2.1 Evolutionary diversification of MADS-box genes

The MADS-box gene family has undergone extensive expansion and diversification throughout plant evolution, primarily driven by gene duplication events such as whole-genome duplications and tandem duplications. Phylogenetic analyses indicate that ancestral MADS-box genes existed prior to the divergence of major eukaryotic lineages, followed by lineage-specific expansions in plants. In angiosperms, the diversification of MIKC-type genes has enabled the evolution of complex floral structures. Functional divergence following duplication has resulted in subfunctionalization and neofunctionalization, allowing paralogous genes to acquire specialized roles in distinct developmental pathways. Comparative genomics studies reveal conservation of core regulatory functions alongside species-specific innovations, highlighting the evolutionary plasticity of this gene family. Genome-wide analyses have further refined classification and functional annotation of MADS-box genes across plant species. Recent phylogenomic studies have revealed dynamic patterns of gene retention and loss, emphasizing the evolutionary plasticity of MIKC-type MADS-box genes (Gramzow and Theissen, 2015; Ruelens et al., 2017).

3 Functional Roles in Plant Development

3.1 Floral development

MADS-box transcription factors are central regulators of floral organ identity through the ABC model, where combinatorial gene activity determines the formation of sepals, petals, stamens, and carpels. Key genes such as APETALA1, PISTILLATA, and AGAMOUS coordinate organ specification via transcriptional regulation. The regulatory role of AGAMOUS and associated complexes has been further elucidated through recent molecular studies (Ó'Maoiléidigh et al., 2013). Furthermore, experimental evidence supporting the quartet model suggests that higher-order protein complexes provide an additional level of specificity. Recent evidence further suggests that quartet complex activity may depend on local chromatin accessibility and developmental stage, indicating that floral organ identity is regulated through dynamic integration of transcription factor assembly and epigenetic context rather than fixed combinatorial codes alone. However, the stability, composition, and in vivo dynamics of these complexes remain insufficiently characterized.

Importantly, discrepancies between mutant phenotypes and predicted ABC model outcomes highlight that redundancy and network buffering play significant roles in floral development. This suggests that floral organ identity is governed not by linear gene interactions, but by a robust and highly interconnected regulatory network.

3.2 Seed and ovule development

B-sister MADS-box genes have been implicated in ovule and seed coat development; however, their functional conservation across species remains only partially understood. While studies in *Arabidopsis* demonstrate a clear role in endothelium differentiation, comparative analyses suggest divergence in regulatory function in crop species.

This raises an important limitation in current research: the over-reliance on *Arabidopsis* as a model system. Although it provides valuable mechanistic insights, translating these findings to agriculturally relevant species is not straightforward due to differences in gene regulation and developmental context.

Consequently, future studies must adopt a comparative framework to determine whether observed functions represent conserved mechanisms or species-specific adaptations.

3.3 Fruit development

MADS-box genes also regulate fruit formation and ripening processes. They control tissue differentiation, cell expansion, and hormonal signalling pathways, contributing to fruit morphology and reproductive success.

3.4 Regulatory networks and epigenetic control

MADS-box transcription factors function within complex gene regulatory networks (GRNs), integrating multiple signalling pathways to control plant development. Their activity is modulated through protein-protein interactions, enabling the formation of dimers and higher-order complexes such as those described in the quartet model. Epigenetic mechanisms further refine MADS-box gene regulation. Chromatin remodelling, histone modifications, and DNA methylation influence gene accessibility and transcriptional activity. Additionally, long non-coding RNAs