

柿性别分化及性别连锁标记研究进展

张萌¹, 张平贤¹, 徐莉清^{1,2}, 郭大勇^{1,2}, 罗正荣^{1,2}, 张青林^{1,2*}

(¹华中农业大学园艺植物生物学教育部重点实验室, 武汉 430070; ²黄冈师范学院大别山特色资源开发湖北省协同创新中心·经济林木种质改良与资源综合利用湖北省重点实验室, 湖北黄冈 438000)

摘要: 柿 (*Diospyros kaki* Thunb.) 起源中国, 按其花性表现可分为雌株、雄株、雌雄同株和三全同株等类型。笔者对柿及其部分近缘植物的性别类型、花芽分化类型及特点、性别决定细胞学及分子机制、雌雄性别遗传规律及与雄性性别连锁的分子标记等研究进展进行扼要总结。此外, 还对原产我国中部大别山区的完全雄性柿种质的起源及其利用价值进行初步探讨, 以期对柿性别调控机制研究以及完全甜柿遗传改良中的亲本选育提供科学依据。

关键词: 柿; 性别分化; 性别连锁标记; 完全雄株; 遗传改良

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Research progress of sex determination and sex-linked markers in persimmon

ZHANG Meng¹, ZHANG Pingxian¹, XU Liqing^{1,2}, GUO Dayong^{1,2}, LUO Zhengrong^{1,2}, ZHANG Qinglin^{1,2*}

(¹Key Laboratory of Horticultural Plant Biology, Huazhong Agricultural University, Wuhan 430070, Hubei, China; ²Hubei Collaborative Innovation Center for the Characteristic Resources Exploitation of Dabie Mountains · Hubei Key Laboratory of Economic Forest Germplasm Improvement and Resources Comprehensive Utilization, Huanggang Normal University, Huanggang 438000, Hubei, China)

Abstract: Persimmon (*Diospyros kaki* Thunb.) is originated and domesticated in China. Artificial control of sexuality is an important issue for both fruit production and breeding of leading persimmon cultivars. Complex sexualities occurred in *Diospyros* L., and individual persimmon floral phenotypes can be generally divided into four types: gynoeceous (bearing female flowers only, most cultivars), androeceous (bearing male flowers only, few cultivars), monoecious (bearing both male and female flowers, few cultivars) and trimonoecious (bearing both bisexual and unisexual flowers, rare cultivars). Some gynoeceous genotypes, such as Japanese PCNA (pollination constant non-astringent) cultivars ‘Fuyuu’ and ‘Jirou’, can occasionally generate male flowers under certain circumstance. The goal of this article is to review the nature of sexual dimorphism in *Diospyros* L. and discuss the androeceous genotypes benefits in persimmon orchard production. The genetic control of sex determination is gradually understood in *Diospyros* L.. Sex expression was influenced by many factors including hormone, tree age, nutrient status and environment, etc.. Low concentration of indoleacetic acid (IAA), abscisic acid (ABA), the ratio of ABA to IAA, and high levels of gibberellin A3 (GA₃) in male floral buds in the middle of June were correlated to the formation of three-flower cymes. In addition, high levels of the ratio of ABA to zeatin (ZT) might promote the differentiation of male floral buds. Benzylaminoadenine (BA) treatment leads staminate flowers to hermaphroditic flowers. The balance between cytokinins, auxins and abscisic acid plays a vital role in persimmon sex expression. Persimmon pistillate and staminate floral primordia in

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作者简介: 张萌, 女, 在读博士研究生, 研究方向为果树生物技术和种质创新。Tel: 027-87282677, E-mail: zhangmeng2015430@163.com

*通信作者 Author for correspondence. Tel: 027-87282677, E-mail: zhangqinglin@mail.hzau.edu.cn

the buds of current shoots initiated to differentiate in early June, then quiescent and overwintered. The floral structure restarted to differentiate shortly before blooming in the following season. The presence of flower was observed on current shoots derived from adventitious buds, plantlets *in vitro* or *Diospyros* sp. Deyangshi. Floral morphogenesis of ‘Gongcheng Shuishi’ *in vitro* was observed after continuous subculture using stem-segment explant. The exhibition of early flowering in Deyangshi seedling after sown three months later suggested it might enhance the transgenic or genetic research. Under the analysis of RNA-Seq data or genomic sequence, recent great advance in commercial persimmon related diploid species *D. lotus* revealed that the action of a Y-encoded sex determinant pseudogene called *OGI* (*Oppressor of MeGI*), which produces small RNAs targeting the autosomal gene *MeGI* (*Male Growth Inhibitor*), generated separated male and female individuals (dioecy, XY homomorphic). Moreover, in hexaploid persimmon with Y-chromosome-carrying, the expression of *OGI* is silenced by the presence of a SINE (short interspersed nuclear element)-like insertion in the *OGI* promoter, named *Kali*. At the same time, DNA methylation of the *MeGI* promoter facilitates *smMeGI* production to inhibit the expression of *MeGI*, which in turns leads to male flower development. Those gene functions were just characterized in heterogenous plants. None early flowering was observed after the regenerated transgenic shoots were sub-cultured *in vitro* over 2 years, which used *FLOWERING LOCUS T* as donor gene for transformed persimmon. Early elimination of male progeny has been regarded as an important strategy for enhancing breeding efficiency. Two male sex-linked molecular markers, DISx-AF4S and *OGI* locus were validated in a relatively large-scale of *Diospyros* L. germplasms, the accuracy of the two SCAR markers were nearly over 90%. Male plants including androecious and monoecious genotypes could be distinguished from female individuals at an early stage. Special androecious persimmon genotypes were collected in Dabie Mountains located in central China. Those androecious genotypes presented stable male characteristics even after two consequent top-grafting. Androecious persimmon germplasm could produce high amount of pollen and its germination on stigma of leading cultivars was well. Both the outer or inner fruit quality after pollination using “Male 8” exceeded those of non-pollination. A novel pollination constant non-astringent genotype “H8-2” was derived from the artificial cross between ‘Huashi 1’ (pollination variant and astringent, PVA) and “Male 8”. This suggested that androecious genotype “Male 8” carries the dominant gene locus *CPCNA* that controls the trait of natural de-astringency. The potentiality of androecious persimmon genotypes as pollen donor for the genetic improvement of PCNA persimmon requires further investigation. This review focused on the recent development of sex determination, sex-linked molecular markers and germplasm utilization in persimmon. Systematic collection, preservation and reasonable utilization for the special germplasms with male gender should be carried out in the future.

Key words: Persimmon; Sex differentiation; Sex-linked markers; Androecious genotype; Genetic improvement

植物可着生雌花、雄花和完全花中的一种或多种,根据单花组合分类存在雌株(仅开雌花)、雄株(仅开雄花)、完全花株(仅着生完全花)、雌雄同株(雌花和雄花共存于1株)、雌全同株(雌花和完全花共存于1株)、雄全同株(雄花和完全花共存于1株)及三全同株(雌花、雄花和完全花共存于1株)等个体;根据单株组合分为完全花植株、雌雄同株(雌雄

同株异花植株)、雌雄异株(雌株和雄株共存)等性别系统^[1]。植物界中完全花植物约占90%,雌雄异株植物占5%~6%^[2]。植物性别分化既是基础生物学现象,也是影响作物产量和育种效率的重要因素,但迄今植物性别调控机制尚存许多未知领域^[3-4]。

柿属植物(*Diospyros* L.)包含500余种,其中柿(*D. kaki* Thunb.)、君迁子(*D. lotus* L.)、油柿(*D.*

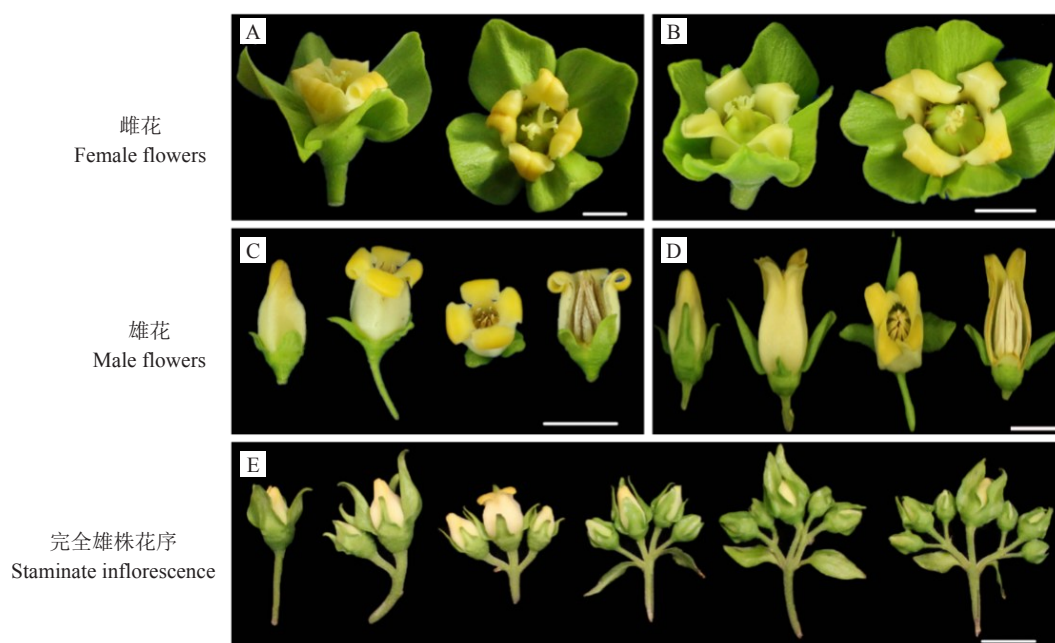
oleifera Cheng)、老鸦柿(*D. rhombifolia* Hemsl.)及美洲柿(*D. virginiana* L.)是常见的果树植物,柿为该属植物作为果树栽培的代表种^[5]。经过长期的人工选择,目前绝大多数柿品种仅着生雌花,少数品种表现为雌雄同株,极少数品种为雌、雄和完全花共存^[6-9]。近年来,华中农业大学柿研究团队在我国大别山区收集到 10 余份仅开雄花的完全雄性柿种质资源^[10],其作为专用授粉品种及育种亲本的应用潜力正被系统评价^[11]。日本京都大学 Akagi^[12-15]教授团队在柿属植物性别决定基因和表观遗传调控机制及其分子连锁标记开发等方面取得重要进展。笔者拟对柿(*D. kaki* Thunb.)及其部分近缘种植物性别决定的细胞学和分子机制以及相关分子标记开发等研究进展进行扼要总结。此外,还对原产我国的完全雄性柿种

质的起源及其利用价值进行初步探讨。

1 柿性别类型

柿单花有雄花、雌花和完全花 3 种花型,雌花单生,雄花和完全花属聚伞花序(cyme)。雌花通常仅有 1 朵发育良好的中心小花(central flower),其边侧小花(lateral flower)通常退化(图 1-A~B)。雄花序通常有 3~5 朵小花,由 1 朵中心小花和 2~4 朵边侧小花结构组成,以 3 朵小花结构最为常见^[7,16]。当雄花序(一般位于当年生新梢基部)发育不良时,可见 1~2 朵小花的结构,有时可见 7 朵小花组成的花序结构(图 1-C~E)。完全花与雄花花序结构类似,完全花的雌蕊正常可育。

日本学者将柿性别在个体水平划分为雌株(仅



A. 上西早生; B. 太秋; C. 雄株 8 号; D. 鄂柿 1 号; E. 完全雄株花序。标尺为 1 cm。
A. Uenishiwase; B. Taishuu; C. Male 8; D. Eshi 1; E. Staminate inflorescence. Bar = 1 cm.

图 1 部分柿品种花型

Fig. 1 Flower type of some persimmon cultivars

开雌花)、雌雄同株(雌花和雄花共存于 1 株)和完全花株(同时着生雌花、雄花及完全花)^[17]。绝大多数现存柿品种仅开雌花,如‘阳丰’‘磨盘柿’‘恭城水柿’和‘富平尖柿’等;少数品种为雌雄同株异花,如‘禅寺丸’‘太秋’和‘襄阳牛心柿’等;极少数品种既着生完全花,又着生雌花和雄花,如‘夫妇柿’和‘华柿 1 号’等(表 1)。在雌雄同株品种中,不同品种间雄花和雌花着生比例及其产生的花粉量不同,如‘禅

寺丸’雄花着生较多且花粉量大,是栽培上主要的授粉品种;此外,同一品种在不同树龄或生长状态下着生雌花和雄花的比例也不同,如‘太秋’在树势较弱或受逆境胁迫时易着生较多的雄花,成为影响其产量的重要因素。华中农业大学柿研究团队近年来在湖北省大别山区收集到仅着生雄花的系列完全雄性柿单株,并对其作为专用授粉品种及完全甜柿育种亲本的应用潜力进行了研究^[18-21]。

表 1 柿性别类型

Table 1 Classification of persimmon sexuality

性别水平 Sexuality level	性别类型 Sexuality phenotypes	描述 Description	代表种质 Genotype
单花水平 Individual flower level	完全花 Hermaphrodite	具有雄蕊和雌蕊的两性花 Bisexual flower with both stamens and pistil	夫妇柿、华柿 1 号 Meotogaki, Huashi 1
	雌花 Pistillate	只有雌蕊的单性花 Unisexual flower with pistil only (female flower)	阳丰、磨盘柿 Youhou, Mopanshi
	雄花 Staminate	只有雄蕊的单性花 Unisexual flower with stamens only (male flower)	雄株 8 号 Male 8
单株水平 Individual plant level	雌株 Gynoecious	仅有雌花 Plant bears only pistillate flowers	阳丰、磨盘柿 Youhou, Mopanshi
	雄株 Androecious	仅有雄花 Plant bears only staminate flowers	雄株 8 号 Male 8
	雌雄同株 Monoecious	雌花和雄花共存于 1 株 Both pistillate and staminate flowers on the same plant	太秋、襄阳牛心柿 Taishuu, Xiangyang Niuxinshi
	三全同株 Trimonoecious	雌花、雄花和完全花共存于 1 株 Female, male and hermaphrodite flowers on the same plant	夫妇柿、华柿 1 号 Meotogaki, Huashi 1
种群水平 Population level	杂性 Polygamous	种内单株性别类型复杂 Complex individuals gender (intersexuality)	

虽然绝大多数柿品种为雌株,但少数雌性品种在某些年份可在少数枝条上发生雄花,如日本原产完全甜柿‘富有’和‘次郎’^[22]、日本原产完全涩柿‘西条’^[23]以及中国原产完全甜柿‘罗田甜柿’(数据未发表)和‘鄂柿 1 号’^[24]。日本学者 Yakushiji 等^[22]报道,将偶开雄花的‘次郎’枝条高接至‘富有’后,其 2 a (年)内先开雄花而后仅开雌花,经同工酶检测‘富有’和‘次郎’的偶开雄花枝条与其母株并无差异。张平贤等^[24]对‘鄂柿 1 号’雄花枝与母株间的基因组进行 AFLP 检测,结果显示‘鄂柿 1 号’雄花枝与母株遗传背景一致,说明‘鄂柿 1 号’雄花枝与母株基因组 DNA 水平可能并无差异,推测此种现象可能受表观遗传调控或环境因素的影响。雌性品种所开雄花亦可用于杂交育种,山根弘康等^[25]利用‘次郎’的偶发雄花花粉授粉于‘富有’雌花后,成功地培育出综合性状优良的主栽完全甜柿新品种‘阳丰’。笔者经过多年田间观测也发现,部分雌雄同株种质可偶然发生完全花,如‘禅寺丸’‘太秋’和‘华柿 1 号’等,但该类完全花形成的果实比正常雌花获得的果实小一半左右,且种子退化(未发表数据)。

2 柿花芽分化过程及其特点

植物花芽分化过程可分 5 个阶段:分化初期、萼片分化期、花瓣分化期、雄蕊分化期、雌蕊分化期。柿花芽分化过程包括 2 种类型,一种类型较为常见,称为“跨年分化类型”,该类花芽原基在当年 6 月中旬开始孕育,7 月中旬后分化出萼片或花瓣原基,之

后花芽分化处于停顿状态,至翌年春季继续分化形成花瓣、雄蕊和雌蕊原基,直到花器官形态建成和开花^[26-27];另一种类型是花芽分化进程在当年即可完成,拟称为“当年分化类型”,其特点是花芽分化开始后可持续分化出花的各部分原基,并可当年正常开花。张平贤等^[28]曾观察到‘上西早生’‘富有’‘君迁子’及‘华柿 1 号’×‘罗田甜柿’ F_1 部分单株有多年生枝萌蘖当年开花的现象;‘恭城水柿’茎段外植体获得的试管苗可见成花现象。此外,西北农林科技大学 Zhang 等^[29]也观察到德阳柿(*Diospyros* sp. Deyangshi, $2n=4x=60$)无论高接还是 1 a 生实生苗均可在当年开花的现象。

日本学者对柿雌、雄花芽分化时期的研究表明,‘禅寺丸’雄花和雌花的花原基分化早期即可区分^[30-31],其雄花成熟时通常 3 朵合生,中间 1 朵略大,雌花为单花;雄花花期一般略早于雌花。Yonemori 等^[17]观察到‘花御所’和‘柿山柿’的雌蕊、雄蕊花原基 6 月开始分化直到 8 月份开始有差异。同时发现,当年着生雌花枝条的混合芽在翌年抽生枝条中,雌花着生率高于雄花,而从当年着生雄花枝条分化的混合芽,翌年抽生枝条的雄花着生率则高于雌花。Kajita 等^[32]利用扫描电镜观察‘禅寺丸’和君迁子发现,其花芽 6 月中旬到 6 月底分生组织开始分化。李加茹等^[33]通过扫描电镜与组织切片也观察到‘禅寺丸’雌、雄花发育初期并没有明显差异,在 6 月中旬萼片发生期,其雌、雄花的中间花原基两侧的小花原基的发育开始出现不同,虽然此时雌、雄花芽外部形

态仍没有明显差异,但解剖可见雌花单生、雄花3朵合生的现象,翌年4月中旬的大小孢子发生期,雌花单生、雄花3朵合生的外部形态已经完全显现;此后,雌花中的雄蕊原基发生败育,而雄花中的雌蕊发生败育,最终形成单性花。

3 柿属植物性别决定

早期研究表明,雌雄同株柿树上的雌、雄花比例受营养水平、树势强弱、前一年产量、激素水平、枝条类型、芽着生位置的影响较大^[7,34]。一般情况下,在树势较强时雌花发生多,反之则雄花多。少数柿品种,如‘太秋’在树龄较大或逆境下,雄花数量增加。部分雌株品种会偶开雄花,如‘罗田甜柿’‘鄂柿1号’和‘次郎’等;一些雄性单株也会偶开雌花,如“雄株10号”等。

植物性别分化受多种激素调节。Chailakhyan^[35]曾认为赤霉素(GAs)是影响花性别分化的重要因素,用赤霉素处理马齿苋可诱导其雌株雄性化。Irish等^[36]发现生长素(如IAA)和细胞分裂素(CTKs)也可参与植物性别调控。米森敬三等^[37]用苄氨基嘌呤(BA)处理‘台湾正柿’和‘甘四沟’,可促进花芽分化向雌性转变;此外,BA处理还有使雄花转变为完全花的效果。Sun等^[38]研究也发现,当年6月和翌年4月是性别分化的2个关键时期,高水平ABA/ZT比率可促进雄花芽分化,而高水平IAA和ABA可能刺激雄蕊原基的败育而促进雌蕊原基的发育。因此,细胞分裂素和生长素的平衡在柿品种花性别转变中有重要作用。

尽管植物进化亦产生特异的性染色体,但其性别表达的控制因素未见报道。2014年,日本京都大学Akagi等^[13]对雌雄异株的柿属植物二倍体种君迁子(*D. lotus* L.)F₁代性别分离的2个群体(32个雌株、25个雄株)基因组深度测序结合RNA-Seq测序定位时发现,其雄株Y染色体上包含有雄性性别决定区域,分离获得1个位于Y染色体上的性别决定基因*OGI*(*Oppressor of MeGI*),其编码小RNA特异剪切*MeGI*(*Male Growth Inhibitor*)基因从而使植株表现雄性;*MeGI*则可调节花药育性(柿植株通常表现雌性),其位于常染色体上并与*OGI*同源异型,并属于一类具有剂量依赖型的转录因子基因。Akagi等^[15]进一步对六倍体栽培柿(*D. kaki*, 2n=6x=90)研究发现,*OGI*启动子区域的*Kali*片段(268 bp)插入可导

致*OGI*表达沉默,但*Kali*同时可引起*MeGI*启动子发生甲基化,促进*smMeGI*产生,从而使*MeGI*表达受到抑制,最终表现为雄花发育。这2项突破性研究深入解析了柿属植物性别分化的分子调控机制(图2)。由于小RNA和DNA甲基化受环境影响,导致雌雄同株柿品种雌雄花发生比例不稳定^[15],雌株或雄株上也能观察到分别偶发雄花或雌花,如杨树中miRNA172b的甲基化与花发育相关,其可促使雄性基因在两性花中的表达^[39];在棕榈树中曾发现因DNA甲基化程度减弱导致的异常性别表型,推测由小RNA调控甲基化发生以及染色质构象变化进而改变花发育^[40]。

MADS-box家族大部分成员在植物花器官发育中起着重要的作用,丁燕等^[41]首次在‘阳丰’雌花中克隆获得了1个*DkMADS1*基因,其蛋白序列与很多物种中报道的MADS-box蛋白具有很高的相似性;其在‘阳丰’雌花器官以及‘禅寺丸’的雄蕊中均表达,在叶片中则不表达。Viaene等^[42]通过对多种双子叶植物MADS-box基因中的*AGL6*表达进行分析,认为其对生殖结构功能发挥起作用,在营养枝的发育转向中也可能起到了一定的作用。Gao等^[43]将拟南芥中控制早花性状的基因*AtFT*以及果梅中的1个同源基因*PmTFL1*利用农杆菌介导法转入日本完全甜柿‘次郎’,但经过2a观察,并未发现有早花现象发生。

4 柿属植物雄性性别的连锁标记及其应用

柿品种一般仅开雌花且单性结实能力强,生产上要求主栽品种能够稳定着生雌花;实生柿树童期为5~8a,杂交育种过程中早期筛选剔除雄性后代是提高育种效率的重要措施。Akagi等^[12]利用君迁子F₁代雄性/雌性性状分离群体,通过BSA-AFLP技术筛选到1个与雄性性状紧密连锁的分子标记DISx-AF4S。Kajita等^[44]利用该标记对39份雄性柿属试材进行鉴定,表明除3份试材以外,其余种质分子标记鉴定与花性调查结果相同。张平贤等^[45]利用该标记对268份柿属相关试材进行通用性鉴定,结果表明,116份试材中有112份的鉴定结果与其田间花性调查结果吻合(鉴定成功率为96.6%);在已知花性的46份F₁代个体中鉴别率可达90.2%。

Zhang等^[46]利用在君迁子(*D. lotus* L.)中获得的

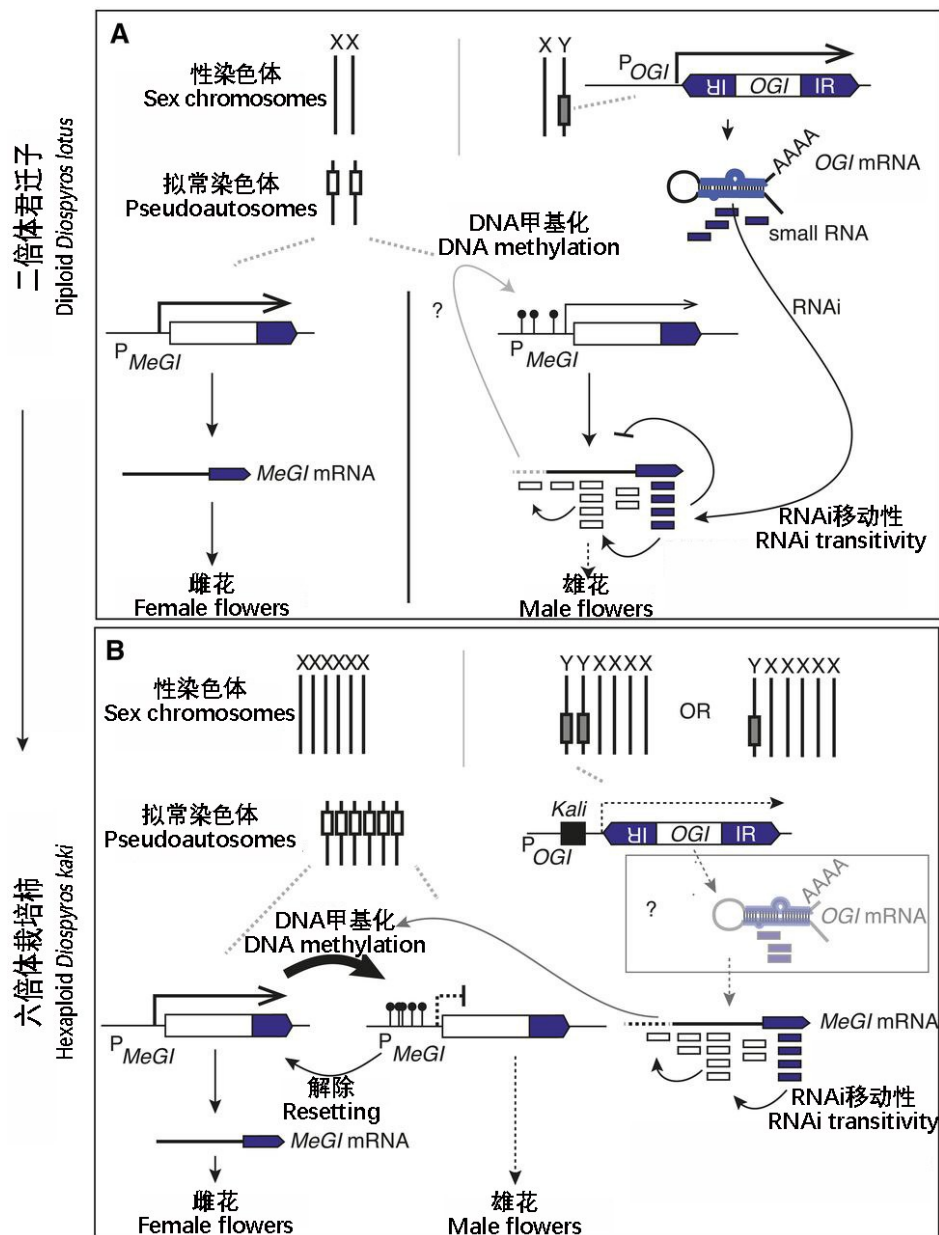


图 2 柿属植物性别进化模型图^[15]

Fig. 2 Evolutionary model of sex determination in *Diospyros*^[15]

*OGI*为雄性连锁标记,对50份柿相关试材进行性别鉴定(鉴定率为100%);143份F₁代单株中有95份进入成花状态,*OGI*标记检测鉴别率为89.5%。此外,Akagi^[14]等对172份柿相关材料进行雄性性别决定基因*OGI*进行扩增,其中35份雌雄同株材料均显示此标记,137份雌性个体中113份未扩增出此标记。说明*OGI*标记在柿属植物间具有较高的通用性。

5 中国原产完全雄性种质的发现及其应用价值

完全甜柿新品种选育是目前世界范围内遗传改

良和产业化应用的重点。目前可用于完全甜柿育种的亲本较少,长期近亲杂交已面临近交退化的瓶颈^[47],亟需在育种亲本和杂交组合选配方面引入新的遗传资源。

现有完全甜柿根据其自然脱涩性状遗传特点,分为受显性单基因位点控制的中国原产完全甜柿(简称中国甜柿或CPCNA)和受隐性基因位点控制的日本原产完全甜柿(简称日本甜柿或JPCNA)^[48]。日本甜柿间杂交,或者亲本之一是中国甜柿时,F₁代才会出现完全甜柿。我国中部大别山区分布着中国甜柿及部分完全雄性柿种质^[10]。Zhang等^[21]研究表

明,完全雄性柿种质与中国甜柿的关系非常密切。Pei 等^[49]发现部分单株带有与中国甜柿自然脱涩性状显性位点连锁的RO2 标记,说明以完全雄株为父本,与完全甜柿和非完全甜柿杂交,有望获得新的完全甜柿种质。Zhang 等^[20]以‘华柿 1 号’(不完全涩

柿)为母本、“雄株 8 号”为父本,获得具中国甜柿自然脱涩特点的完全甜柿新种质“H8-2”。证明中国原产完全雄性柿种质“雄株 8 号”带有控制自然脱涩性状的显性基因,具有作为完全甜柿育种亲本的应用潜力(表 2)。

表 2 柿品种雄花着生程度及花粉量

Table 2 The degree of male flower production and the amount of pollen of persimmon

雄花着生量 Male flowers production	花粉量 Pollen amount			
	无 None	少 Few	中 Middle	多 More
稀 Rare	清道柿、堂上蜂屋 Seidoushi, Doujouchachiya	西条木练、守屋、保山烘柿 Saijokinuri, Moriya, Baoshan Hongshi	甘百目、衣纹、柏原御所、伽罗、上丸、 长野御所、投鸟帽子、红重井、四沟 Amahyakume, Emon, Kaibaragoshō, Kyara, Uemaru, Naganogoshō, Na- geeboshi, Benishigei, Yotsumizo	会津御所 Aizugoshō
少 Few	三郎座、红柿 Saburouza, Benigaki	晚御所、西村早生、杭州小油柿、黑 心柿、盘县水柿、商城水柿、什样景 柿、树头红、小八胡、偃师天生柿 Okugoshō, Nishimurawase, Hang- zhou Xiaoyoushi, Heixinshi, Panx- ian Shuishi, Shangcheng Shuishi, Shiyang Jingshi, Shutouhong, Xiao- bahu, Yanshi Tianshengshi	出石大御所、久保、丹城柿、天神御 所、东洋一、福御所、袋御所、藤原御 所、花御所、村屋、吉本御所 Izushiogoshō, Kubo, Danseongsi, Ten- jingoshō, Touyouichi, Fukugoshō, Fu- kurogoshō, Fujiwaragoshō, Hanago- shō, Muraya, Yoshimotogoshō	
中 Middle			赤柿、太秋、甘四沟、高丽水柿、御所、 正月、清白寺、笔柿、麓田御所、吉田 御所 Akagaki, Taishuu, Amayotsumizo, Koreiseishi, Goshō, Shougatsu, Sei- hakuji, Fudegaki, Mushirodagoshō, Yoshidagoshō	素人擬 Shiroutodamashiya
多 More		夫妇柿、华柿 1 号 Meotogaki, Huashi 1	岩濑户、绘御所、台湾正柿、藤八、河 西大果、襄阳牛心柿 Iwasedo, Egoshō, Taiwan Zhengshi, Tohachi, Hexi Daguō, Xiangyang Niuxinshi	大宫早生、柿山柿、猩猩、禅寺 丸、天龙坊、水田富有 Oomiyawase, Kakiyamagaki, Sho- ujou, Zenjimarū, Tenryoubou, Suitafuyū
很多 Abundant			水种 Seijon	久保型带仕、丰冈、雄株 1 号、雄 株 2 号、雄株 3 号、雄株 8 号、雄株 9 号、雄株 10 号、雄株 11 号 Kubogataobishi, Toyoka, Male 1, Male 2, Male 3, Male 8, Male 9, Male 10, Male 11

注:根据广岛县果树试验场(1979)^[51]有增补。

Note: The table was modified on Hiroshima Prefecture Fruit Tree Research Station(1979)^[51].

黄瑜芳^[50]发现 7 份完全雄性柿种质处于不同的聚类分支中,可能具有 2 个或多个进化起源,其中大部分由‘罗田甜柿’变异而来,“雄株 11 号”可能由中国原产完全涩柿进化而来,完全雄株开花量较大且很稳定,5 份种质的花粉量均高于常见日本柿授粉品种,各完全雄株花粉离体萌发率均高于‘禅寺丸’和‘西村早生’,并且其与大多数柿品种具有较强的授粉亲和性。经授粉后,不同的柿品种间杂交坐果率和品质有较大差异^[52-53],刘佳^[11]的研究结果也揭示了“雄株 8 号”作为完全甜柿专用授粉品种的可能

性。

6 结 语

柿属植物花性复杂,是研究植物性别决定机制的理想材料。Akagi 等^[13-15]发现了二倍体君迁子中性别性别决定基因 *OGI* 的性别调控机制以及六倍体栽培柿中性别决定基因 *MeGI* 的表观遗传学调控机制,为进一步开展柿属植物性别调控及遗传改良奠定了良好的理论基础。同时,与柿属植物雄株性状紧密连锁的 *DISx-AF4S* 和 *OGI* 标记的发现和应

有望在杂种性别早期鉴定中广泛应用,从而显著缩短育种周期和降低管理成本。此外,中国甜柿遗传改良技术体系正在不断优化^[54],在湖北省大别山区分布的完全雄株是十分宝贵的柿属种质资源,已知其带有与中国甜柿自然脱涩性状紧密连锁的显性基因位点,不仅是性别决定研究的重要试材,也是完全甜柿遗传改良的可选亲本。德阳柿(*Diospyros* sp. Deyangshi, $2n=4x=60$)早花现象的发现使其有望成为稳定遗传转化的理想试材,从而在柿属植物经济性状相关功能基因的筛选中发挥重要作用。目前对本植物性别调控的分子机制研究较少,以形态、生理和相关分子标记开发研究较多,今后应着重利用基因组、转录组、蛋白质组和降解组学数据以及分子生物学技术对植物性别控制的分子调控网络进行研究,以期为遗传改良与性别调节实践提供科学依据。

参考文献 References:

- [1] DELLAPORTA S L, CALDERON-URREA A. Sex determination in flowering plants[J]. *The Plant Cell*, 1993, 5(10): 1241-1251.
- [2] MING R, BENDAHMANE A, RENNER S S. Sex chromosomes in land plants[J]. *Annual Review of Plant Biology*, 2011, 62(1): 485-514.
- [3] CHARLESWORTH D. Plant contributions to our understanding of sex chromosome evolution[J]. *New Phytologist*, 2015, 208(1): 52-65.
- [4] CHARLESWORTH D. Plant sex chromosomes[J]. *Annual Review of Plant Biology*, 2016, 67: 397-420.
- [5] LUO Z, WANG R. Persimmon in China: Domestication and traditional utilizations of genetic resources[J]. *Advances in Horticultural Science*, 2008, 22(4): 239-243.
- [6] YANG Y, RUAN X, WANG R. Indigenous persimmon germplasm resources in China[J]. *Acta Horticulturae*, 2013, 996: 89-96.
- [7] GEORGE A P, MOWAT A D, COLLINS R J, MORLEY-BUNKER M. The pattern and control of reproductive development in non-astringent persimmon (*Diospyros kaki* L.): a review[J]. *Scientia Horticulturae*, 1997, 70(2/3): 93-122.
- [8] KAJIURA I, BLUMENFELD A. *Diospyros kaki* [M]//HALEVY A H. CRC Handbook of Flowering. Boca Raton, Florida, USA: CRC Press, 1989: 298-306.
- [9] 李高潮,王仁梓,杨勇. 柿种植物雄性种质资源[J]. *果树科学*, 1996, 13(3): 199-200.
LI Gaochao, WANG Renzi, YANG Yong. The staminate germplasm of *Diospyros kaki* in China[J]. *Journal of Fruit Science*, 1996, 13(3): 199-200.
- [10] 张青林,罗正荣. 部分柿属雄性种质巨大花粉、花粉离体及其在罗田甜柿柱头上的萌发率[J]. *果树学报*, 2006, 23(2): 293-296.
ZHANG Qinglin, LUO Zhengrong. Observation of giant pollen and pollen germination ability *in vitro* of some *Diospyros* spp. and their pollen germination on the stigma of *Diospyros kaki* cv. Luotiantianshi[J]. *Journal of Fruit Science*, 2006, 23(2): 293-296.
- [11] 刘佳. 部分完全雄性种质作为柿专用授粉种质的评价研究[D]. 武汉:华中农业大学,2015.
LIU Jia. Evaluation of some androecious germplasm for professional pollinizer in persimmon[D]. Wuhan: Huazhong Agricultural University, 2015.
- [12] AKAGI T, KAJITA K, KIBE T, MORIMURA H, TSUJIMOTO T, NISHIYAMA S, KAWAI T, YAMANE H, TAO R. Development of molecular markers associated with sexuality in *Diospyros lotus* L. and their application in *D. kaki* Thunb.[J]. *Journal of the Japanese Society for Horticultural Science*, 2014, 83(3): 214-221.
- [13] AKAGI T, HENRY I M, TAO R, COMAI L. A Y-chromosome-encoded small RNA acts as a sex determinant in persimmons[J]. *Science*, 2014, 346(6209): 646-650.
- [14] AKAGI T, KAWAI T, TAO R. A male determinant gene in diploid dioecious *Diospyros*, *OGI*, is required for male flower production in monoecious individuals of oriental persimmon (*D. kaki*)[J]. *Scientia Horticulturae*, 2016, 213: 243-251.
- [15] AKAGI T, HENRY I M, KAWAI T, COMAI L, TAO R. Epigenetic regulation of the sex determination gene *MeGI* in polyploid persimmon[J]. *The Plant Cell*, 2016, 28(12): 2905-2915.
- [16] NAMIKAWA I, SISA M, ASAI K. On the flower types of *Diospyros kaki*[J]. *Japanese Journal of Botany*, 1932, 6: 139-172.
- [17] YONEMORI K, SUGIURA A, TANAKA K, KAMEDA K. Floral ontogeny and sex determination in monoecious-type persimmons[J]. *Journal of the American Society for Horticultural Science*, 1993, 118(2): 293-297.
- [18] HUANG Y, LUO Z, ZHANG Q. Phylogenetic analysis of some androecious genotypes native to China and related *Diospyros* spp. using chloroplast fragments[J]. *Acta Horticulturae*, 2013, 996: 103-109.
- [19] 何欢. 甜柿专用授粉种质的筛选及其 F₁ 代鉴定[D]. 武汉:华中农业大学,2016.
HE Huan. Screening of professional PCNA pollinizer and F₁ progenies sexuality identification in persimmon[D]. Wuhan: Huazhong Agricultural University, 2016.
- [20] ZHANG N, XU J, MO R, ZHANG Q, LUO Z. Androecious genotype 'Male 8' carries the CPCNA gene locus controlling natural deastringency of Chinese PCNA persimmons[J]. *Horticultural Plant Journal*, 2016, 2(6): 309-314.
- [21] ZHANG Q, GUO D, LUO Z. Identification and taxonomic status of Chinese *Diospyros* spp. (Ebenaceae) androecious germplasms[J]. *Acta Horticulturae*, 2009, 833: 91-96.

- [22] YAKUSHIJI H, YAMADA M, YONEMORI K, SATO A, KIMURA N. Staminate flower production on shoots of 'Fuyu' and 'Jiro' persimmon (*Diospyros kaki* Thunb.) [J]. Journal of the Japanese Society for Horticultural Science, 1995, 64(1): 41-46.
- [23] 江角智也, 渡辺諄, 小杉友華菜, 大畑和也, 板村裕之. カキ'西条'における雄花の発見[J]. 島根大学生物資源科学部研究報告, 2015, 20: 3-8.
ESUMI T, WATANABE A, KOSUGI Y, OHATA K, ITAMURA H. Staminate flowers on 'Saijo' persimmon (*Diospyros kaki* Thunb.) [J]. Bulletin of the Faculty of Life and Environmental Science, Shimane University, 2015, 20: 3-8.
- [24] 张平贤, 张娜, 郑洁, 黄金盟, 张青林, 徐莉清, 罗正荣. 中国甜柿'鄂柿1号'偶开雄花现象及其杂交后代早期鉴定[J]. 果树学报, 2017, 34(3): 288-294.
ZHANG Pingxian, ZHANG Na, ZHENG Jie, HUANG Jinmeng, ZHANG Qinglin, XU Liqing, LUO Zhengrong. Occasional staminate flowering in Chinese PCNA persimmon 'Eshi 1' and identification of early de-astringency and sexuality in F_1 progenies by RO2 and DISx-AF4S [J]. Journal of Fruit Science, 2017, 34(3): 288-294.
- [25] 山根弘康, 栗原昭夫, 永田賢嗣, 山田昌彦, 岸光夫, 吉永勝一, 松本亮司, 金戸橋夫, 角利昭, 平林利郎, 小澤俊治, 広瀬和栄, 山本正幸, 角谷真奈美. カキ新品種'陽豊'[J]. 果樹試験場報告, 1991, 20: 49-61.
YAMANE H, KURIHARA A, NAGATA K, YAMADA M, KISHI T, YOSHINAGA K, MATSUMOTO R, KANATO K, SUMI T, HIRABAYASHI T, OZAWA T, HIROSE K, YAMAMOTO M, KAKUTANI M. New Japanese persimmon cultivar 'Youhou' [J]. Bulletin of the Fruit Tree Research Station, 1991, 20: 49-61.
- [26] 王永慧, 韩其谦, 周吉柱, 王文江, 郑利华. 柿树花芽分化的观察[J]. 烟台果树, 1985(3): 14-16.
WANG Yonghui, HAN Qiqian, ZHOU Jizhu, WANG Wenjiang, ZHENG Lihua. Observation of persimmon flower bud differentiation [J]. Yantai Fruit, 1985(3): 14-16.
- [27] 沈瑞骞. 柿树花芽分化的观察与研究[J]. 山西果树, 1992(4): 25-27.
SHEN Ruiqian. Observation and study on persimmon flower bud differentiation [J]. Shanxi Fruits, 1992(4): 25-27.
- [28] 张平贤, 张青林, 徐莉清, 郭大勇, 罗正荣. 部分柿属植物的早花现象观察[J]. 落叶果树, 2017, 49(3): 24-26.
ZHANG Pingxian, ZHANG Qinglin, XU Liqing, GUO Dayong, LUO Zhengrong. Early flowering phenomenon in *Diospyros* L. [J]. Deciduous Fruits, 2017, 49(3): 24-26.
- [29] ZHANG Y, YANG Y, GUO J, HU C, ZHU R. Taxonomic status of Deyangshi based on chromosome number and SRAP markers [J]. Scientia Horticulturae, 2016, 207: 57-64.
- [30] 西田光夫, 池田勇. カキの花芽分化に関する研究[J]. 東海近畿農試研報. 園芸, 1961, 6: 15-32.
NISHIDA T, IKEDA I. Flower-bud formation and development in the Japanese persimmon [J]. Tokai-Kinki Agricultural Experiment Station Horticulture Division Bulletin, 1961, 6: 15-32.
- [31] 原田久. カキの生育に関する生態的研究[J]. 静岡大学農学部園芸学研究室特別報告, 1985, 9: 1-66.
HARADA H. Studies on flower initiation and bud dormancy in annual growth cycle of Japanese persimmon [J]. Technical Bulletin of Department of Horticulture, Faculty of Agriculture, Shizuoka University, 1985, 9: 1-66.
- [32] KAJITA K, TAO R, YAMANE H. Sex expression in *Diospyros*: phenological control of flower bud development in *D. kaki* and *D. lotus* [J]. Acta Horticulturae, 2014, 1059: 89-96.
- [33] 李加茹, 孙鹏, 韩卫娟, 李芳东, 傅建敏, 刁松峰. 不完全甜柿'禅寺丸'花性别分化形态学关键时期的研究[J]. 园艺学报, 2016, 43(3): 451-461.
LI Jiaru, SUN Peng, HAN Weijuan, LI Fangdong, FU Jianmin, DIAO Songfeng. Morphological key period study on floral sex differentiation in pollination-constant and non-astringent persimmon 'Zenjimaruru' [J]. Acta Horticulturae Sinica, 2016, 43(3): 451-461.
- [34] 米森敬三, 亀田克巳, 杉浦明. カキの雌花, 雄花の着花特性について[J]. 園芸学会雑誌, 1992, 61(2): 303-310.
YONEMORI K, KAMEDA K, SUGIURA A. Characteristics of sex expression in monoecious persimmons [J]. Journal of the Japanese Society for Horticultural Science, 1992, 61(2): 303-310.
- [35] CHAILAKHYAN M K. Genetic and hormonal regulation of growth, flowering, and sex expression in Plants [J]. American Journal Botany, 1979, 66(6): 717-736.
- [36] IRISH E E, NELSON T. Sex determination in monoecious and dioecious plants [J]. The Plant Cell, 1989, 1(8): 737-744.
- [37] 米森敬三, 四方康範, 杉浦明. カキの雌雄性に関する研究(第2報) サイトカイニンによる雌性化の誘導 [J]. 園芸学会雑誌, 1990, 59(別冊2): 230-231.
YONEMORI K, YOMO Y, SUGIURA A. Sexuality in Japanese persimmon. 2. Induction of sex conversion in male flower by cytokinin treatment [J]. Journal of the Japanese Society for Horticultural Science, 1990, 59 (Suppl. 2): 230-231.
- [38] SUN P, LI J, DU G, HAN W, FU J, DIAO S, SUO Y, ZHANG Y, LI F. Endogenous phytohormone profiles in male and female floral buds of the persimmons (*Diospyros kaki* Thunb.) during development [J]. Scientia Horticulturae, 2017, 218: 213-221.
- [39] SONG Y, TIAN M, CI D, ZHANG D. Methylation of microRNA genes regulates gene expression in bisexual flower development in andromonoecious poplar [J]. Journal of Experimental Botany, 2015, 66(7): 1891-1905.
- [40] JALIGOT E, ADLER S, DEBLADIS E, BEULE T, RICHAUD F, ILBERT P, FINNEGAN E J, RIVAL A. Epigenetic imbalance and the floral developmental abnormality of the *in vitro*-regenerated oil palm *Elaeis guineensis* [J]. Annals of Botany, 2011, 108(8): 1453-1462.

- [41] 丁燕,韩振海,许雪峰,李天忠. 柿花发育相关的 MADS-box 基因克隆与表达[J]. 园艺学报, 2007, 34(1): 39-42.
DING Yan, HAN Zhenhai, XU Xuefeng, LI Tianzhong. Molecular cloning and expression of flowering-related MADS-box gene in *Diospyros kaki*[J]. Acta Horticulturae Sinica, 2007, 34(1): 39-42.
- [42] VIAENE T, VEKEMANS D, BECKER A, MELZER S, GEUTEN K. Expression divergence of the *AGL6* MADS domain transcription factor lineage after a core eudicot duplication suggests functional diversification[J]. BMC Plant Biology, 2010, 10(1): 148.
- [43] GAO T M, TAKEISHI H, KATAYAMA A, TAO R. Genetic transformation of Japanese persimmon with *FLOWERING LOCUS T (FT)* gene and *TERMINAL FLOWER 1 (TFL1)* homologues gene[J]. Acta Horticulturae, 2013, 996: 159-164.
- [44] KAJITA K, AKAGI T, YAMANE H, TAO R, YONEMORI K. The relationship between a maleness-associated region in *Diospyros lotus* L. and maleness of persimmon (*D. kaki* Thunb.) cultivars[J]. Horticultural Research, 2015, 14(2): 121-126.
- [45] 张平贤,何欢,罗正荣,杨勇,王仁梓,张青林. DISx-AF4S 标记在柿及其杂交后代性别鉴定中的有效性研究[J]. 园艺学报, 2016, 43(1): 47-54.
ZHANG Pingxian, HE Huan, LUO Zhengrong, YANG Yong, WANG Renzi, ZHANG Qinglin. Validation of male sex-linked DISx-AF4S marker in persimmon and F₁ progenies[J]. Acta Horticulturae Sinica, 2016, 43(1): 47-54.
- [46] ZHANG P X, YANG S C, LIU Y F, ZHANG Q L, XU L Q, LUO Z R. Validation of a male-linked gene locus (*OGI*) for sex identification in persimmon (*Diospyros kaki* Thunb.) and its application in F₁ progeny[J]. Plant Breeding, 2016, 135(6): 721-727.
- [47] YAMADA M. Persimmon genetic resources and breeding in Japan[J]. Acta Horticulturae, 2005, 685: 51-64.
- [48] AKAGI T, KATAYAMA-IKEGAMI A, YONEMORI K. Proanthocyanidin biosynthesis of persimmon (*Diospyros kaki* Thunb.) fruit[J]. Scientia Horticulturae, 2011, 130: 373-380.
- [49] PEI X, ZHANG Q, GUO D, LUO Z. Effectiveness of the RO2 marker for the identification of non-astringency trait in Chinese PCNA persimmon and its possible segregation ratio in hybrid F₁ population[J]. Scientia Horticulturae, 2013, 150: 227-231.
- [50] 黄瑜芳. 完全雄性柿种质的分类学地位及其作为花粉供体的应用潜力研究[D]. 武汉: 华中农业大学, 2013.
HUANG Yufang. The analysis of taxonomic status and application value as pollen donor of some androecious genotypes native to China[D]. Wuhan: Huazhong Agricultural University, 2013.
- [51] 広島県果樹試験場. 種苗特性分類調査報告書(カキ)[R]. 1979: 1-436.
Hiroshima Prefecture Fruit Tree Research Station. Investigation of classification on seedlings characteristics (persimmon) [R]. 1979: 1-436.
- [52] WOODBURN K R, ANDERSEN P C. Pollination and pollen source influence fruit of oriental persimmon 'Fuyu' and 'Tane-nashi'[J]. HortScience, 1996, 31(2): 218-221.
- [53] KIM J C, CHAE Y S, KANG S M. Selection of economic pollinizers for Fuyu sweet persimmon (*Diospyros kaki*)[J]. Acta Horticulturae, 1997, 436: 395-401.
- [54] 徐君驰,干建平,项俊,张青林,徐莉清,郭大勇,罗正荣. 中国甜柿遗传改良技术体系研究进展[J]. 园艺学报, 2017, 44(5): 987-998.
XU Junchi, GAN Jianping, XIANG Jun, ZHANG Qinglin, XU Liqing, GUO Dayong, LUO Zhengrong. Advance of genetic improvement technology for Chinese PCNA persimmon[J]. Acta Horticulturae Sinica, 2017, 44(5): 987-998.