禾本科/豆科间作促进豆科共生固氮机理研究进展

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摘要 综述以往研究发现, 大多数禾本科/豆科间作都会显著增加豆科作物的固氮比例(%Ndfa), 并认为这种增加是由于禾本科作物对豆科作物根际氮素的耗竭作用引发的. 但豆科作物体内通常会在氮磷之间保持稳定的化学计量关系, 豆科作物共生固氮的增加会使作物生长受到磷的限制. 在这种磷素限制条件下豆科作物会被激发出潜在的磷吸收策略, 即通过根系分泌物或丛枝菌根真菌增加对土壤磷素的获取满足其自身固氮对磷素的大量需求, 进而增强豆科作物自身的结瘤及固氮. 此外, 随着分子生物学技术的发展, 新近研究发现禾本科作物根系分泌物能上调豆科根部关键结瘤基因 *NODL4*, *ENODL2*, *ENOD93*, 固氮基因 *FixI3*, 查尔酮-黄酮异构酶 *CFI* 基因和生长素响应基因 *GH3.1* 的表达, 这些基因超量表达可促进豆科作物类黄酮物质的分泌、根毛卷曲、结瘤并促进豆科作物固氮. 间作作物间通过根际氮素耗竭作用、磷素活化作用以及固氮基因分子调控等三个层面上互相偶联来促进间作系统氮素吸收、优化氮素利用效率和提升作物产量, 这些研究对促进和发展禾本科/豆科间作固氮理论体系具有重要指导意义.

关键词 共生固氮, 根系分泌物, 丛枝菌根真菌, 间作, 根际过程

氮(nitrogen, N)是陆地生态系统植物生产力最主要的限制因素, 仅次于光和水^[1]. 施用氮肥是提高土壤养分、保证作物高产的关键手段. 然而, 过量氮肥投入会导致土壤酸化、生物多样性丧失、水体破坏性污染和温室气体过度排放等一系列严重的环境问题^[2]. 大多数作物依赖于根系从土壤吸收氮素来满足自身生长和发育的需求, 而豆科作物还能够通过与根瘤菌的共生关系从大气中固定氮素^[3]. 豆科作物共生固氮是自然生态系统和人工生态系统的宝贵氮源, 可在减少或不使用工业氮肥的情况下提高可持续性农业生产力^[4]. 因此为了满足国家日益增长的肥料需求, 缓解环境污染的压力, 通过豆科作物共生固氮而非人工合成肥料向农业生态系统提供氮素已成为一个更加有吸引力的

选择.

1 不同禾本科/豆科间作系统对豆科作物共生固氮的影响

禾本科/豆科间作被广泛认为是一种可持续的农业生产体系, 它可以通过改善豆科作物的共生固氮提高作物的产量, 减少化学肥料的投入. 固氮比例(%Ndfa)是指豆科作物从空气中固定氮素占其体内全部氮素的百分比^[5]. 豆科作物固氮量取决于固氮比例和地上生物量. 在一年生禾本科/豆科间作系统中, 由于禾本科对土壤养分的竞争能力较强, 故种间竞争通常会降低豆科作物地上生物量, 从而影响豆科作物固氮的绝对含量^[6,7]. 这往往会产生间作降低或者没有显著增加豆科

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作物固氮的误解。相比之下,固氮比例是固氮量占植物氮素累积量相对含量,能更直观地反映间作豆科作物对大气氮源的补偿性吸收利用情况。如在豌豆/大麦间作系统中,间作豌豆固氮比例提高了138%,说明间作显著增加豌豆共生固氮,但由于强烈的种间竞争使豌豆地上生物量减少了153%,使豌豆固氮量没有发生显著变化^[8]。综述国内外文献发现不同禾本科/豆科间作对豆科作物固氮比例的影响不尽一致(图1)。60%左右的研究表明间作会显著增加豆科作物的固氮比例。如大田试验中,米豆和玉米间作系统中米豆的固氮比例增加最高可达121%^[9]。在盆栽试验中,蚕豆/小麦间作使蚕豆的固氮比例增加最高可达108%^[10]。也有30%左右的研究表明,间作对豆科作物固氮比例没有显著的影响。如在花生/旱作水稻的间作系统中,在15和75 kg N ha⁻¹的施氮量下,间作花生的固氮比例变化范围为-6%~5%^[11]。在豌豆/玉米间作系统中,间作使豌豆的固氮比例减少了4%~6%,作用并不显著^[12]。还有10%左右的研究发现间作豆科作物的固氮比例显著降低。如在蚕豆/高粱间作系统中,间作蚕豆的固氮比例降低13%^[13]。在豌豆/燕麦间作系统中,间作豌豆固氮比例降低13%^[14]。不同豆科作物在不同禾/豆间作体系中固氮能力的巨大差异主要受到豆科作物本身的种类、根系分泌物和丛枝菌根真菌等生物因素的影响^[10,15,16],其次还会受到一些环境因子即土壤水分、土壤矿质营养

和土壤pH等非生物因素的限制^[17~19]。为了挖掘间作系统中豆科作物固氮潜力并应用于农业生产,需要更好地了解禾本科/豆科间作提高豆科作物共生固氮的机理。

2 禾本科/豆科间作提高豆科作物共生固氮的机理

目前间作提高豆科作物共生固氮的主要理论解释为根际氮素耗竭作用,这种现象存在于豌豆/小麦、豌豆/大麦、花生/水稻、大豆/小麦等间作系统中^[11,20~27]。新近一些研究发现,在玉米/紫花苜蓿、早稻/绿豆间作系统中豆科作物可通过根系分泌物和丛枝菌根真菌(AMF)增加对土壤磷素的获取进而促进其自身的共生固氮^[28,29]。此外,随着分子生物学技术的发展,在蚕豆/玉米间作系统中发现玉米根系分泌物能上调蚕豆根部与固氮相关基因的表达从而增加结瘤和固氮^[15](表S3)。

2.1 根际氮素耗竭作用

一般来说,禾本科作物生长早于豆科作物,并且禾本科作物具有更快的根系生长速度和更广泛的根系分布,在最初的生长阶段逐渐主导资源获取,因此豆科作物的竞争能力往往低于禾本科作物^[30,31]。这主要表现为禾本科作物可以通过耗竭豆科作物根际土壤矿质氮,降低豆科作物根际氮含量,从而促进豆科共生固氮。如

图1 (网络版彩色)不同间作系统中豆科作物固氮比例的相对增加量。(a) 大田实验; (b) 盆栽实验。数据来自世界各地16个不同间作系统的35篇文献(表S1和S2)

Figure 1 (Color online) Relative increase of %Ndfa in different cereal/legume intercropping systems. (a) and (b) represent the field and greenhouse experiments, respectively. Data were collected from 35 literatures of 16 different intercropping systems around the world (Table S1 and S2)

在小麦和蚕豆间作系统中,根系相互作用使得蚕豆吸收来自施入土壤中的 ^{15}N 量降低80%,而间作小麦吸收来自于施入土壤中的 ^{15}N 量比单作增加79%,说明小麦对土壤和肥料氮素的竞争能力比蚕豆更强,但这并没有导致蚕豆地上部总吸氮量发生显著下降,主要是由于间作蚕豆体内来自大气的氮素比单作增加57%^[32].在豌豆/大麦间作田间试验研究表明,间作中的大麦对土壤氮素的竞争比豌豆大30倍,间作模式下大麦吸收氮素的65%来自于肥料氮,豌豆仅有9%来自于肥料氮,这导致间作豌豆的固氮比例比单作平均提高32%^[33].然而也有研究表明,禾本科/豆科间作并没有促进禾本科作物氮素吸收,如甘蔗/大豆间作模式下甘蔗的氮素累积量没有显著提高,这可能是导致间作大豆固氮比例没有增加的主要原因^[34,35].综上所述,在禾本科/豆科间作体系中,根际氮素耗竭作用能促进豆科作物的结瘤和固氮,从而实现了禾本科作物和豆科作物在氮素利用上的生态位分离,降低种间竞争,两种作物均获得高产.

2.2 根际磷素的活化作用

在禾本科/豆科间作系统,由根际氮素耗竭作用引起共生固氮的增加往往会使豆科作物生长受到磷的限制,这主要是由于根瘤共生固氮是高耗能过程,其所需能量由三磷酸腺苷(ATP)直接提供.因此间作豆科作物为了维持体内稳定氮磷化学计量关系,而采取不同根

际磷吸收策略(图2),即通过增加根系分泌物分泌活化豆科作物根际中难溶含磷化合物,或通过丛枝菌根真菌(AMF)菌丝长度和面积增加对土壤磷素的获取,从而满足其自身固氮对磷的大量需求^[36].

2.2.1 根系分泌物对豆科共生固氮的影响

根系诱导的酸碱度变化是影响土壤磷有效性的主要过程之一.植物阴阳离子吸收不平衡是造成土壤酸碱度变化的主要原因^[30].当植物以铵态氮为主要氮源时,由于阳离子的吸收占优势,为维持植物体内电荷平衡,根系溢出的 H^+ 多于 HCO_3^- 或 OH^- ,致使根际土壤呈酸性;反之,当植物以硝态氮为主要氮源时,根际土壤呈碱性^[30].有研究表明,在玉米/紫花苜蓿间作系统中豆科作物根际 $\text{NO}_3^-/\text{NH}_4^+$ 的降低使豆科作物分泌更多的质子,通过酸化土壤促进作物磷素的吸收从而提高豆科固氮^[28].当豆科作物与禾本科作物间作时,豆科作物与单作相比能够产生更多的有机酸进而活化难溶性含磷化合物,但是禾本科没有表现出这种反应^[37].这主要是由于禾本科根系对豆科作物根际土壤磷素的竞争较为强烈,使得豆科作物根际生物有效磷浓度降低,导致豆科作物根部细胞内磷酸烯醇式丙酮酸羧化酶(PEPC)的表达和活性增强,进而诱导更多有机酸的生物合成^[38].同时嵌入质膜中的阴离子通道蛋白在土壤磷含量较低条件下也会显著增加有机酸(苹果酸、柠檬酸和草酸)的被动流出^[39],因此间作能够使豆科作物产生更多的有机酸进而活化土壤难溶性含磷化合物.

图2 (网络版彩色)禾本科/豆科间作提高豆科结瘤和共生固氮的机理

Figure 2 (Color online) The mechanisms of improving legume nodulation and N fixation in the different cereal/legume intercropping systems

如在玉米/蚕豆间作系统中, 间作能显著提高豆科作物根系分泌物中柠檬酸的分泌, 而根际中柠檬酸可以降低根际pH, 提高土壤根际磷的生物有效性, 促进豆科作物磷的吸收^[37]。类似的在玉米/苜蓿间作研究中也发现, 与单作相比间作玉米和苜蓿根际总有机阴离子浓度分别高28%和30%, 促进玉米和苜蓿根际磷浓度显著增加21%和41%^[40]。此外在不同环境下生长的豆科植物, 其根系分泌物组成存在很大的差异^[41]。如在酸性土壤中, 木豆根部主要分泌酒石酸通过螯合铁铝等元素促进铁磷和铝磷中磷素的释放, 供给植物吸收利用^[42]。而在石灰性土壤中生长的白羽扇豆, 其根系会分泌大量的柠檬酸和苹果酸, 通过螯合和酸化作用溶解土壤中的钙磷, 促进植物磷素吸收。土壤磷素有效性的提高不仅可以满足豆科植物固氮对养分的需求, 还能促进豆科植物生长, 并将更多的碳(C)源分配给根和根瘤, 从而促进豆科植物固氮。然而以上的研究并没有将豆科植物有机酸分泌与其共生固氮能力直接联系起来。新近研究发现在紫花苜蓿/玉米间作系统中, 根系交互作用使紫花苜蓿根际柠檬酸的分泌显著增加, 且柠檬酸含量与紫花苜蓿的共生固氮比例显著正相关^[28], 这说明根系分泌的有机酸对豆科共生固氮有重要的影响。但由于植物分泌有机酸需要消耗植物体内大量的碳, 且植物获取碳的能力也会受到植物总的光合能力的限制, 这使得豆科作物在碳投入和磷素回报之间需要进行经济权衡^[43], 当植物满足自身磷素需求后不会继续增加土壤磷素的吸收进而增加固氮, 所以豆科植物对土壤磷素获取的增加不能无限制的增加固氮, 这并不是一个“永动机”的逻辑。

2.2.2 丛枝菌根真菌对豆科共生固氮的影响

大约80%的陆生植物物种与丛枝菌根真菌建立了共生关系, 丛枝菌根真菌在促进植物养分吸收方面起着至关重要的作用^[44]。丛枝菌根真菌可以延伸到根际以外的区域, 将根系与周围土壤微生物连接起来, 扩大根系吸收营养的面积, 并增强土壤磷酸酶和有机酸分泌帮助植物活化土壤中难溶性含磷化合物, 从而改善共生植物磷养分状况^[16]。作物磷素吸收利用的提高可以促进植物生长, 有利于根瘤的形成, 从而提高豆科共生固氮。如在低磷有效性条件下, 接种AMF可以显著提高蚕豆根部根瘤数量、根瘤重量和固氮酶活性, 这是由于丛枝菌根真菌可以通过外部菌丝体增加植物根系吸收面积, 来加强蚕豆对土壤不可利用的磷素获取^[45]。在缺乏AMF的情况下, 低磷有效性可减少甚至完全抑

制豆科作物氮固定。然而在高磷有效性条件下, 植物与AMF共生体系的碳成本变得越来越高, 因此丛枝菌根真菌通常不会改善寄主植物的磷营养来增加生物固氮^[46]。在禾本科/豆科间作系统中, 豆科作物共生固氮的增加会使作物生长受到磷的限制, 并且豆科作物在养分和其他资源的竞争方面均弱于禾本科作物, 因此间作豆科作物会增加对丛枝菌根共生体的依赖以克服自身养分需求的局限性^[10,16]。如在绿豆和水稻间作系统中, 根系交互作用增加绿豆根部丛枝菌根真菌的侵染, 使总磷和氮吸收量分别增加了65%和64%, 进而使绿豆结瘤率增加了54%^[29]。

2.3 豆科作物固氮基因的表达

在禾本科/豆科间作系统中, 豆科作物根瘤的形成受到禾/豆互作双方分子对话的影响, 而这些分子对话过程直接与根际氮素水平密切相关^[47]。大量的研究表明土壤中高浓度的硝态氮和铵态氮会抑制豆科作物结瘤固氮^[48,49]。这些抑制作用可以分为早期下调固氮酶活性和后期诱发根瘤衰老。在施用硝酸盐的早期阶段, 结瘤基因表达发生了强烈的转变, 最显著的作用是早期结瘤蛋白基因*ENOD93*和根瘤特异性富含半胱氨酸肽(*NCR*)基因表达的下调, 从而影响根瘤菌的新陈代谢和固氮酶功能^[50]。同时硝酸盐还能引起豆血红蛋白基因表达的下调, 而豆类血红蛋白基因沉默已被证明会导致根瘤内固氮酶活性的丧失和氧含量的增加^[51]。此外, 高浓度硝酸盐中生长的野生型百脉根在受结瘤因子(NF)诱导后无早期结瘤素基因*NIN*的表达; 而对硝酸盐具有耐受性的超结瘤突变株来说, 即使在培养基中添加高浓度硝酸盐的情况下, *NIN*基因仍能被高水平诱导, 说明*NIN*基因在硝态氮作用早期抑制信号途径中起关键作用^[52]。在施用硝酸盐的后期阶段, 根瘤活性进一步降低, 差异表达基因的数量几乎增加了3倍, 特别是新出现了一些与根瘤衰老相关基因的差异表达^[53]。土壤中较低浓度的矿质氮能促进豆科植物的结瘤和固氮^[11,23]。新近研究发现在蚕豆根际氮含量较低时, 添加玉米的根分泌物在早期阶段可上调查尔酮-黄酮异构酶*CFT*基因表达刺激类黄酮的生物合成, 并迅速触发结瘤过程。同时豆科作物早期结瘤素基因*ENODL2*, *ENOD93*和生长素响应基因*GH3.1*表达显著增加, 说明植物在生长素信号的调节下开始结瘤。根瘤成熟时, 玉米根系分泌物继续上调蚕豆根部关键结瘤基因*NODL4*和固氮基因*FixI3*从而促进蚕豆结瘤及固氮作用^[15]。

(图2).

3 总结与展望

禾本科/豆科间作能提高豆科作物氮素固定,减少无机氮肥的使用,从而保证粮食安全、促进可持续农业的发展. 禾本科/豆科间作系统提高豆科作物固氮的3种主要途径是根际氮素耗竭作用、根际磷素活化作用、固氮基因表达的提升. 这3种途径在固氮增加的过程中层层递进,相辅相成(图2). 目前间作提高豆科植物共生固氮的主要理论解释为根际氮素耗竭作用,即禾本科作物通过耗竭豆科作物根际土壤矿质氮,降低豆科作物根际氮含量,从而促进豆科共生固氮. 然而根际氮素耗竭作用只是间作促进豆科作物固氮过程中普遍

存在的表面现象,其背后还蕴藏着化学计量学(如氮磷耦合)和分子调控机理(图2). 一方面,间作豆科作物为了维持体内稳定氮磷化学计量关系,而采取不同根际磷吸收策略,即通过根系分泌物或丛枝菌根真菌增加对土壤磷素的获取满足其自身固氮对磷的大量需求. 另一方面,禾本科作物对豆科作物根际氮素耗竭能解除高浓度硝态氮对早期结瘤素基因(*NIN*, *ENOD93*)表达的抑制,激活关键结瘤和固氮基因(*NOD*, *Fix*)的表达,从而促进豆科作物的结瘤和共生固氮. 综上所述,将间作作物的根际氮素耗竭作用和根际磷素活化作用与共生固氮基因的分子调控过程相联系起来,有助于丰富和发展间作体系固氮理论,为优化间作系统氮素吸收和利用提供了最有前景的途径.

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补充材料

表S1 不同禾本科/豆科间作系统对氮固定的影响(大田试验)

表S2 不同禾本科/豆科间作系统对氮固定的影响(盆栽试验)

表S3 不同禾本科/豆科间作提高豆科结瘤和共生固氮的机理

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Summary for “禾本科/豆科间作促进豆科共生固氮机理研究进展”

Advances in the mechanism of cereal/legume intercropping promotion of symbiotic nitrogen fixation

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Biological nitrogen fixation by rhizobia in symbiotic association with legumes is a valuable N source for agroecosystems, and thus it is crucial for increased sustainability of agricultural production. Previous studies found that legume and cereal intercropping can promote symbiotic nitrogen fixation mainly due to rhizosphere nitrogen depletion by the cereal and that soil mineral nitrogen is absorbed more by cereals than legumes, which forces the legume to increase its reliance on symbiotic nitrogen fixation. However, in addition to the nitrogen depletion theory in the rhizosphere, stoichiometry (such as nitrogen and phosphorus coupling) and molecular regulation mechanisms are also important in understanding legume nitrogen fixation. The legume usually maintains a stable stoichiometric relationship between nitrogen and phosphorus. Symbiotic nitrogen fixation of legumes tends to have a high demand for phosphorus, mainly because nitrogen fixation by legumes is a high energy-consuming process, and the required energy is directly provided by ATP. Therefore, when the phosphorus obtained by the intercropped legume is insufficient to meet the increase in symbiotic nitrogen fixation, legume growth is limited by phosphorus. Under phosphorus limitation, the phosphorus uptake strategy of the legume can be stimulated, i.e. by increasing the infection of arbuscular mycorrhizal fungi (AMF) and secretion of root exudates. The AMF mycelia of legumes can extend beyond the rhizosphere, linking the roots to the surrounding soil microhabitats and expanding the area of phosphorus absorbed by the roots. On the other hand, mobilizing rhizosphere insoluble phosphorus through root exudates is also an important pathway for improving phosphorus utilization. Increased soil phosphorus availability promotes plant growth and increased allocation of carbon (C) sources to roots and nodules, resulting in a larger root system or greater nodule formation, finally higher symbiotic nitrogen fixation. In addition, the formation of root nodules is influenced by rhizosphere talk between the cereal and legume, which are directly related to the level of nitrogen. A recent study found that under low N concentration conditions, cereal root exudates stimulated flavonoid biosynthesis and secretion partly through upregulating expression of the Chalcone-flavanone isomerase gene (*CFI*) in legumes. Meanwhile, an auxin-responsive *GH3* family gene (*GH3.1*) and the nodulation gene *ENODL2* displayed significant increases, suggesting that nodulation continues under the regulation of auxin signaling and the nodulin-like protein. At nodule maturity, cereal root exudates continue to up-regulate key nodulation genes, such as *NODL4*, *ENOD93* and the N fixation gene *FixI3*, finally promoting symbiotic nitrogen fixation. Moreover, a high concentration of soil mineral N significantly suppressed expression of the early nodulin gene *NIN* in *Lotus corniculatus*, which inhibited nodulation and symbiotic nitrogen fixation. In the cereal/legume intercropping system, rhizosphere nitrogen depletion can relieve the inhibition of nitrate on the expression of key nodulation and N fixation genes, thus promoting nodulation and symbiotic nitrogen fixation. In conclusion, intercropping can promote nitrogen uptake and optimize nitrogen use efficiency through the coupling effects of rhizosphere nitrogen depletion, phosphorus mobilization and the regulation of nitrogen fixation genes. This review will help improve our understanding of the mechanism of symbiotic nitrogen fixation in the cereal/legume intercropping system.

symbiotic nitrogen fixation, root exudates, arbuscular mycorrhizal fungi, intercropping, rhizosphere process

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