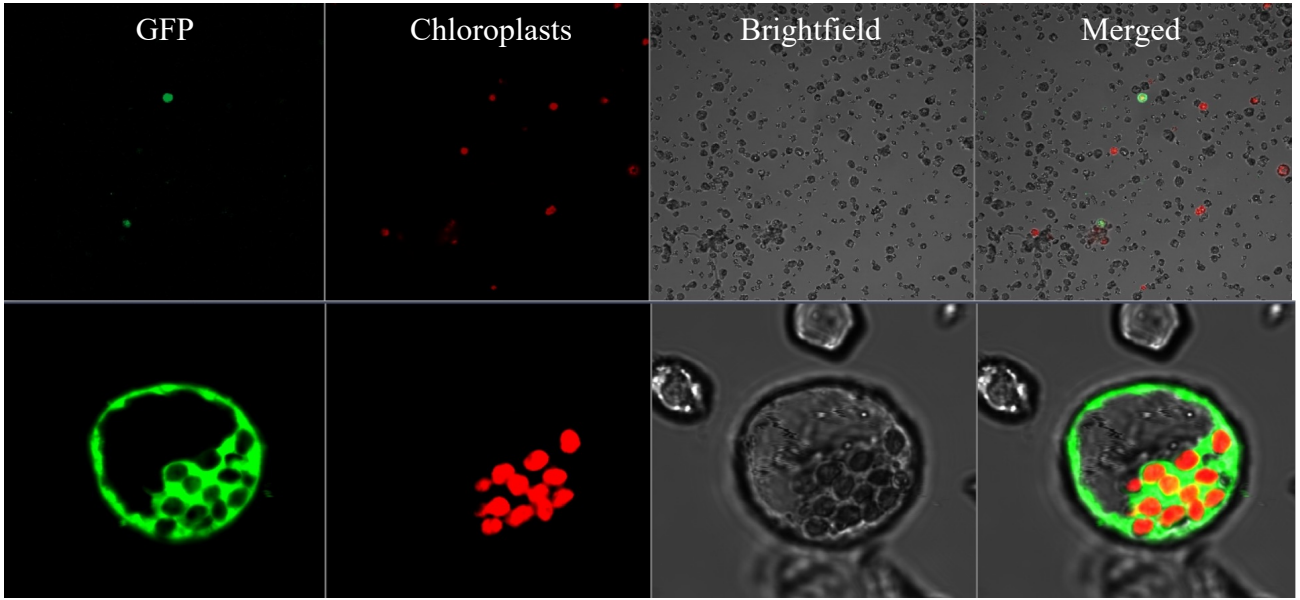
Confocal images obtained through mentor.

OsPP2C68



Nuclear localization of the gRNA-Cas9-GFP complex was visualized through confocal microscopy under GFP, red channel (chloroplasts), brightfield, and merged images. Images for OsPP2C09, OsPP2C49, and OsPP2C68 genes respectively.

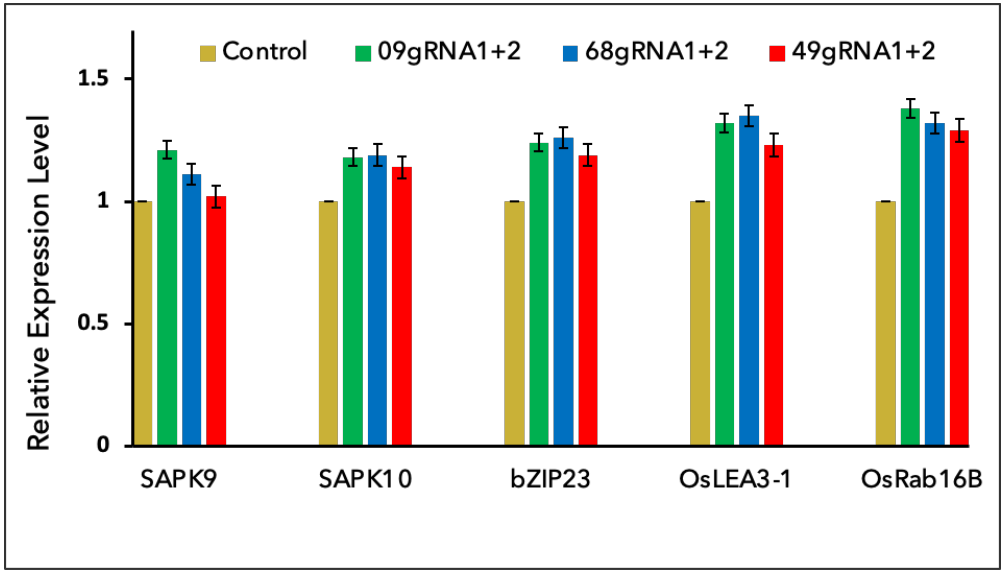
Confocal Microscopy of eGFP-Control



Confocal images obtained through mentor.

Localization of eGFP-control throughout the cell was visualized using the confocal microscope under GFP, red channel, brightfield, and merged images.

Identification of Downstream Genes Affected by PP2C Gene Knockouts



Total RNA isolated from transformed and control protoplasts was used for qRT-PCR analysis of downstream ABA signaling pathway genes.

It was observed that the expression of SAPK9, SAPK10, bZIP23, OsLEA3-1, and OsRab16B genes upregulated in comparison to control.

Discussion

Abiotic Stress Testing

- Stress-Specific Regulation: Differential expression patterns of PP2C genes under different stress treatments indicate that these genes are regulated in a stress-specific manner. This suggests that the PP2C genes may play distinct roles in response to different abiotic stresses.
- Differential Response to Stressors: Expression of PP2C genes varies depending on the type of stress treatment. For instance, ABA stress induces significantly higher expression levels of PP2C genes than cold stress.
- Late Induction of PP2C Genes: The significantly increased expression of PP2C genes at 24 hr and 48 hr under various stress treatments suggests that the induction of these genes occurs relatively late in response to stress. In addition, when considering the five stress conditions not including cold stress, we see that 44% of the time the expression of PP2C genes decreases between 24 and 48 hours. This indicates that gene expression in response to stress is dynamic.
- Over the 5 genes, ABA, Mannitol, and PEG produced the most pronounced results. From this subset, the three genes that showed the largest change in expression were chosen for further research: OsPP2C09, OsPP2C49, and OsPP2C68.

CRISPR Gene Knockouts

- In-vitro cleavage of target amplicon was validated and downregulation of target PP2C genes was confirmed.
- Confirmation of ribonucleoprotein (RNP) complex localization in rice protoplast through eGFP signal was visualized and confirmed through confocal microscopy for OsPP2C09, OsPP2C49, and OsPP2C68 genes.
- Analysis of downstream ABA signaling pathway genes shows upregulation in the expression of SAPK9, SAPK10, bZIP23, OsLEA3-1, and OsRab16B genes, meaning the ABA pathway was successfully activated through the knockouts.

Conclusion

- Conventional crop improvement approaches (mutagenesis and hybridization) are time-consuming, tedious, prone to human biases, and require large mutant screens.
- CRISPR gene editing offers hope to enhance abiotic stress resilience in rice to fight climate change more robustly, precisely, and quickly. This is a requirement to meet rice demand by the ever-growing world population.
  - Genome editing can increase yield by up to 35% in rice edited for stress-resilience compared to wild type.
- The scarcity of functionally characterized genes or validated targets is a major bottleneck in unlocking the CRISPR potential for developing climate-smart stress-resilient rice.

This research led to the novel characterization of selected PP2C genes in rice for their involvement in abiotic stress tolerance. The outcome of this study opens up the scope for developing abiotic stress-resistant rice varieties and ensuring sustainable rice production for the growing global population.

Future Work

- I would like the opportunity and resources to grow genetically edited rice in comparison to wild type to see if edited rice produce higher yields under stress in cultivation. It would be fulfilling to see this research through from theory to practice.
- There are a multitude of pathways that respond to abiotic and biotic stressors. I would like to analyze the changes in gene expression when the seedlings experience different stresses, such as intensive heat, overwatering, and exposure to different levels of heavy metals caused by pollution.
- Investigate the stress responses in wheat, corn, and soybeans, and find commonalities among their signaling pathways.

Important References

ABA Inducible Rice Protein Phosphatase 2C Confers ABA Insensitivity and Abiotic Stress Tolerance in Arabidopsis
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4401787/

Abiotic Stress in Agricultural Crops Under Climatic Conditions
https://link.springer.com/chapter/10.1007/978-981-13-6830-1_3

Applications and Potential of Genome-Editing Systems in Rice Improvement: Current and Future Perspectives
https://www.mdpi.com/2073-4395/11/7/1359

CRISPR-Cas System, a Possible “Savior” of Rice Threatened by Climate Change: An Updated Review
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10492775/

Effects of abiotic stress on plants: a systems biology perspective
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3252258/

Evolution of ABA signaling pathways
https://doi.org/10.1016/bs.abr.2019.06.003

Genome-wide and expression analysis of protein phosphatase 2C in rice and Arabidopsis
https://pubmed.ncbi.nlm.nih.gov/19021904/

Molecular and Physiological Mechanisms to Mitigate Abiotic Stress Conditions in Plants
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9605384/

Protein phosphatase 2C (PP2C) function in higher plants
https://pubmed.ncbi.nlm.nih.gov/9869399/

The Importance of Rice
http://www.knowledgebank.irri.org/ericeproduction/bodydefault.htm#Importance_of_Rice.htm

【評語】060016

1. This study proposes an innovative strategy for using modern gene editing technology to address the challenges of rice yield and climate change, and has high application potential.

2. The precision and efficiency of CRISPR technology make it an ideal tool for regulating the ABA pathway. Deleting the class A PP2C gene to improve stress tolerance is a practical solution. However, the molecular mechanism of specific gene functions in the PP2C gene family has not been fully elucidated, which may lead to unintended effects of gene editing. In addition, overexpression of the ABA pathway may have a negative impact on rice growth and development, and further exploration of the best strategy for balancing is needed.