

胁迫下的细胞程序性死亡鉴定有助于植物抗逆性

徐凌翔, 陈壬杰, 许海锦, 周元昌*, 吴为人*

福建农林大学作物遗传育种与综合利用教育部重点实验室, 福州 350002

* 联系人, E-mail: zwy_2002@163.com; wuwr@fafu.edu.cn

2015-09-11 收稿, 2015-11-30 修回, 2015-12-01 接受, 2016-01-05 网络版发表

国家科技支撑计划(2013BAD01B0107)资助

摘要 细胞程序性死亡(PCD)在植物生存和进化上有重要的意义. 近几年, 遗传学、生理学、分子与细胞生物学技术的发展以及动物细胞理论的平行性对植物PCD的鉴定起了很大推动作用, 但胁迫相关的PCD的鉴定仍有较大的复杂性. 当今植物PCD的鉴定主要以探针染色分析核形为主, 多种方法结合, 但还需要更加可靠的表征和表征的量化. 逆境下PCD的鉴定主要在3个方面有潜在应用价值: (i) 多重抗性间的关联研究; (ii) 抗性遗传资源使用; (iii) 种质资源保存. 本文主要结合实践中的PCD鉴定的应用前景来综述当前胁迫条件下植物PCD研究的进展.

关键词 坏死, 类半胱天冬酶, DNA 片段化, 类病斑突变体, 自噬, 活性氧

细胞程序性死亡(programmed cell death, PCD)是生物体可遗传的主动清除自身细胞的一种机制. 这种机制既能作为雄性不育、抗病性等作物育种关键性状的表型, 又能用于理解种质资源保存过程中劣变的机理, 还与多种生物和非生物抗性的调控网络有所关联, 因此是当前植物遗传学和生理生化研究的热点问题之一. 近5年来, 关于植物PCD的研究取得了显著的进展, 国内外学者从多方面对PCD现象的鉴别、检测方法、成因和育种上的应用等开展了研究, 最突出的进展分别体现在现象鉴定和机理研究上.

植物PCD鉴定上, Van Doorn等人^[1]提出的液泡型PCD的概念近几年被逐步采纳^[2], 液泡形态变化以及液泡合成酶的作用越来越多地被作为植物PCD的依据, 标志性事件是液泡酸化、中央大液泡形成、液泡中产生自噬体或自噬小体, 以及液泡破裂释放水解酶等^[1]. 液泡对逆境作出响应和清除细胞内容物的作用被肯定. 而在机理方面对Metacaspase(类半胱天冬酶^[3]家族酶)的系统性研究^[4,5]更为PCD的成因提供了依据, 这在拟南芥(*Arabidopsis thaliana*)中尤为突

出. Metacaspase是动物细胞中的半胱天冬酶(Caspase)的类似物, 在两个主要亚基和反应催化结构上具有保守性^[6]. 并有Tsiatsiani等人^[5]的研究表明, Metacaspase在很多情况下是发生PCD所必需的, 其中拟南芥的Metacaspase-1和2(AtMC1-2)分别促进和抑制PCD, 两者相互起拮抗作用^[4], 在识别病原体后的作用规律类似于细胞焦亡(pyroptosis^[7,8]), 动物细胞PCD的一种, 是动物细胞死亡的4个主要类型之一^[8,9]中的caspase-1和caspase-12. 此外Metacaspase还是细胞死亡调控网络上的关键节点, 对细胞是否死亡或采取何种形式死亡有决定作用^[10], 例如, AtMC1后来被发现具有促存活的作用^[11].

然而, 对植物PCD, 当前很多学者认识仍不全面. 例如, 认为植物中存在细胞凋亡, 且较容易混淆细胞坏死(necrosis)和非程序性细胞死亡(non-programmed cell death)的概念, 而正确认识植物PCD对植物遗传资源的保护和利用有重要价值. 因此, 本文综述胁迫条件下的植物PCD在定义、分类和成因上的研究进展, 旨在为植物PCD更加准确的鉴定提供依

引用格式: 徐凌翔, 陈壬杰, 许海锦, 等. 胁迫下的细胞程序性死亡鉴定有助于植物抗逆性. 科学通报, 2016, 61: 809–818

Xu L X, Chen R J, Xu H J, et al. Identifying stress-related programmed cell death improves research on plant resistance (in Chinese). Chin Sci Bull, 2016, 61: 809–818, doi: 10.1360/N972015-01046

据,使之更好地与作物育种和遗传资源保存及利用相结合.

1 植物PCD的鉴别及其复杂性

植物PCD的研究进展,特别是对其现象和机理的研究,包括词汇的使用都源于动物细胞学,但两者截然不同,其中调控PCD的基因鲜有保守型可言,因此用于植物PCD鉴定的标志性事件很大程度上依赖的是动物细胞PCD理论的平行性^[12]. PCD在1964年由Lockshin在动物上详细描述,而“凋亡”(apoptosis)一词,由Kerr等人^[13]在1972年提出. Clarke^[14]于1990年将动物PCD分为3类,凋亡、自噬型死亡(autophagic cell death)和非溶酶体性囊泡分解(non-lysosomal vesiculate degradation),这是非常权威的分类,为当今科学家广泛接受. 细胞学家多将上述三类的前两类加上坏死并称为三大类细胞死亡^[15-17].

“类似凋亡”(apoptosis-like)这一描述在植物学中被长期广泛使用以表述植物PCD中存在着DNA片段化(DNA laddering),类似于动物细胞中一种现象: Caspase-3将DNA切割成核小体整倍长度的片段,从而使电泳呈现间隔180~200 bp的等距离阶梯状条带. 这在植物细胞学上也长期被作为PCD的标志性事件^[1]. 但因为植物细胞有细胞壁的阻隔,不可能像动物细胞那样能产生凸出于细胞之外的凋亡小体(apoptotic body),也没有清除凋亡产物的吞噬细胞(phagocyte)^[1,18],植物中也就不存在凋亡,因此“类似凋亡”这一描述不够准确.

随着植物PCD的作用和机理上的研究逐步深入,PCD的分类变得愈加重要,而且不断出现新的归类方法^[18]. 较为权威的是van Doorn等人^[1]将植物PCD分为3个大类:液泡型PCD和坏死型PCD(动物细胞死亡中对应的表述为necroptosis,但植物细胞中未见使用)以及两者的中间型(表1). 液泡型PCD通常在植物发育中产生,长期被误认为是植物细胞凋亡. van Doorn和Woltering^[18]提出发育过程中的PCD接近于动物中的自噬型死亡,而凋亡的存在是无法证明的.

PCD主要出现在植物发育、抗逆和衰老过程中. 因植物发育而产生的PCD属于液泡型PCD,又称发育型PCD,包括胚柄、胚乳、子叶、维管束中发生的PCD,如玉米(*Zea mays*)种子发育过程中PCD仅限于发育成熟个体上消失的部分^[20]. 跟发育型PCD相比逆境下的植物PCD较为复杂,原因是这种PCD和多

表1 细胞死亡类型和特征

Table 1 Cell death types and traits

细胞死亡类型	简要特征	
	形态	生理生化
液泡型PCD ^{[11]①}	自噬体 (autophagosome) 累积; 溶解性液泡形成; 细胞器膨大但在液泡膜破裂前依然完整; 液泡膜破裂; 空的细胞壁残骸的出现	液泡酸化, 液泡合成酶(vacuole processing enzyme, VPE)激活
坏死型PCD ^{[11]②}	线粒体膨胀破裂; 细胞膜在早期破裂; 细胞质浓缩; 细胞残骸未被清除	呼吸速率和ATP下降, 活性氧和活性氮积累
中间型PCD ^{[11]③}	兼有①和②的特征	
非程序性死亡④	蛋白质修饰(包括二硫键交联和羰基化); 核酸的随机损伤如链断裂、共价键修饰和点突变; 脂类过氧化 ^[19]	
细胞坏死	包含②③④	
胁迫下的PCD	包含②③以及①中的少数案例	

种细胞死亡方式之间有交集(表1).

与PCD在概念上对立的是非程序性细胞死亡(又称突发性细胞死亡(accidental cell death, ACD)^[15]). 其在动物上的鉴定也存在一定难度,不断出现新的研究成果. 细胞死亡命名委员会(Nomenclature Committee on Cell Death, NCCD)对其定义为: 由于极端的物理、化学或机械刺激引起的失控的伴随细胞结构快速崩溃的细胞死亡^[15]. NCCD同时指出^[15], 由于可调控的细胞死亡形式不断被发现, ACD的范围随之缩小. 植物中的非程序性细胞死亡同样难以鉴定, 种子学家Kranner等人^[19]所提供的标准(表1)目前还未在高等植物中实现原位检测. 相对而言较被认可的划分来源于CdSO₄对烟草(*Nicotiana benthamiana*)悬浮细胞的处理结果^[21,22], 高浓度的CdSO₄体现了ACD的特征而低浓度时则体现了PCD的特征, 尽管受限于当时的认知, 两者分别被定性为细胞坏死和凋亡, 但这种比较^[21]仍被作为鉴定PCD的经典参照.

非程序性死亡理论上可以用死亡细胞数和发生PCD的细胞数的差值来判断, 但尚无科学实验证明这种可行性, 因为统计细胞数的准确性存在两个困难, 荧光探针标记的可靠性和细胞计数的准确性. El-Maarouf-Bouteau等人^[23]尝试了荧光标记死亡细胞和PCD细胞并对数量进行了估算, 但在原文中未能显示出死亡细胞多于PCD细胞. Minina等人^[24]也尝试了

用两种以上探针标记不同死亡细胞类型, 但仅对坏死型细胞计数. 自噬型死亡细胞虽然通过自噬体数量来体现但未能计量自噬型死亡细胞的数量.

胁迫下的植物PCD难以鉴定, 不仅是因为细胞死亡的多种形式难于区分, 还因为胁迫所致的PCD和老化、发育中的PCD从机理上并不互斥, 因为老化和发育过程中有机体不需要的细胞必然受到养分不足的胁迫. 又如木质部形成过程中的PCD是发育型^[1], 但植物免疫反应同样能促使木质部形成^[25].

由于鉴定的复杂性, 实际研究中为了保证研究逻辑的严密性, 表1中的划分不一定被采用, 植物病理学家将“过敏性(hypersensitive response, HR)死亡”^[26-28]视作规范用法. NCCD认为鉴于细胞死亡鉴定的复杂性以及越来越多的特例被发现, PCD应通过更加具体的词汇描述^[17]. 但概要地划分仍然对当前的科学研究和实践有很重要的价值, 而且PCD信号传导和调控网络方面更进一步的研究, 及遗传学和生物化学上新的细胞原位检测手段的出现和应用也会对PCD的鉴定产生新的推动作用.

2 植物PCD的检测方法

鉴定植物PCD需要检测, 检测方法主要分为分子生物学方法如琼脂糖电泳检测DNA片段化, 形态学方法如电子显微镜(electron microscope, EM)观察细胞膜、线粒体和核质结构, 生理学研究方法如检测Caspase活性抑制剂对代谢产物的影响, 以及形态学与分子生物学的结合法如组织原位染色或悬浮细胞染色等. DNA片段化的现象在种子PCD研究中并不常见: Kranner对4种老化作物的种子检测了DNA片段化但仅在豌豆(*Pisum sativum*)中成功^[29], 也有研究证明植物PCD产生的DNA片段长度不是核小体长度的整数倍, 而是160~180 bp或更低^[30,31]. 很多已发表的通过荧光染色验证PCD的研究^[24,31]也是通过DNA片段化以外的方法来验证. 同时, 通过液氮冻伤这一典型的非程序性细胞死亡的发生条件处理的烟草悬浮(*Nicotiana benthamiana*)细胞, 形态上虽然符合坏死的特征, 却产生了DNA片段化^[22]. 因此植物体中产生DNA片段化的条件仍然需要探索. 片段化的存在还和细胞所处周期有关^[32]. 而植物中有液泡参与的PCD的关键酶活性除了类Caspase-3活性之外还有类Caspase-1活性^[33], 产生这两种活性的酶不同, 前者是通过蛋白酶亚单元PBA1, 后者则是液泡合成酶

(vacuole processing enzyme, VPE).

与DNA链结合从而产生荧光或显色的探针结合显微镜观察是当前最主要的检测方法, 据检测目的的不同可将探针分为3个大类: (i) PCD指示探针, 其中最常用的是TUNEL(脱氧核苷酸末端转移酶(terminal deoxynucleotidyl transferase, TdT)介导的dUTP缺口末端标记(TdT-mediated dUTP nick-end labeling, TUNEL)), 对DNA断裂后的3'-OH黏性末端特异性结合从而显色或发出荧光; (ii) 标记死亡细胞的探针, 原理是通过细胞死亡后细胞膜选择性透过功能的丧失而实现的, 这种探针标记的结果需要和其他手段结合作为PCD的凭据; (iii) 以4',6-二脒基-2-苯基吲哚(4',6-diamidino-2-phenylindole, DAPI)为主, 标记视野内所有细胞核作对照(图1(a)), 但有时也用于观察细胞核形态(图1(b)). 一种PCD指示探针与一种核标记探针复染在实验中较常见, 最普遍的是TUNEL/DAPI复染^[34,36,37], 以及吖啶橙/溴化乙锭(acridine orange/ethidium bromide, AO/EB)复染^[38], 此外还能将荧光标记与明场观察相结合, 如在明场下观察细胞膜的完整性结合细胞核是否发生形变(由DAPI标记)^[37], 或PI/FDA/TUNEL(PI: propidium iodine, 碘化丙啶; FDA: fluorescein diacetate, 二乙酸荧光素)多种探针结合^[39](更多探针的使用见表2). 其中Lee和Chen^[42]通过TUNEL证明水稻(*Oryza sativa*)衰老叶片中存在PCD, 又通过DNA片段化的缺失证明类Caspase-3活性不存在, 在当时是非常有意义的发现, 尽管作者仍将类Caspase-3活性作为凋亡存在的依据. 类Caspase活性虽然已经可以在悬浮细胞中实现可视化荧光标记^[43], 但作为植物PCD分类的凭据仍存在可研究的空间.

死亡细胞伴随自噬体(autophagic body)或自噬小体的形成也能作为PCD的证据. 当前主要的探针有红色或绿色荧光蛋白(red/green fluorescent protein, RFP/GFP)^[24,44,45]和LysoTracker(一种溶酶体示踪剂)^[45,46]及单丹磺酰尸胺(monodansylcadaverine, MDC)^[44,45,47], 前两者的难点在于需要将荧光蛋白导入宿主细胞, 后两者的难点在于标记的特异性不佳^[45].

电子显微镜(以下简称电镜)观察仍然是目前的形态学方法中最为可靠的手段之一^[1], 可以在电镜下观察液泡结合激光扫描共聚焦显微镜下的TUNEL阳性判明液泡型PCD存在^[30,48], 此外还可以在电镜下

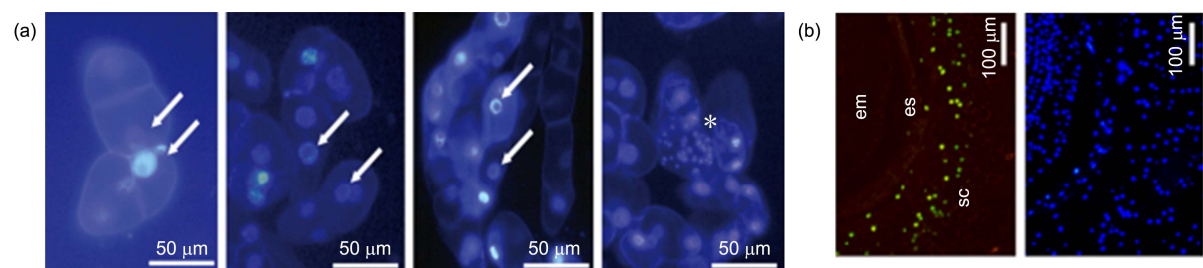


图 1 (网络版彩图)DAPI标记降解细胞核和标记全部细胞核的示例((a)和(b)分别引自文献[34]和[35]). (a) DAPI在烟草细胞培养液中标记异常细胞核^[34], 白色箭头指示正在降解的细胞核, 比未降解的细胞核亮度更大或出现了空心化, *指示已经解体的细胞核; (b) DAPI在西红柿 (*Lycopersicon esculintum*)胚发育子叶折叠阶段中标记全部细胞核^[35], 以证实(b)中左图的TUNEL未标记的细胞核是存在从而排除假阴性. em: embryo, 胚; es: endosperm, 胚乳; sc: seed coat, 种皮. 本图的(a)和(b)分别经该图版权所有Elsevier出版社和美国植物生物学会授权

Figure 1 (Color online) Application of DAPI in marking degraded nuclei and total nuclei. (a) DAPI labbled abnormal nuclei in Tabacco (*Nicotiana benthamiana*) suspension cells^[34], white arrows indicated degrading nuleus, which is brighter and more vacuolated compared with normal nuclei, yellow arrow indicated disassembled nucleus. DAPI labelled nuclei in curled cotyledon stage of tomato (*Lycopersicon esculintum*)^[35] to prove the existence of the non-labelled nuclei in the left image thus eliminate false negative. (a) and (b) were authorized respectively by Elesvier Publisher and American Society of Plant Biologists.

表 2 常见PCD检测荧光探针汇总^{a)}

Table 2 Profile of usual fluorescent probe for detecting PCD^{a)}

探针名称	标记的特征	标记的部位	反应条件	透过性	参考文献
DAPI	全部细胞核	细胞核	InS Sus	是	[34,35]
TUNEL	PCD	细胞核	InS	否	[19]
AO			InS Sus	是	[34]
EB		细胞核	Sus	否	[38]
PI	细胞死亡、生活力		InS	否	[34]
Evans Blue		细胞质	Sus	否	[37]
FDA			InS Sus	否	[34,37]

a) InS: *In situ*, 原位, 表明该探针常用于原位标记; Sus: suspension, 悬浮液, 表明该探针常用于细胞悬浮液中的标记; AO: acridine orange, 吖啶橙; PI: propidium iodine, 碘化丙啶; Evans Blue: 伊文氏蓝; FDA: fluorescein diacetate, 二乙酸荧光素. 透性一列中“是”和“否”分别表示荧光探针能和不能通过活细胞的细胞膜. DAPI除标记全部细胞核外, 还可标记降解的核酸, 但仅在细胞悬浮液上有应用. 同类染液还有性质相似的Hoechst. PI同类探针还有Sytox和Dead/live细胞活性分析试剂盒, 其中后者染色后固定依然能保持荧光, 但试剂盒的推出时间较短所以在高等植物中未见应用; PI同类型染液在细胞固定后也能起到DAPI标记全部细胞核的作用^[23], 但若标记死亡细胞则事先不能进行固定否则会因固定液能够杀死细胞而呈现假阳性. 用DAPI来标记已降解的核酸的例子仅在悬浮液中可见^[34]. Annexin细胞核荧光探针可用于流式细胞仪, 高等植物中不常用. 另, TB: trypan blue, 台盼蓝作用和PI相似^[40], 但可以直接显色无需荧光激发; TUNEL也有无荧光的试剂盒, 依靠辣根过氧化物酶来显色^[41]

直接观察亚细胞层面的PCD标记, 如EM-TUNEL法^[49]. 但如要大量取样则时间消耗和成本都很大, 为电镜所呈现的PCD类型找到具有表征性的探针或者特定的多个探针的组合有可能成为大量取样的可行办法.

随着遗传学的发展, 原位PCD相关基因表达将有可能通过显微切割结合原位杂交、qPCR、免疫组化以及转录组和蛋白质组等技术来实现. 此外, 随着对Metacaspase研究的深入, Metacaspase的表达变化会在将来成为有力证据.

3 PCD是研究作物抗性及其机理和调控网络的重要因素

胁迫下的植物PCD目前研究得最充分的是叶片在病原体侵染下形成的HR-PCD, 但PCD在生理学和分子生物学上并不局限于植物和病原体互作而是和大量其他抗逆机制相关联.

HR-PCD的理论基础主要有基因对基因假说^[26], 简而言之就是植物针对病原体的第一道防线——病

原相关分子模式触发的免疫(PAMPs (pathogen-associated molecular patterns)-triggered immunity, PTI): 一旦被病原体利用效应因子(effector)——一种有毒(virulent)蛋白抑制后, 植物体有可能通过R基因识别这种效应因子形成第二道防线——效应因子触发的免疫反应(effector-triggered immunity, ETI): 此时的效应因子就成了无毒(avirulent)蛋白从而被宿主的抗病(R)蛋白识别引起联级反应在局部产生HR-PCD来限制病原体扩散. 而其他细胞则产生系统获得性抗性(system acquired resistance, SAR). 病原体又可以产生新的效应因子致病, 而宿主亦可形成新的R基因. 这种进化被称为“军备竞赛”.

通常ETI被认为是专化抗性, 又被称为垂直抗性, 会在局部产生PCD, 持久性差. PTI则通常是基础抗性, 又称水平抗性, 抗谱较宽, 抗性持续时间久, 但抗性不如ETI彻底. 新近的研究进展表明, PCD并不专属于ETI^[27,28], PAMPs可以触发PCD或在不触发PCD的情况下形成SAR, ETI也有可能不伴随PCD, 如在抗谱相对较广的非寄主抗性中. 而从抗性的效果来看PCD伴随的抗性是多重的, 可以是针对跨真菌和细菌界的不同病原体^[50], 也可以是同时针对生物和非生物胁迫^[24]. 遗传学上, 近年Lee等人^[51]的研究证明R基因非但对基础抗性有所贡献, 且调控的基础抗性的蛋白与专化抗性中的一致.

随着基因组关联分析技术(genome-wide association study, GWAS)的发展, 跟PCD相关的基因位点研究越来越多地被应用于作物抗逆遗传当中, 特别是在模式植物水稻^[52-54]和玉米^[55,56], 以及双子叶作物大豆(*Glycine max*)^[57,58]中, 此外还有菜豆(*Phaseolus vulgaris*)^[59]和栗属(*Castanea mollissima*和*Castanea dentata*)^[60]. Olukolu等人^[2]通过对3381份玉米重组自交系作GWAS发现44个HR相关候选基因, 除了与PCD、自噬、防御和钙信号(PCD中的关键信号)相关外还有和氧化还原内平衡相关的基因. 在生物和非生物胁迫中, 控制抗性的遗传多为数量遗传, 遗传分析通常以转录组的方式进行, 这些结果中涉及的表达变化多属于控制PCD的信号传导、调控网络的基因位点, 此外还包括活性氧(reactive oxygen species, ROS)、水杨酸、茉莉酸代谢相关基因^[60]和氧化还原内平衡的相关基因, 这些位点也和PCD息息相关. Zhang等人^[52]意外发现在水稻抗寒品种中的10个比非抗寒品种有着更高的基本表达量的基因都和PCD

有关.

由此可见, 诸多因素能引起PCD, 而不同成因下的PCD也相应呈现不同的格局. 例如, 病毒引起的PCD通过液泡膜破裂释放水解酶来抑制核酸中的病毒; 而细菌与宿主的相互作用往往发生在膜外空间, 质壁融合是宿主抑制细菌的表现^[1]. 相同因素引起的PCD时间进程上也有不同表现^[61]. 因此, 对PCD细分和鉴定有利于更好地把握植物逆境下的表型, 从而推断遗传和表达机理, 有利于设计育种目标、制定植物材料处理的策略, 通过对信号的放大或抑制还有植物内平衡的改变来优化抗性.

4 PCD鉴定是抗性遗传资源利用的凭据

PCD的鉴定不仅有助于深入研究作物抗性, 还有助于抗性遗传资源在生产上的应用. 带有自发抗性的类病斑突变体(lesion mimic mutant, LMM)是农业中的宝贵遗传资源, 得名的原因是叶片上会出现类似HR-PCD现象的斑点^[62,63]. 其中大麦(*Hordeum vulgare* L.)*mlo*^[62]突变体是被学者们认可的最早用于农业实践的LMM, 来源于诱变剂诱变, 对禾谷白粉病菌既有垂直抗性也有水平抗性. LMM中的HR-PCD的时空格局通常和野生型有所不同. 这些PCD很多不是由病原体触发的, 而是环境因子, 特别是光照^[64]和温度^[65], 此外湿度也有作用^[66]. LMM中的PCD的复杂性除了和野生型中的HR-PCD产生的时间、规模、机理不同外, 还因为扩散型细胞死亡(runaway cell death, RCD)的存在^[67]. RCD是通过拟南芥的突变体表现的: 抑制细胞死亡的基因突变后, 在HR中产生了面积远大于野生型的死亡叶片细胞, 有时甚至覆盖全部叶片, 这就是RCD得名的原因. LMM中的PCD表型和作物产量、抗性性状有很大关系. 产生抗性的LMM通常产量比起野生型有所下降, 但也有下降不显著的^[64], 这类材料会被推荐到育种当中^[68], 特点是病斑较少, 是否是因为RCD到了控制需要进一步验证.

Minina等人^[10]的研究表明, HR-PCD和RCD存在空间特异性, 在ROS和水杨酸的调控作用下自噬基因(*autophagy*, *ATG*)在感染中心促进PCD, 从而起到限制病原体的作用, 而随着位置由感染中心向外围扩散, *ATG*的作用逐步由促进PCD转为抑制PCD, 从而保证细胞的存活. 这和其他研究进展相映射: Coll等人^[11]发现*AtMC-1*同样存在促存活和促死亡作用并

存,从而推断PCD是对病原体的抵抗作用(通常伴随着ROS浓度增加)中产生的副作用而不是直接影响抗性的.这些进展有可能进一步加快叶片中的PCD机理研究,为LMM材料的应用提供理论依据,促使人们更好地把握表型,分解不同病原体 and 环境的贡献.

除了空间特异性,Zuppini等人^[61]证明细胞死亡存在时间特异性.随着胁迫的加剧由液泡型死亡特征逐步过渡为坏死型.van Doorn^[69]列举了包括衰老的黄叶返绿等依据证明PCD存在不可逆点(当细胞死亡的进程通过这一节点后细胞死亡变得不可逆),这体现了时间上的特异性.

从动态的格局来说,个体不同位置的细胞处于变化中,无论选取时间还是空间因素,其死亡方式都会呈现一定的特异性,只有准确把握时空特异性才能从整体研究PCD及其相关的事件和过程.对LMM而言,鉴定PCD的类型、规模、各主要事件的时间顺序有利于准确选择符合育种目标的遗传资源.

5 PCD鉴定对种质资源保存的意义

叶片中的PCD在较为匀质的材料中进行;而种质资源,如种子和离体保存的组织具有一定的异质性,PCD的时空格局相对更加复杂多变.PCD的时空特异性在种质保存抵御逆境方面受到了越来越多的重视.

种子在高温高湿条件下劣变相关的PCD近几年取得了突出的研究进展^[23,29,41,70].Hu等人^[41]人利用荧光示踪技术发现ROS在家榆(*Ulmus pumila*)种子外皮、子叶和下胚轴中的扩散顺序和规模与PCD的发生以及种子活力的大幅下降等关键事件时间节点上高度一致.向日葵(*Helianthus annuus*)种子的研究^[23]通过区域细胞程序性死亡格局推断种子群体老化的不可逆点(point of no return)在50%萌发率水平上.沈嵘等人^[71]也证实通过外源添加锌离子来抑制ROS可以延缓PCD,此外van Doorn^[69]的论述支持了Kranmer的“不可逆点”假说.Kranmer认为PCD在胁迫中最基本的角色是选择性去除植物体“不需要”的细胞,是生物体对胁迫产生预警后发生的抵御,但随着胁迫强度的增加,细胞死亡的程度逐渐加剧,种子由阻抗

阶段进入衰竭阶段,因出现PCD的数量较多、位置较关键(特别是胚中)而产生了不可逆转的变化,最终导致死亡,因此PCD具有双重意义^[19].

除了种子,离体保存的胞组织和细胞中的PCD同样取得了重大的研究进展^[72,73],结果和种子保存相似,离体保存的组织或细胞的生活力减退与发生PCD的细胞数量成正相关.Wesley-Smith等人^[72]等提出了低温保存的冰晶损伤对组织的影响主要是以PCD的方式进行,而非此前人们认为的ACD.该研究证明糖槭(*Acer saccharum*)的低温保存过程中死亡细胞能被补充从而保持组织的活力,这是通过由形成层和基本分生组织等生长点的细胞增殖来实现的,并表明离体保存组织中多种细胞死亡方式并存.

如果能进一步在种质资源保存中判明死亡的数量、位置和方式与个体活力之间的关系,有助于细化和规范化种质保存的技术和措施.

6 展望

时空特异性决定了无论在细胞还是个体层面都存在不可逆点.在LMM应用上强调的是细胞或组织的不可逆点;种质保存上则强调个体的不可逆点.种质保存中的PCD研究局限在于研究主要强调细胞死亡的负面影响,而PCD和抗性之间的关联性可以探索PCD的正面作用.LMM揭示了PCD调控网络中关键基因的作用^[63,74],促进了多重抗性之间关联性的研究;反之,PCD相关的抗逆性和种质保存上的时空特异性的研究进展又有望为LMM的利用提供依据.因此,和PCD鉴定有关的多种应用之间是相辅相成的.加强对PCD的鉴定包括PCD的类型、成因、所处阶段和PCD对其他调控网络及性状的影响无论在育种还是种质资源保存方面都有积极的作用.全球气候变化和人口、经济增长引起的人地矛盾加剧是当今农业面临的重要问题^[75],由此产生的土地退化、极端气象和频发的病虫害,以及遗传资源的生物多样性损失使抗病、广适的作物培育以及遗传资源的保存迫在眉睫.植物PCD的鉴定有望促进上述问题的预防 and 解决,意义深远.

致谢 河南农业大学硕士生林一欣、中国科学院武汉植物园博士操瑜、江红生为本文语句和行文提供了宝贵意见,在此一并致谢.

参考文献

- 1 van Doorn W G, Beers E P, Dangel J L, et al. Morphological classification of plant cell deaths. *Cell Death Differ*, 2011, 18: 1241–1246
- 2 Olukolu B A, Wang G F, Vontimitta V, et al. A genome-wide association study of the maize hypersensitive defense response identifies genes that cluster in related pathways. *PLoS Genet*, 2014, 10: 761–768
- 3 Zhan J, He H Y, Li W, et al. Progress in caspase-like proteases and their regulatory roles in programmed cell death in plants (in Chinese). *Plant Physiol J*, 2012, 48: 435–441 [詹洁, 何虎翼, 李文, 等. 植物类 Caspase 及其调控细胞程序性死亡的研究进展. *植物生理学报*, 2012, 48: 435–441]
- 4 Coll N S, Dominique V, Andrea S, et al. *Arabidopsis* type I metacaspases control cell death. *Science*, 2010, 330: 1393–1397
- 5 Tsiatsiani L, Van B F, Gallois P, et al. Metacaspases. *Cell Death Differ*, 2011, 18: 1279–1288
- 6 Dominique V, Wim D, Peter V, et al. Are metacaspases caspases? *J Cell Biol*, 2007, 179: 375–380
- 7 Coll N S, Epple P, Dangel J L. Programmed cell death in the plant immune system. *Cell Death Differ*, 2011, 18: 1247–1256
- 8 Dickman M B, Fluhr R. Centrality of host cell death in plant-microbe interactions. *Annu Rev Phytopathol*, 2013, 51: 543–570
- 9 Duprez L, Wirawan E, Berghe T V, et al. Major cell death pathways at a glance. *Microbes Infect*, 2009, 11: 1050–1062
- 10 Minina E A, Bozhkov P V, Daniel H. Autophagy as initiator or executioner of cell death. *Trends Plant Sci*, 2014, 19: 692–697
- 11 Coll N S, Smidler A, Puigvert M, et al. The plant metacaspase AtMC1 in pathogen-triggered programmed cell death and aging: Functional linkage with autophagy. *Cell Death Differ*, 2014, 21: 1399–1408
- 12 Danon A, Delorme V, Mailhac N, et al. Plant programmed cell death: A common way to die. *Plant Physiol Bioch*, 2000, 38: 647–655
- 13 Kerr J F, Wyllie A H, Currie A R. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*, 1972, 26: 239–257
- 14 Clarke P G. Developmental cell death: morphological diversity and multiple mechanisms. *Anat Embryol*, 1990, 181: 195–213
- 15 Galluzzi L, Pedro B S, Vitale I, et al. Essential versus accessory aspects of cell death: Recommendations of the NCCD 2015. *Cell Death Differ*, 2014, 22: 58–73
- 16 Gozuacik D, Kimchi A. Autophagy as a cell death and tumor suppressor mechanism. *Oncogene*, 2004, 23: 2891–2906
- 17 Kroemer G, Galluzzi L, Vandenabeele P, et al. Classification of cell death: Recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ*, 2008, 16: 3–11
- 18 van Doorn W G, Woltering E J. Many ways to exit? Cell death categories in plants. *Trends Plant Sci*, 2005, 10: 117–122
- 19 Kranner I, Minibayeva F V, Beckett R P, et al. What is stress? Concepts, definitions and applications in seed science. *New Phytol*, 2010, 188: 655–673
- 20 Giuliani C, Consonni G, Gavazzi G M, et al. Programmed cell death during embryogenesis in maize. *Ann Bot*, 2002, 90: 287–292
- 21 Fojtova M A. Genotoxic effect of cadmium is associated with apoptotic changes in tobacco cells. *Plant Cell Environ*, 2000, 23: 531–537
- 22 Kuthanova A, Opatrny Z, Fischer L. Is internucleosomal DNA fragmentation an indicator of programmed death in plant cells? *J Exp Bot*, 2008, 59: 2233–2240
- 23 El-Maarouf-Bouteau H, Mazuy C, Corbineau F, et al. DNA alteration and programmed cell death during ageing of sunflower seed. *J Exp Bot*, 2011, 62: 5003–5011
- 24 Minina E A, Filonova L H, Kazutake F, et al. Autophagy and metacaspase determine the mode of cell death in plants. *J Cell Biol*, 2013, 203: 1803–1809
- 25 Kissoudis C, van de Wiel C, Visser R G, et al. Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. *Front Plant Sci*, 2014, 5: 207
- 26 Jones J D G, Dangel J L. The plant immune system. *Nature*, 2006, 444: 323–329
- 27 Thomma B P, Nurnberger T, Joosten M H. Of PAMPs and effectors: The blurred PTI-ETI dichotomy. *Plant Cell*, 2011, 23: 4–15
- 28 Tsuda K, Katagiri F. Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. *Curr Opin Plant Biol*, 2010, 13: 459–465
- 29 Kranner I, Birtic S, Anderson K M, et al. Glutathione half-cell reduction potential: A universal stress marker and modulator of programmed cell death? *Free Radical Bio Med*, 2006, 40: 2155–2165
- 30 Wojtyla L, Kosmala A, Garnczarska M. Lupine embryo axes under salinity stress. II. Mitochondrial proteome response. *Acta Physiol Plant*, 2013, 35: 2383–2392
- 31 Mittler R, Simon L, Lam E. Pathogen-induced programmed cell death in tobacco. *J Cell Sci*, 1997, 110: 1333–1344
- 32 Kuthanova A, Fischer L, Nick P, et al. Cell cycle phase-specific death response of tobacco BY-2 cell line to cadmium treatment. *Plant*, 2008, 31: 1634–1643
- 33 Hara-Nishimura I, Hatsugai N. The role of vacuole in plant cell death. *Cell Death Differ*, 2011, 18: 1298–1304

- 34 Lytvyn D I, Yemets A I, Blume Y B. UV-B overexposure induces programmed cell death in a BY-2 tobacco cell line. *Environ Exp Bot*, 2010, 68: 51–57
- 35 Goetz S, Hellwege A, Stenzel I, et al. Role of *cis*-12-oxo-phytodienoic acid in tomato embryo development. *Plant Physiol*, 2012, 158: 1715–1727
- 36 Vuosku J, Sarjala T, Jokela A, et al. One tissue, two fates: Different roles of megagametophyte cells during Scots pine embryogenesis. *J Exp Bot*, 2009, 60: 1375–1386
- 37 Petrusa E, Bertolini A, Casolo V, et al. Mitochondrial bioenergetics linked to the manifestation of programmed cell death during somatic embryogenesis of *Abies alba*. *Planta*, 2009, 231: 93–107
- 38 Kuzniak E, Kazmierczak A, Wielanek M, et al. Involvement of salicylic acid, glutathione and protein S-thiolation in plant cell death-mediated defence response of *Mesembryanthemum crystallinum* against *Botrytis cinerea*. *Plant Physiol Bioch*, 2013, 63: 30–38
- 39 Lu H X, Wan Q, Wang H H, et al. Oxidative stress and mitochondrial dysfunctions are early events in narciclasine-induced programmed cell death in tobacco Bright Yellow-2 cells. *Physiol Plantarum*, 2012, 144: 48–58
- 40 Hu X Y, Fang J Y, Cai W M, et al. Induction of hypersensitive response of rice suspension cells by nitrogen-monoxide-mediated non-affinity elicitor (in Chinese). *Chin Sci Bull*, 2003, 48: 157–161 [胡向阳, 方建颖, 蔡伟明, 等. 一氧化氮介导非亲和性激发子诱发水稻悬浮细胞过敏反应. *科学通报*, 2003, 48: 157–161]
- 41 Hu D, Ma G, Wang Q, et al. Spatial and temporal nature of reactive oxygen species production and programmed cell death in elm (*Ulmus pumila* L.) seeds during controlled deterioration. *Plant Cell Environ*, 2012, 35: 2045–2059
- 42 Lee R H, Chen S C G. Programmed cell death during rice leaf senescence is nonapoptotic. *New Phytol*, 2002, 155: 25–32
- 43 Elbaz M, Avni A, Weil M. Constitutive caspase-like machinery executes programmed cell death in plant cells. *Cell Death Differ*, 2002, 9: 726–773
- 44 Wang Y, Yu B J, Zhao J P, et al. Autophagy contributes to leaf starch degradation. *Plant Cell*, 2013, 25: 1383–1399
- 45 Merkulova E A, Guiboileau A, Naya L, et al. Assessment and optimization of autophagy monitoring methods in *Arabidopsis* roots indicate direct fusion of autophagosomes with vacuoles. *Plant Cell Physiol*, 2014, 55: 715–726
- 46 Hofius D, Schultz-Larsen T, Joensen J, et al. Autophagic components contribute to hypersensitive cell death in *Arabidopsis*. *Cell*, 2009, 4: 773–783
- 47 Liu Y M, Burgos J S, Deng Y, et al. Degradation of the Endoplasmic Reticulum by Autophagy during Endoplasmic Reticulum Stress in *Arabidopsis*. *Plant Cell*, 2012, 24: 4635–4651
- 48 Graner E M, Brondani G E, Almeida C V D, et al. Study of senescence in old cultures of *Bactris gasipaes* Kunth *in vitro*. *Plant Cell Tiss Organ Cult*, 2015, 120: 1169–1189
- 49 Yao N, Eisfelder B J, Greenberg J T, et al. The mitochondrion—an organelle commonly involved in programmed cell death in *Arabidopsis thaliana*. *Plant J*, 2004, 40: 596–610
- 50 Yin Z C, Chen J, Zeng L R, et al. Characterizing rice lesion mimic mutants and identifying a mutant with broad-spectrum resistance to rice blast and bacterial blight. *Mol Plant Microbe Interact*, 2000, 13: 869–876
- 51 Lee J, Feng J, Campbell K B, et al. Quantitative proteomic analysis of bean plants infected by a virulent and avirulent obligate rust fungus. *Mol Cell Proteomics*, 2009, 8: 19–31
- 52 Zhang F, Huang L Y, Wang W S, et al. Genome-wide gene expression profiling of introgressed indica rice alleles associated with seedling cold tolerance improvement in a japonica rice background. *BMC Genomics*, 2012, 13: 237–243
- 53 Fujino K, Matsuda Y. Genome-wide analysis of genes targeted by qLTG3-1 controlling low-temperature germinability in rice. *Plant Mol Biol*, 2010, 72: 137–152
- 54 Shabala S. Physiological and cellular aspects of phytotoxicity tolerance in plants: The role of membrane transporters and implications for crop breeding for waterlogging tolerance. *New Phytol*, 2011, 190: 289–298
- 55 Zila C T, Samayoa L F, Santiago R, et al. A genome-wide association study reveals genes associated with Fusarium ear rot resistance in a maize core diversity panel. *G3-Genes Genomes Genetics*, 2013, 3: 2095–2104
- 56 Ye J R, Guo Y L, Zhang D F, et al. Cytological and molecular characterization of quantitative trait locus *qRfg1*, which confers resistance to gibberella stalk rot in maize. *Mol Plant Microbe Interact*, 2013, 26: 1417–1428
- 57 Kandoth P K, Ithal N, Recknor J, et al. The soybean *rhg1* locus for resistance to the soybean cyst nematode *Heterodera glycines* regulates the expression of a large number of stress- and defense-related genes in degenerating feeding cells. *Plant Physiol*, 2011, 155: 1960–1975
- 58 Radwan O, Liu Y, Clough S J. Transcriptional analysis of soybean root response to *Fusarium virguliforme*, the causal agent of sudden death syndrome. *Mol Plant Microbe Interact*, 2011, 24: 958–972
- 59 Hannah M A, Kramer K M, Geffroy V, et al. Hybrid weakness controlled by the dosage-dependent lethal (*DL*) gene system in common bean (*Phaseolus vulgaris*) is caused by a shoot-derived inhibitory signal leading to salicylic acid-associated root death. *New Phytol*, 2007, 176: 537–549

- 60 Barakat A, Staton M, Cheng C H, et al. Chestnut resistance to the blight disease: Insights from transcriptome analysis. *BMC Plant Biol*, 2012, 12: 1–15
- 61 Zuppini A, Gerotto C, Baldan B. Programmed cell death and adaptation: Two different types of abiotic stress response in a unicellular chlorophyte. *Plant Cell Physiol*, 2010, 51: 884–895
- 62 McGrann G R, Steed A, Burt C, et al. Differential effects of lesion mimic mutants in barley on disease development by facultative pathogens. *J Exp Bot*, 2015, 66: 3417–3428
- 63 Lorrain S, Vaillau F, Balaque C, et al. Lesion mimic mutants: Keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci*, 2003, 8: 263–271
- 64 Yao Q, Zhou R H, Fu T H, et al. Characterization and mapping of complementary lesion-mimic genes *lm1* and *lm2* in common wheat. *Theor Appl Genet*, 2009, 119: 1005–1012
- 65 Matin M N, Pandeya D, Baek K H, et al. Phenotypic and genotypic analysis of rice lesion mimic mutants. *Plant Pathol J*, 2010, 26: 159–169
- 66 Mosher S, Moeder W, Nishimura N, et al. The lesion-mimic mutant *cpr22* shows alterations in abscisic acid signaling and abscisic acid insensitivity in a salicylic acid-dependent manner. *Plant Physiol*, 2010, 152: 1901–1913
- 67 Jabs T, Dietrich R A, Dangel J L. Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. *Science*, 1996, 273: 1853–1856
- 68 Kamlofski C A, Antonelli E, Bender C, et al. A lesion-mimic mutant of wheat with enhanced resistance to leaf rust. *Plant Pathol*, 2007, 56: 46–54
- 69 van Doorn W G. Plant programmed cell death and the point of no return. *Trends Plant Sci*, 2005, 10: 478–483
- 70 Wang Y, Li Y, Xue H, et al. Reactive oxygen species-provoked mitochondria-dependent cell death during ageing of elm (*Ulmus pumila* L.) seeds. *Plant J*, 2015, 81: 438–452
- 71 Shen R, Liu X Y, Zhang H X, et al. Effects of Zn^{2+} on rice root tip cells programmed cell death under high salt stress or UV-induced (in Chinese). *J Nanjing Agr Univ*, 2010, 33: 13–18 [沈嵘, 刘晓宇, 张红晓, 等. Zn^{2+} 对高盐和紫外线胁迫条件下水稻根尖细胞程序性死亡的影响. *南京农业大学学报*, 2010, 33: 13–18]
- 72 Wesley-Smith J, Walters C, Pammenter N W, et al. Why is intracellular ice lethal? A microscopical study showing evidence of programmed cell death in cryo-exposed embryonic axes of recalcitrant seeds of *Acer saccharinum*. *Ann Bot*, 2015, 115: 991–1000
- 73 Graner E M, Brondani G E, de Almeida C V, et al. Study of senescence in old cultures of the *Bactris gasipaes* Kunth *in vitro*. *Plant Cell Tiss Org*, 2015, 120: 1169–1189
- 74 Love A J, Milner J J, Sadanandom A. Timing is everything: Regulatory overlap in plant cell death. *Trends Plant Sci*, 2008, 13: 589–595
- 75 Meyer R S, Purugganan M D. Evolution of crop species: Genetics of domestication and diversification. *Nat Rev Genet*, 2013, 14: 840–852

Identifying stress-related programmed cell death improves research on plant resistance

XU LingXiang, CHEN RenJie, XU HaiJin, ZHOU YuanChang & WU WeiRen

Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, Fujian Agriculture & Forestry University, Fuzhou 350002, China

Programmed cell death (PCD) is essential to the survival and evolution of plants. Recently, the advance in the technology of plant genetics, physiology, molecular and cytological biology supports the study on the phenomena and mechanisms of plant PCD. In respect of phenomena, the significant progress includes the classification of two distinguish types of plant PCD: vacuolar PCD and necrotic PCD. The outstanding progress in mechanisms includes the discovery of the structure of a proteinase family: metacaspases, along with their role in survival/death decision or cell-death type controlling.

However, complications in recognizing plant PCD still persist for little is genetically conserved between plants' and animals' PCD-regulation, so recognition of plant PCD depends on its parallels with more adequately studied animal PCD. Stress-related PCD is even more complicated comparing with developmental PCD because it contains briefly necrotic PCD and features of vacuolar PCD, while developmental PCD can be exclusively attributed to vacuolar PCD. Moreover, stress-related PCD is likely to be confused with non-programmed cell death.

Identification of plant PCD consists of multiple methods including molecular biological methods, morphological methods and physiological methods. Up to date, fluorescent probe staining nuclei, which is a combination of molecular and morphological method becomes the most popular technique to label plant PCD and its effect is reinforced by other probes which label cell death or autophagic activity, morphological observation with electron microscope and DNA agarose gel electrophoresis. Still, both more reliable markers and quantification of marked events is required.

Stress-related PCD is becoming more important against both biotic and abiotic stress and its identification would potentially improve the research in three respects: (i) correlating the resistances to multiple stresses; (ii) the utilization of disease-resistant genetic resources and (iii) the preservation of germplasm. These applications assist each other and would reciprocally improve the comprehension of the patterns and mechanisms in plant PCD.

Stress-related PCD in plant is most profoundly studied in the hypersensitive response (HR) during effector-triggered-immunity (ETI) with recognition of pathogens. However, PCD is by far not confined only within ETI but also associated with a great variety of resistance. Loci related to PCD or other PCD-related process like salicylic or jasmonic metabolism, reactive oxygen species (ROS) scavenging, redox homeostasis and calcium signaling often contribute to resistance to a great variety of factors. Therefore PCD identification has the potentiality to help in selection in crop breeding and to decipher regulatory pathways and network for stress resistance.

One popular application of PCD in crop breeding is the lesion mimic mutants (LMMs). LMMs exhibit intrinsic PCD and can be disease resistant usually at the cost of production. The identification of phenotype concerning PCD could help selecting most fitable LMM genotype to balance production and resistance. LMMs in turn help deciphering PCD pathways.

Spatial and temporal heterogeneity exists in LMMs and such heterogeneity is comparatively well studied in PCD in germplasm preservation including the storage of seeds, tissues or cells. Decline in vigor of germplasm correlates with the proliferating of PCD cells, which probably include a process of survival/death decision. Therefore timing, counting and locating PCD cells would assist in monitoring germplasm vigor, understanding the process of deterioration and deciding whether and when to rescue germplasm resources.

As a result of global climate change and population growing pressure and various secondary problems derived from these, breeding stress-resistant crops and preserving germplasm become urgent and emphasize the identification of stress-related PCD.

necrosis, metacaspase, DNA laddering, lesion mimic mutant, autophagy, reactive oxygen species

doi: 10.1360/N972015-01046