

泛素 - 蛋白酶体系统影响植物农艺性状的研究进展

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摘要: 当前人口激增、环境污染、生态破坏等问题层出不穷。改良农艺性状, 如提高产量、增强逆境耐受能力, 是作物遗传改良的重要目标, 是推动农业高质量发展的基础。泛素 - 蛋白酶体系统 (UPS) 是一种快速的选择性水解植物体产生的冗余蛋白和被损坏蛋白的体系。目前, 研究发现泛素 - 蛋白酶系统影响植物的发育、生殖和重要的农艺性状: 如应对环境胁迫、开花诱导和种子大小等。综述了近年来的研究成果, 阐述了蛋白酶体 UPS 组分和各亚基的重要功能, 并描述了泛素 - 蛋白酶系统是如何影响植物农艺性状的。最后, 讨论了未来的研究热点和利用 UPS 改良作物的潜力。

关键词: 泛素 - 蛋白酶系统; 亚基; 植物; 农艺性状

DOI: 10.13560/j.cnki.biotech.bull.1985.2022-0160

Research Progress in the Effects of Ubiquitin-proteasome System on Plant Agronomic Traits

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Abstract: At present, problems such as population explosion, environmental pollution and ecological destruction emerge one after another. Improving agronomic traits, such as increasing yield and enhancing stress tolerance, is the most important goal of crop genetic improvement and the basis of promoting high-quality agricultural development. The ubiquitin-proteasome system (UPS) is a rapid and selective system for the hydrolysis of redundant and damaged proteins produced by plant. Now, studies have found that the ubiquitin-proteasome system affects plant development, reproduction, and important agronomic traits, such as pathogen responses, induction of flowering, seed size, and so on. This review focuses on recent studies illustrating the important functions of the UPS components and subunits of the proteasome and describes how the UPS affect plant agronomic traits. Finally, the review discusses the future research hotspots and the potential for crop improvement with UPS.

Key words: UPS; subunits; plant; agronomic traits

当前人口、环境、气候、生态平衡等问题层出不穷, 改良植物农艺性状, 提高产量, 增强品质, 维持植物内稳态至关重要。目前一些研究已经揭示了植物蛋白质分子的识别和分子作用机制。活细胞内的蛋白质通过调节特定的生化或代谢途径控制细

胞活动和生理过程。构象的改变, 亚细胞定位或稳定性变化都会影响蛋白质的活性。蛋白质的降解与合成一样重要。除了线粒体和细胞质中的蛋白酶及溶酶体, 泛素 - 蛋白酶体系统 (UPS) 是蛋白质降解的主要途径, 负责细胞中大多数蛋白质的降解

收稿日期: 2022-02-09

基金项目: 北京电子科技职业学院校级重点课题项目资助 (2021Z025-KXZ)

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(80%–90%)^[11]。UPS 提供了一种有效和快速的方法来选择性降解蛋白达到广泛调节哺乳动物和植物细胞的细胞学和生理过程的目的。近年来,遗传和细胞生物学方法揭示了 UPS 在植物内稳态的几乎所有方面的作用,包括植物生长发育、对植物激素的反应以及对非生物和生物刺激的信号传递^[2-4]。本文主要讨论了近年来 UPS 调控农艺性状方面的研究进展和未来的发展方向。

1 E2 结合酶的功能

成千上万的蛋白质参与泛素化、识别和处理泛素化蛋白质以及 26S 蛋白酶体的调控,表明泛素在植物细胞生理学和植物对环境条件的反应中具有广泛的作用^[5]。泛素通过泛素激活酶(E1)、一种或多种泛素结合酶(E2)和泛素连接酶(E3)附着在底物蛋白上^[6]。E1 启动共轭级联,对靶标特异性影响不大。拟南芥中只有两种 E1 亚型,其中一种可能是核定位的^[7]。相比之下,在拟南芥基因组中,E2 亚型是一个由至少 37 个 E2 基因组成的大家族,这些基因分为 12 个亚家族和 8 个 E2-like 基因^[8]。有趣的是,许多 E2 编码基因是应激诱导的。如大豆 *GmUBC2* (Ub conjugating enzyme E2)、花生 *AhUBC2* 和拟南芥 *AtUBC32* 在干旱或盐胁迫下转录水平上调^[9-11]。*atubc32* 突变体植株更耐盐胁迫,超表达 *AtUBC32* 使植株对盐胁迫敏感^[9]。此外,在拟南芥中过表达豇豆 *Vignaradiata UBC1* (*VrUBC1*)、*AhUBC2* 或 *GmUBC2* 植株具有更强的干旱耐受性^[12]。Pan 等^[13]还发现拟南芥中 *AtUBC27* 正向调控 ABA 信号转导和抗旱性,*AtUBC8* 和 *AtUBC24* 分别是应对氮限制或磷缺乏所必需的^[14]。最近研究发现,*OsUBC26*,一种水稻泛素结合酶,可以防御稻瘟病菌。通过接种稻瘟病菌和茉莉酸甲酯处理可以诱导 *OsUBC26* 的表达。*OsUBC26* 基因沉默的水稻对稻瘟病菌的抗性减弱^[15]。

2 E3 连接酶的功能

蛋白质降解在植物生长发育中起着至关重要的作用。越来越多的研究表明,UPS 也是植物适应环境胁迫的一个组成部分,如干旱、盐度、寒冷、营养剥夺和病原体。根据植物 E3 连接酶的结构组成和激活的泛素(Ub)片段的结合过程,可以将植物

E3 连接酶分为三大类: PUB (plant U-box)、HECT (homologous to the E6-AP carboxyl terminus), 以及 RING-type E3 连接酶^[16-19]。与 PUB 和 RINGs 连接酶不同,HECT E3s 是 E3 酶中唯一一组在识别并催化底物泛素化之前被 E2 - 泛素结合酶泛素化的酶。在拟南芥中,7 种 HECT 酶已被鉴定^[20]。PUB 蛋白与 HECT 酶类似,在非植物真核生物中构成一个小型 E3 连接酶家族,如人类中含 2 个和酵母中含 21 个^[21]。然而,它们在植物中数量更多^[22]。例如,对番茄进行全基因组分析发现,62 种 E3 连接酶含有一个 U-box 结构域^[23]。RING E3 连接酶是植物基因组中最复杂、最神秘的一组蛋白质。它由单亚基和多亚基 E3 酶组成。一个单亚基 RING 型 E3 连接酶包含一个 RING 基序,用于与 E2 泛素结合酶相互作用,以及一个底物识别区域来招募底物。多亚基 RING 型 E3 酶可分为大组 CRL E3 (Cullin (Cul) - RING) 连接酶^[24-25]和小组后期促进酶复合物 APCs (anaphase-promoting complexes)^[26-27]。植物 CRL E3 连接酶可以根据底物受体的不同进一步分为 3 个主要的亚组:(1) Skp1-Cul1-F-box (SCF) 复合物,其中大量的 F-box 蛋白作为底物受体;(2) Broad complex-Tramtrack-Bric a brac (BTB) -Cul3a/b 复合体,利用其 BTB 蛋白识别底物;(3) DDB1-binding/WD40-Cul4 复合物,通过 DDB1-binding/WD40 蛋白引导蛋白泛素化^[28]。2019 年有研究表明 RING 型 E3 连接酶基因在单子叶和双子叶植物基因组中都有编码(表 1)^[29]。植物基因组中如此大的 E3 连接酶扩展群,为植物应对各种环境挑战提供了必要的基因组资源,如病原体响应、干旱敏感性、营养平衡和耐热性等。

2.1 E3 连接酶影响植物对干旱和盐度的响应调控

苹果中 RING-type E3 连接酶 MIEL1 (MYB30-interaction E3 ligase) 可以通过 26S 蛋白酶体途径介导 *MdBBX7* (B-BOX 7) 的泛素化降解,是干旱胁迫响应的负调控因子。*MdBBX7* 的过表达增强了其抗旱性,而敲除 *MdBBX7* 则降低了其抗旱性^[30]。RING-type E3 连接酶 DRIP1 和 DRIP2 功能冗余,通过泛素介导的蛋白水解,降解 DREB2A (dehydration-responsive element binding protein 2A),一种调控干

表 1 在选定的 14 个植物基因组中预测的 E3 连接酶数量
Table 1 Number of E3 ligases predicted in 14 selected plant genomes

物种 Species	BTB	F-box ^a	HECT	RING ^b
鼠耳芥 <i>Arabidopsis halleri</i>	63	850	9	480
琴叶拟南芥 <i>Arabidopsis lyrata</i>	77	989	10	542
拟南芥 <i>Arabidopsis thaliana</i>	64	697	7	516
无油樟 <i>Amborella tricapoda</i>	56	230	7	314
二穗短柄草 <i>Brachypodium distachyon</i>	178	813	10	554
白菜 <i>Brassica rapa</i>	97	975	10	802
荸荠 <i>Boechera stricta</i>	75	505	9	473
芥菜 <i>Capsella rubella</i>	69	970	8	527
假稻属 <i>Leersia perrieri</i>	110	542	9	460
花药野生稻 <i>Oryza brachyantha</i>	78	264	12	391
斑点稻 <i>Oryza punctata</i>	111	535	8	485
稻 <i>Oryza sativa</i>	156	732	8	532
高粱 <i>Sorghum bicolor</i>	158	678	9	563
玉米 <i>Zea mays</i>	95	331	20	699

注：a：只分析了两个 F-box 亚族，F-box 和 F-box-like。3 种罕见的 F-box 亚族，F-box-like-2，F-box-4 和 F-box-5 没有被包括在内。b：数量表示单亚基 RING 型 E3 连接酶的个数
Note：a: Only two F-box subfamilies, F-box and F-box-like were analyzed. Three rare F-box subfamilies, F-box-like-2, F-box-4, and F-box-5, were not included. b: The number indicates the group of mono-subunit RING E3s

旱和盐胁迫诱导基因表达的转录因子，负向调节干旱胁迫响应^[31-32]。drip1、drip2 双突变体表现出比单突变体更好的干旱耐受性。在双突变体中增加 DREB2A 基因的表达，导致干旱响应基因的表达量进一步升高^[32]。进一步研究发现，DREB2A 中一个 30-aa 的负调控域（NRD）的缺失会使 DREB2A 转化为一种稳定的、组成性的活性形式，Cullin3（CUL3）E3 连接酶的作用底物 BPM（BTB/POZ and math domain proteins），可与 DREB2A 的 NRD 相互作用。因此 BPM 表达的缺失增强了 DREB2A 的稳定性使拟南芥耐热性提高^[33]。3 个 RING 型 E3 连接酶拟南芥 RGLG1、RGLG2 和水稻 OsDIS1 的功能缺失也能提高植物的抗旱性^[34-35]。目前 RGLG1 和 RGLG2 影响植物抗旱性的作用机制包括两点：RGLG1 和 RGLG2 介导了 AtERF53（ethylene response factor 53）的蛋白酶体降解，AtERF53 是调控干旱诱导的基因表达的转录因子；泛素连接酶 RGLG1 和 RGLG2 在赖氨酸残基 K32 和 K154 处泛素化 MAPKKK18（mitogen

activated protein kinase kinase kinase 18），并促进其降解，负向调控 MAPKKK18 介导的拟南芥的耐旱性^[36]。OsDIS1 通过调节蛋白 OsNek6（一种与微管蛋白复合物相关的丝氨酸/苏氨酸蛋白激酶）的数量，从而影响与干旱胁迫相关基因的转录。气孔运动的调节是植物适应环境胁迫的关键。拟南芥中的泛素 E3 连接酶 MREL57（microtubule-related E3 ligase 57）和微管稳定蛋白 WDL7（wave-dampened2-like7）相互互作，泛素化并降解 WDL7，MREL57-WDL7 模块通过调节微管的分解来调节气孔在干旱胁迫和 ABA 处理下的关闭^[37]。

在辣椒中，最近几项研究表明 CaATBZ1，一个负调控脱落酸（ABA）信号转导的 bZIP 转录因子，受 RING E3 连接酶 CaASRF1（*Capsicum annuum* ABA sensitive RING finger E3 ligase 1）^[38]和 CaTIR1（*Capsicum annuum* ATBZ1-interacting RING finger protein 1）的调节，正向调控脱落酸信号通路和干旱响应^[39]。CaASRF1 也可通过调节 CaAIBZ1（*Capsicum annuum* ASRF1-interacting bZIP transcription factor 1）的稳定性，正向调控 ABA 信号转导和干旱胁迫响应^[40]。

由于盐胁迫和渗透胁迫与耐旱性密切相关，许多 E3s，如 AtPUB18/19/22/23、SDIR1、AtAIRP3/LOG2、AtATL61 和 TaPUB1 等参与 ABA 介导的盐、渗透和干旱响应^[41-46]。UPS 组件调节 ABA 生物合成或 ABA 信号转导。脱落酸（ABA）在干旱情况下可以激活植物抗旱性^[47]。除干旱外，ABA 还与各种不利的盐度和温度条件导致的非生物胁迫相关^[48]。ABA 在幼苗发育和植物开花中也发挥着重要作用^[49]。UPS 通过调节 ABA 信号通路，在很大程度上影响应激的感知和转导^[50]。E3 Ub 连接酶靶向不同的 ABA 信号传导核心组分，即 ABA 受体、PP2C、SNRK2 和 ABFs/ABI5 等转录因子。

2.2 E3连接酶影响植物对寒冷、营养缺乏的响应调控

植物对冷胁迫的反应有些是由转录级联介导的。转录因子 ICE1 及相关蛋白促进 CBFs（C-repeat（CRT）-binding factors）的表达，导致下游效应基因表达增加。过表达 RING-type E3 连接酶 AtHOS1

(osmotically responsive gene), 介导 ICE1 的降解, 使拟南芥植株对温度波动更加敏感^[51]。E3 泛素连接酶在泛素介导的热相关蛋白降解途径中起重要作用。C3HC4 锌指泛素 E3 连接酶 AtPPRT1 过表达株系增强了幼苗的基础和获得性耐热性, 且热处理后 AtPPRT1 和几个热相关基因 (*AtZAT12*、*AtHSP21* 和 *AtHSPA7a*) 的转录水平大幅上调^[52]。而 *atpprt1* 突变体在高温胁迫下的萌发率和存活率降低。辣椒 U-box E3 泛素连接酶 CaPUB1 (*Capsicum annuum* putative U-box protein 1) 增强了转基因水稻的抗寒性, 降低了其抗旱性^[53]。另外还有一些具有非蛋白水解功能的 E3 连接酶, 如 AtCHIP、OsHCI1。在低温条件下, 过表达 PUB E3 连接酶 AtCHIP 的植物中可以观察到较高的磷酸酶活性, AtCHIP 介导的蛋白磷酸酶 2A (PP2A) 泛素化具有非蛋白水解功能^[54]。另一个在温度胁迫信号转导过程中具有非蛋白水解功能的 E3 连接酶是 OsHCI1 (*Oryza sativa* heat and cold induced 1), OsHCI1 介导的核蛋白 (如 OsbHLH065) 重新定位促进了植物对高温胁迫的耐受性^[55]。热诱导的 OsHIRP1 (*Oryza sativa* heat-induced RING finger protein 1) 在热处理后定位于细胞核, 并直接泛素化 OsAKR4 (主要在细胞核中) 和 OsHRK1 (主要在胞质中) 并将其降解^[56]。

除了干旱、盐、冷胁迫, 植物还能感知营养缺乏引起的胁迫。RING-type E3 连接酶 NLA (nitrogen limitation adaptation) 是植物对低氮响应的正调控因子。在拟南芥 *nla* 突变体中, NLA 亚细胞定位的改变破坏了 NLA 与泛素共轭酶 8 (AtUBC8) 的相互作用。与野生型相比, 突变型植株对氮素缺失和衰老具有超敏感性^[14]。具有泛素 E3 连接酶活性的 SDEL1 和 SDEL2 的生化功能一致, SDEL1 或 SDEL2 的过表达导致无机磷酸盐 (Pi) 的过度积累, 即使在 Pi 充足的条件下也会诱导 Pi 饥饿信号。相反, 功能缺失的突变体则表现出 Pi 积累减少和 Pi 饥饿信号减弱。研究表明 SDEL1 和 SDEL2 促进 SPX4 (SPX-domain-containing protein 4) 的降解, 从而调节 PHR2 (phosphate starvation response protein 2) 活性, 调节 Pi 稳态和 Pi 信号, 以响应外源 Pi 的有效性^[57]。碳 (C) 和氮 (N) 的利用比例对植物生长至关重要。碳/氮 (C/N) 比值, 但 C/N 平衡和 C/N 信号转

导的感知机制仍不清楚。FERONIA (FER) 是一种受体激酶, FER 的突变导致拟南芥对高碳氮比过敏。FER 磷酸化 E3 泛素连接酶 ATL6, 通过调节 14-3-3 蛋白的稳定性响应碳氮比^[58]。

2.3 E3连接酶影响植物对生物胁迫响应的调控

在自然环境中, 植物经常暴露于各种病原体, 包括细菌、病毒、真菌和昆虫。植物已经进化出多种机制来保护自己免受病原体和昆虫的入侵, 特别是通过茉莉酸 (JAs) 和水杨酸 (SA) 等激素介导的途径。与 CSN (COP9 signalosome) 相互作用的 CRL-type E3 泛素连接酶包括 SCF^{TIR1}^[59] 和 SCF^{COI1}, 它们分别作用于生长素和 JA 信号通路。CSN 在依赖 JA 的植物防御反应中发挥重要作用, 正调控植物对草食性烟草天蛾幼虫和坏死性真菌病原菌葡萄孢属灰霉菌的抗性^[60]。SCF^{COI1} 是茉莉基 -I- 异亮氨酸受体 (JA-Ile)^[61], JA-Ile 促进 COI1-JAZ 相互作用并触发 JAZ 的降解, 释放结合的转录因子 (如 MYC2), 激活下游防御基因的转录^[62-63]。拟南芥 coronatine insensitive1 (*coi1*) 突变体无法表达保护性病原体诱导的基因, 导致对昆虫和病原体攻击的敏感性增加^[64]。COI1 还与组蛋白去乙酰化酶 RPD3b (reduced potassium dependency 3b) 相互作用, 通过去乙酰化调节靶基因的转录^[65]。

NPR1 (nonexpressor of pathogenesis-related genes 1) 组装到 BTB E3 连接酶中, 通过 SA 介导对病原体的系统获得性抗性 (SAR)^[66]。NPR1 是免疫应答的正向调节因子。而 NPR1 的两个类似序列 NPR3 和 NPR4 负向调节对病原体的应答^[67]。NPR3/NPR4 作为转录共抑制因子, SA 通过抑制其活性促进下游免疫调节因子的表达。U-box- E3 泛素连接酶 CMPG1 在宿主免疫相关 PCD 中发挥积极作用。研究发现烟草中超表达 *NtCMPG1* 增强对黄分枝霉的抗性^[68]。小麦亲缘种 *Haynaldia villosa* L. 中 CMPG1-V, 是 CMPG1 的同源物, 具有对白粉病的广谱抗性^[69], 表明 CMPG1 也参与了单子叶植物先天免疫的调控。最新研究发现泛素 E3 连接酶 *SINAT4* 基因超表达可削弱干旱诱导的对丁香假单胞菌 *Pseudomonas syringae* pv. Tomato DC3000 (Pst-DC3000) 的免疫^[70]。非生物胁迫和生物胁迫的相互作用, 干旱诱导免疫

的分子机制在很大程度上仍需进一步研究。

2.4 E3连接酶对植物生长发育的影响

2.4.1 泛素化是激素介导的植物生长发育的前提条件 Skp1-Cullin-F-box (SCF) E3 连接酶参与激素感知。生长素与 F-box TIR1 结合形成一个活性复合物, 靶向转录抑制因子 Aux 和 IAA 降解^[71]。在相同的模式下, JA 作为氨基酸结合 COI1 导致转录抑制因子 JAZ 家族的降解^[72]。GA (gibberellic acid) 与 GID1 结合导致构象改变, 从而与 DELLA 结合, 该复合物随后被 SCF^{SLY} 检测, 引导 DELLA 蛋白降解, 释放对下游转录因子的抑制^[73]。乙烯途径通过一个非常有趣的新基因 (RING) - E3 连接酶, 即 SDIR1, 它正调控乙烯的反应, 并促进 EIN3 的积累。研究表明, SDIR1 通过介导温度诱导的 EBF1/EBF2 降解和 EIN3 积累, 调控乙烯对温度变化的响应^[74-75]。

2.4.2 泛素化影响花发育的昼夜节律 花的发育是一个受多重调控的复杂过程, 在过去的几十年里吸引了大量的研究。本文着重对近几年的研究进行综述。F-box 蛋白 ZTL、FKF1 和 LKP2 在昼夜节律调节中发挥重要作用, 并存在功能重叠^[76]。这些 F-box 蛋白都包含一个独特的光-氧-电压域, 使它们能够检测蓝光^[77]。作为 SCF 复合体的一部分, ZTL 导致组分 TOC1 降解^[78]。ZTL 也调控 PRR5^[79]。PRR5 通过影响 TOC1 磷酸化和定位对昼夜节律钟有调节作用^[80], 并调节开花时间^[81]。

RING E3 连接酶 SINAT5 促进 LHY 的降解, 在开花过程中发挥了不可或缺的作用^[82]。而另一种蛋白质 DET1, 保护 LHY 免受 SINAT5 的降解^[83], 但 DET1 的作用机制尚不清楚。这 3 种蛋白之间的相互作用似乎在决定开花时间方面很重要, 因为 *sinat5* 突变体和 *LHY* 超表达植株都表现为开花延迟^[84]。FLC (flowering locus C) 的抑制是拟南芥从营养阶段过渡到生殖阶段的关键。FLC 抑制数个参与花诱导的基因的表达, 研究发现 SINAT5 通过泛素介导的 FLC 蛋白水解来调控开花时间^[85]。FLC 位点组蛋白 H2B 的单泛素化和去泛素化都是 FLC 适当表达所必需的。在一些作物物种中, HUB (histone monoubi quitination) 同源基因在组蛋白 H2B 泛素化过程中发挥了类似的作用。番茄的同源基因 *SIHUB1*

和 *SIHUB2* 在体外分离得到了单硫喹酸 H2B^[86]。*FRRP1* (flowering-related RING protein 1) 是 *HUB2* 在水稻中的同源基因, 它能单泛素化 H2B 并影响水稻的开花^[87]。

FRIGIDA (FRI) 是一个转录激活复合物中的支架蛋白, 通过促进 *FLC* 的表达来抑制开花。FRI 同样影响油料作物甘蓝型油菜、芥菜叶类蔬菜和芸薹类作物的花期^[88-89]。水稻 RING E3 连接酶 HAF1 (heading date-associated factor 1) 负向调节 *ELF3* (early flowering 3), 这是一个在长日照下促进开花的转录因子。HAF1 在体外泛素化 *ELF3* 并促进其在水稻中的降解^[90]。另外 *haf1* 突变体在短日和长日条件下均表现出较晚的开花抽穗期, *haf1* 主要通过调控 Hd1 (heading date 1) 决定抽穗期^[91]。泛素系统调节许多其他影响开花时间的蛋白质的丰度, 特别是与调节昼夜节律时钟和激素合成有关的蛋白质^[92]和调节开花信号的激素, 尤其是赤霉素^[93]。

2.4.3 泛素化影响果实品质 泛素化对果实成熟, 品质等方面的研究目前还较少。葡萄中 U-Box E3 泛素连接酶 VIPUB38 通过促进脱落醛氧化酶降解负调控果实成熟^[94]。AtARRE, 环型 E3 泛素连接酶, 通过控制蜡类生物合成酶 ECERIFERUM1 和 ECERIFERUM3 蛋白水平, 负调控拟南芥表皮蜡质合成^[95]。这为果实的品质调控提供一些参考。植物的角质层的主要组分为角质和蜡质。果实的角质层对果实的生理和品质具有重要的影响, 它能够影响果实的外观 (色泽、质地、均一性), 采后处理效果, 贮藏、运输和货架期等。乙烯激活的 U-box 型 E3 泛素连接酶 MdPUB24 直接与 MdBEL7 相互作用并泛素化 MdBEL7。MdBEL7 的降解导致 *MdCLH*、*MdPPPH2* 和 *MdRCCR2* 的表达增强, 从而导致叶绿素在苹果果实贮藏过程中的降解。研究表明, Ethylene- MdPUB24 - MdBEL7 模块在苹果果实贮藏过程中通过翻译后修饰调控叶绿素降解^[96]。

2.4.4 泛素化影响种子发育 种子在脂质体中积累淀粉、三酰基甘油酯和蛋白质, 以维持幼苗生长, 直到它们能够通过光合作用产生自己的能量。除了为幼苗提供能量外, 种子也是包括人类在内的动物的主要营养来源。种子大小是一个重要的农艺性状。首次发现泛素系统参与决定种子大小是在一个

水稻的数量性状位点上,该位点编码环型 E3 连接酶 Grain WIDTH 2 (GW2), GW2 负向调节种子大小和产量^[97]。随后的研究表明,泛素系统调控多种影响种子大小的蛋白质^[98-99],主要路径有 3 条:(1) E3 连接酶 UPL3 (ubiquitin-protein ligase 3) 路径。在甘蓝型油菜中,HECT E3 连接酶 UPL3 负向调控种子大小,并以 LEC2 (leafy cotyledon 2) 蛋白为降解目标^[100]。(2) DA1 路径。拟南芥最近的研究表明,泛素特异性蛋白酶 UBP12 和 UBP13 将 DA1、DAR1 和 DAR2 去泛素化,降低了它们的肽酶活性。同样,组蛋白去泛素酶 OTU1 (ovarian tumor domain (OTU)-containing dub 1) 也可以抑制 DA1 和 DA2 的表达^[101]。小麦 DA1 抑制细胞增殖,并与拟南芥 DA2 的同源 E3 连接酶 GW2 相互作用^[102]。研究发现,泛素特异性蛋白酶 OsUBP15 促进水稻的大粒化,也与 DA1 密切相关^[103]。DA1 路径通过抑制细胞增殖负向调节种子大小,但确切机制尚不清楚。(3) F-box 蛋白 SAP 路径。拟南芥中 SAP 靶向转录因子 PPD1 和 PPD2 (PEAPOD 1 和 2) 进行降解;在豆科植物中,SAP (sterile petala) 的同源基因 *SLB1* (small leaf and bushy 1),是 E3 连接酶复合体的一个组成部分,靶向 BS1 (big seeds 1) 降解影响种子大小^[104]。

3 26S 蛋白酶体对植物农艺性状的影响

3.1 蛋白酶体亚基在环境胁迫耐受性中的作用

26S 蛋白酶体功能的缺失或抑制也会改变植物的农艺性状。26S 蛋白酶体是由 20S CP 和 19S RP 组成的复合体。19S RP 亚基的突变导致复合物积累减少,靶蛋白依赖泛素化的蛋白水解速率降低,从而影响植物对非生物或生物胁迫的响应^[105-106]。例如,拟南芥 *rpn10-1* 突变体植株对盐胁迫和热休克的耐受性较差,对紫外线辐射和 DNA 损伤剂过敏^[107]。RPN10 调节 ABA 诱导的对环境胁迫的响应^[106]。20S CP 既以泛素依赖的方式降解蛋白质也通过不依赖泛素化的蛋白质降解^[105]。如氧化蛋白的降解是不依赖泛素化的^[108],这与 *rpt2a*, *rpn10* 和 *rpt12a* 突变体对氧化应激的耐受性增强观点相一致^[105, 109]。

对 20S CP 亚基的研究表明, $\alpha 2$ 亚基 OgTT1 有助于提高非洲水稻的耐热性和适应性^[110]。与水稻 OsTT1 相比,OgTT1 似乎在去除热应激变性的细胞

毒性蛋白方面更有效,这与 OgTT1 有不同的 SNP (单核苷酸多态性) 位点有关。*ars5* 编码 $\alpha 6$ 亚基,基因敲除的拟南芥突变体在砷胁迫下,砷和硫化化合物积累增加,但 ARS5 抑制转录反应的靶向蛋白仍有待鉴定^[111]。最新研究表明,砷诱导的蛋白毒性胁迫导致拟南芥同源 PUB22 和 PUB23 U-box E3 泛素连接酶上调,*pub22*、*pub23* 双突变体表现出砷敏感性种子萌发和根生长表型。PUB22/PUB23 通过负调控 26S 蛋白酶体完整性在亚砷酸盐诱导的蛋白毒性应激反应中发挥关键作用^[112]。

$\beta 1$ 、 $\beta 2$ 和 $\beta 5$ 亚基分别具有半胱天冬酶、胰蛋白酶和胰凝乳蛋白酶活性^[113]。外源病原菌信号增强了这 3 个亚基的活性,参与植物对细菌感染的抗性,拟南芥中 $\beta 1$ 负调控内质网应激导致的程序性细胞死亡^[114]。然而,这些表型背后的分子机制尚不清楚。最近的一项研究报道, $\beta 5$ 亚基 PBE1 的缺失影响了盐胁迫下蛋白酶体的组装,表明 PBE1 对完整的蛋白酶体组装至关重要^[115]。此外,研究发现 PBE1 可以减少转录因子 ABI5 的蛋白积累,从而调节 ABA 介导的植物盐胁迫信号。

除了蛋白水解活性,20S 蛋白酶体也被证明具有核糖核酸酶 (RNase) 活性,可在体外降解莬苣花叶病毒 (LMV) 和烟草花叶病毒衍生的 RNAs,例如拟南芥 $\alpha 5$ 亚基敲除突变体对 LMV 感染的易感性显著增强^[116]。

3.2 蛋白酶体亚基在植物正常生长发育中的作用

在拟南芥中,19S RP 中 RPT2a 亚基,对植物细胞大小、抗氧化胁迫能力、芽尖及根尖分生组织的维持有十分重要的作用。同源基因 RPT2b 在基尖及根尖分生组织中也有表达,但是突变体却没有任何表型上的变化^[117]。*rpt2a*, *rpt2b* 双突变体会造成雄配子和雌配子的死亡^[118]。单个 *rpn5b* 突变体表现为野生型,而单个 *rpn5a* 突变体则表现出大量的形态缺陷,包括胚胎发生异常、在黑暗条件下去黄化发育、在光照下生长时出现严重的侏儒表型以及不育^[119]。在拟南芥中过表达 RPN5a 则会引起植株提前衰老。在 *rpn12a* 突变体中,两个细胞分裂素诱导基因 *CYCD3* 和 *NAI1* 被上调,使植株对细胞分裂素的响应敏感,影响植株根系的延长和叶子的形

成^[120]。*rpn10-1* 突变体除了改变植物环境胁迫耐受性,还会导致种子萌发率降低、生长速率降低、生殖发育不良^[121]。研究发现,拟南芥 RPN10 还可以作为一种选择性自噬受体,通过与泛素化的蛋白酶体亚基/靶标和包裹自噬膜的脂化 ATG8 (autophagy-related protein 8) 同时相互作用,靶向失活的 26S 蛋白酶体^[122]。细胞内成分的自噬转换对于真核生物的细胞管理、营养循环和生长发育的各个方面都是至关重要的^[123]。RPN11 与去泛素化有关,从蛋白酶体的靶向底物上去除泛素链是底物加工的先决条件,由去泛素酶 Rpn11 完成。在盖子与蛋白酶体结合之前, Rpn11 去泛素酶活性被抑制,以防止多泛素化蛋白的非必要去泛素化^[124],对植物正常生长发育至关重要。

4 总结与展望

泛素-蛋白酶系统在植物生长发育和响应环境胁迫中起着关键作用。这里我们总结了泛素化影响的农艺性状包括:诱导开花、种子大小、果实品质和病原体反应等。

鉴于大量基因参与泛素系统,剖析具体的相互作用和这些相互作用的后果,这仍然是一个重大挑战。迄今为止编码 UPS 组件的基因数量非常多,但只有少数基因及其功能得到了阐明。进一步鉴定 E2s,不同的 E3 底物,单亚基 E3 连接酶和多亚基 E3 连接酶,蛋白酶体亚基和蛋白酶体调节器将有助于阐明植物在蛋白质水平上影响植物农艺性状的调控机制,并为提高作物育种、对生物和非生物胁迫的耐受性提供有用策略。

基于异体或同源基因表达的遗传操作可以提高作物的农艺性状。UPS 中与种子大小相关的正调节因子,如 SLB1 和 OsUBP15 等,可以尝试通过超表达转基因技术提高水稻、玉米和小麦等作物的产量^[103]。相反, GW2 和 UPL3 等负向调控种子大小,可以通过基因敲除等手段提高作物产量^[97, 100]。此外,最近发展起来的基因组编辑技术 (TALENs、Crispr/Cas9 等) 允许相关蛋白酶体亚基基因的新等位基因的创建,这可能赋予更强的酶活性或促进蛋白酶体组装。新的等位基因可以用于分子育种,改进植物基因型。例如,与 OsTT1 相比, OgTT1 在

去除热应激变性的细胞毒蛋白方面的功能更有效, OsTT1 的 SNP 位点与 OgTT1 不同,因此,用 CrispR/Cas9 替代水稻中的 SNP 位点,可能有助于培育出更耐热的水稻。

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