

whether target loci can be transcriptionally activated or repressed. Thus, regulatory specificity emerges from the combined effects of protein complex composition, cis-regulatory context, and chromatin state. Insights from *Arabidopsis* have therefore provided a foundational framework for understanding conserved transcriptional regulatory mechanisms in plants, with increasing relevance for translational studies aimed at improving flowering behaviour, seed development, fruit traits, and environmental adaptability in crop species.

3.5 Applications in crop improvement

Understanding MADS-box gene function has significant implications for agricultural biotechnology. Manipulation of these genes can enhance traits such as flowering time, fruit quality, seed development, and yield. For example, modification of floral regulators can optimise flowering for different climatic conditions. Targeting fruit-related MADS genes can improve ripening and shelf life. Engineering seed development pathways can increase productivity. Advances in genome editing technologies, particularly CRISPR/Cas systems, have enabled precise functional studies and targeted manipulation of MADS-box genes. These approaches provide powerful tools for developing improved crop varieties with enhanced agronomic traits.

4 Knowledge Gaps and Future Perspectives

4.1 Challenges in understanding MADS-box regulatory specificity

Despite substantial conceptual and technological advances in understanding MADS-box transcription factors in *Arabidopsis thaliana*, several fundamental mechanistic questions remain unresolved that limit a comprehensive understanding of their regulatory mechanisms and functional specificity.

One of the major unresolved questions is the specificity of CARG-box binding. Although the consensus sequence CC (A/T)₆ GG is well established, it is widely distributed throughout the genome, raising questions about how MADS-box proteins selectively regulate target genes. Emerging evidence suggests that binding specificity is influenced by chromatin context, cofactor interactions, and higher-order protein complex formation; however, these factors remain insufficiently characterized. Collectively, current research has firmly established that MADS-box transcription factors act through combinatorial protein interactions, higher-order complex formation, and binding to CARG-box cis-elements to regulate floral and reproductive development. It is also well supported that chromatin accessibility and epigenetic modifications influence transcriptional activity. However, major uncertainties remain regarding how these regulatory layers are integrated *in vivo* to achieve developmental specificity. In particular, the dynamic assembly of transcription factor complexes, the contribution of local chromatin architecture to target selection, and the extent to which these mechanisms are conserved across plant species remain incompletely understood. Emerging approaches including single-cell multi-omics, spatial transcriptomics, live-cell imaging, chromatin conformation analyses, and AI-assisted network modelling are expected to provide deeper mechanistic insights into these unresolved questions.

Another important limitation is the lack of high-resolution spatiotemporal data. Most transcriptomic studies are based on bulk tissue analysis, which obscures cell-type-specific regulatory dynamics. The application of single-cell RNA sequencing and spatial transcriptomics could provide deeper insights into the precise roles of MADS-box genes during different developmental stages, particularly in floral organogenesis and seed development.

Furthermore, while protein-protein interactions and regulatory networks have been extensively proposed, functional validation of these networks remains incomplete. Advanced genome editing technologies, such as CRISPR/Cas systems, offer powerful tools for targeted mutagenesis and gene function analysis, yet their application in systematically dissecting MADS-box regulatory circuits is still limited.

The role of epigenetic regulation and chromatin architecture also represents an emerging frontier. Although studies have highlighted the importance of histone modifications, DNA methylation, and chromatin remodelling in modulating MADS-box gene activity, the integration of these epigenetic layers with transcription factor binding and gene regulatory networks is not fully understood.