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·专题论坛·

活性氧在植物种子休眠释放和萌发中的作用研究进展

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摘要 活性氧(reactive oxygen species, ROS)在植物种子休眠释放和萌发中发挥重要调控作用。低水平的ROS促进种子萌发, 而过高水平的ROS则会降低种子活力和萌发率。穗发芽(pre-harvest sprouting, PHS)是作物的一种重要性状, 严重影响作物产量和品质。穗发芽与种子休眠紧密相关, 为同一性状的两种极端表现。因此, 研究ROS在种子休眠和萌发中的双重作用可为揭示穗发芽的内在分子机制开辟新视角。该文总结了种子萌发时ROS的产生部位和途径, 重点介绍了ROS与生物大分子、植物激素和其它小分子互作参与植物种子萌发以及萌发过程中ROS参与的信号通路, 以期为ROS在作物穗发芽过程中的作用机制研究提供理论依据。

关键词 活性氧, 植物激素, 萌发, 休眠, 信号转导

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在农作物驯化过程中, 由于人类更多考虑高产、优质、抗病虫和耐逆境等有利性状, 以确保在播种时种子具有较好的萌发特性, 常常忽视对种子适度休眠性的保留, 从而导致许多禾本科作物常发生穗发芽(pre-harvest sprouting, PHS)现象。尤其是近年来, 由于全球气候变暖, 作物成熟后期的高温阴雨天气常导致种子在穗上萌发, 即PHS。PHS作为农业生产中的一种不良性状, 严重影响水稻(*Oryza sativa*)等农作物的种子活力、谷物质量和产量。在中国, PHS的发生使长江流域和西南地区的稻谷产量减少10%–50% (Tao et al., 2007)。近年来, 在寒凉地区, 如中国黑龙江寒带水稻生产区, PHS也呈现上升势头(董慧雪和王际睿, 2024)。据测算, 在全球范围内, 每年因PHS造成了约1×10^9美元的经济损失(Tai et al., 2021)。可见, PHS已成为制约全球农业产业发展的重要问题。挖掘耐穗发芽的优异基因资源, 明确穗发芽的遗传特性和调控机理, 进而培育休眠度适宜、耐穗发芽的新种质是提高作物穗发芽抗性的最有效途径(唐思敏等, 2024)。

种子休眠和萌发是植物适应外界环境并确保其生存与繁衍的重要特性, 穗发芽与种子休眠紧密相关(Matilla, 2024)。前人已从穗发芽的生理基础、数量性状位点(quantitative trait locus, QTL)、关键功能基因以及防控穗发芽措施等方面开展了大量研究。已有研究表明, 穗发芽和种子休眠性是微效多基因控制的复杂数量性状。利用不同的遗传群体, 已定位到200多个与水稻种子休眠性或穗发芽抗性相关的QTL, 这些QTL广泛分布于水稻的12条染色体上, 且在第3、5、6、7和11号染色体较为集中(林鑫焱等, 2024)。这些位点的基因主要受内源激素、环境和复杂遗传网络等多种因素调控。其中, 脱落酸(abscisic acid, ABA)和赤霉素(gibberellic acid, GA)是水稻休眠释放和萌发调控网络中最重要的2种激素; 大多数相关基因均通过直接或间接调控ABA和GA的合成或信号转导途

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径来介导种子的休眠释放或萌发, 如*OsDOG1* (*delay of germination 1*)、*Sdr4* (*seed dormancy 4*)、*PHS8* (*pre-harvesting sprouting 8*)、*OsMFT2* (*mother of FT and TFL2*)、*SD6* (*seed dormancy 6*)、*OsVP1*、*Rc* (*red pericarp*)、*OsC1*以及bZIP转录因子(卢心明和牛百晓, 2024)。

活性氧(reactive oxygen species, ROS)是一类高活性的含氧分子, 包括超氧阴离子($\cdot O_2^-$)、过氧化氢(H_2O_2)、羟基自由基($\cdot OH$)和单线态氧($^1O_2$)等(Nathan and Ding, 2010)。除了已知的毒性效应外, 它们还是植物生长发育和应激反应的重要信号分子(Kumar et al., 2015)。在种子生物学中, 低水平的ROS作为信号分子可促进生理休眠释放, 并触发种子萌发(Kumar et al., 2015; Considine and Foyer, 2021); 而高水平的ROS常通过影响脂质过氧化、膜通透性、蛋白质缺陷、抗氧化系统、线粒体降解以及DNA和RNA损伤而导致种子劣化(Kurek et al., 2019; Wang et al., 2024)。近年来, 也发现ROS在作物穗发芽调控中发挥关键作用。例如, 已有证据表明, 穗发芽基因*PHS9*在水稻种子胚胎发育晚期特异性表达, 该基因编码一类谷氧还蛋白, 可与ABA受体(regulatory component of ABA receptors, RCAR)结合, 整合ROS信号和ABA信号, 从而调控水稻穗发芽(Xu et al., 2019), 但其精细调控机制尚不清楚。目前, 以作物为研究材料, 解析ROS参与种子休眠释放和萌发的报道比较有限。总结ROS在植物种子休眠释放和萌发中的双重作用, 可为研究和揭示作物穗发芽的分子机制提供新视角。本文总结了调控植物种子休眠释放和萌发过程中ROS的产生、与激素和关键分子相互作用以及信号转导等调控机制, 以丰富对该领域的认识。

1 种子萌发过程中ROS的产生

1.1 组织分布

种子中ROS积累的部位与种子内含水量密切相关(Bailly, 2019)。由于ROS检测技术的限制, 干种子中ROS代谢和产生的相关研究很少(Bailly, 2004)。拟南芥(*Arabidopsis thaliana*)种子的定量相位断层扫描揭示, 在干燥的种子中, 存在于细胞间隙的单线态氧可还原产生各种形式的ROS (Cloetens et al., 2006), 但ROS对于种子休眠释放和萌发的作用尚缺乏研究。种子吸胀后, 细胞伸长时产生的ROS首先出现在细胞质, 其次是细胞核, 最后是细胞壁(Leymarie et al., 2012)。在细胞质中, ROS调节氧化还原稳态, 触发蛋白质氧化。mRNA合成是种子萌发的第1个明显标志(Bahin et al., 2011), 细胞核中的抗氧化系统通过与负责氧化还原状态的转录因子直接或间接相互作用实现ROS水平的调节。最后, 位于细胞壁的NADPH氧化酶(NADPH oxidase, NOXs)有助于细胞间ROS含量的增加, 从而诱导种子萌发(Chandrakuntal et al., 2010)。在休眠表型中, 适量的ROS从细胞质扩散到细胞核和细胞壁, ROS含量较高且分散(Bailly et al., 2008), 具有时空积累的特征(Leymarie et al., 2012)。利用细胞成像技术, 可清晰展示在拟南芥种子萌发中沿胚轴产生ROS的动态过程: 在萌发早期(6小时), ROS主要位于胚根的分生组织区域; 随着吸胀作用的持续进行, 其逐渐占据下胚轴伸长区, 成为积累ROS的最大区域。此动态过程显示种子萌发过程中ROS的产生有“发育波”, 提示特定的细胞区域即分生组织区域可以感知环境信号, 并启动ROS信号沿胚胎轴传播, 最终导致下胚轴区域的细胞伸长(Stamm et al., 2017)。研究表明, 植物种子萌发细胞中ROS的产生可能与ABA和GA代谢及其信号转导过程中的时空扩增有关(Bailly, 2019)。与休眠种子中ROS不均匀分布不同, 在非休眠种子中, $\cdot O_2^-$和H_2O_2均匀分布在胚根内, 不呈现时空变化, 保持适当的氧化还原稳态, 从而有利于种子萌发(Seo et al., 2006)。

1.2 合成途径

植物种子中ROS的合成途径与种子的含水量密切相关。干种子含水量较低(8%–10%), ROS通过非酶促反应产生(主要通过脂质自氧化途径) (Bewley et al., 2013)。随着种子含水量增加, 种子内的脂质氧化减弱(Singh et al., 2014)。当种子含水量从8%–10%增加至50%以上时, ROS的产生途径从非酶系统转向酶促抗氧化酶系统(Bailly, 2019)。种子内的线粒体是ROS的主要来源(Bailly, 2004)。在线粒体基质中, 电子传递过程中消耗的氧通过呼吸电子传递链(respiratory electron transfer chain, RETC)还原为超氧化物, 超氧化物再通过Mn超氧化物歧化酶(Mn-superoxide dismutase, Mn-SOD)或Cu/Zn超氧化物歧化酶

(Cu/Zn-superoxide dismutase, Cu/Zn-SOD)转化为H_2O_2(Møller et al., 2020)。叶绿体是光合作用细胞中ROS的另一个重要来源，但其一般不涉及种子萌发(Considine and Foyer, 2021)。NOXs作为细胞质ROS产生的主要催化酶，介导电子从细胞质NADPH通过黄素腺嘌呤二核苷酸(flavin adenosine dinucleotide, FAD)穿过膜，再通过血红素传递到氧气，在外质体中产生超氧化物，超氧化物则迅速转化为H_2O_2(Krumova and Cosa, 2016)；在外泌体中，亚精胺和精胺催化合成过程中，H_2O_2有不同的产生方式，即可通过多胺氧化酶(polyamine oxidase, PAO)产生H_2O_2(Moschou et al., 2008)，也可通过含铜胺氧化酶(amine oxidase copper-containing, AOC)脱胺产生H_2O_2(Kärkönen and Kuchitsu, 2015)。另外，草酸氧化酶(oxalate oxidases, OxO)催化草酸氧化生成CO_2和H_2O_2 (Hu et al., 2003)。过氧化物酶(peroxidase, POD)和醌还原酶(quinone reductase, QR)也会产生ROS (Liszkay et al., 2003; Schopfer et al., 2008; 陈思羽等, 2016)。这些ROS可能在细胞伸长之前的细胞壁削弱中起关键作用。另外，外质体系统中分泌的抗坏血酸和III类含血红素过氧化物酶(POD III)，可由·O_2^-和H_2O_2转化成·OH (Schopfer, 2001; Miura, 2012)。有趣的是，在所有线粒体、叶绿体和细胞质中，H_2O_2也可以通过金属基(Fe^{2+}、Cu^{+}或Mn^{2+}等)的Haber Weiss或Fenton反应产生·OH (Pospíšil, 2009)。过氧化氢反应基因*HRG1*和*HRG2* (*H_2O_2 Response Gene 1 and 2*)在H_2O_2水平升高之前保持较低的蛋白含量，通过调节该基因的表达感知和响应H_2O_2，并微调H_2O_2水平，有助于保护根尖分生组织的微环境稳定性，支持分生组织细胞的正常活动，包括细胞分裂和伸长，从而促进种子萌发(Gong et al., 2021; 宋松泉等, 2024) (图1)。然而，以上多是在拟

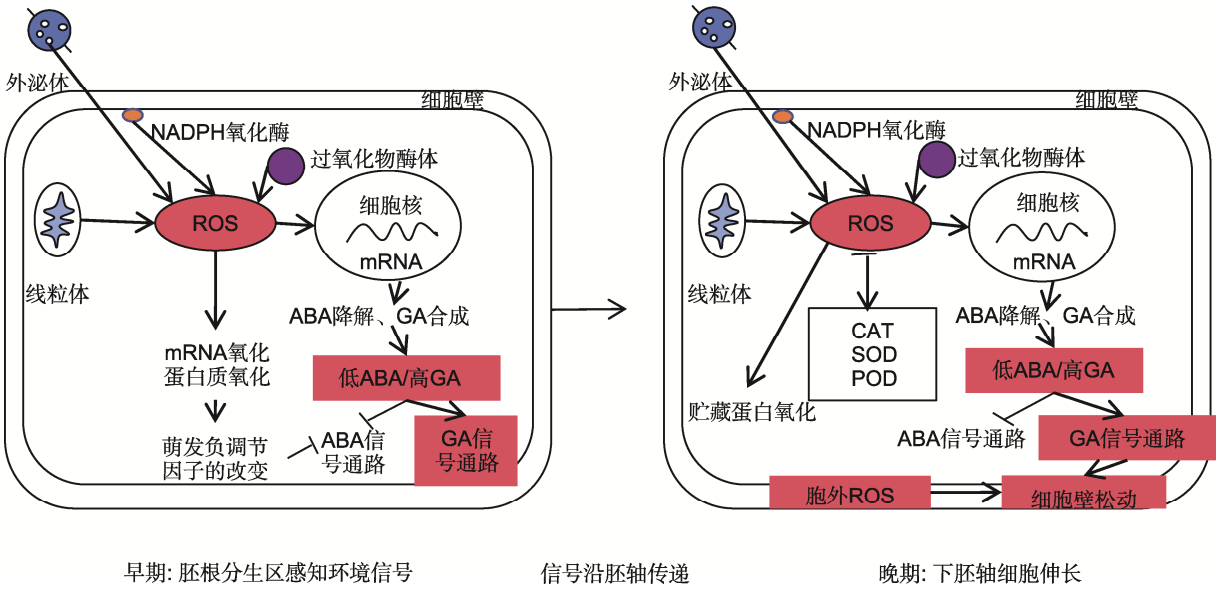


图1 活性氧(ROS)在植物器官和细胞水平上的产生模型

在器官水平上，吸胀作用刚开始时，胚根的分生区感知环境萌发信号，并沿胚轴向下胚轴区域传递，最终使下胚轴区域的细胞伸长。在细胞水平上，线粒体、NADPH氧化酶、外泌体和过氧化物酶体都可以通过各自的方式产生ROS，这些ROS首先定位在细胞质，然后是细胞核，最后是细胞壁，通过激活赤霉素(GA)信号以及抑制脱落酸(ABA)信号使植物种子萌发。CAT: 过氧化氢酶; SOD: 超氧化物歧化酶; POD: 过氧化物酶。箭头和钝线分别表示正向和负向作用。

Figure 1 Models of reactive oxygen species (ROS) production at the organ and cellular levels in plants

At the organ level, at the beginning of imbibition, the meristem of the radicle senses the environmental germination signal, and the signal is transmitted along the plumular axis down to the hypocotyl region, then finally causes the cell elongation in the hypocotyl. At the cellular level, mitochondria, NADPH oxidase, exosomes, and peroxisomes all produce ROS in their own ways, localized first in the cytoplasm, then in the nucleus, and finally in the cell wall, activating gibberellic acid (GA) signaling and inhibiting abscisic acid (ABA) signaling for plant seed germination. CAT: Catalase; SOD: Superoxide dismutase; POD: Peroxidase. The arrows and bars represent positive and negative effects, respectively.

南芥等模式植物中的研究结果，且目前已报道的大多数成果是在萌发后期(胚根突出的始期)取样获得。已有研究显示，许多外泌体分泌的氧化酶在细胞壁削弱时可能即发挥作用，但相关的直接证据并不多，尤其是对作物种子萌发过程中非酶促反应产生的ROS仍然知之甚少(Bailly, 2019)。今后，了解作物种子萌发过程中每个组织以及不同细胞器，如线粒体、过氧化物酶体、叶绿体、细胞质、外质体和外泌体对ROS生成的贡献，对于准确阐明ROS参与作物种子萌发的功能至关重要。

2 种子休眠释放与萌发中ROS的生物学功能

植物种子休眠释放和萌发中ROS的生物学功能已有较多报道(Bailly et al., 2008)。例如，在种子萌发过程中广泛观察到H_2O_2、·OH和$\cdot O_2^-$的积累(Kumar et al., 2015)；水稻PAO氧化多胺(polyamine, PAs)并释放H_2O_2，参与胚根鞘限制的种子发芽(Chen et al., 2016)；NOXs产生的ROS参与水稻种子萌发过程中自由基的积累和根伸长(Li et al., 2017)。ROS在种子萌发不同阶段的作用可能不同，包括与细胞质信号通路的相互作用(萌发早期)、细胞核内基因表达的氧化调节(萌发中期)和细胞壁削弱(萌发后期) (Bailly, 2019)。干燥种子中ROS的生成有助于其在储藏过程中释放休眠，在该过程中可能由于生物分子的直接氧化，即ROS过量引起种子内脂肪等大分子的氧化而导致种子老化或劣变(这一点本文不深入涉及)。ROS参与生物分子的直接氧化通常发生在萌发早期，此时ROS含量极低，而在种子吸胀过程中，种子萌发则通过控制ROS的产生水平，间接参与外界环境感知以及调控发芽相关的信号转导，这通常发生在萌发中后期以及未成熟种子后熟期的生物学过程中(Li et al., 2017)。胚乳弱化是种子萌发的先决条件，可为种子萌发提供重要的物质和能量，其受多种内外因素驱动，胚乳的降解和响应外部环境信号均与ROS的产生直接相关(Zhang et al., 2019)。在种子萌发过程中，H_2O_2执行信号功能，可穿越生物膜，在种子内部适量累积，并使种皮松弛，利于种子萌发(Hajihashemi et al., 2020)。在生菜(*Lactuca sativa*)种子萌发过程中，外源ROS和ROS产生诱导剂均能增加胚乳帽的破裂比例，有利于种子萌发。但若过量累积H_2O_2，则将破坏细胞内部的细胞器，产生细胞毒害。此时，种子细胞内部的氧化防御机制被激活，清除过量的H_2O_2，维持种子内ROS的稳态(Zhang et al., 2014b)。种子中ROS维持在特定范围内的现象，被称为“氧化窗口”，即允许种子内的细胞事件按顺序发生，从而诱导种子发芽，较低和较高水平的ROS均会抑制种子萌发(Anand et al., 2019) (图2)。

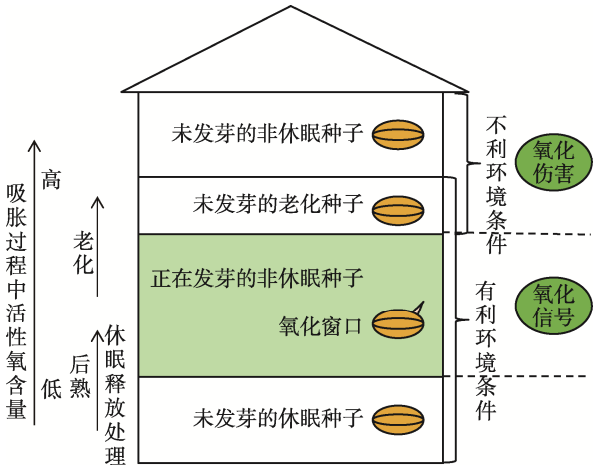


图2 活性氧(ROS)的氧化窗口模型

种子中的ROS只有维持在一定的范围，即在氧化窗口内时，种子才能发芽。在这个窗口之下，种子吸胀过程中ROS的含量过低，导致无法发芽。在氧化窗口内，由于ROS的信号作用，休眠释放导致种子吸胀过程中ROS的含量增加，从而使种子发芽。而在这个窗口之上，由于种子在吸胀过程中老化或被放置在不利的环境条件下，导致ROS含量过高，产生毒害作用，从而阻止或延迟发芽。

Figure 2 The oxidative window model in reactive oxygen species (ROS)

A seed can germinate only when the level of ROS in the seed is maintained within a certain range, i.e. within the oxidative window. Below the lower limit of the oxidative window, i.e. in dormant seeds, the low ROS content during imbibition leads to seed germination failure. Dormancy release leads to an increase in ROS content during imbibition, thereby enabling seed germination. However, above the upper limit of the oxidative window, due to seed aging or being placed in unfavorable environmental conditions during imbibition, high ROS content occurs, at which point ROS becomes toxic, thereby inhibiting or delaying germination.

3 种子休眠释放和萌发中ROS的作用机制

3.1 与生物大分子直接互作

植物种子中ROS调控的休眠释放可能涉及mRNA氧化(Bazin et al., 2011)、蛋白质羰基化(Oracz et al., 2009)和氧化稳态(Bailly et al., 2008) (图3)。

在种子后熟过程中，种子中的mRNA被ROS氧化，ROS作用于不同位置的核酸从而影响其稳定性。其中，编码休眠基因的核酸常在鸟嘌呤的C-8位置被氧化，产生8-羟基鸟苷(8-hydroxyguanosine)，氧化的核酸导致核糖体功能受损，核酸降解不通过衰变途径，经过翻译可能形成无功能蛋白，这些蛋白随后被降解以减轻休眠(Bazin et al., 2011)。此外，向日葵(*Helianthus annuus*)和小麦(*Triticum aestivum*)种子中储存的mRNA会经历氧化，并编码α-淀粉酶和胰蛋白酶等，促进休眠释放和种子萌发(Katsuya-Gaviria et al., 2020)。通过种子蛋白质组和转录组实验，观察到向日葵和小麦种子成熟后，其中储存的24种mRNA，包括丝裂原活化蛋白激酶磷酸酶(mitogen-activated protein kinase phosphatase, MAPK)和苯基氨裂解酶(phenyl ammonia lyase, PAL)等的mRNA明显被ROS氧化，从而引发信号转导途径，促进种子休眠释放和萌发或者穗发芽(Bazin et al., 2011; Bykova et al., 2011)。

种子中贮藏蛋白是高度敏感的易被氧化部分，蛋白质氧化修饰作为有效的休眠缓解系统和ROS清除系统，能保护其细胞成分免受ROS引起的氧化损伤，这也是种子休眠释放的先决条件。种子中ROS的积累使贮藏蛋白发生不同类型的氧化修饰，从而缓解休眠。例如，精氨酸(Arginase, Arg)、苏氨酸(Threonine, Thr)、赖氨酸(Lysine, Lys)和脯氨酸(Proline, Pro)等氨基酸，易在一些蛋白质(如糖酵解酶、线粒体ATP合酶、醛糖还原酶、蛋氨酸合酶、翻译因子及分子伴侣等)侧链上插入羰基，这些羰基化的蛋白质不能逆转且不可修复，积累后会产生毒性，进一步通过细胞质中20S蛋白体复合物降解，从而释放休眠并促进种子萌发(Nyström, 2005)。另外，种子中ROS的氧化并不随机，而是定向作用于靶蛋白，主要包括糖酵解酶、线粒体ATP合酶、醛缩酶、蛋氨酸合酶、翻译因子和分子伴侣等贮藏蛋白，从而帮助和保护种子萌发中的其它细胞免受ROS毒害(El-Maarouf-Bouteau et al., 2013)。糖酵解酶的氧化可能会导致磷酸戊糖途径(pentose phosphate pathway, PPP)的活化，以NADPH形式为种子萌发提供还原力，从而有利于种子的休眠释放(Barba-Espín et al., 2011)。

在种子萌发过程中，ROS也可直接与细胞壁多糖相互作用，促进胚根中的细胞伸长(Bailly, 2019)。例如，·OH通过破坏细胞壁上多糖的氢键而影响胚乳弱化，促进胚根突出(Müller et al., 2009)。简言之，植物种子中适宜的ROS水平可通过与生物大分子直接相互作用释放种子休眠，促进种子萌发。需要指出的是，上述多是采用拟南芥等模式植物进行研究得到的结果，而涉及大多数作物种子诱导休眠释放或种子发芽过程中ROS水平的阈值，尚不清楚。

3.2 间接作用

3.2.1 与植物激素互作

3.2.1.1 与GA和ABA互作

ABA和GA是植物种子休眠释放和萌发调控网络中最重要的2种激素(林鑫焱等, 2024) (图3)。ROS促进GA合成和ABA降解，进而促进种子休眠释放和萌发。例如，H_2O_2的积累通过影响ABA催化酶导致ABA降解，促进种子萌发(Ye et al., 2012)。Müller等(2006)发现，H_2O_2可消除ABA对胚乳弱化的抑制作用。H_2O_2通过提高GA生物合成基因(*GA3ox1*)和ABA分解代谢ABA8-羟化酶基因(*ABA-H*)的表达而降低ABA/GA_3比值，从而提高番茄(*Solanum lycopersicum*)种子的萌发能力(Anand et al., 2019)。在大麦(*Hordeum vulgare*)种子萌发过程中，H_2O_2可以通过激活GA_3的生物合成解除种子休眠，进而促进发芽(Bahin et al., 2011)，也可以通过影响ABA分解代谢酶的活性来调节种子胚胎中的ABA含量，从而诱导种子萌发(Ishibashi et al., 2017)。Bahin等(2011)认为，H_2O_2通过激活GA信号而不是影响ABA代谢来中断大麦种子的休眠。外源H_2O_2可增强大麦中*KAO1*和*HvGA3ox1*的表达，从而促进GA合成和种子萌发(Kai et al., 2016)。同样，H_2O_2可增强GA诱导的膨胀素基因*HvExpA11*和GA生物合成基因*HvGA20ox1*的表达，抑制GA分解代谢基因*HvGA2ox3*的表达，促进GA在大麦种子中积累，从而促进种子萌发(Bahin et al., 2011)。在拟南芥中，外源H_2O_2处理可以激活GA合成相关基因

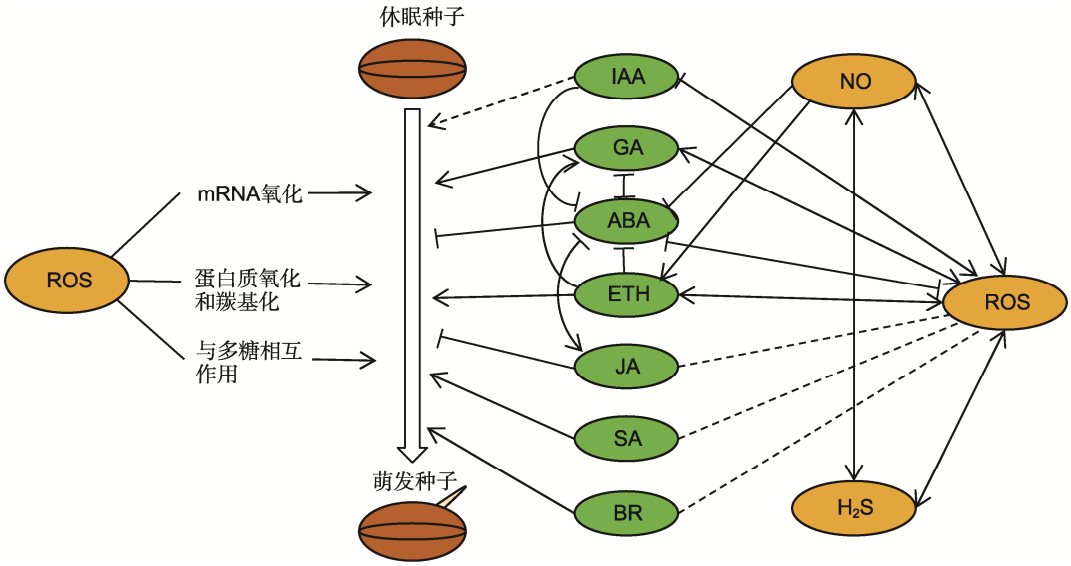


图3 活性氧(ROS)与生物大分子、植物激素和其它小分子在调节种子休眠和萌发中的互作

在种子休眠向萌发的转变过程中, ROS通过mRNA氧化、蛋白质氧化和羰基化以及与多糖相互作用来促进休眠释放。此外, 其它小分子, 如NO和H_2S, 也通过与植物激素联合导致种子从休眠向萌发调节。ROS主要通过增高赤霉素(GA)和乙烯(ETH)水平, 同时降低吲哚乙酸(IAA)和脱落酸(ABA)水平, 调节植物激素的网络平衡。ROS与茉莉酸(JA)、水杨酸(SA)和油菜素甾醇(BR)在种子萌发过程中的作用机制还需进一步研究。箭头和钝线分别表示正向和负向作用, 虚线表示假定作用。

Figure 3 Interaction of reactive oxygen species (ROS) with biomacromolecules, plant hormones and other small molecules in regulation of seed dormancy and germination

In the transition from dormant seeds to germinating seeds, ROS promote dormancy release by promoting mRNA oxidation, protein oxidation and carbonylation, and interactions with polysaccharides. Additionally, other small molecules such as NO and H_2S also regulate seed transition from dormancy to germination through interactions with plant hormones. ROS mainly regulate the balance of plant hormone networks by increasing the levels of gibberellic acid (GA) and ethylene (ETH), while decreasing the levels of indole-3-acetic acid (IAA) and abscisic acid (ABA). The mechanisms of jasmonic acid (JA), salicylic acid (SA), and brassinosteroid (BR) interacting with ROS in the seed germination process still need further research. The arrows and bars represent positive and negative effects, respectively, while the dashed lines represent hypothetical effects.

*GA3ox*和*GA20ox*以及ABA代谢相关基因*CYP707A*的表达, 从而增强GA合成和ABA分解, 促进种子萌发(Liu and Stone, 2010)。在水稻种子萌发过程中也观察到ROS介导的ABA与GA之间的拮抗作用(Ye and Zhang, 2012)。

种子内GA和ABA信号转导途径也参与调控ROS的稳态, 最终参与种子萌发。例如, 研究表明GA诱导普通大麦种子糊粉层产生H_2O_2, 而ABA抑制其产生(Ishibashi et al., 2013); 外源GA处理可诱导野生燕麦(*Avena sativa*) (Cembrowska-Lech et al., 2015)和白菜(*Brassica rapa* var. *glabra*) (Chen et al., 2021)产生ROS, 并促进种子萌发; 在种子吸胀早期, GA_3处理使野生燕麦中颖果、胚和糊粉层的H_2O_2和$\cdot O_2^-$含量增加(Cembrowska-Lech et al., 2015); 同样, 外源GA_3和GA_{4+7}处理也能有效促进油菜(*B. campestris*)种子萌发过程中内源ROS的产生(Chen et al., 2021)。然而, 在向日葵休眠和非休眠种子中, ABA处理均抑制H_2O_2和$\cdot O_2^-$的产生(El-Maarouf-Bouteau et al., 2015)。虽然植物中ROS参与休眠释放和种子萌发已有许多报道, 但是ROS、ABA和GA三者结合调控植物种子休眠释放和萌发的精细分子机制尚不清楚, 尤其在作物中的报道较少。

3.2.1.2 与其它植物激素互作

其它植物激素也可以与ROS相互作用进而参与种子休眠释放与萌发(图3)。在大豆(*Glycine max*)种子中,

外源H_2O_2处理可以促进吸胀后胚胎轴产生ROS，从而诱导内源乙烯(ethylene, ETH)的合成，促进根尖细胞伸长，诱导种子萌发(Ishibashi et al., 2013)。用ROS产生诱导剂甲基紫精处理，向日葵休眠种子胚胎中ETH受体基因*ETR2*和ETH响应基因*ERF1*的表达量增加，从而促进种子萌发(Oracz et al., 2009)。外源ETH处理向日葵种子也可通过内源ETH激活NOXs，促进休眠胚胎轴的ROS积累，从而促进休眠释放(El-Maarouf-Bouteau et al., 2015)。此外，乙烯还通过调节ABA/GA激素平衡以及控制参与ABA和GA生物合成及信号转导基因的表达，在种子休眠缓解和萌发中发挥重要作用(Corbineau, 2024)。例如，ROS积累和乙烯信号转导促进钻果大蒜芥(*Sisymbrium officinale*)储存种子中CYP707A的活性，导致ABA降解，反过来促进GA的生物合成，从而释放休眠，促进种子萌发(Bazin et al., 2011)。生化分析显示，H_2O_2的增加和过氧化物酶(peroxidase, POD)的激活促进了生长素吲哚乙酸(indole-3-acetic acid, IAA)的氧化降解(Gazarian et al., 1998)。对拟南芥、大豆和番茄等的研究均显示，内外源IAA通过调节NOXs、CAT和SOD等产生超氧化物，同时降低过氧化氢酶(catalase, CAT)和抗坏血酸氧化酶(ascorbic acid oxidase, ASO)等抗氧化酶基因的表达和活性，调节H_2O_2代谢和下胚轴伸长(Morre et al., 1995; Tyburski et al., 2009; Iglesias et al., 2010; Krishnamurthy and Rathinasabapathi, 2013)，提示ROS与IAA互作可能通过影响ROS稳态和IAA水平来调控种子休眠释放或萌发(Li et al., 2022)。

茉莉酸(jasmonic acid, JA)主要通过调节ABA代谢或信号通路控制种子休眠和萌发。例如，JA通过抑制小麦ABA生物合成的9-顺式-环氧类胡萝卜素双加氧酶基因*TaNCED1*和*TaNCED2*的表达促进休眠释放(Xu et al., 2016)。ABA促进JA的生物合成，协同抑制水稻种子萌发(Wang et al., 2020)。然而，ROS与JA的互作参与作物种子休眠释放和萌发的精细协同调控机制还需深入研究。水杨酸(salicylic acid, SA)通过调节抗氧化酶活性促进高盐条件下拟南芥的种子萌发(Lee et al., 2010)。在向日葵种子从休眠到发芽的过程中观察到SA和H_2O_2的时空变化(Vigliocco et al., 2020)。外源油菜素内酯(brassinolide, BRs)也可促进胁迫条件下芥菜(*B. juncea*)种子的萌发(Soares et al., 2020)。另外，ROS与GA和ABA互作，调节Ca^{2+}的内流和外排，增加胞质中的Ca^{2+}含量，促进GA诱导的钙调素信号，激活α-淀粉酶，从而使胚根伸长，促进种子萌发(Bethke and Jones, 1994)。淹水条件下，可通过*OsCBL10*触发Ca^{2+}信号转导，其介导OsMYBS1磷酸化以增加α-Amy的表达，使淀粉降解，促进淹水条件下水稻种子发芽(Ye et al., 2018)。可见，ROS参与植物种子休眠释放和萌发涉及的激素分子众多，其内在的分子机制也非常复杂，不同激素分子之间以及与ROS之间的互作是否有交集鲜有报道，尚未见涉及作物种子休眠释放与萌发的相关研究。

3.2.2 与其它小分子互作

在种子休眠释放和萌发过程中，ROS亦可与种子内的小分子互作共同发挥作用，这些小分子包括活性氮(reactive nitrogen species, RNS)、氰化氢(hydrogen cyanide, HCN)和硫化氢(hydrogen sulfide, H_2S)等(图3)。ROS与RNS、HCN以及H_2S互作，并以精准的含量对植物种子萌发和发育调控发挥至关重要的作用(Krasuska and Gniazdowska, 2012; Wojtyla et al., 2016)。

3.2.2.1 活性氮

一氧化氮(nitric oxide, NO)是RNS的重要分子之一。用NO供体硝普钠(sodium nitroprusside, SNP)处理种子，可减少或解除拟南芥、大麦和小麦的种子休眠，而使用NO清除剂2-(4-羧基苯基)-4,4,5,5-四甲基咪唑啉-1-氧-3氧化物(2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3 oxide, c-PTIO)则可有效维持种子休眠(Bethke et al., 2004; Jacobsen et al., 2013)。NO、ROS和植物激素在种子萌发过程中存在复杂的相互作用(Zhang et al., 2023)。在拟南芥吸胀种子中，NO诱导ABA快速减少，这与编码ABA8′-羟化酶的其中1个基因*CYP707A2*的转录水平增高有关。SNP处理不能提高*cyp707a2*突变体种子的发芽率，表明在拟南芥种子萌发过程中，*CYP707A2*基因在NO介导的ABA含量调控中发挥重要作用(Matakiadis et al., 2009)。在野生燕麦中，NO可通过降低ABA含量以及ABA/GA的比率促进种子休眠释放(Kępczyński et al., 2023)。NO和H_2O_2通过与

ABA相互作用, 在种子休眠释放和萌发中发挥平行作用, 即种子吸胀增高H_2O_2和NO水平, H_2O_2上调ABA分解代谢, 同时也促进GA的生物合成, 从而促进种子萌发(Liu et al., 2010; Arc et al., 2013b)。为此, Liu等(2010)提出了H_2O_2可以通过2种途径中断拟南芥种子休眠的模型: 第1种途径依赖于增强ABA分解代谢和GA生物合成, 该途径中NO不直接调节GA的生物合成, 而是作为临时的信号分子, 参与H_2O_2调节ABA分解代谢; 第2种途径则假设通过H_2O_2与NO互作, 负调节ABA对GA的生物合成, 参与种子萌发, 此途径中ROS和NO均起主要作用。然而, 关于以上2种途径的生化证据不多。

3.2.2.2 氰化氢

在苹果(*Malus pumila*)、拟南芥和向日葵种子萌发过程中, 观察到通过ROS分别与HCN和RNS的下游信号通路互作, 激活ETH生物合成, 以依赖或不依赖HCN的途径缓解种子休眠, 促进种子萌发(Oracz et al., 2009; Gniazdowska et al., 2010; Arc et al., 2013a)。Krasuska和Gniazdowska (2012)在“氧化窗口”模型(Bailly et al., 2008)的基础上, 结合RNS的研究进展, 进一步提出了“亚硝化”假说, 明确了RNS在种子萌发中的作用, 提示RNS和ROS含量受到ROS清除酶的严格调控, 参与种子萌发过程。

3.2.2.3 硫化氢

在种子萌发期, H_2S分子与H_2O_2和NO的互作也发挥信号转导作用(Hancock and Whiteman, 2016)。经H_2S处理的普通菜豆(*Phaseolus vulgaris*)、玉米(*Zea mays*)、小麦和豌豆(*Pisum sativum*)种子的萌发率提高, 且萌发周期缩短(Dooley et al., 2013)。还观察到在H_2S和H_2O_2联合处理的绿豆(*Vigna radiate*)种子萌发过程中, H_2O_2可能作为H_2S的下游信号分子, 通过动员贮藏蛋白促进绿豆种子萌发(Li and He, 2015)。Li等(2012)研究发现, 经H_2O_2浸泡的麻疯树(*Jatropha curcas*)种子发芽率提高, 同时L-半胱氨酸脱硫酶(L-cysteine desulfhydrase)活性增加, 从而诱导H_2S积累, 表明在种子萌发过程中H_2O_2与H_2S之间存在互作。氯化钠胁迫处理下的紫花苜蓿(*Medicago sativa*)种子, 经H_2S供体(氢硫化钠)和硝普钠处理, 均可通过防止氧化损伤而显著减弱种子萌发和幼苗生长抑制(Wang et al., 2012) (图3)。

4 种子休眠释放和萌发中的ROS信号通路

关于ROS作为信号分子参与植物生命过程的研究已有很多报道。种子中的ROS信号通路是参与种子休眠释放或萌发调节的前提, 然而, 迄今为止种子中的ROS信号通路尚不完全清楚。种子在水合(发芽)和干燥(成熟、脱落)状态下如何感知ROS是一个有趣的问题。目前的研究推测, 植物主要通过ROS清除与稳态调节其水平, 进而引导ROS进入信号转导系统, 从而诱发种子的不同生命过程(Jhanji et al., 2024)。种子中的ROS稳态不仅取决于ROS产生部位, 还受酶和非酶抗氧化机制的效率调节(Bailly, 2004)。抗氧化酶的适当激活也可能引导ROS进入信号转导过程。在萌发过程中, 种子吸收水分导致细胞质黏度和自由水的可用性较低, 由于不同ROS分子的反应性不同, 发挥的生物学功能也不同。例如, ·OH半衰期短, 不能进行长距离运输, 通常只能与周围的分子发生反应。而H_2O_2是半衰期最长的分子, 则可以在细胞内移动更长的距离, 发挥更大作用。在干燥状态下, 细胞质黏度很高, 缺乏可用的自由水, 可能会阻碍反应性较低的ROS形式(如H_2O_2)或迁移(Møller, 2001)。为此, 科学家提出了种子休眠和萌发过程中ROS流动性及其感知的不同假设。一种假设认为, 干燥的种子具有受限的水化区, 允许H_2O_2有限流动, 可以参与水化区域的细胞信号转导; 另一种假设则认为, H_2O_2可以在干燥阶段积累, 在种子吸胀后, ROS库可以发出诱导基因表达变化的信号, 从而调节种子萌发(El-Maarouf-Bouteau and Bailly, 2014)。种子萌发过程中转录组和蛋白质组水平的研究结果显示, ROS可能通过协调H_2O_2和ABA介导的信号转导途径, 并通过下游MAPK途径或活化色氨酸-天冬氨酸重复蛋白家族的成员C激酶1受体(receptor for activated C kinase 1, RACK1)途径来实现种子萌发(Barba-Espín et al., 2011; Zhang et al., 2014a)。脂肪酸环氧化酶CYP77A4是一种有效的H_2O_2传感器, 在种子萌发过程中, CYP77A4可催化不饱和脂肪酸的环氧化, 也可被H_2O_2激活介导亚磺酰化, 从而平衡脂质动员和ROS产生(Xiang et al., 2023)。

有研究表明, H_2O_2调控拟南芥种皮和胚乳水解酶的基因表达, 使胚从种子包膜的控制中解放出来, 促进种子萌发(Lariguet et al., 2013)。据此提出假设: 种子萌发过程中H_2O_2和ROS的产生通过对贮藏蛋白的氧化修饰促进储备动员, 从而可能被储存器官识别, 并将储备动员信号传递至快速生长轴的信号(Verma et al., 2015)。线粒体基质热休克蛋白GhHSP24.7通过调节细胞色素C/C1诱导ROS产生, 促进植物种子发芽(Ma et al., 2019)。植物特异性丝氨酸/苏氨酸激酶-蔗糖非发酵蛋白激酶2 (sucrose non-fermenting 1-related protein kinase 2, SnRK2)参与植物非生物胁迫响应和ABA依赖的植物发育(张金飞等, 2017)。研究者对拟南芥气孔保卫细胞中ABA、NO和ROS的相互作用进行研究, 发现NO通过*S*-亚硝基化SnRK2蛋白(SnRK2.2、SnRK2.3和SnRK2.6/OST1)负调控ABA信号, 参与气孔关闭、抑制种子萌发和幼苗生长。据此提出, SnRK2s蛋白的*S*-亚硝基化是调节拟南芥种子萌发过程中ABA与NO之间信号互作的关键组分, 内源和外源NO均可通过*S*-亚硝基化抑制SnRK2.2和SnRK2.3的激酶活性, 从而阻断ABA信号转导(Wang et al., 2015; 宋凝曦等, 2020)。*OsUBC2*是一种E2泛素结合酶, 可促进OsSnRK的降解, 增强粳稻的低温发芽活力(Zhang et al., 2024)。H_2O_2还可以抑制ABA信号转导途径关键元件ABI1 (abscisic acid-insensitive 1)和ABI2的磷酸酶活性, 从而抑制拟南芥种子萌发(Meinhard and Grill, 2001; Meinhard et al., 2002)。在拟南芥中的研究发现, ABI5直接与*CAT1*启动子结合激活其表达, 改变ROS稳态(Bi et al., 2017)。在盐胁迫下, ABI4直接与拟南芥种子萌发过程中ROS产生和清除的2个关键基因NADPH氧化酶基因(*NADPH oxidase gene*, *RbohD*)和维生素C缺陷2基因(*Vitamin C Defective 2*, *VTC2*)结合, 从而调控ROS代谢(Luo et al., 2021)。水稻中1个主效QTL *qSE3*编码K^+转运基因*OsHAK21*, 通过增加ABA生物合成和激活ABA信号响应, 降低盐胁迫下萌发种子的H_2O_2水平, 进而促进种子萌发和幼苗生长(He et al., 2019)。在拟南芥和豌豆的生长发育过程中, 已观察到一些ROS分子可以激活MAPK级联作用, 或抑制蛋白磷酸酶活性, 并激活Ca^{2+}通道或Ca^{2+}结合蛋白(Diaz-Vivancos et al., 2013)。在GA的生物合成过程中, ROS也可通过MAPK发挥级联作用, 但其参与种子萌发的精细机制尚需进一步研究(Kumar et al., 2015)。因此, 植物种子的氧化还原状态与其它信号通路之间的互作, 将是未来ROS分子参与植物种子休眠释放和萌发调节分子机制研究的重要方向(Matilla, 2024; 宋松泉等, 2024)。进一步加强作物种子萌发过程中ROS信号转导研究, 也将是未来改良作物休眠或穗发芽特性的新方向。

5 结论与展望

种子休眠和萌发是作物生产的重要过程。已有研究表明, 在细胞水平上, ROS主要在线粒体、细胞质、细胞核和细胞壁中产生; 在器官水平上, ROS从胚根的分生组织区域沿胚轴产生。ROS在干种子中通过非酶促反应产生, 种子吸水后则转变为通过酶促反应产生。低水平的ROS有助于种子休眠释放和萌发, 而高水平的ROS则会损伤种子的萌发能力。因此, 维持ROS的稳态对于植物种子休眠释放和萌发至关重要。虽然在植物种子休眠释放和萌发中, ROS与其它生物大分子、植物激素和其它小分子的互作已有较多研究(Li et al., 2022; Zhao et al., 2024), 但由于ROS检测技术的不稳定性, 尤其是在植物种子中的检测面临较大的困难和挑战, 相关论点多为假设, 直接的证据较少, 而且多是来自模式植物的研究结论, 对大宗作物的研究相对较少(Wang et al., 2015; Bailly, 2019)。随着极端天气的频繁发生, 寒凉地区水稻的穗发芽危害不断显现。因此, 解释作物休眠释放和种子萌发的机理, 有效调节作物休眠与种子萌发的平衡, 以减少因穗发芽而导致损失的生产需求也越来越强烈。穗发芽是影响作物产量的重要问题, 与种子休眠和萌发关系密切, 是一个由多基因控制的遗传特性, 而且与外界环境条件息息相关, 利用单个基因很难有效控制作物的休眠和萌发。通过多学科交叉, 深入研究作物种子休眠释放和萌发中共性的生命过程和调控机制, 将有助于丰富对休眠与萌发生命过程的认识。植物中ROS作为信号分子参与生命过程的研究已有诸多报道, 但种子休眠释放与萌发的作用机制研究较为分散, 获得的直接证据并不多, 尤其是对作物穗发芽发生中ROS的生理功能以及内在的分子调节机制的关注更少。

针对目前ROS在种子休眠释放和萌发中的作用

研究进展, 今后可在如下方面进一步开展工作。首先, 创新种子中ROS检测手段, 如细胞成像技术和特异荧光标记的开发, 将是精确研究ROS功能的前提; 第二, 通过多学科交叉, 进一步验证和区分ROS在种子休眠释放和萌发中的生物学功能, 将是精准调控和改良植物种子萌发特性的理论基础; 第三, ROS作为信号转导和细胞毒害的“双刃剑”, 精准定量其调控含量并解析其分子机制, 是有效利用ROS途径的重要研究内容, 将为植物的生命过程研究提供新思路; 第四, 种子中ROS如何感受外界环境因子, 并转化为体内信号, 也是植物信号转导机制研究的重要方向之一; 第五, 作为体内的重要信号分子, ROS如何与其它内源信号分子互作, 协同调节种子休眠和萌发, 是丰富植物信号转导途径理论的重要方面; 第六, 验证和研究ROS在作物穗发芽中的作用机制, 将为有效减少穗发芽提供新的技术途径, 通过改良作物穗发芽特性, 最终提高作物产量和质量。随着基因组学、转录组学、蛋白质组学、磷酸组学、表观遗传组学以及基因组编辑等新技术的快速发展, 相信将为解析ROS在作物种子休眠释放与萌发以及作物穗发芽中的作用机制提供新技术和新视角, 并丰富对种子生理的认识, 最终促进作物的安全高效生产。

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Research Progress of the Function of Reactive Oxygen Species in Plant Seed Dormancy Release and Germination

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Abstract Reactive oxygen species (ROS) play an important role in the regulation of seed dormancy release and germination. Low levels of ROS can promote seed germination, while excessively high levels of ROS can reduce seed vigor and germination rate. Pre-harvest sprouting (PHS) is an important agronomic trait that can cause severe decrease of crop yield and grain quality. Pre-harvest sprouting and seed dormancy are two manifestations of the same trait. Therefore, studying the dual role of ROS in seed dormancy and germination can reveal the molecular mechanisms underlying pre-harvest sprouting. For this purpose, this paper summarized the subcellular locations and metabolic pathways of ROS production during seed germination, focusing on the interaction of ROS with biomacromolecules, plant hormones and other small molecules and the signaling pathways of ROS functionalization. This review provides a theoretical basis for understanding the mechanisms of ROS in the process of crop pre-harvest sprouting.

Key words reactive oxygen species, plant hormones, germination, dormancy, signal transduction

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