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评 述

种子萌发调控的研究进展

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郴州国家可持续发展议程创新示范区建设省级专项(批准号: 2022sfq06)和郴州市人民政府及湘南学院人才项目资助

摘要 种子萌发是植物生命周期的起始. 在萌发过程中, 干燥种子从静止状态迅速恢复代谢活性, 导致胚突破周围结构以完成萌发. 种子萌发是一个复杂的多步骤过程, 受许多内源和外源因子的调控, 在物种生存与繁衍和作物生产中具有重要的生物学和经济意义, 但其分子调控机制目前还不完全清楚. 近年来, 种子萌发及其调控的研究已取得了许多重要的成就. 本文在此基础上综述了种子萌发调控的研究进展, 主要包括种子萌发的生理调节、植物激素特别是脱落酸和赤霉素的作用、基因转录和转录后控制、特异性调控因子以及表观遗传的调控, 并提出了在本领域需要进一步研究的科学问题, 试图为深入理解种子萌发的分子机制, 从而为提高种子萌发活力和防止穗萌发提供参考.

关键词 表观遗传, 生理, 植物激素, 调控, 种子萌发, 转录和转录后

种子萌发(seed germination)是植物特别是一年生和二年生植物生命周期中的起始步骤, 是实现种子芯片功能的前提. 种子能够感知环境信号的变化, 使萌发和整个植物生命周期的完成与环境变化相适应; 这在物种生存与繁衍和作物生产中具有重要的生物学和经济意义. 种子萌发从吸收水分(吸胀作用)开始, 随着胚轴的伸出结束, 通常是胚根突破周围结构[1]. 种子萌发是一个复杂多步骤的生物学过程, 在这个过程中干燥静止的种子迅速恢复代谢活性, 如呼吸活性增强、能量产生、修复机制激活、从储存和新合成的mRNA合成蛋白, 以及储藏物的动员. 这些事件促进了胚轴的伸长和胚周围组织的弱化, 从而导致种皮(和胚乳)破裂、胚根伸出周围结构和幼苗形成[1,2].

研究发现, 许多内源和外源因子调控种子的萌发过程. 线粒体(mitochondria)在种子萌发中的主要功能是为萌发提供能量和碳骨架[1]; 它们也是活性氧(reactive oxygen species, ROS)产生[3]和多种维生素[4]合成的主要部位, 在种子萌发中起重要作用. 胚乳弱化(endosperm weakening)是胚根突破周围组织(狭义萌发)的前提[5]. 植物激素脱落酸(abscisic acid, ABA)和赤霉素(gibberellin, GA)是调控种子萌发的主要因素, ABA诱

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导和维持休眠, 而GA释放休眠和促进萌发, 具有拮抗ABA的作用[6]; 其他植物激素也在种子萌发与休眠中起重要作用[7,8]. 另外, 种子萌发还受基因转录(transcription)和转录后(post-transcription)以及表观遗传(epigenetic)等的调控[8,9]. 除内源因子外, 种子也可以感知光照、温度和土壤条件等环境信号的变化, 以调节其萌发时间[10~12].

近年来, 种子萌发及其调控研究已取得了许多重要成就[8,13~17], 本文主要综述了种子萌发调控的研究进展, 主要包括种子萌发的生理调节、植物激素的作用、基因转录和转录后控制、特异性调控因子以及表观遗传的调控, 并提出了在本领域需要进一步研究的科学问题, 试图为深入理解种子萌发的分子机制, 从而为提高种子萌发活力(germination vigor)和防止收获前萌发(pre-harvest sprouting, PHS)提供参考.

1　种子萌发的生理调节

1.1　线粒体的作用

线粒体是种子中的主要细胞器, 其重要的功能是通过氧化磷酸化(oxidative phosphorylation)以ATP的形式为细胞的各种生命活动提供能量以及为生物大分子的合成提供碳骨架[18,19]. 萌发开始的显著标记是成熟干燥的种子中简单和静止的前线粒体(promitochondria)发育成为代谢活跃、富含能量和嵴的细胞器. 利用透射电子显微镜对向日葵(*Helianthus annuus*)种子的研究表明, 干种子的线粒体基质其电子密度低、内部结构(嵴)缺乏, 通过氧化磷酸化产生的ATP数量少[20]. 从水稻(*Oryza sativa*)种子中分离出的前线粒体富含蛋白质, 种子吸胀前代谢活性很低. 当水稻种子吸胀30 min时, 线粒体的代谢和蛋白输入功能恢复. 因此, 吸胀是前线粒体发育成为成熟线粒体的先决条件[21]. 水稻[22]、玉米(*Zea mays*)[23]和豌豆(*Pisum sativum*)[24]种子中, 成熟线粒体具有大量连续的嵴结构、基质的电子密度高以及具有丰富的电子传递链(electron transfer chain, ETC)组分, 使种子能够通过增加呼吸作用产生大量的ATP. 在加杨(*Populus×canadensis*)种子吸胀过程中, 其呼吸速率迅速增加; 种子萌发显著地被KCN (细胞色素c氧化酶(cytochrome c oxidase)抑制剂)、水杨基羟肟酸(salicylhydroxamic acid, 交替氧化酶(alternative oxidase)抑制剂)和2,4-二硝基苯酚(2,4-dinitrophenol, 氧化磷酸化解偶联剂)抑制(宋松泉等, 未发表数据), 这些结果表明呼吸作用和/或者能量产生在种子萌发中起关键作用.

此外, 线粒体参与维生素(如抗坏血酸(ascorbic acid)、叶酸(folic acid)和生物素(biotin))和氨基酸的产生, 从而使细胞的生理过程持续进行[4,25]. 线粒体也诱导ROS的产生, ROS在细胞网络的信号途径中发挥重要作用[26].

1.2　胚乳弱化

在许多物种中, 当胚的周围组织的机械阻力或者抑制能力超过胚的生长潜能时, 活性胚仍然不能完成萌发[1,27]. 研究表明, 在种子萌发完成之前, 胚乳的机械阻力逐渐降低, 如胚乳帽(endosperm cap)的弱化被认为是促进番茄(*Solanum lycopersicum*)和洋茄(*Solanum lycocarpum*)种子萌发的一种机制; 在一些物种中, 胚根顶端的珠孔端胚乳(micropylar endosperm)在吸胀过程中变弱; 以及在胚根伸出之前内-β-甘露聚糖酶(endo-β-mannanase, MAN)的活性增加[5,28,29]. 胚乳弱化的关键酶主要包括细胞壁松弛的水解酶(hydrolytic enzyme), 果胶降解的果胶甲基酯酶(pectin methylesterase, PME)和多聚半乳糖醛酸酶(polygalacturonase, PG).

(1) 细胞壁松弛的水解酶. 在种子萌发过程中, 内源分泌的水解酶对细胞壁多糖的降解是种子中胚乳弱化的主要机制[30]. 普遍认为, 珠孔端胚乳的弱化和胚根的伸长都需要几种细胞壁重塑酶(cell wall remodeling enzyme, CWRE)的作用[29,31]. Morris等人[32]表明, 胚乳的弱化被来自胚的早期信号所诱导, GA的生物合成对于随后的弱化过程是必需的, 从而影响胚乳中*CWRE*基因的转录和靶向蛋白的降解. CWRE有2种关键类型, 如番茄中的MAN以及番茄和烟草(*Nicotiana tabacum*)中的β-1,3-葡聚糖酶(β-1,3-glucanase)[31]. 珠孔端胚乳中的甘露聚糖(mannan)被降解, 以促进胚乳的弱化[33]. 在莴苣(*Lactuca sativa*)中, 许多细胞壁水解酶如纤维素酶(cellulase)和一些半纤维素降解酶(hemicellulose-degrading enzyme), 包括MAN、α-半乳糖苷酶(α-galactosidase)、β-甘露糖苷酶(β-mannosidase)和内-β-木聚糖酶(endo-β-xylanase)在珠孔端胚乳弱化中起作用[34]. 研究表明, MAN也有助于调控拟南芥(*Arabidopsis thaliana*)种子的萌发[35,36]. 与拟南芥野生型种子

相比, *AtMAN5*、*AtMAN6*和*AtMAN7*基因的功能缺失突变体都表现出萌发延迟, 这表明MAN参与了胚乳弱化和胚生长促进[35,37]. 此外, 木糖苷酶(xylosidase, Xyl), α-Xyl1和β-Xyl3在阿拉伯聚糖(arabinan)的水解以及在胚乳细胞壁的木葡聚糖(xyloglucan, XyG)的重塑中起重要作用; *xyl3*突变体表现出种子萌发延迟的表型. Xyl1通过加强胚乳细胞壁中的XyG链, 作为种子萌发的负调控因子起作用[38]. 扩展蛋白(expansin, EXPA)在胚乳破裂过程中发挥重要作用[5,39], 被认为是在纤维素和半纤维素界面上通过破坏非共价键(如氢键)起细胞壁松弛因子的作用[40].

(2) 果胶甲基酯酶. 双子叶植物种子初生细胞壁的聚合物成分由大约35%的果胶、30%的纤维素、30%的半纤维素和5%的蛋白组成[41]. 在大多数双子叶植物种子的细胞壁中, 半纤维素与果胶结合并交联, 形成甲酯化的水合基质. 在体内, 当PME去除甲基时, 果胶基质发生溶解, 从而提供带阴离子电荷的基质以及细胞壁的机械特性发生改变[42]. 在细胞壁中PME被蛋白果胶甲基酯酶抑制剂(proteinaceous pectin methylesterase inhibitor)拮抗调节[43]. PME在甲基化羧基(-COOCH3)的果胶基质脱脂化中的活性是非常重要的, 因为它们能够与果胶聚合物链中存在的底物结合[43].

(3) 多聚半乳糖醛酸酶. 细胞壁果胶的分解也与PG的作用密切相关[44]. PG水解果胶的多聚半乳糖醛酸聚糖(polygalacturonan)网络中的*O*-糖苷键和*O*-乙酰键, 导致细胞壁弹性降低[45]. 由*ADPG1*(*Arabidopsis dehiscence zone polygalacturanas 1*)和*ADPG2*基因编码的2种内-PG与角果开裂密切相关, 特别是与角果细胞壁中的果胶解聚有关[46]. 然而, 这2种内-PG在胚乳弱化过程中的表达模式和定位还不清楚. PG抑制蛋白(polygalacturonase-inhibiting protein, PGIP)是一种与植物细胞壁中的PG相互作用的胞外蛋白[47], 在果胶分解过程中起负调控因子的作用. 果胶分解是各种植物组织中细胞壁分离的一个必需过程. 在拟南芥中, 过氧化物酶体蛋白复合物(peroxisomal protein complex) PED3/CTS/PXA1抑制编码*PGIP*基因的表达并增加*ABI5* (*ABA Insensitive 5*)基因的表达[48]. 在*cts* (*COMATOSE*)背景下, *abi5*突变与*PGIP*表达和胚乳破裂呈正相关, 表明CTS通过抑制ABI5介导的*PGIP*来促进胚乳破裂和种子萌发[48].

1.3 活性氧的作用

在种子中, ROS产生的主要部位是线粒体、过氧化物酶体(peroxisome)和质膜NADPH氧化酶(NADPH oxidase, NOX)[49]. 种子吸胀后, 线粒体呼吸作用的恢复导致部分电子传递给O_2 (电子受体), 从而产生ROS. ROS包括自由基, 如单线态氧($^1O_2$)、超氧化物自由基($\cdot O_2^-$)或羟基自由基($\cdot OH$)[3]. NOX又称为呼吸爆发氧化酶同源物(respiratory burst oxidase homolog, Rboh), 是细胞壁空间ROS产生的主要驱动因素[50]. Rboh将电子从NADPH或NADH转移到质外体的O_2, 从而产生$\cdot O_2^-$, 这些自由基可以直接裂解多糖, 从而使植物细胞壁松弛[51]. 过氧化氢(H_2O_2)是一种活性分子, 在种子萌发过程中起信号转导功能, 并具有穿过生物膜的能力[52]. 在种子吸胀后, 非休眠的大麦(*Hordeum vulgare*)胚比休眠的胚产生更多的H_2O_2, 而H_2O_2的添加与胚中高水平的ABA-8′羟化酶(ABA-8′ hydroxylase, ABA8ox)和低水平的ABA有关; 同样, 在拟南芥种子吸胀过程中, H_2O_2通过激活*CYP707A*的表达增加ABA的分解代谢, 导致ABA含量降低[26]. 种子萌发时, 内源H_2O_2在种子内部积累, 因而促进种皮松弛和种子萌发. 当H_2O_2的产生开始破坏内部细胞器时, 细胞内的防御机制将被激活. *HRG1* (*hydrogen peroxide responsive gene 1*)和*HRG2*被认为在控制细胞内H_2O_2的产生中起重要作用[53]. *HRG1*和*HRG2*的特征是保持较低的蛋白含量, 直到H_2O_2的水平升高, 这为H_2O_2的感知和反应提供了一条新的途径. 此外, *HRG1*和*HRG2*基因的表达可保护根尖分生组织的微环境稳定性, 以维持分生组织细胞在细胞分裂和伸长中的正常活性[53].

种子吸胀过程中最显著的变化之一是胚乳中细胞壁聚合物被ROS分解[3,5,54]. 在水芹(*Oenanthe javanica*)种子中, 质外体的ROS (apoplast ROS, apROS)通过裂解多糖(如纤维素和半纤维素)链来诱导细胞壁松弛[5,31]. 从水芹种子珠孔端胚乳产生的组织特异性cDNA文库中已鉴定到许多高丰度过氧化物酶(peroxidase, POD)的转录物[55]. 这些POD与从NADPH产生H_2O_2有关, 导致胚乳细胞壁的破裂[31]. NOX是水稻种子萌发过程中ROS产生的主要酶促途径[56]. NOX mRNA在大麦种子的胚和糊粉细胞中表达[57], 这些表达位点与吸胀后种子中ROS产生的位点一致[58]. 在植物组织的质外体空间产生的·OH可以作为一种特定位

点的氧化剂，促进细胞扩大过程中的细胞壁松弛[59]. ·OH引起的细胞壁聚合物裂解不仅参与胚乳破裂，而且在胚根伸长中起作用[31]. Grainge等人[60]利用GPAW (gas plasma-activated water)在拟南芥中的研究表明, 产生的ROS可通过直接的化学作用(骨架多糖的断裂)和诱导*CWRP* (*CELL WALL-REMODELLING PROTEIN*)基因(如编码EXPA的基因*EXPA2*和*EXPA8*, 以及编码木葡聚糖内转葡糖苷酶/水解酶(xyloglucan endotransglycolase/hydrolase)的基因*XTH*)的表达，从而导致胚乳的弱化.

胚根鞘(coleorhiza)是一种非维管化(non-vascularization)的多细胞胚组织，覆盖单子叶植物种子的胚根, 在保护新生根中发挥作用；同时，它也参与单子叶植物种子萌发时胚根伸出的调控[61]. 胚根鞘在单子叶植物中的作用与珠孔端胚乳在双子叶植物中的作用相同，即它们作为胚根伸出的屏障[62]. 水稻种子吸胀6 h后, 胚根鞘发生破裂, 因此, 胚根鞘的破裂被认为是种子萌发的先决条件[63]. ROS的产生与萌发的增加直接相关，尤其是萌发种子的胚根鞘和胚根中·O_2^-、·OH和H_2O_2的产生和积累比胚芽鞘更高[64].

此外, ROS的积累也导致蛋白氧化增加, 其中羰基化(carbonylation)是最典型的事件. 在向日葵种子休眠释放过程中，几种伴侣蛋白(chaperone protein)被鉴定出发生羰基化[65]. 羰基化可能是由ROS启动的信号转导途径的一种元件，它参与了不同细胞过程的调控, 特别是在胚乳弱化期间. 值得注意的是，ROS能够与种子中储存的几乎所有大分子反应，当浓度较高时负调控种子萌发，引起氧化损伤和细胞壁中多糖链的裂解[14].

2　植物激素的调控

2.1　ABA对种子萌发与休眠的影响

(1) ABA代谢影响种子萌发与休眠. ABA是抑制种子萌发和维持种子休眠的核心因子[8,13,17,66]. 细胞中的ABA水平是由其生物合成和分解代谢的动态平衡所决定的[67](图1). 玉米黄质环氧化酶(zeaxanthin epoxidase, ZEP)在ABA生物合成途径中催化玉米黄质(zeaxanthin)转化为紫黄质(violaxanthin). 在水稻中，已经分离到一种称为*Osaba1*的ZEP明显缺陷的胎生突变体(viviparous mutant). 9-顺式环氧类胡萝卜素双加氧酶(9-*cis*-epoxycarotenoid dioxygenase, NCED)将紫黄质和新黄质(neoxanthin)的顺式异构体裂解为黄氧素(xanthoxin). 内源ABA水平的变化与*NCED*转录本的表达呈正相关. 在吸胀过程中, 过表达*AtNCED6*的种子增加ABA的水平，足以阻止萌发[68]. ABA的分解代谢主要依赖于由*CYP707A*基因编码的ABA8ox，该酶催化ABA 8′位置的羟基化[67]. 最丰富的ABA羟基化分解代谢产物是红花菜豆酸(phaseic acid, PA). 拟南芥中*CYP707A2*基因的突变导致干燥和吸胀种子中ABA的水平增加，从而降低萌发能力. 活性ABA也可以通过与葡萄糖结合形成ABA葡糖酯(ABA glucosyl ester, ABA-GE)而被失活[67,69]. 几种ABA葡糖基转移酶(glucosyl-transferase, UGT)能够催化该反应. ABA-GE也能够被β-葡糖苷酶(β-glucosidase)裂解形成活性ABA[67,69]. Sun等人[70]报道，一个MYB转录因子(transcription factor, TF)成员在吸胀初期介导ABA-GE解离成为ABA, 并抑制水稻种子萌发.

*NCED*和*ABA8ox*基因的表达控制ABA的水平，从而调控种子的萌发与休眠. 水稻*NCED*基因家族共有5个成员. *OsNCED*转录本的表达水平常常被用作ABA水平的标记，促进萌发或减少休眠的表型通常伴随着*OsNCED*的下调(除*OsNCED1*以外)[71~74]. 同样, 分解代谢基因*OsABA8ox1*、*OsABA8ox2*和*OsABA8ox3*的表达下降也将抑制种子萌发[75,76].

ABA是种子成熟和休眠维持所必需的. 在拟南芥种子的成熟中期和后期分别有2个ABA的积累峰值, 其中成熟后期生物合成的ABA在种子休眠的诱导和维持以及阻止胎萌中起关键作用[77,78]. 然而，在水稻中，ABA的积累峰值发生在种子发育的早期阶段，随后下降，并在发育后期保持在较低的水平[79]. 深休眠品种的ABA水平在种子发育早期和中期(授粉后10~20 d)高于低休眠品种[79]. 在杂交水稻种子中也发现了类似的结果，即ABA的积累峰值出现在授粉后10 d[80]，表明拟南芥和水稻之间诱导种子休眠的机制不同. 除了在发育中的种子中诱导休眠外，ABA也参与吸胀过程中种子休眠的维持[67]. 萌发是由吸胀种子中内源ABA水平降低引起的，这是抑制从头合成和激活分解代谢的结果[78]. Liu等人[79]表明, ABA水平从吸胀开始后下降, 这种ABA水平的下降主要与*OsABA8ox*基因的表达增加有关，而不是与生物合成*OsNCED*基因的下调有关[75,81]. 此外，外源ABA显著降低水

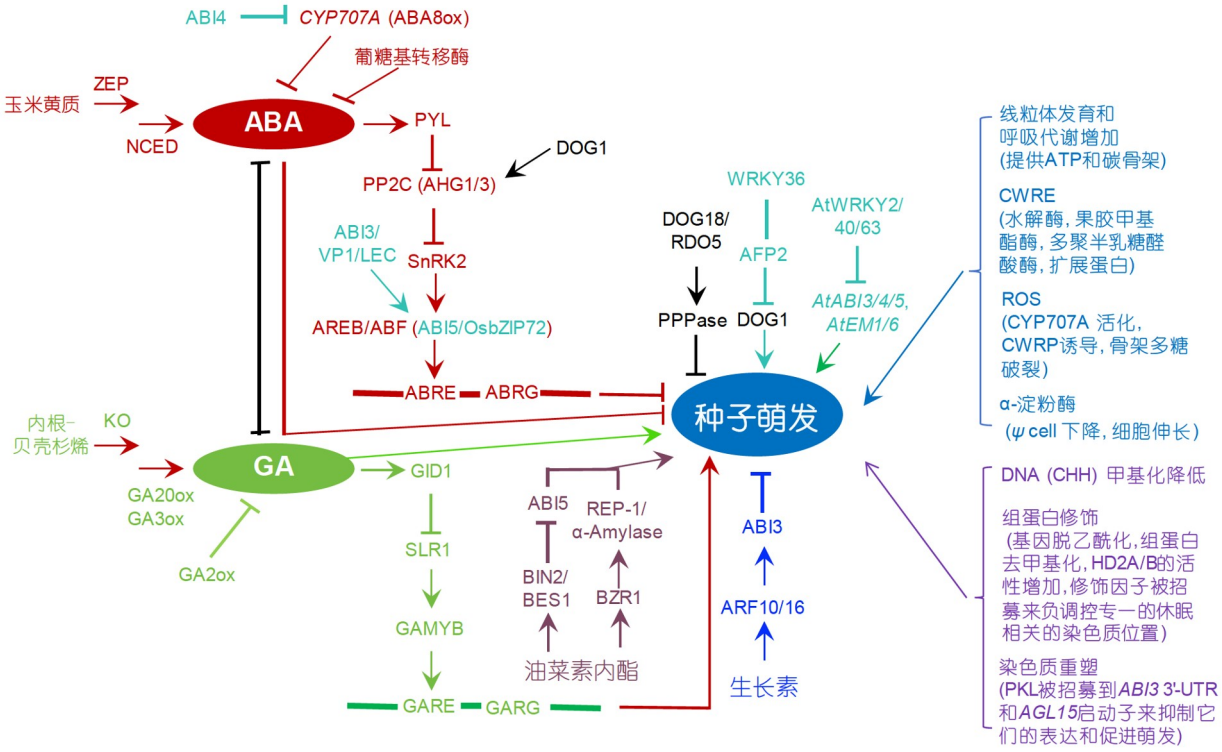

图 1 种子萌发调控的模式图. 种子萌发的调控主要包括生理调节, 植物激素的作用, 基因转录和转录后控制, 特异性调控因子以及表观遗传的调控. →和⊣分别表示促进和抑制. ABA, 脱落酸; ABA8ox, ABA 8′羟化酶; ABI3/4/5, ABA不敏感3/4/5; ABRE/ABF, ABA反应元件/ABA反应元件结合因子; ABRG, ABA反应基因; AFP2, ABI5结合蛋白2; AGL15, 类无性生殖15; AHG1/3, ABA 过敏感萌发1/3; ARF10/16, 生长素反应因子10/16; AtEM1/6, 拟南芥胚胎发生晚期丰富1/6; AtWRKY2/40/63, 拟南芥WRKY2/40/63; BIN2/BES1, 油菜素内酯不敏感2/BRI1-EMS-抑制物1; BZR1, 抗芸苔素唑1; CWRE, 细胞壁重塑酶; CWRP, 细胞壁重塑蛋白; DOG1, 萌发延迟1; DOG18, 萌发延迟18; GA, 赤霉素; GA2ox, GA 2氧化酶; GA20ox, GA 20氧化酶; GA3ox, GA 3氧化酶; GARE, GA反应元件; GARG, GA反应基因; GID1, 赤霉素不敏感矮化1; HD2A/2B, 组蛋白脱乙酰酶2A/2B; KO, 内根-贝壳杉烯氧化酶; LEC, leafy cotyledon; NCED, 9-顺式环氧类胡萝卜素双加氧酶; OsbZIP72, 水稻碱性亮氨酸拉链72; PKL, PICKLE; PP2C, 蛋白磷酸酶2C; PPPase, 伪磷酸酶; PYL, PYR/PYL/RCAR; ROS, 活性氧; RDO5, 减少休眠5; REP-1, 半胱氨酸蛋白酶; SnRK2, SNF1相关的蛋白激酶2; SLR1, SLENDER RICE 1; VP1, 胎生1; ZEP, 玉米黄质环氧化酶; Ψcell, 细胞水势

Figure 1 Diagram of seed germination regulation. The regulation of seed germination mainly includes physiological regulations, the actions of phytohormones, controls of gene transcription and post-transcription, as well as regulations of specific regulatory factors and epigenetics. →and ⊣represent promotion and inhibition, respectively. ABA, abscisic acid; ABA8ox, ABA 8′hydroxylase; ABI3/4/5, ABA insensitive 3/4/5; ABRE/ABF, ABA-responsive element/ABA-responsive element binding factor; ABRG, ABA-responsive gene; AFP2, ABI5-BINDING PROTEIN 2; AGL15, AGAMOUS-LIKE15; AHG1/3, ABA HYPERSENSITIVE GERMINATION 1/3; ARF10/16, AUXIN RESPONSE FACTOR 10/16; AtEM1/6, LATE EMBRYOGENESIS ABUNDANT 1/6; AtWRKY2/40/63, *Arabidopsis thaliana* WRKY2/40/63; BIN2/BES1, brassinosteroid insensive 2/BRI1-EMS-SUPPRESSOR 1; BZR1, BRASSINAZOLE-RESISTANT 1; CWRE, cell wall remodeling enzyme; CWRP, cell wall remodeling protein; DOG1, DELAY OF GERMINATION 1; DOG18, DELAY OF GERMINATION 18; GA, gibberellin; GA2ox, GA 2-oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; GARE, GA-responsive element; GARG, GA-responsive gene; GID1, GIBBERELLIN INSENSITIVE DWARF 1; HD2A/2B, histone deacetylase 2A/2B; KO, ent-kaurene oxidase; LEC, leafy cotyledon; NCED, 9-*cis*-epoxycarotenoid dioxygenase; OsbZIP72, *Oryza sativa* basic LEUCINE ZIPPER 72; PKL, PICKLE; PP2C, protein phosphatase 2C; PPPase, pseudophosphatase; PYL, PYR/PYL/RCAR; ROS, reactive oxygen species; RDO5, REDUCED DORMANCY 5; REP-1, cysteine proteinase; SnRK2, SNF1-related protein kinase 2; SLR1, SLENDER RICE 1; VP1, VIVIPAROUS 1; ZEP, zeaxanthin epoxidase; Ψcell, water potential of cell

稻[82]、莴苣[83]和黑黄檀(*Dalbergia fusca*)[84]种子的萌发速率和萌发率.

(2) ABA信号转导负调控种子萌发. ABA信号转导由3个主要成分组成: ABA受体PYR/PYL/RCAR (pyrabactin resistance 1/pyrabactin resistance 1-like/regulatory components of ABA receptor; 以下简称PYL)蛋白、蛋白磷酸酶2C (protein phosphatase 2C, PP2C; 负调控因子)和SNF1相关的蛋白激酶2 (SNF1-

related protein kinase 2, SnRK2; 正调节因子)[29,66](图1). ABA信号转导从ABA与PYL的结合开始, 并与PP2C形成复合物, 从而抑制PP2C介导的SnRK2的去磷酸化. SnRK2的活化形式随后磷酸化ABRE(BA-responsive element)结合蛋白(ABRE-binding protein, AREB)/ABRE结合因子(ABRE-binding factor, ABF)的TF, 进而激活ABA反应基因的转录. 在下游TF中, ABI5 (bZIP (basic LEUCINE ZIPPER)类型TF家族的一个成员)在激活种子的ABA反应中起核心作用[8,13,85]. 除此之外, 2个其他的TF ABI3 (ABSCISIC ACID INSENSITIVE 3, B3结构域TF)和ABI4 (AP2结构域TF)与ABI5一起来诱导ABA反应基因. 这两个TF在ABI5的上游起作用, 它们是种子萌发过程中ABI5表达的正调控因子[8].

基于AtPYL的氨基酸序列分析, 在水稻中已鉴定出13个PYL的直系同源基因(ortholog gene), 并对其中的3个成员进行了功能研究[86,87]. 过表达*OsPYL3*、*OsPYL5*和*OsPYL9*的转基因水稻种子在萌发过程中对ABA过敏感, 表明它们是萌发过程中ABA信号转导的正调控因子. OsPYL5与OsPP2C30 (一种AHG3 (ABA-hypersensitive germination 3)的同源物)相互作用, 其中OsPP2C30是一种已知的在拟南芥种子萌发中专一地以ABA依赖的方式起作用的PP2CA[86]. 其他的OsPP2CA以不同的方式和不同的强度与OsPYL结合[87]. 在PP2CA基因中, *OsPP2C51*主要在种子中表达, 并正调控水稻种子萌发[88]. 值得注意的是, OsPYL5是与OsPP2C51相互作用的靶向ABA受体[88], 这表明OsPYL5是种子萌发中ABA信号转导的主要PYL. 在水稻中, 下游的SnRK被称为SAPK (osmotic stress/ABA-activated protein kinase). 相对于其他的SAPK, SAPK2似乎主要介导ABA信号转导, 因为SAPK2与OsPP2C30和OsPP2C51物理相互作用[86,88]. 研究表明, SAPK10过表达系表现出种子萌发延迟和对ABA过敏感[89]. SAPK10在丝氨酸177上的自动磷酸化对其功能是重要的, 这使其能够激活下游的TF. bZIP是典型的AREB或ABF TF, 可被SnRK2磷酸化, 它们构成ABA信号转导的下游核心组分. ABI5是一个种子专一的bZIP TF激活的ABA信号转导成员, 负调控拟南芥种子的萌发. 在水稻中, 已经鉴定到几个ABA调控的ABI5同源物, 如TRAB1和OsbZIP10[90]. OsABI5能够与G-box元件结合并反式激活下游基因的表达. 萌发试验表明, 在ABA信号转导途径中*OsABI5*具有类似于*AtABI5*的功能[90]. OsABI5能够被SAPK2磷酸化[86,88], 或被OsPP2C51绕过SAPK2去磷酸化而失活[88]. 作为A组bZIP因子, OsABF2 (OsbZIP46)与ABRE结合, 以及*Osabf2*突变体在萌发阶段表现出对高水平ABA的敏感性显著降低[91]. 对水稻种子的酵母双杂交试验发现, OsbZIP72与SAPK10相互作用[89]. *bzip72*突变体及其最近的同源基因*trab1*/*osbzip66*突变体的萌发率没有变化, 而其过表达系对ABA的敏感性增强, 表明OsbZIP72负调控种子萌发. 上述研究表明, AREB、ABF和ABI5是水稻种子萌发过程中ABA信号转导的主要下游正调控因子. 除此之外, ABI3和ABI4对种子萌发也是重要的, 因为它们是ABI5的正调控因子[8].

2.2　GA对种子萌发的影响

(1) GA代谢对种子萌发的影响. 内根-贝壳杉烯氧化酶(ent-kaurene oxidase, KO)参与GA生物合成的早期步骤, 将内根-贝壳杉烯转化为内根-贝壳杉烯酸(ent-kaurenoic acid)[92]. *OsKO1*基因主要在种子萌发和幼苗阶段起作用, 该基因的缺陷引起GA含量降低, 并表现出萌发延迟和半矮化(semi-dwarfism, SD)表型[93]. 此外, OsLOL1 (rice lesion simulating disease 1-like 1)通过上调*OsKO2*来促进种子萌发[94], 表明*OsKO*在水稻种子萌发过程中起重要作用. 生物活性GA主要被GA 20氧化酶(GA 20-oxidase, GA20ox)和GA 3氧化酶(GA 3-oxidase, GA3ox)催化形成, 而它们的失活主要由GA 2氧化酶(GA 2-oxidase, GA2ox)控制[8](图1). 水稻中内源生物活性GA的含量和种子萌发率与这些酶的编码基因的表达水平有关. *OsGA3ox2*在胚中的表达对于水稻种子萌发过程中α-淀粉酶(α-amylase)的诱导是重要的, 而GA失活基因(*GA2ox*)在非休眠水稻种子吸胀过程中的表达被抑制[81]. *qSD1-2* (*seed dormancy1-2*)位点包含GA合成基因*OsGA20ox2*, 该基因的功能缺失突变导致种子中的GA水平降低和休眠增强[95]. 同样, *OsGA2ox3*被认为是控制种子萌发的候选基因[96]. 在*gd1* (*germination-defective 1*)突变体中, GA生物合成基因*OsGA20ox1*、*OsGA20ox2*和*OsGA3ox2*的表达受到抑制, 而失活基因*OsGA2ox3*的表达被显著上调, 导致内源GA_4含量降低和种子萌发被抑制[97]. 此外, GA_3能够降低莴苣种子萌发的热抑制(thermoinhibition), GA生物合成抑制剂多效唑(paclobutrazol)增加莴苣种子萌发的热抑制[83]. GA_3也能够显著地解除黄栌(*Cotinus*

coggygria var. *cinerea*)种子的休眠和促进萌发[98].

(2) GA信号转导对种子萌发的调控. 核心的GA信号转导组分包括GA受体GID1 (GIBBERELLIN INSENSITIVE DWARF 1, 正调控因子)、DELLA (Asp-Glu-Leu-Leu-Ala)蛋白(负调控因子)和F-box蛋白GID2 (正调控因子)[9,17]. 生物活性GA被其受体GID1感知, 导致GA-GID1-DELLA复合物的形成[9]. 该复合物与F-box蛋白结合, F-box蛋白是SCF^{GID2} E3泛素连接酶的核心组分, 通过泛素-26S蛋白酶体途径导致DELLA的降解. DELLA蛋白的降解激活下游GA反应的TF, 通常是GAMYB, 从而介导GA信号转导效应[9](图1). 作为GA信号转导的正调控因子, 拟南芥*GID1*和*GID2*基因促进种子萌发[99]. 水稻中DELLA蛋白的同源物被鉴定为SLR1 (SLENDER RICE 1), 但没有直接的证据表明SLR1在种子萌发中的作用. 对小麦(*Triticum aestivum*)种子的研究表明, 抑制非休眠种子的萌发与*RHT1* (小麦DELLA)的表达增加有关[100]. 因此, SLR1的降解是水稻种子萌发所必需的.

杨晓颖等人[101]研究了水稻GA类受体家族基因*OsGRL1* (*Gibberellin receptor like 1*)的功能, 发现该基因在水稻穗茎长度和产量性状方面发挥重要的调控作用. *OsGRL1*在茎、叶鞘及茎顶端组织中高表达, 不受外源GA的诱导; 与已知的水稻GA受体蛋白OsGID1定位于细胞核中不同, OsGRL1蛋白定位在细胞膜上, 且不随外源GA的诱导而改变[101]. 与野生型相比, *OsGRL1*敲除突变体表现出穗颈伸长、籽粒变小和千粒重降低的表型, 而过表达转基因株系穗颈缩短、籽粒变大且千粒重增加; *OsGRL1*基因突变使GA信号转导途径中的抑制基因*SLR1*的表达下调, 提高了突变体对GA的敏感性[101]. GA类受体蛋白OsGRL1是否类似于GA受体蛋白OsGID1, 从而影响种子的萌发与休眠还不清楚, 值得进一步研究.

糊粉细胞(aleurone cell)中α-淀粉酶基因对GA的反应被认为是最典型的GA反应[102]. 许多参与这一反应的关键顺式作用元件(*cis*-acting element)和TF被发现, 其中GAMYB起核心作用. GAMYB是一种典型的MYB TF, 在对GA信号的反应中, 与α-淀粉酶的GA反应元件(GA-responsive element, GARE)结合, 正调控水稻种子糊粉层中该基因的表达. 此外, 转录因子DOF (DNA binding with one finger)蛋白被证明与GAMYB协同作用, 作为水稻种子中GA反应*RAmy1A*基因转录调控的一个功能单元; 而2个WRKY结构域TF OsWRKY51和OsWRKY71通过抑制OsGAMYB激活的*Amy32b*表达, 作为GA信号转导的转录阻遏物起作用[103,104]. 总之, 水稻种子萌发过程中的GA信号转导包括以下步骤. 在GA缺乏时, SLR1与GAMYB结合, 导致α-淀粉酶的抑制. 当GA存在时, 促进GA-GID1-SLR1复合物的形成, 这通过泛素-蛋白酶体途径促进了SCF^{GID2}介导的SLR1的降解. 从SLR1中释放的GAMYB随后与α-淀粉酶启动子中的GARE元件结合, 并激活它们的表达, 从而导致淀粉胚乳储存物的水解(图1).

2.3 ABA和GA平衡控制种子萌发

种子休眠释放或萌发通常伴随着ABA水平的降低和GA水平的增加, 这是由合成代谢和分解代谢基因的表达模式发生改变所引起的. 研究表明, 在水稻种子发育过程中, 胚的休眠主要由ABA/GA的比值所决定. 深休眠表型的水稻品种具有高的ABA/GA比值[79]. 在拟南芥中, ABA抑制GA的生物合成并促进GA的失活, 二者都导致种子中GA含量和种子萌发率降低. 相反, GA负调控ABA的生物合成. 在GA缺陷的突变体中, ABA生物合成基因的表达增加, 导致较高水平的ABA积累[105]. 在水稻中也观察到类似的结果. 在*Osko1*和*Osko2* (编码GA合成酶)的突变体中, 大多数GA生物合成基因和ABA分解代谢基因下调, 而ABA生物合成基因上调[93]. 此外, 这些基因的相应转基因水稻或突变体通常伴随着ABA和GA合成代谢和分解代谢基因表达模式的变化[106,107].

ABA/GA平衡也受信号转导水平的相互拮抗调节的控制. TE (tiller enhancer)是一个APC/C^{TE} E3泛素连接酶复合物的激活子, 能够降解水稻ABA受体OsPYL[108]. ABA的存在通过激活SnRK2磷酸化TE来抑制APC/C^{TE}的活性, 而GA则抑制SnRK2的活性, 并能促进OsPYL的降解. 因此, *te*功能缺陷突变体表现出种子对ABA的敏感性降低和萌发率增加. Shu等人[109]发现, AP2结构域TF (对ABA反应的重要TF之一)在ABA和GA拮抗作用中起关键作用. 在拟南芥中, ABI4通过抑制ABA失活基因*CYP707A1*和*CYP707A2*的表达来促进ABA积累, 但在种子萌发过程中负介导GA的生物合成[110]. 尽管未检测到ABI4介导的GA代谢基因的直接靶点, 但在高粱(*Sorghum bicolor*)中, ABI4能够直接与

*SbGA2ox3*的启动子结合来激活其表达，从而导致GA降低并维持种子休眠[111]. 与ABI4一样，水稻AP2结构域TF OsAP2-39通过调节ABA和GA水平之间的平衡来调控萌发[106]. OsAP2-39上调ABA生物合成基因*OsNCED1*的转录，并增加GA失活基因*OsEUI*的表达. 此外，在水稻GA缺陷突变体中，*ARAG1* (*ABA responsive AP2-like 1*)基因的转录增加[93].

另一方面, GA信号转导途径中的调控因子也影响ABA生物合成的调节. DELLA蛋白除了在GA信号转导中的负作用外，也在萌发过程中作为GA和ABA互作的调控因子起作用. DELLA蛋白RGL2是拟南芥种子萌发过程中GA信号转导的主要抑制因子. RGL2对种子萌发的抑制可能是通过增强ABA生物合成和ABI5活性来实现的[112]. *XERICO*是一个编码促进ABA积累的RING-H2锌指(zinc finger)因子基因, 当GA水平低时，RGL2刺激*XERICO*的表达[113]. 另外，增加内源ABA生物合成对于在RNA和蛋白水平上提高ABI5是必需的. 增加的ABI5蛋白最终负责阻止种子萌发[112]. 同样，在水稻种子萌发过程中，*XERICO*的过表达表现出对外源ABA过敏感; 转基因系表现出内源ABA含量以及*OsNCED*和*OsABI5*的表达水平显著增加[114].

2.4　其他植物激素对种子萌发的调控

(1) 油菜素内酯(brassinosteroid，BR)促进种子萌发. 在豌豆种子萌发过程中, 生物活性BR的水平和BR生物合成基因的表达量增加[115]. 在拟南芥中, BR能够挽救严重的GA缺陷和GA不敏感突变体的萌发表型[8]. BR也促进水稻种子的萌发; 在BR生物合成抑制剂芸苔素唑(brassinazole, BRZ)存在下, 种子的萌发率降低, 类似于BR缺乏的萌发条件[116]. *nbg4* (*notched belly grain 4*)是一个编码细胞色素P450 (CYP724B1) *Dwarf 11*的等位基因，参与BR的生物合成，其突变也表现出萌发延迟[117]. BR信号转导途径的一个关键下游TF BZR1 (BRASSINAZOLE-RESISTANT 1)通过与*RAmy3D* (*α-Amylase 3D*)的启动子结合来介导水稻种子萌发，并激活α-淀粉酶的表达和活性. 该BZR1-RAmy 3D转录模块调控胚乳中淀粉的降解，从而促进种子萌发[118]. BR和GA能够在种子萌发过程中促进半胱氨酸蛋白酶(cysteine proteinase, REP-1)的表达, 导致胚乳中储藏谷蛋白(glutelin)的降解来产生更多的氨基酸，从而促进胚的生长[119]. BR也通过拮抗ABA的活性来促进种子萌发. 在萌发过程中，BR生物合成突变体*det2* (*de-etiolated-2*)和BR不敏感突变体*bri1* (*brassinosteroid insensitive 1*)比野生型对ABA更敏感[120]. 此外，一种BR信号转导途径中的关键抑制因子BIN2 (brassinosteroid insensive 2)与ABI5相互作用并稳定ABI5, 而BR在种子萌发过程中抑制BIN2–ABI5途径以拮抗ABA活性. BES1 (BRI1-EMS-SUPPRESSOR 1)直接与ABI5相互作用，并抑制ABI5与其下游基因的结合，导致下游基因的表达降低, 从而促进种子萌发[121].

此外，BR也通过抑制种子成熟程序(seed maturation program)基因的表达来促进种子萌发[122]. B3结构域蛋白VAL1 (VIVIPAROUS 1/ABSCISIC ACID-INSENSITIVE3-LIKE 1)参与抑制种子成熟程序. 用BR生物合成抑制剂处理的*val1-2*突变体幼苗形成胚状结构，而BR信号转导功能获得突变能挽救胚的结构特征. BR激活转录因子BES1和BZR1，它们直接与*AGL15* (*AGAMOUS-LIKE 15*)的启动子结合并抑制其表达. *AGL15*编码参与激活种子成熟程序的转录因子. 遗传分析表明，BR信号转导对*AGL15*具有上位性，并通过下调*AGL15*来抑制种子成熟程序. BR介导的途径与VAL1/2介导的途径协同作用来完全抑制种子成熟程序, 从而促进萌发[122].

(2) 生长素(auxin)维持种子休眠和抑制种子萌发. 生长素几乎参与植物生长发育的每一个过程，是最重要的植物激素之一. 生长素通过生物合成、代谢、极性运输和信号途径协同建立生长素的浓度梯度和局部浓度差异，决定了植物器官的发生、极性建立和对环境的适应[123]. 外源施用生长素吲哚-3-乙酸(indole-3-acetic acid, IAA)延迟小麦和大豆(*Glycine max*)种子的萌发[7]. 共轭修饰(conjugate modification)是有效控制活性生长素水平的重要过程之一，生长素糖基转移酶(auxin glycosyltransferase)通过共轭修饰催化糖转移到活性生长素上[124]. IAA糖基转移酶(IAA glycosyltransferase，IAGLU)已经在水稻中被鉴定，该酶参与催化IAA的糖基化(glycosylation)[125]. 与野生型相比，*osiaglu*突变体的种子萌发率较低，萌发种子中具有较高水平的游离IAA (free IAA). 同样，UGT74E2 (UDP-glucosyltransferase 74E2)将葡萄糖转移到吲哚-3-丁酸(indole-3-butyric acid，IBA)上，其过表达能够增加水稻种子的萌发，部分原因是游离IBA水平降低[126]. 生长素控制种子萌发的机制可能是通过与ABA的相互

作用来调控种子休眠与萌发. 生长素响应TF ARF10 (AUXIN RESPONSE FACTOR 10)和ARF16通过间接地促进ABI3的转录在其上游起作用, 从而维持种子休眠和抑制萌发[127]. *OsIAGLU*的失活提高了IAA和ABA的含量, 并持续诱导*OsABI*的表达[125]. 因此, 在*UGT74E2*过表达的植株中, IBA和ABA的水平均下降, 其信号转导途径也受到影响, 包括*ARF*、*ABI3*和*ABI5*下调[126].

(3) 茉莉酸(jasmonic acid, JA)抑制种子萌发. 茉莉酸甲酯(methyl jasmonate, MeJA)能有效地抑制水稻种子萌发[82]. 与野生型相比, 在过量积累JA的转基因水稻植株中, 种子萌发显著延迟[128]. 此外, 水稻*coi1* (JA受体的关键组分)突变体表现出迅速萌发的表型, 表明其在种子萌发中的负调控作用[129]. JA对萌发的抑制作用通常是由于其与ABA的协同作用. OPDA (*cis*(+)-12-oxophytodienoic acid)是JA生物合成过程中的一种关键的代谢中间产物, 与ABA一起共同抑制拟南芥种子的萌发[130]. JAZ (jasmonate-ZIM domain)蛋白是JA信号转导的抑制因子. 在拟南芥和面包小麦(*Triticum aestivum*)中, JAZ通过与ABI5的相互作用来负调控ABA抑制的种子萌发[131]. 在面包小麦中, JAZ3与ABI5相互作用并抑制其转录活性, 从而抑制ABA信号, 因此促进种子萌发[131]. JA和ABA在抑制水稻种子萌发中具有协同作用[89,132]. 田苗苗等人[133]从已有的数据中筛选到*GhCYP94C1*(Cytochrome P450 94C1)基因; 发现其启动子区域含有多种激素的响应元件; 0.5~2.5 μmol/L MeJA处理可以促进棉花种子萌发; 构建过表达载体转化拟南芥后, 发现该基因可以促进种子萌发、主根伸长和提前开花. MeJA抑制水稻种子萌发和促进棉花种子萌发的原因还不清楚.

3 转录和转录后调控

3.1 转录调控

转录因子在连接上游信号和下游转录网络中起关键作用, B3、AP2、bZIP、WRKY和NAC等类型的TF在种子萌发过程中调控基因的表达[8].

(1) B3结构域TF. *ABI3*是玉米*VP1* (*VIVIPAROUS 1*)的直系同源基因, 是拟南芥对ABA反应抑制种子萌发的一个典型的B3结构域TF成员. ABI3/VP1在ABA信号转导中的正作用是通过与AREB/ABF/ABI5的相互作用实现的, AREB/ABF/ABI5直接与ABA调控基因中的ABRE结合. ABI3在ABI5的上游起作用, 对于ABI5的表达以阻止萌发胚的生长是必需的. *OsDSG1* (*Oryza sativa delayed seed germination 1*)编码一种负调控ABI3的环指E3连接酶(RING finger E3 ligase)[134]. *osdsg1*突变体表现出萌发延迟的表型, 同时, 也表现出ABA信号转导基因如*OsABI3*和*OsABI5*的转录水平显著增加[134]. 因此, 当水稻种子萌发时需要OsDSG1介导的OsABI3降解, 随后ABA信号级联通过OsABI3-OsABI5途径被阻断, 最终ABA抑制的基因如编码水解酶的基因被重新激活, 从而促进萌发. 除了ABI3外, B3结构域TF的ABI3/VP1相关的亚家族还包括一个VAL (VP1/ABI3-like)因子家族和2个控制胚成熟的LEC (leafy cotyledon)类型的调节因子成员: LEC2和FUS3 (FUSCA3)[69]. 这些成员在控制从胚胎发生到萌发的发育调控中起核心作用. LEC2、FUS3和ABI3作为种子发育的激活因子[135], 而VAL抑制种子发育[136]. 水稻突变体*gd1* (*germination-defective 1*)在种子萌发和幼苗发育中存在缺陷[97]. *GD1*编码含有B3结构域的TF, 与拟南芥的*VAL*具有高度相似性. 在*gd1*中, 萌发抑制是由于内源GA_4水平降低所引起的[97].

(2) AP2结构域TF. ABI4是一种含有AP2结构域的TF, 通过直接与编码ABA分解代谢酶的基因(*CYP707A1*和*CYP707A2*)的启动子结合, 促进ABA的积累, 进而正调控拟南芥种子的初生休眠[110]. 在AP2结构域家族中, ABI4与DREB (drought response element binding)亚家族的关系最密切[69]. 一个类DREB基因*ARAG1* (*ABA responsive AP2-like 1*)被证明在水稻种子萌发中起作用[137]. *ARAG1*的敲除赋予种子萌发和幼苗生长过程中对ABA的抑制过敏感, 表明其在ABA信号转导途径中的负调控作用[137]. 此外, 转录组研究发现, 含有AP2结构域的TF, 特别是一些*OsDREB*在水稻种子萌发的最初吸胀中富集[138]. 由于AP2结构域TF被认为在种子休眠调控中起关键作用[77], 因此, AP2家族在水稻种子萌发和休眠中的调控作用需要进一步的研究. 遗传研究表明, *abi5*突变能够挽救*ap2*突变体的ABA敏感表型, 支持AP2在ABA信号转导和ABA介导的种子萌发抑制中拮抗ABI5的作用. 此外, 在核斑(nuclear speckle)中观察到AP2与SnRK2.2、SnRK2.3和SnRK2.6的相互作用, 表明AP2在ABA信号转导途径中起多方面的作用. 这些结果表明, AP2与SnRK2和

ABI5的相互作用对于ABA信号转导在控制种子萌发中至关重要[139].

(3) bZIP TF. 在bZIP大家族成员中, AREB/ABF/ABI5亚家族与ABRE结合, OsABI5 (OsbZIP10)在抑制ABA介导的种子萌发中起关键作用. 此外, 其他的一些bZIP成员也参与ABA介导的对水稻种子萌发和休眠的调控. OsMFT2 (MOTHER OF FT AND TFL 2)被发现是水稻种子萌发的一个负调节因子, 它通过与3种bZIP (OsbZIP23、66和72)相互作用并增强它们与*Rab16A* (一个典型的ABA响应基因)的ABRE结合来延迟萌发[140]. 值得注意的是, *OsbZIP23*过表达能够挽救*osmft2*敲除系的收获前萌发表型[140]. OsbZIP75直接与*DOG1* (*DELAY OF GERMINATION 1*, 一个种子休眠的主要控制基因)的启动子结合, 并促进OsDOG1L-3的积累[141]. OsDOG1L-3的高表达随后诱导ABA合成基因的表达和增加内源ABA含量, 从而增强ABA信号转导, 最后抑制种子萌发[141]. 通常, OsbZIP负调控水稻种子萌发, 但也有研究表明, OsbZIP09正促进种子萌发[142]. 进一步的证据显示, OsbZIP09通过与ABA信号转导相互作用来协调下游的共同靶基因 (即通过抑制萌发的负调控因子*OsLEA*和增强萌发的正调控因子*OsLOX2*) 的表达来调控种子萌发[142].

OsbZIP58与*OsKO2*启动子的G-box顺式元件结合并激活其表达, 从而促进GA生物合成和种子萌发[94]. OsABF1是bZIP TF的一个成员, 以ABA依赖的方式参与许多非生物胁迫反应, 包括盐胁迫和干旱胁迫[143,144]. 然而, OsABF1在种子萌发中也作为GA合成的一种关键阻遏物起作用[145]. *OsABF1*的过表达表现出典型的GA缺陷表型, 具有半矮化和种子萌发延迟的特性. 此外, OsABF1直接与*SD1* (编码GA生物合成中的GA20ox2)启动子中的G-box结合, 并抑制其转录, 从而降低种子中的内源GA水平[145].

(4) WRKY TF. 该TF参与种子萌发或休眠是由于它们对ABA的响应所决定的. 在拟南芥中, *AtWRKY41*的沉默导致种子初生休眠降低, 并下调*AtABI3*在成熟和吸胀种子中的转录. 此外, *AtWRKY41*直接与*AtABI3*启动子中的3个相邻的W-box结合, 并正调节其表达[146]. 在水稻中, *OsWRKY29*功能缺陷突变增强种子休眠, 而*OsWRKY29*的过表达则具有相反的作用, 表明*OsWRKY29*是一个休眠的负调控因子. 在ABA缺乏时, OsWRKY29与水稻*OsVP1*和*OsABF1*的启动子结合来下调它们的表达. ABA的存在抑制*OsWRKY29*的表达, 从而导致*OsVP1*和*OsABF1*的转录增加, 最终引起ABA响应增强和种子休眠增加[146]. AtWRKY2、AtWRKY40和AtWRKY63通过下调ABA响应基因*AtABI3*、*AtABI4*、*AtABI5*、*AtEM1* (*LATE EMBRYOGENESIS ABUNDANT 1*)和*AtEM6*的表达来降低ABA信号转导以及促进种子萌发和萌发后生长[146]. OsWRKY50通过直接抑制水稻ABA生物合成基因*OsNCED5*的表达来负调控ABA依赖的种子萌发和幼苗生长. 相反, 几种拟南芥WRKY TF (包括AtWRKY6、AtWRKY18、AtWRKY43和AtWRKY60)通过激活ABA响应基因的表达来抑制种子萌发. *AtWRKY2*敲除突变体的种子在ABA存在时表现出萌发延迟增加, 以及在萌发过程中ABA诱导的WRKY2积累需要ABI3和ABI5的参与[147]. 在ABA处理水稻种子时, OsWRKY72和OsWRKY77增强了ABA响应基因*HVA22*启动子的转录. OsWRKY53直接与*OsABA8ox1*和*OsABA8ox2* (ABA分解代谢基因)的启动子结合来抑制它们的表达, 导致ABA积累和进一步抑制种子萌发[148]. 然而, WRKY TF在调控种子萌发中具有功能差异, 因为一些WRKY蛋白被发现通过负调控ABA响应基因来促进种子萌发[149]. 同样, *OsWRKY29*的敲除系和RNAi系增加种子休眠, 而其过表达系显著增加种子萌发[150]. 进一步研究表明, OsWRKY29能够与*OsABF1* (ABI5类型TF)和*OsVP1* (ABI3类型TF)的启动子结合来抑制它们的表达, 从而导致ABA反应降低和种子萌发促进[150].

WRKY36通过AFP2 (ABI5-BINDING PROTEIN 2)作为ABA信号的负调控因子, 逐渐沉默*DOG1*的表达, 以打破种子休眠. AFP2与WRKY36相互作用, WRKY36识别*DOG1*启动子中的W-box以抑制其表达; 过表达*WRKY36*打破初生种子休眠, 而*WRKY36*突变体表现出强烈的初生种子休眠. 此外, AFP2招募转录共阻遏物TPR2 (TOPLESS-RELATED PROTEIN 2)来减少*DOG1*位点的组蛋白乙酰化, 最终介导WRKY36依赖的对*DOG1*表达的抑制, 以打破种子初生休眠[151]. 这些结果表明, WRKY36-AFP2-TPR2模块在表观遗传上逐渐沉默*DOG1*的表达, 从而调控种子的初生休眠.

*WRKY*基因在种子萌发过程中调控ABA和GA信号转导的互作. OsWRKY71通过OsGAMYB阻断*Amy32b*启动子的激活, 作为GA信号转导的转录抑制因子起作用. OsWRKY71也与另一个OsWRKY51一

起共同介导GA和ABA之间的互作. 受ABA诱导的和GA抑制的OsWRKY51和OsWRKY71 TF形成一种异源四聚体, 该四聚体与*Amy32b*的启动子结合并阻止OsGAMYB对启动子的激活[103]. 此外, 在*IPA1* (*Ideal Plant Architecture 1*)突变体中, 种子萌发和早期幼苗生长受到抑制; 同时, *OsWRKY51*和*OsWRKY71*的表达显著上调. 进一步研究发现, IPA1直接与OsWRKY51和OsWRKY71结合并激活它们的表达, 从而干扰GA诱导的OsGAMYB的结合亲和力, 抑制α-淀粉酶基因的表达[104]. 此外, OsWRKY72通过"LRK1-OsKO2"(糊粉层中的途径)被鉴定为水稻种子萌发的负调控因子[152]. WRKY72直接靶向LRK1 (a leucine-rich repeat receptor-like kinase)并激活其转录, 抑制内根-贝壳杉烯氧化酶OsKO2, 从而导致内源GA水平降低和抑制萌发[152]. AtWRKY43也可能有助于其他细胞过程, 如脂肪酸去饱和以及储藏蛋白表达, 以调节种子的萌发[146].

(5) *SD6* (*Seed Dormancy 6*). *SD6*编码一个bHLH (basic helix-loop-helix) TF, 该TF通过专一性识别ABA分解代谢基因*ABA8OX3*启动子中的G-box基序来负调控水稻种子休眠, 并激活*ABA8OX3*的表达. 而SD6相互作用伴侣ICE2 (inducer of C-repeat binding factors expression 2)专一性识别*ABA8OX3*启动子中的E-box基序, 并抑制*ABA8OX3*表达, 从而增强种子休眠. SD6和ICE2以温度依赖的方式拮抗控制种子休眠. 当温度适合萌发时(高于25°C), *SD6*的表达水平显著升高, 而*ICE2*的表达水平急剧降低, 从而促进*ABA8OX3*和*OsbHLH048*基因的表达, 进而抑制ABA生物合成基因*NCED2*的表达; 因此, 种子中的ABA含量降低, 最终引起萌发. 当种子处于不合适的温度(低于20°C)条件下, *SD6*的表达水平略有降低, 而*ICE2*的表达升高, 因此抑制了*ABA8OX3*和*OsbHLH048*基因的表达, 这反过来又促进了ABA生物合成基因*NCED2*的表达, 导致种子中的ABA含量增加, 种子处于休眠状态[153]. *SD6*的弱休眠等位基因在栽培稻中很常见, 但在野生稻中经历了负选择[153]. 通过基因组编辑*SD6*及其小麦同源基因, Xu等人[153]证明SD6是在田间条件下缓解禾谷类作物收获前萌发的有用育种靶标.

(6) NAC (NAM-ATAFCUC) TF. 水稻NAC家族成员OsNAC2负调控种子萌发[72]. 尽管OsNAC2与乙烯生物合成基因的启动子具有结合能力, 但它可能通过ABA途径而不是乙烯和GA途径抑制萌发[77].

3.2 转录后调控

转录后过程严格调控数千个基因的表达, 以及基因表达的许多其他方面[154]. 下面主要介绍RNA剪接(RNA splicing)、miRNA (microRNA)活性和翻译后修饰(post-translation modification)对种子萌发的调控.

(1) RNA剪接. RNA剪接是一种共转录(co-transcription)的分子事件, 由剪接体(spliceosome)大分子复合物进行; 剪接体识别并删除一些区域(内含子, intron), 同时连接其他区域(外显子, exon)[155]. 可变剪接(alternative splicing)是真核生物中的一个重要的基因调控过程; 在细胞核中mRNA前体(pre-mRNA)的转录过程中发生, 它从单个基因产生多种mRNA变体来调节转录组的多样性(transcriptome diversity). 这些转录物能够被翻译成为多种蛋白, 产生的蛋白在细胞中具有相似或不同的功能[8,156](图2).

在拟南芥中, *HAB1* (PP2C)有两个剪接变异体(*HAB1.1*和*HAB1.2*), 在种子萌发中具有相反的功能[157]. PP2C与亚类III SNF1相关的蛋白激酶(subclass III SNF1-related protein kinase; SnRK2.2、2.3和2.6)相互作用来去磷酸化并抑制其激酶活性, 从而关闭ABA的信号转导. 虽然*HAB1.1*促进种子萌发, 但*HAB1.2*是ABA信号转导的正调控因子, 并阻止种子萌发. 有趣的是, RBM25 (含有RNA结合基序RBM的蛋白)是*HAB1*可变剪接的一种关键调控因子. *rbm25*幼苗具有的高ABA敏感性是由于*HAB1.1*异构体的表达减少引起的[157].

对4个大麦品种的萌发胚进行的RNA-seq研究表明, 在吸胀后的24和48 h分别鉴定到2200和3900个可变剪接转录本; 其中具有最多可变剪接转录本的途径包括转录后调控、植物激素信号转导和细胞壁修饰, 表明这些过程在调节种子萌发中具有重要作用[158]. *RGH3* (*rough endosperm 3*)编码一种与剪接体有关的RNA剪接因子. *rgh3*玉米突变体表现出胚发育和胚乳细胞分化缺陷. 分子克隆表明, 它有19个剪接变异体, 以组织和发育阶段专一的方式被调节[159]. Gault等人[160]证明, *rgh3*突变体蛋白破坏了与U2AF2的共定位, 并引起胚乳细胞的分化和增殖异常. RRM_RBM48类型RNA结合蛋白DEK42通过与其他剪接体组分的相互作用参与mRNA前体剪接的调

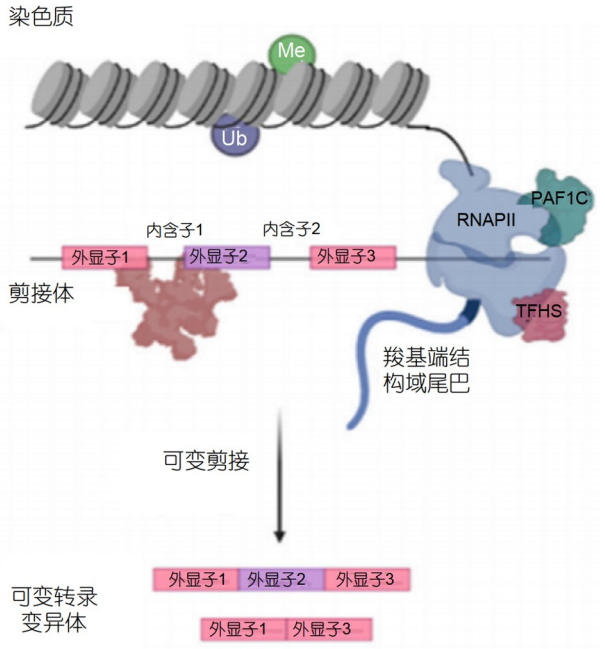

图 2　种子中可变剪接的调控. RNA剪接是由剪接体进行的. 受到可变剪接调控的基因编码具有调控功能的蛋白. 染色质压缩和修饰也影响转录和剪接. 主要的转录变化连同染色质重塑一起调控种子的成熟、休眠和萌发. Me, 甲基化; PAF1C, 聚合酶II相关因子1复合物; RNAPII, RNA聚合酶II; TFIIS, 转录因子IIS; Ub, 泛素化

Figure 2　Alternative splicing regulation in seeds. RNA splicing is carried out by the spliceosome. Genes subjected to alternative splicing regulation encode proteins with regulatory functions. Chromatin compaction and modification also affect transcription and splicing. Major transcriptional changes, together with chromatin remodeling, regulate seed maturation, dormancy, and germination. Me, methylation; PAF1C, polymerase II-associated factor 1 complex; RNAPII, RNA polymerase II; TFIIS, transcription factor IIS; Ub, ubiquitination

控[161]. 由于可变剪接的变化, *dek42*玉米突变体产生小的有缺陷的籽粒和致死的幼苗[161]. Qi等人[162]发现, P类型PPR核蛋白DEK2参与线粒体编码的mRNA剪接. *dek2*突变降低线粒体*nad1*内含子1的剪接效率, 并表现出呼吸复合物I的组装和线粒体功能的严重受损[162]. *OsABI5*的可变剪接产物(*OsABI5-1*和*OsABI5-2*)同时存在于水稻组织中, 但表达模式不同[163]. 在种子成熟过程中, *OsABI5-1*和*OsABI5-2*之间的物理相互作用与*OsVP1*一起共同调控下游基因, *OsABI5-1*或*OsABI5-2*的组成性表达可以挽救*abi5-1*种子的ABA不敏感性[163].

Xu等人[164,165]发现, *OsbZIP58*的可变剪接在高温下发生改变. 授粉后, 在35°C光照12 h和28°C黑暗12 h的周期下培养数天的水稻植株降低了*OsbZIP58-β*剪接变异体的活性, 减少了与粒重、厚度、淀粉和脂肪含量有关的种子质量[165]. 在番茄幼苗中, 热休克因子(heat-shook factor) *HsfA2-I*的剪接变异体增加了短期高温适应性, 从而增强耐热性; 而*HsfA2-II*变异体, 其截短的C端激活基序缺乏核输出信号, 被迅速降解[166]. 在对28种不同的大豆组织包括种子发育和萌发阶段的RNA-seq比较研究中, Shen等人[167]发现, 63%的基因(27764个基因)被可变剪接, 可变剪接在给定组织类型的年幼发育阶段比年老发育阶段更普遍. 可变剪接事件与RNA剪接体的基因有关, 至少1/3的剪接事件在组织间的表达不同. 可变剪接的频率与内含子的长度和外显子的数量呈正相关[167]. 利用发育中的大豆胚进行的另一项RNA-seq分析表明, 转录后的剪接事件与碳氮代谢、休眠和剪接过程有关, 也与种子成熟过程中耐脱水性的获得有关[168]. 一些RNA异构体表现出结构域发生变化, 这可能有助于蛋白的亚细胞定位或活性变化[168].

(2) miRNA. miRNA是一个由20~24个核苷酸组成的非编码RNA分子, 通过其互补的序列调控编码TF和关键调控蛋白的基因表达[169]. 植物miRNA由RNAPII (RNA polymerase II)转录, 并被顺式作用和反式作用调节元件调控[170,171]. 初级*MIR*转录本被加工成为发夹前体(hairpin precursor), 该过程由DCL1 (DICER-LIKE 1)酶和2种RNA结合蛋白HYL1 (HYPONASTIC LEAVES 1)和SE (SERRATE)催化. miRNA前体经过另外一次DLC介导的裂解并形成双链(duplex). 该双链的3′端随后被HEN1 (HUA ENHANCER 1)甲基化, 并运输到细胞质中, 进一步掺入miRNA诱导的沉默复合物中. 然后, miRNA能够在转录后的基因沉默(gene silencing)或翻译抑制中起作用, 或者它们能够触发转录本的裂解以产生另一种类型的miRNA. AGO1 (ARGONAUTE 1)蛋白是一种miRNA诱导的沉默复合物的核心组分, 它引导复合物进入靶RNA中的序列, 这些序列几乎与miRNA完全互补[172].

在拟南芥种子中, miR163被光高水平诱导, 并促进种子萌发和胚根生长[173]. 此外, 在胁迫条件下, miR402通过miRNA指导的对DML3 (DEMETER-LIKE PROTEIN 3)的调节正调控种子萌发和幼苗生长, DML3参与DNA的去甲基化[174]. 许多与萌发过程相关的激素信号转导途径也被miRNA调节. 例如, miR159

通过调节GA和ABA的信号转导参与种子萌发; 它靶向编码与GA反应元件相互作用的GAMYB TF的mRNA. miR159的表达由GA和ABA共同控制[175]. 在GA缺乏时, miR159的表达可能通过DELLA蛋白的作用被抑制. 生长素相关途径也受miRNA调节. miR393靶向*TIR1*生长素受体和其他3种相关的F-box蛋白. miR160靶向*ARF10*、*ARF16*和*ARF17*, 以及miR167靶向*ARF6*和*ARF8*. Liu等人[176]表明, *ARF10*被miR160抑制; 在萌发和萌发后过程中, 其调控在生长素和ABA途径之间的互作中起关键作用. *ARF10*的去阻遏增加种子对ABA的敏感性, 而*MIR160a*的过表达降低拟南芥种子对ABA的敏感性[176]. 这些证据表明, miR160在种子萌发期间作为生长素和ABA介导的互作汇聚点起作用[176]. 此外, 在高温下, 高水平的miR156抑制种子萌发、而miR172增加种子的萌发. miR159控制TF MYB33和MYB101, 它们是ABA信号转导的正调控因子[177].

在水稻中, *OsmiR156*由12个基因编码, 这些基因产生3种*OsmiR156-5p*异构体和4种*OsmiR156-3p*异构体[178]. *OsmiR156*调控*SPL*和*IPA1*基因的表达. 其中*SPL13*、*SPL16*和*IPA1*是种子大小的正调控因子[179], *SPL12*和*IPA1*通过直接调控GA途径中的多个基因来增强种子休眠[180,181]. 在水稻中, *OsmiR396*基因家族是高度保守和丰富的. 它由8个基因组成, 编码5个成熟的异构体, 这些异构体靶向11个*GRF* (*GROWTH REGULATING FACTOR*) TF基因[182]. Wang等人[157]发现, OsmiR156和OsmiR396在水稻籽粒大小中起作用, 表明miRNA的转录后修饰有助于开发出更好的遗传材料来提高产量. 另一项研究表明, 抗OsmiR160的*OsARF18* (一种籽粒大小的负调节因子)的表达产生较小的籽粒, 减少淀粉粒的积累, 这表明miRNA可以精确地调节种子发育[183]. OsmiR397在幼穗和籽粒中高度表达, 其过表达增加籽粒的体积并促进穗分枝[184].

(3) 翻译后修饰. 水稻种子萌发过程中蛋白磷酸化(phosphorylation)水平的变化主要发生在吸胀后最初的12 h. 这一阶段不仅对转录过程而且对翻译后过程和代谢变化都至关重要. 因为这一时期是决定水稻种子萌发的关键时期[185]. 此外, 种子萌发也受磷酸化/去磷酸化(dephosphorylation)模式变化的显著影响[185]. 除了ABA信号转导途径的磷酸化状态发生变化外, 其他的磷酸化事件也影响种子萌发. FyPP1和FyPP3PP6磷酸酶与SnRK2激酶起拮抗作用, 使ABI5去磷酸化和去稳定[186]. Raf10和Raf11 MAP3K是休眠以及ABI3和ABI5表达的正调控因子[187]. 特别是Raf10磷酸化亚类III SnRK2, 依次磷酸化ABI5、ABF2和ABI3 TF以增强它们的活性[188]. TAP46是一种PP2A磷酸酶相关的蛋白, 它与ABI5的活性磷酸化形式结合并使其稳定, 阻止其PP2A介导的去磷酸化[189]. 磷酸化也影响GA的信号转导. DELLA的稳定性也与磷酸化有关, 因为TOPP4 PP1磷酸酶直接与DELLA蛋白RGA和GAI 结合并使它们去磷酸化, 促进其GA依赖的不稳定[190]. MYB44 TF对萌发的活性也依赖于其被MPK3和MPK6激酶的磷酸化[191], 与RAV1 TF相反, 它在被SnRK2激酶磷酸化后失活[192].

蛋白泛素化(protein ubiquitination)是另一种重要的蛋白修饰, 萌发过程中负影响起重要作用的蛋白的稳定性, 如DELLA、ABI3和ABI5[193]. 在水稻种子萌发过程中, 多于1000个蛋白发生泛素化变化, 其中大多数变化发生在吸胀后12 h[194].

苏木化(sumoylization, SUMO)保护ABI5不被降解, 但使其失活[195], 表明在ABA缺乏时通过维持ABI5的抗降解失活池起保护作用[196]. MYB30是ABA反应的一个负调控因子, 似乎为ABI5所产生的正调控提供了一种平衡. 有趣的是, 这2种调控因子在专一的氨基酸残基上被同一SUMO E3连接酶(SUMO E3 ligase, SIZ1) SUMO化[197]. ABI5的SUMO化位点与KEG-E3-连接酶依赖的转换(KEG-E3-ligase-dependent turnover)所需的赖氨酸残基(K344)位于相同的结构域(K391)中[196]; 这表明ABI5的SUMO化或泛素化依赖于酶活性的直接物理竞争.

4 特异性调控因子

4.1 DOG1

萌发延迟1 (*DOG1*)最初被报道为拟南芥种子休眠遗传调控的主要数量性状位点(quantitative trait locus, QTL)[198,199]. 在拟南芥种子中DOG1的积累引起休眠增加和萌发延迟. 与野生型拟南芥种子相比, 功能缺失的*dog1-3*突变体种子萌发较早, 而功能获得的*dog1-5*突变体种子萌发延迟[177,200]. 在种子成熟过程中, 低温增加*DOG1*的表达并诱导种子休眠[201]. 低温条件下, 种子发育通过增加bZIP67 TF的表达和蛋白丰度来触

发*DOG1*的表达, bZIP67直接靶向*DOG1*启动子并激活其表达, 导致种子休眠增强[202]. DOG1也影响miRNA的产生和吸胀的拟南芥种子萌发热抑制[177]. 有趣的是, DOG1影响编码miRNA加工蛋白的基因的表达: *DCL1*和*HYL1*的转录本被DOG1诱导, 而干种子中的*SE*被DOG1抑制, 这表明DOG1可以通过调控miRNA的加工来控制种子萌发[177].

DOG1与ABA信号转导的两个关键负调控因子AHG1 (ABA HYPERSENSITIVE germination 1)和AHG3物理相互作用, 在功能上阻断其下游的作用[17,203~205]; 以及DOG1调控种子休眠需要 PP2C磷酸酶[203]; 这些研究结果表明, ABA核心途径和DOG1途径在PP2C磷酸酶处汇合[203,205,206]. 在*dog1-1*中, 一些ABA合成基因(*NCED2*、*3*、*5*、*9*)的表达被显著降低[207], 而ABA分解代谢基因(*CYP707A1*、*A3*)和GA合成基因(*GA20ox1*–*3*和*GA3ox1*、*2*)的表达被显著增加[208]; 与基因表达相一致, *dog1-1*突变体中的ABA和GA含量也发生了相应的变化[201]. 此外, ERF12 (ETHYLENE RESPONSE FACTOR 12)及其下游靶子ETR1 (ETHYLENE RESPONSE 1)直接与*DOG1*启动子结合并招募TPL (TOPLESS), 导致*DOG1*被抑制, 表明乙烯也通过ETR1–ERF12/TPL–DOG1模块调控种子休眠[209]. Li等人[210]的研究表明, 在*dog1-2*背景下, ABA和IAA的内源信号强度明显降低, 而GA、BR和细胞分裂素的内源信号强度显著增加; 这可能是其休眠缺陷表型的重要原因. 他们强调了DOG1在平衡植物激素相关基因表达中的作用, 并提供了DOG1可能整合植物激素网络来控制种子休眠的证据. DOG1也可以通过影响miR156和miR172的水平来调控种子萌发和开花时间[177,206].

此外, *DOG1*也被一些复杂的机制调控, 包括组蛋白修饰(histone modification)、可变多聚腺苷酸化(alternative polyadenylation)、可变剪接和顺式作用反义非编码转录物(*cis*-acting antisense noncoding transcript, *asDOG1*) [29,200,205,206,211]. HSI2 (HIGH-LEVEL EXPRESSION OF SUGAR INDUCIBLE 2)和HSL1 (HSI2-LIKE 1)二聚体与*DOG1*启动子中的RY (CATGCA (TG))元件相互作用. B3结构域和类PHD (plant homeodomain-like)结构域对于在*DOG1*启动子上富集HSI2和HSL1都是必需的. HSI2和HSL1招募多梳家族蛋白(polycomb-group protein)的成分, 包括CLF (CURLY LEAF)和LHP1 (LIKE HETERCHROMATIN PROTEIN 1), 从而沉积H3K27me3标记, 导致*DOG1*表达的抑制. 这些研究结果表明, 在种子萌发和幼苗早期生长过程中, HSI2和HSL1依赖的组蛋白甲基化在种子休眠的调控中起关键作用[212].

4.2 DOG18

*DOG18*是拟南芥种子休眠的自然变异因子. *DOG18*编码A分支2C型蛋白磷酸酶家族(clade A of the type 2C protein phosphatases family)的一个成员, 先前被鉴定为*RDO5* (*REDUCED DORMANCY 5*)基因[213,214]. 然而, RDO5不与分支A的磷酸酶聚类, 并且在*rdo5*突变体中ABA的水平和敏感性不发生改变. RDO5转录本仅在种子中被检测到, 且在干种子中丰度最高. RDO5存在于整个胚的细胞中, 并定位于细胞核[213]. RDO5被证明是一种伪磷酸酶(pseudophosphatase), 在种子吸胀过程中抑制去磷酸化[214]. *DOG18*/*RDO5*在欧洲西北部的天然材料中存在广泛的序列变异, 表现出相对高频率的功能丧失等位基因, 其中的休眠丧失可以通过遗传因素如*DOG1*和*DOG6*以及环境因素如低温来补偿. 对干燥和吸胀种子中磷酸化蛋白质组的分析显示, 在种子吸胀过程中蛋白磷酸化普遍降低, 而*rdo5*突变体中的磷酸化作用增强[214].

4.3 OsSDR4和AtSDR4L

对水稻收获前萌发的QTL分析表明, *SDR4* (*SEED DORMANCY 4*)促进成熟中的种子休眠发育[215]. OsSDR4定位于细胞核以及*OsSDR4*突变体的发育胚中*DOG1L-1*的表达降低表明, OsSDR4是水稻种子休眠主控因子的正调控因子[215]. 在拟南芥基因组中已经发现了7个*OsSDR4*的同源基因, 其中同源性最大的基因被命名为*SFL1* (*SDR FOUR LIKE 1*)[215]. 与*OsSDR4*相比, *SFL1* (先前被报道为*AtSDR4L*或*ODR1*)被认为是拟南芥干燥成熟种子休眠的负调控因子[216,217]. SFL1被证明与bHLH57结合, 并抑制bHLH57诱导的ABA生物合成基因*NCED6*和*NCED9*的表达[217]. 对ABA处理的拟南芥幼苗中的21个ABA相关转录因子靶标的全基因组分析发现了7个*OsSDR4*同源基因中有6个*SFL* (*SFL2*–*SFL7*)[218]. 6个*OsSDR4*同源基因中的2个同源基因*DIG1* (*Dynamic Influencer of Gene expression 1*)和*DIG2*被证明影响几百个基因的表达[218]. 诱导表达研

究发现, DIG是转录调控因子, 可增强幼苗对ABA和盐的敏感性. *OsSDR4*同源基因*ATIR1–ATIR6*的功能缺失突变体分析也表明, 这6个基因是ABA诱导的转录调控因子, 正调控幼苗对ABA和干旱的反应[219,220]. 然而, 除*SFL1*外, 还没有其他*SFL*对种子休眠作用的报道.

Zheng等人[221]分析了拟南芥直系同源基因*SFL1*的功能缺失突变体, 发现*sfl1-1*种子类似于水稻*Ossdr4*突变体的种子, 表现出在成熟中期到成熟后期提早萌发, 但在成熟干燥过程中变得比野生型种子的休眠更强. 与休眠水平一致, *sfl1-1*种子中种子成熟和休眠的主控基因*ABI3*、*FUS3*和*DOG1*的表达水平在成熟早期和中期低于野生型种子, 但在成熟后期高于野生型种子. 除了种子休眠表型外, *sfl1-1*幼苗表现出生长停滞表型以及幼苗中*LAFL* (*LEC1*、*ABI3*、*FUS3*、*LEC2*)和*DOG1*异时表达(heterochronic expression). 这些研究结果表明, *SFL1*是一个种子休眠程序启动和终止的正调控因子, 也是种子成熟和萌发过程中从生长停滞到生长恢复的特异性调控因子, 其旁系同源基因(paralog gene) *SFL2–SFL4*增强了*SFL1*的功能, 并影响从胚成熟到幼苗生长阶段转变的时间[221].

5 表观遗传调控

表观遗传机制(epigenomic mechanism)调控植物生长和发育的许多过程, 包括种子成熟、休眠和萌发相关基因的表达. DNA甲基化(DNA methylation)、组蛋白修饰和染色质重塑(chromatin remodeling)是种子萌发过程中表观遗传调控的重要内容[8,17,154].

5.1 DNA甲基化

在种子发育过程中CHH甲基化明显增加, 而在萌发过程中CHH甲基化显著减少. 这些结果表明, DNA甲基化重编程事件(DNA methylation reprogramming event)可能是调控这2个发育阶段的机制[222]. 拟南芥ATXR7是一种H3K4甲基转移酶(methyltransferase), 当发生突变时, 种子的休眠减少[223]. 对具有厚糊粉层表型的水稻籽粒的筛选发现了一种负责限制糊粉细胞层数量的DNA脱甲基酶(DNA demethylase) OsROS1[224]. *OsROS1*参与胚乳中CG和CHG的去甲基化, 调控RISBZ1 bZIP和RPBF Dof TF的表达. 对保持在42°C下24或48 h的发育中的水稻种子的研究发现, 由于编码PRC2 (polycomb repressive complex 2)成员的*OsFIE1* (*FERTILIZATION ENDOSPERM INDEPENDENT 1*)的H3K9甲基化缺陷, 热处理减少种子的体积和促进过早的细胞化[225]. 对拟南芥和大豆种子的甲基化组(methylome)的比较研究发现, CHH甲基化在发育和休眠过程中增加, 但在随后的萌发种子中急剧下降. 相反, CG和CHG的甲基化在同一发育时期不发生变化. 虽然在缺乏CHH和CHG甲基化的拟南芥*ddcc*突变体中观察到种子的发育正常, 但有106个转座子(transposon)被转录去抑制, 这表明CHH甲基化的增加可能是一种增加转座子沉默的故障安全机制(failsafe mechanism) [226]. 此外, 大豆基因组中CHH甲基化区域被转录下调, 并与成熟种子中的DNA复制和细胞分裂有关[227].

5.2 组蛋白修饰

HUB1/*RDO4* E3泛素连接酶基因, 与其同源基因HUB2一样, 是H2B组蛋白单泛素化(histone monoubiquitination)和休眠相关基因表达所必需的[228]. H2B单泛素化是一种与促进转录起始和早期延伸事件有关的染色质修饰[229]. *RDO2*编码一种转录延伸因子(transcription elongation factor, TFIIS), *rdo2*单突变体减少休眠[223]. 研究表明, 基因的脱乙酰化(deacetylation)机制对种子休眠具有负影响. 与ABA、乙烯和生长素途径有关的关键基因被SNL (SWI-INDEPENDENT 3 (SIN3)-LIKE)脱乙酰化复合物的成员调控. SNL1和SNL2的表达在胚发育和种子成熟过程中逐渐增加, 引起*CYP707A1*和*CYP707A2* (ABA降解基因)以及*ACO1* (*1-Aminocyclopropane-1-carboxylic acid oxidase 1*)和*ACO4* (乙烯生物合成基因)的乙酰化水平(H3K9/K18/K14)降低; 从而提高ABA水平并阻断乙烯途径[230]. SNL1和SNL2的表达在后熟(after-ripining)种子的吸胀过程中下降, 引起*AUX1* (*Auxin importer 1*)的乙酰化水平增加; 随后*AUX1*的转录被激活, 导致生长素水平和信号转导增加, 促进种子萌发[231].

组蛋白H3K27me3去甲基化酶(histone H3K27me3 demethylase) REF6 (RELATIVE OF EARLY FLOWERING 6)通过诱导种子中的ABA分解代谢来抑制种子休眠. *ref6*功能缺失突变体的种子表现出休眠增强, 这与内源ABA含量的增加有关. 在种子发育和萌发过程中, 是*ref6*突变体中ABA分解代谢的两个关键基因

*CYP707A1*和*CYP707A3*的转录本，而不是ABA生物合成基因的转录本显著降低. REF6直接与*CYP707A1*和*CYP707A3*基因中的CTCTGYTY (Y代表C或T)基序结合，并负责降低它们的H3K27me3水平. *ref6*中种子休眠和ABA浓度的增加主要取决于*CYP707A1*和*CYP707A3*的表达减少; 相反, *CYP707A1*的过表达可能抵消*ref6*增强的种子休眠[232].

H3.3是组蛋白H3的变体. 在拟南芥中，H3.3与H3.1有4个氨基酸不同[233]，它们的表达分别是DNA复制不依赖的和DNA复制依赖的[234]. 拟南芥H3.3由3个基因*HTR4*、*HTR5*和*HTR8*编码，主要富集于转录基因体(transcribed gene body)[234]. 拟南芥H3.3 (*h3.3kd*)的敲除导致轻度发育缺陷，包括叶片锯齿状、早花和育性轻度降低[235,236]. Zhao等人[237]发现，H3.3对种子形成不是必需的，但H3.3的缺失会导致胚后期发育和萌发严重受损. H3.3表现出种子专一的5′基因末端分布，并促进种子中调控区域的染色质开放. 在萌发过程中，H3.3是正确的基因转录调控所必需的. 此外, H3.3在3′基因末端不断加载，这与基因体DNA甲基化以及该区域染色质可及性(chromatin accessibility)和隐性转录(cryptic transcription)的限制有关. 这些研究结果表明，H3.3在启动种子调控区域的染色质可及性以及赋予胚向胚后期过渡中起重要作用.

组蛋白脱乙酰酶(histone deacetylase, HDA)从组蛋白中去除乙酰基[238]，而组蛋白乙酰转移酶(histone acetyltransferase)将乙酰基转移到组蛋白N端区域的赖氨酸残基上[239]. 组蛋白脱乙酰酶是催化组蛋白和非组蛋白脱乙酰化的酶. 组蛋白脱乙酰化导致染色质致密，从而使转录不活跃. 研究表明，2种植物专一的组蛋白脱乙酰酶HD2A和HD2B的功能丧失导致拟南芥种子休眠增强. 有趣的是, HD2A和HD2B的沉默引起DOG1位点的过乙酰化，并在种子成熟和吸胀过程中促进DOG1的表达. *DOG1*的敲除可以减少种子的休眠，以及部分减少*hd2ahd2b*紊乱的发育表型. *hd2ahd2b*系的转录组学分析表明，许多与种子发育有关的基因受损[240]. HD2B脱乙酰酶对H2B的脱乙酰作用与吸胀种子中休眠减少和GA水平增加有关. 在浅休眠的拟南芥材料(Columbia-0)中，*HD2B*的表达被冷或后熟处理上调，并且与GA失活基因(*GA2ox2*)的表达水平降低和与GA生物合成基因(*GA3ox1/2*)的表达水平增加有关. 在深休眠的拟南芥材料(Cape Verde Islands)中，这种*HD2B*表达的上调被显著地抑制[241]. 脱乙酰酶HDA9负影响种子萌发和促进休眠. HDA9可能通过H3K9的脱乙酰化作用参与种子发育过程中与从种子向幼苗转变相关的基因的转录抑制[242].

组蛋白精氨酸脱甲基酶(histone arginine demethylase) *JMJ20* (*JUMONJI C DOMAIN-CONTAINING PROTEIN 20*)和*JMJ22*在SOM (SOMNUS)下游起作用，在对光的反应中正调控种子萌发[243]. 研究证明，在拟南芥种子萌发过程中，SUVH5作为光介导的转录调控网络的正调控因子起作用[244]. 在染色质水平上的研究发现，组蛋白脱乙酰酶HDA19与SUVH5相互作用，并通过抑制种子休眠基因的表达促进种子萌发[71]. 在吸胀的二穗短柄草(*Brachypodium distachyon*)胚中，H4K16ac和H3K18ac的活性增加[245].

AL6和AL7能够在H3K4me3标记周围与PRC1多梳蛋白(PRC1 polycomb protein)相互作用并形成复合物，导致与种子发育相关的基因(如*ABI3*、*DOG1*、*CRU3*、*CHO1*)在种子萌发过程中从H3K4me3相关的转录活性状态转变为H3K27me3相关的转录抑制状态[246]. 在萌发的种子和营养器官中发现了另外的种子成熟基因的抑制因子, 包括与SDG8甲基转移酶结合的多梳EMF2-PRC2复合物(维持幼苗中H3K27me3抑制标记所必需的)[247]，2种ZRF蛋白(通过与单泛素化的H2A和H3K27me3结合有助于PCR1介导的抑制) [248]，SUVH5甲基转移酶(介导H3K9的抑制性二甲基化)[244]和LDL1/2去甲基化酶(demethylase) (可能从种子休眠基因中去除激活的组蛋白修饰(H3K4me2/3)) [249].

一些TF可以招募修饰因子来负调控专一的休眠相关的染色质位置. BES1 TF能够与TPL辅阻遏物和*ABI3*位点的HDA19形成转录抑制因子复合物[250]，SCL15 TF在营养组织中的胚特异性位点的亚群中招募HDA19 [251]，HSI2 TF在萌发后招募HDA6来抑制种子成熟基因[252]. 此外，HSI2和HSL1可能将HD2A和HD2B招募到*DOG1*上来负调控*DOG1*的表达并减少种子休眠, 从而在种子成熟过程中影响种子发育, 在吸胀过程中促进种子萌发[240].

在萌发过程中，含有SANT (SWI3/DAD2/N-CoR/tfff-b)结构域的蛋白POWERDRESS (PWR)通过促进组蛋白H3脱乙酰化水平和H2A.Z在*SOM*位点的沉积来抑制ABI3依赖的*SOM*转录. 种子在高温胁迫下吸胀阻断*PWR*的转录和触发次生休眠[253]. *SOM*的H3脱乙

酰化也是一氧化碳(carbon monoxide, CO)信号转导的一个靶点. CO信号转导通过H3脱乙酰化作用招募HDA6到*SOM*的启动子来降低其表达[254].

5.3 染色质重塑

染色质重塑因子也调控基因表达. PKL (PICKLE)是一种CHD3 (chromodomain helicase DNA-binding domain 3)类型的染色质重塑因子[255], 有助于维持种子吸胀过程中对*LEC1*和*FUS3*的抑制, 因为*pkl*突变体种子在吸胀时异常表达*LEC1*和*FUS3* [256]. PKL与B3家族转录因子VAL1和VAL2直接结合, 并通过VAL1和VAL2被招募到*ABI3* 3′UTR和*AGL15*启动子上, 抑制它们的表达, 促进种子萌发[255]. 在拟南芥中, *PKL*在种子吸胀时被迅速激活, 它可能在萌发过程中介导染色质重塑事件, 抑制早期胚胎基因表达程序, 并使发育向萌发后生长转变[257]. 在*pkl*突变体种子中, *DOG1*在种子吸胀过程中下调, 表明PKL抑制了DOG1的表达; 染色质免疫沉淀(chromatin immunoprecipitation)分析表明, PKL与*DOG1*的染色质区域结合, 而不是与启动子区域结合, 表明PKL与*DOG1*的基因体相关[258]. 有趣的是, PKL通过抑制*ABI3*和*ABI5*的表达来抑制ABA的信号转导, 从而促进种子萌发. *pkl*突变体种子中这2个基因的高丰度表达与被抑制的染色质的组蛋白标记中的低甲基化程度相关[259].

染色质结构也能够通过依赖ATP的重塑复合物的改变而发生变化[260]. BRM (BRAHMA)是一种SWI2/SNF2染色质重塑ATP酶, 其功能缺陷突变体导致ABA过敏感反应, 并直接抑制*ABI5*[261]. 此外, BRM通过激活*GA3ox1*的启动子在GA生物合成的正向调控中起直接作用, *brm-1*和 *brm-3*突变体种子的活性GA水平显著降低[262].

6 结束语

种子萌发和/或休眠研究的主要目的之一是指导农林业生产实践中的种苗繁育和防止种子的PHS, 而种子萌发是一个复杂的生物学过程, 涉及将环境信号和物种的遗传信息整合到包括萌发表型及其生理过程、转录和转录后调控以及表观遗传调控的机制中. 到目前为止, 这些过程及其调控机制尚未完全理解, 需要更多的研究来充分阐明种子萌发及其调控的机制[13].

在拟南芥、油菜(*Brassica napus*)、玉米、苜蓿(*Medicago sativa*)等种子萌发之前, 胚轴细胞的伸长是显著的, 但未观察到明显的细胞分裂[2]. 研究发现, 拟南芥种子的萌发不是由于胚根本身的延长引起的, 而是由于下胚轴以及下胚轴和胚根之间的过渡区的少量细胞伸长引起的[263]. 下胚轴细胞伸长或者下胚轴和胚根之间的过渡区的少量细胞伸长的机制及其与萌发完成的关系尚不清楚.

可变剪接调控是由转录和剪接之间的功能耦联指导的[154], 它们之间的潜在分子机制以及与种子萌发和休眠的关系是不清楚的. 因此, 探索种子中可变剪接调控和表观遗传修饰之间的联系将具有重要的意义, 这可以增加我们在特定条件下对复杂调控机制的理解, 指导我们制定更准确的策略, 选择在亚适(suboptimum)和真实环境条件下表现更好的重要作物基因型.

确定一些关键基因在不同的环境条件下可能负责萌发的机制将有助于深入了解作物种子的其他重要经济参数, 如萌发率和种子活力. 因此, 深入了解与种子萌发活力有关的参数, 如生化标记、特定蛋白图谱和DNA/RNA水平的变化, 将有助于确定不同因素对种子萌发特性的影响.

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Research progress in regulation of seed germination

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Seed germination is the initiation of plant life cycle. The dry seeds quickly recover their metabolic activities from a quiescent state during germination, which causes embryos to break through the surrounding structures during the completion of seed germination. Seed germination is a complex multiple-step process. It is regulated by many endogenous and exogenous factors, and has an important biological and economic significance in species survival, reproduction, and crop production. The molecular regulatory mechanism of seed germination, however, is now not fully understood. In recent years, many significant achievements have been made in the studies of seed germination and its regulation. In this article, the research progress in the regulation of seed germination was reviewed, mainly including physiological regulations, the roles of phytohormones (especially abscisic acid and gibberellins), controls of gene transcription and post-transcription, regulations of specific regulators, as well as epigenetic regulation. In addition, we also propose some scientific issues that need to be further investigated in this field, which will help to deeply understand the molecular mechanism of seed germination, increase seed germination vigor, and prevent pre-harvest sprouting.

epigenetics, physiology, phytohormone, regulation, seed germination, transcription and post-transcription

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