

Impact of Rice PPR Protein YSA on Early Chloroplast Development



Pranav Mettu

Rocky Mount, North Carolina

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Personal Reflection

The summer I spent in Changsha, China, immersed in third-generation hybrid rice research, felt like stepping into a living story, one that combined the rhythm of pipetting with the pulse of an unfamiliar culture. My days began in the sweltering heat of the paddies, where the shimmer of green rice plants stretched endlessly, and ended with late-night conversations in the lab, where I learned the science behind one of the world's most important crops. The challenges I faced while living in a foreign country tested both my endurance and adaptability, but they also revealed new strengths I didn't know I had.

The most immediate challenge was the sheer physicality of the research. Researching hybrid rice is not confined to the controlled sterility of a lab—it demands patience, precision, and stamina under the sun. Hours spent crossing plants, collecting data, and recording observations required a discipline beyond what I had encountered in classrooms. At first, I found myself overwhelmed by the scale and pace of the work, but I managed by establishing small rituals: taking careful notes at every stage, hydrating frequently with Chinese Redbull, and breaking the tasks into manageable steps. Slowly, what seemed exhausting became meditative, and I came to respect the persistence required to translate science into sustenance.

Equally daunting were the cultural and linguistic barriers. Even though many of my lab members spoke English, the day-to-day life of the lab and the city unfolded in Mandarin. The first time I tried ordering food at a local night market, fumbling through tones and words, I felt the weight of my outsider status. But over time, I discovered that communication did not always require perfect grammar or vocabulary. Patience, gestures, and an open heart bridged gaps more

powerfully than I expected. Sharing delicious meals with graduate students and my PI, laughing over mispronunciations, and listening to their stories gave me insights far beyond language: that humility is the first step to understanding, and that respect forms the true foundation of collaboration.

Scientifically, the summer transformed me. I learned practical lab skills like pipetting, writing lab reports, and the rigor of precise data collection. But more importantly, I gained perspective. Standing in the very fields where hybrid rice was first developed, I was reminded that science is not just theory; it is rather a lifeline that holds the power to feed millions, and that the pursuit of resilient crops is not just a technical challenge but a moral one.

As I look back, I am filled with gratitude. Gratitude for the rice fields that became my classroom, for the mentors who guided me with generosity, and for the city that welcomed me with open arms. This summer did not simply sharpen my scientific skills; it expanded my vision of what it means to be a global citizen and a researcher. I left Changsha carrying not just data and techniques, but a deeper sense of purpose: to contribute, however I can, to a world where science continues to nourish both people and possibility.

Acknowledgments

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Introduction

When Yuan Longping first developed hybrid rice in the 1970s, he altered the course of global agriculture. Known today as the “Father of Hybrid Rice,” Yuan’s pioneering work proved that carefully crossing rice varieties could produce hybrids with higher yields, vigor, and resilience than their parents. This discovery was not a technical achievement, with the ability to help millions. In the decades since, hybrid rice has fed hundreds of millions of people, reshaping food security in China and providing a model that has spread across Asia, Africa, and beyond (Yuan, 1998; Virmani, 2003). Rice became a symbol of innovation, demonstrating how the advancement of science and genetic diversity can transform hunger into abundance.

Yet, the challenge Yuan Longping sought to address remains far from resolved. Rice still serves as the staple food for more than half of the world’s population, and the demand for higher yields grows each year as populations expand and climates shift (Khush, 2013). Hybrid rice, while truly revolutionary, is not a static solution. Its continued success depends on deepening our understanding of the plant at the molecular level; its genetics, its physiology, and the subtle processes that determine its growth. This is where research at the intersection of molecular biology and crop science becomes truly vital.

In recent years, academic attention has turned to the role of nuclear-encoded proteins that regulate chloroplast development in rice. Chloroplasts, the green organelles responsible for photosynthesis, are not only the engines of plant growth but also the primary drivers of yield. Their function determines how efficiently a plant can capture light, fix carbon, and ultimately support grain production. Within this intricate system, pentatricopeptide repeat (PPR) proteins

have emerged as key regulators. These proteins are involved in RNA editing, splicing, and stabilization processes within chloroplasts, and defects in their function can disrupt chloroplast development, leading to reduced photosynthetic capacity and compromised plant performance (Barkan & Small, 2014).

The Young Seedling Albino (YSA) gene represents one such critical PPR protein. Mutations in YSA can lead to striking chloroplast developmental defects, visible as pale or albino seedlings unable to produce sufficient chlorophyll under certain conditions (Su et al., 2012). These mutants reveal just how essential proper RNA metabolism in chloroplasts is for sustaining plant vitality. At the same time, they offer an entry point for researchers to better understand how rice plants regulate their photosynthetic systems under both normal and stressful environments. As global temperatures continue to rise, understanding how genes like YSA respond to environmental stressors such as heat becomes increasingly important. A future in which hybrid rice continues to sustain billions will require varieties that are not only high in yield but also resilient to a changing climate.

Working in the birthplace of hybrid rice, this study investigates the role of YSA in early chloroplast development, with an emphasis on how temperature fluctuations affect its localization and expression. Hybrid rice has already transformed global food security, but its future depends on advancing our molecular understanding of plant development. By studying the YSA gene and its role in chloroplast formation under variable temperature conditions, this research highlights detailed genetic insights that inform the next generation of resilient, high-yielding rice varieties, ensuring that Yuan Longping's vision continues to flourish in a changing world.

The importance of research such as this study stems from the nature of need; food security is truly inseparable from human security. The lessons of hybrid rice are reminders that scientific innovation can change the fate of nations, but that innovation must be continual. The rice fields of the past century answered the question of “how to produce more.” The rice fields of the next century must answer the question of “how to produce sustainably, under harsher conditions, with fewer resources.” By exploring the molecular underpinnings of chloroplast development, this research aims to contribute small but critical pieces to that puzzle.

Methods

Participants

This study utilized rice (*Oryza sativa*) plants as the primary participants in order to investigate the role of the Young Seedling Albino (YSA) gene in chloroplast development. Both wild-type and mutant lines were included in the experiment. The mutants displayed an albino or pale phenotype during early seedling development, a characteristic associated with disruptions in the YSA gene.

The inclusion of both wild-type and mutant participants was essential for comparative analysis. Wild-type plants served as a control group, providing a baseline for chlorophyll content, growth morphology, and gene stability. The YSA mutant plants represented the experimental group, allowing for the assessment of phenotypic deviations and molecular differences attributable to the gene mutation.

Participants were selected to represent uniform age and growth stage cohorts, thereby

minimizing confounding variables. Seeds were germinated and grown under standardized environmental conditions, ensuring that observed differences could be attributed primarily to genetic background and experimental manipulation rather than uncontrolled external variation.

Apparatus and Materials

The apparatus and materials used in this study combined bioinformatics resources, molecular biology tools, and plant physiology equipment. To begin, the NCBI and Rice Annotation Project Database (RAP-DB) were utilized to identify and characterize the YSA gene sequence, while bioinformatics software supported analyses of exon–intron organization, conserved PPR motifs, and phylogenetic relationships. Genomic DNA amplification was carried out using thermocyclers, and the resulting amplicons were validated through agarose gel electrophoresis under UV illumination.

For plant growth, controlled-environment chambers were employed to maintain seedlings under defined temperature conditions of 20°C, 28°C, and 35°C, with regulation of light intensity, photoperiod, humidity, and nutrient availability to ensure consistency. Phenotypic differences between wild-type and mutant lines were systematically documented using digital imaging equipment. Chlorophyll quantification was performed with a spectrophotometer, which measured absorbance at 663 nm and 645 nm following pigment extraction in 80% acetone. Supporting these materials, a range of reagents and consumables such as plant growth media, DNA extraction kits, primers, acetone, and sterile collection tools, were essential for the smooth execution of both molecular and physiological assays.

Procedure

The study began with the identification and characterization of the YSA gene. Using publicly available databases such as NCBI and RAP-DB, the full-length sequence of YSA was retrieved. Exon–intron structure analysis was performed to clarify the gene’s genomic context. Conserved PPR motifs were identified using domain prediction software, which suggested a likely role in RNA binding and regulation.

A phylogenetic analysis was conducted to situate YSA among homologous PPR proteins from other species. This evolutionary perspective helped infer whether YSA is highly conserved, indicating essential function, or divergent, suggesting potential specialization. Subcellular localization predictions indicated a chloroplast-targeting signal, aligning with its proposed role in photosynthetic regulation.

To validate mutations in YSA, primers were designed to flank suspected mutation sites. Primer efficiency and specificity were optimized to avoid non-specific amplification. Genomic DNA was extracted from both wild-type and -17bp mutant seedlings using a standard plant DNA isolation protocol. PCR amplification was conducted with the designed primers, and products were analyzed on agarose gels to verify size and integrity. PCR products were subsequently sequenced, enabling identification of precise mutations such as point mutations, insertions, or deletions. These results confirmed the genetic basis of the observed albino seedling phenotype.

Wild-type and YSA mutant plants were cultivated under controlled environmental conditions across three temperature regimes: 20°C, 28°C, and 35°C. These settings simulated moderate, optimal, and heat-stressed environments, respectively. Light intensity, humidity, and nutrient access were standardized across groups.

Phenotypes were recorded at multiple developmental stages, including the coleoptile, one-leaf, and two-leaf stages. Plants were photographed systematically to document visible differences in leaf coloration, morphology, and growth vigor. Comparisons across temperature treatments were used to evaluate whether YSA's role in chloroplast development is temperature-sensitive.

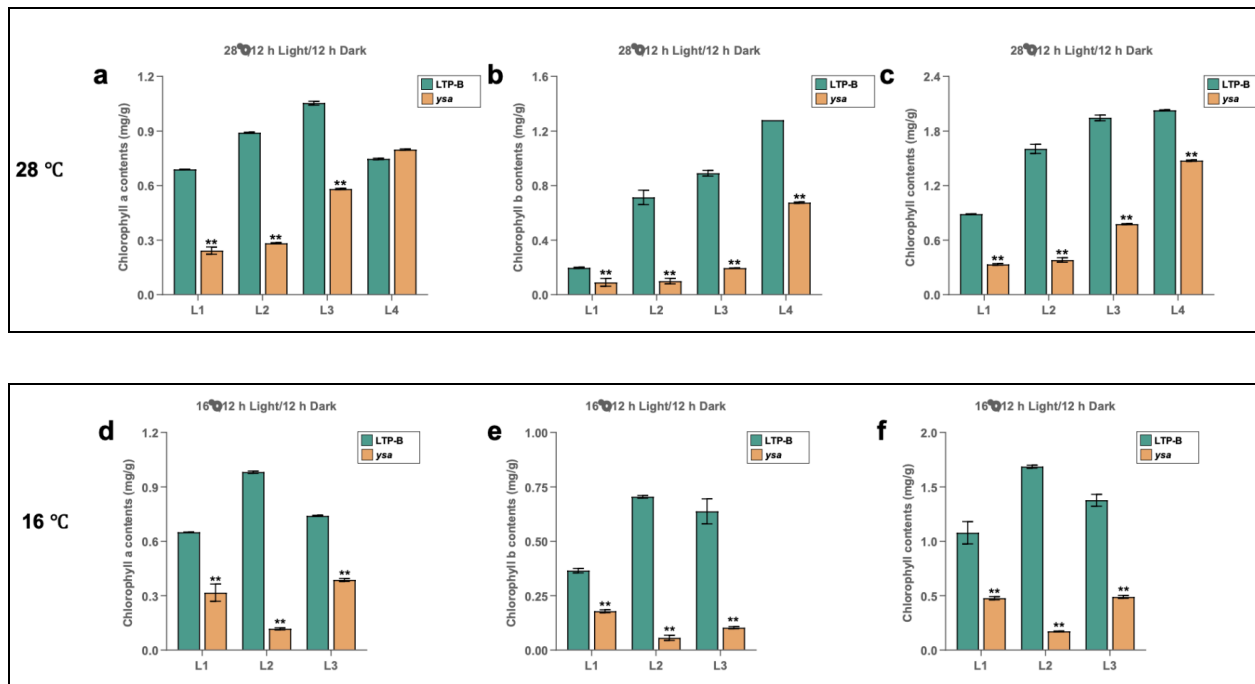
The independent variable was temperature condition, while the dependent variables were leaf coloration, morphology, and overall growth performance. Control variables included light cycle, humidity, soil composition, and watering schedule.

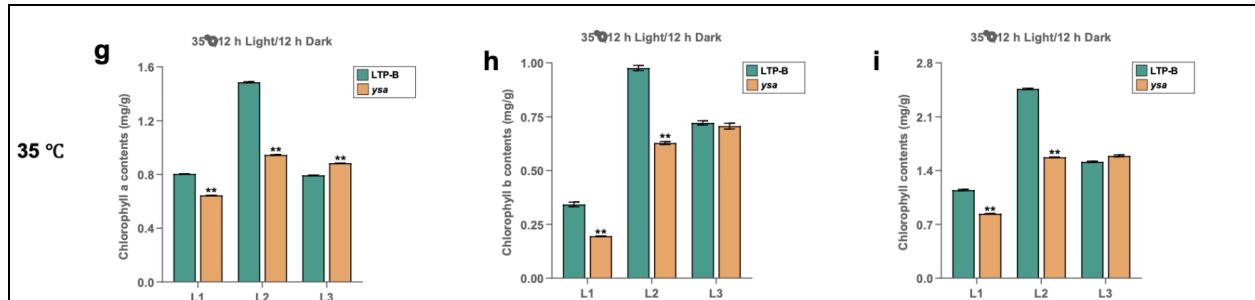
In order to quantify phenotypic differences more precisely, chlorophyll extraction and measurement were performed. Leaf tissue samples were collected from both wild-type and mutant seedlings at defined developmental stages. To ensure reliability, all samples were collected at the same time of day and processed immediately to minimize degradation.

Chlorophyll was extracted using liquid nitrogen and 80% acetone, and absorbance was measured at 663 nm and 645 nm using a spectrophotometer. These values were used to calculate the concentrations of chlorophyll a and chlorophyll b, as well as total chlorophyll content. The chlorophyll a/b ratio was also determined, providing further insight into chloroplast development and function. Data was analyzed by comparing chlorophyll levels across wild-type and mutant groups at different temperature conditions. Trends in chlorophyll accumulation were correlated with visual phenotypic differences, allowing for a dynamic analysis of how YSA mutation disrupts pigment biosynthesis under environmental stress.

Results

The growth performance of wild-type and YSA mutant rice seedlings differed markedly across the three temperature treatments (28 °C, 16 °C, and 35 °C). Among these conditions, 28 °C emerged as the most favorable environment for plant development. Seedlings grown at this temperature displayed vigorous growth, deep green pigmentation, and the highest chlorophyll accumulation. In contrast, plants grown at 16 °C exhibited delayed growth, paler leaves, and reduced chlorophyll levels, while those grown at 35 °C displayed signs of heat stress, including chlorosis and reduced biomass. These results indicate that 28 °C represents an optimal balance between metabolic activity and stress tolerance, consistent with rice's adaptation to warm but not extreme climates.





Temperature variance for different leaves (columns 1, 2, and 3 are 1, 2, and 3 leaves, respectively)

At 28 °C, clear distinctions between wild-type and YSA mutant plants became evident. Wild-type plants showed robust green leaves with uniform morphology, while YSA mutants exhibited pale or albino seedlings at early developmental stages. This difference was particularly striking during the coleoptile and one-leaf stages, where mutant plants lagged significantly in pigment development compared to the controls. However, by later developmental stages, some YSA mutant plants partially recovered pigmentation, though they never achieved the chlorophyll density observed in wild-type lines. These results suggest that YSA plays a critical role during the early establishment of chloroplasts, particularly under conditions favorable for growth.

The differences became even more pronounced under suboptimal temperatures. At 16 °C, both wild-type and mutant plants showed stunted growth, but the albino phenotype of YSA mutants was exaggerated, with seedlings remaining pale across multiple stages. At 35 °C, wild-type plants displayed moderate reductions in pigmentation, while YSA mutants showed severe chlorosis, curling leaves, and, in some cases, failure to progress beyond early seedling stages. Together, these results underscore the importance of YSA in conferring stability to chloroplast development under both cool and hot stress conditions.

Quantitative analysis of chlorophyll content reinforced the phenotypic observations. At 28 °C, wild-type seedlings accumulated the highest levels of chlorophyll a, chlorophyll b, and total chlorophyll across all developmental stages measured (L1–L3). For example, wild-type seedlings at the two-leaf stage displayed chlorophyll a concentrations approaching 1.0 mg/g fresh weight, whereas YSA mutants showed only about half that amount. Chlorophyll b and total chlorophyll followed the same pattern, with mutants consistently underperforming relative to wild-type lines.

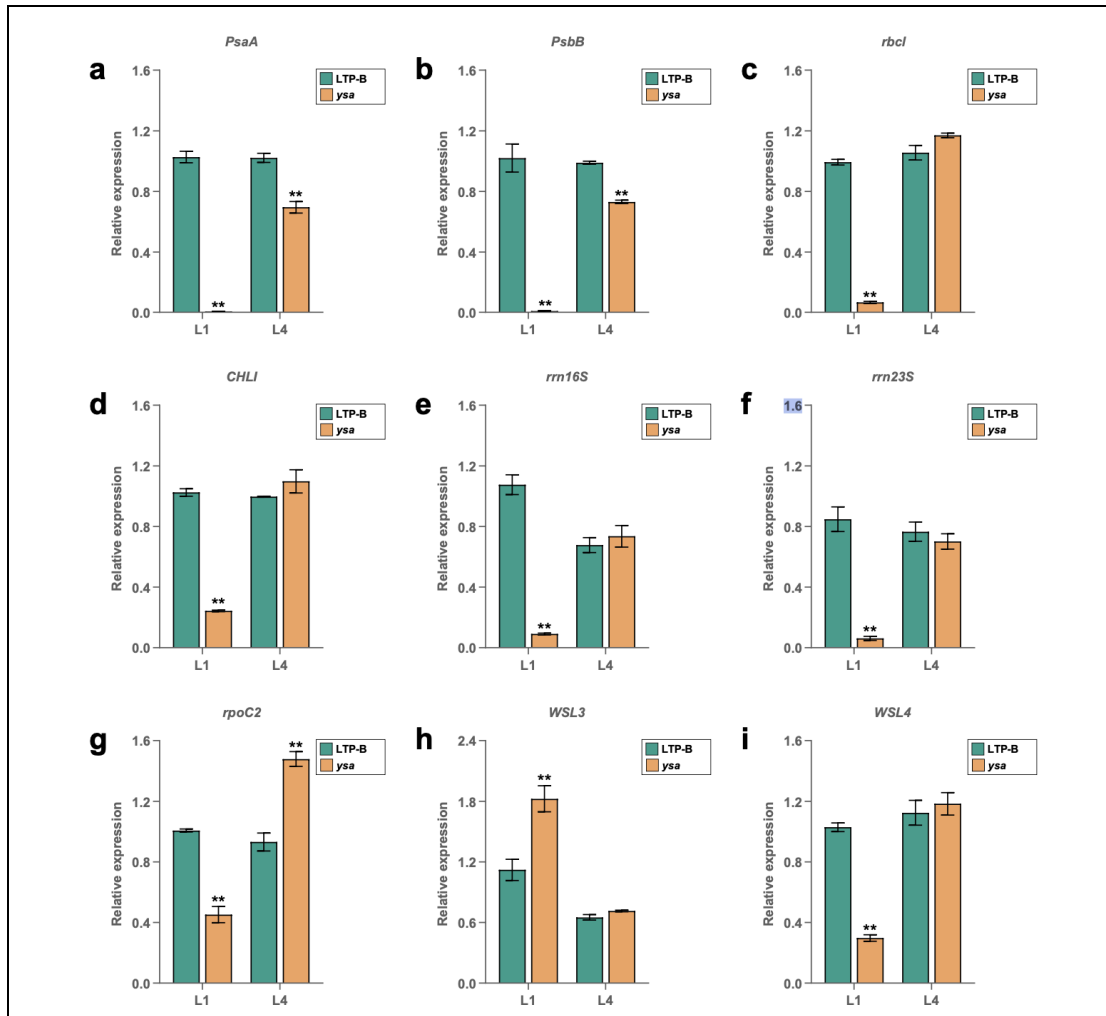
The ratio of chlorophyll a to b remained relatively stable in wild-type plants, reflecting balanced photosynthetic machinery development. In contrast, YSA mutants showed altered ratios, suggesting disruptions in the assembly of light-harvesting complexes. These findings align with the visual observation of pale leaves and support the conclusion that YSA mutations interfere with the proper regulation of chloroplast biogenesis under otherwise optimal growth conditions.

Growth at 16 °C resulted in a substantial reduction in chlorophyll accumulation for both wild-type and YSA mutant plants. Wild-type seedlings displayed lower pigment concentrations than at 28 °C, with values dropping by nearly 30–40%. YSA mutants at this temperature fared even worse, often failing to accumulate measurable levels of chlorophyll until later developmental stages. This lack of pigment corresponded with prolonged pale phenotypes, delayed growth, and smaller leaf area.

Interestingly, the difference between wild-type and mutant seedlings was most striking during the early stages (L1 and L2), where YSA mutants exhibited almost no detectable chlorophyll a or b. These results suggest that YSA's role in chloroplast development becomes even more critical when temperature slows down metabolic processes. In cooler environments, the inability of mutants to establish chloroplast functionality early in development leaves them unable to recover efficiently.

Seedlings exposed to 35 °C demonstrated stress-induced reductions in chlorophyll content, though the trends differed from those observed at 16 °C. Wild-type plants accumulated more chlorophyll than they did at 16 °C, but values remained well below those achieved at 28 °C. YSA mutants, however, showed severe reductions across all developmental stages, with total chlorophyll levels frequently less than half of wild-type counterparts.

Phenotypically, this reduction manifested as leaf curling, bleaching, and in extreme cases, seedling death. Unlike at 16 °C, where mutants showed gradual but delayed pigment development, at 35 °C their chlorophyll levels remained persistently low, suggesting that high temperatures may directly destabilize chloroplast integrity in the absence of functional YSA. These results highlight YSA as a potential genetic factor in thermotolerance during early seedling development.



Calculated relative expression of chloroplast-related developmental genes in mutant vs. wild-type leaves

Across all three temperature regimes, 28 °C produced the healthiest plants with the highest chlorophyll levels, while 16 °C and 35 °C imposed significant stress. Wild-type plants displayed flexibility, adjusting to both cooler and hotter environments with reduced but still functional chloroplast development. YSA mutants, however, consistently struggled outside of optimal conditions. At 16 °C, they were unable to establish sufficient chloroplasts during early

development, while at 35 °C, they suffered from persistent pigment loss and visible stress symptoms.

These results strongly support the hypothesis that YSA is a critical regulator of chloroplast development during early seedling stages. Furthermore, they indicate that the impact of YSA mutations is magnified under environmental stress, particularly at non-optimal temperatures. Taken together, the findings suggest that YSA not only influences chloroplast formation under standard growth conditions but also contributes to resilience against temperature fluctuations, a trait of growing importance as climate change continues to challenge crop stability.

Discussion

The central question of this study was how the Young Seedling Albino (YSA) gene influences chloroplast development in rice under different temperature conditions. Specifically, the research sought to determine whether YSA mutations impair chloroplast biogenesis and pigment accumulation, and if such effects are magnified under environmental stress. To address this, gene information was analyzed, mutations were confirmed through genotyping, phenotypic differences were observed, and chlorophyll content was quantified in both wild-type and mutant seedlings across three controlled temperature regimes (16 °C, 28 °C, and 35 °C).

This study found that 28 °C is optimal for chloroplast development and plant growth. Wild-type rice at this temperature accumulated the highest levels of chlorophyll a and b, displayed robust leaf morphology, and exhibited strong early growth. YSA mutants consistently underperformed across all temperatures. Mutants showed pale or albino seedlings in early stages,

with lower pigment accumulation, delayed development, and reduced adaptability compared to wild-type plants. Temperature stress magnified the impact of YSA mutations. At 16 °C, YSA mutants failed to accumulate detectable pigments during early growth, while at 35 °C they exhibited severe chlorosis, leaf curling, and seedling mortality.

In plain terms, these findings demonstrate that YSA is required for normal chloroplast development during the earliest stages of rice growth, and that its absence makes seedlings particularly vulnerable to environmental extremes. The observed patterns reinforce the hypothesis that YSA encodes a pentatricopeptide repeat (PPR) protein essential for RNA processing in chloroplasts. Prior studies have shown that mutations in chloroplast-targeted PPR proteins often cause seedling-lethal or albino phenotypes because they disrupt RNA editing, splicing, or translation within plastids (Barkan & Small, 2014). The results of this study are consistent with this model: YSA mutants accumulated significantly less chlorophyll, and the pale phenotype was most severe at non-optimal temperatures, where chloroplast function is already under stress.

At 28 °C, wild-type plants displayed balanced growth and pigment accumulation, while mutants struggled but sometimes partially recovered. This suggests that YSA is not the sole determinant of chloroplast biogenesis, but rather one of several factors necessary for efficiency. At cooler (16 °C) and warmer (35 °C) temperatures, the demand for robust chloroplast regulation increases, and the absence of YSA pushes mutant seedlings past a functional threshold, resulting in exaggerated or irreversible defects.

While this study focused on gene characterization, genotyping, phenotypic observation,

and chlorophyll measurement, the broader framework for YSA investigation includes additional approaches such as subcellular localization, transcript profiling, and protein interaction analysis. Although these experiments were not performed here, they provide critical avenues for future research and contextualize the findings.

Subcellular Localization

The prediction that YSA localizes to chloroplasts is consistent with the phenotypes observed in this study. Confirming this through GFP-tagging and confocal microscopy would strengthen the argument that YSA directly regulates plastid RNA metabolism rather than acting indirectly. Such localization studies could also reveal whether YSA distribution changes under heat or cold stress, providing a mechanistic link to the temperature sensitivity of the phenotypes.

RNA Expression Profiling

Mutations in PPR proteins often disrupt expression of chloroplast-related genes, including photosystem proteins (e.g., *psaA*, *psbB*) and components of the transcription/translation machinery. Future qRT-PCR analysis of wild-type versus YSA mutants could determine whether reduced chlorophyll accumulation correlates with decreased expression of these genes, thereby validating the functional role of YSA in chloroplast gene regulation.

Protein Interaction Analysis

PPR proteins often function within multi-protein complexes. Identifying YSA's interaction partners via Bimolecular Fluorescence Complementation (BiFC) or co-immunoprecipitation would provide insight into whether YSA collaborates with other

RNA-binding proteins or enzymes in regulating chloroplast biogenesis. This could explain why YSA mutants exhibit incomplete lethality at 28 °C; other proteins may partially compensate for their loss under optimal conditions.

By situating these results within this broader framework, it becomes clear that this study represents a foundational step. Phenotypic and biochemical evidence of YSA's role has been established, and future molecular studies can uncover the exact pathways through which it operates.

The findings align closely with prior studies on PPR proteins in rice and Arabidopsis. Su et al. (2012) reported that YSA mutations result in an early albino phenotype, with seedlings recovering green coloration at later stages under favorable conditions. The results presented here are consistent with this, showing partial pigment recovery at 28 °C but severe defects under stress conditions. Similarly, Khush (2013) emphasized the necessity of temperature-resilient traits in rice breeding, and these findings support the idea that genes like YSA could be targets for enhancing stress tolerance.

Where the results differ slightly from the literature is in the severity of heat-induced phenotypes. While previous reports focused primarily on albino phenotypes under standard conditions, the findings of this study suggest that high temperatures may exacerbate chloroplast instability in YSA mutants, pointing to a possible role for YSA in thermotolerance. This nuance may reflect differences in experimental design, such as growth chamber settings, or it may reveal a previously underappreciated environmental sensitivity.

Limitations

As with any internship research project, there were limitations. Time constraints prevented the execution of localization or expression assays, which would have provided direct mechanistic evidence of YSA's role. The study also relied primarily on chlorophyll measurements and visible phenotypes, which, while compelling, provide an indirect view of molecular processes. Additionally, because rice is genetically diverse, the findings in one background may not fully capture variability across cultivars. However, these limitations do not diminish the significance of the results. Instead, they highlight clear next steps: validating YSA localization, assessing downstream gene expression, and expanding studies across additional rice varieties and environments.

Broader Implications

This research underscores the critical intersection of molecular genetics and food security. Chloroplast development may seem like a narrow area of study, but its regulation determines the efficiency of photosynthesis, which in turn underpins global crop productivity. The discovery that YSA mutants fail under temperature extremes suggests that genes of this class could be leveraged to breed rice varieties with enhanced resilience. For example, identifying alleles of YSA or related PPR proteins that confer stability under heat stress could help future breeders adapt hybrid rice to warming climates.

More broadly, this study raises important questions for future work: Can YSA interactors or homologs compensate for its loss in certain genetic backgrounds? How does YSA-mediated

chloroplast regulation intersect with other stress response pathways in rice? Could gene editing tools like CRISPR be used to engineer temperature-resilient variants of YSA to support food security initiatives? Addressing these questions will require collaboration across molecular biology, genetics, and agronomy; an approach very much in the spirit of Yuan Longping's hybrid rice vision, which combined basic science with real-world application.

Conclusion

In summary, this study demonstrated that the YSA gene is essential for proper chloroplast development in rice seedlings, particularly during early growth stages. Wild-type plants thrived at 28 °C, while YSA mutants displayed persistent defects that worsened under 16 °C and 35 °C stress conditions. These findings support the role of YSA as a chloroplast-targeted PPR protein critical for RNA metabolism and pigment biosynthesis, and they point to its importance in temperature resilience. While this research addressed the phenotypic and biochemical outcomes, future studies expanding into localization, expression, and protein interaction analysis will be necessary to fully define YSA's function. Ultimately, this research contributes to the larger story of how molecular insights can inform hybrid rice breeding, ensuring that the crop continues to sustain global food security in the face of climate change.

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