江西农业大学学报　2024,46(3)　　http://xuebao.jxau.edu.cn
Acta Agriculturae Universitatis Jiangxiensis　　https://doi.org/10.3724/aauj.2024050

叶佳伟,高晓磊,周璇,等.攸县油茶远缘杂交亲和性的解剖学研究[J].江西农业大学学报,2024,46(3):558-571.
YE J W,GAO X L,ZHOU X,et al.Anatomical study on the compatibility of distant hybridization in *Camellia yuhsienensis*[J].Acta agriculturae universitatis Jiangxiensis,2024,46(3):558-571.

攸县油茶远缘杂交亲和性的解剖学研究

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摘要:【目的】为了探究攸县油茶(*Camellia yuhsienensis*)远缘杂交亲和性的生物学机制。【方法】研究以石果毛蕊山茶[*C. mairei* var. *lapidea*(Y.C.Wu)Sealy](SG)、海南油茶'海油3号'(*C. hainanica* 'Haiyou 3')(HN-3)和普通油茶'德油6号'(*C. oleifera* 'Deyou 6')(DY-6)为父本,采用常规授粉和混合授粉方式对攸县油茶(YX)进行自交和杂交授粉。采用流式细胞仪测定亲本倍性,通过离体萌发法测定父本花粉活力,运用显微技术观察花粉萌发及花粉管生长动态以及早期果实和胚珠发育动态,并田间调查各授粉组合的座果率和落果率。【结果】(1)YX为六倍体,SG为二倍体,HN-3为八倍体,DY-6为六倍体。(2)4种油茶花粉具有较强的活力和育性,其中花粉活力最高的是DY-6,为70.13%;花粉活力最低的是YX,为40.68%。(3)自交和杂交所有组合的花粉均能在柱头上正常萌发,且花粉管在授粉后12 h均生长到花柱基部,但自交和YX×SG的花粉管进入子房后生长受阻,无法到达胚珠;YX×HN-3和YX×DY-6的花粉管则在授粉后72h到达胚珠。(4)自交和杂交授粉后100 d内,YX×DY-6的果实横径、纵径最大,YX×SG的果实横径、纵径最小;YX×DY-6的败育胚珠数最少,平均为3.83枚/果;YX×SG的败育胚珠数最多,平均为6.00枚/果。(5)自交和杂交授粉后100 d内,YX×DY-6的座果率最高,为68.0%;YX×SG的座果率最低,为4.0%;YX×HN-3的座果率为16.0%,其落果高峰主要集中在授粉后0~30 d和40~70 d;混合授粉组合YX×SD的座果率为22.0%。【结论】自交和YX×SG的杂交障碍主要为受精前障碍,YX×HN-3的杂交障碍为受精前和受精后障碍;攸县油茶远缘杂交亲和性也与亲本倍性有关,其亲和性大小为YX(六倍体)×DY-6(六倍体)>YX×HN-3(八倍体)>YX×YX>YX×SG(二倍体);混合授粉方式能在一定程度上提高攸县油茶远缘杂交亲和性。研究结果为攸县油茶远缘杂交亲和性机理研究提供了细胞学基础,也为油茶远缘杂交育种工作提供理论参考。

关键词:攸县油茶;远缘杂交;亲和性;花粉管生长;座果率
中图分类号:S759.3　　**文献标志码:**A　　**开放科学(资源服务)标识码(OSID):**
文章编号:1000-2286(2024)03-0558-14

Anatomical study on the compatibility of distant hybridization in *Camellia yuhsienensis*

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收稿日期:2023-09-21　　**修回日期:**2024-01-24
基金项目:湖南省"揭榜挂帅"重大科技项目(2021NK1007)
Project supported by the Special Funds for Construction of Innovation Provinces in Hunan Province(2021NK1007)
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Abstract:[**Objective**]In order to explore the biological mechanism of compatibility of distant hybridization in *Camellia yuhsienensis*.[**Method**] In this study, *C. mairei* var. *lapidea* (Y. C. Wu) Sealy (SG), *C. hainanica* 'Haiyou 3' (HN-3) and *C. oleifera* 'Deyou6' (DY-6) were used as male parent, self- and cross-pollination of *C. yuhsienensis* were studied by conventional pollination and mixed pollination methods.The parental ploidy was measured by the flow cytometry, and the pollen viability of the male parent was examined by in vitro germination.The pollen germination, pollen tube growth and early fruit and ovule development were observed by microscopy, and the fruit setting rate and dropping rate of each pollination combination were investigated in the field.[**Result**](1)YX was hexaploidy, SG was diploid, HN-3 was octaploid and DY-6 was hexaploidy.(2)The pollen of four samples had strong viability and fertility, and the highest pollen viability was DY-6(70.13%), the lowest pollen viability was YX (40.68%).(3) Pollen of all combinations of self- and cross-pollination could germinate normally on stigma, and their pollen tubes grew to the base of style at 12h after pollination, but pollen tubes of self-pollination and YX×SG were blocked after entering ovary and could not reach ovule.The pollen tubes of YX×HN-3 and YX×DY-6 reached the ovule 72 h after pollination.(4) Within 100 d after self-and cross-pollination, transverse and longitudinal diameter of fruit of YX×DY-6 were the largest, while transverse and longitudinal diameter of fruit of YX×SG were the smallest.The number of abortive ovule of YX×DY-6 was the lowest, with an average of 3.83 ovule/fruit, and the number of abortive ovule of YX×SG was the highest, with an average of 6.00 ovule/fruit.(5) Within 100d after self- and cross-pollination, the fruit setting rate of YX×DY-6 was the highest(68.0%).The fruit setting rate of YX×SG was the lowest(4.0%).The fruit setting rate of YX×HN-3 was 16.0%, and the peak of fruit dropping was mainly concentrated on 0-30 d and 40-70 d after pollination, respectively. The fruit setting rate of mixed pollination combination YX×SD was 22.0%.[**Conclusion**]The hybridization barriers of self-pollination and YX×SG were mainly pre-fertilization barriers, while the hybridization barriers of YX×HN-3 were pre-and post-fertilization barriers. The compatibility of distant hybridization in *C. yuhsienensis* was also related to parental ploidy, and the compatibility size was YX (hexaploid) ×DY-6 (hexaploid) >YX×HN-3 (octaploid) >YX×YX>YX×SG (diploid). The mixed pollination method can improve the compatibility of distant hybridization in *C. yuhsienensis*. These results provided a cytological basis for the compatibility mechanism of distant hybridization of *C. yuhsienensis*, and also laid a theoretical reference for the work of distant hybridization breeding of oil tea.

Keywords: *Camellia yuhsienensis*; distant hybridization; compatibility; pollen tube growth; fruit setting rate

【研究意义】油茶(*Camellia oleifera*)为山茶科(Theaceae)山茶属(*Camellia*)常绿小乔木或灌木,是我国特有的重要木本食用油料树种[1]。近年来,国家对油茶产业政策支持力度持续加大,极大地助推油茶产业健康发展[2]。良种是油茶产业可持续发展的基础[3-4],是实现油茶高产、优质、高效的重要前提。经过前人的不懈努力,我国在油茶育种方面取得了巨大的进步,选育出了一大批油茶优良品种[5-9]。目前,我国油茶杂交育种研究主要集中在种内不同性状互补间的杂交[10],对油茶种间杂交障碍的研究相对较少。种间杂交障碍严重影响了油茶远缘杂交育种的研究。因此,开展油茶种间杂交障碍研究,对破解油茶远缘杂交育种技术难题具有重要的意义。【前人研究进展】杂交是油茶种质创新及新品种培育的重要途经,不少研究者从事杂交育种实践并对其杂交亲和性开展了研究。如幸伟年等[11]选用12个油茶良种为亲本,开展不同种间杂交育种试验,发现各杂交组合间亲和性存在较大差异,并确定了座果率高达90%的杂交组合。韦晓娟等[12]通过金花茶(*C. chrysantha*)与其他山茶属植物的杂交育种试验,发现普通金花茶与宛田红花油茶(*C. polyodonta*)具有较高的亲和性,与越南抱茎山茶(*C. amplexicaulis*)亲和性不高,但金花茶与广宁红花油茶(*C. semiserrata*)、普通油茶'岑软3号'(*C. oleifera* 'Cenruan 3')、黑牡丹茶花(*C. japonica* 'Heimudan')具有一定的亲和性。韩国勇[13]对茶梅(*C. sasanqua*)品种间杂交亲和性进行研究,发现'晚霞'×'银元'的杂交亲和性最好,结实率达100%,另有6对杂交组合无法获得杂交果实,表现出严重不亲和性。杂交不亲和性主要分为受精前障碍和受精后障碍[14]。如柴弋霞等[15]研究发现紫花含笑(*Mi-*

chelia crassipes)×阔瓣含笑(*M. platypetala*)主要存在受精前障碍,导致其座果率低;薛金燕[16]研究发现喀西茄(*Solanum aculeatissimum* Jacquem.)花粉在栽培茄柱头上能正常萌发并进入子房完成受精,但在原胚形成及分裂期出现败育,为受精后障碍。

【本研究切入点】虽然前人开展了攸县油茶生殖生物学研究[17],但是其远缘杂交亲和性研究报道较少,为进一步阐明攸县油茶远缘杂交亲和性的生物学机制。【拟解决的关键问题】研究选择不同倍性的杂交亲本并采用不同授粉方式对攸县油茶进行自交和杂交授粉,以期分析攸县油茶远缘杂交存在的杂交障碍,也为油茶杂交亲本选配及育种工作提供理论依据。

1 材料与方法

1.1 试验材料

在湖南省长沙市中南林业科技大学油茶科研基地开展杂交授粉试验,母本为16 a生攸县油茶(代号:YX),其春季2—3月开花,树形矮小,果壳薄,出籽率高,含油率高,抗炭疽病,是一种非常优良的育种种质资源[17]。父本均为15 a生,分别为石果毛蕊山茶(代号:SG)、'海油3'油茶(代号:HN-3)和'德油6号'油茶(代号:DY-6)。石果毛蕊山茶在春季2—3月开花,花为红色,果大、皮厚;'海油3号'在冬季11-12月开花,果大、皮褐色;'德油6号'在冬季12月至翌年1月开花,果大、皮薄、出籽率高。各授粉亲本的花粉于盛花期采集后放于-20 ℃冰箱贮藏备用。

表1 攸县油茶自交和杂交组合

Tab.1 Self-pollination and cross-pollination combinations of *C. yuhsienensis*

组合编号 Combination number	母本×父本 Female parent×Male parent
1	YX×YX
2	YX×SG
3	YX×HN-3
4	YX×DY-6

1.2 试验方法

1.2.1 亲本花器官形态特征及其倍性测定

分别于盛花期随机采摘各亲本20朵完全开放的花,用Nikon D7500照相机对各亲本花器官进行拍照,并测量其花瓣长度、花瓣宽度、花瓣数、花冠直径、花丝长度、花柱长度和雄蕊数。

亲本倍性的测定采用流式细胞仪,采集各亲本油茶嫩叶,具体参照叶天文等[18]的方法,用流式细胞仪(Sysmex,Germany)检测5 000~10 000个细胞,记录每个样品DNA含量峰值及变异系数(CV,%),样品倍性分析图像由Flomax软件(Sysmex,Germany)生成。

1.2.2 亲本花粉活力测定

花粉活力测定方法为离体萌发法[19]:将待测花粉均匀撒播于1%琼脂+10%蔗糖+0.01%硼酸的培养基上,置于25 ℃恒温箱中培养,4 h后于Olympus BX-51型显微镜下观察花粉的萌发状况。

1.2.3 花粉萌发及花粉管在花柱和子房中的生长

(1)荧光压片观察法。于2023年2月26日至27日08:30~11:30选择攸县油茶含苞待放的花朵且雌蕊发育正常的材料,去雄后采用人工点授的方法,用棉签蘸取授粉试验品种的花粉,抹到已去雄的攸县油茶柱头上,再套袋并挂牌标记[20]。分别采集自交和杂交授粉后2,6,10,12,24,48,72,96 h的雌蕊10个(花柱和子房),用卡诺氏固定液(乙醇:冰醋酸=3:1)固定12 h后转入70%酒精中4 ℃保存备用。参照邹锋[17]的方法并稍作修改,将固定雌蕊经50%、30%酒精复水后用8 mol/L NaOH软化2 h,蒸馏水冲洗2遍,0.5%苯胺蓝染色4 h,压片后在Olympus BX-51型荧光显微镜下观察柱头上花粉的萌发及花粉管在花柱和子房中的生长情况并拍照。

(2)切片荧光法。具体参照高超[20]的方法并稍作修改:根据花粉管的荧光压片观察结果选取授粉后72 h所采样的雌蕊材料,进行常规石蜡切片,切片厚度为12 μm。将切片脱蜡、复水后,用0.3%苯胺蓝溶液染色2 h,后将切片直接在Olympus BX-51型荧光显微镜下观察并拍照。

1.2.4　早期果实及胚珠发育

分别采集各授粉组合授粉后0,7,14,21,30,40,50,60,70,85,100 d的幼果10个,利用体视显微镜拍照并用游标卡尺测量其横径、纵径;将授粉后30,40,50,60,70,85,100 d果实中的胚珠取出,采用体视显微镜拍照后,运用Image J软件测量其面积,并统计每个授粉组合果实中的平均败育胚珠数。

1.2.5　田间座果率和落果率统计

授粉试验于2023年3月1日至2日08:30—11:30进行,具体有两种方法:(1)常规授粉:选择攸县油茶含苞待放且雌蕊发育正常的材料,去雄后采用人工点授的方法,用棉签蘸取授粉试验品种的花粉,抹到已去雄的攸县油茶柱头上,再套袋并挂牌标记,7 d后拆袋[20]。(2)混合授粉[21]:用有活力的SG花粉和无活力的DY-6花粉混合后的花粉(代号:SD)进行授粉。分别调查不同授粉组合在授粉后30,40,50,60,70,85,100 d的座果率及落果率。

1.2.6　数据分析

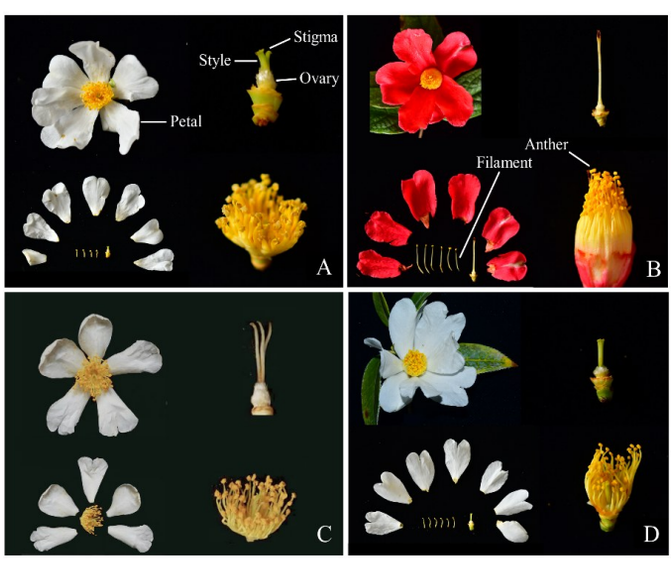
采用Excle 2016进行数据处理,利用SPSS 26.0软件进行数据分析和检验并运用Origin 2021软件绘图。

2　结果与分析

2.1　亲本花器官形态特征及其倍性

成熟的油茶雌蕊由柱头、花柱和子房组成,雄蕊由花药和花丝组成,其中SG的花朵为红色,YX、HN-3和DY-6的花朵为白色(图1)。由表2可知,各亲本花朵由大到小排序为SG>DY-6>HN-3>YX,其中YX花柱最短,为2.83 mm,SG花柱最长,为36.68 mm,且SG花柱比花丝长,YX、HN-3和DY-6花柱比花丝短;SG雄蕊数最多,为170.63个,YX雄蕊数最少,为54.75个。

流式细胞仪测定结果表明:YX为六倍体,SG为二倍体,HN-3为八倍体,DY-6为六倍体,且各样品峰值的变异系数(CV)都在5%以内,说明该检测结果较为准确。



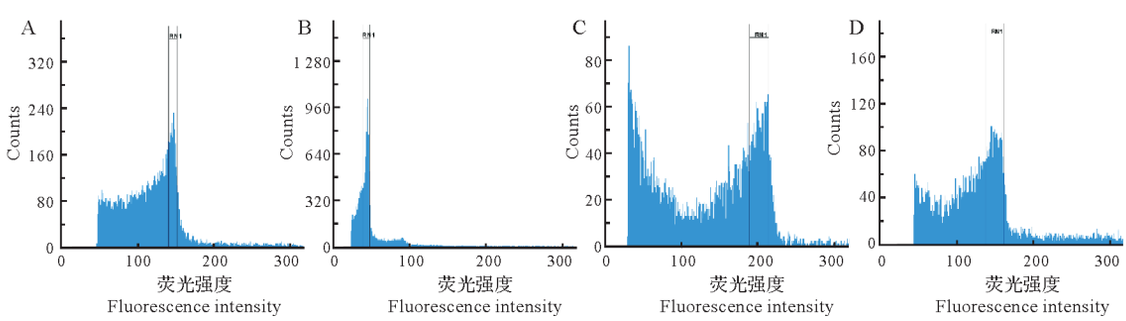
A:YX;B:SG;C:HN-3;D:DY-6。A:YX;B:SG;C:HN-3;D:DY-6.

图1　亲本花器官的形态特征

Fig.1　Morphological characteristics of floral organs of parents

2.2　亲本花粉活力

培养4 h后,DY-6花粉活力最高,为70.13%;YX花粉活力最低,为40.68%;SG和HN-3花粉活力分别为46.04%、58.94%(图3E)。花粉活力测定结果表明4种油茶花粉具有较强的活力和育性,可用于人工授粉。



A:YX为六倍体;B:SG为二倍体;C:HN-3为八倍体;D:DY-6为六倍体。

A:YX was hexaploid;B:SG was diploid;C:HN-3 was octaploid;D:DY-6 was hexaploidy.

图2　亲本倍性

Fig.2　Ploidy of parents

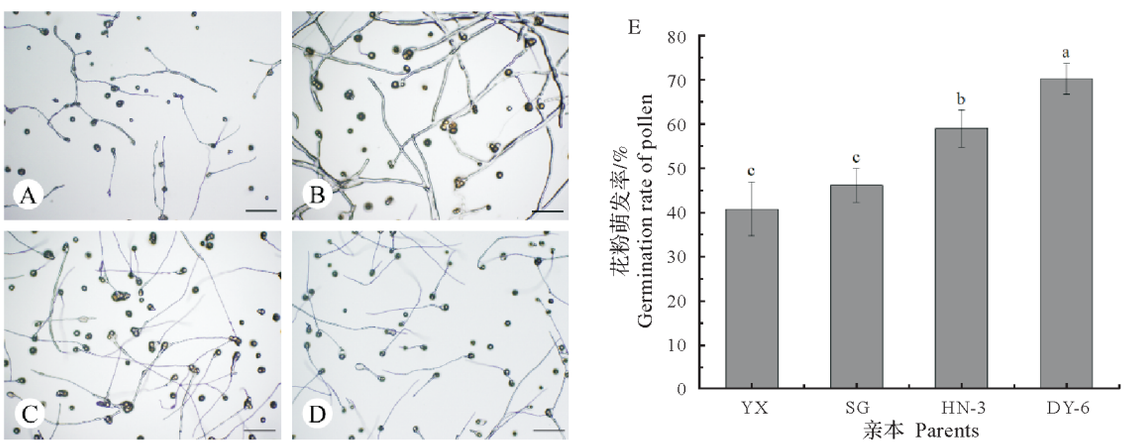
表 2 亲本花器官形态特征

Tab.2 Morphological characteristics of floral organs of parents

亲本 Parents	花瓣长度/mm Petal length	花瓣宽度/mm Petal width	花瓣数 Number of petal	花冠直径/mm Corolla diameter	花丝长度/mm Filament length	花柱长度/mm Style length	雄蕊数 Number of Stamens
YX	25.64±1.96^{c}	19.89±1.56^{c}	6.70±0.48^{b}	48.17±3.18^{c}	5.76±1.07^{c}	2.83±0.09^{d}	54.75±4.46^{d}
SG	59.71±6.76^{a}	34.07±4.58^{a}	6.90±0.32^{ab}	94.02±4.19^{a}	29.20±5.38^{a}	36.68±1.12^{a}	170.63±11.99^{a}
HN-3	39.68±3.63^{b}	25.65±2.10^{b}	6.80±0.42^{ab}	64.64±4.42^{b}	15.59±0.82^{b}	10.85±1.03^{b}	80.38±6.19^{c}
DY-6	40.10±3.90^{b}	26.37±2.33^{b}	7.20±0.42^{a}	67.72±4.12^{b}	14.76±3.25^{b}	7.98±0.98^{c}	111.38±4.66^{b}

同列不同字母表示差异显著(P<0.05)。

The different lowercase letter in the same column indicated significant difference(P<0.05).



A:YX;B:SG;C:HN-3;D:DY-6;E:花粉萌发率;比例尺=500 μm。

A:YX;B:SG;C:HN-3;D:DY-6;E:The germination rate of pollen;bars=500 μm.

图 3 亲本花粉活力

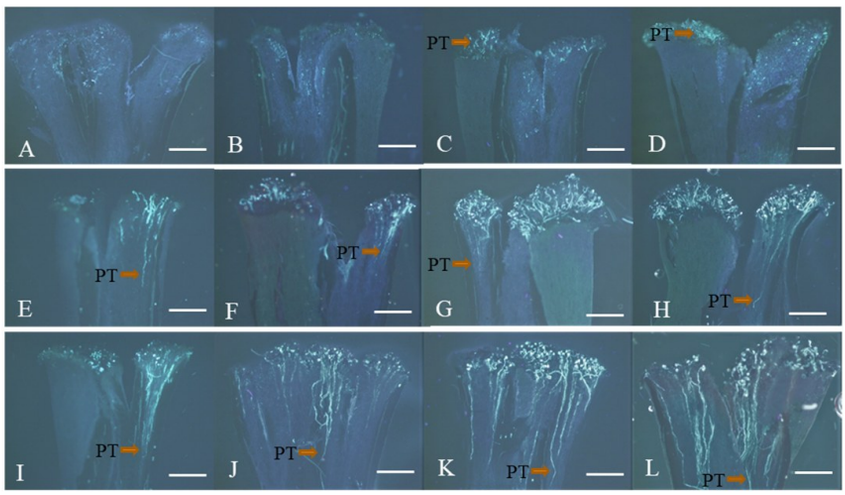
Fig.3 Pollen viability of parents

2.3 攸县油茶自交、杂交授粉后花粉萌发及花粉管生长观察

自交和杂交授粉后,各组合的花粉粒均能在柱头上萌发并产生花粉管,但花粉管的萌发时间和生长速度存在差异。在自交后 2 h,花粉粒未在柱头上萌发(图 4A);在杂交后 2 h,YX×HN-3(图 4C)和 YX×DY-6(图 4D)的柱头上有少量花粉粒萌发,但在 YX×SG(图 4B)的柱头上花粉粒未萌发。随后,在自交后 6 h,有少量花粉粒在柱头上萌发,一些花粉管已进入花柱道并生长到花柱长度的约 1/3(图 4E);在杂交后 6 h,YX×HN-3 和 YX×DY-6 的柱头上有大量花粉粒萌发,其中 YX×HN-3 的花粉管生长到花柱长度的约 1/4(图 4G),YX×DY-6 的花粉管生长到花柱长度的约 1/2(图 4H);在 YX×SG 中只有少量花粉粒在柱头上萌发,花粉管生长到花柱长度的约 1/4(图 4F)。在授粉后 10 h,自交(图 4I)和 YX×SG(图 4J)的花粉管生长到花柱长度的约 1/2;YX×HN-3(图 4K)和 YX×DY-6(图 4L)的花粉管生长到花柱长度的约 3/4。

在授粉后 12 h,自交(图 5A)和杂交所有组合(图 5B、图 5C、图 5D)的花粉管均生长到达花柱基部,且 YX×DY-6 有少量花粉管已经穿过花柱基部,继续向下生长(图 5D)。在授粉后 24 h,自交和杂交所有组合的花粉管均已穿过花柱基部进入子房,但自交(图 5E)和 YX×SG(图 5F)的花粉管生长速度慢于 YX×HN-3(图 5G)和 YX×DY-6(图 5H)。在授粉后 48 h,自交(图 5I)和 YX×SG(图 5J)的花粉管生长变缓甚至停滞,而 YX×HN-3(图 5K)和 YX×DY-6(图 5L)的花粉管继续生长,逐渐向胚珠靠近。

在授粉后 72 h,自交花粉管生长到达子房室上端(图 6A、图 6E、图 6J),YX×SG 的花粉管则生长停滞(图 6B、图 6F、图 6K),而 YX×HN-3(图 6C、图 6G、图 6L)和 YX×DY-6(图 6D、图 6H、图 6M)的花粉管已经生长到达胚珠。在授粉后 96 h,自交(图 7A、图 7E)和 YX×SG(图 7B、图 7F)的花粉管任没到达胚珠,其花粉管末端出现膨大、卷曲、波浪状等不亲和现象(图 7E、图 7F);YX×HN-3(图 7C、图 7G)和 YX×DY-6(图 7D、图 7H)的花粉管已经通过胚珠珠孔端进入胚囊。

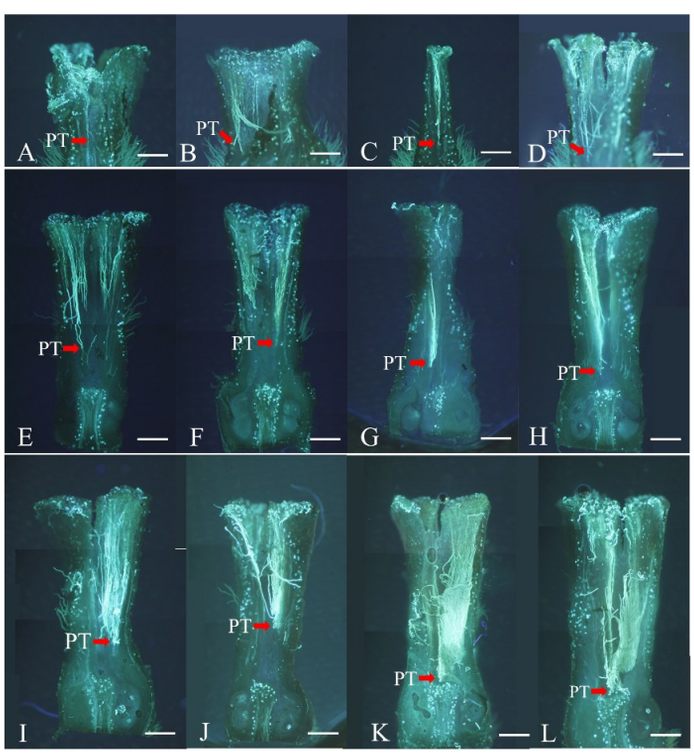


A,B,C,D 分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 2 h 花粉管生长;E,F,G,H 分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 6 h 花粉管生长;I,J,K,L 分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 10 h 花粉管生长;PT=花粉管;比例尺=500 μm。

A,B,C,D respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 2 h after pollination; E, F, G, H respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 6 h after pollination; I, J, K, L respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 10 h after pollination; PT=pollen tube; bars=500 μm.

图 4　攸县油茶自交、杂交授粉后 2,6,10 h 花粉萌发及花粉管生长观察

Fig.4　Observation of pollen germination and pollen tube growth 2, 6, 10 h after self-pollination and cross-pollination in *C. yuhsienensis*

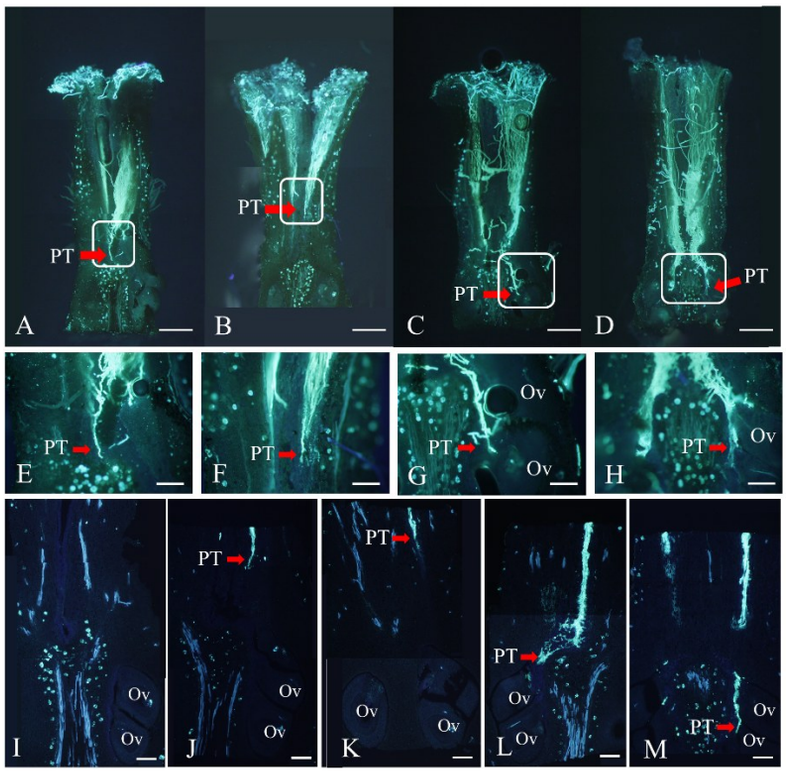


A,B,C,D 分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 12 h 花粉管生长;E,F,G,H 分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 24 h 花粉管生长;I,J,K,L 分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 48 h 花粉管生长;PT=花粉管;比例尺=500 μm。

A,B,C,D respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 12 h after pollination; E, F, G, H respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 24 h after pollination; I, J, K, L respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 48 h after pollination; PT=pollen tube; bars=500 μm.

图 5　攸县油茶自交、杂交授粉后 12,24,48 h 花粉管生长观察

Fig.5　Observation of pollen tube growth 12, 24, 48 h after self-pollination and cross-pollination in *C. yuhsienensis*

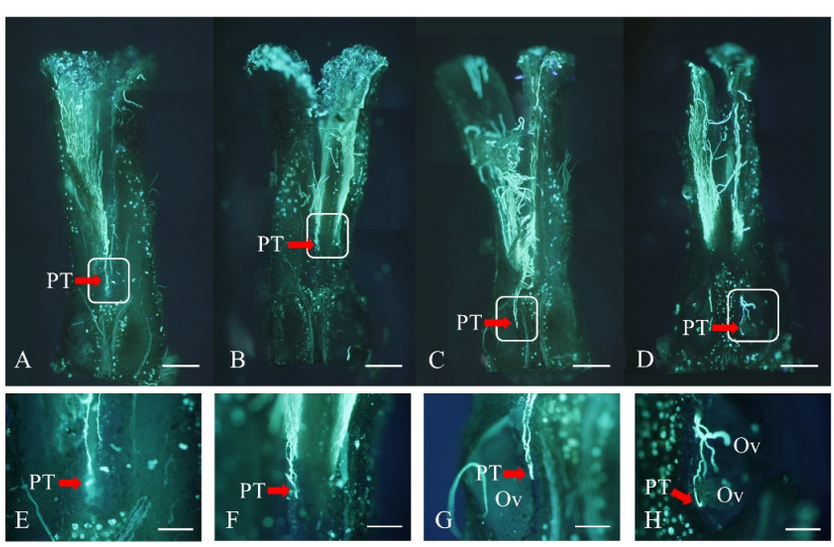


A,B,C,D 分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 72 h 花粉管生长;E,F,G,H 分别为图 A、B、C、D 中白色方框区域内的放大视图;I,J,K,L,M:分别为不授粉、YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 72 h 花粉管在花柱基部及子房中的生长;PT=花粉管;Ov=胚珠;比例尺:A,B,C,D=500 μm;E-M=200 μm。

A,B,C,D respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 72 h after pollination; E,F,G,H respectively are the enlarged views in the white circle area in A,B,C and D; I,J,K,L,M respectively are the growth of pollen tubes of non-pollination, YX×YX, YX×SG, YX×HN-3 and YX×DY-6 at the base of style and ovary 72 h after pollination; PT=pollen tube; Ov=ovule; bars: A,B,C,D=500 μm; E-M=200 μm.

图 6 攸县油茶自交、杂交授粉后 72 h 花粉管生长观察

Fig.6 Observation of pollen tube growth 72 h after self-pollination and cross-pollination in *C. yuhsienensis*



A,B,C,D:分别为 YX×YX、YX×SG、YX×HN-3 和 YX×DY-6 授粉后 96 h 花粉管生长;E,F,G,H:分别为图 A、B、C、D 中白色方框区域内的放大视图;PT=花粉管;Ov=胚珠;比例尺:A,B,C,D=500 μm;E,F,G,H=200 μm。

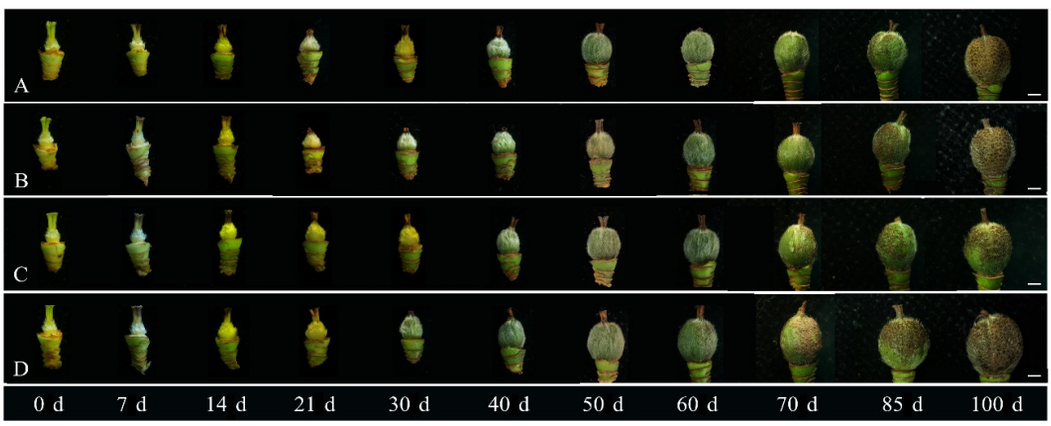
A,B,C,D: Respectively are the growth of pollen tubes of YX×YX, YX×SG, YX×HN-3 and YX×DY-6 96 h after pollination; E,F,G,H: Respectively are the enlarged views in the white circle area in A,B,C and D; PT=pollen tube; Ov=ovule; bars: A,B,C,D=500 μm; E,F,G,H=200 μm.

图 7 攸县油茶自交、杂交授粉后 96 h 花粉管生长观察

Fig.7 Observation of pollen tube growth 96h after self-pollination and cross-pollination in *C. yuhsienensis*

2.4　攸县油茶自交、杂交授粉后果实及胚珠发育观察

自交、杂交授粉后 100 d 内,各授粉组合果实的横径和纵径生长呈“慢-快-慢”的生长趋势,且纵径生长速度比横径生长速度快,其中 YX×DY-6 果实生长最快,其横径为 8.17 mm,纵径为 8.93 mm;YX×SG 果实生长最慢,其横径为 6.17 mm,纵径为 6.91 mm(图 8 和图 9)。

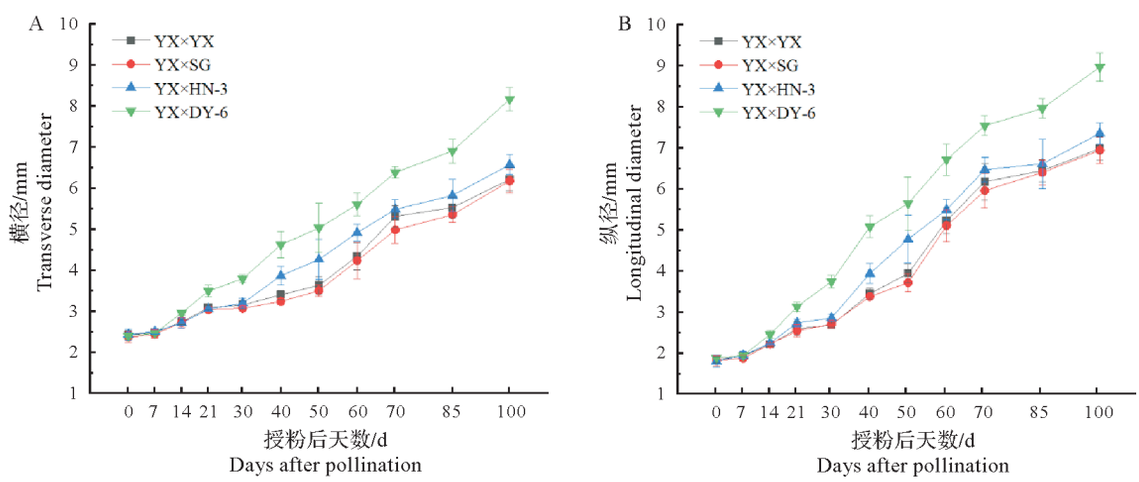


A:YX×YX;B:YX×SG;C:YX×HN-3;D:YX×DY-6;比例尺=2 mm。

A:YX×YX;B:YX×SG;C:YX×HN-3;D:YX×DY-6;bars=2 mm.

图 8　攸县油茶自交、杂交授粉后果实发育情况

Fig.8　Development of fruit after self-pollination and cross-pollination in *C. yuhsienensis*



A:果实横径;B:果实纵径。

A:The transverse diameter of fruit;B:The longitudinal diameter of fruit.

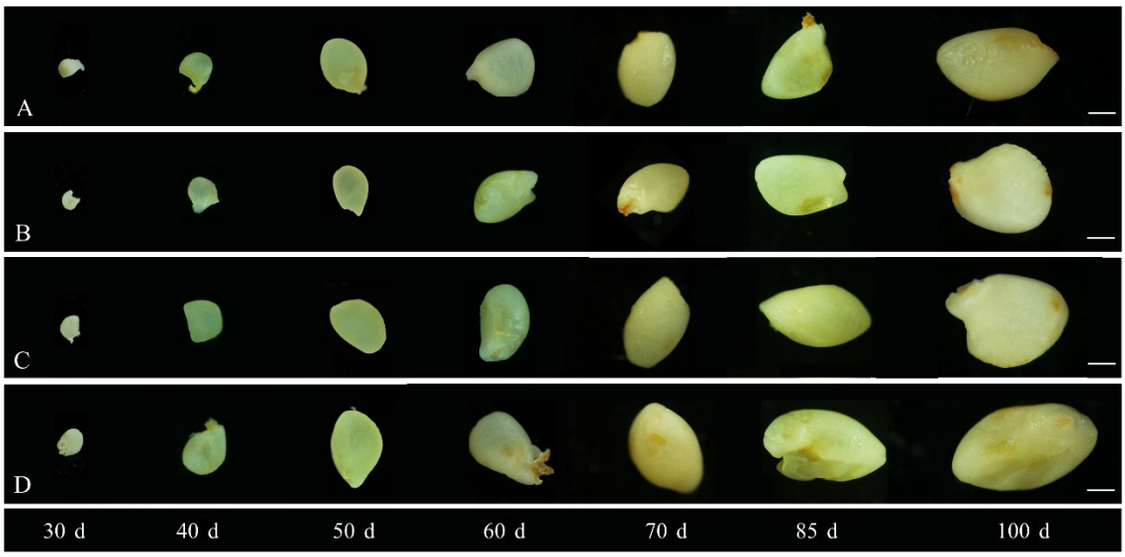
图 9　攸县油茶自交、杂交授粉后果实大小动态观测

Fig.9　Dynamic observation of fruit size after self-pollination and cross-pollination in *C. yuhsienensis*

在果实生长发育过程中,各授粉组合的胚珠随着果实一起不断膨大(图 10),其中 YX×DY-6 的胚珠最大,授粉后 100 d 时,其面积为 3 835 504.37 μm^2;YX×SG 的胚珠最小,授粉后 100 d 时,其面积为 2 803 642.70 μm^2(图 11)。此外,各授粉组合果实中均有败育胚珠,但出现的时间不同,且败育胚珠数随着果实生长发育不断增多,到授粉后 100 d 时,YX×SG 败育胚珠数达到了 6.00 枚/果,YX×DY-6 败育胚珠数最少,为 3.83 枚/果(表 3)。

2.5　攸县油茶自交、杂交授粉后座果率和落果率动态变化

通过对攸县油茶自交和杂交授粉后的座果率调查发现,授粉后 100 d 内,座果率最高的授粉组合是 YX×DY-6,为 68.0%;座果率最低的授粉组合是 YX×SG,仅为 4.0%;YX×YX 和 YX×HN-3 的座果率分别为 8.0%、16.0%。混合授粉组合 YX×SD 的座果率较 YX×SG 有明显提高,为 22.0%(图 12A)。落果率调查结果表明(图 12B),除 YX×DY-6 外,其余组合在授粉后 0~40 d 的落果率均达到 40% 以上,其中 YX×SG 的落果率最高,为 90.0%;授粉后 40~70 d,YX×HN-3 的落果率最高,为 32%;授粉后 70~100 d,YX×HN-3 和 YX×SD 的落果率最高,为 6%。



A：YX×YX；B：YX×SG；C：YX×HN-3；D：YX×DY-6；比例尺=500 μm。

A：YX×YX；B：YX×SG；C：YX×HN-3；D：YX×DY-6；bars=500 μm.

图 10 攸县油茶自交、杂交授粉后胚珠发育情况

Fig.10 Development of ovule after self-pollination and cross-pollination in *C. yuhsienensis*

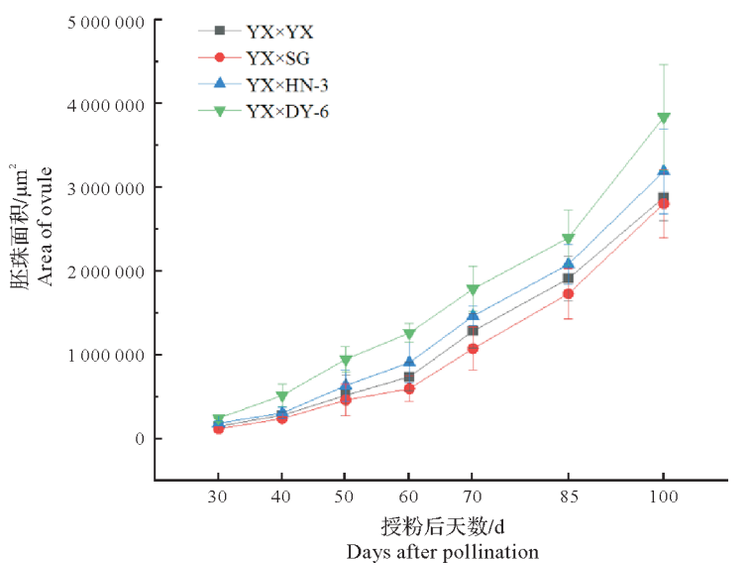


图 11 攸县油茶自交、杂交授粉后胚珠面积大小动态观测

Fig.11 Dynamic observation of ovule area size after self-pollination and cross-pollination in *C. yuhsienensis*

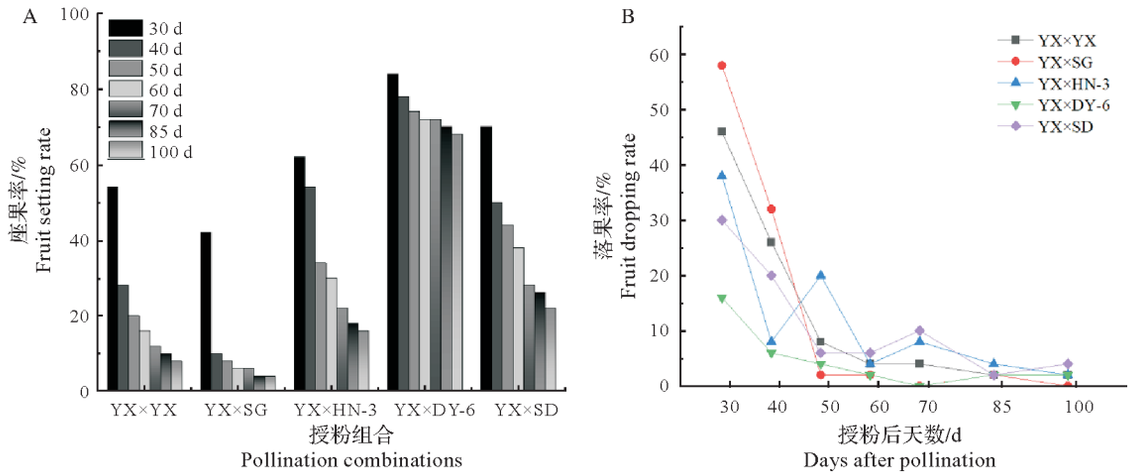
表 3 攸县油茶自交、杂交授粉后的每果平均败育胚珠数

Tab.3 The average number of abortive ovule per fruit after self-pollination and cross-pollination in *C. yuhsienensis*

授粉组合 Pollination combinations	授粉后不同时期败育胚珠数 The number of abortive ovule in different periods after pollination						
	30 d	40 d	50 d	60 d	70 d	85 d	100 d
YX×YX	0.17±0.41^{a}	0.40±0.55^{b}	1.40±0.55^{b}	2.80±0.45^{b}	4.17±0.41^{ab}	5.00±0.63^{ab}	5.50±0.55^{a}
YX×SG	0.33±0.52^{a}	2.20±0.45^{a}	2.80±0.45^{a}	4.40±0.55^{a}	5.00±0.63^{a}	5.33±0.52^{a}	6.00±0.63^{a}
YX×HN-3	0	0.20±0.45^{b}	1.40±0.55^{b}	2.60±0.55^{b}	3.83±0.75^{b}	4.50±0.55^{b}	5.33±0.52^{a}
YX×DY-6	0	0	0.40±0.55^{c}	1.00±0.71^{c}	1.67±0.52^{c}	2.67±0.52^{c}	3.83±0.41^{b}

同列不同字母表示差异显著（P<0.05）。

The different lowercase letter in the same column indicated significant difference（P<0.05）.



A:座果率;B:落果率。

A:Fruit setting rate;B:Fruit dropping rate.

图 12　攸县油茶各授粉组合的座果率和落果率动态观测

Fig.12　Dynamic observation of fruit setting rate and dropping rate of each pollination combination in *C. yuhsienensis*

3　结论与讨论

3.1　攸县油茶杂交亲和性分析

在植物远缘杂交过程中,不能完成受精作用并发育形成合子的障碍称为受精前障碍[22]。受精前障碍具体可分为三类:首先,花粉不能萌发或只有极少一部分萌发[23-24];其次,花粉萌发后花粉管生长受阻或生长异常[24-26];最后,没有完整的双受精过程[27-28]。受精前障碍在作物、果树及花卉等植物的远缘杂交中普遍存在。何丽等[29]对白菜型油菜(*Brassica campestris*)与芥菜型油菜(*B. juncea*)进行远缘杂交时发现花粉粒不能附着在柱头上、花粉粒不能正常萌发。美味猕猴桃(*Actinidia chinensis* var.*diliciosa*)'徐香'与长果猕猴桃(*A. longicarpa*)进行远缘杂交时,其花粉管在花柱中生长出现波纹状弯曲、停止生长等异常现象[30]。李娜等[31]在榆叶梅(*Prunus triloba*)与李属(*Padus*)的杂交中发现其花粉萌发延迟,花粉管缠绕、末端膨大等现象,结实率总体较低,主要受到受精前障碍的影响。欧哲等[32]研究发现以单叶蔷薇(*Rosa persica*)为母本与月季组进行远缘杂交时,发现其存在受精前障碍。在本研究中,自交和杂交所有组合的花粉均能在柱头上萌发且其花粉管在授粉后 12 h 到达花柱基部,但自交和 YX×SG 2 个组合的花粉管在进入子房后生长受阻,无法到达胚珠,存在受精前障碍,从而导致其座果率较低。同样,杨志玲等[33]观察到长瓣短柱茶×浙江红山茶(*C. chekiangoleosa*)杂交后其花粉管虽能在柱头上正常萌发,但花粉管在花柱中生长弯曲,伸长缓慢,表现为受精前障碍。胡禾丰等[25]以连蕊茶组为母本进行远缘杂交时也发现花粉管萌发后伸长缓慢且难以定向生长,花粉管出现扭曲或先端膨大等异常现象,并且各杂交组合座果率为 0,表明存在严重的受精前障碍。

在植物远缘杂交中,受精过程完成后却经常会有影响种子发育或结实的异常现象发生,统称为受精后障碍[34]。受精后障碍是植物杂交结实率低的原因之一。王丹等[35]发现白菜(*B. pekinesis*)×芥菜型油菜的杂种胚和胚乳败育。孙春青等[36]发现栽培菊花'奥运天使'(*Dendranthema grandiflorum* 'Aoyuntianshi')×野路菊(*D. japonense*)杂交授粉后大量胚胎在其发育早期异常降解,导致结实率低。本研究中,YX×HN-3 授粉后 0~30 d 的落果率为 38%,主要受到受精前障碍的影响,可能原因是其花粉管与胚珠结合率低或受精失败;授粉后 40~70 d 落果率为 32%,且从授粉后 40 d 开始不断出现败育的胚珠,这可能受到受精后障碍的影响。这与杂交石竹(*Dianthus hybridus*)[28]、梅(*P. mume*)与山桃(*P. davidiana*)[37]、黄菖蒲(*I. pseudacorus*)与西伯利亚鸢尾(*I. sibirica*)[38]授粉后都观察到存在受精前和受精后障碍的结果类似。

植物杂交亲和性可能与杂交亲本的染色体倍性有关[39-40]。远缘杂交时亲本材料的选择至关重要[22],研究发现杂交亲本的染色体倍性与杂交亲和性密切相关,亲本染色体倍性越接近的杂交越容易成功,差

异越大的生殖障碍越严重[41-42]。赵丹等[43]在柿(*Diospyros*)的研究中发现相同倍性的父本与母本杂交亲和性大于不同倍性间父本与母本杂交。朱炜等[44]对不同倍性芍药(*Paeonia lactiflora* Pall.)品种间杂交时,也表明相同倍性亲本的杂交亲和性高于不同倍性亲本。本研究中,YX为六倍体,SG为二倍体,二者杂交后亲和性较低;而DY-6和YX均为六倍体,二者杂交后亲和性较高。因此,在油茶远缘杂交育种时,可考虑选择倍性接近或相同、配合力高的材料作亲本。

3.2 植物远缘杂交障碍的克服

利用不同杂交授粉方式能在一定程度上提高植物远缘杂交的亲和性。辐射授粉能在一定程度上提高浙江红花油茶果实的出籽率[45]。延迟授粉可有效克服牡丹(*P. suffruticosa* Andr.)、芍药远缘杂交育种的受精前障碍[46]。切割花柱授粉可较好地克服紫斑百合(*Lilium nepalense*)与东方百合和OT百合远缘杂交的受精前障碍[47]。重复授粉对月季(*R. hybrida*)的受精前障碍克服效果最佳[40]。混合授粉时,亲和花粉被认为可有效降低柱头对不亲和花粉的识别和抑制反应,为不亲和花粉的花粉管生长创造有利环境,从而克服远缘杂交的障碍[48-49],提高结实率。如刘青林[21]将无活力的亲和花粉与有活力的不亲和花粉混合后进行授粉,发现这种方法可以提高梅花(*P. mume*)远缘杂交的结实率。在地被菊(*D. morifolium* Ramat)育种过程中,用混合授粉方法可使原本不亲和的杂交组合变为亲和[50]。本研究中,混合授粉组合YX×SD在授粉后100 d时的座果率较YX×SG组合提高了18%,表明混合授粉方式能在一定程度上提高攸县油茶远缘杂交的亲和性,可应用于今后油茶远缘杂交育种工作。

致谢:长沙市"揭榜挂帅"重大科技项目课题(Kq2102007-02)同时对本研究给予了资助,谨致谢意!

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