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Computational Identification and Evolutionary Characterization of PISTILLATA Gene in Carpel (Whorl 4) Development in Fabaceae using Medicago Sativa as Model

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ABSTRACT: The PISTILLATA gene plays a pivotal role in floral organ development, primarily regulating petal and stamen formation as part of the B-class genes in the ABCDE model of flower development. However, recent research has shown that PISTILLATA is also expressed in Whorl 4, contributing to carpel formation. This study utilizes *Medicago sativa* PISTILLATA (AIT11843.1) as a model to computationally identify and characterize homologs across Fabaceae species, focusing on its involvement in carpel development. Sequence alignment, phylogenetic analysis, and 3D structural predictions provide insights into the evolutionary role of PISTILLATA in flower morphology diversification in Fabaceae.

KEYWORDS: PISTILLATA, Fabaceae, carpel development, Whorl 4, ABCDE model, evolutionary characterization, computational biology.

I. INTRODUCTION

The ABCDE Model and Floral Organ Development

Floral organ identity in angiosperms is governed by the ABCDE model, which explains how five classes of genes interact to define the identity of sepals, petals, stamens, and carpels (Coen & Meyerowitz, 1991). This model, based on work in *Arabidopsis thaliana* and *Antirrhinum majus*, defines B-class genes (PISTILLATA and APETALA3) as essential for specifying petal and stamen identity. However, new findings have suggested that PISTILLATA plays a role in carpel (Whorl 4) development as well, challenging the traditional framework of the ABCDE model (Zahn et al., 2005).

Expanding the Role of PISTILLATA in Carpel Development

Traditionally, C-class (AGAMOUS) and D-class (SEEDSTICK) genes were considered the primary regulators of carpel identity. However, emerging research has identified PISTILLATA expression in Whorl 4, indicating its involvement in carpel formation (Becker & Theißen, 2003). This expanded role suggests that PISTILLATA, while maintaining its classical function in petals and stamens, has acquired additional functions in certain plant families, such as Fabaceae.

Fabaceae and Floral Diversity

The Fabaceae family is one of the largest and most morphologically diverse plant families. Flower development plays a crucial role in its evolutionary success, with distinct gene expression patterns contributing to variations in floral structure. Understanding how PISTILLATA contributes to carpel formation in Fabaceae species can provide insights into the genetic mechanisms driving floral diversity.

Computational Approach to Gene Identification

Computational tools have revolutionized the study of gene identification and characterization. Methods such as sequence alignment, phylogenetic analysis, and structural modeling allow for the exploration of gene function and evolution across species. In this study, we employ these techniques to investigate the role of PISTILLATA in carpel development across Fabaceae, using *Medicago sativa* as a model.

II. LITERATURE REVIEW

The ABCDE Model and Its Evolutionary Framework

The ABCDE model of flower development, initially proposed in the early 1990s, defines how combinations of gene classes specify the identity of floral organs (Coen & Meyerowitz, 1991). A-class genes specify sepals, A and B-class genes together define petals, B and C-class genes determine stamens, and C-class genes specify carpels. D-class genes play a role in ovule development, and E-class genes (such as SEPALLATA) are necessary for the formation of all floral organs by forming complexes with A, B, C, and D-class genes (Riechmann & Meyerowitz, 1997).

PISTILLATA and Its Classical Role in Flower Development

As a B-class gene, PISTILLATA (PI) has been extensively studied for its role in specifying petal and stamen identity. In *Arabidopsis*, PI forms heterodimers with APETALA3 (AP3), another B-class gene, and together they regulate the identity of petals (Whorl 2) and stamens (Whorl 3) (Theißen & Saedler, 2001). In species where PI is non-functional, flowers develop with sepals in place of petals and carpels in place of stamens.

Carpels are crucial components of a flower because they are the reproductive structures responsible for producing and housing the female gametophytes, which are essential for sexual reproduction in plants. Here are some key reasons why carpels are important:

1. Reproductive Function

Ovule Production: The carpel contains the ovary, where ovules are produced. Ovules develop into seeds after fertilization.

Fertilization Site: Carpels provide the site for fertilization. The stigma, located at the top of the carpel, captures pollen, which then travels down the style to fertilize the ovules in the ovary.

Seed Formation: After fertilization, the ovules develop into seeds, and the ovary often develops into a fruit, which helps in seed dispersal.

2. Protection of Reproductive Structures

The ovary within the carpel protects the ovules from environmental damage and herbivores before fertilization. The carpel ensures that the delicate gametophytes are protected throughout the reproductive process, from pollen reception to seed formation.

3. Fruit Formation

After fertilization, the carpel often develops into a fruit, which plays a critical role in the protection and dispersal of seeds. Fruits attract animals that eat them and disperse the seeds, helping plants colonize new areas.

4. Pollination and Species Diversity

Carpels contribute to the reproductive isolation and evolution of plant species. The specific shape, size, and function of the stigma, style, and ovary often play a role in determining which types of pollen can fertilize the plant, thereby maintaining species diversity and promoting evolutionary adaptations.

5. Structural Significance in Flower Development

As one of the defining characteristics of angiosperms (flowering plants), the presence of carpels marks a key evolutionary development in plant reproduction, distinguishing angiosperms from non-flowering plants.

In summary, carpels are essential for the reproductive success of flowering plants, playing roles in pollination, fertilization, seed formation, protection, and species evolution. Their contribution to fruit and seed dispersal further highlights their importance in plant survival and diversity. In this biological process, PISTILLATA play important role in Whorl 4, giving rise to carpels.

Emerging Role of PISTILLATA in carpels Whorl 4

Recent studies have challenged the view of PI as a gene limited to the development of petals and stamens. Evidence suggests that PISTILLATA is also expressed in Whorl 4, contributing to carpel development (Whorl 4) in some plant families, including Fabaceae (Zahn et al., 2005). In *Medicago sativa*, a model Fabaceae species, PI has been found to play a role in the formation of carpels alongside C and D-class genes. This expanded role of PI may result from

evolutionary co-option, where genes acquire new functions without losing their original roles (Theißen & Saedler, 2001).

Fabaceae-Specific Studies on PISTILLATA

The Fabaceae family, which includes species such as *Medicago sativa*, *Glycine max* (soybean), and *Phaseolus vulgaris* (common bean), has been the subject of extensive research due to its economic and ecological significance. Studies have demonstrated that the expression of PISTILLATA in Fabaceae species is not confined to petals and stamens, but also includes carpel tissues (Becker & Theißen, 2003). This suggests that PI may have undergone functional diversification, allowing for greater morphological variation in Fabaceae flowers.

Computational Approaches to Gene Evolution

The use of computational methods has become essential for studying the evolution of genes involved in flower development. Sequence alignment tools like ClustalW, phylogenetic analysis using MEGA software, and structural prediction platforms such as SWISS-MODEL have been instrumental in identifying conserved domains and predicting the evolutionary relationships of floral genes (Zahn et al., 2005). These tools allow researchers to explore the functional divergence of genes like PISTILLATA across plant families.

III. MATERIALS AND METHODS

Gene Identification and Homology Search

The *Medicago sativa* PISTILLATA gene (AIT11843.1) was used as the model sequence for this study. Homologous sequences in other Fabaceae species were identified using BLAST searches in the NCBI database. Sequences with high similarity to AIT11843.1 were selected for further analysis.

Sequence Alignment and Conserved Motif Analysis

Multiple sequence alignment was performed using ClustalW to identify conserved regions across Fabaceae species. Particular attention was paid to the MADS-box and K-box domains, as these are critical for the function of B-class genes in floral development.

Phylogenetic Analysis

A phylogenetic tree was constructed using MEGA software, employing the neighbor-joining method with 1000 bootstrap replicates. The tree was used to infer evolutionary relationships between the PISTILLATA homologs in Fabaceae and related plant families.

Functional Domain and Structure Prediction

InterProScan was used to identify functional domains in the PISTILLATA protein sequences. The SWISS-MODEL server was employed to predict the 3D structure of the *Medicago sativa* PISTILLATA protein. The structure was assessed for reliability using Ramachandran plot analysis, and potential functional sites were identified.

IV. RESULTS

Sequence Alignment and Conserved Regions

The sequence alignment of PISTILLATA homologs from various Fabaceae species revealed high conservation in the MADS-box and K-box domains (**Figure 1**), which are essential for DNA binding and protein-protein interactions. However, variations in the C-terminal region were observed, suggesting functional diversification of PI in Fabaceae.

Phylogenetic Analysis

The phylogenetic tree constructed from the PISTILLATA sequences showed a clear clustering of Fabaceae species, indicating that the gene has been conserved across the family. Bootstrap values supported the monophyletic grouping of PISTILLATA within Fabaceae, and close relationships between species such as *Medicago sativa* and *Glycine max* were observed (**Figure 2**).

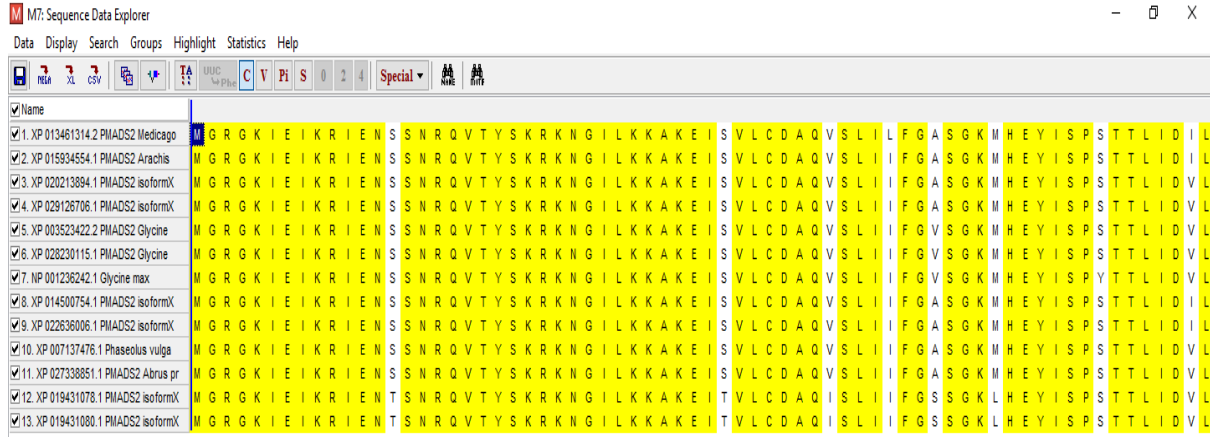


Figure 1: Sequence Alignment using MEGA

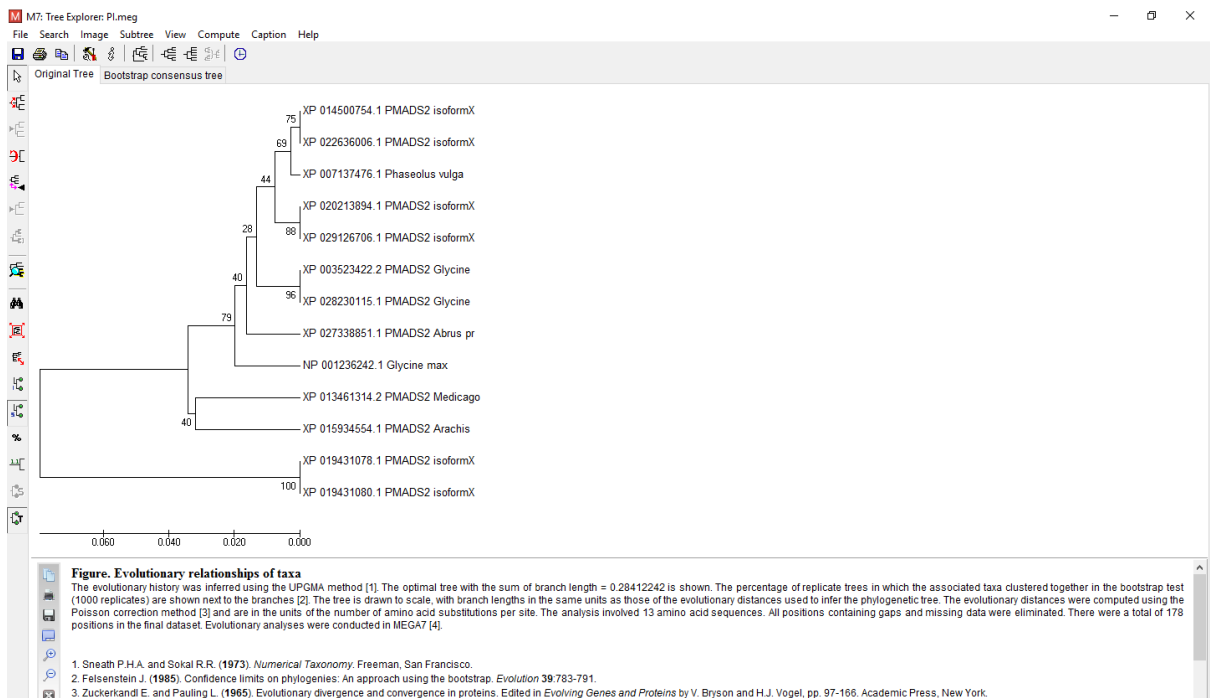


Figure 2: Phylogenetic tree construction using MEGA

Domain Analysis and Structural Prediction

The functional domain analysis confirmed the presence of conserved MADS-box and K-box domains in all PISTILLATA sequences (Figure 3). The 3D structure of Medicago sativa PI was consistent with known MADS-box proteins, with 95% of residues falling within favored regions of the Ramachandran plot (Figure 4). Functional site prediction identified residues critical for DNA binding in the MADS-box domain.

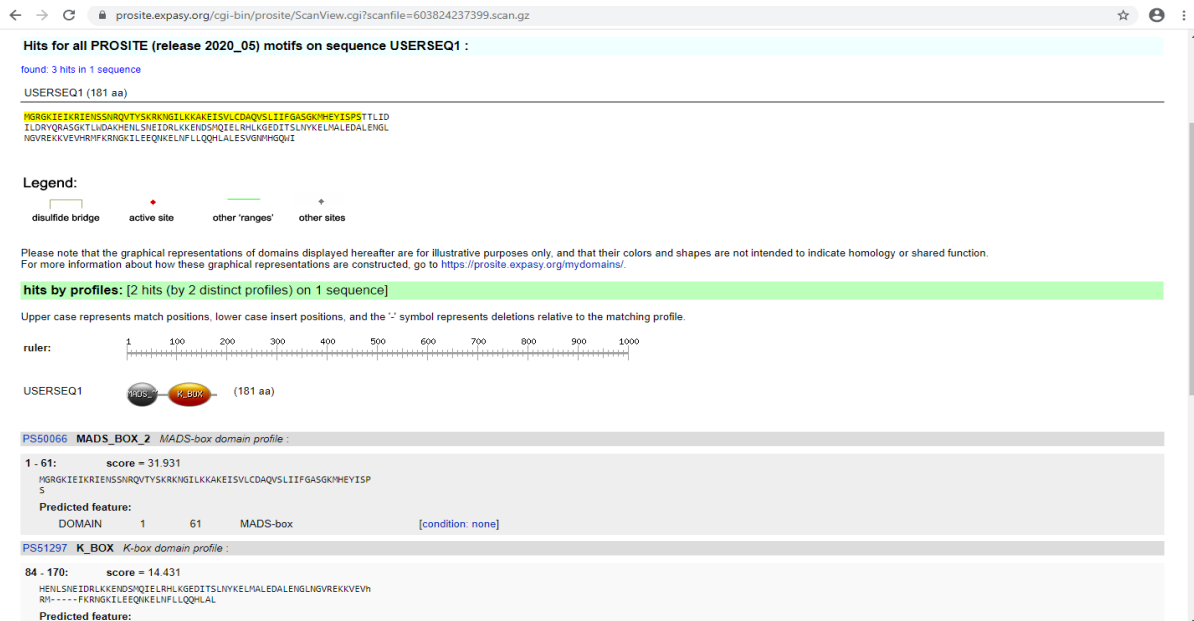


Figure 3: Functional domain analysis using Prosite

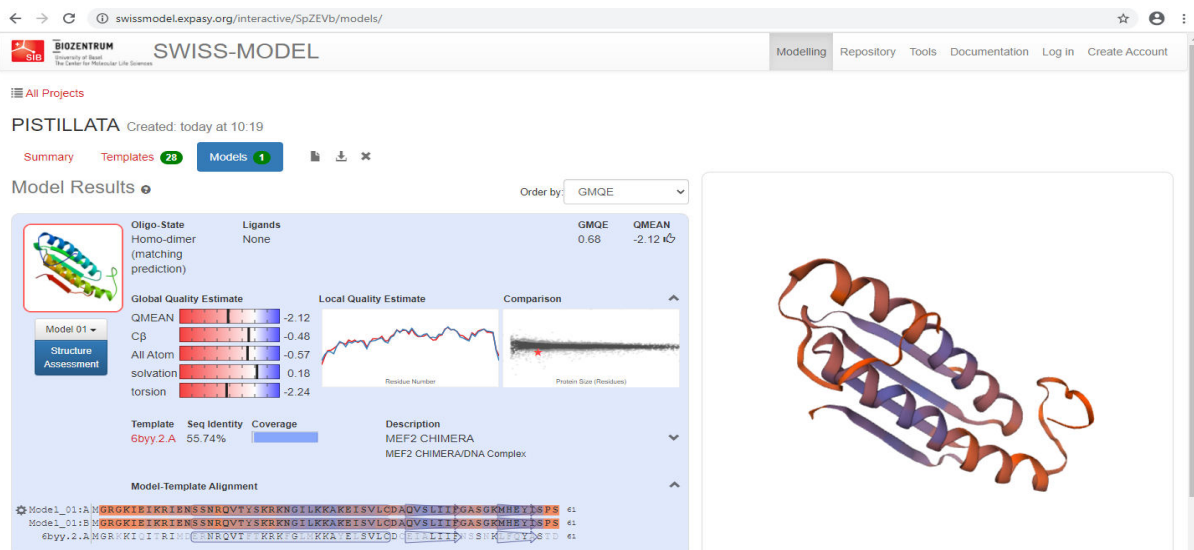


Figure 4: Structure prediction using SWISS-MODEL

V. DISCUSSION

Expanding the Role of PISTILLATA in Floral Development

The findings of this study provide strong evidence for the involvement of PISTILLATA in carpel development (Whorl 4) in Fabaceae. While traditionally considered a B-class gene that regulates petal and stamen identity, PI appears to have acquired new functions related to carpel formation in some species. This dual role of PI in floral organ development expands the classical ABCDE model and suggests that the gene has undergone functional diversification in Fabaceae.

Evolutionary Implications

The high degree of sequence conservation in the MADS-box and K-box domains across Fabaceae species indicates that PISTILLATA has maintained its core function in floral development. However, the observed sequence variations in the C-terminal region suggest that PI has also evolved species-specific functions, possibly related to carpel formation. This diversification may have contributed to the wide range of floral morphologies observed in Fabaceae.

Functional Diversification of B-Class Genes

The expanded role of PISTILLATA in carpel development reflects a broader trend of functional diversification in B-class genes. Gene duplication events and subsequent divergence have allowed B-class genes to acquire new roles while retaining their original functions. In Fabaceae, this has likely played a significant role in the evolution of floral diversity.

VI. CONCLUSION

This study has provided valuable insights into the role of the PISTILLATA gene in carpel development within Fabaceae. Using computational tools, we identified and characterized homologs of the *Medicago sativa* PISTILLATA gene across Fabaceae species, revealing that PI is expressed in Whorl 4 and contributes to carpel formation. These findings challenge the traditional view of B-class genes and suggest that PISTILLATA has undergone functional diversification in Fabaceae. Further research is needed to explore the molecular mechanisms underlying this expanded role of PI in floral development.

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