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植物细胞质雄性不育与线粒体

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摘　要：细胞质雄性不育（cytoplasmic male sterility，CMS）是由于核基因组与细胞质基因组之间不协调互作导致的一种雄性器官异常而雌器官可以接受外来花粉正常结实的自然现象。在生产上，CMS是植物杂交制种的有力工具和杂种优势利用的重要途径。对CMS分子机制的解析是其有效利用的基础，一直以来都是研究的热点，然而其机制研究却相对滞后。该文从线粒体嵌合基因（*orfs*）形成、特征及分类，基因转录后修饰（RNA编辑）的特点及与CMS的关系，基因翻译产物特征、分类及其与CMS的关系等3个方面综述了近年来植物胞质雄性不育的机制研究进展，以期为进一步深入解析其分子机制提供理论参考。

关键词：胞质雄性不育；线粒体嵌合基因/开放阅读框；RNA编辑；能量赤字；毒性蛋白；程序性细胞死亡

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Cytoplasmic Male Sterility (CMS) and Mitochondria in Plants

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Abstract: Cytoplasmic male sterility (CMS) is a natural phenomenon caused by the disorder interaction between the nuclear genome and the cytoplasmic genome, in which the male organs are abnormal and the female organs can accept the foreign pollen and develop. CMS is a powerful tool for plant hybrid seed production and an important way to utilize plant heterosis. Molecular mechanism of CMS, the basis of its effective use, has been a hot research, but its mechanism research is relatively lagging. In this paper, the formation, characteristics and classification of mitochondrial chimeric genes (*orfs*), the characteristics of post-transcriptional modifications (RNA editing), their relationship with CMS, and the characteristics and classification of gene translation products and their relationship with CMS were reviewed in order to provide theoretical reference for further analysis of its molecular mechanism.

Key words: cytoplasmic male sterility (CMS); mitochondrial chimeric genes/open reading frame (ORF); RNA editing; energy deficiency; cytotoxic protein; programmed cell death (PCD)

植物细胞质雄性不育（CMS），即核-质互作雄性不育，是一种由于雄蕊退化、花粉败育或功能性不育等原因导致雄蕊不能正常授粉的母性遗传现象，是核-质互作、花药和柱头发育研究的重要材料[1]。利用CMS不育系、保持系和恢复系三系配套方法生产杂交种子，不仅节省人工去雄、隔离等成本，确保杂交种子的纯度，还能避免亲本流失，保护新品种知识产权，是农作物杂种优势利用的重要途径[2]。自胞质雄性不育现象发现以来就被广泛应用于作物杂交种子生产，对其发生机制的研究也从未间断。从

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细胞形态、生理生化、分子生物学等方面对 CMS 的发生机制都进行了探索，大量研究结果表明，植物线粒体基因组频繁地插入、丢失及重组产生嵌合基因/开放阅读框(open reading frame，ORF)，其编码产物通过各种形式影响线粒体正常功能，从而导致 CMS 的发生。本研究将近年来与线粒体有关的植物 CMS 研究进行综述，为其深入研究和利用提供参考。

1 线粒体嵌合基因(*orfs*)与 CMS

线粒体基因组与自身基因的侧翼区域、叶绿体基因或未知来源的序列发生频繁的非同源重组产生嵌合区域，形成嵌合基因(新 *orfs*)[2-3]。新 *orfs* 通常靠近线粒体功能基因，与其共转录，甚至包含其部分或全部序列，进而影响线粒体电子传递链的正常功能。迄今为止，已有诸多 CMS 相关基因被鉴定，其中大多数基因与线粒体电子传递链复合物基因，如复合物Ⅰ(*nad*2、*nad*5、*nad*9)、复合物Ⅳ(*cox*1、*cox*2)、复合物Ⅴ(*atp*1、*atp*4、*atp*6、*atp*8、*atp*9)有关(表 1)。

(1)与复合物Ⅰ相关的 CMS 基因。在油菜胞质雄性不育系(CMS-nap)中，*orf*222 与反式拼接的 *nad*5 的第 3 个外显子和开放阅读框 *orf*139 共转录[4]。茎用芥菜中，位于 *nad*2 下游的 *orfB* 与其共转录[5]。除了与功能基因共转录，水稻 CMS-RT98 的 CMS 基因 *orf*113 的 5′侧翼序列－151 至＋11 区域与 *nad*9 的同一区域序列完全相同[6]。

(2)与复合物Ⅳ相关的 CMS 基因。水稻中的 *orf*79(CMS-BT)5′区域与 *cox*1 序列相似[7]，而 *orfH*79(CMS-HL)与 *cox*1 和 *cox*2 序列同源[8]。矮牵牛融合基因 *Pcf* 包含了 *cox*2 的部分编码序列[9]，而洋葱 CMS 基因 *orf*725 的 5′端包含了 *cox*1 几乎全部序列[10]。在甜菜中，CMS 基因 *G-cox*2 是一个 C 端缩短了的 *cox*2[11]。小麦 CMS 基因 *orf*256 和 *orf*260^{cra} 除了与 *cox*1 在 5′侧翼序列－1 至－228 区域序列同源外，还与其共转录[12-13]。在辣椒 CMS 中，*orf*456 和 *orf*507 与位于其上游的 *cox*2 共转录[14-15]。

(3)与复合物Ⅴ相关的 CMS 基因。在复合物Ⅴ相关的基因中，*atp*6、*atp*8 和 *atp*9 频繁地涉及 CMS 基因的形成。首先，*atp*6 多与 *orfs* 共转录。如在水稻 CMS-BT、CMS-HL 和 CMS-LD 中，*atp*6 均位于 CMS 基因 *orf*79(*orfH*79)的上游，并与 CMS 基因共转录[7-8]；油菜 *orf*288(CMS-hau)与其上游的 *atp*6 共转录形成 2.3 kb 特殊的转录产物[16]，而在 CMS-pol 中，*orf*224 与下游 *atp*6 共转录产生 2.2 kb 和 1.9 kb 的双顺反子转录本[17]；此外，*atp*6 还与其他 *orfs* 部分或全部序列同源，如甜菜 *preSatp*6[18]、玉米 *atp*6-*C*[19] 及辣椒 *orf*456 和 *orf*507[14-15]。其次，*atp*8 与芸薹属作物 CMS 基因的形成有密切关系。源于萝卜胞质的 CMS 基因 *orf*125(CMS-Kosena)和 *orf*138(CMS-Ogura)与 *atp*8 共转录[20-21]；在油菜 CMS-pol 和 CMS-nap 中，*orf*224 与 *orf*222 有 79%的序列相似，其中 *orf*224 包含了与 *atp*8 同源的胡萝卜 *orfB* 的编码区域和 5′侧翼序列[4, 22]；另外，向日葵(CMS-PET1)*orfH*522 包含 *atp*8 前 57 bp 的序列[23]。再次，*atp*9 与 CMS 基因主要是序列同源。玉米 *orf*77 与 *atp*9 的编码区序列相似[24]，高粱 *orf*107 编码产物氨基末端的 31 个氨基酸残基与 ATP9 有 84%相似[25]；香葱 *orfA*501 和洋葱 *orf*725 包含了 *atp*9 的部分序列[10, 26]，矮牵牛 *Pcf* 基因融合了 *atp*9 的 5′侧翼序列和氨基末端的跨膜区[9]。此外，*atp*1(*A*)和 *atp*4 均与 CMS 基因共转录而产生特殊的转录产物(表 1)。

(4)与其他来源或未知序列相关的 CMS 基因。除了与线粒体电子传递链复合物基因同源的 CMS 基因外，一些 *orfs* 包含其他来源或未知的序列。水稻 CMS 基因 *WA*352(CMS-WA)和 *orf*352(CMS-RT102)包含 3 个推测的线粒体开放阅读框 *orf*284、*orf*224、*orf*288 和一段未知来源序列[27-28]。在水稻 CMS-CW 中，*orf*307 包含 *orf*288 位于 N 末端 294 bp 序列和未知来源序列[29]。萝卜 *orf*463(CMS-Don)包含 *cox*1 的 128 bp 序列和 1 261 bp 未鉴定序列[30]。这些包含非线粒体功能基因序列的 *orfs* 被证明可以导致 CMS 的发生[27]。同样，萝卜的 *orf*138(CMS-Ogura)及其同源 CMS 基因 *orf*125(CMS-Kosena)也未找到同源基因序列[20-21]。此外，一些 *orfs* 包含其他来源的序列，如菜豆(CMS-Sprite)的 CMS 基因 *PVS* 包含 3 个开放阅读框 *orf*98、*orf*97 和 *orf*239，其中 *orf*98 包含叶绿体转运 RNA(丙氨酸)的内含子序列[31-32]。

2 线粒体 RNA 编辑与 CMS

RNA 编辑是一个重要的转录后修饰和调控过程，对功能蛋白的产生至关重要。在开花植物线粒体中，RNA 编辑主要是编码区密码子的第二或第三个胞嘧啶核苷(C)转变成尿嘧啶核苷(U)[53]，导致氨基酸的转换，甚至产生新的起始或终止密码子。

表 1　植物中鉴定的 CMS 基因（*orfs*）及其编码蛋白特征

Table 1　The characteristics of CMS genes (*orfs*) and their encoding proteins identified in plants

物种 Species	CMS 类型 CMS type	CMS 基因（*orfs*） CMS gene	编码蛋白特征 Protein property	参考文献 Reference
水稻 *Oryza sativa*	CMS-WA	*rpl5-WA352*	膜蛋白 Membrane protein	[27]
	CMS-BT	*B-atp6-orf79* (*cox1* and *cox2*)	跨膜、毒性蛋白 Transmembrane, cytotoxic protein	[7]
	CMS-HL	*atp6-orfH79* (*cox1* and *cox2*)	毒性蛋白 Cytotoxic protein	[8, 33]
	CMS-CW	*orf307*	UK	[29]
	CMS-LD	*L-atp6-orf79*	UK	[34]
	CMS-RT102	*rpl5-orf352*	膜蛋白（3 个跨膜结构域）Membrane protein with three transmembrane domains	[28]
	CMS-RT98	*orf113-atp4-cox3* (*nad9*)	膜蛋白（1 个跨膜结构域）Membrane protein with one transmembrane domain	[6]
小麦 *T. aestivum*	CMS-*timopheevi*	*orf25*	UK	[35]
		orf256-cox1 (*cox1*)	7 kD 膜蛋白 7 kD membrane protein	[12]
	CMS-(cr)-CSdt7BS	$orf260^{cra}$- *cox1*	UK	[13]
甘蓝型油菜 *B. napus*	CMS-pol	*orf224-atp6* (*atp8*)	26.2 kD 膜蛋白（2 个跨膜结构域）26.2 kD membrane protein with two transmembrane domain	[36]
	CMS-nap	*orf222-nad5c-orf139* (*atp8*)	26 kD 膜蛋白 26 kD membrane protein	[4]
	CMS-ogura	*orf138- atp8*	20 kD 膜蛋白（1 个跨膜结构域）20 kD membrane protein with one transmembrane domain	[21, 37]
芥菜型油菜 *B. juncea*	CMS-hau	*atp6-orf288*	毒性蛋白 Cytotoxic protein	[16]
B. tournefortii	CMS-*tournefortii*	*atp6-orf263*	32 kD 膜蛋白 32 kD membrane protein	[38]
芥菜 *B. juncea*	CMS-8982-4	*orf220* (*atpA*)	26 kD 疏水蛋白 26 kD hydrophobin	[39]
	UK	*nad2-orfB* (*atp8*)	18.1 kD 疏水蛋白 18.1 kD hydrophobin	[5]
玉米 *Zea mays*	CMS-T	*urf13-atp4*	13 kD 毒性膜蛋白 13 kD cytotoxic membrane protein	[40]
	CMS-C	*atp6-C*	UK	[19]
	CMS-S	*orf355-orf77* (*atp9*)	UK	[24]
高粱 *Sorghum bicolor*	CMS-A3	*orf107* (*atp9*)	11.85 kD 疏水蛋白 11.85 kD hydrophobin	[25]
甜菜 *Beta vulgaris*	CMS-Owen	*preSatp6*	35 kD 膜蛋白 35 kD membrane protein	[18]
	CMS-G	*G-cox2*	31 kD 缩短的 COX2 蛋白 31 kD truncated COX2	[11]
	I-12CMS(3)	*orf129* (*cox2*)	13 kD 蛋白（线粒体基质中）31 kD mitochondrial matrix protein	[41]
向日葵 *H. annuus*	CMS-PET1	*atp1-orfH522* (*atp8*)	15 kD 毒性膜蛋白 15 kD cytotoxic membrane protein	[23, 42]
	CMS-PET2	*orf288-orf231* (*atp8* and *atp9*)	11.1 kD（*orf288*）、6.7 kD 毒性蛋白（*orf231*） 11.1 kD for *orf288* and 6.7 kD cytotoxic protein for *orf231*	[43]
		orf228 (*atp9*) *orf285* (*atp9*)	UK	[44]
	CMS-ANN2	*orf1197*	膜蛋白 Membrane proteins	[45]
	CMS-MAX1	*orf1287*	膜蛋白（7 个跨膜结构域）Membrane protein with seven transmembrane domains	[46]
矮牵牛 *P. hybrida*	CMS-petunia	*Pcf* (*atp9/cox2/urfS*)	43 kD（加工后 19.5 kD）线粒体可溶性蛋白 43 kD (19.5 kD after processing) mitochondrial soluble protein	[9, 47]
萝卜 *R. sativus*	CMS-Kosena	*orf125- atp8*	17 kD 膜蛋白 17 kD membrane protein	[20]
	CMS-Don	*orf463* (*cox1*)	膜蛋白（12 个跨膜结构域）Membrane protein with 12 transmembrane domains	[30]
	CMS-Ogura	*orf138- atp8*	20 kD 膜蛋白 20 kD membrane protein	[21, 37]
辣椒 *C. annuum*	CMS-Peterson	*cox2-orf456* (*atp6*)	17 kD 膜蛋白 17 kD Membrane protein	[14]
		cox2-orf507 (*atp6*)	毒性蛋白 Cytotoxic protein	[15, 48]

续表 1 Continued Table 1

物种 Species	CMS 类型 CMS type	CMS 基因(*orfs*) CMS genes	编码蛋白特征 Protein property	参考文献 References
茄子 *S. melongena*	UK	*orf*218-*atp*1	疏水蛋白(2 个疏水域) Hydrophobin with two hydrophobic domains	
		*orf*312-*atp*1	膜蛋白(4 个跨膜结构域) Membrane protein with four transmembrane domains	[49]
		*orf*227-*atp*1	可溶性蛋白 Soluble protein	
胡萝卜 *D. carota*	CMS-petaloid	*orfB* (*atp*8)	ATP8 类似蛋白 Similar to ATP8 protein	[50]
		*rrn*5-*atp*9	UK	[51]
洋葱 *Allium cepa*	CMS-S	*orf*725 (*cox*1 and *atp*6 and *atp*9)	UK	[10]
香葱 *A. schoenoprasum*	CMS1	*orfA*501 (*atp*6 and *atp*9)	18 kD 膜蛋白 18 kD membrane protein	[26, 52]
菜豆 *P. vulgaris*	CMS-Sprite	*orf*97	UK	[31-32]
		*orf*98 (叶绿体转运 RNA Chloroplast tRNA)	10.9 kD 膜蛋白 10.9 kD membrane protein	
		*orf*239	26.7 kD 膜蛋白 26.7 kD membrane protein	

注:UK 表示 CMS 类型或编码蛋白未知;－表示 *orfs* 与其它基因共转录;括号中的基因表示 *orfs* 包含其部分或全部序列

Note: UK represents unknown CMS type or encoding protein; － represents that *orfs* are co-transcribed with other genes; The genes in brackets indicates *orfs* contains some or all of its sequence

诸多研究表明 CMS 的发生与线粒体基因的转录产物减少、缺失或错误的 RNA 编辑有关,其改变了基因的表达模式、翻译产物的功能特性而导致 CMS 的发生[53-54]。在高粱雄性不育系中,*atp*6 转录产物的 RNA 编辑减少导致 CMS,而 F_1 和 F_2 代的育性恢复与 *atp*6 转录本增加的 RNA 编辑有关[55],况且这种 RNA 编辑的异常具有组织特异性,只发生在花药中[56]。大豆不育系 NJCMS2A 的 *atp*9 转录产物没有发生 RNA 编辑,而在保持系 NJCMS2B 中检测到了位于保守区的 2 个编辑位点,使亲水的丝氨酸转变成疏水的亮氨酸,进而导致蛋白跨膜方向相反[57]。同样,水稻 *atp*9 转录本存在 2 个 RNA 编辑位点,在保持系中编辑将精氨酸密码子变成了终止密码子,而不育系的 *atp*9 转录本没有编辑位点和终止密码子,无法翻译成正常功能的蛋白[58]。此外,玉米胞质雄性不育系(CMS-S)的 *orf*77 与 *atp*9 序列相似,在不育系小孢子中 *atp*9 所有的编辑位点均发生了 RNA 编辑,而 *orf*77 未编辑或部分编辑,这些编辑的 *orf*77 翻译成了与 ATP9 具有竞争性的 C-端跨膜结构域相似的 17 个氨基酸的多肽[59]。

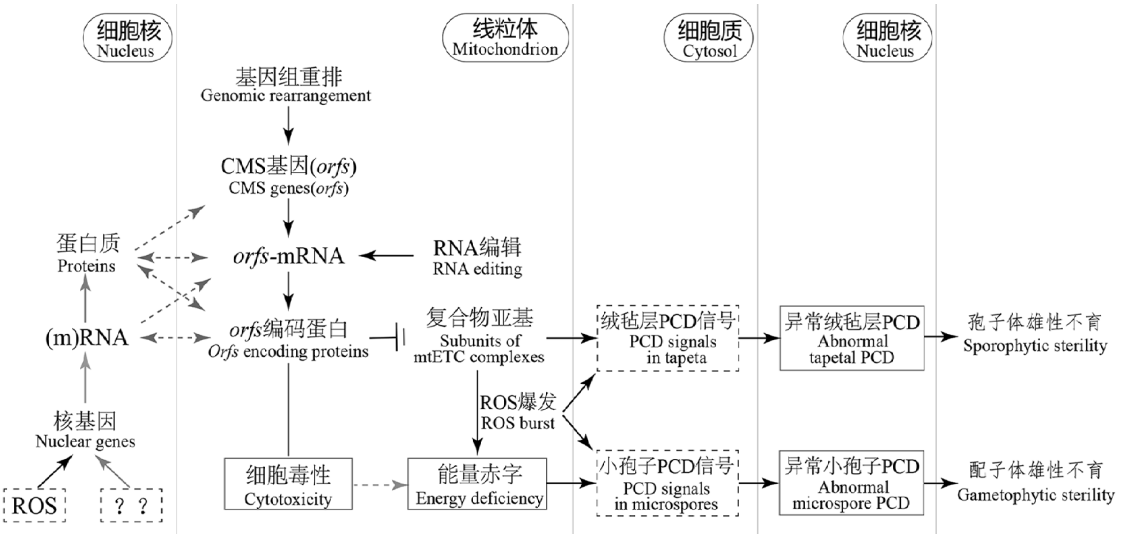
然而,在水稻野败型不育系(CMS-WA)中,即使未编辑的 *orfB* 转录本过表达可导致 CMS 形成,但 CMS 的形成与否依赖于未编辑的 *orfB* 转录本的表达量[60]。也有研究表明 CMS 相关的基因可能被编辑,但编辑程度与 CMS 的发生没有必然关系[61]。在转 RNA 编辑的 *atp*9、空载体的烟草和对照植株中均表现出可育,而未编辑的 *atp*9 转基因植株则出现不育、半不育和可育植株[62]。在比较了 8 个线粒体基因(*atp*1、4、6、8、9 和 *cox*1、2、3)在棉花 CMS、保持系及恢复系中 RNA 编辑频率后,Suzuki 等发现 8 个基因编辑频率在 3 个不同胞质中的差异均未达到显著水平[63]。因此,RNA 编辑在 CMS 中的差异可能并不一定是引发 CMS 的原因,植物线粒体中的 RNA 编辑受核-质互作的影响[64]。

3 线粒体嵌合 *orfs* 编码蛋白质与 CMS

在植物 CMS 中,线粒体嵌合基因经表达、RNA 编辑最终翻译成功能异常的蛋白,其积累影响线粒体功能,进而影响能量的产生;其次,这些蛋白可能具有细胞毒性,在花药组织细胞(绒毡层或造孢细胞)中特异积累而影响花药发育[65]。此外,线粒体功能异常伴随活性氧(reactive oxygen species, ROS)的增加,ROS 触发或直接参与异常的绒毡层和小孢子程序性细胞死亡(programmed cell death, PCD),分别产生孢子体和配子体雄性不育(图 1)。本章节从能量赤字、毒性蛋白及最终的异常 PCD 对 CMS 的产生机制进行分类综述。

3.1 能量赤字模型

花药发育过程需要大量的能量,通常造孢细胞、绒毡层细胞中线粒体数量会迅速增加以满足较高的能量需求[66],线粒体功能障碍将影响 ATP 产生进而



黑色箭头表示本文综述的内容，其中虚线表示跨膜传递；灰色箭头表示后期需开展的研究，其中虚线表示可能的互作关系或未知的机制；实线框表示 CMS 发生的模型；虚线框表示信号分子；mtETC 为线粒体电子传递链

图 1　胞质雄性不育(CMS)发生的可能机制

The black arrows represent the content of this review, with dashed lines representing transmembrane transmission; The grey arrows indicate later studies need to be carried out, with the dotted lines indicating possible interactions or unknown mechanisms; The frames with solid line represent the CMS models; The dashed frames represent the signal molecules; The mtETC indicates the mitochondrial electron transfer chain

Fig. 1　Possible mechanism of cytoplasmic male sterility (CMS)

影响雄性配子发育[2]。线粒体是细胞中最重要的供能单位，提供细胞活动 90%的能量。其利用内膜两侧的质子(H^+)梯度，通过电子传递链偶联磷酸化产生 ATP，此过程依赖呼吸链复合物和内膜的完整性。

(1)影响呼吸链复合物的正常功能。呼吸链复合物Ⅰ(NADH 脱氢酶)、复合物Ⅲ(泛醇-细胞色素 *c* 还原酶)、复合物Ⅳ(细胞色素 *c* 氧化酶)具有质子泵的功能，负责将 H^+ 泵出到内膜外形成跨膜 H^+ 梯度，H^+ 顺梯度经复合物Ⅴ(F_0F_1-ATP 合酶)进入膜内并偶联磷酸化合成 ATP。线粒体基因组重排产生的 *orfs* 通常与线粒体呼吸链复合体基因序列同源或共转录，其编码蛋白可能与复合体竞争而影响其正常的功能。低表达量的水稻 ORFH79 蛋白(CMS-HL)可抑制大肠杆菌和酵母的生长，同时检测到呼吸速率和 ATP 产量的降低[33, 67]，其与线粒体复合物Ⅲ互作，导致泛醇-细胞色素 *c* 还原酶活性降低[68]。在甜菜中，C 端缩短的 COX2 蛋白(复合物Ⅳ)导致细胞色素 *c* 氧化酶活性降低了 50%[11]；辣椒 *orf*507 与 *cox*2 共转录，其低表达增加了细胞色素 *c* 氧化酶活性[69]，同时 ORF507 与 ATP6 亚基互作导致 ATP 合酶活性降低[48]。线粒体 F_0F_1-ATP 合酶除了 ATP 合成外，还具有 ATP 水解活性[70]。辣椒 Ψ*atp*6-2 是一个 3′端编码区缩短了的 *atp*6-2，其表达增加了 F_0F_1-ATP 合酶活性，进而促进 ATP 的水解[69, 71]。CMS 基因 *orfB* 与 *atp*8 同源，其编码 F_1F_0-ATP 合酶的核心亚基，向日葵 *orf*522(CMS-PET1)编码蛋白 ORF522 与 ORFB 的 N 端 19 个氨基酸相同，其表达导致 ATP 合酶活性降低[72]。此外，油菜 ORF222 (CMS-nap)[4]、ORF224(CMS-pol)[36]，萝卜 ORF125(CMS-Kosena)[20]、ORF138(CMS-Ogura)[37]，向日葵 ORF288 (CMS-PET2)[43]及芥菜[5]和胡萝卜[50]均与 ORFB 同源，可能也是通过影响 ATP 合酶活性降低 ATP 水平，最终由于能量赤字而导致 CMS 的发生。

(2)影响线粒体内膜完整性。线粒体内膜两侧的质子梯度为 ATP 合成提供了动力，内膜完整性是保证 H^+ 梯度形成的根本。玉米 CMS-T 特异的基因 *T-urf*13 编码一个 13 kD，包含 3 个跨膜结构域的线粒体内膜蛋白 URF13，其与 T 毒素互作可在 CMS-T 玉米线粒体膜上产生亲水孔而影响内膜的透性[73-74]。此外，很多 CMS 基因编码蛋白具有跨膜结构域(表 1)，其与内膜结合可能会影响内膜的结构和稳定性，从而破坏膜内外质子梯度，影响 ATP 合成[75]。

3.2　毒性蛋白模型

研究表明，许多线粒体 *orfs* 编码的蛋白，如玉

米 URF13(CMS-T)[76]、向日葵 ORF522[77]、萝卜 ORF138(CMS-Ogura)[78]、油菜 ORF288(CMS-hau)[16]、水稻 ORF79(CMS- Boro Ⅱ &CMS-BT)[7,79]/ORFH79(CMS-HL)[33]等对大肠杆菌或酵母有毒性影响(表 1),其在植物中的积累可导致 CMS 发生。在烟草中表达向日葵 ORF522 基因(*orf*522)导致转基因烟草植株雄性不育[80],而将 *orf*522 沉默后,转基因不育植株的育性恢复[81]。同样,转 *orf*79(CMS- Boro Ⅱ)的水稻植株表现雄性不育[7],其细胞毒性依赖于与线粒体靶向序列的结合[82]。在细菌中,玉米 URF13 与 T-toxin 结合在线粒体内膜产生孔洞[75],当孔打开时,会导致线粒体膨胀、膜电位丧失和细胞死亡[83],然而在植物细胞中,毒性蛋白积累导致 CMS 发生的机制仍然未知。

3.3 异常 PCD 模型

任何由 CMS 相关基因、修饰而导致的线粒体功能缺陷,最终都可能导致绒毡层或孢子体细胞的程序性细胞死亡(PCD)。在植物中,PCD 是由线粒体信号触发的,类似哺乳动物细胞凋亡的一种细胞过程。ROS 的过量积累导致线粒体细胞色素 *c* 从线粒体释放到细胞质是 PCD 产生的重要信号[84]。

植物雄性配子在花药中发育依赖配子体(小孢子)和孢子体(花药壁)之间的协调,而花药壁最内层的绒毡层细胞最为重要[85]。其通过适时的 PCD 降解对小孢子的发育和成熟花粉的散出至关重要[86],提早或延迟的 PCD 均会导致雄性不育[27,87-89]。在向日葵 CMS-PET1 花药中,细胞色素 *c* 从线粒体释放到细胞液中,触发了绒毡层细胞提早发生 PCD,随后向花药其他组织蔓延[88]。ORF522 蛋白在 CMS-PET1 花药中积累能够影响 F_1F_0-ATP 酶和细胞色素 *c* 氧化酶活性[72,88],其是否通过影响细胞色素 *c* 氧化酶导致细胞色素 *c* 释放,进而触发提早的 PCD 尚不明晰。诸多研究表明线粒体功能缺陷通常影响呼吸代谢[33],进而影响 ROS 代谢[90-91],ROS 可以作为信号触发或参与 PCD 过程[87,90]。水稻 CMS 花药中 WA352 蛋白与核编码的 COX11(OsCOX11)互作阻碍了其在过氧化代谢中的功能,导致 ROS 的爆发和细胞色素 *c* 的释放,进而触发了提早的绒毡层 PCD 导致孢子体雄性不育[27]。在小麦中,PCD 的提早和延迟发生与 ROS 的动态变化趋势一致[87]。同样,在小麦和油菜中,PCD 的延迟启动均伴随着 ROS 变化[89,92]。

4 问题与展望

线粒体基因组重排产生的 CMS 基因(*orfs*),其编码蛋白具有细胞毒性可导致 CMS 的发生,但机制尚不清楚;另一方面 *orfs* 编码蛋白与线粒体复合物亚基互作,影响正常的线粒体功能:(1)导致 ATP 合成降低,能量赤字,最终影响花药发育;(2)同时伴随 ROS 增加产生氧化胁迫,损坏细胞质膜、核酸、蛋白等;(3)ROS 诱导细胞色素 *c* 的释放,产生 PCD 信号,异常的 PCD 导致 CMS 发生(图 1)。然而植物的其他组织和器官并未出现明显的异常表型,是什么机制调控 CMS 基因的组织(器官)特异性表达? 此外,序列的插入和重组产生的嵌合 *orfs* 大多包含线粒体基因或叶绿体基因序列和未知来源序列,这些未知序列在 CMS 发生过程中的作用尚未明晰。最后,线粒体基因组频繁的基因片段插入、丢失及重排等导致其大小、结构的巨大变异性,然而其功能基因的种类和数目却很保守,其中必定存在某种机制来协调或调控序列的插入、丢失及重排而不会影响功能基因。

自 CMS 现象发现以来,对其发生机制的研究取得了丰硕成果,然而前期大量研究多以线粒体为目标,从线粒体嵌合 *orfs* 鉴定、克隆及表达等来揭示 CMS 发生机制。研究发现,大量核基因参与 CMS 的发生过程,同时 DNA 甲基化模式的改变也与 CMS 有关[93-94],然而核基因和 DNA 甲基化在植物 CMS 发生过程中的作用机制仍有待进一步研究。CMS 表型是胞质与核互作、多基因参与、多种修饰机制共同作用的结果。随着高通量组学技术的快速发展,综合利用多组学数据,从基因、RNA、蛋白、代谢等,甚至 DNA 修饰(甲基化)、RNA 修饰、蛋白修饰等多层面对 CMS 的发生机制进行系统深入的研究,将有助于阐明 CMS 发生的分子机制。

参考文献:

[1] CHEN L T, LIU Y G. Male sterility and fertility restoration in crops[J]. *Annual Review of Plant Biology*, 2014, **65**(1): 579-606.

[2] BOHRA A, JHA U C, ADHIMOOLAM P, *et al*. Cytoplasmic male sterility (CMS) in hybrid breeding in field crops [J]. *Plant Cell Reports*, 2016, **35**(5): 967-993.

[3] CHASE C D. Cytoplasmic male sterility: a window to the

world of plant mitochondrial-nuclear interactions [J]. *Trends in Genetics*, 2007,**23**(2): 81-90.

[4] L'HOMME Y, STAHL R J, LI X Q, *et al*. *Brassica nap* cytoplasmic male sterility is associated with expression of a mtDNA region containing a chimeric gene similar to the *pol* CMS-associated *orf*224 gene[J]. *Current Genetics*, 1997, **31**(4): 325-335.

[5] YANG J H, ZHANG M F, YU J Q. Mitochondrial *nad*2 gene is co-transcripted with CMS-associated *orfB* gene in cytoplasmic male-sterile stem mustard (*Brassica juncea*)[J]. *Molecular Biology Reports*, 2009,**36**(2): 345-351.

[6] IGARASHI K, KAZAMA T, MOTOMURA K, *et al*. Whole genomic sequencing of RT98 mitochondria derived from *Oryza rufipogo*n and northern blot analysis to uncover a cytoplasmic male sterility-associated gene [J]. *Plant and Cell Physiology*, 2013,**54**(2): 237-243.

[7] WANG Z H, ZOU Y J, LI X Y, *et al*. Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing [J]. *The Plant Cell*, 2006,**18**(3): 676-687.

[8] YI P, WANG L, SUN Q, *et al*. Discovery of mitochondrial chimeric-gene associated with cytoplasmic male sterility of HL-rice [J]. *Chinese Science Bulletin*, 2002,**47**(9): 744-747.

[9] YOUNG E G, HANSON M R. A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated [J]. *Cell*, 1987,**50**(1): 41-49.

[10] KIM S, LEE E T, CHO D Y, *et al*. Identification of a novel chimeric gene, *orf*725, and its use in development of a molecular marker for distinguishing among three cytoplasm types in onion (*Allium cepa* L.) [J]. *Theoretical and Applied Genetics*, 2009,**118**(3): 433-441.

[11] DUCOS E, TOUZET P, BOUTRY M. The male sterile G cytoplasm of wild beet displays modified mitochondrial respiratory complexes [J]. *The Plant Journal*, 2001,**26**(2): 171-180.

[12] SONG J S, HEDGCOTH C. A chimeric gene (*orf*256) is expressed as protein only in cytoplasmic male-sterile lines of wheat [J]. *Plant Molecular Biology*, 1994, **26** (1): 535-539.

[13] ZHU Y, SARAIKE T, YAMAMOTO Y, *et al*. *orf*260*cra*, a novel mitochondrial gene, is associated with the homeotic transformation of stamens into pistil-like structures (pistillody) in alloplasmic wheat [J]. *Plant and Cell Physiology*, 2008,**49**(11): 1 723-1 733.

[14] KIM D H, KANG J G, KIM B D. Isolation and characterization of the cytoplasmic male sterility-associated orf456 gene of chili pepper (*Capsicum annuum* L.)[J]. *Plant Molecular Biology*, 2007,**63**(4): 519-532.

[15] GULYAS G, SHIN Y, KIM H, *et al*. Altered transcript reveals an *Orf*507 sterility-related gene in chili pepper (*Capsicum annuum* L.)[J]. *Plant Molecular Biology Reporter*, 2010,**28**(4): 605-612.

[16] JING B, HENG S P, TONG D, *et al*. A male sterility-associated cytotoxic protein ORF288 in *Brassica juncea* causes aborted pollen development[J]. *Journal of Experimental Botany*, 2012,**63**(3): 1 285-1 295.

[17] SINGH M, BROWN G G. Characterization of expression of a mitochondrial gene region associated with the *Brassica* "Polima" CMS: developmental influences [J]. *Current Genetics*, 1993,**24**(4): 316-322.

[18] YAMAMOTO M P, KUBO T, MIKAMI T. The 5′-leader sequence of sugar beet mitochondrial *atp*6 encodes a novel polypeptide that is characteristic of Owen cytoplasmic male sterility [J]. *Molecular Genetics and Genomics*, 2005,**273**(4): 342-349.

[19] DEWEY R E, TIMOTHY D H, LEVINGS C S III. Chimeric mitochondrial genes expressed in the C male-sterile cytoplasm of maize[J]. *Current Genetics*, 1991,**20**(6): 475-482.

[20] IWABUCHI M, KOIZUKA N, FUJIMOTO H, *et al*. Identification and expression of the kosena radish (*Raphanus sativus* cv. Kosena) homologue of the ogura radish CMS-associated gene, *orf*138[J]. *Plant Molecular Biology*, 1999,**39**(1): 183-188.

[21] KRISHNASAMY S, MAKAROFF C A. Organ-specific reduction in the abundance of a mitochondrial protein accompanies fertility restoration in cytoplasmic male-sterile radish [J]. *Plant Molecular Biology*, 1994,**26**(3): 935-946.

[22] HANDA H, GUALBERTO J M, GRIENENBERGER J M. Characterization of the mitochondrial *orfB* gene and its derivative, *orf*224, a chimeric open reading frame specific to one mitochondrial genome of the "Polima" male-sterile cytoplasm in rapeseed (*Brassica napus* L.) [J]. *Current Genetics*, 1995,**28**(6): 546-552.

[23] HANS KÖHLER R, HORN R, LÖSSL A, *et al*. Cytoplasmic male sterility in sunflower is correlated with the co-transcription of a new open reading frame with the *atpA* gene [J]. *Molecular and General Genetics*, 1991, **227** (3): 369-376.

[24] WEN L Y, CHASE C D. Pleiotropic effects of a nuclear restorer-of-fertility locus on mitochondrial transcripts in male-fertile and S male-sterile maize[J]. *Current Genetics*, 1999, **35**(5): 521-526.

[25] TANG H V, PRING D R, SHAW L C, *et al*. Transcript processing internal to a mitochondrial open reading frame is correlated with fertility restoration in male-sterile sorghum [J]. *The Plant Journal*, 1996,**10**(1): 123-133.

[26] ENGELKE T, TATLIOGLU T. A PCR-marker for the CMS1 inducing cytoplasm in chives derived from recombination events affecting the mitochondrial gene atp9[J]. *Theoretical and Applied Genetics*, 2002,**104**(4): 698-702.

[27] LUO D P, XU H, LIU Z L, *et al*. A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice[J]. *Nature Genetics*, 2013,**45**(5): 573-577.

[28] OKAZAKI M, KAZAMA T, MURATA H, *et al*. Whole mitochondrial genome sequencing and transcriptional analysis to uncover an RT102-type cytoplasmic male sterility-associated candidate gene derived from *Oryza rufipogon* [J]. *Plant and Cell Physiology*, 2013, **54**(9): 1 560-1 568.

[29] FUJII S, KAZAMA T, YAMADA M, *et al*. Discovery of global genomic re-organization based on comparison of two newly sequenced rice mitochondrial genomes with cytoplasmic male sterility-related genes[J]. *BMC Genomics*, 2010, **11**(1): 209.

[30] PARK J Y, LEE Y P, LEE J, *et al*. Complete mitochondrial genome sequence and identification of a candidate gene responsible for cytoplasmic male sterility in radish (*Raphanus sativus* L.) containing DCGMS cytoplasm[J]. *Theoretical and Applied Genetics*, 2013, **126**(7): 1 763-1 774.

[31] JOHNS C, LU M Q, LYZNIK A, *et al*. A mitochondrial DNA sequence is associated with abnormal pollen development in cytoplasmic male sterile bean plants[J]. *The Plant Cell*, 1992, **4**(4): 435-449.

[32] CHASE C D, ORTEGA V M. Organization of ATPA coding and 3′ flanking sequences associated with cytoplasmic male sterility in *Phaseolus vulgaris* L. [J]. *Current Genetics*, 1992, **22**(2): 147-153.

[33] PENG X J, LI F H, LI S Q, *et al*. Expression of a mitochondrial gene orfH79 from the CMS-HongLian rice inhibits *Saccharomyces cerevisiae* growth and causes excessive ROS accumulation and decrease in ATP[J]. *Biotechnology Letters*, 2009, **31**(3): 409-414.

[34] ITABASHI E, KAZAMA T, TORIYAMA K. Characterization of cytoplasmic male sterility of rice with Lead Rice cytoplasm in comparison with that with Chinsurah Boro II cytoplasm [J]. *Plant Cell Reports*, 2009, **28**(2): 233-239.

[35] BONEN L D, BIRD S, BELANGER L. Characterization of the wheat mitochondrial orf25 gene[J]. *Plant Molecular Biology*, 1990, **15**(5): 793-795.

[36] SINGH M, BROWN G G. Suppression of cytoplasmic male sterility by nuclear genes alters expression of a novel mitochondrial gene region [J]. *The Plant Cell*, 1991, **3**(12): 1 349-1 362.

[37] TANAKA Y, TSUDA M, YASUMOTO K, *et al*. A complete mitochondrial genome sequence of Ogura-type male-sterile cytoplasm and its comparative analysis with that of normal cytoplasm in radish (*Raphanus sativus* L.) [J]. *BMC Genomics*, 2012, **13**(1): 352.

[38] LANDGREN M, ZETTERSTRAND M, SUNDBERG E, *et al*. Alloplasmic male-sterile *Brassica* lines containing *B. tournefortii* mitochondria express an ORF 3′ of the *atp6* gene and a 32 kDa protein [J]. *Plant Molecular Biology*, 1996, **32**(5): 879-890.

[39] ZHANG M F, CHEN L P, WANG B L, *et al*. Characterization of *atpA* and *orf220* genes distinctively present in a cytoplasmic male-sterile line of tuber mustard[J]. *The Journal of Horticultural Science and Biotechnology*, 2003, **78**(6): 837-841.

[40] DEWEY R E, TIMOTHY D H, LEVINGS C S. A mitochondrial protein associated with cytoplasmic male sterility in the T cytoplasm of maize[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1987, **84**(15): 5 374-5 378.

[41] YAMAMOTO M P, SHINADA H, ONODERA Y, *et al*. A male sterility-associated mitochondrial protein in wild beets causes pollen disruption in transgenic plants [J]. *The Plant Journal*, 2008, **54**(6): 1 027-1 036.

[42] LAVER H K, REYNOLDS S J, MONEGER F, *et al*. Mitochondrial genome organization and expression associated with cytoplasmic male sterility in sunflower (*Helianthus annuus*) [J]. *The Plant Journal*, 1991, **1**(2): 185-193.

[43] REDDEMANN A, HORN R. Recombination events involving the atp9 gene are associated with male sterility of CMS PET2 in sunflower[J]. *International Journal of Molecular Sciences*, 2018, **19**(3): 806.

[44] MAKARENKO M S, KORNIENKO I V, AZARIN K V, *et al*. Mitochondrial genomes organization in alloplasmic lines of sunflower (*Helianthus annuus* L.) with various types of cytoplasmic male sterility [J]. *Peer Journal*, 2018, **6**: e5266. doi:10.7717/peerj.5266

[45] MAKARENKO M S, USATOV A V, TATARINOVA T V, *et al*. Organization features of the mitochondrial genome of sunflower (*Helianthus annuus* L.) with ANN2-type male-sterile cytoplasm[J]. *Plants*, 2019, **8**(11): 439.

[46] MAKARENKO M S, USATOV A V, TATARINOVA T V, *et al*. Characterization of the mitochondrial genome of the MAX1 type of cytoplasmic male-sterile sunflower[J]. *BMC Plant Biology*, 2019, **19**(S1): 51.

[47] NIVISON H T, HANSON M R. Identification of a mitochondrial protein associated with cytoplasmic male sterility in *Petunia*[J]. *The Plant Cell*, 1989, **1**(11): 1 121-1 130.

[48] LI J J, PANDEYA D, JO Y D, *et al*. Reduced activity of ATP synthase in mitochondria causes cytoplasmic male sterility in chili pepper [J]. *Planta*, 2013, **237**(4): 1 097-1 109.

[49] YOSHIMI M, KITAMURA Y, ISSHIKI S, *et al*. Variations in the structure and transcription of the mitochondrial atp and *cox* genes in wild *Solanum* species that induce male sterility in eggplant (*S. melongena*) [J]. *Theoretical and Applied Genetics*, 2013, **126**(7): 1 851-1 859.

[50] NAKAJIMA Y, YAMAMOTO T, MURANAKA T, *et al*. A novel *orfB*-related gene of carrot mitochondrial genomes that is associated with homeotic cytoplasmic male sterility (CMS) [J]. *Plant Molecular Biology*, 2001, **46**(1): 99-107.

[51] SZKLARCZYK M, SZYMAŃSKI M, WÓJCIK-JAGŁA M, *et al*. Mitochondrial atp9 genes from petaloid male-sterile and male-fertile carrots differ in their status of heteroplasmy, recombination involvement, post-transcriptional processing as

well as accumulation of RNA and protein product[J]. *Theoretical and Applied Genetics*, 2014, **127**(8): 1 689-1 701.

[52] ENGELKE T, TATLIOGLU T. The fertility restorer genes X and T alter the transcripts of a novel mitochondrial gene implicated in CMS1 in chives (*Allium schoenoprasum* L.) [J]. *Molecular Genetics and Genomics*, 2004, **271**(2): 150-160.

[53] GRAY M W, HANIC-JOYCE P J, COVELLO P S. Transcription, processing and editing in plant mitochondria[J]. *Annual Review of Plant Physiology and Plant Molecular Biology*, 1992, **43**(1): 145-175.

[54] BONEN L D, BROWN G G. Genetic plasticity and its consequences: perspectives on gene organization and expression in plant mitochondria[J]. *Canadian Journal of Botany*, 1993, **71**(5): 645-660.

[55] HOWAD W, KEMPKEN F. Cell type-specific loss of atp6 RNA editing in cytoplasmic male sterile *Sorghum bicolor*[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1997, **94**(20): 11 090-11 095.

[56] KEMPKEN F, HOWAD W, PRING D R. Mutations at specific atp6 codons which cause human mitochondrial diseases also lead to male sterility in a plant[J]. *FEBS Letters*, 1998, **441**(2): 159-160.

[57] JIANG W, YANG S P, YU D Y, *et al*. A comparative study of ATPase subunit 9 (*Atp9*) gene between cytoplasmic male sterile line and its maintainer line in soybeans[J]. *African Journal of Biotechnology*, 2011, **10**(51): 10 387-10 392.

[58] WEI L, YAN Z X, DING Y. Mitochondrial RNA editing of F0-ATPase subunit 9 gene (*atp9*) transcripts of Yunnan purple rice cytoplasmic male sterile line and its maintainer line [J]. *Acta Physiologiae Plantarum*, 2008, **30**(5): 657-662.

[59] GALLAGHER L, BETZ S, CHASE C. Mitochondrial RNA editing truncates a chimeric open reading frame associated with S male-sterility in maize [J]. *Current Genetics*, 2002, **42**(3): 179-184.

[60] CHAKRABORTY A, MITRA J, BHATTACHARYYA J, *et al*. Transgenic expression of an unedited mitochondrial *orfB* gene product from wild abortive (WA) cytoplasm of rice (*Oryza sativa* L.) generates male sterility in fertile rice lines [J]. *Planta*, 2015, **241**(6): 1 463-1 479.

[61] STAHL R, SUN S, L'HOMME Y, *et al*. RNA editing of transcripts of a chimeric mitochondrial gene associated with cytoplasmic male-sterility in *Brassica*[J]. *Nucleic Acids Research*, 1994, **22**(11): 2 109-2 113.

[62] HERNOULD M, SUHARSONO S, LITVAK S, *et al*. Male-sterility induction in transgenic tobacco plants with an unedited atp9 mitochondrial gene from wheat[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1993, **90**(6): 2 370-2 374.

[63] SUZUKI H, YU J W, NESS S A, *et al*. RNA editing events in mitochondrial genes by ultra-deep sequencing methods: a comparison of cytoplasmic male sterile, fertile and restored genotypes in cotton[J]. *Molecular Genetics and Genomics*, 2013, **288**(9): 445-457.

[64] HU J H, YI R, ZHANG H Y, *et al*. Nucleo-cytoplasmic interactions affect RNA editing of *cox2*, *atp6* and *atp9* in alloplasmic male-sterile rice (*Oryza sativa* L.) lines[J]. *Mitochondrion*, 2013, **13**(2): 87-95.

[65] CONLEY C A, HANSON M R. Tissue-specific protein expression in plant mitochondria[J]. *The Plant Cell*, 1994: 85-91.

[66] WARMKE H E, LEE S L J. Pollen abortion in T cytoplasmic male-sterile corn (*Zea mays*): A suggested mechanism [J]. *Science*, 1978, **200**(4 341): 561-563.

[67] DING X, CHEN Q S, BAO C M, *et al*. Expression of a mitochondrial gene orfH79 from CMS-Honglian rice inhibits *Escherichia coli* growth via deficient oxygen consumption [J]. *SpringerPlus*, 2016, 5: 1 125.

[68] WANG K, GAO F, JI Y X, *et al*. ORFH79 impairs mitochondrial function via interaction with a subunit of electron transport chain complex III in Honglian cytoplasmic male sterile rice [J]. *New Phytologist*, 2013, **198**(2): 408-418.

[69] JI J J, HUANG W, YIN C C, *et al*. Mitochondrial cytochrome *c* oxidase and F_1F_0-ATPase dysfunction in peppers (*Capsicum annuum* L.) with cytoplasmic male sterility and its association with *orf*507 and Ψ*atp*6-2 genes [J]. *International Journal of Molecular Sciences*, 2013, **14**(1): 1 050-1 068.

[70] CABEZON E, BUTLER P J G, RUNSWICK M J, *et al*. Modulation of the oligomerization state of the bovine F1-ATPase inhibitor protein, IF1, by pH[J]. *Journal of Biological Chemistry*, 2000, **275**(33): 25 460-25 464.

[71] JI J J, HUANG W, LI D W, *et al*. A CMS-related gene, Ψatp6-2, causes increased ATP hydrolysis activity of the mitochondrial F_1F_0-ATP synthase and induces male sterility in pepper (*Capsicum annuum* L.)[J]. *Plant Molecular Biology Reporter*, 2014, **32**(4): 888-899.

[72] SABAR M, GAGLIARDI D, BALK J, *et al*. ORFB is a subunit of F1F(O)-ATP synthase: insight into the basis of cytoplasmic male sterility in sunflower [J]. *EMBO Reports*, 2003, **4**(4): 381-386.

[73] LEVINGS C S, SIEDOW J N. Molecular basis of disease susceptibility in Texas cytoplasm of maize [J]. *Plant Molecular Biology*, 1992, **19**(1): 135-147.

[74] RHOADS D M, KASPI C I, LEVINGS C S, *et al*. N, N'-dicyclohexylcarbodiimide cross-linking suggests a central core of helices II in oligomers of URF13, the pore-forming T-toxin receptor of cms-T maize mitochondria[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1994, **91**(17): 8 253-8 257.

[75] RHOADS D M, LEVINGS C S III, SIEDOW J N. URF13, a ligand-gated, pore-forming receptor for T-toxin in the inner membrane of cms-T mitochondria[J]. *Journal of Bioenergetics and Biomembranes*, 1995, **27**(4): 437-445.

[76] DEWEY R E, SIEDOW J N, TIMOTHY D H, *et al*. A 13-kilodalton maize mitochondrial protein in *E. coli* confers sensitivity to *Bipolaris maydis* toxin [J]. *Science*, 1988, **239** (4 837): 293-295.

[77] NAKAI S, NODA D, KONDO M, *et al*. High-level expression of a mitochondrial *orf*522 gene from the male-sterile sunflower (*Helianthus annuus*) is lethal to *E. coli* [J]. *Breeding Science*, 2010, **45**(2): 233-236.

[78] DUROC Y, GAILLARD C, HIARD S, *et al*. Biochemical and functional characterization of ORF138, a mitochondrial protein responsible for Ogura cytoplasmic male sterility in Brassiceae [J]. *Biochimie*, 2005, **87**(12): 1 089-1 100.

[79] KAZAMA T, NAKAMURA T, WATANABE M, *et al*. Suppression mechanism of mitochondrial ORF79 accumulation by Rf1 protein in BT-type cytoplasmic male sterile rice [J]. *The Plant Journal*, 2008, **55**(4): 619-628.

[80] NIZAMPATNAM N R, DOODHI H, KALINATI NARASIMHAN Y, *et al*. Expression of sunflower cytoplasmic male sterility-associated open reading frame, orfH522 induces male sterility in transgenic tobacco plants[J]. *Planta*, 2009, **229** (4): 987-1 001.

[81] NIZAMPATNAM N R, DINESH KUMAR V. Intron hairpin and transitive RNAi mediated silencing of *orfH*522 transcripts restores male fertility in transgenic male sterile tobacco plants expressing *orfH*522 [J]. *Plant Molecular Biology*, 2011, **76**(6): 557-573.

[82] KOJIMA H, KAZAMA T, FUJII S, *et al*. Cytoplasmic male sterility-associated ORF79 is toxic to plant regeneration when expressed with mitochondrial targeting sequence of ATPase γ subunit [J]. *Plant Biotechnology*, 2010, **27**(1): 111-114.

[83] WISE R P, BRONSON C R, SCHNABLE P S, *et al*, The genetics, pathology, and molecular biology of T-cytoplasm male sterility in maize [J]. *Advances in Agronomy*, 1999, **65**: 79-130.

[84] VIANELLO A, ZANCANI M, PERESSON C, *et al*. Plant mitochondrial pathway leading to programmed cell death[J]. *Physiologia Plantarum*, 2007, **129**(1): 242-252.

[85] MA H. Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants[J]. *Annual Review of Plant Biology*, 2005, **56**(1): 393-434.

[86] LUO X D, DAI L F, WANG S, *et al*. Male gamete development and early tapetal degeneration in cytoplasmic male-sterile pepper investigated by meiotic, anatomical and ultrastructural analyses[J]. *Plant Breeding*, 2006, **125**(4): 395-399.

[87] LIU Z H, SHI X Y, LI S, *et al*. Oxidative stress and aberrant programmed cell death are associated with pollen abortion in isonuclear alloplasmic male-sterile wheat[J]. *Frontiers in Plant Science*, 2018, 9: 595. doi: 10. 3389/fpls. 2018. 00595.

[88] BALK J, LEAVER C J. The PET1-CMS mitochondrial mutation in sunflower is associated with premature programmed cell death and cytochrome c release[J]. *The Plant Cell*, 2001, **13**(8): 1 803-1 818.

[89] LIU Z H, SHI X Y, LI S, *et al*. Tapetal-delayed programmed cell death (PCD) and oxidative stress-induced male sterility of *Aegilops uniaristata* cytoplasm in wheat[J]. *International Journal of Molecular Sciences*, 2018, **19**(6): 1 708.

[90] LI S Q, WAN C X, KONG J, *et al*. Programmed cell death during microgenesis in a Honglian CMS line of rice is correlated with oxidative stress in mitochondria [J]. *Functional Plant Biology*, 2004, **31**(4): 369-376.

[91] WAN C X, LI S Q, WEN L, *et al*. Damage of oxidative stress on mitochondria during microspores development in Honglian CMS line of rice[J]. *Plant Cell Reports*, 2007, **26** (3): 373-382.

[92] DU K, XIAO Y Y, LIU Q E, *et al*. Abnormal tapetum development and energy metabolism associated with sterility in SaNa-1A CMS of *Brassica napus* L. [J]. *Plant Cell Reports*, 2019, **38**(5): 545-558.

[93] ZHANG M, GUO L P, QI T X, *et al*. Integrated methylome and transcriptome analysis between the CMS-D2 line ZBA and its maintainer line ZB in upland cotton[J]. *International Journal of Molecular Sciences*, 2019, **20**(23): 6 070.

[94] LIU Z W, LIU Y F, SUN Y H, *et al*. Comparative transcriptome analysis reveals the potential mechanism of abortion in tobacco sua-cytoplasmic male sterility[J]. *International Journal of Molecular Sciences*, 2020, **21**(7): 2 445.

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