



一氧化氮对豆科植物结瘤及固氮的影响机制

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摘要 豆科植物-根瘤菌共生过程受双方基因复杂且精细的调控, 能够产生特异的根瘤结构并可将大气中的惰性氮气(N₂)转化为可被植物直接利用的氨态氮。结瘤与固氮受多种因素影响, 其中, 一氧化氮(NO)作为一种自由基反应性气体信号分子, 可参与调节植物的许多生长发育过程, 如植物的呼吸、光形态建成、种子萌发、组织和器官发育、衰老以及响应各种生物及非生物胁迫。在豆科植物中, NO不仅影响寄主与菌共生关系的建立, 还参与调控根瘤菌对氮气的固定并提高植株氮素营养利用效率。该文主要从豆科植物及共生菌内NO的产生、降解及其对结瘤、共生固氮的影响和对环境胁迫的响应, 阐述了NO调控豆科植物共生体系中根瘤形成和共生固氮过程的作用机制, 展望了NO信号分子在豆科植物共生固氮体系中的研究前景。

关键词 一氧化氮(NO), 结瘤, 共生固氮, 血红蛋白(Hbs)

张卫勤, 邹杭, 张妮娜, 林雪媛, 陈娟 (2020). 一氧化氮对豆科植物结瘤及固氮的影响机制. 植物学报 55, 623–633.

氮(N)是植物生长发育所必需的大量营养元素之一。据统计, 每年全球粮食作物需施用 5.30×10^7 t氮肥, 而氮肥利用率只有50%–75%, 未完全利用的部分最终造成全球N循环失衡、地下水污染以及大气中一氧化二氮(nitrous oxide, N₂O)增加等一系列环境问题(Smil, 1999; Garg and Geetanjali, 2007)。根瘤菌侵染豆科植物形成根瘤, 将大气中的氮气(nitrogen, N₂)还原为氨气(ammonia, NH₃)供植物吸收利用(Hichri et al., 2016b)。据统计, 豆科植物根瘤菌共生体系的固氮量占生物固氮总量的60%以上(李欣欣等, 2016)。根瘤菌与豆科植物的共生固氮作用是目前效率最高的生物固氮体系(何恒斌和贾桂霞, 2013)。在农业环境中, 根瘤菌的存在为生物固定N₂提供了一个生态位, 有效提高了生态系统的N素利用效率。

豆科植物根瘤菌共生体系受多种信号分子和转录因子调控, 其中一氧化氮(nitric oxide, NO)信号分子在豆科植物微生物互作过程中发挥重要作用。共生体早期的转录组学分析表明, NO可调节细胞脱分化和器官发生(Ferrarini et al., 2008; Boscari et al., 2013), 并抑制植物防御反应(Gonzalez-Rizzo et al.,

2006), 在建立植物与菌共生关系中发挥重要调控作用(Hichri et al., 2016b)。而在成熟根瘤中, NO既可抑制植物体固氮(Shimoda et al., 2005; Kato et al., 2010; Cam et al., 2012), 又可诱发根瘤衰老(Horchani et al., 2011; Cam et al., 2012; Blanquet et al., 2015), 亦可作为能量代谢调节器维持低氧(oxygen, O₂)状态下的能量平衡(Kato et al., 2010; Hichri et al., 2016b)。尽管目前关于NO在植物体内的生理功能已进行了充分研究, 但国内对于其在豆科植物根瘤菌共生体系中的作用机制并未进行系统的阐述。本文综述了豆科植物根瘤菌共生体系中NO的产生和降解, 及其对根瘤形成与共生固氮影响的研究进展, 并对NO在共生体系中对非生物胁迫的响应及其与激素的相互作用进行了阐述。

1 豆科植物根瘤菌共生体系内NO的产生与降解

1.1 共生体系内NO的产生

植物和根瘤菌内NO的来源丰富, 主要通过硝酸还原

收稿日期: 2020-03-03; 接受日期: 2020-06-05

基金项目: 国家自然科学基金(No.31501822)

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酶(nitrate reductase, NR)和一氧化氮合酶(nitric oxide synthase, NOS)介导以及线粒体电子传递链(electron transport chain, ETC)的还原产生。在植物体内, NO既可由质外体中的亚硝酸根离子(NO_2^-)转化(Bethke et al., 2004), 又可通过以精氨酸(arginine)、多胺(polyamines)或羟胺(hydroxylamine)作为底物的氧化途径产生(Hichri et al., 2015)。在低氧环境下, 还可在还原途径中通过质膜结合亚硝酸盐, 然后通过NO还原酶(nitric oxide reductase, Nor)和黄嘌呤氧化还原酶(xanthine oxidoreductase, XOR)或利用ETC的作用将 NO_2^- 还原为NO (Gupta et al., 2011; Mur et al., 2013; Hichri et al., 2015)。而Horchani等(2011)发现, 植物及其共生菌中的NR和ETC均可产生NO, 其中NR在植物体内的主要功能是将硝酸盐转化为亚硝酸盐以同化 N_2 (Neill et al., 2008)。但早期的研究发现, 在NAD(P)H作为电子供体时, 大豆(*Glycine max*)中的NR可将亚硝酸盐转化为NO (Dean and Harper, 1988)。且NR抑制剂钨酸盐(tungstate, Tg)也能够抑制NO的产生, 表明NO的合成依赖于NR活性, 但并不是由其直接产生(Horchani et al., 2011)。在类菌体中, NO则主要产生于反硝化途径(Hichri et al., 2015), 该途径涉及NR、亚硝酸还原酶(nitrite reductase, NiR)、Nor和NOS (Horchani et al., 2011; Sánchez et al., 2011; Calvo-Begueria et al., 2018) (图1)。Berger等(2018)发现NOS也是植物NO的来源, 但目前关于共生菌内NO产生途径的机制研究仍较少。

Cueto等(1996)在白羽扇豆(*Lupinus albus*)的根和根瘤中鉴定到NO合酶类似酶(nitric oxide synthase-like enzymatic, NOS-like)。Baudouin等(2006)认为这种酶可能就是根瘤菌侵染细胞时产生NO的主要原因。此外, 有研究表明, NOS-like和多胺氧化酶(polyamine oxidase, PAOX)均可在正常条件下介导成熟根瘤中氧化性NO产生(Mur et al., 2013; Hichri et al., 2015)。上述结果表明, 植物和根瘤菌中产生NO的过程并非完全各自独立, 而是通过相关途径或转导系统相互联系。例如, NR、ETC和NOS是植物及其共生菌中NO产生的共同来源(图1)。

在根瘤菌侵染豆科植物的每个阶段(包括接种4小时后、根毛卷曲、侵染线的产生、根瘤原基的形成和根瘤成熟)都伴有NO的产生(Hichri et al., 2015),

即从豆科植物与根瘤菌的早期共生到根瘤成熟与衰老, NO的产生贯穿始终(Meilhoc et al., 2011)。尽管目前对于NO的产生已经进行了许多研究, 但由于气体分子本身的性质以及与其它体系反应的复杂性, 使人们对植物中NO产生机制的研究受限, 因此关于豆科植物与根瘤菌共生的各个阶段中NO产生的机理还有待深入探索。

1.2 共生体系内NO的降解

豆科植物根瘤中包含一种与其它蛋白性质和功能不同的血红蛋白(hemoglobins, Hbs), 能够清除机体产生或累积的NO (Gupta et al., 2011)。基于其序列同源性和对氧的亲合力, 可将植物体内的Hbs划分成3类: 非共生血红蛋白(non-symbiotic hemoglobins, ns-Hbs, 1类)、豆血红蛋白(leghemoglobins, Lbs, 2类)和截短血红蛋白(truncated hemoglobin, Tr-Hbs, 3类) (Gupta et al., 2011; Hill, 2012)。Lbs作为高等植物中发现的第1种豆血红蛋白, 在豆科植物中主要负责将根瘤中的 O_2 运输至根瘤菌(Trevaskis et al., 1997)。而Hbs对 O_2 和NO的亲合力非常强, 能够有效清除 O_2 和NO并将其转化为硝酸盐。有研究表明, Hbs能够维持植物细胞在缺氧状态下的氧化还原能力和能量状态(Igamberdiev and Hill, 2009)。除植物体蛋白外, 根瘤菌内也含有3类血红蛋白: 黄素血红蛋白(flavo-haemoglobins, fHbs/Hmp)、单域血红蛋白(single-domain haemoglobins, sd-Hbs)和截短血红蛋白(Sánchez et al., 2011)。Hmp是蒺藜苜蓿(*Medicago truncatula*)中主要的NO清除剂, 而NO可诱导hmp基因表达以影响自身生成量(Meilhoc et al., 2011; Cam et al., 2012) (图1)。另有研究表明, 在蒺藜苜蓿hmp突变的根瘤中, NO水平显著高于野生型, 且固氮效率降低和根瘤衰老提前(Cam et al., 2012; Hichri et al., 2016a)。因此, 植物中的Hbs与菌内的Hmp对于维持共生体NO水平至关重要。

共生体内NO的降解还受相关基因及其它蛋白调控。苜蓿中华根瘤菌(*Sinorhizobium meliloti*)中包含1个与短链脱氢酶相关且位于nrrR下游的nrrS基因家族, nrrS的表达依赖于转录调节器NnrR (De Bruijn et al., 2006)。当nrrS1突变时, 根瘤内NO的水平升高(Blanquet et al., 2015)。NnrS是一种haeme-Cu膜蛋白(Bartnikas et al., 2002), NnrS家族的2种蛋白

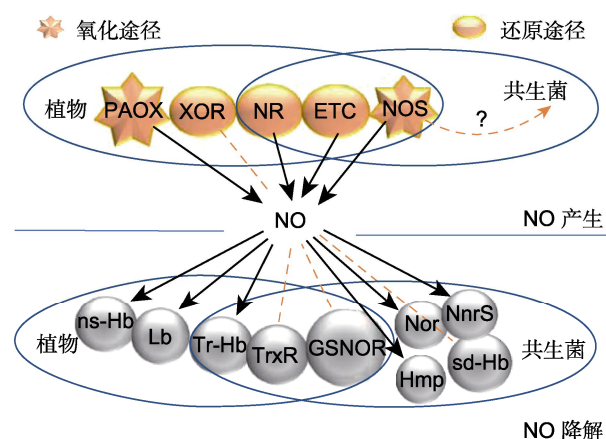


图1 共生体系中NO的产生与降解示意图(改编自Hichri et al., 2016a)

图中包含上下2部分, 分别对应植物和共生体NO的产生与降解。其中, 七角星图示代表氧化途径, 椭圆形图示代表还原途径。实线表示已有研究证实, 虚线表示还有待考证。ETC: 线粒体电子传递链; GSNOR: 亚硝基谷胱甘肽还原酶; Hmp: 黄素血红蛋白; Lb: 豆血红蛋白; NnrS: 含血红素和铜的膜蛋白; Nor: NO还原酶; NOS: NO合酶; ns-Hb: 非共生血红蛋白; NR: 硝酸还原酶; PAOX: 多胺氧化酶; sd-Hb: 单域血红蛋白; Tr-Hb: 截短血红蛋白; TrxR: 硫氧还蛋白还原酶; XOR: 黄嘌呤氧化还原酶

Figure 1 The schematic diagram of NO production and degradation in the symbiotic system (modified from Hichri et al., 2016a)

The figure contains the upper and lower parts, which correspond to the production and degradation of NO from plants and symbionts, respectively. The seven horns star diagrams refer to the oxidation pathway and the oval diagrams refer to the reduction pathway. The lines indicate that studies have been confirmed, and the dashed lines indicate that it is yet to be studied. ETC: Mitochondrial electron transport chain; GSNOR: S-nitrosoglutathione reductase; Hmp: Flavin hemoglobin; Lb: Leghemoglobin; NnrS: Haem- and copper-containing membrane protein; Nor: NO reductase; NOS: NO synthase; ns-Hb: Nonsymbiotic hemoglobin; NR: Nitrate reductase; PAOX: Polyamine oxidase; sd-Hb: Single domain hemoglobin; Tr-Hb: Truncated hemoglobin; TrxR: Thioredoxin reduction enzymes; XOR: Xanthine oxidoreductase

(NnrS1和NnrS2)和Nor能够直接或间接参与NO的降解并有效地维持共生固氮(Meilhoc et al., 2013; Blanquet et al., 2015)。

除根瘤菌内的血红蛋白以外, 还有其它细菌蛋白调控根瘤中的NO水平。例如, 硫氧还蛋白(thioredoxin reductase, TrxR)或亚硝基谷胱甘肽还原酶(S-nitrosoglutathione reductase, GSNOR)都可能间接参与NO的降解和信号传递过程(Lee et al., 2010)。而

细菌的NO降解蛋白不仅能保护其自身蛋白免受NO的负面影响, 还能使共生植物蛋白免受NO介导的失活(Blanquet et al., 2015; Hichri et al., 2016b)。综上, 鉴于NO的影响, 共生菌可能需要更多不同的系统来降低自身的NO水平。

2 NO影响豆科植物根瘤的形成和发育

2.1 NO影响根瘤和结瘤过程中的能量代谢

根瘤中的NO可通过调控酶的翻译后修饰调节碳(C)、氮(N)及能量代谢(Chaki et al., 2014; Hu et al., 2015)。NO的产生是植物应对缺氧胁迫的一种响应, 与呼吸循环过程相关联, 形成“Hb/NO”循环。缺氧条件下, Hb/NO循环可维持细胞内的氧化还原和能量平衡状态(Igamberdiev and Hill, 2004; Gupta et al., 2011)。Hb/NO呼吸循环包括4个步骤: (1) 硝酸根离子(nitrate ion, NO_3^-)被NR还原为 NO_2^- ; (2) NO_2^- 从细胞质转移到线粒体基质; (3) 通过线粒体ETC将 NO_2^- 还原为NO, 使ATP再生; (4) 最终NO通过被动扩散到胞质溶胶中, 由Hb将其氧化成硝酸盐(Gupta et al., 2011; Horchani et al., 2011; Hichri et al., 2015)。因此, 在低氧环境下, 植物线粒体可以保持其氧化NADH的能力, 从而合成ATP。相关研究也表明, 缺氧环境下, 大豆和苜蓿的成熟根瘤通过硝酸还原途径和细菌反硝化途径均能产生NO, 参与Hb/NO呼吸循环, 为机体提供能量(Meakin et al., 2007; Horchani et al., 2011; Sánchez et al., 2011)。此外, 在缺氧条件下, 结瘤的能量状态几乎全部取决于NR的功能(Horchani et al., 2011)。值得注意的是, 在Hb/NO循环过程中, 由于多数情况下处于缺氧状态, 因此最终电子受体是亚硝酸盐而非 O_2 (Gupta et al., 2011; Igamberdiev et al., 2014)。Hichri等(2015)发现, NO在结瘤过程中具有双重作用: 一方面作为维持基础能量代谢所必需的中间物; 另一方面作为C、N代谢的调节剂, 降低微氧环境下的能量需求。由于缺氧对于植物的生长发育影响很大, 因此当 O_2 不足时, NO对维持豆科植物与根瘤菌共生结瘤的氧化还原和能量平衡状态至关重要。

2.2 NO通过调控共生相关基因的表达影响根瘤的形成和发育

NO在影响相关基因的表达、调控豆科植物防御反应

进而促进共生和结瘤中发挥重要作用。转录组分析表明, NO影响多种植物基因的表达, 如编码富含半胱氨酸蛋白(cysteine-rich proteins, NCRs)、核糖体蛋白、肽酶、结瘤发育细胞周期蛋白及细胞周期转换蛋白的基因(Vinardell et al., 2003; del Giudice et al., 2011; Boscari et al., 2013), 进而调控细胞脱分化、诱导组织形成及抑制植物防御反应(Hichri et al., 2016b)。LjHb1是百脉根(*Lotus japonicus*) ns-Hbs的编码基因, 外源添加NO、缺氧和低温均可诱导其表达(Shimoda et al., 2005)。在根瘤形成的早期阶段, NO的产生可以上调LjHb1的表达, 反之LjHb1的表达又下调NO的水平, 以降低植物防御反应, 促进根瘤菌和宿主植物建立共生关系(Nagata et al., 2008; Murakami et al., 2011)。植物也可通过降低Hb的表达促进内源NO产生, 调控自身机体内相关防御基因的表达(Wally et al., 2013) (图2)。而NO的清除会引起基因*MtCRE1*和*MtCCS52A*的下调表达, 从而延缓结瘤(del Giudice et al., 2011)。通过对接种中华根瘤菌的蒺藜苜蓿外源添加NO清除剂(2-(4-carboxyphenyl)-4,4,5,5-tetramethyl imidazoline-1-oxyl-3-oxide, cPTIO), 发现受NO调节的3种基因(*Contig525*、*Contig3307*和*Medtr5g010350*)表达下调(Boscari et al., 2013)。其中, 前2种基因分别与ns-Hbs和茉莉酮酸代谢途径(Palmieri et al., 2008)相关, 而最后1种基因可调控还原型谷胱甘肽(glutathione, GSH)的合成(Innocenti et al., 2007)。转录组分析显示, 蒺藜苜蓿接种中华根瘤菌后, 外源添加cPTIO或NADPH氧化酶抑制剂二苯烯碘铵(diphenylene iodide, DPI), 导致细胞壁形成和发育过程中相关基因下调表达, 而植物防御和二次代谢相关基因上调表达(Puppo et al., 2013)。hmp的过表达和外源添加cPTIO都可降低蒺藜苜蓿的结瘤效率(Kato et al., 2010; del Giudice et al., 2011), NO则通过调节上述基因的表达, 抑制相关防御反应从而促进共生关系的建立。此外, 在根瘤菌内也鉴定出数百个依赖NO表达的基因, 如中华根瘤菌中编码Hmp的*hmp*基因(del Giudice et al., 2011; Cam et al., 2012)、编码haeme-Cu蛋白的*nnrS1*和*nnrS2*基因(Blanquet et al., 2015)及编码NiR的*nirKV*基因(Meilhoc et al., 2011), 但关于根瘤菌内受NO影响且与共生结瘤相关的基因报道很少。

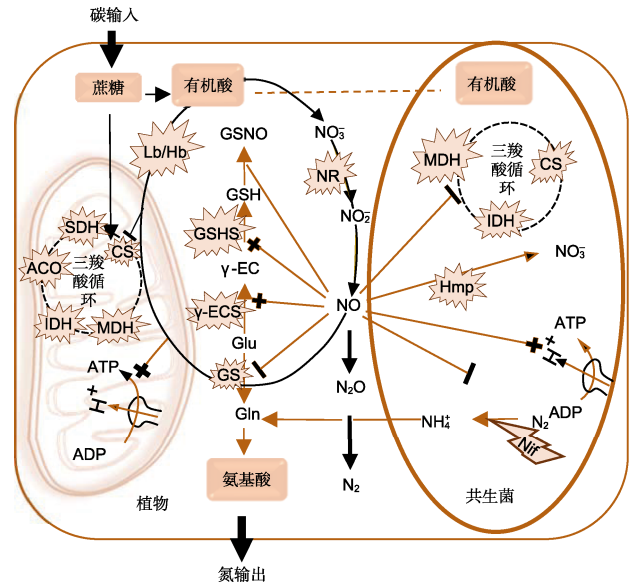


图2 NO在共生固氮中的作用示意图(改编自Boscari et al., 2013; Hichri et al., 2015, 2016b)

一方面, NO抑制固氮和C、N代谢; 另一方面, NO调控细胞氧化还原和保持低氧水平下的能量状态。带+的细线表示NO的活化、诱导和保持效果; 带有-的细线表示NO的抑制作用。椭圆粗线箭头表示NO主要的代谢途径。爆炸型图示指来自植物和菌共生体的酶, 闪电型图示表示根瘤菌内的基因。ACO: 乌头酸; CS: 柠檬酸合酶; Gln: 谷氨酰胺; Glu: 谷氨酸; GS: 谷氨酰胺合成酶; GSH: 谷胱甘肽; GSHS: 谷胱甘肽合成酶; GSNO: S-亚硝基谷胱甘肽; Hb: 血红蛋白; IDH: 异柠檬酸脱氢酶; MDH: 苹果酸脱氢酶; NH_4^+ : 铵根离子; Nif: 固氮酶; SDH: 琥珀酸脱氢酶; γ -EC: γ -谷氨酰半胱氨酸; γ -ECS: γ -谷氨酰半胱氨酸合成酶

Figure 2 Schematic diagram of the role of NO in symbiotic nitrogen fixation (modified from Boscari et al., 2013; Hichri et al., 2015, 2016b)

On the one hand, NO inhibits nitrogen fixation and carbon and nitrogen metabolism; on the other hand, it regulates cellular redox status and maintains the energy state under low oxygen levels. A thin line with + indicate the activation, induction, and retention effects of NO; a thin line with - indicate the inhibition of NO. The oval thick line arrows indicate the main metabolic pathways of NO. Explosive type diagrams refer to enzymes from plants and bacterial symbionts, and lightning type diagram represents genes within rhizobium. ACO: Aconitic acid; CS: Citrate synthase; Gln: Glutamine; Glu: Glutamic acid; GS: Glutamine synthetase; GSH: Glutathione; GSHS: Glutathione synthetase; GSNO: S-nitrosoglutathione; Hb: Hemoglobin; IDH: Isocitrate dehydrogenase; MDH: Malate dehydrogenase; NH_4^+ : Ammonium ion; Nif: Nitrogenase; SDH: Succinate dehydrogenase; γ -EC: γ -glutamylcysteine; γ -ECS: γ -glutamyl cysteine synthetase

2.3 NO与激素相互作用影响结瘤

脱落酸(abscisic acid, ABA)是植物适应环境胁迫的关键激素(Yoshida et al., 2015), 可通过抑制根毛变形及侵染线的形成影响豆科植物结瘤(Suzuki et al., 2004; Ding et al., 2008)。Tominaga等(2010)在百脉根中发现, ABA不仅可调节根瘤的固氮酶活性, 还可通过影响结瘤过程中NO的产生来影响结瘤。在根瘤形成过程中, NO通过酪氨酸(tyrosine, Tyr)硝化调控ABA信号传递, 而ABA又在信号转导过程中决定NO的产生与积累(Castillo et al., 2015)。Tyr硝化是NO介导的翻译后修饰, 其特征在于将-NO₂添加到Tyr残基芳环的等价邻位C上, 产生3-硝基酪氨酸(3-nitrotyrosine, 3-NT), 并由此改变蛋白质构象(Radi, 2004)。作为硝化分子的前体, NO通过Tyr硝化改变细胞溶质谷氨酰胺合成酶(glutamine synthetase 1, GS1)和Lb等关键结瘤蛋白的活性(Melo et al., 2011; Navascués et al., 2012; Li et al., 2014)。豆科植物结瘤时Lb发生Tyr硝化, 可保护共生体免受过氧化硝酸盐毒害, 因此3-NT的形成既是硝基氧化应激的标志, 也是功能性结瘤活跃代谢的结果(Sainz et al., 2015)。此外, NOS抑制剂N-硝基-L-精氨酸甲酯(N^G-nitro-L-arginine methyl ester, L-NAME)和cPTIO处理可减弱豌豆(*Pisum sativum*)表皮由ABA诱导的气孔关闭(张绪成等, 2005)。

NO还参与生长素(indole-3-acetic acid, IAA)信号通路调控的不定型瘤的形成过程(del Giudice et al., 2011)。Breakspear等(2014)研究发现, IAA在根瘤菌侵染蒺藜苜蓿根毛过程中能够影响细胞的分裂和扩张。在IAA过剩的苜蓿中, 根瘤表现出NO的富集, 且植株的侧根发育能力较强, 这一过程受IAA和NO共同调控, 通过添加cPTIO可显著抑制野生型和IAA过剩菌株诱导的结瘤(Pii et al., 2007)。Matamoros等(2015)还发现, 百脉根根瘤中存在2个高表达的Gpx基因(*LjGpx1*和*LjGpx3*), *LjGpx1*存在于根瘤细胞的质体和细胞核中, *LjGpx3*则存在于细胞质和内质网中。这2个基因都可以参与氧化应激反应, 抵抗盐胁迫和膜损伤, NO可上调根瘤中依赖于硫氧还蛋白的*LjGpx1*, 而*LjGpx3*受细胞分裂素(cytokinin, CK)与乙烯合成前体1-氨基环丙烷羧酸(1-aminocyclopropane-1-carboxylic acid, ACC)诱导上调表达。上述结果表明, 在豆科植物根瘤菌共生结瘤过程中, NO与

激素对根瘤形成的影响很大, 但二者的交互机制还有待深入探究。

3 NO在共生体系中固氮、抗氧化及衰老的作用

3.1 NO影响共生体固氮

研究表明, 外源添加NO可抑制大豆与百脉根根瘤的固氮酶活性(Kato et al., 2010; Cam et al., 2012; Boscari et al., 2013)。Cam等(2012)报道, 成熟根瘤中NO的积累不仅可降低固氮效率, 还会影响植物的生长适应性, 而通过降解NO可增强植物体固氮能力和延长植物整体固氮周期。研究发现, *hmp*的过表达降低了根瘤中NO的含量, 却增强了乙炔还原活性(acetylene-reducing activity, ARA)和延迟根瘤衰老(Meilhoc et al., 2011; Cam et al., 2012)。但NO的毒性、信号功能或代谢效应取决于其所在作用部位的NO浓度(Boscari et al., 2013; Mur et al., 2013)。例如, 在百脉根中, 高浓度的NO可抑制氮的固定, 适当低浓度的NO则可增强固氮作用(Kato et al., 2010)。因此, 较低且稳定浓度的NO是维持豆科植物根瘤形成和发育的重要因素(Shimoda et al., 2005; Cam et al., 2012; Calvo-Begueria et al., 2018)。

NO可通过调节共生体的能量代谢影响N₂固定。固氮过程中会产生铵根离子(ammonium ion, NH₄⁺), 当其达到一定浓度时即具有毒性并损害植物的生长(Li et al., 2014)。GS可同化NH₄⁺, 解除这种离子胁迫, 而NO通过Tyr硝化改变其活性, 因此在N代谢中NO与GS起着至关重要的作用(Melo et al., 2011; Sainz et al., 2015)。S-亚硝基化也是植物体内NO信号转导的关键机制。研究发现蒺藜苜蓿成熟根瘤中约有80种S-亚硝基化蛋白, 且多数与C、N代谢及固氮酶有关, 如果糖激酶、固氮酶钼铁(MoFe)蛋白nifK与铁(Fe)蛋白nifH(Puppo et al., 2013)。NO可能通过影响根瘤中与S-亚硝基化蛋白相关的酶活性, 利用三羧酸循环(tricarboxylic acid cycle, TCA cycle)和糖酵解(glycolysis)等共生体的能量代谢过程调控固氮酶活性(Igamberdiev and Hill, 2009; Boscari et al., 2013; Li et al., 2014; Igamberdiev et al., 2014)(图2)。但对于NO究竟是直接抑制固氮酶活性还是通过间接调节C、N代谢影响豆科植物体固氮, 目前尚不清楚(Hichri

et al., 2016b)。

3.2 NO影响根瘤的抗氧化水平和衰老过程

NO可改变豆科植物根瘤菌共生体的抗氧化水平。豆科植物具有产生GSH同系物高谷胱甘肽(homogluthathione, hGSH)的特殊性。GSH是植物中的主要抗氧化剂之一,而NO可调控细胞氧化还原基因,如 γ -谷氨酰半胱氨酸合成酶(γ -glutamylcysteine synthetase, γ -ECS)基因 γ -ecs和谷胱甘肽合成酶(gluthathione synthetase, GSHS)基因 $gshs$ (Innocenti et al., 2007) (图2)。在百脉根根瘤中,NO可调节谷胱甘肽过氧化物酶(gluthathione peroxidase, GPX)基因 Gpx 的表达,进而影响共生体内的氧化应激反应(Matamoros et al., 2015)。

尽管目前对于豆科植物与根瘤菌共生固氮的初始过程已有较多研究,但因发育或应激引起根瘤衰老的分子机制却报道较少。根瘤衰老区域的表型是由粉红色变为绿色,其实质源于植物Hbs的降解;在转录水平上,衰老表现为编码蛋白质降解,核酸、膜脂和糖类的相关基因被激活;同时伴有衰老根瘤中氧化还原平衡的改变,以及抗氧化防御的整体减弱等现象(Van de Velde et al., 2006; Loscos et al., 2008)。NO在植物的衰老过程中具有重要作用(Procházková and Wilhelmová, 2011)。例如,NO是水稻(*Oryza sativa*)光依赖性叶细胞死亡的关键介质(Lin et al., 2012);蒺藜苜蓿根瘤内NO含量的增加会导致根瘤过早衰老,且衰老程度与NO水平相关(Cam et al., 2012; Meilhoc et al., 2013; Blanquet et al., 2015),表明NO对于维持共生体系和避免根瘤过早衰老极为重要。此外,施加硝酸盐可导致豌豆表现出典型的衰老特征(Escuredo et al., 1996)。对蒺藜苜蓿添加硝酸盐后,其自身也可通过还原硝酸盐促使根瘤中生成NO,然而硝酸盐引起的根瘤衰老是否依赖于NO目前尚不清楚(Horchani et al., 2011)。此外,NO也是一种有效的呼吸抑制剂,能够抑制线粒体和细菌末端的呼吸氧化酶,并增加活性氧(reactive oxygen species, ROS)和活性氮(reactive nitrogen species, RNS)的积累,而NO和ROS均为共生发育所必需(Shimoda et al., 2005; Cam et al., 2012; Igamberdiev et al., 2014; Arjona et al., 2015),因此衰老也可能是固氮时呼吸抑制的间接结果。

4 NO参与调控豆科植物根瘤菌共生体系的非生物胁迫响应

NO不仅能调节植物生长发育,还可参与植物对非生物胁迫的响应(Moreau et al., 2010)。目前的研究发现,在不同非生物胁迫(如干旱和缺氧)下,共生有机体中的NO均会产生响应(Desalvo et al., 2010; Sánchez et al., 2011; Hawkins et al., 2014; Iarullina et al., 2014; Zimmer-Prados et al., 2014)。同时,在豆科植物中NO还可能参与调控机体对金属元素镉(Cd)和铝(Al)的胁迫反应以及应对非金属元素砷(As)的胁迫(Singh et al., 2015; 尚玉婷等, 2018),但都缺乏在结瘤和共生固氮体系中的实验证据(Pérez-Chaca et al., 2014)。Meilhoc等(2011)提出,宿主中任何应激反应下NO的产生都会直接影响共生,在成熟根瘤中,除了NO专用调节器NnrR外,多数根瘤菌的基因通过双组分系统(FixLJ)来调节NO,其中FixLJ是O₂限制反应的主要调节因子。*nnrR*是Nor结构基因*norCB*的上游基因,可防止NO这种高活性氮氧化物物的积累,其插入失活可抑制亚硝酸盐的产生以及亚硝酸盐和NO的还原(De Bruijn et al., 2006)。NO还能够可逆地结合光系统II (photosystem II, PSII)并调节电子的转移和猝灭,对光合作用至关重要(Wodala et al., 2008)。从农业和进化的角度来看,NO在植物与根瘤菌共生体系中的调控作用,可以改善植物对病原体的防御状态、保护植物免受虫害以及增强植物对非生物胁迫的适应性(Chadha et al., 2015)。

4.1 黑暗胁迫

研究表明,长期黑暗处理能显著诱导根瘤衰老,同时降低固氮酶活性和Hbs水平(Romanov et al., 1980; Matamoros et al., 1999; Swaraj et al., 2001; Pérez Guerra et al., 2010)。持续的暗处理会干扰根瘤功能,导致根瘤完全衰老(Pérez-Guerra et al., 2010),而通过降解根瘤中的NO可以延缓黑暗诱导的衰老,但关于NO在根瘤衰老过程中的作用机制还需进一步研究(Cam et al., 2012)。She等(2004)发现在光/暗条件下,对蚕豆(*Vicia faba*)外源添加cPTIO和L-NAME可减弱H₂O₂对NO产生的诱导效应和改变人为光照引起的气孔关闭。气孔关闭由多种生物和非生物因素引起,如渗透胁迫、黑暗、高浓度的CO₂和机械压力,其中光是调节气孔运动最重要的环境因素之一(Zeiger,

1983; Kearns and Assmann, 1993; Herold and Puppo, 2005; Sánchez et al., 2010)。但关于黑暗条件下NO调节豆科植物共生固氮的作用机制还未见报道。

4.2 缺氧胁迫

NO还参与豆科植物对缺氧胁迫的反应。缺氧胁迫可诱导百脉根根瘤产生亚硝酰基豆血红蛋白(nitrosylhemoglobin, LbNO)以减少根瘤内的硝酸盐,同时LbNO的形成也有利于解除NO对植物体的毒害(Meakin et al., 2007)。根瘤中LbNOs的增加不会抑制植物的固氮作用,其实质源于Lb在根瘤中既可清除由细菌反硝化作用产生的NO和亚硝酸盐,又可保护淹水条件下的固氮酶活性(Sánchez et al., 2010)。Sánchez等(2010)发现,在水淹缺氧条件下,野生型和norC突变体根瘤中编码固氮酶Fe蛋白的nifH基因表达量降低、固氮酶活性下降,通过清除NO可抵消这种影响。而当从常氧转为缺氧时,根瘤内NO迅速增加,表明根瘤中不仅能产生NO,还具有响应缺氧胁迫而迅速上调表达的基因(Sánchez et al., 2010; Horchani et al., 2011)。因此,无论在转录水平还是翻译水平,固氮酶都是NO抑制N₂固定的关键靶标(Sánchez et al., 2010),同时也表明在微氧环境中,NO主要通过影响固氮酶活性来调节植物体固氮。

5 总结与展望

NO在豆科植物根瘤菌共生体系的能量代谢、防御调节、功能结瘤、共生固氮、衰老及响应非生物胁迫等方面发挥重要作用,但对于NO在豆科植物及共生体中结瘤和固氮的作用机制仍需深入研究。例如:(1) 尽管许多研究表明,NO在共生体中产生的阶段可被追溯,但关于共生过程中NO产生的具体部位、时间及关键因素的调控机制还未见报道;(2) 在结瘤方面,NO既可作为C、N代谢的调节剂,又是能量代谢所必需的中间物,在低氧条件下保证共生有机体的能量供给,同时还可调节相关基因的表达及与激素协同作用影响根瘤形成和发育,但目前还缺乏NO与植物激素及其它信号分子在根瘤形成中的作用机制研究;(3) NO在固氮方面的作用取决于其浓度,因此,应严格控制NO稳态浓度,以限制其毒性,促进NO信号和功能的发挥,然而目前关于NO如何调节植物体固氮及在根

瘤衰老过程中的作用模式都需进一步探究;(4) 虽然在分子水平上已经证明Hbs(ns-Hbs、Lbs和Tr-Hbs)、Hmp、Nor以及NnrS参与NO的信号和代谢功能调节,但植物与细菌GSNOR和TrxR系统的参与机制仍有待研究。

目前,关于豆科植物及共生体内NO信号分子转导的机制、与其它信号分子间的互作、与酶系统的关联及调控网络、实时检测技术和仪器的开发等都是未来研究的重点。随着不断优化气体信号分子研究技术,逐步利用NO改善豆科植物的结瘤能力、固氮效率和延迟衰老等,以及进行生理与分子遗传学研究也是未来亟须解决的焦点问题。而在实际生产中,在保持生态平衡的前提下如何将NO的研究成果转换为有利的生产工具,进而改良作物品种,提高作物抗性和生产效益,仍然是当今研究领域面临的严峻挑战。相信随着各项研究的不断深入,以及监测技术的不断成熟,最终NO有望应用于高效的农业生产中。

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Influence Mechanisms of Nitric Oxide on Nodulation and Nitrogen Fixation in Legumes

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Abstract Legume-rhizobium symbiosis is genetically co-regulated by the genes of both partners. The symbiosis process involves the formation of special nodule structure where the inert nitrogen (N₂) from the atmosphere is converted into ammonia nitrogen that can be directly used by plants. Nodulation and nitrogen fixation are affected by many factors. As a free radical reactive gas signaling molecule, nitric oxide (NO) participates in the regulation of many plant growth and development processes, such as respiration, photomorphogenesis, seed germination, tissue and organ development, aging, and response to various biotic and abiotic stresses. In the legumes, it has been found that NO not only affects the establishment of the symbiotic relationship between the host and the bacteria, but also is involved in regulating the fixation of nitrogen by the rhizobia and increases the efficiency of nitrogen nutrition utilization. Here we review the mechanism of NO regulating nodule formation and symbiotic nitrogen fixation in legume-rhizobium symbiosis system, including the production and degradation of NO in legumes and rhizobia and its effect on nodulation, symbiotic nitrogen fixation and their response to environmental stress. We discuss the prospects and challenges of studying NO signaling molecule in symbiotic nitrogen fixation system of legume-rhizobium.

Key words nitric oxide (NO), nodule, symbiotic nitrogen fixation, hemoglobins (Hbs)

Zhang WQ, Zou H, Zhang NN, Lin XY, Chen J (2020). Influence mechanisms of nitric oxide on nodulation and nitrogen fixation in legumes. *Chin Bull Bot* **55**, 623–633.

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