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接种华癸中慢生根瘤菌AW-8对紫云英氮含量 及根际土壤养分的影响

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摘要 为探究接种华癸中慢生根瘤菌(*Mesorhizobium huakuii*)对田间种植紫云英的效应,对华癸中慢生根瘤菌7653R进行复壮,获得菌株AW-8与AW-10,进行回接试验,验证其共生结瘤固氮能力,以AW-8为核心菌株,通过土培盆栽比较4株华癸中慢生根瘤菌的共生表型;通过田间试验,在施加0、3、6 kg/667 m²尿素氮水平下比较华癸中慢生根瘤菌AW-8、AW7R-1、AB-21及AZ-21的接种效应,结果显示:接种AW-8的紫云英地上部鲜质量和结瘤固氮指标均高于接种出发菌株AW7R-1,且AW-8、AB-21、AZ-21均为优良菌株,其中信紫1号紫云英接种AW-8后,地上部分鲜质量、根瘤数、根瘤质量及根瘤固氮酶活性分别提升41.3%、87.8%、34.2%和6.3%;施加3 kg/667 m²尿素并接种AW-8时,紫云英地上部干草氮含量与氮积累量分别提高9.5%和4.1%,同时根际土壤有效磷、速效钾及有机质含量分别增加2.6%、35.9%和0.5%。综合分析表明,接种华癸中慢生根瘤菌AW-8配合施用3 kg/667 m²尿素,可协同提升紫云英植株氮含量及氮积累量,并改善根际土壤养分状况。因此,华癸中慢生根瘤菌AW-8接种与适量氮肥配施在紫云英种植中具有增效作用。

关键词 绿肥;紫云英;共生固氮;华癸中慢生根瘤菌;植株氮含量;根际土壤养分

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在我国农业可持续发展和化肥农药双减的时代背景下,农业生产正从依赖化学肥料向生态友好型的绿肥种植转变,这一转变旨在提高农业生产效率的同时,减少对环境的负面影响。绿肥种植不仅能够改善土壤微生物群落结构和营养结构,还能显著提高作物产量。研究表明,绿肥的种植可使玉米作物的产量提高31.3%,这充分体现了绿肥在可持续农业实践中的重要性^[1]。

紫云英(*Astragalus sinicus* L.)作为一种豆科植物,能够形成高效的共生固氮体系,广泛种植于长江中下游和华南地区,主要用作水稻绿肥,同时也是重要的蜜源植物和饲料。紫云英在亚洲多个国家和地区,如日本、越南和朝鲜半岛等也有分布。研究证明紫云英绿肥在提高作物生产力方面具有巨大潜力^[2-3]。在水稻种植中,将紫云英与水稻种子以适当比例混播,可替代部分化学肥料,在减少化学氮肥用

量的同时,水稻产量也能显著提升^[4-5]。紫云英与水稻的混作和轮作模式在中国长江中下游地区较为常见,紫云英衰老后还田处理,不仅有利于后茬作物生长,还能显著改善土壤质量,这是化学氮肥难以比拟的^[3-5]。

在初次种植紫云英的土壤中,有效接种根瘤菌是种植成功的关键。即使在多年种植紫云英的地区,人工接种优良菌株也能进一步提高紫云英地上部产量。陈华癸首次在中国分离出紫云英根瘤菌的有效菌株^[6-7]。Chen等^[8]、Jarvis等^[9]基于根瘤菌系统发育研究,将这一物种归入新建立的*Mesorhizobium*属,命名为华癸中慢生根瘤菌(*Mesorhizobium huakuii*)。

华癸中慢生根瘤菌7653R具有高效的固氮能力,能够与紫云英形成不定型根瘤,且具有相对较窄的宿主范围和共生物质^[10]。自20世纪80年代以来,

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学者们对紫云英根瘤菌 7653R 接种应用^[11]、生物学、生态学^[12]、生理学、遗传学^[12-13]和菌植互作分子机制^[14-18]等开展了深入研究,结果表明 7653R 菌株与多数紫云英品种共生时,在提升作物产量和固氮能力方面具有显著优势^[19]。目前关于接种华癸中慢生根瘤菌对田间种植紫云英含氮量及紫云英根际土壤养分的影响,特别是在不同氮肥水平下华癸中慢生根瘤菌的接种效应研究较少,本研究以华癸中慢生根瘤菌复壮菌株 AW-8 为核心菌株,比较华癸中慢生根瘤菌原始菌株和复壮菌株对于紫云英生长表型的影响,利用田间小区试验验证复壮菌株对紫云英植株氮含量、氮积累量和改善根际土壤养分状况的作用,旨在为优良华癸中慢生根瘤菌+适量氮肥施用的应用模式提供一定的科学依据和技术指导。

1 材料与方法

1.1 华癸中慢生根瘤菌

AW7R-1、AW7R-2 和 AH-2 为华中农业大学农业微生物资源发掘与利用全国重点实验室保存的常用华癸中慢生根瘤菌菌株,AW-8 和 AW-10 是本研究复壮的华癸中慢生根瘤菌菌株。华癸中慢生根瘤菌菌株 AB-21 由中国农业大学陈文峰博士惠赠,华癸中慢生根瘤菌菌株 AZ-21 由郑州轻工业大学张俊杰博士惠赠。

1.2 紫云英品系

本研究中采用 2 个紫云英(*Astragalus sinicus*)品系,即信紫 1 号和弋江品系。

1.3 华癸中慢生根瘤菌复壮

配制 SM 或 YMA 固体培养基,在培养基中加入微量结晶紫溶液(约 1/100 000),以培养基略呈紫色为宜。将待分菌的根瘤从根上剪下时应略带一点根系,投入灭菌 PA 瓶中,用 5% (V/V) NaClO 表面清洗 1~2 min,再用无菌水洗涤 8~10 次。用火焰灼烧过的小镊子夹出根瘤,放 SM 或 YMA 平板上,用镊子夹破,使根瘤汁液涂于平板上,用封口膜封口后置于 28 ℃ 培养约 2~4 d,长出菌苔,用无菌水稀释后涂布 SM 固体平板培养基,进行染色镜检、生理生化鉴定。采用质粒快检方法对紫云英复壮根瘤菌中的共生固氮大质粒进行检测。采用通用引物 27F: AGAGTTGATCMTGGCTCAG; 1492R: TACG-GYTACCTGTTACGACTT 对紫云英复壮根瘤菌进行 16S rDNA 测序分析,确认其种属地位。

1.4 复壮根瘤菌回接试验

选用双层钵种植紫云英,对河沙进行清洗、灭菌处理,保证种植过程严格无菌,在 16 h 光照/8 h 暗期的光照培养室培养。在种植后约第 6 天接种根瘤菌,在接菌后第 28 天收获紫云英,对紫云英植株长势、根系及根瘤进行拍照,同时测定紫云英植株地上部鲜质量、根瘤数、根瘤质量。采用乙炔还原法测定紫云英根瘤的固氮酶活性。

1.5 土培盆栽试验

采用普通花盆种植紫云英,模拟田间生态环境,选用水稻土作为基质,在 16 h 光照/8 h 暗期光照培养室培养。按照盆栽回接试验的相同收获方式,收获植株并统计和分析共生固氮表型指标。

1.6 田间小区试验

田间试验在湖北省随州市曾都区(113°14'46"E、31°19'58"N)进行。2 个试验因素为华癸中慢生根瘤菌和不同氮肥水平。华癸中慢生根瘤菌包括 4 个菌株 AW-8、AW7R-1、AB-21 和 AZ-21,不接种根瘤菌为对照。土壤施氮水平设置 3 个,分别为施氮(尿素)量 0、3、6 kg/667 m²,分别记为 N1、N2 和 N3。所有处理均施 3 kg/667 m² 磷肥做基肥。采用完全随机区组方案,共 15 个处理,每个处理 3 次重复,每个重复 20 m²。共 2 块试验田,试验田 1 为重复 1,试验田 2 为重复 2 和重复 3。试验田 1:南侧共 12 个小区,规格为 4 m×5 m,北侧共 3 个小区,规格为 2 m×10 m;试验田 2:西侧 15 个小区为重复 2,东侧 15 个小区为重复 3,规格为 4 m×5 m。紫云英采用信紫 1 号品系,播种量 2 kg/667 m²。华癸中慢生根瘤菌接种的培养物活菌数为 2×10⁹ CFU/mL,接种量为 20 mL/667 m²,拌种紫云英种子应用。

1.7 土壤养分测定

紫云英根际土壤样品的有机质、氮磷钾等养分指标测定由农业农村部微生物产品质量检验测试中心(武汉)完成。采用凯氏定氮法对根际土壤含氮量进行测定。联合浸提-比色法对根际土壤有效磷和速效钾含量进行测定。采用重铬酸钾容量法对根际土壤有机质含量进行测定。

1.8 数据分析

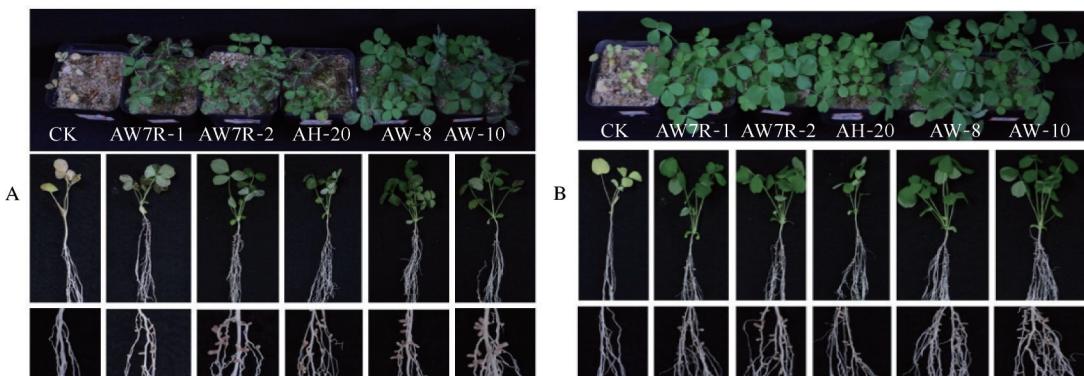
采用 Excel 2010 和 Prism Graphpad 进行数据统计分析。采用多重比较方法对平均值进行差异显著性分析。

2 结果与分析

2.1 复壮根瘤菌 AW-8 结瘤固氮能力的鉴定

在前期研究中,基于盆栽试验和从根瘤中分离纯化目标根瘤菌,得到2个优良的复壮紫云英根瘤菌候选菌株AW-8、AW-10。基于科赫法则思想,采用盆栽试验进一步测定AW-8、AW-10菌种的共生表型。选用复壮前菌株AW7R-1及笔者所在实验室保存的另外2个紫云英根瘤菌菌株AW7R-2、AH-20作

为对照菌株。由图1可见,接种AW-8和AW-10的紫云英植株地上部分长势较好、根系相对发达,形成根瘤较多。信紫1号品系紫云英接种AW-8、AW-10与接种AW7R-1、AW7R-2、AH-20处理相比较,地上部分鲜质量均显著增加;弋江品系同样如此。无论是信紫1号品系还是弋江品系的紫云英,在接种AW-8、AW-10菌株后,其根瘤数均高于接种其他菌株处理。2个品系紫云英接种AW-8、AW-10的根瘤固氮酶活性也高于其他菌株(图2)。



A: 接种信紫1号品系紫云英的盆栽表型;B: 接种弋江品系紫云英的盆栽表型。A: Phenotype of the Xinzi No. 1 variety of *A. sinicus* in pot system inoculation with different rhizobia strains. B: Phenotype of the Yijiang variety of *A. sinicus* in pot system inoculation with different rhizobia strains.

图1 不同华癸中慢生根瘤菌株的盆栽回接紫云英共生表型

Fig.1 Symbiotic phenotypes of *Astragalus sinicus* inoculated with the reinvigorated *Mesorhizobium huakuii* strains in pot system

盆栽回接试验结果表明,接种复壮紫云英根瘤菌株后紫云英的共生表型均优于接种出发菌株和对照菌株。结果还显示,根瘤菌AW-8与信紫1号品系紫云英共生效应相对较好,AW-10与弋江品系紫云英共生效应相对较好。由于笔者所在实验室常以信紫1号品系紫云英为试验材料,因此,选用AW-8菌株开展后续研究。

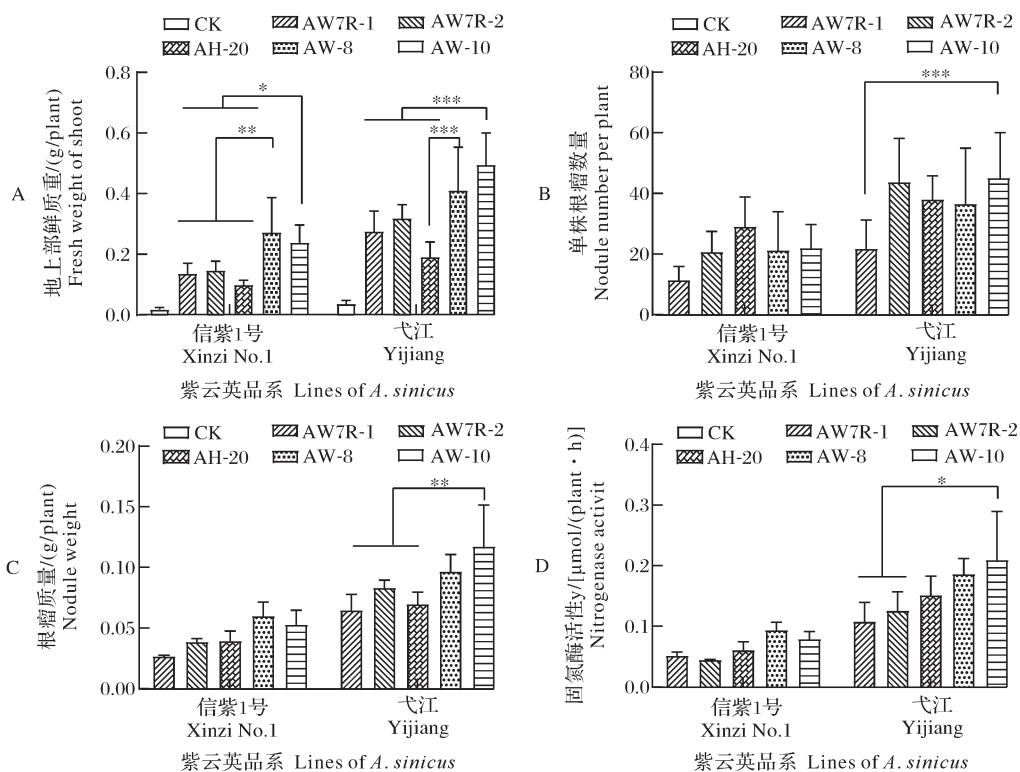
2.2 土壤盆栽系统中根瘤菌 AW-8 结瘤固氮能力评价

选取水稻田自然土壤不做灭菌处理,信紫1号和弋江2个品系紫云英分别接种AW-8、AW7R-1、AB-21、AZ-21菌株,于28 d后收获紫云英植株测定结瘤固氮表型。由于土壤未经灭菌处理,其中含有土著根瘤菌,未接菌的空白对照组(CK)植株能够结瘤固氮,但长势明显弱于接种其他根瘤菌处理组。人工接种4个紫云英根瘤菌的紫云英叶片呈绿色且生长旺盛,根系上形成有固氮能力的红色根瘤(图3)。

对接种4个不同根瘤菌的紫云英结瘤固氮表型进行测定,结果(图4)显示,信紫1号品系紫云英接种

根瘤菌AW-8的植株地上鲜质量为0.277 g/plant,相较于接种AW7R-1处理(植株地上鲜质量为0.196 g/plant)增加41.3%。弋江品系紫云英接种根瘤菌AW-8、AB-21、AZ-21的植株地上部鲜质量均高于接种根瘤菌AW7R-1。信紫1号和弋江品系紫云英接种AW-8的根瘤数和根瘤质量均高于接种AW7R-1。其中,信紫1号品系紫云英接种AW-8的根瘤数和根瘤质量分别为13.9个/plant和0.051 g/plant,比接种AW7R-1的根瘤数(7.4个/plant)和根瘤质量(0.038 g/plant)分别增加87.8%和34.2%。接种根瘤菌AW-8的紫云英根瘤固氮酶活为0.0051 $\mu\text{mol}/(\text{plant}\cdot\text{h})$,相较于接种AW7R-1的根瘤固氮酶活[0.0048 $\mu\text{mol}/(\text{plant}\cdot\text{h})$]提升6.3%。

综上可知,土培盆栽试验结果表明,接种根瘤菌AW-8的2个品系紫云英的地上部分生物量、结瘤固氮表型均高于接种出发菌株AW7R-1,表明AW7R-1菌种的共生固氮能力得到了有效复壮。同时也可看出AW-8、AB-21和AZ-21均是比较优良的紫云英根瘤菌菌株。



A:紫云英植株地上部鲜质量;B:结瘤数;C:根瘤质量;D:根瘤固氮酶活。*表示在不同处理之间存在显著差异(*;P<0.05;**;P<0.01;***;P<0.001;****;P<0.0001),误差线表示平均值±标准差,下同。A: Fresh weight of the aerial parts of *A. sinicus* plants; B: Number of nodules on the plants; C: Weight of root nodules on the plants; D: Nitrogenase activity of root nodules on the plants. Asterisks denote significant differences in different treatments (*; P<0.05; **; P<0.01; ***; P<0.001; ****; P<0.0001). Error bars represent means ± SD. The same as below.

图2 接种不同华癸中慢生根瘤菌株的紫云英盆栽共生表型定量分析

Fig. 2 Quantitative analysis of symbiotic phenotypes of *A. sinicus* inoculated with the reinvigorated *M. huakui* strains in pot experiments

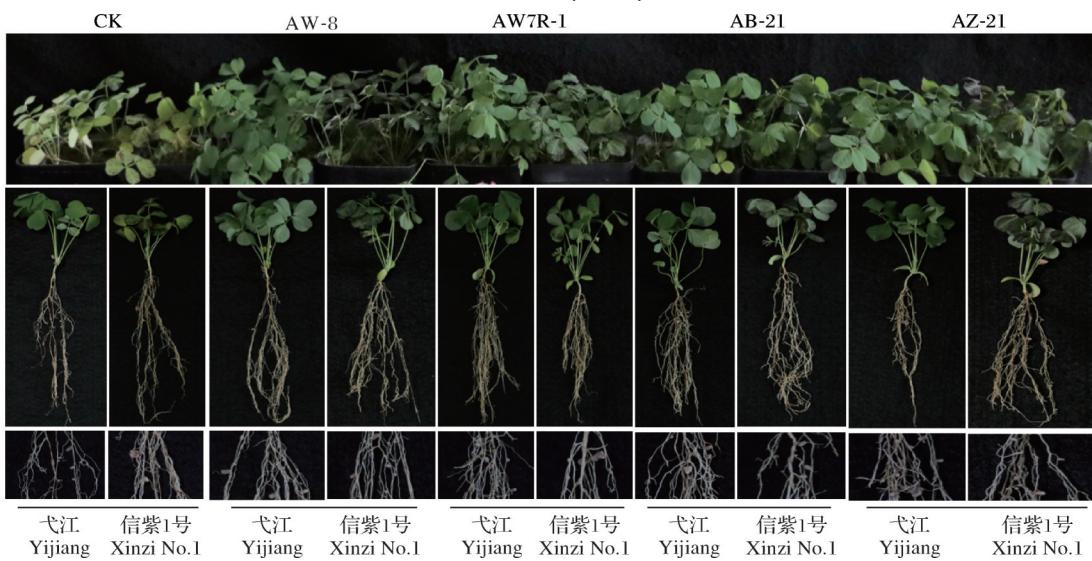


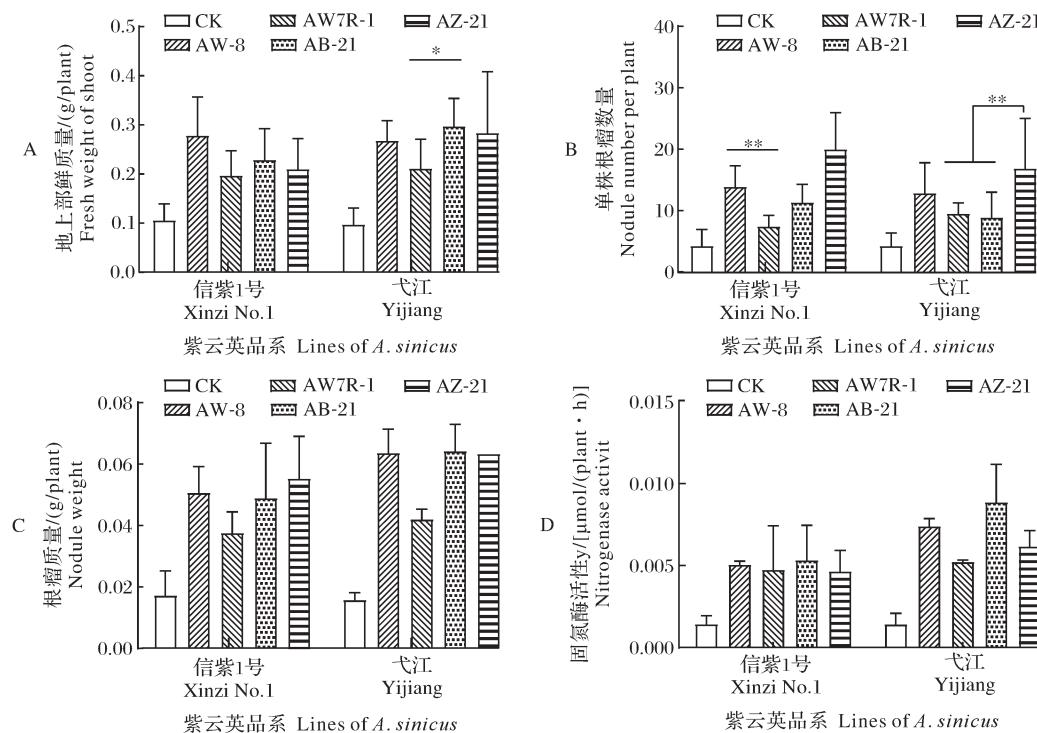
图3 在土壤盆栽系统中紫云英接种华癸中慢生根瘤菌AW-8的共生表型

Fig. 3 Symbiotic phenotypes of *A. sinicus* inoculated with *M. huakui* strain AW-8 in a natural soil pot system

2.3 田间试验中华癸中慢生根瘤菌AW-8对紫云英含氮量和氮积累量的影响

为探究华癸中慢生根瘤菌接种在田间应用中对

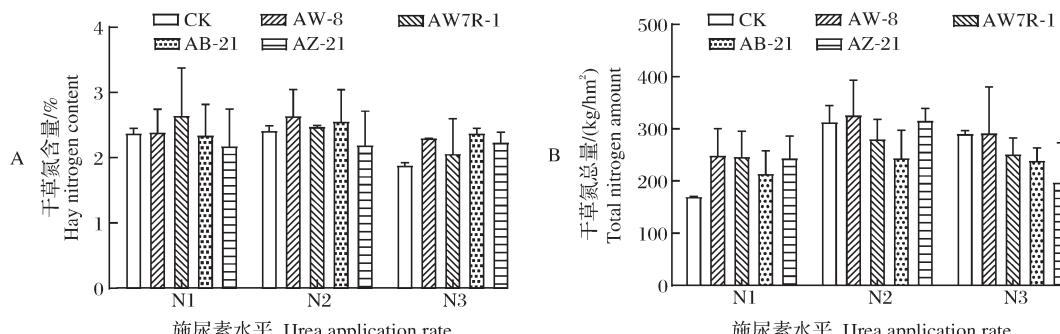
紫云英含氮量和氮积累量的作用及不同氮肥水平对这种作用的影响差异,开展了田间小区试验。由图5可见,在不施加氮肥条件下(N1),接种华癸中慢生根



A:紫云英植株地上部鲜质量; B:植株结瘤数; C:植株根瘤质量; D:植株根瘤固氮酶活。A: Fresh weight of the aerial parts of *A. sinicus* plants; B: Number of nodules on the plants; C: Weight of root nodules on the plants; D: Nitrogenase activity of root nodules on the plants.

图4 在土壤盆栽系统中紫云英接种不同华癸中慢生根瘤菌株的共生表型定量分析

Fig. 4 Quantitative analysis of symbiotic phenotypic indicators of *A. sinicus* inoculated with *M. huakuii* strains in a soil pot system



N1 表示小区底肥尿素施加水平为 0 kg/667 m², N2 表示小区底肥尿素施加水平为 3 kg/667 m², N3 表示小区底肥尿素施加水平为 6 kg/667 m²。下同。A:紫云英地上干草氮含量;B:紫云英的总含氮量。N1 indicates a urea application rate of 0 kg/667 m² as the basal fertilizer in the plot, N2 indicates a urea application rate of 3 kg/667 m² as the basal fertilizer in the plot and N3 indicates a urea application rate of 6 kg/667 m² as the basal fertilizer in the plot. The same as below. A: Hay nitrogen content of *A. sinicus*; B: Total nitrogen amount of *A. sinicus*.

图5 接种华癸中慢生根瘤菌及不同氮肥水平下紫云英地上干草氮含量和氮积累量

Fig. 5 The nitrogen content and total nitrogen amount of the aerial hay of *A. sinicus* in field experiments under inoculation with *M. huakuii* strains and different nitrogen fertilizer levels

瘤菌 AW-8 等 4 个菌株的紫云英总氮含量都高于对照, 证明了华癸中慢生根瘤菌的共生固氮和促生长作用。在施加 3 kg/667 m² 尿素条件下(N2), 接种 AW-8 菌株的紫云英干草含氮量为 2.65%, 氮积累量为 326.63 kg/hm², 均高于对照组(干草含氮量为 2.42%, 氮积累量为 313.63 kg/hm²), 分别增加 9.5%

和 4.1%。表明接种根瘤菌 AW-8 同时配施适量氮肥能够进一步提高紫云英植株的氮积累量。在施加 6 kg/667 m² 尿素条件下(N3), 接种 AW-8、AB-21 和 AZ-21 菌株的紫云英干草含氮量都高于对照组, 但接种 AW-8 菌株的紫云英氮积累量与对照组处于相同水平, 提示在高氮肥条件下, 不利于紫云英氮积累。

2.4 华癸中慢生根瘤菌AW-8对田间试验紫云英根际土壤养分含量的影响

1)田间试验不同处理下紫云英根际土壤氮含量。由图6可见,在不施加氮肥条件(N1)下,接种华癸中慢生根瘤菌的紫云英根际氮含量高于不接菌对照组。在施加3 kg/667 m²尿素条件(N2)下,对照组紫云英根际土壤氮含量高于接种根瘤菌的处理。在施加6 kg/667 m²尿素条件(N3)下,接种AW-8、AW7R-1及AZ-21菌株的紫云英根际土壤氮含量均高于对照组,且略高于N2氮肥水平。结果表明,在N1和N3水平条件下,接种华癸中慢生根瘤菌有助于提高共生宿主紫云英根际土壤氮含量;在N2条件下接种华癸中慢生根瘤菌可能间接促进紫云英对土壤氮素的吸收。

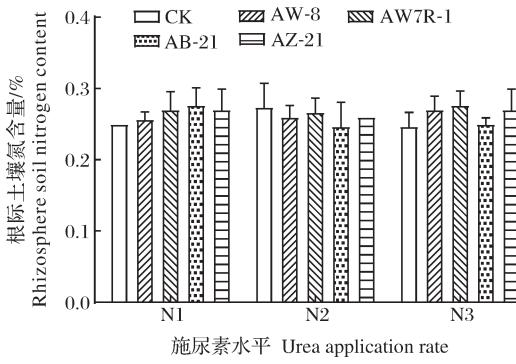


图6 接种华癸中慢生根瘤菌及不同氮肥水平下紫云英根际土壤氮含量

Fig.6 Nitrogen content in the rhizosphere soil of *A. sinicus* in the field experiments under inoculation with *M. huakuii* strains and different nitrogen fertilizer levels

2)田间试验不同处理下紫云英根际土壤磷含量。由图7可见,在3种氮肥水平条件下,未接种根瘤菌对照组的紫云英根际土壤有效磷含量没有变化,在不施加氮肥的条件(N1)下,接种AW-8菌株的紫云英根际土壤有效磷含量高于对照组;在施加3 kg/667 m²尿素条件(N2)下,接种华癸中慢生根瘤菌AW-8的紫云英根际土壤有效磷含量为71.6 mg/kg,相较于对照组(根际土壤有效磷含量69.8 mg/kg)增加2.6%;在施加6 kg/667 m²尿素条件(N3)下,接种华癸中慢生根瘤菌AW-8的紫云英根际土壤有效磷含量最高,根际土壤有效磷含量为79.5 mg/kg,相较于对照组(根际土壤有效磷含量为67.4 mg/kg)提高了18.0%。表明在不同氮肥条件下接种AW-8均可促进宿主紫云英根际土壤有效磷的积累。

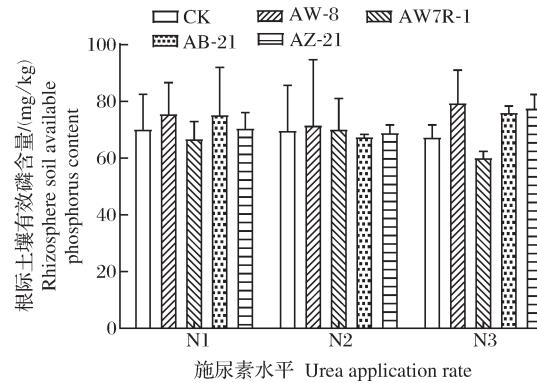


图7 接种华癸中慢生根瘤菌及不同氮肥水平下紫云英根际土壤有效磷含量

Fig.7 Available phosphorus content in the rhizosphere soil of *A. sinicus* in the field experiments Under inoculation with *M. huakuii* strains and nitrogen fertilizer levels

3)田间试验不同处理下紫云英根际土壤速效钾含量。由图8可见,在不施加氮肥的条件(N1)下,与对照组相比,接种AW-8等4个华癸中慢生根瘤菌均可提高紫云英根际土壤速效钾的含量;在施加3 kg/667 m²尿素条件(N2)下,接种AW-8菌株的紫云英根际土壤速效钾含量最高,根际土壤速效钾含量为628 mg/kg,相较CK对照组(根际土壤速效钾含量为462 mg/kg)提高35.9%;在施加6 kg/667 m²尿素条件(N3)下,接种AW-8的紫云英根际土壤速效钾含量亦最高,根际土壤速效钾含量为674 mg/kg,相较于CK对照组(根际土壤速效钾含量376 mg/kg)提高了79.3%。结果表明接种华癸中慢生根瘤菌AW-8在3个氮肥水平下均可提高紫云英根际土壤速效钾含量,在N2和N3条件下的促进效应更强,且相较于

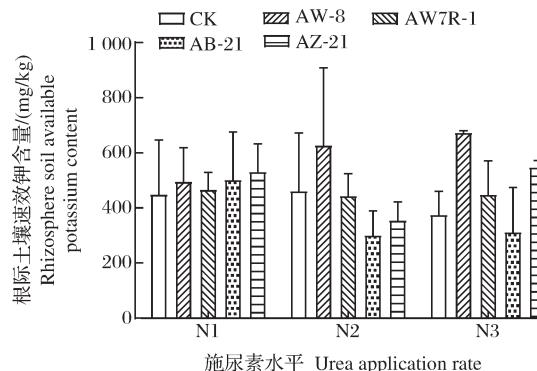


图8 接种华癸中慢生根瘤菌及不同氮肥水平下紫云英根际土壤速效钾含量

Fig.8 Available potassium content in the rhizosphere soil of *A. sinicus* in field experiments under inoculation with *M. huakuii* strains and nitrogen fertilizer levels

其他根瘤菌,接种AW-8的紫云英根际土壤速效钾的含量更高。

4)田间试验不同处理下紫云英根际土壤有机质含量。由图9可见,在不接种根瘤菌的对照组,紫云英根际土壤有机质含量在3个氮肥水平(N1、N2和N3)条件下比较稳定。在不施加氮肥条件(N1)下,与对照组相比,接种AZ-21菌株可提高紫云英根际土壤有机质含量;在施加3 kg/667 m²尿素条件(N2)下,与CK对照组相比,接种AW-8、AW7R-1的紫云英根际土壤有机质含量均有所提高,其中接种AW-8的紫云英根际土壤有机质含量为37.7 g/kg,相比较对照组(根际土壤有机质含量为37.5 g/kg)提高0.5%;在施加6 kg/667 m²尿素条件(N3)下,接种AW-8等4个华癸中慢生根瘤菌的紫云英根际土壤有机质含量高于对照组。

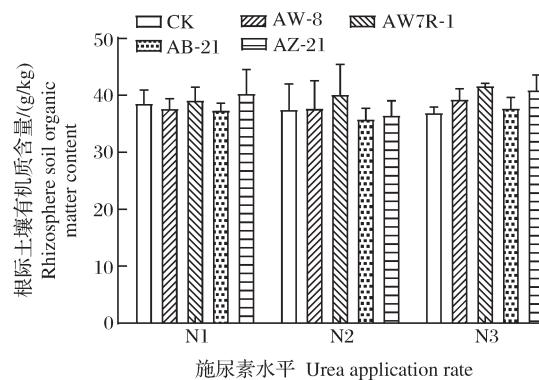


图9 接种华癸中慢生根瘤菌及不同氮肥水平下紫云英根际土壤有机质含量

Fig.9 The organic matter content in the rhizosphere soil of *A. sinicus* in the field experiments under inoculation with *M. huakuii* strains and nitrogen fertilizer levels

3 讨论

根瘤菌经过长期人工培养与转接或在非共生环境中,其优良性状可能会逐渐退化,导致其侵染能力、结瘤能力和固氮酶活性下降。原因可能是根瘤菌共生相关基因突变^[20]或共生产质粒发生部分缺失或质粒丢失。通过植物盆栽试验,采用宿主体内根瘤菌复壮是特定有效的方法,将根瘤菌液体培养物接种到相应的豆科宿主植物上侵染结瘤,选择长势旺盛的宿主植物,从其根系上所结根瘤中分离出根瘤菌,其结瘤固氮性能可以恢复甚至提高。本研究采用此方法,将笔者所在实验室长期使用的优良华癸中慢生根瘤菌7653R进行复壮,获得了复壮菌株

AW-8和AW-10。

本研究结果表明,接种复壮后的华癸中慢生根瘤菌AW-8与紫云英共生,能够提高紫云英地上部干草含氮量以及氮积累量,表明AW-8具有高效固氮能力;它能够与紫云英根系互作形成根瘤,通过根瘤的共生固氮作用,将大气中的氮气转化为植物可利用的氨态氮^[21],为紫云英提供充足的氮源。同时,接种华癸中慢生根瘤菌AW-8对紫云英根际土壤中氮磷钾养分也产生一定的促进作用,这可能是通过促进土壤根际其他有益微生物的生长,间接提高了土壤肥力。AW-8等华癸中慢生根瘤菌可能与解磷菌^[22]、解钾菌协同作用,促进土壤中磷和钾的释放,或可能诱导植物紫云英根系分泌更多的有益物质,从而促进土壤中氮磷钾和有机质的积累。这种诱导作用因菌种而异,导致增效作用的差异。

本研究还发现不同氮肥水平下,接种华癸中慢生根瘤菌对紫云英植株地上部含氮量增效作用不同。原因可能是高氮施用对根瘤菌结瘤固氮能力具有一定抑制作用^[23];另外,不同根瘤菌菌株的共生固氮能力存在差异^[24],可能导致氮素增效效果不同,固氮能力强的菌种能够更有效地增加宿主紫云英的氮含量。固氮效率不仅取决于菌种本身,还与土壤环境和植物根系分泌物有关,不同菌种对这些环境因素的适应性不同^[25],从而影响其固氮效率。

综上,华癸中慢生根瘤菌AW-8接种与适量氮肥配施在紫云英绿肥种植中具有良好的应用前景。在该模式下,接种华癸中慢生根瘤菌AW-8能够提高紫云英地上部干草含氮量及氮积累量,一定程度上促进紫云英根际土壤氮磷钾养分累积。这可能是多种因素综合作用的结果,这些因素包括根瘤菌的固氮能力、根际微生物群落的相互作用及根系分泌物的影响等。未来将进一步探究华癸中慢生根瘤菌与绿肥作物共生增效作用机制,从而进一步优化豆科绿肥根瘤菌的应用技术。

参考文献 References

- [1] TAO J M, LIU X D, LIANG Y L, et al. Maize growth responses to soil microbes and soil properties after fertilization with different green manures[J]. Applied microbiology and biotechnology, 2017, 101(3):1289-1299.
- [2] XIE Z J, ZHOU C H, SHAH F, et al. The role of Chinese milk vetch as cover crop in complex soil nitrogen dynamics in rice rotation system of South China[J/OL]. Scientific reports, 2018, 8: 12061 [2025-06-11]. <https://doi.org/10.1038/>

- s41598-018-30239-6.
- [3] LV M H, WANG Y D, CHEN X F, et al. The moderate substitution of *Astragalus sinicus* returning for chemical fertilizer improves the N cycle function of key ecological bacterial clusters in soil [J/OL]. *Frontiers in microbiology*, 2022, 13: 1067939 [2025-06-11]. <https://doi.org/10.3389/fmicb.2022.1067939>.
- [4] CHEN J R, QIN W J, CHEN X F, et al. Application of Chinese milk vetch affects rice yield and soil productivity in a subtropical double-rice cropping system [J]. *Journal of integrative agriculture*, 2020, 19(8): 2116-2126.
- [5] XIE J, LIANG F, XIE J J, et al. Yield variation characteristics of red paddy soil under long-term green manure cultivation and its influencing factors [J/OL]. *International journal of environmental research and public health*, 2022, 19 (5) : 2812 [2025-06-11]. <https://doi.org/10.3390/ijerph19052812>.
- [6] CHEN H K, SHU M K. Note on the root-nodule bacteria of *Astragalus sinicus* l[J]. *Soil science*, 1944, 58(4): 291-294.
- [7] 陈华癸. 微生物学[M]. 北京: 高等教育出版社, 1959. CHEN H G. Microbiology [M]. Beijing: Higher Education Press, 1959 (in Chinese).
- [8] CHEN W X, LI G S, QI Y L, et al. *Rhizobium huakuii* sp.nov. Isolated from the root nodules of *Astragalus sinicus* [J]. *International journal of systematic bacteriology*, 1991, 41 (2) : 275-280.
- [9] JARVIS B D W, VAN BERKUM P, CHEN W X, et al. Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. Nov. [J]. *International journal of systematic and evolutionary microbiology*, 1997, 47 (3) : 895-898 (in Chinese with English abstract).
- [10] 周俊初, 张忠明, 黄诚金, 等. 紫云英根瘤菌质粒的研究: II. 紫云英根瘤菌(*Rhizobium astragali*)经高温和吖啶橙处理后结瘤和固氮突变株的产生和质粒的消除 [J]. 华中农业大学学报, 1987, 6 (2) : 156-164. ZHOU J C, ZHANG Z M, HUANG C J, et al. Studies on *Rhizobium* plasmids: II .Tests of plasmid elimination of *R. astragali* [J]. *Journal of Huazhong Agricultural University*, 1987, 6(2): 156-164 (in Chinese with English abstract).
- [11] CHEN H K, LI F D, CAO Y Z. Characteristics, distribution, ecology, and utilization of *Astragalus sinicus*-rhizobia symbiosis [M]//HONG G F. The nitrogen fixation and its research in China. Berlin, Heidelberg: Springer, 1992: 439-455.
- [12] ZHANG X X, GUO X W, TEREFEWORK Z, et al. Genetic diversity among rhizobial isolates from field-grown *Astragalus sinicus* of Southern China [J]. Systematic and applied microbiology, 1999, 22(2): 312-320.
- [13] 张学贤, 李阜棣, 曹燕珍, 等. 紫云英根瘤菌分子遗传学研究进展 [J]. 华中农业大学学报, 2003, 22(1): 77-83. ZHANG X X, LI F D, CAO Y Z, et al. Molecular genetics of *Astragalus sinicus* rhizobia [J]. *Journal of Huazhong Agricultural University*, 2003, 22(1): 77-83 (in Chinese with English abstract).
- [14] PENG J L, HAO B H, LIU L, et al. RNA-seq and microarrays analyses reveal global differential transcriptomes of *Mesorhizobium huakuii* 7653R between bacteroids and free-living cells [J/OL]. *PLoS One*, 2014, 9 (4) : e93626 [2025-06-11]. <https://doi.org/10.1371/journal.pone.0093626>.
- [15] WANG S M, HAO B H, LI J R, et al. Whole-genome sequencing of *Mesorhizobium huakuii* 7653R provides molecular insights into host specificity and symbiosis island dynamics [J/OL]. *BMC genomics*, 2014, 15(1): 440 [2025-06-11]. <https://doi.org/10.1186/1471-2164-15-440>.
- [16] ZHOU D L, LI Y N, WANG X T, et al. *Mesorhizobium huakuii* HtpG interaction with nsLTP AsE246 is required for symbiotic nitrogen fixation [J]. *Plant physiology*, 2019, 180 (1): 509-528.
- [17] SI Z Y, GUAN N, ZHOU Y, et al. A methionine sulfoxide reductase B is required for the establishment of *Astragalus sinicus* - *Mesorhizobium* symbiosis [J]. *Plant and cell physiology*, 2020, 61(9): 1631-1645.
- [18] TANG Z D, CAI S Y, LIU Y, et al. A lipopolysaccharide O-antigen synthesis gene in *Mesorhizobium huakuii* plays differentiated roles in root nodule symbiotic compatibility with *Astragalus sinicus* [J]. *Molecular plant-microbe interactions*, 2023, 36(10): 623-635.
- [19] LIU F, YI M X, LIU X B, et al. Symbiotic performances of three *Mesorhizobium huakuii* strains inoculated to Chinese milk vetch varieties [J/OL]. *Frontiers in plant science*, 2020, 11: 599400 [2025-06-11]. <https://doi.org/10.3389/fpls.2020.599400>.
- [20] LONG S H, SU M, CHEN X H, et al. Proteomic and mutant analysis of hydrogenase maturation protein gene hypE in symbiotic nitrogen fixation of *Mesorhizobium huakuii* [J/OL]. *International journal of molecular sciences*, 2023, 24(16) : 12534 [2025-06-11]. <https://doi.org/10.3390/ijms241612534>.
- [21] MUS F, CROOK M B, GARCIA K, et al. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes [J]. *Applied and environmental microbiology*, 2016, 82: 3698-3710.
- [22] 胡倡, 李慧明, 伍惠, 等. 解磷菌和根瘤菌复合接种对大豆和紫云英共生固氮的影响 [J]. 华中农业大学学报, 2020, 39 (4): 38-45. HU C, LI H M, WU H, et al. Effects of co-inoculation of phosphate-solubilizing bacteria and *Rhizobium* on symbiotic nitrogen fixation of soybean and *Astragalus sinensis* [J]. *Journal of Huazhong Agricultural University*, 2020, 39(4): 38-45 (in Chinese with English abstract).
- [23] YAMASHITA N, TANABATA S, OHTAKE N, et al. Effects of different chemical forms of nitrogen on the quick and reversible inhibition of soybean nodule growth and nitrogen fixation activity [J/OL]. *Frontiers in plant science*. 2019, 19: 10: 131 [2025-06-11]. <https://doi.org/10.3389/fpls.2019.00131>.
- [24] 鲁伟, 王凤杰, 汪小旵. 不同大豆-根瘤菌共生组合的根系构

- 型及共生固氮能力[J].农业工程学报,2025,41(7):121-128.LU W, WANG F J, WANG X C. Root architecture and symbiotic nitrogen fixation potential of different soybean rhizobia symbiotic combinations [J]. Transactions of the CSAE, 2025, 41(7): 121-128(in Chinese with English abstract).
- [25] 张宗祥,王军,赵强,等.不同类型土壤接种根瘤菌对大豆共生固氮和根际氮转化的影响[J].植物营养与肥料学报,2024,30(10):1883-1897.ZHANG Z X, WANG J, ZHAO Q, et al. Effects of rhizobial inoculation on soybean nodule N fixation and rhizospheric N transformation in different soil types [J]. Journal of plant nutrition and fertilizers, 2024, 30 (10):1883-1897(in Chinese with English abstract).

Synergistic effect of *Mesorhizobium huakuii* AW-8 inoculation on content of nitrogen in Chinese milk vetch and nutrients in rhizosphere soil

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Abstract The *Mesorhizobium huakuii* 7653R (slow growing rhizobium) was revitalized to obtain strains AW-8 and AW-10 to study the synergistic effects of *M. huakuii* on planting Chinese milk vetch (*Astragalus sinicus*) in the field. The symbiotic nodulation and nitrogen-fixing capabilities of the strains obtained were verified with re-inoculation experiments. *M. huakuii* AW-8 was used as the core strain to compare the symbiotic phenotypes of four *Mesorhizobium* strains through a soil pot cultivation experiment. Results showed that the aboveground biomass, nodulation, and nitrogen-fixation indexes of *M. huakuii* AW-8-inoculated Chinese milk vetch were higher than those of the original strain AW7R-1, and AW-8, AB-21, and AZ-21 were identified as all elite strains. The aboveground biomass, nodule number, nodule weight, and the activity of nodule nitrogenase of the Xinzi No.1 variety of Chinese milk vetch inoculated with AW-8 increased by 41.3%, 87.8%, 34.2% and 6.3%, respectively. The results of comparing the inoculation effects of strain AW-8, AW7R-1, AB-21, and AZ-21 at urea nitrogen levels of 0 kg/667 m², 3 kg/667 m², and 6 kg/667 m² showed that the content of nitrogen and the accumulation of total nitrogen in the above ground hay of Chinese milk vetch increased by 9.5% and 4.1% when urea nitrogen level of 3 kg/667 m² was applied and AW-8 was inoculated, while the content of available phosphorus, available potassium, and organic matter in rhizosphere soil increased by 2.6%, 35.9%, and 0.5%, respectively. The results of comprehensive analyses showed that co-application of *M. huakuii* AW-8 inoculation and 3 kg/mu urea synergistically enhanced the accumulation of nitrogen and the total nitrogen in Chinese milk vetch plants, and improved nutrients in rhizosphere soil as well. It is indicated that the inoculation of *M. huakuii* AW-8 and the application of appropriate nitrogen fertilizer has a synergistic effect on planting Chinese milk vetch.

Keywords green manure; Chinese milk vetch (*Astragalus sinicus*); symbiotic nitrogen fixation; *Mesorhizobium huakuii*; content of nitrogen in plants; nutrients in rhizosphere soil

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