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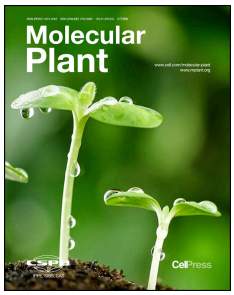

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PII: S1674-2052(26)00003-1

DOI: https://doi.org/10.1016/j.molp.2026.01.002

Reference: MOLP 2070

To appear in: *Molecular Plant*

Received Date: 17 December 2025

Revised Date: 31 December 2025

Accepted Date: 7 January 2026

Please cite this article as: Yin W., Yang Y., and Tong H. (2026). Decoding dichotomous regulation for decoupling yield traits. Molecular Plant doi: https://doi.org/10.1016/j.molp.2026.01.002.

Decoding dichotomous regulation for decoupling yield traits

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Rice grain yield per unit area is jointly determined by panicle number per unit area, grain number per panicle, and grain weight. Among these components, grain number per panicle is widely regarded as the most effective contributor to yield improvement. However, enhancement of grain number is commonly coupled with penalties in other yield traits, such as grain size and spikelet fertility, posing a major challenge for yield improvement. Therefore, elucidating the molecular and genetic mechanisms underlying the coupling and trade-offs among yield components is essential for understanding crop yield formation and for enabling rational molecular design in crop improvement.

During rice panicle morphogenesis, the active reproductive meristem produces cell-specific EPIDERMAL PATTERNING FACTOR-LIKE (EPFL) small peptides, which are perceived by the receptor-like kinase OsERECTA1 (OsER1) (Guo et al., 2023). Upon ligand perception, OsER1 activates the well-established OsMKKK10–OsMKK4–OsMPK6 cascade, leading to phosphorylation and activation of the zinc-finger transcription factor DST. Activated DST enhances the expression of *CYTOKININ OXIDASE2* (*OsCKX2*), thereby modulating cytokinin homeostasis to control cell proliferation and division, ultimately shaping spikelet number and panicle architecture (Guo et al., 2018; Guo et al., 2020). Collectively, these findings establish the OsER1–OsMKKK10–OsMKK4–OsMPK6–DST–OsCKX2 signaling module as a central regulatory pathway that negatively regulates spikelet number. To prevent excessive signaling, the protein phosphatase GRAIN SIZE AND NUMBER1 (GSN1) counterbalances the MAPK cascade by dephosphorylating and inactivating MPK6, maintaining the pathway at an appropriate signaling intensity (Guo et al., 2018). Although OsER1 clearly functions as the signal entry point of this pathway, the mechanisms governing its receptor homeostasis remain largely elusive.

Building on these foundations, a recent study fills this critical knowledge gap by uncovering how OsER1 homeostasis is maintained to control panicle morphogenesis in rice (Lu et al., 2025). The authors identified a family of rice SEVEN IN ABSENTIA E3 ubiquitin ligases, named SINARs, that mediate OsER1 degradation through the endosome-to-vacuole pathway. Rice contains six SINAR family members, all of which can physically interact with OsER1 and promote its ubiquitination. Intriguingly, single mutants of *sinar1* and *sinar6* exhibit increased spikelet number per panicle, a phenotype resembling that of the *oser1* mutant, suggesting functional divergence of *SINAR1* and *SINAR6* from *SINAR2*, *SINAR3*, *SINAR4*, and *SINAR5* during spikelet development. Through the generation and analysis of a large set of overexpression lines and higher-order null mutants, the authors revealed a striking dichotomous regulation among the SINAR members: SINAR2–SINAR5 act

redundantly to promote OsER1 ubiquitination and degradation, whereas SINAR1 and SINAR6 function as molecular brakes that synergistically antagonize the ubiquitination activity of SINAR2/3/4/5 in a feedback manner, thereby protecting OsER1 from excessive vacuolar degradation. Mechanistically, SINAR1 and SINAR6 competitively interact with SINAR2/3/4/5 to form heterodimers, preventing SINAR2/3/4/5 homodimer formation and attenuating its binding to OsER1. This dichotomous regulation of SINARs establishes a finely balanced regulatory system that maintains OsER1 homeostasis and ensures precise spatiotemporal control of OsER1 signaling, preventing signal overactivation while preserving responsiveness to dynamic developmental and environmental cues (Figure 1). Altogether, this study deepens our understanding of how balanced regulation and robustness are achieved within plant signaling networks during growth and development.

Notably, a similar antagonistic mechanism is also discovered at the ligand perception level of OsER1. Multiple OsEPFL small peptides competitively bind OsER1, with OsEPFL6, OsEPFL7, OsEPFL8, and OsEPFL9 acting as positive regulators that activate OsER1 signaling, whereas OsEPFL5 suppresses OsER1 activity. EPFL5 further competitively inhibits the binding of OsEPFL6/7/8/9 to OsER1, suggesting the existence of a negative feedback loop that fine-tunes receptor signaling (Guo et al., 2024).

OsER1 emerges as a central negative regulator of rice panicle morphogenesis. Although knockout of *OsER1* increases spikelet number, it simultaneously reduces grain length and spikelet fertility, making direct manipulation of OsER1 unsuitable for yield improvement. In contrast, precise manipulation of specific antagonistic regulatory components offers a promising strategy to overcome the trade-offs between spikelet number and other yield traits. For example, the simultaneous knockout of *OsEPFL6*, *OsEPFL7*, and *OsEPFL9*, or specific overexpression of *OsEPFL5*, significantly increases spikelet number without compromising grain size or fertility (Guo et al., 2024). Importantly, recent work by Lu et al. (2025) provides the first compelling evidence that manipulating dichotomous SINAR ligases offers a promising strategy to mitigate trade-offs among complex yield traits. Targeted suppression of *SINAR1* and *SINAR6* in the *japonica* variety Zhonghua-11 (ZH11) markedly enhances spikelet number and grain size while maintaining spikelet setting percentage. In a high-yield *indica* variety Fengaizhan-1 (FAZ1), this manipulation also increases spikelet number, resulting in approximately 29.2% and 11.7% increases in grain yield per plant and plot yield, respectively. Together, these findings support a rational molecular design strategy in which the precise deployment of antagonistic regulatory components decouples complex yield traits and optimizes panicle architecture, thereby providing new molecular modules for future crop improvement.

Members of the same protein family often display functional redundancy, antagonism, diversification, or dominancy. Targeted suppression of antagonistic SINAR members markedly increases spikelet number, underscoring that rational manipulation of homologous proteins with distinct functions is an effective strategy for yield optimization. Notably, in some cultivars, SINARs coordinately regulate spikelet number and grain weight, thereby exerting a pronounced impact on overall grain yield.

Beyond cytokinin homeostasis, emerging evidence suggests that the OsER1–OsMKKK10–OsMKK4–OsMPK6 pathway also intersects with brassinosteroid (BR) pathway, which plays a key role in regulating grain number per panicle (Zhang et al., 2024). Both OsMKK4 and OsMPK6 have been shown to modulate BR homeostasis and signaling (Duan et al., 2014; Liu et al., 2015). Moreover, OsMPK6 directly phosphorylates the transcriptional factors OsWRKY53 and OsWRKY72, which in turn promote BR signaling to regulate plant architecture (Tian et al., 2021; Wang et al., 2025). It will therefore be particularly intriguing to further elucidate the crosstalk between the OsER1–MAPK cascade and phytohormone signaling networks during panicle morphogenesis.

Given that OsER1 has been implicated in thermotolerance (Shen et al., 2015), it will be of particular interest to determine whether SINARs also participate in stress-responsive pathways. An important future question is whether simultaneous improvement of yield and stress tolerance can be achieved through tissue-specific manipulation of OsER1 or SINARs, thereby mitigating the trade-off between productivity and stress adaptation.

FUNDING

The authors' work was supported by grants from the National Natural Science Foundation of China (32425042, 32201704), the National Key Research and Development Program of China (2023YFF1000400) and the Agricultural Science and Technology Innovation Program (CAAS-CSNCB-202301).

ACKNOWLEDGMENTS

The authors declare no conflict of interest.

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Figure Legends

Figure 1 Regulatory mechanisms of OsER1-mediated signaling pathway in rice panicle morphogenesis.

During rice panicle development, small secreted peptides of the OsEPFL family bind to the OsER1 receptor. Among them, OsEPFL6/7/8/9**–**OsER1 activates the OsMKKK10–OsMKK4–OsMPK6 cascade, whereas OsEPFL5**–**OsER1 inactivates the MAPK signaling. In addition, OsEPFL5 competitively displaces OsEPFL6/7/8/9 from OsER1, thereby antagonizing pathway activation. Moreover, OsER1 protein stability is modulated by SINAR E3 ligase-mediated ubiquitination, followed by endosome-to-vacuole degradation. SINAR1 and SINAR6 attenuate the E3 ligase activity of SINAR2, SINAR3, SINAR4, and SINAR5 through competitive heterodimer formation. This dichotomous regulation of SINAR homologs ensures the fine-

tuned activation of the MAPK cascade, which is further negatively regulated by GSN1. Activated MAPK signaling leads to phosphorylation of downstream substrates such as DST, thereby inducing *OsCKX2* expression to modulate cytokinin homeostasis and ultimately shape panicle architecture. Figure created in BioRender (https://BioRender.com/3n0wdtg).