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Bioinformatics-Guided CRISPR/Cas9 Editing of *OsPYL9* Improves Drought Tolerance through ABA and Circadian Rhythm Regulatory Networks

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Abstract : Rice (*Oryza sativa* L.) is a major staple crop worldwide, yet its productivity is severely constrained by increasing drought stress driven by climate change and water scarcity. Abscisic acid (ABA) signaling plays a central role in regulating plant responses to drought, with the PYR/PYL/RCAR family of ABA receptors acting as key molecular regulators. Among these, *OsPYL9* has emerged as an important candidate gene due to its involvement in drought tolerance and circadian rhythm–associated stress regulation. This study aims to enhance drought resilience in rice through precise genome editing of *OsPYL9* using CRISPR/Cas9 technology. A locally adapted, high-yielding rice variety will be subjected to targeted gene modification, followed by molecular validation, physiological screening under controlled drought stress, and comprehensive agronomic evaluation. The expected outcomes include improved water-use efficiency, enhanced antioxidant activity, optimized stomatal regulation, and stable grain yield under water-limited conditions. This research provides a sustainable and precise breeding strategy for developing climate-resilient rice varieties and offers significant potential for strengthening food security in drought-prone regions.

Keywords: CRISPR/Cas9, OsPYL9, Drought tolerance, Abscisic acid signaling, Rice genomics

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and serves as a primary staple food for more than half of the global population. The genus *Oryza* derives from the classical Latin word for rice, while the term *sativa* means “cultivated.” Among the two cultivated rice species, *O. sativa* (Asian rice) is far more widely grown than *O. glaberrima* (African rice). Archaeological and genetic evidence indicates that *O. sativa* was first domesticated in the Yangtze River basin of China approximately 13,500–8,200 years ago. It belongs to the family Poaceae and possesses a relatively small and well-characterized genome of approximately 430 million base pairs distributed across 12 chromosomes, making it an ideal model organism for genetic, genomic, and molecular studies in cereals and monocots.[1]

Oryza sativa consists of two major subspecies: japonica and indica. Japonica rice is typically short-grained and sticky in nature, having been domesticated in the Yangtze Valley 6,000–9,000 years

[1] Gross, B. L., & Zhao, Z. (2014). Archaeological and genetic insights into the origins of domesticated rice. *Proceedings of the National Academy of Sciences, 111*(17), 6190–6197. https://doi.org/10.1073/pnas.1308942110

ago, and is mainly cultivated in temperate East Asia, upland Southeast Asia, and at higher elevations in South Asia. In contrast, indica rice is long-grained and non-sticky, domesticated around the Ganges basin 4,500–8,500 years ago, and is widely grown in lowland, submerged environments throughout tropical Asia. Rice grains exhibit considerable diversity in color, including white, brown, red, and black, reflecting wide genetic and nutritional variation.[2]

Despite its global importance, rice is a highly water-intensive crop and is extremely sensitive to drought stress. Climate change–induced alterations in rainfall patterns, declining freshwater availability, rising temperatures, and increasing frequency of drought events pose serious threats to sustainable rice production. Drought stress disrupts rice growth at multiple developmental stages, leading to reduced photosynthesis, impaired stomatal regulation, oxidative damage, poor grain filling, and substantial yield losses. These challenges are particularly severe in South Asia, including Pakistan, where rice is both a staple food and a key export commodity.[3]

In Pakistan, rice plays a central role in national food security, rural livelihoods, and economic stability. However, increasing water scarcity due to climate variability and competition for irrigation water has intensified the vulnerability of rice production systems. Conventional breeding approaches have achieved limited success in developing highly drought-tolerant rice varieties because of the

[2] Goff, S. A., et al. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. japonica). *Science, 296*(5565), 92–100.
https://doi.org/10.1126/science.1068259
[3] Huang, X., et al. (2012). A map of rice genome variation reveals the origin of cultivated rice. *Nature, 490*(7421), 497–501.
https://doi.org/10.1038/nature11432

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complex genetic nature of drought resistance and its frequent trade-off with yield. Therefore, there is a critical need for modern, precise, and efficient breeding strategies that can improve drought tolerance without compromising grain yield or quality.[4]

At the molecular level, abscisic acid (ABA) signaling represents a central regulatory mechanism governing plant responses to drought stress. ABA controls stomatal closure, osmotic balance, antioxidant defense, and the expression of numerous stress-responsive genes. A key component of this pathway is the PYR/PYL/RCAR family of ABA receptors, which act as molecular switches that initiate downstream drought signaling. Among these receptors, the *OsPYL9* gene has emerged as a particularly promising candidate due to its dual regulatory role in both ABA-mediated drought signaling and circadian rhythm–associated stress responses.[5]

Recent studies have demonstrated that *OsPYL9* enhances drought tolerance by improving stomatal regulation, activating antioxidant defense systems, coordinating osmoprotectant pathways, and regulating circadian rhythm–linked stress genes. This unique integration of hormonal and circadian regulation allows plants to optimize water-use efficiency in a time-dependent manner during critical growth phases. However, despite its strong potential, significant knowledge gaps remain regarding how precise genome editing of *OsPYL9* can be used to engineer rice varieties that

[4] Kumar, R., Singh, P., & Verma, R. (2024). Drought stress and its impact on rice productivity: A comprehensive review. *Journal of Agricultural Science and Technology, 26*(2), 101–118. https://doi.org/10.1016/j.jast.2023.11.004

[5] Hussain, S., Khan, M. A., & Raza, M. A. (2021). Analysis of rice production, export performance and growth trends in Pakistan. *Journal of Asian Business and Economic Studies, 28*(3), 219–232. https://doi.org/10.1108/JABES-04-2020-0039

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maintain high yield and grain quality under water-limited conditions.[6]

The emergence of CRISPR/Cas9 genome-editing technology has revolutionized crop improvement by enabling precise, efficient, and targeted modification of specific genes. Unlike conventional breeding and random mutagenesis, CRISPR/Cas9 allows direct manipulation of key regulatory genes such as *OsPYL9* without introducing undesirable genetic changes. This technology therefore provides a powerful platform for developing climate-resilient rice varieties capable of withstanding drought stress while sustaining productivity.

The present study proposes the development of drought-resilient rice through precise CRISPR/Cas9-mediated genome editing of the *OsPYL9* gene. The edited rice lines will be evaluated for their physiological, molecular, and agronomic responses under drought stress, including water-use efficiency, expression of stress-responsive genes, interaction with circadian rhythm pathways, grain yield, and grain quality.[7]

To develop drought-resilient rice (*Oryza sativa* L.) with enhanced yield stability under water-limited conditions through precise genome editing of key stress-responsive regulatory genes, with a primary focus on *OsPYL9*.

[6] Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., & Abrams, S. R. (2010). Abscisic acid: Emergence of a core signaling network. *Annual Review of Plant Biology, 61*, 651–679. https://doi.org/10.1146/annurev-arplant-042809-112122

[7] Wei, H., Wang, X., & Chen, Z. (2023). Circadian regulation of abscisic acid signaling in rice: Functional characterization of ABA receptors and downstream targets. *Plant Physiology and Biochemistry, 195*, 152–164. https://doi.org/10.1016/j.plaphy.2023.02.019

1. To investigate the functional role of *OsPYL9* in regulating drought stress responses and its molecular interaction with circadian rhythm and other abiotic stress–responsive pathways in rice.
2. To design and apply CRISPR/Cas9-based genome-editing strategies for precise modification of the *OsPYL9* gene to enhance drought tolerance without compromising agronomic performance.

The expected outcomes of this research include the development of rice plants capable of improved survival and growth under drought stress, enhanced water-use efficiency, healthier leaf physiology, and the maintenance or improvement of grain yield and quality under water-limited conditions.[8]

In the short term, this study is expected to generate CRISPR-edited rice lines ready for controlled testing and provide clear molecular insights into the role of *OsPYL9* in drought tolerance. In the long term, the successful development of drought-resilient rice varieties will contribute to sustainable rice production, strengthen food security in drought-prone regions such as Pakistan, and offer a transferable genome-editing strategy for improving drought tolerance in other important cereal crops.[9]

Materials and Methods

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[8] Li, J., Manghwar, H., Sun, L., Wang, P., Wang, G., Sheng, H., Zhang, X., & Jin, S. (2022). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Frontiers in Genome Editing, 4*, 812546. https://doi.org/10.3389/fgeed.2022.812546

[9] Fidler, A., Biesiada, T., & Borkowski, K. (2022). The PYR/PYL/RCAR family of ABA receptors: Structure, function, and regulation in abiotic stress tolerance. *Plant Molecular Biology, 108*(1–2), 1–18. https://doi.org/10.1007/s11103-021-01169-0

A high-yielding and locally adapted rice (*Oryza sativa* L.) variety will be selected for genetic modification. Certified, disease-free, and genetically uniform seeds will be used to ensure experimental reliability. Seeds will be surface-sterilized and germinated under controlled growth conditions prior to transformation. The full-length genomic and coding sequences of the *OsPYL9* gene will be retrieved from publicly available rice genome databases, including NCBI and EnsemblPlants. Conserved functional domains will be identified using bioinformatics tools, and specific guide RNA (gRNA) target sites will be selected based on high on-target efficiency and minimal off-target potential. Selected gRNA sequences will be synthesized and cloned into a CRISPR/Cas9 plant expression vector under suitable promoters. The recombinant plasmid will be transformed into *Agrobacterium tumefaciens*, and correct vector construction will be confirmed through restriction digestion and sequencing.

Agrobacterium-mediated transformation will be employed to introduce the CRISPR/Cas9–*OsPYL9* construct into embryogenic rice calli. Transformed calli will be selected on antibiotic-containing media and regenerated into whole plants under controlled tissue culture conditions. Putative transgenic plants will be transferred to greenhouse conditions for acclimatization. Genomic DNA will be extracted from regenerated plants for molecular validation. Successful editing of *OsPYL9* will be confirmed using polymerase chain reaction (PCR), DNA sequencing, and restriction enzyme analysis. Quantitative real-time PCR (qRT-PCR) will be conducted to evaluate *OsPYL9* expression levels and the regulation of downstream ABA-responsive stress-related genes.

Both genome-edited and wild-type rice plants will be grown under controlled environmental conditions for drought stress experimentation. Drought stress will be imposed at the vegetative and reproductive growth stages by withholding irrigation for a defined period, while control plants will be maintained under optimal watering conditions. Physiological parameters, including relative water content, leaf wilting index, stomatal conductance, and chlorophyll content, will be recorded at regular intervals. Growth and yield-related traits such as plant height, root length, biomass, panicle number, grain number per panicle, thousand-grain weight, and total grain yield per plant will be measured at maturity. Grain quality traits will also be assessed to ensure that genome editing does not adversely affect nutritional or processing characteristics. All experiments will be conducted using a completely randomized design with appropriate biological replicates. Statistical analyses will be performed using standard software such as SPSS or R. Data will be subjected to analysis of variance (ANOVA), and mean differences will be evaluated using post hoc tests at a significance level of $p < 0.05$.

Economic Importance in Pakistan:

Rice occupies a central position in Pakistan's economy and plays a critical role in ensuring national food security, generating foreign exchange, promoting rural employment, and supporting agricultural sustainability. As the second most important staple crop after wheat, rice contributes substantially to the daily caloric intake of millions of people and sustains livelihoods across multiple agricultural and industrial sectors.

From an economic perspective, rice is one of Pakistan's most valuable cash crops and a major contributor to agricultural export

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revenues. Pakistan ranks among the leading rice-exporting countries globally, with premium Basmati rice commanding high prices in international markets. Annual rice exports generate billions of US dollars in foreign exchange, which is essential for maintaining trade balance, stabilizing the national economy, and financing the import of critical commodities such as fuel, fertilizers, and agricultural machinery.

Rice cultivation also serves as a major source of employment, particularly in Punjab and Sindh, where production is most concentrated. Millions of farmers, farm laborers, mill workers, transporters, and traders depend directly or indirectly on the rice sector. The crop supports a vast value chain that includes seed production, cultivation, harvesting, milling, storage, processing, transportation, and international marketing, making it a cornerstone of rural development and poverty alleviation.

Furthermore, rice contributes significantly to Pakistan's agro-based industries, including rice milling, packaging, and export-processing units, thereby promoting industrial growth and job creation. Rice by-products, such as straw and husk, are utilized in livestock feeding, paper manufacturing, and biofuel production, further enhancing its overall economic and industrial utility.

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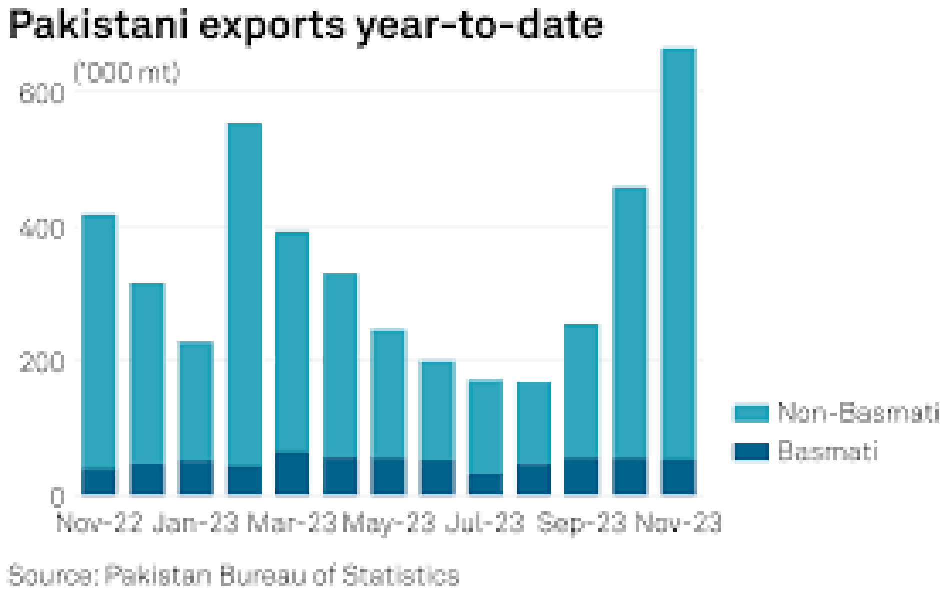

Figure 3: Pakistani rice exports Nov '22 - Nov '23. Non-Basmati vs Basmati.

In terms of employment, rice cultivation provides work for millions of farmers, laborers, mill owners, transporters, and traders, especially in Punjab and Sindh, where rice production is concentrated. The crop supports extensive value chains—ranging from seed production, farming, harvesting, milling, and storage to processing, transportation, and international marketing—making it a cornerstone of rural development and poverty alleviation.

Rice also plays a crucial role in Pakistan's agriculture-based industries, including milling, packaging, and export processing units, leading to further job creation and industrial growth. Additionally, rice straw and husk are used in livestock feeding, paper

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industries, and biofuel production, increasing its overall economic utility.[10]

Nutritional Importance:

Rice (*Oryza sativa* L.) is a primary staple food for a large proportion of Pakistan's population and plays a fundamental role in daily nutrition across all socioeconomic groups. As a rich source of complex carbohydrates, rice serves as a major provider of dietary energy, which is essential for sustaining physical activity, cognitive function, and metabolic processes. In many households, particularly in rural areas, rice constitutes the main component of at least one major meal per day, making it indispensable for meeting daily caloric requirements.

In addition to its high carbohydrate content, rice provides moderate amounts of plant-based protein, contributing to basic amino acid intake, especially in communities where access to animal protein is limited. Although rice is naturally low in fat and cholesterol, it becomes nutritionally more valuable when consumed as brown rice or in fortified forms. Fortified rice varieties are enriched with essential micronutrients such as iron, zinc, folic acid, and B-complex vitamins (thiamine, niacin, and riboflavin), which are critical for red blood cell formation, immune function, nervous system health, and overall metabolic balance.[11]

[10] Rashid, A., & Ahmad, Z. (2020). Economic significance of rice crop and its contribution to rural livelihoods in Pakistan. *Sarhad Journal of Agriculture, 36*(4), 1123–1132. https://doi.org/10.17582/journal.sja/2020/36.4.1123.1132
[11] Fitzgerald, M. A., McCouch, S. R., & Hall, R. D. (2009). Not just a grain of rice: The quest for quality. *Trends in Plant Science, 14*(3), 133–139. https://doi.org/10.1016/j.tplants.2008.12.004

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Rice is also highly valued for its easy digestibility and hypoallergenic nature, making it suitable for individuals of all age groups, including infants, elderly individuals, and patients with gastrointestinal disorders. Its soft texture and low fiber content (particularly in white rice) make it gentle on the digestive system, while brown rice additionally provides dietary fiber that supports gut health and reduces the risk of chronic diseases.

In low-income and food-insecure communities, rice plays an especially vital role due to its affordability, long shelf life, and versatility in preparation. It provides a reliable and accessible source of nutrition that helps reduce hunger, prevent calorie deficiency, and mitigate the risk of malnutrition. Through both direct consumption and fortified food programs, rice contributes significantly to improving dietary quality, maternal health, and childhood nutrition in Pakistan.[12]

[12] Gao, X., Xie, J., Zhang, Y., & Wang, X. (2020). Rice consumption and its role in global food security and nutrition. *Food Security, 12*(4), 673–687. https://doi.org/10.1007/s12571-020-01038-9

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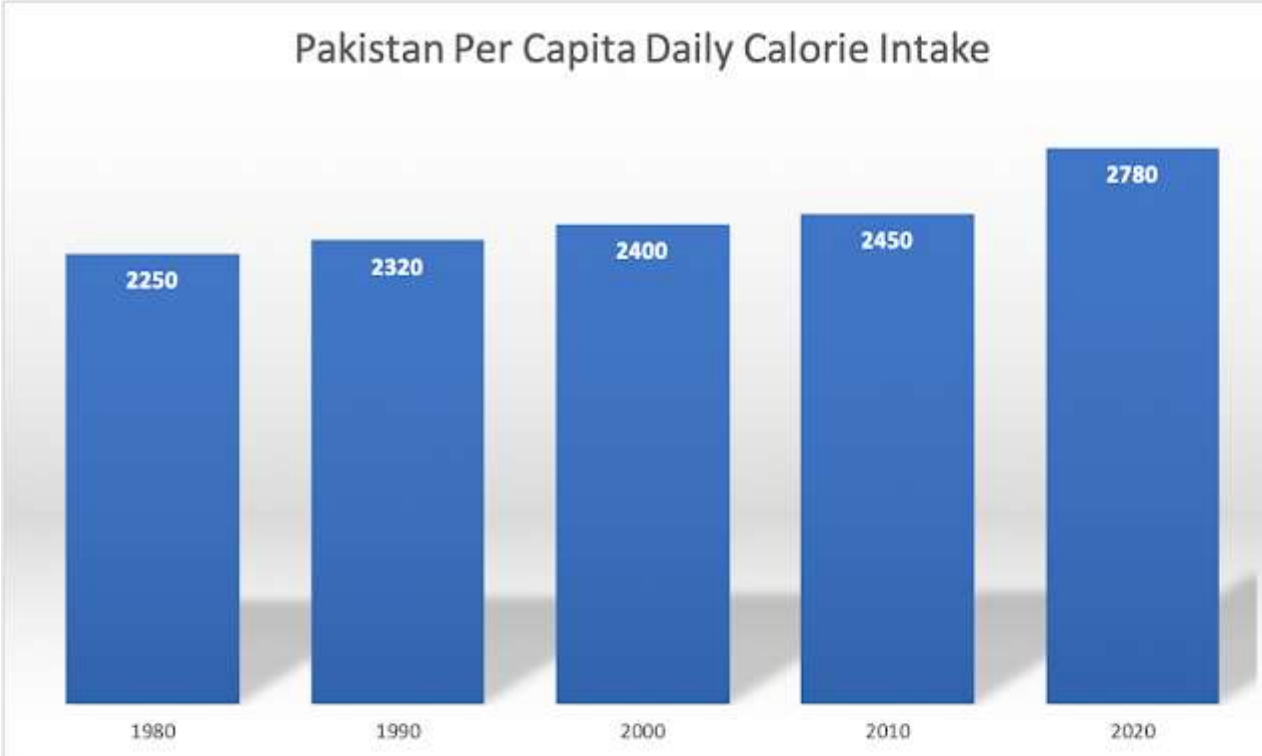

Figure 1: Pakistan Per Capita Daily Calorie Intake Trend (1980-2020)

Agriculture Importance:

Rice (*Oryza sativa* L.) is one of the most important strategic crops in Pakistan's agricultural sector, particularly in the fertile and well-irrigated plains of Punjab and Sindh, where climatic and soil conditions are highly favorable for its cultivation. These two provinces collectively account for the majority of national rice production and serve as the backbone of Pakistan's rice-based rural economy. Rice farming provides direct and indirect employment to millions of farmers, farm laborers, and rural households, thereby playing a critical role in sustaining rural livelihoods and reducing poverty.

Beyond income generation, rice cultivation contributes significantly to agronomic sustainability. It supports crop rotation and diversification systems, particularly in rice–wheat and rice–legume cropping patterns, which enhance soil fertility, improve nutrient cycling, and reduce pest and disease pressure. Flooded rice systems also influence soil physical properties and microbial activity, which can benefit subsequent crops when managed properly.[13]

Rice cultivation further strengthens Pakistan's agro-industrial sector by supplying essential raw materials to rice milling, starch manufacturing, food processing, and export packaging industries. These value-added industries stimulate industrial growth, enhance employment opportunities, and integrate agriculture with national economic development. As a high-value yet water-intensive crop, rice holds a strategically significant position in Pakistan's agricultural planning, particularly in relation to irrigation management, export policy, and food security. Its dual role as both a staple food and a major export commodity makes rice indispensable for national economic stability and long-term agricultural sustainability.[14]

[13] Abedullah, N., Khalid, M., & Kouser, S. (2021). Impact of rice production on farm income and rural employment in Pakistan. *Journal of the Saudi Society of Agricultural Sciences, 20*(4), 273–281.
https://doi.org/10.1016/j.jssas.2021.03.002

[14] Farooq, M., Hussain, M., & Siddique, K. H. M. (2014). Drought stress in wheat during flowering and grain-filling periods. *Critical Reviews in Plant Sciences, 33*(4), 331–349. https://doi.org/10.1080/07352689.2014.875291

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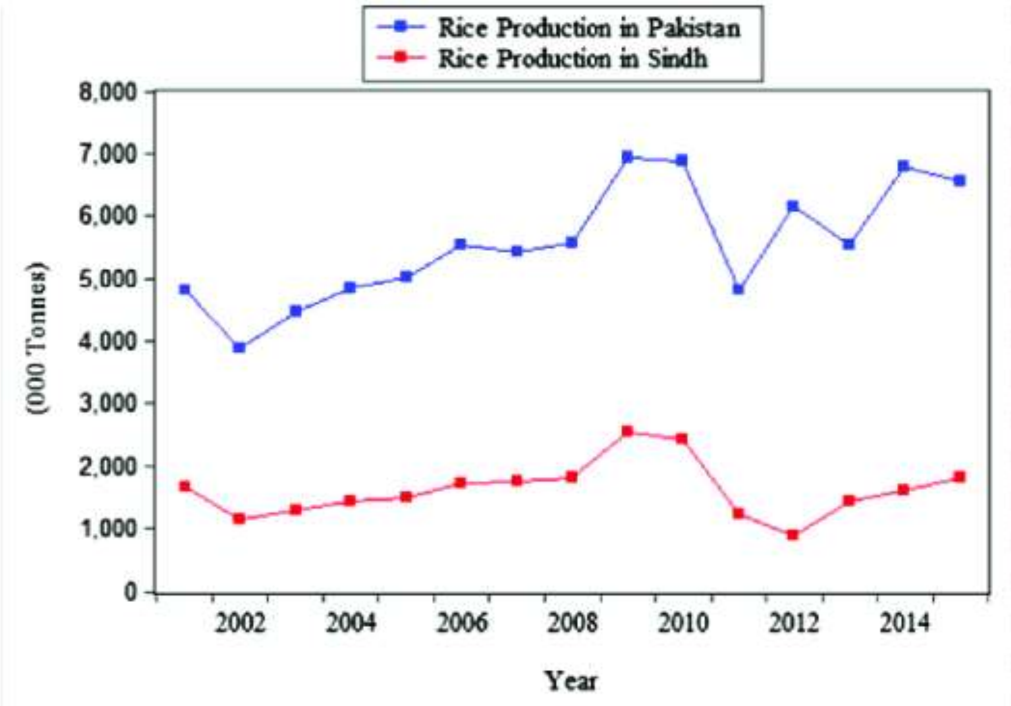

Figure 2: Rice production trends in Pakistan & Sindh.

In figure 2, graph shows Pakistan's total rice production (blue) vs Sindh's production (red) from 2001 to 2015. Sindh contributes a significant chunk to Pakistan's total rice output.

Production Statistics:

Rice (*Oryza sativa* L.) is among the most extensively cultivated cereal crops worldwide and serves as the primary staple food for more than half of the global population. Owing to its adaptability to diverse agro-climatic conditions and its high caloric contribution, rice plays a central role in global food security. According to the Food and Agriculture Organization (FAO, 2023), global rice production reached approximately 787 million metric tons in 2022, with Asia contributing more than 90% of total global output. China and India dominate world rice production, followed by other major producers such as Indonesia, Bangladesh, and Vietnam. These countries collectively account for the majority of the world's rice supply and regional food stability.

In addition to being a staple food crop, rice is also a highly traded agricultural commodity in the international market. Countries such as Thailand, Vietnam, India, and Pakistan are among the world's leading rice exporters, supplying a wide range of premium and non-premium varieties to international markets. Rice trade contributes significantly to export revenues and global food distribution networks, particularly for food-deficit regions.[15]

In Pakistan, rice is a strategically important crop, especially in the irrigated plains of Punjab and Sindh provinces, where favorable climatic conditions and canal irrigation systems support large-scale cultivation. During the 2022–2023 cropping season, Pakistan produced approximately 8 million metric tons of rice, with aromatic Basmati rice contributing a substantial share of export earnings. Basmati rice is internationally recognized for its superior grain quality, aroma, and cooking characteristics, making it one of Pakistan's most valuable agricultural export commodities. The total area under rice cultivation in Pakistan is estimated at around 2.7 million hectares, highlighting its dual importance as both a staple food crop and a key driver of the national agricultural economy.[16]

[15] Shaban, M., Khan, A., & Ahmad, S. (2022). Global rice production, trade, and consumption trends under changing climate. *Climate Risk Management, 36*, 100423. https://doi.org/10.1016/j.crm.2022.100423

[16] Hussain, S., Khan, M. A., & Raza, M. A. (2021). Analysis of rice production, export performance and growth trends in Pakistan. *Journal of Asian Business and Economic Studies,*

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Role in food security and livelihood:

Rice is a cornerstone of food security in Pakistan, providing a staple source of calories and nutrition for millions of people. Its widespread cultivation ensures a steady supply of affordable food, particularly in rural areas where rice forms a significant part of daily meals. By contributing to both domestic consumption and exports, rice helps stabilize the national food supply and supports economic resilience.

Rice cultivation also plays a critical role in livelihoods. Millions of smallholder farmers, agricultural laborers, and workers in rice-related industries, such as milling, transportation, and packaging, depend on rice for income and employment. This makes rice farming a key driver of rural development and poverty alleviation.

Rice (*Oryza sativa* L.) is one of the most important staple food crops globally, serving as the primary source of calories for more than half of the world's population, particularly in South and Southeast Asia. Its productivity is therefore directly linked to global food security. Despite its agronomic importance, rice is highly sensitive to water stress due to its substantial irrigation requirements throughout vegetative and reproductive growth stages. With the increasing impacts of climate change, including irregular rainfall patterns, prolonged dry spells, and declining freshwater availability, drought has emerged as one of the most severe constraints limiting rice productivity. These challenges have intensified the need to develop

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drought-resilient rice varieties capable of sustaining growth and yield under water-limited conditions.

At the molecular level, abscisic acid (ABA) signaling plays a central role in regulating plant responses to drought stress. ABA acts as a key stress hormone that mediates a wide range of physiological and biochemical adaptive mechanisms, including stomatal closure, osmotic adjustment, antioxidant defense activation, and the expression of stress-responsive genes. The ABA signaling pathway is initiated through the perception of ABA by the PYR/PYL/RCAR family of intracellular receptors. These receptors serve as molecular switches that translate drought-induced ABA accumulation into downstream stress signaling cascades.

Upon ABA binding, PYR/PYL/RCAR receptors inhibit type 2C protein phosphatases (PP2Cs), which are negative regulators of ABA signaling. This inhibition activates SNF1-related protein kinases (SnRK2s), leading to the phosphorylation of ABA-responsive transcription factors and the subsequent induction of drought-responsive genes. Numerous studies have demonstrated that modulation of PYR/PYL receptor activity significantly enhances drought tolerance in different crop species, including rice. Consequently, these ABA receptors have become important molecular targets for genetic and biotechnological approaches aimed at improving drought resistance while maintaining agronomic performance.

OsPYL9 and Drought Tolerance in Rice:

The *OsPYL9* gene, a key member of the PYR/PYL/RCAR family of abscisic acid (ABA) receptors in rice, has emerged as a highly promising molecular target for genetic improvement due to

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its pivotal role in drought stress regulation. Previous studies have demonstrated that *OsPYL9* significantly enhances drought tolerance by regulating stomatal closure, modulating circadian rhythm–associated genes, and interacting with a wide range of abiotic stress–responsive proteins. These coordinated molecular and physiological responses strengthen the plant's defense system, thereby improving water-use efficiency and enhancing survival under drought conditions.[17]

With recent advancements in precision genome-editing technologies, particularly CRISPR/Cas9, it has become possible to modify drought-responsive genes such as *OsPYL9* with high specificity and efficiency. Experimental evidence indicates that targeted manipulation of *OsPYL9* can lead to simultaneous improvement in drought tolerance and grain yield, overcoming a major limitation of conventional breeding strategies where stress resistance often compromises productivity. A deeper understanding of the molecular mechanisms underlying *OsPYL9*-mediated stress adaptation will further accelerate the development of climate-resilient rice varieties tailored for drought-prone agro-ecological regions.[18]

[17] Fidler, A., Biesiada, T., & Borkowski, K. (2022). The PYR/PYL/RCAR family of ABA receptors: Structure, function, and regulation in abiotic stress tolerance. *Plant Molecular Biology, 108*(1–2), 1–18.
https://doi.org/10.1007/s11103-021-01169-

[18] Wei, H., Wang, X., & Chen, Z. (2023). Circadian regulation of abscisic acid signaling in rice: Functional characterization of ABA receptors and downstream targets. *Plant Physiology and Biochemistry, 195*, 152–164.
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OsPYL9-Mediated ABA Signaling and Circadian-Regulated Drought Tolerance in Rice

The *OsPYL9* gene functions as a core component of the highly conserved abscisic acid (ABA) signaling cascade that regulates molecular and physiological responses to drought stress in rice. Under water-deficit conditions, endogenous ABA rapidly accumulates in roots and leaves, acting as a primary stress signal that activates ABA receptor proteins, including *OsPYL9*. Upon ABA binding, *OsPYL9* undergoes a conformational change that enables it to interact with and inhibit type 2C protein phosphatases (PP2Cs), which normally act as negative regulators of ABA signaling.[19] This inhibition releases the suppression of SNF1-related protein kinases (SnRK2s), leading to their activation and subsequent phosphorylation of ABA-responsive transcription factors such as AREB/ABFs. These transcription factors regulate a wide spectrum of drought-responsive genes, including those involved in osmoprotectant synthesis, antioxidant defense, stomatal movement, circadian rhythm–associated stress regulation, and ABA-regulated late embryogenesis abundant (LEA) proteins. The coordinated transcriptional activation results in essential physiological adaptations, including stomatal closure to reduce transpirational water loss, enhanced osmotic adjustment, increased antioxidant activity to mitigate oxidative damage, improved cell membrane stability, and maintenance of grain filling and yield under drought conditions. In addition to its central role in ABA signaling, *OsPYL9* also interacts with circadian rhythm–associated genes, enabling time-of-day–specific regulation of stress responses and optimized

[19] Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., & Abrams, S. R. (2010). Abscisic acid: Emergence of a core signaling network. *Annual Review of Plant Biology, 61*, 651–679. https://doi.org/10.1146/annurev-arplant-042809-112122

water-use efficiency during critical growth phases. This integrated ABA–circadian regulatory network significantly enhances drought adaptation and survival in rice.[20]

Projected Outcomes and Biological Significance of CRISPR/Cas9-Mediated OsPYL9 Editing

Following CRISPR/Cas9-mediated genome editing of the *OsPYL9* gene, successful modification is expected to be validated through PCR amplification, Sanger sequencing, and restriction enzyme analysis, confirming precise insertions or deletions at the target site with minimal off-target effects. Under controlled drought-stress conditions, *OsPYL9*-edited rice plants are anticipated to exhibit significantly enhanced physiological performance compared with wild-type controls, including higher relative water content, improved chlorophyll retention, reduced leaf wilting, and more efficient stomatal regulation. At the molecular level, edited plants are projected to show elevated expression of ABA-responsive genes, antioxidant enzymes, and osmoprotectant-related genes, confirming strengthened drought-adaptive mechanisms.[21] Agronomic evaluations are further expected to demonstrate that edited rice lines maintain or surpass control plants in plant height, biomass accumulation, panicle number, and grain yield under water-deficit

[20] Fidler, A., Biesiada, T., & Borkowski, K. (2022). The PYR/PYL/RCAR family of ABA receptors: Structure, function, and regulation in abiotic stress tolerance. *Plant Molecular Biology, 108*(1–2), 1–18.
https://doi.org/10.1007/s11103-021-01169-0

[21] Li, J., Manghwar, H., Sun, L., Wang, P., Wang, G., Sheng, H., Zhang, X., & Jin, S. (2022). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Frontiers in Genome Editing, 4*, 812546.
https://doi.org/10.3389/fgeed.2022.812546

conditions, while grain quality traits such as grain size, weight, and cooking properties remain unaffected. These outcomes collectively highlight the strong potential of *OsPYL9* as a precision breeding target for drought resilience. Drought stress remains one of the most destructive abiotic constraints limiting rice productivity, particularly in South Asia, including Pakistan.[22] Enhanced ABA receptor activity through *OsPYL9* editing is expected to strengthen stomatal control, osmotic balance, and antioxidant defense systems, confirming its role as a key molecular switch in coordinating drought responses at both physiological and transcriptional levels.[23] A unique advantage of *OsPYL9* lies in its interaction with circadian rhythm–associated genes, enabling time-dependent regulation of stress responses and optimized water-use efficiency during critical growth phases. From an agronomic perspective, maintaining yield under drought remains a major breeding challenge; however, unlike conventional breeding approaches that often involve yield penalties, CRISPR/Cas9-based precision editing allows targeted stress tolerance enhancement without compromising productivity. In drought-prone rice-growing regions such as Pakistan, successful deployment of *OsPYL9*-edited rice varieties offers a sustainable strategy for safeguarding rice production under climate-induced water scarcity, with potential scalability to other cereals such as wheat and maize, thereby strengthening regional food security.[24]

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[22] Wei, H., Wang, X., & Chen, Z. (2023). Circadian regulation of abscisic acid signaling in rice: Functional characterization of ABA receptors and downstream targets. *Plant Physiology and Biochemistry, 195*, 152–164. https://doi.org/10.1016/j.plaphy.2023.02.019

[23] Wei, H., Wang, X., & Chen, Z. (2023). Circadian regulation of abscisic acid signaling in rice: Functional characterization of ABA receptors and downstream targets. *Plant Physiology and Biochemistry, 195*, 152–164. https://doi.org/10.1016/j.plaphy.2023.02.019

[24] Fidler, A., Biesiada, T., & Borkowski, K. (2022). The PYR/PYL/RCAR family of ABA receptors: Structure, function, and regulation in abiotic stress tolerance. *Plant Molecular Biology, 108*(1–

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Conclusion

This study presents a precise and sustainable genome-editing strategy for enhancing drought tolerance in rice through CRISPR/Cas9-mediated modification of the ABA receptor gene *OsPYL9*. By strengthening ABA signaling and circadian-regulated stress responses, the edited rice plants are expected to exhibit superior physiological resilience, improved water-use efficiency, and stable grain yield under drought conditions. The integration of molecular validation, physiological screening, and agronomic evaluation provides a comprehensive framework for developing climate-resilient rice varieties. The proposed approach represents a promising advancement in precision agriculture and offers significant potential for improving food security in drought-affected regions such as Pakistan.

References

Gross, B. L., & Zhao, Z. (2014). Archaeological and genetic insights into the origins of domesticated rice. *Proceedings of the National Academy of Sciences, 111*(17), 6190–6197. https://doi.org/10.1073/pnas.1308942110

Huang, X., Kurata, N., Wei, X., Wang, Z. X., Wang, A., Zhao, Q., ... Han, B. (2012). A map of rice genome variation reveals the origin of cultivated rice. *Nature,* 490(7421), 497–501. https://doi.org/10.1038/nature11432

2), 1–18.
https://doi.org/10.1007/s11103-021-01169-0

IJBAI
International Journal of Bioinformatics and Artificial Intelligence
ISSN: XXXX-XXXX
Vol.1 No.1January-June 2026, Pages 01-37

Rashid, A., & Ahmad, Z. (2020). Economic significance of rice crop and its contribution to rural livelihoods in Pakistan. *Sarhad Journal of Agriculture, 36*(4), 1123–1132. https://doi.org/10.17582/journal.sja/2020/36.4.1123.1132

Fitzgerald, M. A., McCouch, S. R., & Hall, R. D. (2009). Not just a grain of rice: The quest for quality. *Trends in Plant Science, 14*(3), 133–139.
https://doi.org/10.1016/j.tplants.2008.12.004

Gao, X., Xie, J., Zhang, Y., & Wang, X. (2020). Rice consumption and its role in global food security and nutrition. *Food Security, 12*(4), 673–687.
https://doi.org/10.1007/s12571-020-01038-9

Abedullah, N., Khalid, M., & Kouser, S. (2021). Impact of rice production on farm income and rural employment in Pakistan. *Journal of the Saudi Society of Agricultural Sciences, 20*(4), 273–281. https://doi.org/10.1016/j.jssas.2021.03.002

Farooq, M., Hussain, M., & Siddique, K. H. M. (2014). Drought stress in wheat during flowering and grain-filling periods. *Critical Reviews in Plant Sciences, 33*(4), 331–349. https://doi.org/10.1080/07352689.2014.875291

Shaban, M., Khan, A., & Ahmad, S. (2022). Global rice production, trade, and consumption trends under changing climate. *Climate Risk Management, 36*, 100423. https://doi.org/10.1016/j.crm.2022.100423

Hussain, S., Khan, M. A., & Raza, M. A. (2021). Analysis of rice production, export performance and growth trends in Pakistan. *Journal of Asian Business and Economic Studies, 28*(3), 219–232. https://doi.org/10.1108/JABES-04-2020-0039

Fidler, A., Biesiada, T., & Borkowski, K. (2022). The PYR/PYL/RCAR family of ABA receptors: Structure, function, and regulation in abiotic stress tolerance. *Plant Molecular Biology, 108*(1–

2), 1–18. https://doi.org/10.1007/s11103-021-01169-

Wei, H., Wang, X., & Chen, Z. (2023). Circadian regulation of abscisic acid signaling in rice: Functional characterization of ABA receptors and downstream targets. *Plant Physiology and Biochemistry, 195*, 152–164. https://doi.org/10.1016/j.plaphy.2023.02.019

Li, J., Manghwar, H., Sun, L., Wang, P., Wang, G., Sheng, H., Zhang, X., & Jin, S. (2022). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Frontiers in Genome Editing, 4*, 812546. https://doi.org/10.3389/fgeed.2022.812546

Wei, H., Wang, X., & Chen, Z. (2023). Circadian regulation of abscisic acid signaling in rice: Functional characterization of ABA receptors and downstream targets. *Plant Physiology and Biochemistry, 195*, 152–164. https://doi.org/10.1016/j.plaphy.2023.02.019

Fidler, A., Biesiada, T., & Borkowski, K. (2022). The PYR/PYL/RCAR family of ABA receptors: Structure, function, and regulation in abiotic stress tolerance. *Plant Molecular Biology, 108*(1–2), 1–18. https://doi.org/10.1007/s11103-021-01169-0

Wei, H., Wang, X., & Chen, Z. (2023). Circadian regulation of abscisic acid signaling in rice: Functional characterization of ABA receptors and downstream targets. *Plant Physiology and Biochemistry, 195*, 152–164. https://doi.org/10.1016/j.plaphy.2023.02.019

Fidler, A., Biesiada, T., & Borkowski, K. (2022). The PYR/PYL/RCAR family of ABA receptors: Structure, function, and regulation in abiotic stress tolerance. *Plant Molecular Biology, 108*(1–

2), 1–18. https://doi.org/10.1007/s11103-021-01169-0.