

From a molecular ecology perspective, understanding drought adaptation requires moving beyond experiments involving individual genotypes under controlled conditions. Instead, ecologically meaningful investigations should integrate natural population sampling, environmental gradient analyses, common garden experiments, and repeated drought treatments with multi-omics profiling. Such approaches enable researchers to distinguish environmentally induced epigenetic variation from genetically determined differences and to evaluate how epigenetic mechanisms contribute to plant performance and fitness under drought conditions (Fortes and Gallusci, 2017; Springer and Schmitz, 2017). Population epigenomics, methylation-expression association studies, methylQTL analyses, and genotype-by-environment interaction models collectively provide powerful tools for linking epigenetic variation to ecological adaptation and evolutionary processes in wild grass species.

3 Case Analysis in *Setaria viridis*

3.1 Ecological significance and model value of *setaria viridis*

Setaria viridis has emerged as one of the most promising model species for investigating epigenetic mechanisms underlying drought adaptation in wild grasses. As a wild C4 grass closely related to several economically important cereal crops, including foxtail millet and other drought-tolerant grasses, *S. viridis* occupies a unique position at the intersection of ecological adaptation, crop evolution, and molecular genetics. Its short life cycle, relatively small genome, ease of transformation, and well-established crossing protocols make it particularly suitable for integrative molecular ecology studies (Brutnell et al., 2010; Jiang et al., 2013; Sebastian et al., 2014).

Unlike many cultivated crop species that have undergone extensive artificial selection, *S. viridis* populations retain substantial natural genetic and ecological variation. This diversity provides an excellent opportunity to investigate how wild grass populations adapt to contrasting environmental conditions and whether epigenetic mechanisms contribute to drought resilience. Consequently, *S. viridis* represents an ideal system for examining the relative contributions of phenotypic plasticity, genetic variation, and epigenetic regulation in shaping adaptive responses to water limitation.

3.2 Experimental framework for investigating drought-induced epigenetic variation

A robust molecular ecology framework for studying drought adaptation in *S. viridis* should incorporate both ecological sampling and multi-omics analyses. Natural accessions collected across aridity gradients can be grown under controlled conditions and subjected to multiple water regimes, including well-watered controls, progressive drought stress, and repeated drought-recovery cycles. Such experimental designs enable researchers to distinguish immediate stress responses from persistent adaptive changes.

Comprehensive epigenomic analyses should include whole-genome bisulfite sequencing (WGBS) to characterize DNA methylation dynamics, RNA sequencing (RNA-seq) to quantify transcriptional responses, ATAC-seq to assess chromatin accessibility, and ChIP-seq or CUT&Tag approaches to examine histone modifications such as H3K4me3 and H3K27me3 (Law and Jacobsen, 2010; Du et al., 2015; Lämke and Bäurle, 2017). The incorporation of small RNA sequencing would further facilitate the identification of RdDM-associated regulatory pathways involved in drought adaptation (Matzke and Mosher, 2014; Erdmann and Lafontaine Picard, 2020). Physiological traits including leaf water potential, stomatal conductance, abscisic acid accumulation, root architecture, and biomass allocation should also be evaluated to establish links between epigenetic variation and adaptive performance.

3.3 Drought-responsive regulatory networks and epigenetic memory

Current knowledge from plant stress epigenetics suggests that drought adaptation in *S. viridis* is likely mediated through coordinated changes in multiple regulatory layers. Immediate responses to water deficit are expected to involve rapid transcriptional activation of genes associated with ABA signaling, osmotic adjustment, reactive oxygen species detoxification, and stomatal regulation. These transcriptional responses are often accompanied by increased chromatin accessibility at stress-responsive loci and dynamic alterations in histone modification patterns (Crisp et al., 2016; Lämke and Bäurle, 2017).