无患子 *MADS-box* 基因家族全基因组鉴定及 *SmAP1* 基因功能研究

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摘 要：【目的】研究无患子的 *MADS-box* 基因家族的成员及其分类，阐明在其生长发育过程中表达规律及功能，并选取关键基因 *SmAP1* 进行功能验证，以期为无患子开花调控分子机制研究奠定基础。【方法】本研究基于无患子的全基因组数据，鉴定出 *MADS-box* 基因家族并进行综合分析，包括基因结构、保守基序、系统进化、顺式作用元件和蛋白理化性质等。同时，分析 *MADS-box* 在各个营养器官和生殖器官的表达模式，并利用 RT-qPCR 技术进行关键基因表达量的验证。利用 Pearson 相关系数构建开花过程中 *SmMADS* 基因的共表达网络，筛选出关键基因 *SmAP1*，并克隆、构建表达载体，进一步在拟南芥（*Arabidopsis thaliana*）中遗传转化，观察表型差异，初步阐述 *SmAP1* 基因在调控开花时间和花器官发育等方面的功能。【结果】在无患子全基因组数据中共鉴定出 106 个 *MADS-box* 基因成员，基于拟南芥（*Arabidopsis thaliana*）、葡萄（*Vitis vinifera*）、大豆（*Glycine max*）、桃子（*Prunus persica*）、苹果（*Malus pumila*）、毛果杨（*Populus trichocarpa*）和无患子（*Sapindus mukorossi*）等物种的 *MADS* 蛋白，构建系统发育进化树，并将其划分为 Type I 类和 Type II 类两大亚家族。Type I 可分为 Mα、Mβ 和 Mγ 三大亚类，而 Type II 则分为 18 个亚类。顺式作用元件分析其能够响应光周期，激素和环境压力等，在响应非生物胁迫方面具有一定功能。不同亚类的 SmMADS 蛋白具有不同的调控功能。*MADS-box* 基因成员在营养器官（如根、茎和叶）和生殖器官（果皮、种子和雌雄小花），特别是雌雄花器官（如花瓣、雄蕊和子房）等 26 个组织部位的表达差异显著。Type I 型 *MADS* 成员主要在根茎叶营养器官表达，在果实的发育过程的子叶生长期表达显著。Type II 型 *MADS* 成员主要参与调控雌雄花和花器官的发育。RT-qPCR 数据表明，*SmSEP4*、*SmPI*、*SmAP3* 和 *SmSOC2* 基因的表达水平在花序分化起始过程中增加。此外，*SmPI* 和 *SmAP1* 基因在雄蕊、雌蕊和花瓣发育中高度表达。值得注意的是，*SmAP3* 基因在不同性别花雄蕊的小孢子形成过程中发挥着至关重要的作用。本研究成功克隆出 *SmAP1* 基因的编码区全长 726 bp。与野生型相比，35S::*SmAP1* 拟南芥植株的 9 个 T_2 代株系，其抽薹和开花时间均呈现出不同程度的显著提前，且其子房发育更粗壮。RT-qPCR 实验的结果表明，35S::*SmAP1* 拟南芥的花组织中，开花时间相关整合因子（*AtFT* 和 *AtSOC1*）、花器官发育相关（*AtAP1*、*AtAP3* 和 *AtPI*）和开花分生组织调节因子（*AtLFY* 和 *AtCAL*）等表达上调，而开花抑制因子（*AtTFL1*、*AtAGL24* 和 *AtSVP*）的表达下调。【结论】*MADS-box* 基因在雄花和雌花的 8 个生长阶段以及花器官发育过程中表现出不同的表达模式。拟南芥中 *SmAP1* 基因的过度表达导致开花整合子和花分生组织调节子的上调。与野生型植物相比，35S::*SmAP1* 转基因拟南芥表现出不同程度的提早抽苔和开花。该结果为进一步研究无患子开花调控的分子机制奠定了基础。

关键词：*MADS-box* 基因家族；无患子；开花时间；花器官, *APETALA1*

Genome-wide Identification of *MADS-box* Gene Family in *Sapindus mukorossi* and functional study of *SmAP1* gene

Abstract:【Objective】To study the members and classification of the *MADS-box* gene family of *Sapindus*, clarify the expression rules and functions during its growth and development, and select the key gene *SmAP1* for functional verification, in order to provide a regulatory molecule for *Sapindus* flowering. foundation for mechanism research. 【Method】Based on the genome-wide data of *Sapindus*, this study identified the *MADS-box* gene family and

conducted a comprehensive analysis, including gene structure, conserved motifs, phylogenetic evolution, prediction of *cis*-acting elements, protein properties and protein interaction networks of *SmMADS* genes. At the same time, the expression patterns of *MADS-box* in various vegetative organs and reproductive organs were analyzed, and RT-qPCR technology was used to verify the expression of key genes. The co-expression network of *SmMADS* genes during the flowering process was constructed by using Pearson correlation coefficient, the key gene *SmAP1* was screened out, and the expression vector was cloned and constructed, and further genetically transformed in *Arabidopsis thaliana* to observe the phenotypic differences and preliminarily elucidate the *SmAP1* gene functions in regulating flowering time and floral organ development.【Result】A total of 106 *MADS-box* gene members were identified in the whole genome data of *Sapindus mukorossi*. Based on *Arabidopsis thaliana*, grape (*Vitis vinifera*), soybean (*Glycine max*), peach (*Prunus persica*), apple (*Malus pumila*), poplar (*Populus trichocarpa*) and *Sapindus mukorossi* MADS protein data to construct a phylogenetic tree, and they were divided into two subfamilies, Type I and Type II. Type I can be divided into three subclass, Mα, Mβ, and Mγ, while Type II is divided into 18 subclass. Analysis of *cis*-acting elements can respond to photoperiod, hormones and environmental stress, and has certain functions in regulating abiotic stress. Different subclasses of *SmMADS* proteins have different regulatory functions. The expression of *MADS-box* gene members was significantly different in 26 tissue parts including vegetative organs (such as roots, stems, and leaves) and reproductive organs (pericarp, seeds, and male and female florets), especially male and female floral organs (such as petals, stamens, and ovaries). Type I *MADS* members were mainly expressed in the vegetative organs of roots, stems and leaves, and were significantly expressed in the cotyledon growth stage of fruit development. Type II *MADS* members are mainly involved in regulating the growth of male and female flowers and the development of floral organs in *Sapindus*. Fluorescence quantitative data indicated that the expression levels of *SmSEP4*, *SmPI*, *SmAP3*, and *SmSOC2* were increased during the initiation of inflorescence differentiation. Additionally, *SmPI* and *SmAP1* were highly expressed in the stamens, pistils, and petals development. Notably, *SmAP3* played a vital role in the microspore formation process of stamens of different gender flowers. In this study, the full-length 726 bp coding region of *SmAP1* gene was successfully cloned. Compared with the wild type, the bolting and flowering time of the 35S::*SmAP1 Arabidopsis* plants of the nine T2 lines were significantly earlier in different degrees, and the ovaries were thicker. The results of RT-qPCR experiments showed that in the floral tissue of 35S::*SmAP1 Arabidopsis thaliana*, flowering time-related integration factors (*AtFT* and *AtSOC1*), floral organ development-related factors (*AtAP1*, *AtAP3* and *AtPI*) and flowering meristem regulators (*AtLFY* and *AtCAL*) were up-regulated, while flowering inhibitors (*AtTFL1*, *AtAGL24* and *AtSVP*) were down-regulated.【Conclusion】The *MADS-box* genes exhibited different expression patterns during the eight growth stages of male and female flowers, as well as floral organ development. The overexpression of the *SmAP1* gene in *Arabidopsis thaliana* results in the upregulation of flowering integrators and floral meristem regulators. Compared to the wild-type plants, the 35S::*SmAP1* transgenic *Arabidopsis* showed varying degrees of early bolting and flowering. This result has laid the foundation for further research on the molecular mechanisms underlying flowering regulation in *Sapindus*.

Key words: *MADS-box* gene family；*Sapindus mukorossi*；flowering time；floral organ；*APETALA1*