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Epigenetic regulation of regrowth in perennial rice

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Millennia of rice (*Oryza sativa*) domestication have optimized grain yield while eroding the perennial growth habit of wild progenitors, whose molecular basis remains elusive. In a recent Science article, Dai et al. reveal that allele-specific epigenetic dynamics at the *Endless Branches and Tillers 1* locus govern post-flowering resetting of the tandem microRNA genes, *MIR156BC*, enabling wild rice to re-enter vegetative growth.[1] These findings establish epigenetic reprogramming of *MIR156BC* as a key determinant of perenniality, offering a framework for designing sustainable perennial rice cultivars.

Annual cropping systems based on staples like rice underpin global food security, yet reliance on recurrent tillage and soil carbon loss reveals a tension between short-term productivity and long-term sustainability. This has renewed interest in perennial grain crops as an alternative. In Brassicaceae, transitions between annual and polycarpic perennial habits are governed by the dosage-dependent regulation of *FLOWERING LOCUS C* (*FLC*), *FLOWERING LOCUS M* (*FLM*), and *MADS AFFECTING FLOWERING* (*MAF*) paralogs.[2] Their epigenetic resetting after vernalization maintains vegetative meristem activity by preserving a subset of meristems in a vegetative state after flowering, enabling repeated reproductive cycles. The

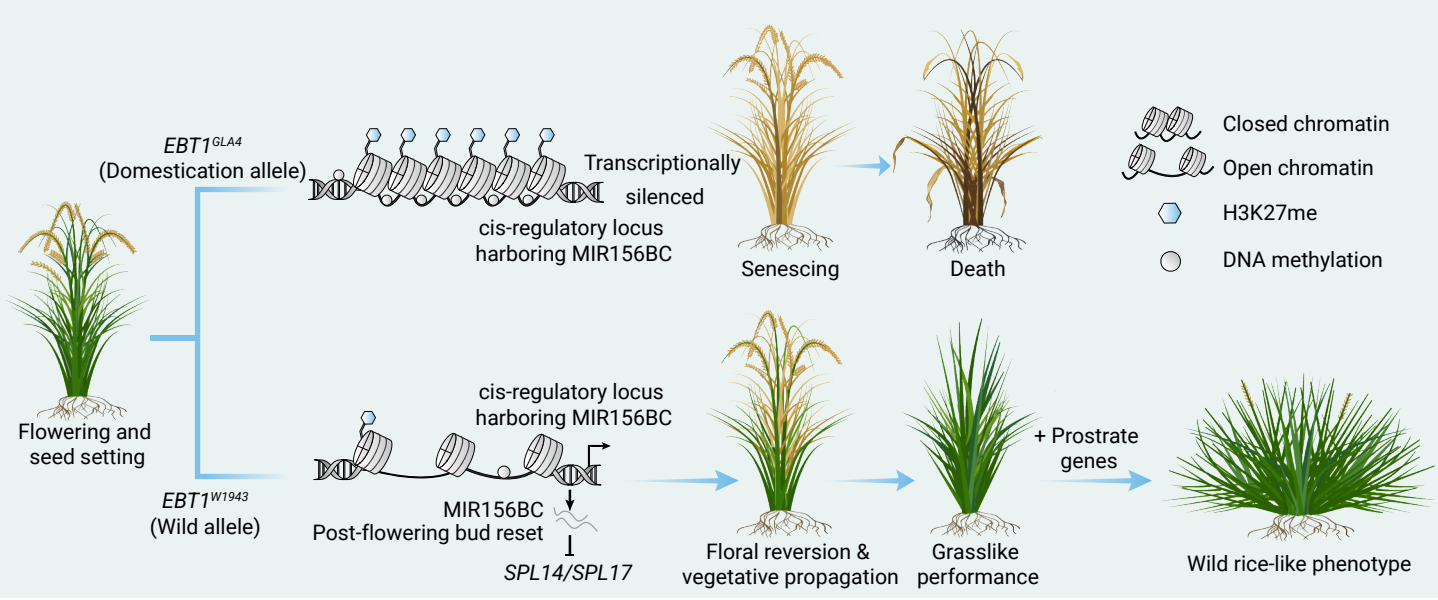

Figure 1. Epigenetic resetting of *EBT1–MIR156BC* regulates post-flowering vegetative reversion in rice The domesticated EBT1GLA4 allele enriches H3K27me3 and DNA methylation at the MIR156BC promoter. This maintains transcriptional silencing and closed chromatin, blocks post-flowering vegetative reversion, and leads to plant senescence. By contrast, the wild *EBT1*^{W1943} allele reduces H3K27me3 occupancy and enhances chromatin accessibility. This promotes post-flowering reset of *MIR156BC* in tiller buds. Elevated miR156 represses *SPL14* and *SPL17*, restoring vegetative meristem identity and promoting post-flowering vegetative reversion together with clonal propagation. Introgression of prostrate genes further reconstructs a typical perennial phenotype resembling wild rice.

absence of a functional *FLC* ortholog in rice precludes direct extrapolation, leaving the regulatory basis of perenniality in this lineage without a conceptual framework. Unlike Brassicaceae, rice perenniality involves post-flowering vegetative reversion and clonal propagation, suggesting that rice relies on distinct developmental programs. Previous efforts to introgress rhizome-associated loci from *Oryza longistaminata* restored underground perennating structures but failed to restore this post-flowering vegetative reversion program characteristic of *Oryza rufipogon*.[3]

Dai et al. address this gap by uncovering an epigenetic mechanism intrinsic to *O. rufipogon* that operates independently of rhizome formation. To define the genetic basis underlying this capacity, the authors employed a population of chromosome single-segment substitution lines (CSSLs) for high-resolution mapping that carry discrete wild rice genomic fragments in a cultivated background. The line G43 (GLA4 × W1943) exhibited robust perennial-like growth and produced sterile secondary tillers after seed maturation. Cytological observations further confirmed that axillary buds developed into leaf primordia rather than spikelet primordia at the shoot apical meristem, providing cytological evidence of post-flowering vegetative reversion. Detached tillers from G43 propagated clonally and survived for multiple years, confirming its strong perennial-like growth capacity. Genetic mapping localized the causal locus, designated *Endless Branches and Tillers 1* (*EBT1*), to a region harboring two tandem microRNA156 (miR156) genes, *MIR156BC*. This tandem organization is conserved across cereals and resembles the Corngrass1 locus in maize, where elevated miR156 drives continuous vegetative growth and branching.[4] These observations identify *MIR156BC* as a strong functional candidate underlying perennial growth in wild rice.

To validate causality and rule out linked variations, Dai et al. performed reciprocal genetic assays.[1] Knockout of *MIR156BC* in G43 abolished tiller outgrowth after seed maturation, while introducing the wild-type *EBT1* allele from W1943 into cultivated rice restored post-flowering vegetative reversion and clonal propagation. Functional divergence was driven by regulatory changes rather than coding differences, as sequence variations in the *MIR156BC* precursor had minimal effect on phenotype. The authors further identified the downstream targets of miR156 that control meristem fate. Expression of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14* (*SPL14*) or *SPL17* from the *EBT1* promoter in G43 suppressed post-flowering vegetative reversion and restored fertility in secondary tillers, with single-plant yield recovering by approximately 30% relative to G43. These results delineate a core regulatory module in which post-flowering reactivation of miR156 represses *SPL14/17* to re-establish vegetative meristem identity (Figure 1).

With the regulatory pathway established, a key question remains as to what distinguishes wild and cultivated *EBT1* alleles to enable this resetting only in wild rice. As the difference did not lie in the microRNA coding sequence, the authors focused on the upstream regulatory region. Chromatin profiling of tiller buds showed similar accessibility in wild and cultivated rice at the seedling stage. However, this region became highly accessible in wild rice during late maturation, whereas it remained condensed and repressively modified in cultivated rice. Consistently, whole-genome bisulfite sequencing further revealed higher DNA methylation levels in the *MIR156BC* promoter region in GLA4 than in G43, linking promoter hypermethylation to transcriptional silencing. To establish causality, the authors generated a series of CRISPR-edited lines with mutations in this regulatory region. Lines maintaining high *MIR156BC* expression produced abundant secondary tillers, whereas lines with reduced expression behaved like annual cultivated rice. These results demonstrate that *EBT1* promoter chromatin state determines epigenetic resetting. Thus, the functional divergence between annual and perennial rice is encoded not in the miR156 sequence itself, but in the epigenetic regulation of its upstream region after flowering (Figure 1).

If *EBT1* underlies the annual-perennial shift, it should have been a domestication target. Population genomic analysis supported this prediction, showing significantly reduced nucleotide diversity in cultivated rice relative to wild rice. Haplotype distribution further revealed that Hap4 and Hap5 were predominantly found in perennial *O. rufipogon* and largely absent from annual cultivated subspecies, which instead carried Hap1, Hap2, and Hap3. These results demonstrate that regulatory changes at *EBT1* underwent selection during rice domestication.

Given that *EBT1* confers post-flowering vegetative reversion, whereas *PROSTRATE GROWTH 1* (*PROG1*) and *TILLER INCLINED GROWTH 1* (*TIG1*) shape the prostrate architecture that enables nodal rooting, the authors hypothesized that combining these loci could reconstruct perenniality in cultivated rice.[5] Lines carrying all three wild alleles exhibited prostrate growth, nodal rooting, clonal propagation, and long-term survival under field conditions, closely resembling the perennial habit of *O. rufipogon*. Although additional loci are still required to restore full seed fertility in regenerated tillers, this work establishes that the core perennial program can be genetically reconstituted through rational breeding.

In summary, this study defines epigenetic reprogramming of the tandem *MIR156BC* locus as a molecular switch governing the annual-perennial transition in rice. By identifying *EBT1* and demonstrating that its integration with *PROG1* and *TIG1* can reconstitute core perennial traits, this work translates developmental mechanisms into a tractable strategy for perennial crop design. Despite the breakthrough of the *EBT1* module, several biological and ecological trade-offs remain to be addressed. The compromised fertility of secondary tillers in current wild-like lines indicates that a fully integrated perennial habit requires additional loci to balance clonal propagation with grain production. Furthermore, the reduced grain number in *EBT1* accessions suggests an inherent competition for resources between perennial post-flowering vegetative reversion and maximum yield. The introduction of competitive, clonally propagating rice also necessitates a proactive assessment of ecological risks including potential weediness and unintended gene flow. Addressing these frontiers will be essential for the rational design of perennial rice systems that balance robust productivity with environmental stewardship.

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AUTHOR CONTRIBUTIONS

J.T. and Y.Z. conceived and drafted the manuscript. J.W. compiled and organized relevant literature resources. J.T. generated the schematic model for this commentary. All authors contributed critically to the writing and approved the final submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.