

# 中国鹅掌楸与北美鹅掌楸 种间杂交的胚胎学研究\*

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**摘要** 应用控制授粉、常规石蜡切片技术研究了中国鹅掌楸与北美鹅掌楸种间杂交后的胚胎发育过程。结果表明:(1)控制授粉后花粉正常萌发,并有较多的花粉管进入柱头,但花粉管在花柱内生长缓慢甚至解体;(2)花粉萌发6d后花粉管经花柱沟、珠孔塞和珠心冠原组织进入胚囊进行珠孔受精,20d时游离核胚乳细胞化,发育形成2~4个细胞厚的狭长组织,35d后可观察到球形胚;(3)随着胚和胚乳的发育,珠被发育形成种皮。授粉时,胚珠具2层珠被,外、内珠被均由外表皮、中层和内表皮组成;胚与胚乳发育期间,珠被各层发生了一系列的变化,最终种皮由外珠被的外表皮细胞形成的膜层和中层细胞形成的气室以及内、外珠被内表皮细胞形成的硬化层组成;(4)受精时成熟胚囊及到达珠孔的花粉管的数目较少,受精频率较低;(5)受精过程基本正常,但胚和胚乳发育不协调,导致胚在发育早期败育,很难观察到后期胚的发生。

**关键词** 中国鹅掌楸、北美鹅掌楸、种间杂交、胚胎发育

杂交育种工作的关键在于能获得具有生活力的杂交种子,了解有性杂交过程中的亲合性及其胚胎发育状况是杂交育种中的一个重要课题。早在50年代,通过杂交手段成功地培育了鹅掌楸属的 $F_1$ 代优良品种,但缺乏系统的胚胎学资料。自1987年以来,作者研究了中国鹅掌楸(*Liriodendron chinense* (Hesml.) Sarg.)与北美鹅掌楸(*Liriodendron tulipifera* L.)的胚胎发生过程,在此基础上进行不同组合的种间杂交的胚胎学研究,以期选择最佳杂交组合,查明发生杂交障碍的发育阶段,为探讨如何提高结籽率提供详实的胚胎学资料。本文报道的是中国鹅掌楸×北美鹅掌楸组合,北美鹅掌楸×中国鹅掌楸组合将另文报道。

## 1 材料和方法

### 1.1 实验材料

以35年生中国鹅掌楸为母本,50年生北美鹅掌楸为父本。

### 1.2 室内水培

采集中国鹅掌楸树冠中上层花枝置于玻璃瓶内水培5d。

### 1.3 控制授粉

分为室内(离体)与室外(活体)授粉两部分。将即将开放的花的雄蕊群摘除,然后授以北美鹅掌楸花粉。

### 1.4 材料固定

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室内花枝于授粉后每隔 2 h 固定一次 (FAA 或 4% 戊二醛溶液) 雌蕊, 直至 128 h; 室外授粉的花枝从第 5 天开始, 以后每逢 15、20、23、26、35、48、53、65、100 d 采样固定。

### 1.5 制片

(1) 常规石蜡切片, 番红—固绿对染, OLYMPUS AHB-LB-2 型显微镜观察拍照。

(2) 4% 戊二醛溶液固定的材料, 经临界点干燥, 喷金制样, 用 PHILIPS 505 扫描电镜观察拍照。

### 1.6 胚乳发育率计算

$$\text{胚乳发育率}(\%) = \frac{\text{有胚乳的胚珠数}}{\text{胚珠总数}} \times 100\%$$

## 2 观察结果

### 2.1 北美鹅掌楸的花粉在中国鹅掌楸柱头上的行为

控制授粉 2 h 后, 北美鹅掌楸的花粉在中国鹅掌楸雌蕊柱头上萌发 (图版 I-1), 萌发率可达 90% 左右 (显微镜镜检目测)。2~4 h 后, 花粉管借助于柱头毛之间的分泌物进入柱头沟 (图版 I-2), 12~14 h, 在花柱沟内可观察到花粉管 (图版 I-3)。花粉管在花柱沟内生长缓慢, 在授粉后 24、36、42、46、53、70 h 的固定材料的花柱沟中均观察到花粉管的顶端 (图版 I-4, 5), 直至授粉后 120~126 h 的材料中才发现花粉管穿过花柱沟、珠孔塞和珠心冠原组织进入胚囊 (图版 I-6), 整个生长过程需 5 d 时间 (以生长最快的花粉管为准)。同时发现生长在花柱沟内的花粉管多数解体破裂, 将内含物泄入花柱沟引导组织的表面, 有些较大的团块显然是有数个花粉管流出的内含物汇成的, 其着色情况与花粉管内物质大致相似 (图版 I-7)。在此过程中花粉管的生长方式有两种: (1) 在柱头毛和柱头沟中是通过胞间分泌物生长的 (图版 I-1, 2), 在花柱沟中是在引导组织表面生长的 (图版 I-4, 5, 8)。

### 2.2 授粉前后中国鹅掌楸雌蕊的形态结构特征

控制授粉前雌蕊柱头及花柱向外伸展并反卷, 表面丛生大量表皮毛细胞 (图版 II-1), 传粉时被大量晶莹透明的分泌物覆盖, 类似裸子植物中的传粉滴, 表明柱头进入可授期。中国鹅掌楸柱头的可授期可延续 2~4 d, 授粉后, 花粉在柱头上吸胀、萌发, 此时柱头毛细胞萎蔫, 花柱沟细胞染色深呈现分泌细胞的典型特征 (图版 I-2)。授粉 6 h 后柱头变褐。授粉时期的子房已发育形成 2 枚胚珠, 胚珠的珠被、珠心组织已分化完善, 但成熟胚囊的数目很少, 多数胚珠的胚囊部位出现空腔 (图版 II-2), 或花粉管到达胚囊时, 胚囊尚未发育完善。

### 2.3 杂种胚和杂种胚乳的发育

控制授粉 6 d 后发生了珠孔受精。分别在授粉 163、168、218 h 和 15 d 的材料中发现游离核胚乳。20 d 时, 游离核胚乳细胞化, 发育形成 2~4 个细胞厚的狭长组织 (图版 II-3), 约占胚囊长度的 2/3 左右。35 d 后观察到球形胚, 此时胚乳充满胚囊腔, 珠心组织解体。另外, 在授粉 20 d 的材料中依然可以观察到游离核胚乳, 此时胚乳的发育率为 63%, 26 d 后降到 44%, 部分胚乳逐渐解体消失。

### 2.4 杂种种皮的形成

中国鹅掌楸的胚珠具有 2 层珠被<sup>[1]</sup>, 授粉时, 胚珠的外珠被由 1 层厚壁细胞组成的外表皮, 2~4 层薄壁细胞的中层和内表皮组成, 内珠被由外表皮, 1~2 层薄壁细胞的中层和内表皮

组成(图版 I-4)。随着胚和胚乳的发育,珠被各层分别以各种方式参与种皮的形成,表现为外珠被的外表皮细胞首先发生径向的延长,细胞壁加厚,后期衍变成切向扁平的细胞构成膜层(图版 I-4,6);中层细胞由排列紧密整齐逐渐变成不规则状态,最后发育成为大型细胞,形成类似气室的结构(图版 I-4,5,6);内表皮细胞在发育后期细胞内充满内含物,参与组成种皮的硬化层。内珠被的外表皮和中层细胞消失;内表皮细胞发生了木质化加厚。最后,杂种种皮由外珠被的外表皮形成的膜层和中层形成的气室以及内、外珠被的内表皮形成硬化层组成(图版 I-6)。

### 3 讨论

(1)北美鹅掌楸的花粉在中国鹅掌楸的柱头上正常萌发,其萌发状况与中国鹅掌楸花粉在北美鹅掌楸柱头上萌发相似,略好于中国鹅掌楸花粉在自身柱头上的萌发<sup>[1,11]</sup>,花粉管可以沿着柱头毛细胞进入柱头沟中,在柱头的表面尚未发现盘曲、扭转等状况,说明在柱头表面不存在杂交障碍<sup>[6~8]</sup>,与其它组合相比,北美鹅掌楸花粉管在中国鹅掌楸花柱沟内生长速度较慢,并且在此阶段多数花粉管解体破裂,通过花柱到达珠孔的花粉管的数量较少<sup>[11,12]</sup>。有关资料表明,花粉管的破裂存在两种结论:①花粉管的破裂是一种生物学上不适应的表现,其后果是导向部分或完全不育;②一部分花粉管的破裂并不妨碍受精的完成,是受精过程的一种自然生理现象,花粉管泄出的内含物对雌蕊组织及正在发育的幼胚具有积极的生物学意义<sup>[2]</sup>。作者认为,北美鹅掌楸花粉管的破裂显然属于前者,因为在本实验中很难观察到进入胚囊的花粉管,由此导致了受精机率的降低。从花粉管生长的状况来看,中国鹅掌楸×北美鹅掌楸组合比北美鹅掌楸×中国鹅掌楸组合杂交效果差一些。

(2)中国鹅掌楸可授期成熟胚囊的数目极少,这一现象与小叶杨×胡杨杂交组合中胡杨的情况相似<sup>[5]</sup>。由于多数胚囊发育迟缓或败育,导致了北美鹅掌楸的花粉管在子房内滞留过久而枯萎死亡,或精、卵不能相遇无法完成受精作用;而且中国鹅掌楸的湿型柱头表面对花粉的选择性不强<sup>[3]</sup>;花柱引导组织细胞活性较低,阻碍了北美鹅掌楸花粉管在花柱区正常生长<sup>[4]</sup>;因此可授期雌蕊的发育状况对受精机率的高低影响很大。此外,室内水培的中国鹅掌楸花枝,可能由于水分和养料供应不足,导致雌性生殖器官发育不良,如何改善雌性器官的发育状况以提高受精机率?这一问题有必要作进一步的研究。

(3)发生杂交障碍的原因很多<sup>[5~7]</sup>,其中胚与胚乳发育不协调是关键因素之一。中国鹅掌楸×北美鹅掌楸杂交组合的受精过程基本正常,但在胚和胚乳发育过程中存在异常现象,表现为胚败育而胚乳发育正常或胚与胚乳均败育,进一步研究发现,胚和胚乳发育不协调,表现在胚乳游离核数目及其细胞化的时间不一致,而且授粉后胚乳的发育率逐渐降低,由于胚乳发育异常,导致胚在发育早期败育,所以很难观察到后期胚的发生过程。

(4)在杂交育种工作中,控制授粉技术是必要的手段,而掌握雌蕊可授期是授粉成功的关键因素之一。由于可授期内柱头分泌的液体,有助于花粉的吸胀、萌发,迅速发育形成花粉管,因而对提高花粉萌发率起到重要作用。同时,由于花粉萌发过程中具有群体效应<sup>[6]</sup>,因此每个柱头上的花粉量也是杂交育种成功与否的主要因素之一。如果花粉量多,相应地其萌发效果也

1)樊汝汶,尤录祥.北美鹅掌楸×中国鹅掌楸种间杂交的胚胎学研究(待发表).

较好。前期实验曾证实花粉萌发形成的花粉管有长短之分,并存在一定的比率<sup>[3]</sup>,若提高整体花粉量,不仅提高了萌发率,而且形成长形花粉管的数量也相应增多,那么,进入花柱沟到达胚珠的花粉管数目增多,由此可以提高受精机率。

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## The Embryological Studies of Interspecific Hybrids between *Liriodendron tulipifera* L. and *L. chinense* (Hesml.) Sarg.

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**Abstract** The embryogenesis of interspecific hybrids between *Liriodendron tulipifera* and *L. chinense* was studied by means of artificial pollination and routine paraffin-embedded sectioning technique. The results were: (1) The pollen of *L. tulipifera* germinated normally after artificial pollination, and more pollen tubes could grow into stigma but a lot of pollen tubes grew slowly and even broke up in the style canal; (2) When the pollen has germinated for six days, the pollen tube reached the embryo sac finally through the style canal, obturator and nucellar cap, then, the porogamy was conducted. After 20 days, the cellular endosperm was formed first as a narrow tissue of 2 to 4 cells in thickness. The spherical embryo could be observed after 35 days; (3) The seed coat was formed by integument following the development of embryo and endosperm. The antipodal has two layers integuments. Both of them were formed exopleura, middle layer and endopleura. Each layer of integument experienced a series of changes when the embryo and endosperm developed. At last, the seed coat was consisted of the membrane layer formed by exopleura of exointegument, air chamber formed by middle layer and hardening layers formed by the endopleura cell of inter integument and outer integument; (4) The number of mature embryo sacs and pollen tubes reaching micropyle was too less to fertilize; (5) The course of fertilization was normal on the whole, but the development of embryo and endosperm was not in harmony, which resulted in the early abortion of embryo, so it was difficult to observe the later development of embryo.

**Key words** *Liriodendron chinense*, *Liriodendron tulipifera*, species cross, embryo development

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the integrity of the financial system and for the ability to detect and prevent fraud. The text notes that without reliable records, it would be difficult to track the flow of funds and to identify any irregularities.

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6. The sixth part of the document discusses the importance of ongoing monitoring and evaluation of financial reporting practices. It notes that the financial reporting environment is constantly evolving, and organizations must stay up-to-date on the latest developments and best practices. The text emphasizes the need for a proactive approach to risk management, with regular assessments of the effectiveness of internal controls and the identification of areas for improvement.

7. The seventh part of the document addresses the role of external auditors in providing independent assurance on financial statements. It discusses the importance of a high-quality audit process, which is based on a thorough understanding of the organization's operations and a commitment to objectivity and integrity. The text notes that external auditors play a critical role in enhancing the credibility of financial reporting and in providing valuable insights to management and stakeholders.

8. The eighth part of the document discusses the importance of communication and stakeholder engagement in financial reporting. It notes that organizations must be transparent about their reporting practices and must engage with stakeholders to understand their needs and expectations. The text emphasizes the need for a clear and consistent message, which is supported by a strong understanding of the organization's financial performance and the challenges it faces.

9. The ninth part of the document concludes by emphasizing the need for a strong leadership commitment to financial reporting. It states that senior management must set the tone at the top and must ensure that financial reporting is a top priority for the organization. The text calls for a clear and consistent message from the top, which is supported by a strong understanding of the organization's financial performance and the challenges it faces.

