大豆结瘤固氮的分子生理研究

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摘 要:有关豆科作物共生固氮的研究已有 170 a 的历史。而豆科结瘤固氮的分子生理研究,仅仅是最近 30 a 的事情。从影响大豆结瘤固氮的生理因子角度,评述了豆科作物的结瘤自动调控机制,进一步在分子水平上阐述了生长素、硝态氮、黄酮类等生理调控物质对大豆结瘤固氮的影响。生长素调节根瘤菌侵染位点和根瘤形成后的生长;可通过控制结瘤的角度来减少土壤硝态氮对大豆结瘤固氮能力的抑制;黄酮类物质在根瘤发育和结瘤基因诱导中起重要作用;乙烯抑制结瘤信号的前期过程,调整根瘤形成的空间分布。详细、系统地从作物-微生物之间关系出发,研究引起豆科作物根瘤形成和固氮系统的整体信号传导、蛋白和代谢过程,是未来大豆共生固氮分子生理研究应该注重的方向。

关键词:结瘤;自动调控;生理活性物质;信号传导

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Molecular Physiology of Nodulation and Nitrogen Fixation in Soybean

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Abstract: Research on symbiotic nitrogen fixation in legumes has been conducted for 170 years, while the aspects of molecular physiology on nodulation and nitrogen fixation of legumes were only investigated in the last 30 years. This review discussed the autoregulation of nodulation from the physiological perspectives and analyzed the effects of auxin, nitrate nitrogen, and physiological active substance like isoflavones on nodulation at molecular level. Auxin regulates the site of rhizobia infection and nodule development. The inhibition of nitrate nitrogen on nodulation and nitrogen fixation can be reduced by adjusting nodulation. Isoflavones play a key role in nodule growth and nod-induced genes. Ethylene inhibits early process of nodulation signal and regulates the distribution of nodules. Further research should focus on the process of signal transduction, protein and metabolism from the relationship between crop and microorganism.

Key words: Nodulation; Autoregulation; Physiological active substance; Signal transduction

从 1838 年 Boussingault 证实豆科作物能够固氮,1886 年德国科学家 Hellriegel 和 Wifarth 明确表明豆科作物固氮由微生物所驱动[1],到 20 世纪末固氮酶化合物结构特征被证实,170 a 以来,科学家们围绕生物固氮这一领域,开展了大量的相关研究,有关固氮的研究工作已经有了质的飞跃。目前,现代分子生物学研究技术的发展已逐步深入到共生固氮的分子遗传学研究领域,从分子生物学角度研究豆科作物固氮机制已成为共生固氮领域研究的热点和未来研究的必然方向。由于生物固氮过程非常复杂,涉及很多豆类作物。该文以大豆为主要对象,重点阐述影响大豆结瘤固氮过程的一些关键因素与基因调控的关系的研究成果和未来发展趋势。

1 结瘤自动调控机制

根瘤形成是需要大量能量的过程,因此豆科作物将反过来调控根瘤的形成,这种负反馈调节系统被称为结瘤自动调节[2-3]。豆科作物结瘤过程所存在的这种自动调节机制或反馈抑制调节,在根和地上部之间存在着长距离信号,早期形成的根瘤将抑制随后根瘤的形成[2,4-5]。因此,作物能够调控结瘤水平和根瘤形成与整体生长发育之间的平衡。这种寄主作物结瘤调节机制最早是在红三叶草上发现的,Nutman[6]去除红三叶草先期形成的根瘤或根尖,促进根瘤形成。Caetano-Anolles 等[1]通过调整硝态氮供给水平发现大豆对根瘤也具有调控机制。豆科作物在表现结瘤自动调节机制时,新形成的根瘤并不是新根瘤菌侵染的结果。对大豆进行

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解剖学研究认为,根瘤菌的早期发展被停滞,通过自动反馈调节机制,休眠的已侵染根瘤菌又重新发挥功能[7-8]。对超结瘤大豆[9]和豌豆[10]根瘤自动调节受体激酶进行基因克隆,结果表明被公认的转换膜-富亮氨酸的受体激酶(LRR)是结瘤自动调节信号途径中的关键调节酶。通用结瘤基因 ABC(NodABC)负责合成结瘤因子的寡糖骨架,这在所有的根瘤菌中都是相同的;专一性的寄主结瘤基因(host-specific nod genes,hsn)负责添加修饰基因,为结瘤因子提供宿主专一性,广宿主范围的根瘤菌能产生多种结瘤因子,其基本结构相同,但取代基不同[11]。即便如此,必须由寄主作物和根瘤菌的特殊基因联合表达来调控根瘤生长。

Delves 等[12]将超结瘤大豆品种与野生品种进行嫁接,结果表明对根瘤数量的控制主要是地上部而不是根系,因此,可以推测根瘤菌侵染后侵染信号的形成是由地上部传导到根系。研究认为,叶片接收到侵染信号后,传到根系控制过量结瘤形成[2-3]。最近的研究发现,HAR1(Hypernodulation and Aberrant Root, 超结瘤和畸形根)和 GmNARK(*G. max* Nodule Autoregulation Receptor Kinase,大豆根瘤自动调控接受激酶)在根瘤自动调节中起着非常重要的作用,它们标记一个富含亮氨酸重复(leucine-rich repeat)的类接受体激酶蛋白(receptor-like kinase protein)[9-10,13],这与地上部表皮分生组织中的 CLV1 是同源基因,控制着细胞分裂。Ito 等[14]利用由 Willians 亲本分离出来的超结瘤突变体 NOD1-3 和 NOD3-7 进行试验,发现开始叶片伸展受到抑制,但是却有较高的叶片生长速率。为了从细胞水平比较超结瘤突变体叶片表现型,Ito 等[15]利用光学显微镜观察大豆第 1 片三出复叶亚表层栅栏组织细胞,结果表明超结瘤突变体完全展开叶片较小是因为叶片细胞数量较少,而不是单个细胞体积减小的缘故。同时推测与根瘤自动调节相关的信号可能不是结瘤所特有的,也可能是植物正常生长的一部分[16-17]。另外,叶片长势的差异也不是光合产物分配的差异引起的,因为直到根瘤出现,根瘤原基和未接菌根系光合产物的分配没有差异[18]。目前对于超结瘤大豆突变体非共生体系条件下地上部的表现型还不清楚。

2 生长素

一些特殊的根瘤菌信号分子,即结瘤因子,诱导根瘤的形成过程,而这些结瘤因子主要是对作物信号分子的反应而相应地分泌出来的[19]。由植物根系分泌的信号分子主要是类黄酮和异类黄酮[20-21]。据报道植物的类黄酮和异类黄酮直接受吲哚乙酸分布情况调控,这是一种天然的植物生长素。植物可以通过调控体内吲哚乙酸水平来调节根瘤菌结瘤因子对根瘤形成的驱动[22-23]。生长素由植物的地上部合成,然后向根尖转移,在植物体内的分布并不均一,正在分裂、延长和有重力及光趋向性的细胞中浓度最高[24],甚至植物木质部薄壁组织的上下层细胞中的含量都有较大的梯度差异。利用生长素对大豆启动子 GH3 进行调控研究,结果发现如果组织中生长素浓度较高,GH3 启动子处于活性状态[25],而且该启动子对生长素浓度变化反应较快[19,26-27]。在根瘤形成早期,生长素向上调控根瘤菌侵染位点,向下调节根瘤形成后的生长过程,但在成熟根瘤中没有这种现象[19]。根系外皮层生长素水平增加的位置可能是有限结瘤作物根瘤原基最先分裂的位点。在无限结瘤的豆科作物上,极性生长素转运抑制调节生长素水平是根瘤形成的关键环节,是否在有限结瘤的豆科作物上,生长素对根瘤原基形成的作用有所不同呢? Lohar 等[28]进行嫁接试验,结果表明有限和无限结瘤豆科作物可以利用不同的信号来启动结瘤。正像大豆具有“生长素起爆假说”[29]一样,最近对蒺藜苜蓿 *M. truncatula* 研究发现高水平的生长素是维持结瘤所必需的[30]。目前还不清楚生长素运输和分布之间的关系,但是接种 *M. loti*(百脉根中慢生根瘤菌)后的第 1 个根表皮细胞分裂时,伴随着接种结瘤因子后生长素运输增强的现象。尽管如此,生长素在结瘤信号传递和调控过程中的确切机制还不十分清楚。为了进一步了解清楚生长素的分布,下一步可以考虑分析作物进行向地性处理后,生长素的分布以及接种根瘤菌和结瘤因子后,生长素的分布方式[31]。

3 硝态氮

共生固定的氮、矿质土壤氮和肥料氮是满足高产大豆氮素需求的主要来源[32]。然而,土壤溶液中的硝态氮浓度和根瘤中的共生固氮过程具有相互拮抗作用。如果豆科作物对氮素的需求超过土壤供氮能力时,将能够固定大量的氮素,近 10 a 来报道的固氮量(0 ~ 450 kg · hm^{-2})和固氮比例(0 ~ 75%,通过固氮作用获得氮的比例)几乎保持不变[33]。大豆在适宜环境和低氮土壤条件下生长能固定大量的氮。尽管已发现通过基因手段可以提高豆科作物的产量,但是对于大豆来讲,提高固氮比例是提高大豆产量的一个很好的途径[32]。在土壤硝态氮含量较高时,大豆利用适量或大量的土壤氮,导致固氮比例和总固氮量都随之降低。土壤硝

态氮对大豆结瘤和固氮量的抑制也降低了大豆产量[34]。

为了减少土壤中硝态氮对大豆固氮比例和固氮量的限制,研究者从作物基因型的角度出发,将共生耐硝态氮的品种与商用品种杂交,培育高固氮能力的大豆品种或品系。结果发现超结瘤突变体在 5 或 5.5 mmol · L^{-1}硝态氮条件下,结瘤量和固氮酶活性分别为野生型的 10 ~ 20 倍,但总生物产量降低了 30% ~ 40%,根生长也受到了限制。Ohyama 等[35]随后研究认为,正是由于根系生长受到了限制,降低了突变体大豆根系吸收硝态氮的能力。但也有些相反的结果,Zhao 等[36]在温室内进行试验,在供给充足氮条件下,回交的超结瘤品系 PS55 和野生品种生长得一样好,认为 PS55 根系生长受到限制和减少产量很可能是对高水平结瘤的生理反应,而不是基因突变或其它突变影响的结果[37]。此外,突变体早期固氮能力提高不能维持到生殖生长后期。据考证,还没有人将超结瘤、限制根系生长和减少产量完全准确地分开。关于超结瘤品系未来的走向,研究者认为超结瘤和极端超结瘤大豆品系的结合将会使大豆产量潜力和固氮优势得以提高[36,38]。截至目前,超结瘤品系材料还没有真正地被投放到市场,最后的成功决定于提高产量、抗病性能等因素[32]。

另一方面研究者从控制结瘤的角度来减少土壤硝态氮对大豆固氮比例和固氮量的限制。早在 1985 年 Pulver 等[39]利用嫁接试验提出,利用范宿主结瘤的大豆可以提高大豆固氮能力。后来国际热带农业研究机构(IITA)的一些研究者也成功地获得了相似的结论[40]。土壤中大量天然存在的根瘤菌,有很多是结瘤低效的或结瘤数量很少。通过鉴定出一些大豆基因型,可以选择性利用土壤中高效根瘤菌株[41-42]。还有一种相关的方法,利用受特殊根瘤菌株或根瘤菌血清限制结瘤的大豆品种来选择土壤中高效结瘤的土著根瘤菌[43-44]。

尽管氮素对结瘤的抑制作用在 100 a 前就已经被研究者们发现,然而迄今为止,其中所包含的确切机制还不十分清楚[45]。对大豆进行不同氮水平处理后,测定类黄酮含量和结瘤能力之间的关系,发现在固氮开始前,供氮水平和大豆根系类黄酮(染料木黄酮和大豆黄酮)含量呈负相关关系[45]。供氮减少大豆根系类黄酮含量,这可能在可利用氮对大豆根瘤生长的影响中起一部份作用。而对耐硝态氮的共生体和超结瘤突变体上的相关关系进行研究发现,根瘤自动调节机制与硝态氮的交叉信号可能控制根瘤数量。

4　类黄酮/异类黄酮

共生固氮系统中共生体间相互信号传递和识别受化学信号物质调控,主要是根系分泌的特殊的类黄酮类化合物。适宜的根瘤菌类菌体能够识别这些类黄酮化合物,诱导结瘤基因[46-47]。结瘤基因产物是一些酶类,合成专一性的脂壳多糖信号分子(结瘤信号),能够被寄主作物识别,激活一系列功能性根瘤器官形成[48-49]。

大豆体内的异类黄酮(包括染料木黄酮和大豆黄酮)被认为是根瘤菌结瘤基因表达的主要诱导剂[50-52],同时能够加强根瘤菌对植物抗毒素防御化合物的抗性[53]。对大豆根系进行生物化学分析,发现根瘤菌处理后的大豆根系异类黄酮水平增加[54],在超结瘤大豆突变体中也检测到较高的异类黄酮含量,表明大豆根系异类黄酮含量受结瘤信号途径所调控[55]。当根瘤菌侵染作物根系以后,内源类黄酮对结瘤起着关键的作用,而外缘类黄酮永远起不到这样的作用[47]。关于作物体内的异类黄酮的作用有二种观点,一种认为在根瘤原基形成位点附近细胞中有异类黄酮化合物积累,表明异类黄酮促进生长素积累,导致根瘤器官发育[22,40]。另一种观点认为异类黄酮在根中细菌结瘤基因诱导中起重要作用。通过 RNA 干扰(RNAi)诱导异类黄酮合成酶稳定化,减少转基因组合大豆植株根毛中异类黄酮水平,结瘤数也将严重减少。奇怪的是加入外缘异类黄酮后根中含有稳定的异类黄酮合成酶的根系结瘤量并没有显著增加[46]。在大豆结瘤过程中,异类黄酮作为根中结瘤基因的诱导物质是必需的,决定着结瘤程度,但对生长素运转的作用并未表现出必需性[46]。进一步的研究发现,类黄酮对生长素运转的抑制调控作用在有限结瘤和无限结瘤的豆科作物上有所不同,在有限结瘤型豆科作物,如大豆和豇豆,对大豆根系进行 RNAi 处理,诱导异类黄酮合成酶稳定,结果表明异类黄酮对大豆体内生长素的运转有抑制作用[47]。对野生型大豆幼苗供给染料木黄酮,同样抑制生长素的运输[46]。此外,利用放射性的生长素 3H-IAA 进行示踪试验,结果在根瘤菌侵染根系的位点上没有检测到类黄酮对该点生长素运输的抑制,因此,对于有限结瘤型的大豆结瘤过程中,异类黄酮对根瘤菌侵染位点生长素运输的抑制作用并不是必需的。在无限结瘤的豆科作物上,如羽扇豆,类似的一些试验恰好获得了相反的结论。

Zhang 和 Smith[56-57]研究了胁迫环境对豆科作物与根瘤菌结瘤信号合成的抑制作用,发现在胁迫

环境条件下,异类黄酮积累量减少可能是豆科作物结瘤和固氮的一个重要限制因子。

目前的研究主要侧重于为异类黄酮在大豆结瘤中的必要性提供证据,证明根中异类黄酮的作用[46]。尤其还没有利用基因研究领域方面的研究结果来证实类黄酮在结瘤过程中的明确作用,这可能是因为改变豆科作物类黄酮数量和轮廓的线性图具有一定的技术挑战[47]。

5 乙烯

根瘤的自动调节机制主要限制根的结瘤区,根瘤的形成受到一些独立过程,包括敏感区荷尔蒙乙烯的控制。研究表明,乙烯处理减少多种豆科作物根瘤形成[58-60]。相反的,乙烯接收和合成抑制剂促进结瘤过程[60-62]。而在大豆上,乙稀的作用有些不同,这可能是因为基因型不同对乙烯的敏感度有差异的缘故。尽管关于乙烯对结瘤影响的确切机制还不十分清楚,但是一些证据表明,乙烯能够抑制结瘤信号的前期过程[63]。乙烯在根系中提供一些位置信息,因此,调整根瘤形成的空间分布[64]。

6 调控结瘤和固氮过程的基因

进行基因克隆发现,根瘤形成过程中的这些表现型是由各不相同而又彼此联系的分子网络调控[64]。根瘤菌侵染豆科作物根系,发出的信号由膜接受器上的 LysM-type 接收蛋白激酶作为结瘤因子受体[65-66],随后是 NBS-LRR-receptor 激酶,分别为 LjNORK, MsSYMRK, MtDMI2 or PsSYM19[67-70]。接下来是根瘤菌驱动信号的接收过程,主要是通过细胞核、细胞质和质粒膜间的通道完成[71-73],引起细胞溶质中 Ca^{2+} 浓度达到峰值[63,69,74-77]。最后通过传导因子完成根瘤菌的侵染过程。侵入根系的根瘤菌通过寄主作物根际接收类菌体信号,诱导结瘤基因序列,调控脂-壳-寡糖(lipo-chito-oligosaccharide)结瘤因子(NF)形成[64]。正是这些结瘤因子诱导前期生理、分子和生长反应,促进根瘤形成[78-79]。NF 是根瘤形成过程中的关键信号分子,结瘤能力也受到 NF 信号传导途径的调节。固氮因子接受激酶基因(NFR1)(nod-factor receptor kinase gene)和 NFR5 是长信号接收器,直接与脂-几丁质结瘤信号相互作用[80]。对非侵染基因,即 DMI1 和 DMI2 基因进行克隆,发现它们分别编码一个假设的阳离子通道和一种富亮氨酸的受体激酶[67]。事实上,DMI2 突变体表现出共生体信号的缺失,这表明 DMI2 基因除了在根瘤菌侵染和结瘤信号识别中的作用外,可能还有其它的功能[80]。在这一工作中,也发现了 LYK3 和 LYJK4 在侵染线发展中的作用,随着一些结瘤信号被识别,LysM RLKs 的功能是识别下游基因,可能通过一个分支途径受 LysM RLKs 调控,但这可能也存在着一些猜测[80]。最近又将 DMI3 基因进行克隆,表明其编码钙蛋白激酶(calcium-calmodulin-dependent)[77],DMI3 被认为对结瘤信号诱导的钙峰值信号起作用[69]。卷毛蛋白(HLC)控制微管组织和诱导根毛卷曲[81],多瘤侵染(LIN)(lumpy infections)可能对结瘤信号途径的起始阶段起到从基因下游开始的作用[67]。有关互惠共生固氮体系的形成,结瘤过程和固氮酶活性的信号传输以及基因调控理论的研究,参见 Kinkema 等[64]和 Cheng[1]的报道。

利用模型作物进行结瘤基因分析,已经为理解结瘤信号识别和以后的信号传递提供了帮助。然而,以上研究大部分还是保持在从基因下游构建侵染线及特殊的根瘤结构分子过程上,而且,有关作物-微生物之间的研究,在整体信号传导、蛋白和代谢的数据还处于起始阶段。研究的目标应该是更详细系统地理解引起豆科作物根瘤形成的那些令人不解的复杂过程[80]。这将对深入理解共生固氮机制,有效利用空气中的氮素、减少化肥氮的投入,提高大豆产量有着极其重要的理论意义。

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