DOI: 10.3724/SP.J.1006.2021.04207

研究简报

甘蓝型油菜温敏细胞核雄性不育系 160S 花药败育的形态学特征和细胞学研究

唐 鑫 李圆圆 陆俊杏 张 涛*

重庆师范大学生命科学学院, 重庆 401331

摘 要: 明确甘蓝型油菜温敏核雄性不育系 160S 花器形态变化、花药败育的时期和细胞学特征, 初步探究败育的原因, 为深入研究不育系 160S 的内在分子调控机制提供理论基础, 也对其在油菜两系杂交育种中的实际应用具有指导意义。本研究在 15℃ 和 28℃ 条件下培养试验材料 160S, 利用体式显微镜分别观察花发育形态特征; 采用醋酸洋红染色方法观察各时期小孢子发育形态; 通过石蜡切片和苏木精-伊红染色对可育植株(Male Fertile/160S-MF)和不育植株(Male Sterile/160S-MS)花药细胞学特征进行显微观察; TUNEL 染色法检测花药发育各时期绒毡层细胞凋亡情况。160S-MF 在 15℃ 表现为可育, 雄蕊正常发育, 成熟的花药呈黄色, 形态饱满, 正常开裂, 表面一层有活性的花粉附着在上面; 28℃ 条件下, 160S-MS 花朵的雌蕊、萼片与 160S-MF 花朵无差异, 但花瓣变小, 花丝变短, 雄蕊明显退化, 花药干瘪呈黄褐色, 无花粉粒附着在花药上, 表现出雄性完全不育。160S-MF 的小孢子能正常发育为成熟有活力的花粉。而 160S-MS 由于雄蕊完全败育, 未观察到小孢子和花粉粒。160S-MS 花药在造孢时期和花粉母细胞时期与 160S-MF 无明显差异, 但在减数分裂期, 160S-MS 花药绒毡层形态和结构出现异常, 绒毡层细胞排列不整齐, 细胞空泡化, 伴随提前解体。同时花粉母细胞发育受阻, 无四分体结构形成, 最终在减数分裂期完成前形成空的花粉囊。TUNEL 检测发现, 160S-MS 花药绒毡层细胞在减数分裂期开始凋亡。本研究结果表明, 160S 属花粉母细胞败育型不育系, 败育时期发生在减数分裂期, 绒毡层异常降解, 绒毡层未向腺质型转化, 不能提供花粉母细胞发育所需要的营养物质, 致使花粉母细胞发育受阻无法形成四分体结构, 从而导致小孢子无法形成, 花药形成空的花粉囊, 产生雄性不育。

关键词: 甘蓝型油菜; 温敏细胞核雄性不育; 花药败育; 细胞学研究

Morphological characteristics and cytological study of anther abortion of temperature-sensitive nuclear male sterile line 160S in *Brassica napus*

TANG Xin, LI Yuan-Yuan, LU Jun-Xing, and ZHANG Tao*

College of Life Sciences, Chongqing Normal University, Chongqing 401331, China

Abstract: The main purpose of this study was to explore the flower morphologies, anther abortion times and cytological characteristics of the thermo sensitive genic male sterile line 160S in *Brassica napus*. The results will provide a theoretical basis for further study on the molecular regulation mechanism and guide significance for its practical application in two-line hybrid breeding of rape. The 160S were grown at 15℃ and 28℃. The flower morphology, microspore, anther structure and tapetum at different stages of fertile plants (Male Fertile/160S-MF) and sterile plants (Male Sterile/160S-MS) were observed by stereomicroscope with carmine acetate staining, paraffin section, hematoxylin eosin staining and TUNEL staining. The anther was yellow, plump and dehiscent with high pollen viability at 15℃. At 28℃, there was no differences in pistil and potato chips between 160S-MS and 160S-MF flowers, but the petals and the filaments of 160S-MS were smaller and shorter. The stamens of 160S-MS degenerated obviously and its anthers were shriveled in yellowish brown without pollen grains, indicating completely male sterility. But inter-

本研究由重庆市自然科学基金项目(cstc2012jjA80010), 重庆市科学技术局基金项目(cstc2017jcyjAX0108)和重庆市教委科学技术项目(KJQN201900533)资助。

This study was supported by the Natural Science Foundation of Chongqing (cstc2012jjA80010), the Science and Technology Bureau Foundation Project Chongqing (cstc2017jcyjAX0108), and the Scientific and Technological Research Program of Chongqing Municipal Education Commission (KJQN201900533).

* 通信作者(Corresponding author): 张涛, E-mail: zht2188@126.com

第一作者联系方式: E-mail: 1047910732@qq.com

Received (收稿日期): 2020-09-09; Accepted (接受日期): 2020-11-13; Published online (网络出版日期): 2020-12-15.

URL: <https://kns.cnki.net/kcms/detail/11.1809.S.20201215.1328.015.html>

estingly, the 160S-MF developed normally with higher pollen viability. However, microspore and pollen grains were not observed in 160S-MS due to complete abortion of stamens. Paraffin section results showed that there was no significant difference between 160S-MS and 160S-MF in sporulation and pollen mother cell stages. The morphology and structure of tapetum of 160S-MS appeared some abnormal appearances, such as irregularly cell arrangement, vacuolization, and premature disintegration at meiosis stage. Meanwhile, the development of pollen mother cells were hindered and no tetrads structure were formed leading to the empty pollen sac before the completion of meiosis. TUNEL assay showed that the tapetum of 160-MS began to apoptosis at meiosis stage. In summary, these results showed that 160S belongs to pollen mother cell abortion type male sterile line, whose abortion stage occurred during meiosis stage and the tapetum cells degraded prematurely and failed to be transformed to the glandular type, so that the nutrients needed for pollen mother cell development were not provided. The tetrad structures and microspores can not be formed, which led to the formation of empty anther sac and resulted in male sterility.

Keywords: *Brassica napus*; thermo-sensitive genic male sterility; anther abortion; cytological study

油菜作为世界上重要的油料作物之一,是食用油和生物燃料的重要来源^[1]。为了满足日益增长的需求,提高油菜产量是育种工作急需解决的问题和长期目标。雄性不育植物是杂交育种所必需的育种材料,能利用杂种优势来提高作物产量,在农业生产上具有重要的经济价值^[2-3]。

细胞质雄性不育(cytoplasmic male sterility, CMS)和细胞核雄性不育(genic male sterility, GMS)已被广泛应用于油菜杂种优势的开发与利用,其中, GMS 类型主要分为受光周期影响的光敏型雄性不育(photoperiod-sensitive genic male sterility, PGMS)和受温度控制的温敏型雄性不育(thermo-sensitive genic male sterility, TGMS)^[4-5]。由于大多数 P/TGMS 由隐形核基因控制,雄性不育基因容易遗传,可以用作父本,具有恢复源广、细胞质来源丰富等优点,被认为是两系杂交育种的高效系统^[6]。目前,已发现的甘蓝型油菜 PGMS 类型主要有 H90S、N196S 和 501-8S; TGMS 类型主要有 Xiangyou 91S、Xiangyou 402S、Huiyou 50S、373S、SP2S、104S、TE5A 等^[7]。自首次报道水稻光敏不育系 58S,并成功用于水稻两系杂交育种以来^[8],在禾本科^[9-11]、豆科^[12]和十字花科^[13]等其他植物中也发现了光温敏雄性不育系材料。近年来,甘蓝型油菜雄性不育材料花药的细胞学特征显微观察已得到广泛的关注。Sun 等^[7]研究发现,373S 花粉败育是由于小孢子在发育过程中出现细胞质浓缩、胞外壁不规则、单核后期绒毡层降解异常引起的。李可琪等^[14]和 Yan 等^[15]观察 TE5A 的花药切片发现,在减数分裂期花粉母细胞同源染色体不能配对、凝聚形成二分体和四分体,导致小孢子发育受阻,最终引起败育。Yu 等^[16]对 SP2S 细胞学观察发现,绒毡层细胞在减数分裂早期高度空泡化变大,小孢子无法从四分体释放出来,并伴随胼胝质降解异常,最终导致败育。总之,植物雄性不育是一个极其复杂的过程,败育时期、方式多种多样,败育特征和程度也各不相同。

前期研究表明^[17],160S 育性受环境温度影响,20℃以下可育(160S-MF),25℃以上不育(160S-MS);温度是影响育性的关键因素,随着温度的升高,花粉活力、角粒数和结角率随之降低;研究还表明,160S 具有恢复源广泛、较强的一般配合力、杂种优势明显等优点,可以应用培育杂

交油菜新品种。前人仅仅开展了温度对不育系 160S 育性转化和杂种优势等方面的研究,但其败育时期、原因和细胞学特征尚未报道。本研究通过观察甘蓝型油菜 160S-MF 和 160S-MS 形态学和细胞学差异,明确 160S-MS 败育的时期和细胞学特征,初步解析其败育原因,旨在为深入研究其雄性不育内在分子调控机理以及在杂种优势的利用等方面奠定基础,同时对甘蓝型油菜两系杂交育种具有指导作用。

1 材料与方法

1.1 材料

甘蓝型油菜温敏细胞核雄性不育系 160S 种子由重庆师范大学油用牡丹种质资源创新与利用重点实验室保存。在室温(20℃)条件下播种,10 d 后将长势良好的幼苗移栽至直径 20 cm 的花盆中,一盆一株苗,春化处理,分别放置在温度为 15℃和 28℃的冷光源植物生长箱(赛福 ZRX-1100E)中培养,光照 16 h/黑暗 8 h,湿度 50%。

1.2 取材与固定

在初花期,取 160S-MF、160S-MS 不同大小花蕾的花药压片,用 1%醋酸洋红染色,在荧光显微镜(SOPTOP RX50)下观察小孢子发育时期。采样后,将样品分组放入 FAA 固定液(50%酒精 90 mL、冰醋酸 5 mL、37%甲醛 5 mL)中,真空干燥箱(SKK ZK-50)抽真空,直至花蕾完全沉入瓶底,室温固定 24 h,70%乙醇 4℃冰箱存放备用。

1.3 花器形态特征观察

分别采集 160S-MF、160S-MS 盛花期的 30 朵花,用游标卡尺测量花冠径、花瓣长、花瓣宽、雌蕊长、四强雄蕊长、二弱雄蕊长、四强花药长、花丝长和花柄长等花器性状。采用 Microsoft Excel 2007 处理数据,用统计软件 SPSS 20.0 分析所测数据。体式显微镜(励扬 LY-WN-OPLNIC)观察花朵形态并照相。

1.4 花药石蜡切片观察

采用常规石蜡切片方法,用 70%、85%、95%和 100%梯度乙醇溶液脱水,二甲苯透明,石蜡浸蜡和包埋,切片厚度 6~8 μm,郝伯特配方粘片剂粘片,苏木精-伊红染色,中性树脂胶封片,待胶完全凝固后,在荧光显微镜(SOPTOP RX50)下观察并照相。

1.5 TUNEL 染色

采用 Dead End Fluorometric TUNEL System (Promega) 试剂盒方法检测细胞凋亡。花药石蜡切片用二甲苯脱蜡, 梯度乙醇脱水洗涤, PBS 洗涤 5 min, 4%多聚甲醛溶液固定 15 min, 然后用 $20\text{ }\mu\text{g mL}^{-1}$ 蛋白酶 K 室温孵育 10 min, 加 rTdT 孵育缓冲液(Equilibration Buffer, Nucleotide Mix, rTdT)对核 DNA 片段进行脱氧三磷酸尿苷缺口末端标记, 37°C 避光孵育 60 min。用碘化丙啶(PI, Sigma)复染。使用荧光显微镜(SOPTOP RX50)观察。在 $(520\pm 20)\text{ nm}$ 处观察荧光素的绿色荧光(TUNEL 信号); 在 $>620\text{ nm}$ 处观察碘化丙啶的红色荧光。

表 1 160S-MF 和 160S-MS 的花器性状比较

Table 1 Comparison of the flower traits between 160S-MF and 160S-MS (mm)

花器特征 Flower trait	育性类型 Types of male sterility	
	160S-MF	160S-MS
花冠径 Diameter of corolla	18.97 $\pm$ 1.68	15.58 $\pm$ 0.78**
花瓣长 Length of petal	13.98 $\pm$ 1.16	12.30 $\pm$ 0.57**
花瓣宽 Width of petal	7.48 $\pm$ 0.78	5.88 $\pm$ 0.37**
雌蕊长 Length of pistil	9.24 $\pm$ 1.13	9.80 $\pm$ 0.72
四强雄蕊长 Length of four stronger stamens	9.38 $\pm$ 0.45	6.11 $\pm$ 1.09**
二弱雄蕊长 Length of two weaker stamens	6.99 $\pm$ 0.81	4.89 $\pm$ 1.20**
四强花药长 Length of four stronger stamens' anther	2.25 $\pm$ 0.15	1.80 $\pm$ 0.08**
花丝长 Length of four long filaments	7.44 $\pm$ 0.46	4.69 $\pm$ 0.89**
花柄长 Length of stalk	29.13 $\pm$ 1.38	16.50 $\pm$ 1.27**
萼片长 Length of sepal	7.10 $\pm$ 0.82	6.61 $\pm$ 0.51

表中所列数据均为平均值 $\pm$ 标准差($n=30$)。**表示在 0.01 水平显著差异。

All data are average $\pm$ standard deviation ($n=30$). ** means significantly different at the 0.01 probability level.

图 1 160S-MF 和 160S-MS 花形态学观察

Fig. 1 Flower morphology of 160S-MF and 160S-MS

A: 160S-MF 盛花期花朵; B: 160S-MF 雄蕊; C: 160S-MF 花瓣和萼片; D: 160S-MS 盛花期花朵; E: 160S-MS 雄蕊; F: 160S-MS 花瓣和萼片。

A: the flower of 160S-MF; B: the flower stamens of 160S-MF; C: the petal and sepal of 160S-MF; D: the flower of 160S-MS; E: the flower stamens of 160S-MS; F: the petal and sepal of 160S-MS.

2 结果与分析

2.1 160S-MF 和 160S-MS 花朵形态学分析

对比观察 160S-MF (15°C)、160S-MS (28°C)花朵的形态发现, 两者在外观形态上有明显差别, 160S-MF 花朵较大(图 1-A), 雄蕊长度高于雌蕊(图 1-B), 花药呈黄色, 形态饱满, 能正常开裂, 释放出有活性的花粉; 160S-MS 能正常开花, 花朵呈浅黄色(图 1-D), 雄蕊 6 枚, 四强二弱, 但花朵整体偏小, 雄蕊长度不及雌蕊 1/2 (图 1-E), 花药褐黄色呈小三角形, 干瘪萎缩, 无花粉。160S-MS 的花器形态也发生显著变化(表 1)。在所测的花器特征参

数中,除雌蕊和萼片外其余花器官都达到极显著水平。与 160S-MF 相比,160S-MS 花瓣长度、宽度显著性变小、变窄(图 1-C, F),雄蕊(四强雄蕊和二弱雄蕊)明显退化,花药和花丝极显著短于可育株的花丝和花药。

2.2 160S-MF 和 160S-MS 小孢子形态观察分析

通过对 160S-MF、160S-MS 不同发育阶段小孢子发育的细胞学观察,研究了花药败育的特征,如图所示,跟大多数植物一样,160S-MF 花粉母细胞经 2 次减数分裂后形成四分体结构(图 2-A),周围有胼胝质;随着胼胝质解体后,进入单核早期(图 2-B),四分体慢慢释放出圆形的单个小孢子,形态较小,未明显观察到细胞核;单核靠边期(图 2-C),明显能观察到细胞核移向细胞一侧,此时的细胞核形态清晰,整个细胞呈三瓣状;之后,随着小孢子继续发育,细胞核经一次有丝分裂(图 2-D),形成一大一小 2 个细胞核,这时进入双核期,营养核较大位于细胞中

央,靠近细胞壁一侧较小的细胞核为生殖核;小孢子继续发育至成熟期(图 2-E),花粉粒染色后整体颜色较深,细胞核不能明显辨别,形态饱满接近圆形。对 160S-MS 花药染色压片,并未观察到小孢子或者花粉粒(图 2-F),表明花药很有可能在小孢子形成前就已败育。

2.3 160S-MF 和 160S-MS 花药细胞学观察

对 160S-MF 花药切片观察发现,在造孢时期,造孢母细胞紧密地排列在药室中央,细胞核清晰可见,形态呈不规则多边形(图 3-A);随着花药的发育,造孢母细胞间稍微分开,此时花药壁由表皮、药室内壁、中层组成,绒毡层的发育不是很清楚(图 3-B);进入花粉母细胞时期,花粉母细胞进一步发育,体积变大,形状呈椭圆形,绒毡层细胞整齐地排列在花药壁最内层,呈长方形,清晰可见(图 3-C);减数分裂期(图 3-D),有的花粉母细胞有两团染色质,表明其在进行核分裂,此时,绒毡层细胞处于双核

图 2 160S-MF 和 160S-MS 小孢子形态

Fig. 2 Microspores morphology of 160S-MF and 160S-MS

A: 160S-MF 四分体; B: 160S-MF 早期小孢子; C: 160S-MF 单核小孢子; D: 160S-MF 双核小孢子; E: 160S-MF 花粉粒; F: 160S-MS 花粉粒。箭头所指为细胞核。

A: tetrads of 160S-MF; B: early microspores of 160S-MF; C: uninucleate microspores of 160S-MF; D: binucleate microspores of 160S-MF; E: pollen grains of 160S-MF; F: pollen grains of 160S-MS. The arrow points to the nucleus of the cells.

状态;四分体时期(图 3-E),小孢子呈四面体型,绒毡层开始出现降解趋势;小孢子细胞核靠近细胞一侧,此时进入单核晚期,小孢子的花粉壁开始增厚(图 3-F);双核期的小孢子细胞壁逐渐加厚且趋于圆球形,绒毡层持续降解(图 3-G);成熟花粉期,绒毡层不复存在,花粉粒呈椭圆形,形态饱满,染色后变深(图 3-H),4 个花粉囊呈蝴蝶型,花粉囊开裂,释放出花粉(图 3-I)。

对 160S-MS 花药切片观察发现,花药也能分化形成蝴蝶型排列的 4 个花粉囊(图 4-A)。160S-MS 在造孢时期和花粉母细胞时期与 160S-MF 无明显差异(图 4-B, C),造孢细胞和花粉母细胞发育均正常,此时组成花药壁的各组织结构在形态和发育也无异常。减数分裂期(图 4-D, E),160S-MS 花药绒毡层发育出现异常,尽管绒毡层为双核细胞,但与同一时期的 160S-MF 花药绒毡层相比形态结构发生变化,细胞排列不整齐,空泡化,出现解体,花药开始出现败育现象。减数分裂期完成之前,绒毡层全部解体(图 4-F),花粉母细胞也逐渐解体,最终剩下空花粉囊。

2.4 TUNEL 染色分析

在植物花药中,绒毡层降解被认为是细胞程序性死亡(programmed cell dead, PCD)的结果,其特征是细胞核 DNA 的断裂。为进一步研究 160S-MS 花药绒毡层发育的异常现象,本研究使用 TUNEL 法观察绒毡层细胞凋亡情况(图 5)。对 160S-MF、160S-MS 不同发育阶段的花药切片进行 TUNEL 染色观察分析表明,在花粉母细胞时期,160S-MF、160S-MS 花药绒毡层细胞的细胞核均为 TUNEL 阴性(图 5-A, B),表明该阶段绒毡层细胞发育正常;减数分裂期,160S-MF 并未检测到绒毡层凋亡信号(图 5-C),相比之下,160S-MS 花药绒毡层细胞在减数分裂期检测到绿色荧光信号(图 5-D),表明其绒毡层核 DNA 断裂,细胞开始凋亡;随着花药发育,绿色荧光信号越强烈(图 5-E),最终只剩下空的花粉囊。四分体时期(图 5-F),160S-MF 花药绒毡层细胞核才出现微弱绿色荧光信号,绒毡层开始降解,分泌小孢子从四分体释放出来所需的物质,并且荧光信号持续到双核期(图 5-G)。表明 160S-MS 花药绒毡层细胞在减数分裂期提前凋亡。

图 3 160S-MF 花药不同发育阶段的显微结构

Fig. 3 Microscopical structure of anthers at different developmental stages in 160S-MF

A 和 B: 造孢时期; C: 花粉母细胞时期; D: 减数分裂时期; E: 四分体时期; F: 单核靠边期; G: 双核期; H 和 I: 成熟花粉粒; Epi: 表皮层; Mid: 中层层; En: 药室内壁; T: 绒毡层; Sp: 造孢细胞; PMC: 小孢子母细胞; Te: 四分体; M: 小孢子; Po: 花粉粒。

A, B: sporogenous cell stage; C: pollen mother cell stage; D: meiotic stage; E: tetrad stage; F: late uninucleate stage; G: binucleate stage; H, I: mature pollen; Epi: epidermal; Mid: middle layer; En: endothecium; T: tapetum; Sp: sporogenous cell; PMC: pollen mother cell; Te: tetrad; M: microspore; Po: pollen.

图 4 160S-MS 花药不同发育阶段的显微结构

Fig. 4 Microscopical structure of anthers at different developmental stages in 160S-MS

A 和 B: 造孢时期; C: 花粉母细胞时期; D 和 E: 减数分裂时期; F: 败育花药药室; Epi: 表皮层; Mid: 中层层; En: 药室内壁; T: 绒毡层; Sp: 造孢细胞; PMC: 小孢子母细胞。

A, B: sporogenous cell stage; C: pollen mother cell stage; D, E: meiotic stage; F: locule of aborted anther; Epi: epidermal; Mid: middle layer; En: endothecium; T: tapetum; Sp: sporogenous cell; PMC: pollen mother cell.

图 5 160S-MF、160S-MS 花药各时期细胞凋亡 TUNEL 检测

Fig. 5 TUNEL detection of anther programmed cell dead at different developmental stages in 160S-MF and 160S-MS

A: 160S-MF 花粉母细胞时期; B: 160S-MS 花粉母细胞时期; C: 160S-MF 减数分裂时期; D 和 E: 160S-MS 减数分裂时期; F: 160S-MF 四分体时期; G: 160S-MF 双核期; T: 绒毡层; PMC: 小孢子母细胞; Te: 四分体; M: 小孢子。碘化丙染色细胞核呈红色荧光, TUNEL 阳性细胞核呈绿色荧光(箭头所指)。

A: 160S-MF at pollen mother cell stage; B: 160S-MS at pollen mother cell stage; C: 160S-MF at meiotic stage; D and E: 160S-MS at meiotic stage; F: 160S-MF at tetrad stage; G: 160S-MF at binucleate stage; T: tapetum; PMC: pollen mother cell; Te: tetrad; M: microspore. The fluorescence of nuclei stained by propidium iodide is red, while green fluorescence is TUNEL-positive nuclei staining (arrow).

3 讨论

在本研究中, 160S-MS 雄蕊明显退化, 花丝变短, 这些形态特征与大多数温敏甘蓝型油菜雄性不育材料相同。对 160S-MS 醋酸洋红染色未观察到小孢子或者花粉粒, 说明 160S-MS 败育彻底, 由于雌蕊发育正常, 可以正常授粉。因此, 不育系 160S 可以用于油菜两系育种, 培育新型杂交油菜品种。

在花粉发育过程中, 任何阶段的异常都会引起雄性不育, 败育发生时期和方式是多样的^[18-19]。余凤群等^[20]根据甘蓝型油菜雄性不育系花药败育时期和方式, 将其败育分为无花粉囊型不育系、花粉母细胞败育型不育系、单核败育型不育系 3 类。本研究发现, 不育系 160S 花药在减数分裂时期出现异常, 属于花粉母细胞败育型不育系。花粉母细胞不能完成减数分裂, 无法形成正常的四分体结构, 这一败育特征与 TE5A^[14-15]败育现象非常相似。160S-MS 花药绒毡层在减数分裂时期出现结构和形态出现异常, 细胞空泡化, 伴随提前解体, 然而, 不育系 TE5A 绒毡层发育正常。从绒毡层发育层面看, 160S-MS 与 373S^[7]、SP2S^[16]和 Huiyou 50S^[21]都出现提前凋亡, 但这些不育系材料在发育过程中能形成正常的四分体结构, 败育发生在小孢子发育过程中, 而 160S-MS 在花粉母细胞减数分裂未完成时, 就形成空的花粉囊, 未观察到四分体结构和小孢子。总之 160S 败育时期和特点与目前报道的甘蓝型温敏核雄性不育材料存在差异。

植物花粉的生长发育需要绒毡层在适当的时期发生细胞程序性死亡^[22]。绒毡层位于花药壁最内层, 它与发育中的雄配子体直接接触, 能向小孢子提供发育需要的营养物质, 如胼胝质、孢粉素和蛋白质等^[23-26]。研究表明, 绒毡层在花药和花粉的发育过程中发挥重要作用, 过早或延迟凋亡会导致雄性不育^[27-29]。目前已在小麦^[27]、拟南芥^[30-31]、水稻^[32-33]、油菜^[34]等植物中发现大量雄性不育材料是由绒毡层发育异常引起的。李六林^[35]根据前人对绒毡层发育的研究, 按照不同的植物种类, 将绒毡层解体分为 2 类: 一种是腺质型类型, 在花粉发育过程中, 绒毡层细胞分泌花粉母细胞和小孢子发育所需的各种营养物质, 绒毡层细胞直到花粉成熟才完全消失; 另一种是变形绒毡层, 绒毡细胞以多核原生质团的形式填充于小孢子之间的空隙中, 供小孢子利用和吸收。腺质型绒毡层多存在于被子植物中。在花药发育早期, 160S-MS 能形成正常的花粉母细胞, 绒毡层细胞整齐地排列在花药壁最内层, 与 160S-MF 无差异。然而, 在减数分裂期, 160S-MS 花药绒毡层细胞形态和结构出现异常, 细胞空泡化并伴随降解现象, 致使花粉母细胞减数分裂受阻, 慢慢解体, 无法形成四分体结构, 最终减数分裂完成前就只剩下空的花粉囊, 导致败育。可能是绒毡层细胞提前凋亡而未向腺质型转化, 不能向花粉母细胞提供发育所需的营养成分, 进而影响四分体小孢子的形成, 无成熟花粉粒产生, 导致 160S 败育。

References

- [1] Tester M, Langridge P. Breeding technologies to increase crop production in a changing world. *Science*, 2010, 327: 818–822.
- [2] Rondanini D P, Gomez N V, Agosti M B, Miralles D J. Global trends of rapeseed grain yield stability and rapeseed-to-wheat yield ratio in the last four decades. *Eur J Agron*, 2012, 37: 56–65.
- [3] Hochholdinger F, Hoecker N. Towards the molecular basis of heterosis. *Trends Plant Sci*, 2007, 12: 427–432.
- [4] Chen L, Liu Y G. Male sterility and fertility restoration in crops. *Ann Rev Plant Biol*, 2014, 65: 579–606.
- [5] Virmani S S, Ilyas-Ahmed M. Environment-sensitive genic male sterility (EGMS) in crops. *Adv Agron*, 2001, 72: 139–195.
- [6] Lei S L, Yao X Q, Yi B, Chen W, Ma C Z, Tu J X, Fu T D. Towards map-based cloning: fine mapping of a recessive genic male-sterile gene (*BnMs2*) in *Brassica napus* L. and syntenic region identification based on the *Arabidopsis thaliana* genome sequences. *Theor Appl Genet*, 2007, 115: 643–651.
- [7] Sun Y, Zhang D, Wang Z, Guo Y, Sun X, Li W, Zhi W, Hu S. Cytological observation of anther structure and genetic investigation of a thermo-sensitive genic male sterile line 373S in *Brassica napus* L. *BMC Plant Biol*, 2020, 20: 8.
- [8] 石明松. 对光照长度敏感的隐性雄性不育水稻的发现与初步研究. *中国农业科学*, 1985, 18(2): 44–48.
Shi M S. The discovery and study of the photosensitive recessive male-sterile rice (*Oryza sativa* L. subsp. *japonica*). *Sci Agric Sin*, 1985, 18(2): 44–48 (in Chinese with abstract).
- [9] Lee D S, Chen L J, Suh H S. Genetic characterization and fine mapping of a novel thermo-sensitive genic male-sterile gene *tms6* in rice (*Oryza sativa* L.). *Theor Appl Genet*, 2005, 111: 1271–1277.
- [10] Tang J H, Fu Z Y, Hu Y M, Li J S, Sun L L, Ji H Q. Genetic analyses and mapping of a new thermo-sensitive genic male sterile gene in maize. *Theor Appl Genet*, 2006, 113: 11–15.
- [11] Xing Q H, Ru Z G, Zhou C J, Xue X, Liang C Y, Yang D E, Jin D M, Wang B. Genetic analysis, molecular tagging and mapping of the thermo-sensitive genic male-sterile gene (*wtms1*) in wheat. *Theor Appl Genet*, 2003, 107: 1500–1504.
- [12] Frasc R M, Weigand C, Perez P T, Palmer R G, Sandhu D. Molecular mapping of 2 environmentally sensitive male-sterile mutants in soybean. *J Hered*, 2011, 102: 11–16.
- [13] Zhu J, Lou Y, Shi Q S, Zhang S, Zhou W T, Yang J, Zhang C, Yao X Z, Xu T, Liu J L, Zhou L, Hou J Q, Wang J Q, Wang S, Huang X H, Yang Z N. Slowing development restores the fertility of thermo-sensitive male-sterile plant lines. *Nat Plants*, 2020, 6: 360–367.
- [14] 李可琪, 曾新华, 袁荣, 闫晓红, 吴刚. 甘蓝型油菜温敏细胞核雄性不育系 TE5A 花药发育的细胞学研究. *中国农业科学*, 2016, 49: 2408–2417.
Li K Q, Zeng X H, Yuan R, Yan X H, Wu G. Cytological researches on the anther development of a thermo-sensitive genic male sterile line TE5A in *Brassica napus*. *Sci Agric Sin*, 2016, 49: 2408–2417 (in Chinese with English abstract).
- [15] Yan X, Zeng X, Wang S, Li K, Yuan R, Gao H, Luo J, Liu F, Wu Y, Li Y, Zhu L, Wu G. Aberrant meiotic prophase I leads to genic male sterility in the novel TE5A mutant of *Brassica napus*. *Sci*

- Rep*, 2016, 6: 33955.
- [16] Yu C Y, Guo Y F, Ge J, Hu Y M, Dong J G, Dong Z S. Characterization of a new temperature-sensitive male sterile line SP2S in rapeseed (*Brassica napus* L.). *Euphytica*, 2015, 206: 473–485.
- [17] 张涛, 沈余亮, 王瑞雪, 邹燕, 赵敬会, 李荣冲, 梁晶龙, 龚慧明. 甘蓝型油菜雄性不育系 160S 育性转换与利用. *西北植物学报*, 2012, 32: 35–41.
- Zhang T, Shen L Y, Wang R X, Zou Y, Zhao J H, Li R C, Liang J L, Gong H M. Fertility alteration and utilization of male-sterile line 160S in *Brassica napus*. *Acta Bot Boreali-Occident Sin*, 2012, 32: 35–41 (in Chinese with English abstract).
- [18] Laser K D, Lersten N K. Anatomy and cytology of microsporo-genesis in cytoplasmic male sterile (CMS) angiosperms. *Bot Rev*, 1972, 38: 425–454.
- [19] Wang S P, Zhang G S, Song Q L, Zhang Y X, Li Z, Guo J L, Niu N, Ma S C, Wang J W. Abnormal development of tapetum and microspores induced by chemical hybridization agent SQ-1 in wheat. *PLoS One*, 2015, 10: e0119557.
- [20] 余凤群, 傅廷栋. 甘蓝型油菜几个雄性不育系花药发育的细胞形态学研究. *武汉植物学研究*, 1990, 8: 209–216.
- Yu F Q, Fu T D. Cytomorphology study on anther development of several male sterile lines of *Brassica napus*. *J Wuhan Bot Res*, 1990, 8: 209–216 (in Chinese with English abstract).
- [21] 葛娟, 郭英芬, 于澄宇, 张国云, 董军刚, 董振生. 甘蓝型油菜光、温敏雄性不育系 Huiyou 50S 花粉败育的细胞学观察. *作物学报*, 2012, 38: 541–548.
- Ge J, Guo Y F, Yu C Y, Zhang G Y, Dong J G, Dong Z S. Cytological observation of anther development of photoperiod/thermo-sensitive male sterile line Huiyou 50S in *Brassica napus*. *Acta Agron Sin*, 2012, 38: 541–548 (in Chinese with English abstract).
- [22] Ma H. Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. *Annu Rev Plant Biol*, 2005, 56: 393–434.
- [23] Jung K H, Han M J, Lee Y S, Kim Y W, Hwang I W, Kim M J, Kim Y K, Nahm B H, An G. Rice undeveloped tapetum1 is a major regulator of early tapetum development. *Plant Cell*, 2005, 17: 2705–2722.
- [24] Zhao D Z, Wang G F, Speal B, Ma H. The excess microsporocytes1 gene encodes a putative leucine-rich repeat receptor protein kinase that controls somatic and reproductive cell fates in the *Arabidopsis* anther. *Genes Dev*, 2002, 16: 2021–2031.
- [25] Bedinger P. The remarkable biology of pollen. *Plant Cell*, 1992, 4: 879–887.
- [26] Xu J, Ding Z W, Vizcay-Barrena G, Shi J X, Liang W Q, Yuan Z, Werck-Reichhart D, Schreiber L, Wilson Z A, Zhang D B. *Aborted microspores* acts as a master regulator of pollen wall formation in *Arabidopsis*. *Plant Cell*, 2014, 26: 1544–1556.
- [27] Liu Z H, Shi X Y, Li S, Hu G, Zhang L L, Song X Y. Tapetal-delayed programmed cell death (PCD) and oxidative stress-induced male sterility of *Aegilops uniariastata* cytoplasm in wheat. *Int J Mol Sci*, 2018, 19: 1708.
- [28] Papini A, Mosti S, Brighigna L. Programmed-cell-death events during tapetum development of angiosperms. *Protoplasma*, 1999, 207: 213–221.
- [29] Li N, Zhang D S, Liu H S, Yin C S, Li X X, Liang W Q, Yuan Z, Xu B, Chu H W, Wang J, Wen T Q, Huang H, Luo D, Ma H, Zhang D B. The rice *tapetum degeneration retardation* gene is required for tapetum degradation and anther development. *Plant Cell*, 2006, 18: 2999–3014.
- [30] Zhang D, Liu D, Lyu X, Wang Y, Xun Z, Liu Z, Li F, Lu H. The cysteine protease CEP1, a key executor involved in tapetal programmed cell death, regulates pollen development in *Arabidopsis*. *Plant Cell*, 2014, 26: 2939–2961.
- [31] Jacobowitz J R, Doyle W C, Weng J K. PRX9 and PRX40 are extensin peroxidases essential for maintaining tapetum and microspore cell wall integrity during *Arabidopsis* anther development. *Plant Cell*, 2019, 31: 848–861.
- [32] Xie X, Zhang Z, Zhao Z, Xie Y, Li H, Ma X, Liu Y G, Chen L. The mitochondrial aldehyde dehydrogenase OsALDH2b negatively regulates tapetum degeneration in rice. *J Exp Bot*, 2020, 71: 2551–2560.
- [33] Uzair M, Xu D, Schreiber L, Shi J, Liang W, Jung K H, Chen M, Luo Z, Zhang Y, Yu J, Zhang D. PERSISTENT TAPETAL CELL2 is required for normal tapetal programmed cell death and pollen wall patterning. *Plant Physiol*, 2020, 182: 962–976.
- [34] 许代香, 贾乐东, 王瑞, 马国强, 段谋正, 曲存民, 李加纳. 甘蓝型油菜显性核不育系 D3A 的细胞学研究. *西南大学学报(自然科学版)*, 2020, 42(1): 16–21.
- Xu D X, Jia L D, Wang R, Ma G Q, Duan M Z, Qu C M, Li J N. Cytological studies of dominant GMS sterile line D3A in *Brassica napus* L. *J Southwest Univ (Nat Sci Edn)*, 2020, 42(1): 16–21 (in Chinese with English abstract).
- [35] 李六林. 植物雄性不育机理研究. 北京: 中国农业科学技术出版社, 2008. p 5.
- Li L L. Study on the Mechanism of Plant Male Sterility. Beijing: China Agricultural Science and Technology Press, 2008. p 5 (in Chinese).