

Rice plants response to modulation of the ERECTA signaling

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ABSTRACT: The ERECTA family of receptors (*Erfs*), encoding leucine-rich repeat receptor-like kinases (LRR-RLKs), can regulate multiple aspects of plant development and abiotic stress responses. The rice (*Oryza sativa*) genome contains three *ERfs*, namely ERECTA 1 (*OsER1*), ERECTA 2 (*OsER2*), and ERECTA-LIKE (*OsERL*). However, there is limited information currently available on their expression pattern during the plant developmental stages. The expression pattern of *OsERfs* genes at reproductive stages from the booting (R2) to milk (R6) stage in Nipponbare cultivar and the transgenic plants expressing a truncated *Arabidopsis* ERECTA (ER) lacking the kinase domain (Δ *Kinase*) were studied. The Δ *Kinase* functions as a dominant-negative receptor and presumably disrupts ER signaling in rice. The highest level of rice *ERfs* (*OsERfs*) expression was observed at the heading (R3) and flowering (R4) stages. Δ *Kinase* expression induced the compensation effect and increased the expression of endogenous *OsERfs* genes, possibly due to the suppression of ER signaling. The response to heat stress was evaluated at the reproductive stages to test the functional effect of the upregulated *OsERfs* on transgenic plants. Although transgenic plants exhibited enhanced growth during the vegetative stages, they succumbed to heat stress during the grain filling stages. Overall, the Δ *Kinase* plants generated in this research serve as essential resources to study the role of *OsERfs* in the response of plants to developmental and environmental stress.

Keywords: ERECTA gene, Δ *Kinase*, receptor-like kinase, heat stress

Introduction

The ERECTA family of receptors (*Erfs*) encoding leucine-rich repeat receptor-like kinases (LRR-RLKs) are found in many species and have evolved to regulate multiple aspects of plant development (Liu et al., 2019). The *ERfs* can control a set of morphological traits and also influence the plant's responses to environmental stresses (Lin et al., 2017; Shpak, 2013; van Zanten et al., 2009; Villagarcia et al., 2012).

In rice (*Oryza sativa* L.), the *ERfs* consist of three members: *OsER1* (LOC_Os06g10230/Os06g0203800) and *OsER2* (LOC_Os02g53720/Os02g0777400), and its paralog *ER-LIKE*, *OsERL* (LOC_Os06g03970/Os06g0130100) (Zhang et al., 2018). *OsER1* affects panicle morphogenesis, impacting the number of spikelets per panicle and the grain size (Li et al., 2023). Conversely, *OsER2* influences the seed setting rate, possibly by regulating the germline cell specification during megasporogenesis (Guo et al., 2020; Zhang et al., 2018; Zhao et al., 2020). The specific functions of *OsERL* have not yet been clarified; nevertheless, it has been identified as an essential gene and its complete loss is considered lethal for the plant (Zhang et al., 2018). Thus, the identification and characterization of the expression pattern of these genes at different growth stages in rice represents an important step to ascertain their roles in terms of yield, quality, and tolerance to be used in breeding programs.

A truncated ERECTA (ER) lacking the cytoplasmic Δ *Kinase* was developed by Shpak et al. (2003) and demonstrated that it suppresses ER signaling in

Arabidopsis. The Δ *Kinase* gene functions as a dominant-negative receptor, exhibiting phenotypic traits similar to those observed in the loss-of-function *erecta* mutants (Shpak et al., 2003). Furthermore, the Δ *Kinase* tomato and soybean lines exhibited enhanced tolerance to drought stress, indicating the potential for ER manipulation to enhance abiotic stress tolerance. Plants that overexpressed the *Arabidopsis* ER gene during vegetative growth and were subjected to heat treatment also exhibited green and lively leaves and tillers, and seed setting rates higher than the wild-type (Shen et al., 2015). The results demonstrate that ER is also important for thermotolerance in rice. However, no further studies have investigated the role of ER in thermotolerance, and the mechanism is not clearly understood. In this study, we identified the expression pattern of the rice ER gene family (*OsERfs*) during reproductive growth stages, studied their modulation in transgenic rice plants by expressing Δ *Kinase*, and analyzed the thermotolerance of the transgenic plants under high temperature stress.

Materials and Methods

Plant growth and reproductive stages

Seeds from cultivar Nipponbare (*O. sativa* japonica) were sown individually in 0.5 L pots containing a mixture of sphagnum peat moss and perlite (9:1), PRO-MIX LP15® and Osmocote® fertilizer (15N-9P-12K). The plants were kept in a greenhouse under normal light conditions, and supplemental light was supplied

between 8h00 and 17h00, when sunlight reduced to below $1,260 \text{ mmol m}^{-2} \text{ s}^{-1}$ and temperature dropped between 22-27 °C. All plants received normal water supply uniformly. The staging of the reproductive tissues was done in accordance with the methodology presented by Moldenhauer and Slaton (2001). In this process, the R2 stage is defined as the booting stage, characterized by panicle growth inside the leaf sheath. The R3 is the heading stage when the panicle emerges from the protective flag leaf sheath. The R4 is the anthesis or flowering stage and refers to the events between the spikelet's opening and closing. The R5 stage occurs five days after the flowering, while the R6, or the milk grain stage, is when developing grains in the kernel are soft, and the inner portion of the kernel is filled with a milky liquid.

Gene expression analysis

A heatmap of tissue-specific expression of *OsERfs* (Figure 1), which was based on the RiceXPro database (Sato et al., 2013), exhibited higher expression of *OsERfs* in inflorescence, in the reproductive organs, and the embryos. Consequently, the identification of expression patterns during the early reproductive stages (R0-R6) is of paramount importance for the guidance of breeding programs. Therefore, total RNA

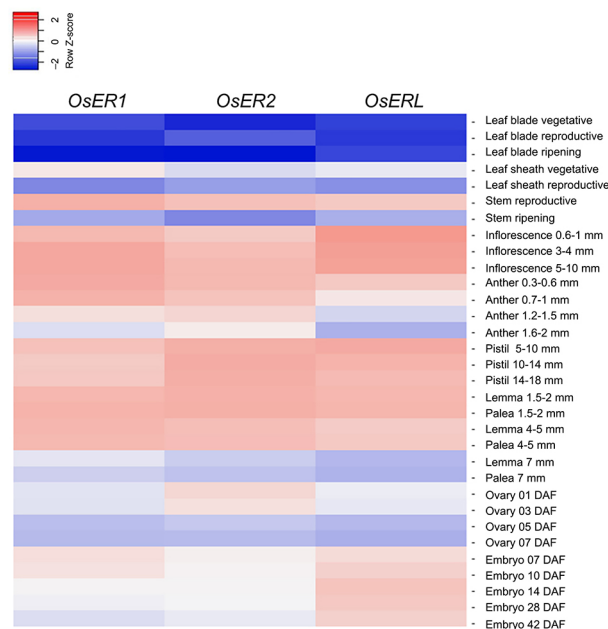


Figure 1 – Expression pattern of rice ERECTA 1 (*OsER1*), ERECTA 2 (*OsER2*) and ERECTA-LIKE (*OsERL*) genes in different tissues and developmental stages. The expression data was retrieved from RiceXPro (Sato et al., 2013) and the heatmap was prepared using Heatmapper (<http://www.heatmapper.ca/expression/>). The gene expression values were standardized using the Z-score. DAF = days after flowering.

was extracted from the spikelet of the first three panicles during different reproductive growth stages, from R2 to R6, using the reagent TRIZol (Invitrogen-Life Technologies), following the manufacturer's instructions. The total RNA was then quantified by Nano-drop 2000 (Thermo-Fisher Inc.). All RNA samples were treated with RQ1-RNase free DNase (Promega Inc.) for 30 min at 37 °C, followed by the inactivation of DNase I by ethylenediaminetetraacetic acid (EDTA). Subsequently, 1 µg DNase-treated RNA was used for cDNA synthesis using the PrimeScript RT reagent kit (Takara Bio). The real-time quantitative polymerase chain reaction (RT-qPCR) was performed on the cDNA using the TB green Premix Ex Taq II kit (Takara Bio) on Bio-Rad CFX 96 C1000 under the following conditions: 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. The melt curve analysis verified the product specificity and the expression of *OsERfs* genes was normalized against *Os7Ubiquitin* fused protein (7UBIQ) as the reference gene. The primers used were 5'-TTCGTGCCGTTTCATCTGGC-3' and 5'-CAGGTGGAC-TATGAGGCC-3' for *OsER1*, 5'-CCTCTGTCTCAAGGTCTG-3' and 5'-GATGGTAGTGCATGGATATC-3' for *OsER2*, 5'-CCTCGCATAATCCACAGAGATG-3' and 5'-AGCACATAAGTGGAGGCATGG-3' for *OsERL*, 5'-GGACTTGTCTTACAATCAGCTAACT-3' and 5'-TTGAACCAGTCAGCTTGTACTGTGC-3' for *ΔKinase*, and 5'-TGGTCAGTAATCAGCCAGTTTG-3' and 5'-CAAATACTTGACGAACAGAGGC-3' for the reference gene, 7UBQ. The Pearson's correlation coefficient between the *ΔKinase* and *OsERfs* expression was applied, and the Student's t-test was performed adopting a 5 % significance level using the fBasics and agricolae packages in R software version 3.3.3.

Generation of transgenic rice plants

The *ΔKinase* transformation vector, pNS37, has been previously described (Shanmugam et al., 2020). It consists of 8.0 kb Arabidopsis *ER* gene (promoter and coding region) with a stop codon before the kinase domain. As a result, it retains the extracellular leucine-rich repeat (LRR) and transmembrane (TM) domain; however, it lacks the cytoplasmic kinase domain (Shpak et al., 2003). Embryogenic calli derived from mature rice seeds of cv. Nipponbare and were subjected to co-bombardment with pNS37 and the selection gene construct, which harbored the hygromycin phosphotransferase gene under the control of 35S promoter, using a Bio-Rad PDS1000/He gene gun. Equal quantities (5 µg each) of the two vectors were combined to create DNA-coated gold particles for the transformation. The bombarded calli were selected on media containing 50 mg L⁻¹ hygromycin, and the selected calli were subjected to plant regeneration in accordance with the protocols described by Nishimura et al. (2006).

High temperature stress analyses

For the analysis of high temperature stress response at the reproductive stage, T₁ progenies from two transgenic plants exhibiting high levels of Δ Kinase expression (1-8 and 5-1) and one non-transgenic control line were sown individually in 2.5 L pots with a soil mixture and greenhouse conditions that were prepared in the same manner as previously described. A completely randomized design in a factorial scheme (3 × 2) was employed to evaluate the thermotolerance. The evaluated treatments were the three genotypes (1-8, 5-1 and control) in two temperature conditions: normal and high temperature stress. Three replicates were employed, with each plot comprising a single pot and a single plant. The plants were kept in the greenhouse until reaching the R2 stage, when the temperature-stressed plants were transferred to a controlled-temperature growth chamber (Conviro E15 Plant Growth Chamber) for 14 days. They were subsequently returned to the greenhouse until the conclusion of the experimental cycle. The specifications of the growth chamber are provided in Table 1. The temperature was set to simulate the daily changes, in accordance with the methodology described by Ye et al. (2012). The phenotypic traits evaluated in the plants were the number of tillers (TIL), the number of panicles (PAN), the grain yield (YLD), and the grain sterility percentage (STR). The phenotypic data was subjected to statistical analysis using the analysis of variance (ANOVA), and the Tukey's test for comparisons among treatment means was applied with 5 % significance level using the fBasics and Agricolae packages in R software version 3.3.3.

Results

Expression pattern of rice ERECTA family genes in reproductive stages

Given the high expression of *OsERf* in reproductive tissue (Figure 1), we focused our investigation on spikelets at different reproductive stages. The expression of *OsERf*s in the spikelets of the cultivar Nipponbare (*O. sativa*) was observed to be higher in the earlier reproductive stages

Table 1 – Settings for the treatment with high temperatures in the growth chamber for 24 h.

Step	Time	Duration	Temperature
		min	°C
1	06h30	07h00	30
2	07h00	07h30	30
3	07h30	08h00	30
4	08h00	14h30	38
5	14h30	15h30	35
6	15h30	16h30	30
7	16h30	17h30	27
8	17h30	18h30	24
9	18h30	06h30	24

(R2-R4) compared to the later stages (R5-R6) (Figure 2). The R3 stage exhibited the highest transcript levels for the three *OsERf*s genes, followed by a gradual decline until the minimum expression at the milk stage (R6), which marks the onset of grain ripening (Moldenhauer and Slaton, 2001). Furthermore, *OsER2* exhibited higher levels of expression between the R3 and R5 stages, followed by *OsER1* and *OsERL*.

Development of transgenic plants expressing Δ Kinase gene

To better understand the role of ER signaling and the *OsERf* genes in thermotolerance, transgenic rice lines expressing the Δ Kinase protein were developed. The expression level of the *Arabidopsis thaliana* (L.) Heynh. Δ Kinase gene, regulated by its native (*AtER*) promoter, was evaluated in six T₀ plants (1-8, 5-1, 5-2, 9-1, 9-4, and 18) at reproductive growth stages from R2 to R6 (Figure 3). Although all plants expressed Δ Kinase compared to the empty-vector control plants, the expression level among them was highly variable. The relative expression of Δ Kinase among the transgenic lines was analyzed, and it was observed that plants 1-8, 5-1, and 5-2 exhibited higher expression levels compared to 9-1, 9-2, and 18. The most significant difference was observed between genotypes 1-8 and 9-1, in which the former showed a ~53-fold higher level of Δ Kinase at the R3 stage (Figure 3).

Moreover, although the pattern of Δ Kinase expression throughout the reproductive stages exhibited some variability between lines, the highest expression levels were typically observed at the R2, R3, and R4 stages. This pattern was similar to that observed for *OsERf* throughout the R2-R6 stages (Figure 3).

Subsequently, four T₀ plants, two each with high (1-8 and 5-1) and low (9-1 and 18) Δ Kinase expression, were selected for evaluation of *OsERf*s expression during

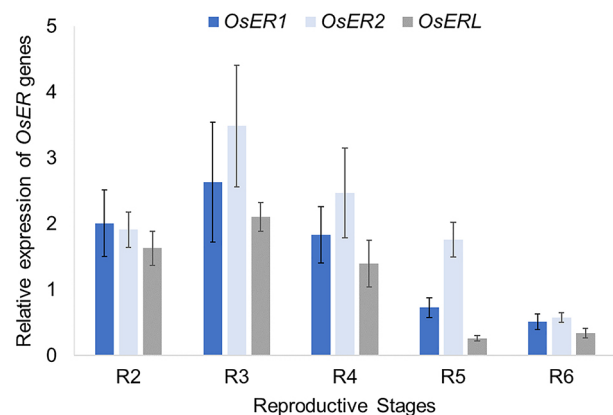


Figure 2 – Expression pattern of rice ERECTA 1 (*OsER1*), ERECTA 2 (*OsER2*) and ERECTA-LIKE (*OsERL*) genes in rice cultivar Nipponbare at different reproductive stages (R2-R6). Expression of *OsER1*, *OsER2*, and *OsERL* relative to the reference gene, 7UBQ. The error bars indicate ± standard error.

the R2, R3, and R4 stages. A genetic compensation effect was observed in all four transgenic lines expressing the $\Delta Kinase$ gene, as evidenced by the significant upregulation of the native *OsERf*s in transgenic plants compared to the control (Figure 4A-C). While all three *OsERf*s exhibited upregulation in the R2-R4 stages, the highest upregulation (up to ~90-fold) was observed for *OsER1* at the R4 stage (Figure 4C).

Correlations between the expression levels of *OsERf*s and $\Delta Kinase$ genes were estimated to obtain greater reliability regarding the compensation effect (Table 2). A high and statistically significant correlation ($p < 0.05$) was observed at the R4 stage (flowering) with correlation values of 0.95, 0.67, and 0.82, respectively, for *OsER1*, *OsER2*, and *OsERL*. Similarly, at the R3 stage, a significant correlation was observed for *OsER1* and *OsERL*, but not for *OsER2*. In conclusion, despite our efforts to disrupt ER signaling by expressing a dominant-negative ER mutation, the transgenic plants exhibited a pronounced genetic compensation effect through the upregulation of the endogenous *OsERf* genes.

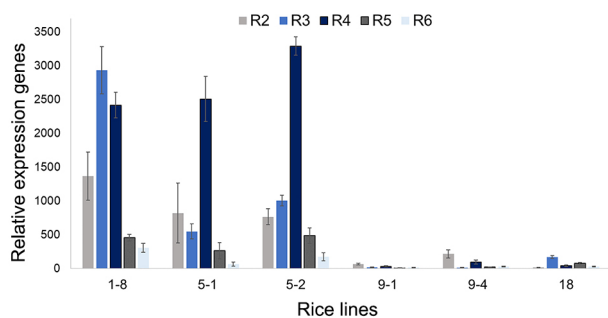


Figure 3 – Quantitative expression of $\Delta Kinase$ in transgenic lines. Expression of $\Delta Kinase$ relative to the reference gene, 7UBQ, in the T_0 plants of transgenic lines, 1-8, 5-1, 5-2, 9-1, 9-4, and 18 at different reproductive stages (R2-R6). The error bars indicate $\pm$ standard error.

Heat response of $\Delta Kinase$ transgenic plants

The effects of genotype and temperature, evaluated under both normal and stressed (high temperature) conditions, were found to be statistically significant ($p < 0.05$) for all traits. However, a significant ($p < 0.05$) genotype $\times$ temperature interaction was observed only for YLD and STR. It is noteworthy that no adverse effects were observed regarding the vegetative development stage of the transgenic plants. Under standard temperature conditions, the $\Delta Kinase$ plants exhibited statistically similar TIL and PAN values compared to the control plants (Figure 5A and B). Although both the control and the transgenic plants exhibited increased TIL under stress conditions, transgenic plants 1-8 and 5-1 demonstrated statistically higher values, with TIL levels 51 and 43 % higher, respectively, compared to the control (Figure 5A). In addition, genotype 1-8 exhibited a higher PAN, with a value that was 86.2 % higher than that of the control (Figure 5B). Notably, genotype 1-8 exhibited a more pronounced upregulation of *OsERf* throughout the R2-R4 stages compared to 5-1, with the most notable difference observed in *OsER1* expression at the R3 stage (Figure 4B). Consequently, the enhanced performance of 1-8 under heat stress correlates with a heightened upregulation of *OsERf* during the R2-R4 stages.

Table 2 – The Pearson's correlation value between expression level of $\Delta Kinase$ and *Oriza sativa* ERECTA family of receptor (*OsERf*s) genes during three different reproductive stages (R2-R4) in T_0 plants.

	$\Delta Kinase$		
	R2	R3	R4
<i>OsER1</i>	0.29	0.60*	0.95*
<i>OsER2</i>	0.20	0.44	0.67*
<i>OsERL</i>	0.33	0.66*	0.82*

*Significant by Student's t-test at 5 %; *OsER1* = rice ERECTA 1, *OsER2* = ERECTA 2, and *OsERL* = ERECTA-LIKE genes.

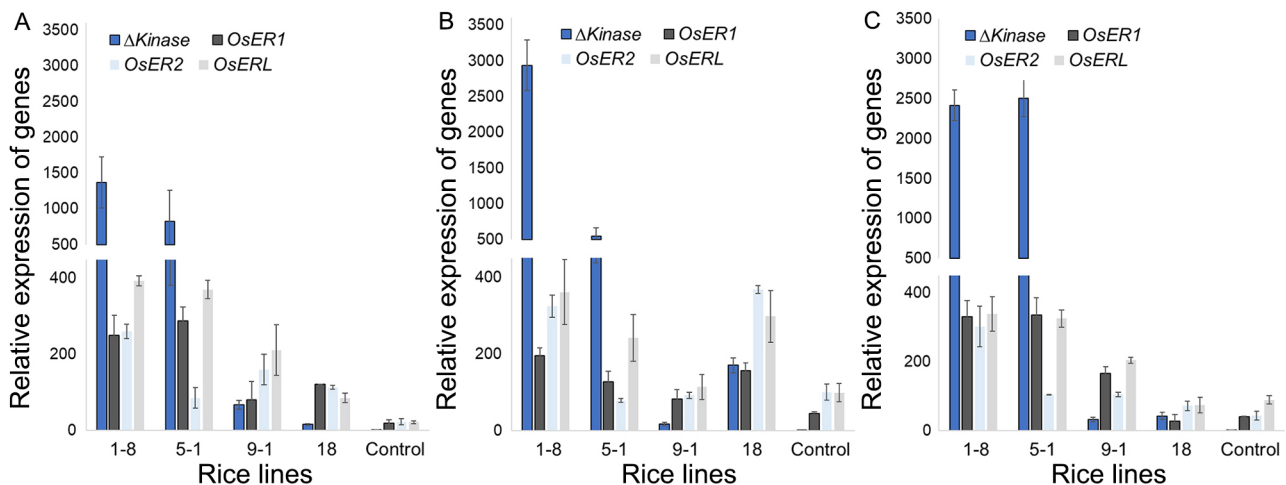


Figure 4 – Relative expression of rice ERECTA 1 (*OsER1*), ERECTA 2 (*OsER2*) and ERECTA-LIKE (*OsERL*) and $\Delta Kinase$ gene in four T_0 (1-8, 5-1, 9-1, and 18) and a control plant at different reproductive stages, A) R2, B) R3, and C) R4. The error bars indicate $\pm$ standard error.

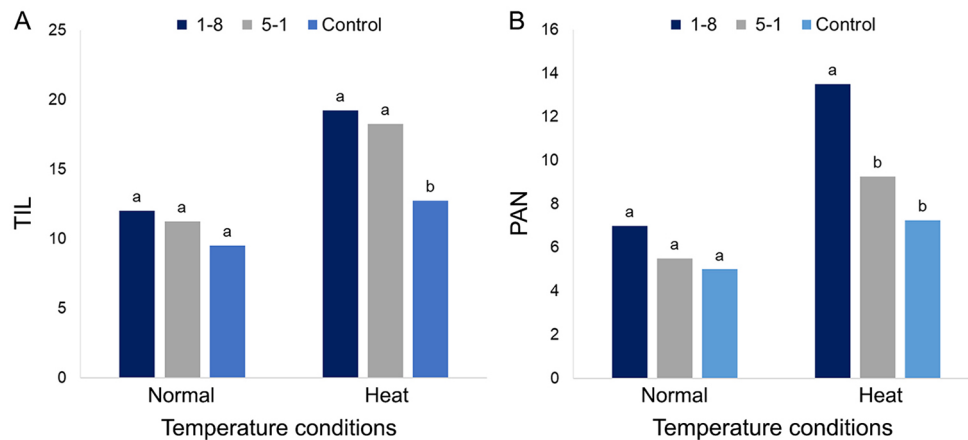


Figure 5 – Effect of heat stress on the vegetative development of rice plants. A) Number of tillers (TIL) and B) number of panicles of transgenic (PAN) (1-8 and 5-1) and the empty-vector control plants under normal and high temperature (heat) conditions. Different letters indicate significant differences ($p < 0.05$) between genotypes by the Tukey's test ($n = 3$) under different temperature conditions.

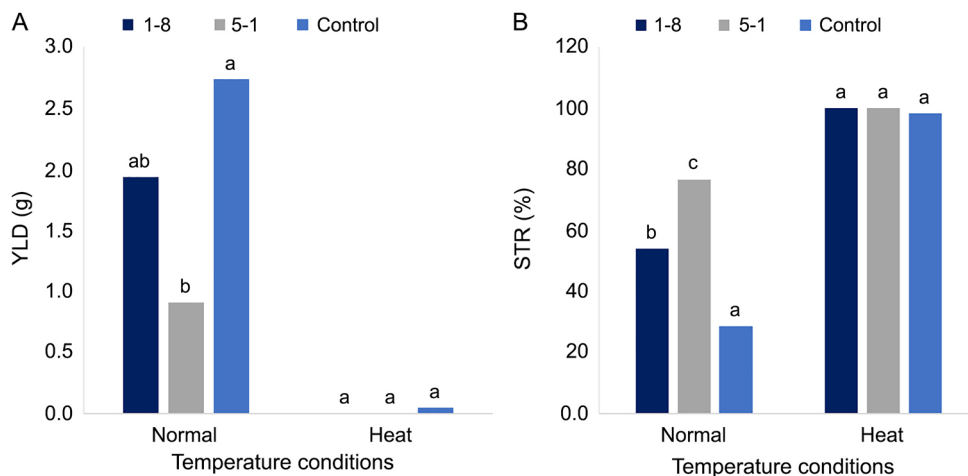


Figure 6 – Grain yield under heat stress. A) Grain yield (YLD) and B) grain sterility percentage (STR) of transgenic (1-8 and 5-1) and the empty vector control plants under normal and high temperature (heat) conditions. Different letters indicate significant differences ($p < 0.05$) between genotypes by the Tukey's test ($n = 3$) under different temperature conditions.

Although transgenic plants initially demonstrated enhanced agronomic performance under heat stressed conditions, this superiority was not sustained for YLD (Figure 6A). Given the high intensity of stress, which reached a maximum of 38 °C, none of the genotypes were able to produce grains, resulting in a STR of 100 %. Under normal conditions, the control plants exhibited superior $\Delta Kinase$ over the transgenic plants, displaying the lowest estimates of STR, while the 5-1 plants exhibited the highest STR (Figure 6A). Accordingly, the YLD in the control plants was 3× higher than in the 5-1 plants, which exhibited the lowest YLD (Figure 6B). In summary, $\Delta Kinase$ transgenic rice plants that displayed the genetic compensation effect by upregulating their *OsERf* showed greater tolerance to heat during the vegetative stages but did not display higher tolerance to heat stress during the flowering or grain filling stages.

Discussion

The *ER* genes and their *ER-LIKE* paralogs exhibit high sequence similarity across plant species, indicating their functional conservation (Zhang et al., 2018). However, to determine their application in rice breeding programs and direct interventions aimed at modulating these genes during the most sensitive stages of abiotic stress, it is imperative to understand the mechanisms by which *ER* genes operate. This can be achieved by examining their expression patterns and disrupting *ER* signaling. The present study identified the specific reproductive stages of rice during which *OsERf* genes are highly expressed. Utilizing the Nipponbare cultivar from the *japonica* subspecies, a model plant for genetic research in rice, a heightened expression of *OsERf* was observed at the R2 until R4 rice reproductive stage, when

one or more spikelets on the main stem panicle have reached anthesis. However, the greatest expression was observed at the R3 stage. These stages are responsible for triggering a series of metabolic activities in the rice plant that directly influence YLD. Microsporogenesis, the process of formation of pollen grains in the male sex organs of a plant, occurs at the R2 stage. The occurrence of abiotic stresses at this stage, such as low moisture and high temperatures, can result in elevated levels of grain sterility (Matsui et al., 2000; Sheoran and Saini, 1996), primarily due to the abortion of male gametes.

The high expression of *OsERfs* genes between the R2 and R4 stages is of significant interest, as they regulate metabolic pathways that influence the plant's response to stress (Meng et al., 2012; Terpstra et al., 2010). In addition, the *ERfs* are involved in the development of reproductive tissues. However, the nature of the signal molecule in cell-to-cell communication enabled by *ERfs* remains unknown (Shpak, 2013). A truncated *Arabidopsis ERECTA* gene, Δ *Kinase*, incorporates a stop codon prior to the cytoplasmic kinase domain (Shpak et al., 2003). The transgenic expression of Δ *Kinase* is a well-established plant approach (Berchembrock et al., 2021; Shanmugam et al., 2020; Shpak et al., 2003; Villagarcia et al., 2012). This approach interferes with the endogenous ER signaling by binding and arresting the signal molecules. The Δ *Kinase* gene was introduced into the rice cultivar Nipponbare to investigate the impact of ER suppression on plant phenotype, the expression of native rice genes (*OsERfs*), and abiotic stress tolerance. The T_0 lines were classified as high-expression (1-8, 5-1, and 5-2) or low-expression (9-1, 9-2, and 18) based on the level of Δ *Kinase* expression observed during the reproductive growth stages. Notably, the expression pattern of the Δ *Kinase* gene was identical to that observed for the *OsERfs* genes in the Nipponbare cultivar, with higher estimates in the reproductive stages (R2, R3, and R4). This overlapping expression pattern suggests that Δ *Kinase* and *OsERfs* participate in the same cell-to-cell signal transduction pathways in transgenic rice plants.

Our transgenic lines did not exhibit any notable morphological effects, prompting us to investigate whether the expression of Δ *Kinase* initiated a genetic compensation. Genetic compensation is the transcriptional upregulations of specific genes occurring in the presence or absence of deleterious mutations (Rossi et al., 2015). To this end, four T_0 lines were subjected to an expression analysis of *OsERfs* genes at the R2, R3, and R4 stages. A strong genetic compensation effect was observed, characterized by transcriptional up-regulation of the *OsERfs* genes in the presence of the Δ *Kinase* gene. Moreover, it indicates that plants attempt to overcome the interference imposed by Δ *Kinase* by enhancing the expression of their endogenous ER proteins. A similar result was observed in tomato (*Solanum lycopersicum* L.) plants by Villagarcia et al. (2012). The expression of the Δ *Kinase* gene in transgenic tomato plants resulted in the upregulation of the two endogenous *ER* family

genes, *ERECTA* (*SIER*) and *ERECTA-LIKE* (*SIERL*). The downregulation of the ER signaling by Δ *Kinase* presumably induced a genetic compensation effect, leading to an increase in the expression of endogenous *ER* genes in different organs, above that observed in wild-type plants. However, whether this genetic compensation is sufficient to restore ER signaling to its original levels is unclear.

Earlier studies have demonstrated that rice plants overexpressing *ER* genes exhibit enhanced heat tolerance (Shen et al., 2015). Specifically, the researchers subjected the plants to prolonged heat stress, allowing them to recover and set seeds. However, given that heat stress is most detrimental to rice during the reproductive stages, it remains unclear whether ER-overexpressing rice can withstand heat stress throughout the reproductive stages. Therefore, the objective was to determine whether the genetic compensation effect observed in the Δ *Kinase* transgenic lines could confer tolerance to heat stress. We selected heat tolerance as the trait of interest due to the association of *OsER1* with heat tolerance in rice (Shen et al., 2015), as well as the observation that *OsER1* is one of the highly upregulated genes of the three *OsERf* in our transgenic lines. In general, the transgenic lines demonstrated superior performance under heat stress, indicating that the genetic compensation observed in rice is not merely a transcriptional effect but is also likely phenotypic. Rice plants typically show an increase in tillers under stress conditions (Baker et al., 1992; Yoshida, 1978). Our transgenic lines exhibited, on average, a 47 % higher TIL than the control under heat stress conditions. The TIL per plant is typically correlated with the PAN (Nuruzzaman et al., 2000), which constitutes a component of YLD. Indeed, a linear relationship was observed between the TIL and panicles under both temperature conditions, except genotype 5-1 under heat stress. However, the transgenic lines exhibited increased sterility and reduced YLDs even under normal conditions, while all genotypes demonstrated absolute sterility and empty panicles under heat stress. Similarly, the modulation of ER signaling by the expression of Δ *Kinase* in soybean or *Arabidopsis* compromised plant fertility (Berchembrock et al., 2021; Shpak et al., 2003). In soybean, an inverse relationship was identified between the level of Δ *Kinase* expression and YLD in transgenic plants, with an estimated correlation of -0.78 (Berchembrock et al., 2021). The reproductive stage in rice is susceptible to high air temperatures, which can impair the dehiscence of the anther, the shedding of pollen, the germination of pollen grains on the stigma, and the elongation of pollen tubes (Krishnan et al., 2011; Prasad et al., 2006). At this stage, stress may impede the fertilization of spikelets, resulting in near-complete sterility and a significant reduction in YLD (Krishnan et al., 2011). Overall, the modulation of ER in transgenic rice has been unable to overcome the detrimental effects of heat on traits associated with fertilization or grain filling.

In conclusion, the *OsERf*s genes exhibited elevated expression levels during the reproductive development stages in rice, with the highest expression levels observed in the spikelets during the R2, R3, and R4 stages. The suppression of ER signaling by Δ *Kinase* in transgenic rice plants resulted in an increase in the expression of the three endogenous rice *ER* family genes, which exhibited a strong correlation with the expression level of the Δ *Kinase* transgene. Despite the absence of diminished vegetative growth in plants expressing Δ *Kinase*, a reduction in seed set and YLD was observed in comparison to the non-transgenic control. Furthermore, during the reproductive stages, the transgenic plants did not tolerate high temperature stress despite the significant upregulation of the *OsERf* genes. A comprehensive understanding of the *ERf*s function in rice will be of great importance in the implementation of biotechnology strategies aimed at modifying their expression in the search for plants with desired agronomic traits and enhanced tolerance to diverse abiotic stresses.

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Author's contributions

Conceptualization: Botelho FBS, Srivastava V. **Funding Acquisition:** Srivastava V. **Investigation:** Berchembrock YV, Botelho FBS, Srivastava V. **Methodology:** Berchembrock YV, Botelho FBS, Pathak B, Zhao S. **Supervision:** Srivastava V. **Validation:** Berchembrock YV, Botelho FBS, Srivastava V. **Visualization:** Berchembrock YV, Botelho FBS, Srivastava V. **Writing-original draft:** Berchembrock YV, Botelho FBS, Pathak B, Srivastava V. **Writing-review & editing:** Berchembrock YV, Botelho FBS, Srivastava V.

Conflict of interest

The authors have no conflicts of interest to declare.

Data availability statement

Data will be made available on request.

Declaration of use of ai technologies

AI technologies were not used in the present study.

References

- Baker JT, Allen Jr. LH, Boote KJ. 1992. Response of rice to carbon dioxide and temperature. *Agricultural and Forest Meteorology* 60: 153-166. [https://doi.org/10.1016/0168-1923\(92\)90035-3](https://doi.org/10.1016/0168-1923(92)90035-3)
- Berchembrock YV, Botelho FBS, Srivastava V. 2021. Suppression of ERECTA signaling impacts agronomic performance of soybean (*Glycine max* (L) Merrill) in the greenhouse. *Frontiers in Plant Science* 12: 667825. <https://doi.org/10.3389/fpls.2021.667825>
- Guo T, Lu ZQ, Shan JX, Ye WW, Dong NQ, Lin HX. 2020. ERECTA1 acts upstream of the OsMKKK10-OsMKK4-OsMPK6 cascade to control spikelet number by regulating cytokinin metabolism in rice. *The Plant Cell* 32: 2763-2779. <https://doi.org/10.1105/tpc.20.00351>
- Krishnan P, Ramakrishnan B, Reddy KR, Reddy, VR. 2011. High-temperature effects on rice growth, yield, and grain quality. *Advances in Agronomy* 111: 87-206. <https://doi.org/10.1016/B978-0-12-387689-8.00004-7>
- Li N, Chen L, Li Y. 2023. Control of grain size and number by MAPK signaling in rice. *Seed Biology* 2: 15. <https://doi.org/10.48130/SeedBio-2023-0015>
- Lin G, Zhang L, Han Z, Yang X, Liu W, Li E, et al. 2017. A receptor-like protein acts as a specificity switch for the regulation of stomatal development. *Genes & Development* 31: 927-938. <https://doi.org/10.1101/gad.297580.117>
- Liu M, Li W, Min Z, Cheng X, Fang Y. 2019. Identification and expression analysis of ERECTA family genes in grape (*Vitis vinifera* L.). *Genes & Genomics* 41: 723-735. <https://doi.org/10.1007/s13258-019-00810-0>
- Matsui T, Omasa K, Horie T. 2000. High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). *Plant Production Science* 3: 430-434. <https://doi.org/10.1626/ppp.3.430>
- Meng X, Wang H, He Y, Liu Y, Walker JC, Torii KU, et al. 2012. A MAPK cascade downstream of ERECTA receptor-like protein kinase regulates *Arabidopsis* inflorescence architecture by promoting localized cell proliferation. *Plant Cell* 24: 4948-4960. <https://doi.org/10.1105/tpc.112.104695>
- Moldenhauer KEWC, Slaton N. 2001. Rice Growth and Development. University of Arkansas, Little Rock, AR, USA.
- Nishimura A, Aichi I, Matsuoka M. 2006. A protocol for Agrobacterium-mediated transformation in rice. *Nature Protocols* 1: 2796-2802. <https://doi.org/10.1038/nprot.2006.469>
- Nuruzzaman M, Yamamoto Y, Nitta Y, Yoshida T, Miyazaki A. 2000. Varietal differences in tillering ability of fourteen japonica and indica rice varieties. *Soil Science and Plant Nutrition* 46: 381-391. <https://doi.org/10.1080/00380768.2000.10408792>
- Prasad PVV, Boote KJ, Allen Jr. LH, Sheehy JE, Thomas JMG. 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research* 95: 398-411. <https://doi.org/10.1016/j.fcr.2005.04.008>
- Rossi A, Kontarakis Z, Gerri C, Nolte H, Hölper S, Krüger M, et al. 2015. Genetic compensation induced by deleterious mutations but not gene knockdowns. *Nature* 524: 230-233. <https://doi.org/10.1038/nature14580>

- Sato Y, Takehisa H, Kamatsuki K, Minami H, Namiki N, Ikawa H, et al. 2013. RiceXPro version 3.0: expanding the informatics resource for rice transcriptome. *Nucleic Acids Research* 41: D1206-D1213. <https://doi.org/10.1093/nar/gks1125>
- Shanmugam S, Zhao S, Nandy S, Srivastava V, Khodakovskaya M. 2020. Modification of soybean growth and abiotic stress tolerance by expression of truncated ERECTA protein from *Arabidopsis thaliana*. *PLoS One* 15: e0233383. <https://doi.org/10.1371/journal.pone.0233383>
- Shen H, Zhong X, Zhao F, Wang Y, Yan B, Li Q, et al. 2015. Overexpression of receptor-like kinase *ERECTA* improves thermotolerance in rice and tomato. *Nature Biotechnology* 33: 996-1003. <https://doi.org/10.1038/nbt.3321>
- Sheoran IS, Saini HS. 1996. Drought-induced male sterility in rice: changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. *Sexual Plant Reproduction* 9: 161-169. <https://doi.org/10.1007/BF02221396>
- Shpak ED. 2013. Diverse roles of *ERECTA* family genes in plant development. *Journal of Integrative Plant Biology* 55: 1238-1250. <https://doi.org/10.1111/jipb.12108>
- Shpak ED, Lakeman MB, Torii KU. 2003. Dominant-negative receptor uncovers redundancy in the Arabidopsis ERECTA leucine-rich repeat receptor-like kinase signaling pathway that regulates organ shape. *The Plant Cell* 15: 1095-1110. <https://doi.org/10.1105/tpc.010413>
- Terpstra IR, Snoek LB, Keurentjes JJ, Peeters AJM, Van den Ackerveken, G. 2010. Regulatory network identification by genetical genomics: signaling downstream of the Arabidopsis receptor-like kinase ERECTA. *Plant Physiology* 154: 1067-1078. <https://doi.org/10.1104/pp.110.159996>
- van Zanten M, Snoek LB, Proveniers MCG, Peeters AJM. 2009. The many functions of ERECTA. *Trends in Plant Science* 14: 214-218. <https://doi.org/10.1016/j.tplants.2009.01.010>
- Villagarcia H, Morin AC, Shpak ED, Khodakovskaya MV. 2012. Modification of tomato growth by expression of truncated ERECTA protein from *Arabidopsis thaliana*. *Journal of Experimental Botany* 63: 6493-6504. <https://doi.org/10.1093/jxb/ers305>
- Ye C, Argayoso MA, Redoña ED, Sierra SN, Laza MA, Dilla CJ, et al. 2012. Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. *Plant Breeding* 131: 33-41. <https://doi.org/10.1111/j.1439-0523.2011.01924.x>
- Yoshida S. 1978. Tropical Climate and its Influence on Rice. IRRI, Los Baños, Laguna, Philippines (IRRI Research Paper Series, 20).
- Zhang Y, Li S, Xue S, Yang S, Huang J, Wang L. 2018. Phylogenetic and CRISPR/Cas9 studies in deciphering the evolutionary trajectory and phenotypic impacts of rice *ERECTA* genes. *Frontiers in Plant Science* 9: 473. <https://doi.org/10.3389/fpls.2018.00473>
- Zhao H, Guo M, Yan M, Cheng H, Liu Y, She Z, et al. 2020. Comparative expression profiling reveals genes involved in megasporogenesis. *Plant Physiology* 182: 2006-2024. <https://doi.org/10.1104/pp.19.01254>