

胡桃科植物花发育研究进展

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摘要:胡桃科植物作为重要的木本油料作物和坚果,具有雌雄同株异花特性。其中核桃属和山核桃属果实具有较高的经济价值,但在实际生产中树体雌、雄花比例失衡,雄花过多,雌花太少,严重制约了果实产量,影响其经济效益。总结了胡桃科植物雌、雄花发育的形态特征,对内源激素、营养物质、矿质元素和位置效应对花芽分化的影响及成花诱导、花发育模型相关基因功能研究进行了综述,以期对胡桃科植物雌、雄花分化机制和果实优质高产栽培研究提供参考。

关键词:核桃;山核桃;花芽分化;成花基因;花器官发育

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Research progress on flower development of Juglandaceae plants

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Abstract: Juglandaceae plants are monoecious and dioecious, and they are major woody oil crops and nuts. *Juglans* and *Carya* fruits are highly valuable economically; however in practice, the ratio of male to female flowers on the tree is out of balance, with an excessive number of male flowers and a deficiency of female flowers. This phenomenon could significantly reduce the tree's economic yield and benefit. The effects of the physical characteristics of male and female flower growth, location, and endogenous hormones on flower bud differentiation, as well as the function of genes linked to floral induction and a flower development model, were reviewed in this paper. Most Juglandaceae plants have unisexual blooms, which typically open once a year. Nonetheless, a few early-bearing *Juglans regia* varieties can produce a large number of secondary flowers and fruits in a unique environment that allows for late-autumn blossoming. The female flower bud differentiation process can be separated into four stages: the emergence stage of the female flower primordium, the formation stage of the involucre and perianth, and the formation stage of the pistil primordium. The flower bud differentiation process can take up to a year. The undifferentiated stage of the male flower, the differentiation stage of the male inflorescence, the differentiation stage of the male flower primordium, the differentiation stage of the stamen primordium, and the formation stage of the anther and pollen grain are the main divisions of the male flower differentiation period. Furthermore, it is possible to determine the stage of flower bud development by looking at the outward morphological traits of the buds, which is useful for managing blooming and floral induction. Numerous plant hormones, including CTK, GA, ABA, IAA, and PAs, significantly impact the differentiation of flower buds. GA promotes the development of male flowers, and spraying proper concentration of PAs can increase the amount of female flower buds. The balance of the endogenous hormones may be more significant for the flower bud differentiation in Juglandaceae plants, even

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if a single exogenous plant growth regulator may control the quantity of male and female flower buds to some extent. It is well known that the material foundation for flower bud differentiation is carbon and mineral nutrition. During the physiological differentiation stage, the soluble sugar concentration rises first in female flower buds. The potassium fertilizer applied during production aids in the differentiation of flower buds, and girdling, branch ringing and root cutting can all be used to control C/N and induce flowering. Plants have developed sexual and reproductive allocation mechanisms over their lengthy evolutionary history. There is conjecture that male flower buds situated at the base of walnut plants, which differentiate early, would prefer obtaining greater nutritional resources. Consequently, there would be significantly more male flower buds than female ones. The *LFY*, *FT*, *CO*, and *API* in the flowering pathway may have favorable effects on the female flower formation in Juglandaceae plants, and the MADS-box family also has a significant impact on flower development, according to research on the flower development of the model plant *Arabidopsis thaliana*. Up to 77 members comprise the MADS-box family of *J. regia*, and some MADS-box genes are strongly expressed in floral organs. Following their heterologous transformation into *A. thaliana*, the *JrAG*, *CiMADS9*, and *CcAGL24* underwent a considerable alteration in their floral phenotype. Genes that are homologous to MADS-box have a variety of regulatory roles. For instance, functional distinctions exist between the homologous genes *CcAGL24a* and *CcAGL24b* in *Carya cathayensis* when controlling carpel formation. *A. thaliana* plants that overexpress *CcAGL24b* have longer carpels, larger sepals, and depression, whereas plants that overexpress *CcAGL24a* have shorter, thicker carpels. The lncRNAs are a type of epigenetics that regulate plant growth and development but do not encode proteins. Analysis was made using the *J. regia* female flower bud and leaf bud development miRNA-seq database. It was shown that female flower buds had larger levels of differentially expressed miRNAs than leaf buds. It was discovered that the target gene of miR156/157 was the flower-promoting gene *SPL*. The current lack of a complete understanding of the genetic system of Juglandaceae plants restricts the study of linked genes' functional properties. We will keep working to find a solution for the genetic transformation system in the future. When combined with additional biological techniques, it should be possible to precisely induce more female flowers, uncover the essential genes for determining the sex of flowers, and enhance the regulatory network map of the blooming pathway.

Key words: *Juglans*; *Carya*; Flower bud differentiation; Flowering gene; Floral organ development

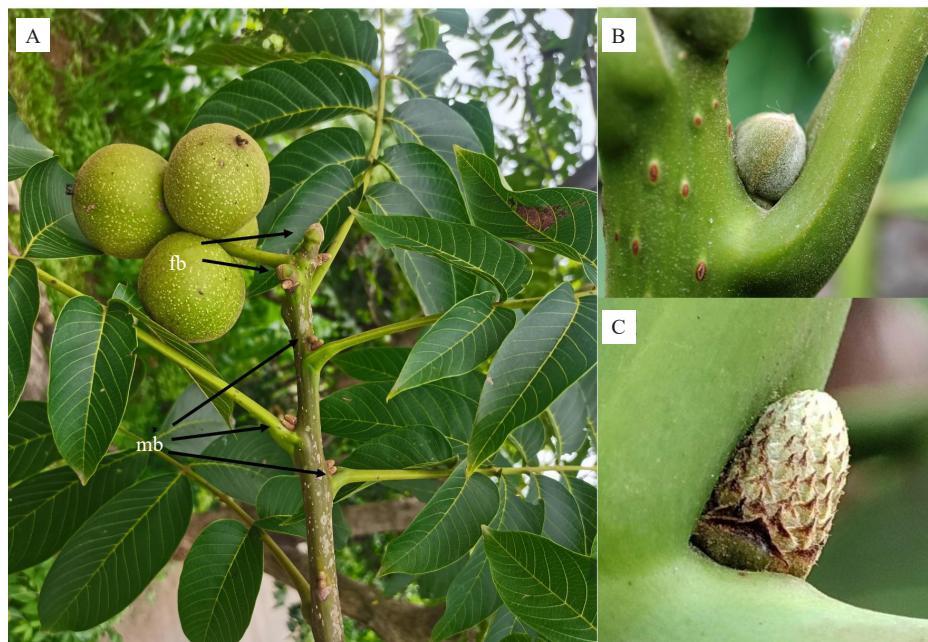
胡桃科(Juglandaceae)植物共有8个属,约60余种,中国产7属25种^[1],其中,山核桃属(*Carya* Nutt.)和核桃属(*Juglans* L.)植物分布范围广、经济价值高,研究利用深入。胡桃科植物雌雄同株,雄花量大,远远超出授粉需求,雄花发育消耗大量树体营养,成为限制核桃和山核桃(胡桃科植物)产量的主要因素^[2-3]。自20世纪60年代,胡桃科植物花发育研究引起广泛关注,前后历经两个发展阶段,实现了从生理形态研究到成花基因的功能研究及调控的跨越,取得了系列成果。同时,生产中为获得优产高质的果实,在胡桃科植物的成花、雌雄比调控等方面也开展了研究,发现通过人工疏雄、环剥、拉枝等措施可有效增加果实产量,提高经济效益。

笔者在借鉴模式植物拟南芥等植物的花发育研究基础上,从胡桃科植物花发育特征、花芽分化及其影响因素、花器官发育的基因调控模型三个方面总结概述了现有研究成果,以期为继续研究胡桃科植物花发育的分子调控机制和分子育种提供借鉴,也为其他雌雄同株植物花发育研究提供参考。

1 胡桃科植物花发育特征

1.1 胡桃科植物雌雄花的形态特征及着生位置

胡桃科植物的花多为单性花,雌雄同株(图1)。雌花单生或呈穗状着生于结果枝的顶端1~3芽(图1-A~B),雌花芽为叶芽和花芽的混合芽^[4-5]。雄花芽为纯花芽(图1-C),除结果枝顶端外,其余叶



fb. 雌花芽; mb. 雄花芽。

fb. Female flower bud; mb. Male flower bud.

图1 泡核桃雌雄花芽着生位置(A)及雌花芽(B)和雄花芽(C)形态特征

Fig. 1 Location of male and female flower buds (A), and morphological characteristics of female flower buds (B) and male flower buds (C) in *J. sigillata*

腋处多有雄花芽分布,于早春开始萌动,伸长成为柔荑花序,每花序着生100~150朵小花,呈螺旋状排列,花序长多为8~12 cm,偶有20~25 cm。胡桃科植物一般每年开花一次,但部分早实型核桃如香玲、温185在晚秋出现适宜开花的特殊气候时具有二次开花结实的特性,其二次花类型丰富,有单性花、雌雄同序(基部着生雌花,上部为雄花)、雌雄同花(多出现在雌雄同序的中部,呈过渡状态)和两性花(中间柱头细弱,外围着生几对花药)等类型^[6]。

1.2 雌雄异熟特性

胡桃科植物虽为雌雄同株,但同株树上雌花的开花与雄花的散粉时间常常不能重合,这种特性被称为雌雄异熟^[7]。胡桃科植物主要有雌先型、雄先型和雌雄同熟型3种类型,其中雌先型和雄先型较为常见,核桃温185雌花先于雄花开放,为雌先型品种;新新2号雄花先于雌花开放,为雄先型品种^[8];而雌雄同熟型较少,一般表现为雌花和雄花各自同步成熟而又互不干扰^[9],但存在部分同熟性^[10]。此外胡桃科雌雄花芽从开始分化到开花所需时间不同,雄花发育期远长于雌花^[11]。研究发现雌先型品种的雌花分化先于雄先型品种,各个分化阶段均具有领先优势,从而为雌花的提早开放奠定了基础^[12]。植

物雌雄异熟可有效避免植物自身的花粉和柱头间的性别干扰,即避免自花授粉现象,防止自交衰退,从而保证物种的长期延续及增强物种的适应性^[13-15],但核桃中雌雄异熟产生机制的相关研究仍非常欠缺,其异熟性研究多用于指导授粉树的配植。

目前对胡桃科植物雌雄异熟特性的稳定性存在争议。杨文衡等^[16]认为雌雄异熟现象受物种、品种、树龄、地区及年份等因素的影响,异熟程度与气候变化存在密切联系,如冷凉气候利于雌蕊先熟,反之则利于雄蕊先熟。张毅萍等^[17]则认为早春气候变化仅影响个别植株雌雄花期的迟早,同一物种的开花顺序是相对稳定的。张志华等^[11]对不同生态条件下同品种核桃的花期调查发现,生态条件差异可导致各地花期相差2周,但雌雄异熟特性相对稳定,对美国山核桃的研究也得到了相同结果^[18]。不同年份间气候条件的变化可改变开花时间,但雌先型与雄先型的次序均无变化^[11, 18]。地区及年份间气候差异所导致的花期不稳定会影响品种的授粉受精效率,但该方面的相关研究仍需深入开展。

由此可见,气候条件的变化对胡桃科植物的花期影响较大,而雌雄异熟顺序基本不会改变。花芽

分化阶段雌雄花芽分化的异步性是异熟性形成的基础^[9,19]。

2 胡桃科植物花芽分化及其影响因素

植物花芽分化即茎尖分生组织(shoot apical meristem, SAM)由叶芽分生组织细胞向花(序)分生组织(inflorescence meristem, IM)的转变,在花序分生组织的周边产生花分生组织(floral meristem, FM),后分化成各轮花器官^[20-21]。植物雌雄花芽分化经历生理分化期、形态分化期和性细胞形成期三个阶段,生理分化期又称成花诱导期,是茎尖分生组织的生理状态向花分生组织的生理状态逐步转化的过程;形态上可以看出花或花序原基时即为花芽形态分化的开始,花芽中性细胞形成不再继续分化新的花器官,标志着花芽分化结束^[22]。

2.1 胡桃科植物花芽分化时期

胡桃科植物花芽分化时间长达一年之久,雌花花芽分化总体可划分为雌花分化初期、雌花原基出现期、总苞及花被形成期和雌蕊原基形成期(图2)^[23-24],其中可见花序原基生长锥标志着花芽分化的开始,随后生长锥顶端突起发育为小花原基,苞片、

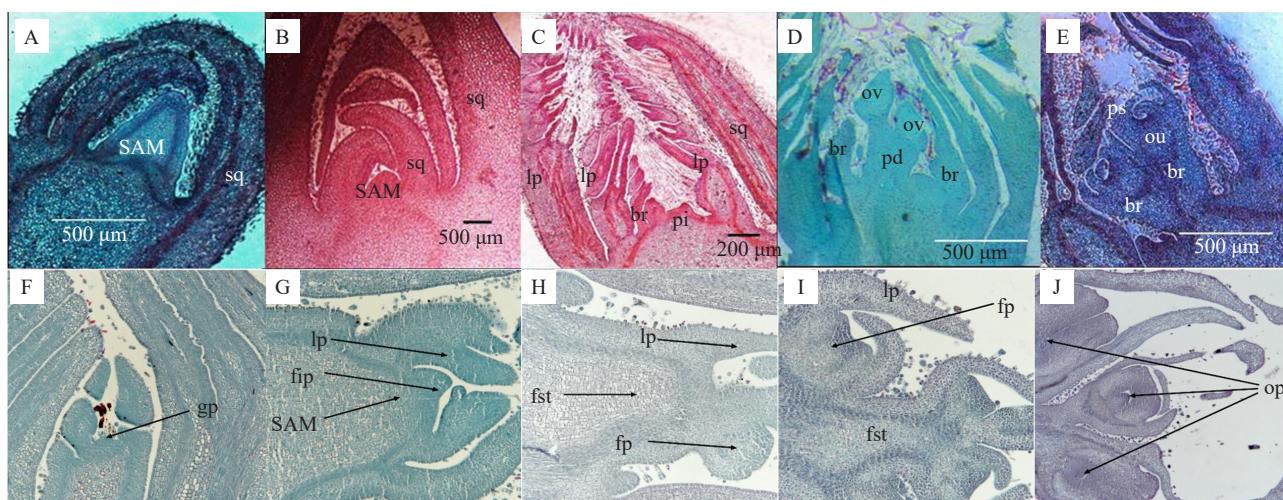
花被及雌蕊原基的形成代表雌花芽分化完成^[25-26]。较为特殊的早实核桃两性花分化在雌蕊原基形成期有所不同,前期花芽会分化出雄蕊原基,后期雄蕊败育消失^[6]。因品种、地理位置和气候条件等差异,雌花分化的时间点也有所不同,胡桃科植物在中短枝停长后3~7周陆续进行雌花芽分化,该时期为生理分化期,是调控雌花分化的关键时期,随后在4~10周进行各花器官的形态分化^[27-28]。

雄花芽与侧叶芽为同源器官,经过冬季休眠,植株开始萌芽,新梢上部分侧芽原基进入雄花芽分化状态,待植株新梢停长时,其芽顶部形态呈现不明显的鳞片状,与此同时,上一年的休眠芽开始萌芽、膨大后散粉(图3)。

整个花发育过程约13个月^[22,27],分化时期可大致分为雄花未分化期、雄花序分化期、雄花原基分化期、雄蕊原基分化期、花药和花粉粒形成期^[29-30](图4)。

2.2 花芽分化外部形态与内部结构的关系

花芽分化的内外结构特征关联性分析有助于从芽的外部形态特征判断花芽发育阶段,便于花期管理和成花诱导^[26,31-32]。胡桃科植物在成花诱导期时,

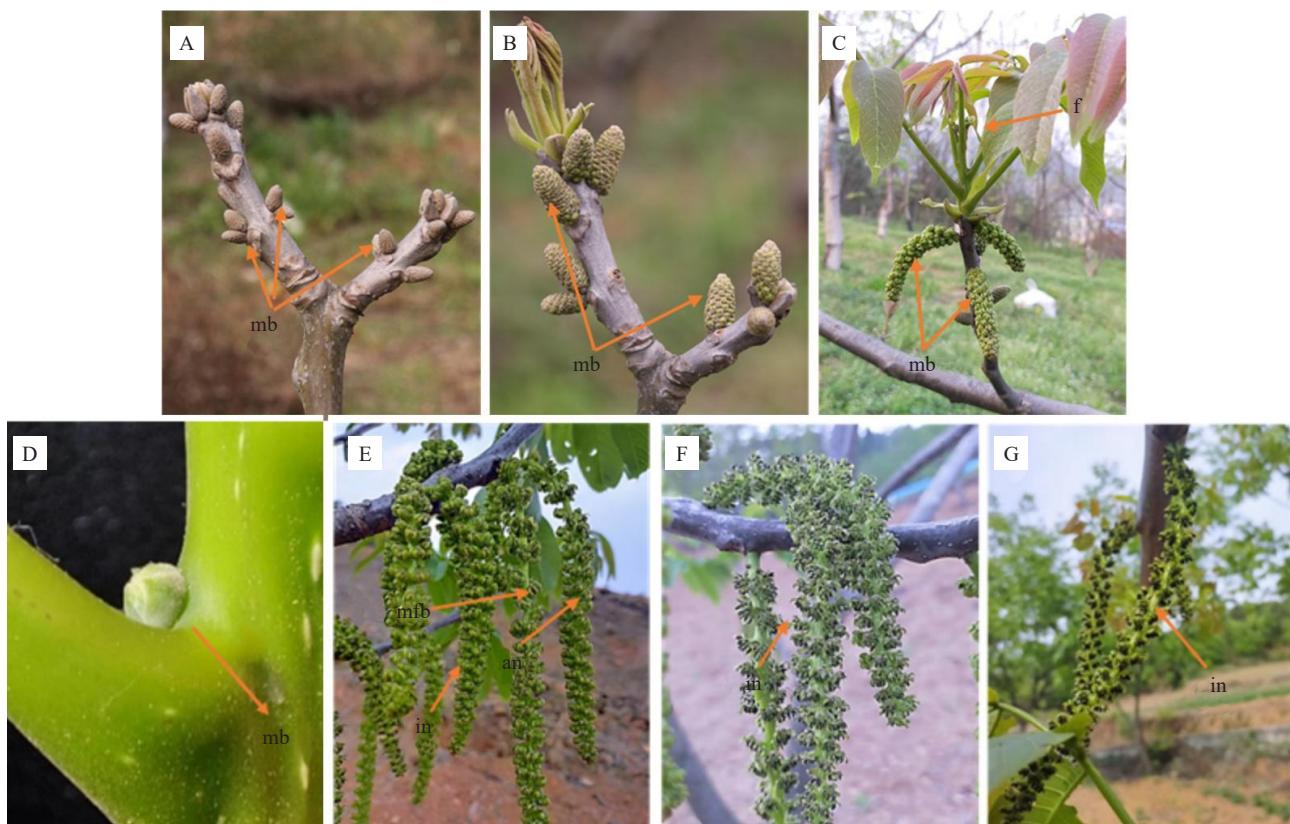


A 和 F. 花芽未分化期;B 和 G. 雌花分化初期即生理分化期;C 和 H. 雌蕊原基及苞片出现;D 和 I. 子房分化;E 和 J. 雌蕊原基形成期;SAM. 生长锥;sq. 鳞片;gp. 生长点;lp. 叶原基;fip. 雌花花序原基;pi. 雌花原基;br. 苞片;fst. 花柄;fp. 花原基;pd. 花梗;ov. 子房;ou. 胚珠;op. 胚珠原基;ps. 雌蕊原基。

A and F. Flower bud undifferentiated stage; B and G. The initial stage of female flower differentiation is the physiological differentiation stage; C and H. Pistil primordium and bracts appear; D and I. Ovary differentiation; E and J. Pistil primordium formation stage; SAM. Growth cone; sq. scales; gp. Growth point; lp. Leaf primordium; fip. Inflorescence primordium of female flower; pi. Female flower primordium; br. Bracts; fst. Petiole; fp. Flower primordium; pd. Pedicel; ov. Ovary; ou. Ovule; op. Ovule primordium; ps. Pistil primordium.

图2 泡核桃(A~E)和薄壳山核桃(F~J)雌花芽在发育过程中的组织结构^[24-25]

Fig. 2 The organizational structure of female flower bud development process of *J. sigillata* (A-E) and *Carya illinoensis* (F-J)^[25-26]

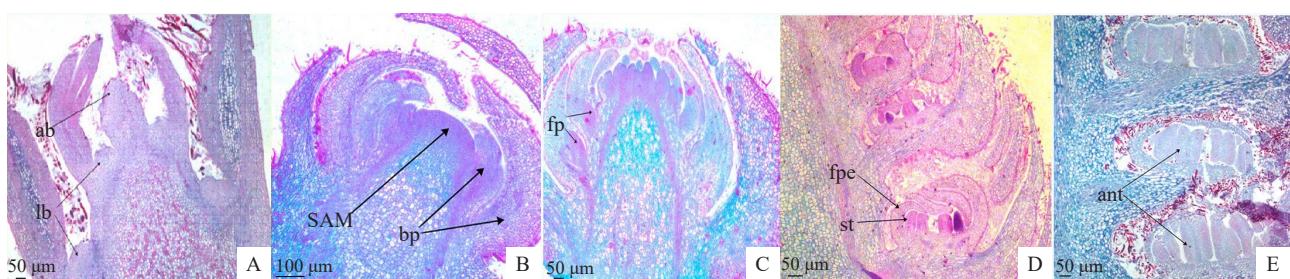


A. 休眠期;B. 萌芽期;C. 膨大期;D. 新梢上当年分化的雄花芽;E. 散粉初期;F. 散粉期;G. 散粉结束。mb. 雄花芽;f. 雌花;mfb. 花蕾;an. 药;in. 花序。

A. Dormant period; B. Germination period; C. Expansion period; D. Male flower buds differentiated in the new shoots; E. Early stage of powder scattering; F. Powder scattering period; G. Powder ends. mb. Male flower bud; f. Female flower; mfb. Flower bud; an. Anther; in. Inflorescence.

图3 泡核桃雄花发育历程^[10]

Fig. 3 Development process of *J. sigillata* male flower^[10]



A. 花芽未分化期;B. 雄花序分化期;C. 雄花原基分化期;D. 雄蕊原基分化期;E. 花药和花粉粒形成期;ab. 顶芽;lb. 侧芽;SAM. 生长锥;bp. 花被原基;fp. 花原基;fpe. 雄花萼片;st. 雄蕊;ant. 花药。

A. Flower bud undifferentiated stage; B. Male inflorescence differentiation stage; C. Male flower primordium differentiation stage; D. Stamen primordium differentiation stage; E. Anther and pollen grain formation stage; ab. Apical bud; lb. Lateral bud; SAM. Growth point; bp. Perianth primordium; fp. Flower primordium; fpe. Male sepals; st. Stamens; ant. Anthers.

图4 早实核桃雄花芽在发育过程中的组织结构^[28]

Fig. 4 The organizational structure of the development process of male flower buds in precocious *J. regia*^[28]

雌花芽外部表现为芽较小,呈扁三角形,颜色为鲜绿色,鳞片3~5枚,内部结构为顶端分生组织逐渐变平,幼叶原基出现;随后芽体变得圆润饱满,鳞片增为6~8枚,内部伸长的顶端分生组织变得扁平,且两

侧突起形成苞片原基;花被分化时,雌花芽变成灰绿色,鳞片为10~12枚且逐渐张开;最后在雌蕊分化期,雌花芽再次增大,鳞片脱落,幼叶展开^[26,31-32]。

雄花序分化期芽为浅绿色,苞片分化期芽体呈

塔形,花序顶部呈馒头状,翌年植株新梢生长,雄花序持续分化,在两侧形成雄蕊原基,雄花的葇荑花序随后产生花药,花药由绿变黄,花粉散出,散粉完成后,花药变黑,雄花花柄处产生离层并脱离(图3)^[30]。

2.3 胡桃科植物花芽分化的影响因素

2.3.1 内源激素与花芽分化 植物激素作为植物生长发育的诱导信号,在胡桃科植物花芽分化中起重要的调控作用。前人研究发现胡桃科休眠期雌花芽中存在较高含量的赤霉素(GA)和脱落酸(ABA)^[33-35],雌花诱导期GA和ABA含量逐渐降低^[34-35],生长素(IAA)含量处于较低的水平,细胞分裂素(CTK)、精胺(Spm)和亚精胺(Spd)含量较高,推测其有利于雌花原基的形成^[34,36],后期较高的腐胺(Put)含量则有利于苞片和花被分化^[36]。与之相反,雄花芽分化初期顶端分生组织积累高含量的IAA和GA来诱导雄花(序)分化^[31,35],雄花芽分化阶段ABA含量呈先下降后上升的趋势,生理分化期最低,推测低浓度的ABA促进雄花芽分化^[37],但随着雄花器官的形成,ABA含量则保持在较高的水平。生产中可通过喷施植物生长调节剂来调控胡桃科植物雌雄花芽的比例,如施用10 mg·L⁻¹油菜素内酯和40 mg·L⁻¹氨基嘌呤可提高核桃的雌花数和果实数^[38];在生理分化期喷施10³ mol·L⁻¹ Put和10⁴ mol·L⁻¹ Spd可增加雌花芽数量^[39-40],减少雄花芽数量,提高雌雄比5.6~6.7倍,达到抑雄促雌的效果^[41-42]。而喷施40 mg·L⁻¹棉氨基嘌呤或适宜浓度的GA则利于雄花形成^[38,43]。

尽管单一外源植物生长调节剂可在一定程度上调控雌雄花芽数量,但内源激素之间的平衡对胡桃科植物花芽分化的影响可能更加重要,研究发现高比值的IAA/ABA、IAA/GA₃、ABA/GA₃更有利于串核桃的雌花芽分化^[36]。常规内源激素含量的检测是成千上万个细胞的平均水平,测定组织范围较宽泛,难以精准检测激素发挥作用的位置,因此原位组织学分析更有利了解内源激素对花芽分化的诱导作用。

2.3.2 营养物质与花芽分化 早在1918年Kraus等^[44]就提出了成花的C/N学说,认为只有当植物体内碳水化合物的积累比含氮化合物在数量上占优势时植物才能开花。时至今日,碳素营养和矿质营养作为花芽分化必需的物质基础已得到普遍认可。研究表明,已成花的湖南山核桃叶片中可溶性总糖、淀粉、碳水化合物含量及碳氮比都比未成花的要高得多^[45];串核桃雌花分化进程中伴随着高含量的可溶

性糖积累^[46];泡核桃花芽分化前碳水化合物以淀粉的形式积累,在雌花芽生理分化期,可溶性糖含量明显提高^[26];在薄壳山核桃上也观察到了相同的现象^[47],上述研究均表明了碳水化合物在胡桃科植物花芽分化中起重要作用。除碳水化合物外,矿质营养在核桃科植物花芽分化过程中也发挥重要作用。在山核桃和泡核桃雌花生理分化期前,植物叶片中易移动的矿质元素N、P、K含量呈快速下降的趋势,不易移动元素Ca和Mg含量快速上升^[48-49],从生理分化期到形态分化期,K含量达到峰值;Ca和Mg在此期间消耗量大于积累量,致使其含量降低,可能参与了花器官的建成^[49];在泡核桃雌花芽生理分化期杂环、芳香和疏水氨基酸含量增加^[26]。因此,生产中增施钾肥有助于花芽分化,也可通过环剥、环割、圈枝、控水和断根等方法调控C/N,促进成花^[50]。但截至目前,营养物质调控胡桃科植物花芽分化的作用机制仍缺乏深入系统的研究,其信号途径、调控通路及关键调控基因需进一步挖掘和研究。

2.3.3 位置效应与花芽分化 在自然生长环境条件下,树体自身产生的资源是有限的,因此,植物长期进化出了繁殖分配和性分配策略。繁殖分配是指不同器官间的资源分配存在差异,在适宜条件下,资源优先供给花、果和种子等器官,保障生殖器官发育,确保植物顺利繁殖,完成生命历程。而性分配则是指资源供应在雌雄同株植物的雌雄花分化中的分配差异,雌雄花芽的分化时间差异及在树体上的位置差异明显影响资源分配,成为性分配差异的主要原因之一^[51]。而雌雄花芽间分配存在权衡^[52],如增加雌性功能的投入必定伴随着雄性功能的投入减少^[53-54],反之亦然。胡桃科植物雌雄花着生位置不同,雄花分化早,位于枝条中下部,雌花芽仅着生于结果枝顶端1~3芽,分化晚于雄花芽,因此在自然分配条件下,分化早且位于基部的雄花芽优先获取更多的营养资源;这种性分配的位置效应由物种遗传所决定,也受环境条件和栽培手段的调控,生产栽培中通过施肥、疏雄、拉枝等方式以保证充足的营养资源或调控资源分配,从而改变雌雄比例^[55-56]。

2.3.4 胡桃科植物的成花诱导基因及其功能 植物的成花诱导是一个复杂的生理过程,是多基因协同表达调控的结果,目前在拟南芥等模式植物上已绘制出初步的调控网络^[57]。植物将不同内源激素和外源环境变化转化为成花诱导信号,最终通过春化途

径、温度途径、光周期途径、赤霉素途径、自主途径和年龄途径将开花信号汇集到少数成花基因 *SOCI* (*SUPPRESSOR OF OVEREXPRESSION OF COI*) 和 *FT* (*FLOWERING LOCUS*) 上^[58], 它们激活 *CAL* (*CAULIFLOWER*)、*LFY* (*LEAFY*)、*API* (*APETALA1*)、*SEPALLATA3* (*SEP3*) 和 *FRUITFULL* (*FUL*) 等花分生组织特征基因的表达^[59-60], 使拟南芥茎尖分生组织转变为花分生组织。

迄今为止, 已有的研究结果尚无法证明胡桃科植物的成花诱导途径与拟南芥等模式植物相似, 但目前利用组学手段挖掘到的胡桃科植物的成花基因及其表达模式与拟南芥相符。研究表明, 胡桃科植物的花芽中 *LFY* 和 *API* 的表达量最高, 调控其由营养生长向生殖生长转变; 伴随雌花芽分化, JrCO (*CONSTANS*) 蛋白通过与 JrNF-YB (NUCLEAR FACTOR Y) 蛋白、JrNF-YC 蛋白结合形成三聚复合蛋白来结合 *JrFT* 启动子^[61-62], 进而调控 *JrFT* 基因的转录表达, 使 *FT* 基因的表达量逐渐上升, 对成花起正调控作用^[63-64]; 胡桃科植物芽休眠结束时, 抑花基因 *TFL1* (*TERMINAL FLOWER1*) 表达下调, 成花基因 *FT* 表达上调, 进而激活 *SOCI*, 促进下游基因 *CAL*、*LFY* 和 *API* 的表达, 从而诱导胡桃科植物开花^[22,65]。在拟南芥中过表达核桃 *LFY*、*FT* 基因后, 植株提早开花, 推测其正向调控核桃成花^[66-67]; 山核桃的 *SVP* (*SHORT VEGETATIVE PHASE*) 基因和核桃的 *FLC* (*FLOWERING LOCUS C*) 基因异源转化拟南芥后, 过表达植株花期延迟, 推测其抑制胡桃科植物的成花^[68-69], 且 *SVP* 的过表达植株还有花瓣缺失、雄蕊减少等表型, 说明 *CcSVP* 对植株的花型和花期均有着重要的影响; 蛋白质互作试验发现 *CcSVP* 与 *CcAPI*、*CcSOCI* 间存在互作^[68-69]。

但由于胡桃科植物多为木本植物, 基因功能研究受童期长、再生困难、遗传转化体系不成熟等众多因素限制, 其成花诱导分子调控网络仍不清楚, 而童期决定机制和雌雄花芽分化差异机制的研究和阐述有助于揭开胡桃科植物的成花奥秘, 为早实品种和高产品种选育奠定理论基础。

3 胡桃科植物的花器官分化及其特征基因

3.1 花发育模型

随着花分生组织的不断发育, 花器官特征基因

(ABCDE类基因)经历时空差异表达, 形成不同的花器官。在“ABCDE”花发育模型中, A+E基因共同调控萼片分化; A+B+E基因调控花瓣形成; B+C+E基因调控雄蕊发育; C+E基因可以调控心皮的发育; 胚珠的发育由B、C、D、E四类基因共同调控^[70], E类基因参与每轮花发育过程中花器官的形成, 并与A、B、C类基因组成“四聚体模型”的复合体^[71]。花器官特征基因除A类基因的AP2是ERF家族外, 其余基因及成花途径中的*FLC*、*SVP*、*SOCI*等基因均为MADS-box转录因子, 利用转录组测序分析山核桃不同发育阶段雌雄花差异基因的表达, 发现多个与花性别分化相关的MADS-box在雌、雄花中高表达, 表明该基因家族在花器官的发育与分化中发挥了重要作用。

3.2 MADS-box基因家族调控胡桃科花器官发育

MADS-box家族成员不仅是开花调控的关键转录因子, 也是花器官发育中重要的决定基因。根据系统进化关系、基因结构和蛋白结构的不同, MADS-box基因在现存的动物、植物和真菌中被分为I型(Type I)和II型(Type II)两大类型。在植物中MIKC^c型基因和MIKC^{*}型基因一起构成II型MADS-box基因^[72]。对被子植物中的MIKC^c型MADS-box基因进行的系统发育分析, 将MADS-box基因主要分为12个主要的亚家族: AGL6、SEP、AP1、SOC1、AGL12、AP3/PI、SVP、AGL15、AGL17、BS、FLC和AG亚家族^[72]。因为花器官“ABCDE”模型的提出, 所以这些亚家族中主要以A类AP1、B类AP3/PI、C类AG、E类SEP四个亚家族在拟南芥花器官研究中最为深入^[72]。目前已对山核桃、核桃、核桃楸基因组中的MADS-box家族成员进行了鉴定分析(表1), 其中核桃基因组中的MADS-box家族成员数量最多, 为77个^[35,72]。

AP1属于A类花器官特征基因, 决定花分生组织的形成及萼片、花瓣花器官原基的发生, 与B类基因相互作用调控第二轮花器官发育^[73]。B类基因包含AP3和PI两个亚家族, 主要负责花瓣和雄蕊的发育。AP3亚家族中有AP3和TM6两个分支。存在两个基因分支的物种中, *AP-3like*和*TM6-like*基因功能出现分化, *TM6-like*基因只参与调控雄蕊的发育^[74]。山核桃 *CcAP3-1*、*CcAP3-2* 在雌雄花中都有表达, 但两者的转录本异构体并不相同, 推测其分别响应不同途径的信号而调控雌雄花不同的成花途

表 1 胡桃科 *MADS-box* 基因家族的基因个数
Table 1 Number of genes in *MADS-box* gene family of Juglandaceae

胡桃科 Juglandaceae	总数 Total numbers	I型 Type I	II型 Type II	AP1	AP3	AG	SEP	参考文献 References
山核桃 <i>C. cathayensis</i> S.	66	16	50	7	3	3	4	[72]
薄壳山核桃 <i>Caraya illinoiensis</i> K.	33	3	30	6	2	2	4	[65]
核桃楸 <i>J. Mandshurica</i> M.	67	19	48	2	3	3	2	[72]
核桃 <i>J. regia</i> L.	77	25	52	6	5	3	5	[72]

径^[72]。AG是目前报道最为广泛的C类基因,调控雄蕊和心皮原基的发育。山核桃中 *CcAG-1* 基因在雄花中不表达,推测该基因可能仅与山核桃雌花形成相关^[72]。在拟南芥中过表达核桃 *JrAG1* 植株雄蕊异常,而过表达 *JrAG2* 基因的植株出现莲座叶较小、早花性状^[66],尽管都为AG基因,但其同源基因调控的功能不同。D类基因包含STK和SHP1/SHP2两个分支,主要在胚珠、果实开裂区域表达,调控胚珠发育、果实发育和开裂^[73]。E类基因主要参与调节花器官、胚珠(珠被)和种子发育,在玉米和水稻的研究中发现 *AGL6* 与 SEP 亚家族关系密切,可能在雌雄配子体发育中发挥作用^[76]。在雌花盛开花期,薄壳山核桃同源基因 *CiAGL6* 表达量最高,对雌花形成可能有重要调控作用^[77]。其他 *MADS-box* 家族成员在胡桃科植物花器官发育中也发挥重要作用,如:拟南芥 *AGL15* 能促进体细胞胚发育^[78],薄壳山核桃同源基因 *CiMADS9* 在生殖器官中的表达量远高于营养器官,雄花中表达量高于雌花;拟南芥中异源过表达 *CiMADS9*,转基因植株表现为叶片数量的增加,且开花延迟,推测 *CiMADS9* 参与了薄壳山核桃的开花期及雌雄器官发育的调控^[79]。*MADS-box* 同源基因的调控功能存在多样性:山核桃中同源基因 *CcAGL24a* 和 *CcAGL24b* 在心皮发育调控中存在功能差异,过表达 *CcAGL24a* 的拟南芥植株心皮变短变粗,而过表达 *CcAGL24b* 的植株心皮变长、萼片变大且呈凹陷状态^[73]。检测山核桃雌花发育过程中 *CcAGL24*、*CcSOC1* 和 *CcAPI* 的表达情况,发现 *CcAGL24* 的表达高峰最先出现, *CcSOC1* 次之, *CcAPI* 最晚,推测三者在山核桃雌花开花网络中的可能顺序位点从上到下依次为 *CcAGL24*、*CcSOC1* 和 *CcAPI*^[80],且 *CcAGL24* 与 *CcAPI* 存在互作关系(表2)^[81]。

因此,胡桃科植物 *MADS-box* 家族基因对开花存在调控作用,其部分基因的表达模式符合经典花发育模型,在模式植物上的过表达会引起花器官的

变化。但与拟南芥等模式植物相比,胡桃科植物基因组信息复杂, *MADS-box* 基因进化明显,B类和C类基因之外的基因在雌雄花器官发育中也表现出特异性;由于同源基因转录中的可变剪切及结构基因的调控元件的差异,其表达模式和调控功能有所不同;且基因功能同源验证仍需突破遗传转化和长童期的瓶颈,这将为深入发掘胡桃科植物的 *MADS-box* 基因功能、进一步完善胡桃科植物花发育理论奠定坚实的基础。

3.3 非编码 RNA 与花器官分化

非编码 RNA(ncRNAs)是一种表观遗传^[84],虽不编码蛋白质,但却在植物生长发育过程中发挥重要的调控作用^[85]。主要包括小 RNA(microRNA, miRNA)、小干扰 RNA(small interfering RNA, siRNA)、长链非编码 RNA(long noncoding, lncRNA)和环状 RNA(circular RNA, circRNA)等^[86]。已报道 miRNA 能够诱导植物成花、调控花发育和雄性不育。lncRNA 能与 miRNA 互作进而控制花发育、生殖结构发育、基因沉默和转录调控等过程^[87]。山核桃花中检测到保守 miRNA 家族的许多成员,其靶基因的表达模式与相关 miRNA 的表达模式相反,表明 miRNA 可能在山核桃的成花中具有重要功能^[88],研究发现雌花中多个 circRNA 可以与 miR167 协作共同调控 FT 的表达^[89]。拟南芥中发现过表达 miR156,会增强其与促花因子 SPL 作用,降低 SPL 表达活性,延迟开花时间。利用核桃雌花芽和叶芽发育的 miRNA-seq 数据库进行分析,鉴定出雌花芽中比叶芽高表达的差异 miRNA(如 miR156、miR157),推测其与雌花发育相关^[90]。早实核桃香玲和西岭的花发育研究均证实 SPL 为 miR156/157 的靶基因,调控其二次梢的花芽分化^[90-91]。山核桃雌花发育中发现 lncRNA 的差异表达及其靶向基因可通过影响 JA 和 ABA 激素信号传导途径进而影响山核桃雌花的发育调控^[35],表明 lncRNAs 可通过多条信号途径参与调控

表2 胡桃科MADS-box基因及其表达调控

Table 2 MADS-box genes and their expressions in Juglandaceae

物种 Species	基因 Genes	类型 Types	表达情况及调控功能 Expression and regulatory function	参考文献 References
山核桃 <i>C. cathayensis</i>	<i>CcSEP-1</i>	E类 Class E	雌花分化后期高表达 High expression at late stage of female flower differentiation	[72]
S.	<i>CcSEP-2/4</i>	E类 Class E	在雌雄花中均有表达,但雄花中表达高于雌花 It was expressed in both male and female flowers, but the expression in male flowers was higher than that in female flowers	
	<i>CcSVP</i>		开花抑制子,雌花分化前期低表达 Flowering inhibitor, low expression in early stage of female flower differentiation	
	<i>CcAPI</i>	A类 Class A	在雌花分化后期和雄花分化初期高表达 It is highly expressed in the late stage of female flower differentiation and the early stage of male flower differentiation	
	<i>CcSOC1</i>		开花促进因子,且雌花中表达高于雄花 Flowering promoting factors, and the expression in female flowers is higher than that in male flowers	
	<i>CcAGL24a</i>		促进植株提早开花,影响心皮发育,与 <i>CcAPI</i> 互作 Promote early flowering of plants, affect carpel development, and interact with <i>CcAPI</i>	[69,81]
	<i>CcAGL24b</i>		促进植株提早开花,影响心皮发育 Promote early flowering of plants, affecting carpel development	[69,81]
薄壳山核桃 <i>C. illinoiensis</i>	<i>CiMADS20/21/37</i> (<i>AG</i>)	C类 Class C	雌花盛花期高表达 High expression of female flowers in full-bloom stage	[65]
K.	<i>CiMADS40(AP3)</i>	B类 Class B	随雌花芽不断分化,基因表达逐渐降低 With the continuous differentiation of female flower buds, gene expression gradually decreased	
	<i>CiMADS9/10/19</i> (<i>SEP</i>)		雌花盛花期高表达 High expression of female flowers in full-bloom stage	
核桃楸 <i>J. Mandshurica</i>	<i>JmMADS28(SVP)</i>		在雌雄花芽的休眠期高表达 Highly expressed in the dormancy period of male and female flower buds	[35]
M.	<i>JmMADS41(PI)</i>	B类 Class B	雄花开花期高表达 High expression of male flowers at flowering stage	
	<i>JmMADS27(AP3)</i>	B类 Class B	雌花开花期高表达 High expression at flowering stage of female flowers	
核桃 <i>J. regia</i> L.	<i>JrFUL</i>		在雄先型品种中高表达,可能对雄花有促进作用 High expression in male-precursor varieties may promote male flowers	[82]
	<i>JrSOC1</i>		分化初期顶芽中高表达,雌雄花中低表达,可能参与核桃花芽形成 At the early stage of differentiation, it was highly expressed in terminal buds and lowly expressed in male and female flowers, which may be involved in the formation of <i>J. regia</i> flower buds	[83]
	<i>JrAPI</i>	A类 Class A	早实核桃中的表达高于晚实核桃,可能促进早花 The expression in early-fruiting <i>J. regia</i> was higher than that in late-fruiting <i>J. regia</i> , which may promote early flowering	[62]

花器官分化。

非编码特性使ncRNAs比蛋白编码基因更快地做出反应,这使其在雌雄花分化中发挥重要作用。此外,时空特异性表达使其能够精细调控植物生长,发挥独特功能。现有研究多在基因组水平对ncRNAs的表达进行研究,基因沉默(VIGS)、基因敲除(CRISPR/CAS9系统)等技术已初步融入到ncRNAs的精确功能研究中^[92],未来可利用更多新技术及研究手段,将RNA结构计算分级、单细胞测序、单分子测序等融合到园艺植物ncRNAs的识

别、鉴定及功能分析中,继续完善成花途径的调控网络图,挖掘花性别决定关键基因,将ncRNAs发展成精准育种的有力工具。

4 讨论与展望

花是高等植物生长发育过程中非常重要的生殖器官,绝大部分被子植物都形成完全花,但也有少部分植物出现雌雄异花,形成单性花。其中雌雄异花植物黄瓜拥有丰富的雌、雄及两性花类型,通过不同组合形成8种性别类型,目前其性别决定的三位点

基因模型及乙烯代谢调控雌花分化研究较为深入,丰富的植株类型及生长周期短使其成为研究性别分化的模式植物之一^[93]。参考葫芦科植物的性别研究史,胡桃科植物花发育历程长,从雌、雄花分化到开花散粉长达一年之多,其遗传背景复杂,受内外多种因素综合调控。目前该科植物花发育的调控研究侧重于花芽分化的形态学解析,基于生物组学数据筛选雌雄花分化差异表达基因及在拟南芥等模式植物上进行异源验证,该通路基因调控网络研究较为薄弱,从分子调控机制研究到调控花芽分化质量和提高雌雄花比例的技术应用还有很长的路要走,未来可从以下几个方面开展工作:

(1) 挖掘创建胡桃科植物雌雄花特异性状资源,建立健全快速表型鉴定体系。寻找早花品种、多雌花品种和性别分化特异资源(如两性花资源),利用基因编辑技术获得特异基因的突变体植株、对特异基因进行数字化标记可克服基因功能验证的表型障碍,快速识别基因作用位点,为进一步挖掘雌花特异表达基因奠定基础。未来利用数量性状遗传定位解析位置效应与雌雄花性别决定,对调控胡桃科植物的雌雄花比率有重大意义。

(2) 利用生物技术手段,联合解析胡桃科作物花发育机制。随着胡桃科植物基因组数据库的建立,单细胞测序和空间转录组学从转录水平上有利对花发育关键基因的聚焦,非编码 RNA 测序从表观遗传和转录调控角度助力基因功能的解析,利用代谢组学探索各激素信号及糖代谢转运,为花发育决定系统的多样化研究奠定基础。针对 *MADS-box* 同源基因具有不同的调控功能,可利用基因编辑技术靶向特定调控区域来达到调节特定功能的目的,从而减少对非目标性状的不良影响。

(3) 多途径进行目的基因的功能验证,完善胡桃科植物遗传转化体系。黄瓜作为研究性别分化的模式植物,通过鉴定其异源转化植株可克服胡桃科植株童期长的障碍。此外,目前胡桃科植物已成功建立单芽茎段及离体种胚快繁体系^[94-95],病毒介导的基因沉默及愈伤的瞬时转化技术实现了基因在细胞中的瞬时表达^[96-97],未来稳定遗传转化体系的建立需突破植株难生根、不成苗等瓶颈,解决植物同源遗传转化问题对基因功能验证有积极影响,一旦性别决定基因和相关调控网络被发现,设计和开发雌雄异株植物将变得可行和具有实用价值。这样,异花授粉

可以用来避免近交衰退,授粉树的配置可以保证雌株植物有足够的生育力和产量。

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