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两株蒜头果内生木霉的物种鉴定 及其对幼苗的促生作用研究

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摘要:【目的】鉴定两株蒜头果内生木霉并探讨其对幼苗生长发育及抗性水平的影响,为蒜头果内生微生物资源利用及优质苗木培育提供参考依据。【方法】结合分子系统学和形态学方法鉴定两株蒜头果内生木霉,并对其产IAA和解钾能力进行定性和定量检测。将供试菌株接种于蒜头果幼苗,6个月后测定植株的生长性状、生物量、叶绿素含量及抗氧化酶活性,并对各测定指标进行相关性分析。【结果】经ITS、TEF和RPB2系统发育树分析以及菌落和微观形态观察,两株内生木霉分别鉴定为 *T. gamsii* 和 *T. koningiopsis*。上述菌株均具有产IAA和解钾能力,其中 *T. gamsii* 的产IAA能力较强,为89.46 μg/mL, *T. koningiopsis* 的解钾能力较强,为29.26 μg/mL。接种 *T. gamsii* 的蒜头果幼苗株高、叶片增量、茎粗和主根长较对照增加了162.16%、140.74%、44.83%和37.06%,且与接种 *T. koningiopsis* 的处理组及对照差异显著。经 *T. koningiopsis* 接种处理的幼苗茎粗较对照增加16.09%,且与对照差异显著。接种 *T. gamsii* 的幼苗根部吸器数量最多,为35.60个,其次是接种 *T. koningiopsis* 的处理组,均与对照差异显著,而对照组的幼苗根部吸器最大直径为1.58 cm,显著高于接菌组。而且接种 *T. gamsii* 的幼苗地上、地下和全株鲜重、干重均与对照差异显著,接种 *T. koningiopsis* 的幼苗全株鲜重显著高于对照。与此同时,接种 *T. gamsii* 的处理组幼苗叶绿素含量最高,为3.37 mg/g,其次是接种 *T. koningiopsis* 的处理组,二者分别较对照高出21.66%和7.58%,且均与对照差异显著。接种 *T. gamsii* 的幼苗叶片过氧化物酶(POD)和超氧化物歧化酶(SOD)活性最高,分别较对照高出40.85%和28.19%,接种 *T. koningiopsis* 的幼苗叶片POD活性较对照高出26.03%,且均与对照差异显著。此外, *T. gamsii* 在蒜头果幼苗根系的定殖率为58.87%, *T. koningiopsis* 的定殖率为52.16%。相关性分析表明,茎粗、吸器数量、全株鲜重、叶绿素含量及叶片POD活性除与最大吸器直径呈显著或极显著负相关外,与其余指标均呈显著或极显著正相关。【结论】*T. gamsii* 和 *T. koningiopsis* 对蒜头果幼苗生长、生物量增加、叶绿素含量提高及抗氧化酶活性增强有积极的促进作用,其中 *T. gamsii* 的促生效果更为显著,为蒜头果理想的促生菌。

关键词:蒜头果;内生木霉;物种鉴定;促生作用

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Identification of Two Endophytic *Trichoderma* Strains in *Malania oleifera* and Their Growth Promoting Effects on Seedlings

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Abstract: [Objective] Two endophytic *Trichoderma* strains from *Malania oleifera* were identified and their effects on the growth and development of seedlings and the level of resistance were explored, thus providing reference for the utilization of microbial resources in *M. oleifera* and the cultivation of high-quality seedlings. [Method] Two endophytic *Trichoderma* strains were identified as *T. gamsii* and *T. koningiopsis* by the molecular systematics and morphological methods, and their producing IAA and potassium resolving abilities were detected qualitatively and quantitatively. After 6 months inoculating with the tested strains, the growth characteristics, biomass, chlorophyll content, and antioxidant enzyme activities of *M. oleifera* seedlings were measured, and the correlation analysis was conducted on the measured indexes. [Result] Through the phylogenetic tree analysis of ITS, TEF and RPB2, and observation of colony and micromorphology, two endophytic *Trichoderma* strains were identified as *T. gamsii* and *T. koningiopsis*. The two strains both have the ability to produce IAA and resolve potassium. The production of IAA was stronger in *T. gamsii*, which was 89.46 $\mu\text{g/mL}$, and the ability to dissolve potassium of *T. koningiopsis* were stronger, which was 29.26 $\mu\text{g/mL}$. The plant height, leaf increment, stem diameter and main root length of the treatment inoculated with *T. gamsii* were 162.16%, 140.74%, 44.83% and 37.06% higher than those of the control, and there was significant differences in growth traits compared from the treatment inoculated with *T. koningiopsis* and the control. The stem diameter of the treatment inoculated with *T. koningiopsis* increased by 16.09%, there was significant differences from that of the control. The number of haustoria in the root of the treatment inoculated with *T. gamsii* was the largest, which was 35.6, followed by the treatment inoculated with *T. koningiopsis*, and there were significant differences from the control. The maximum diameter of root haustorium of *M. oleifera* seedling was 1.58 cm in the control, which was significantly higher than that in the inoculation treatments. Furthermore, there were significant differences in the fresh mass and dry mass between aboveground, underground and the whole plant between the treatment inoculated with *T. gamsii* and the control. There was significant differences in the fresh mass of the whole plant between the treatment inoculated with *T. koningiopsis* and the control. Meanwhile, the chlorophyll content of the seedlings in this treatment inoculated with *T. gamsii* was the highest, which was 3.37 mg/g, followed by that of the treatment inoculated with *T. koningiopsis*. They were 21.66 % and 7.58 % higher than the control, respectively, and both were significantly different from the control. The peroxidase (POD) activities and superoxide dismutase (SOD) activities in the leaves of the treatment inoculated with *T. gamsii* were the highest, which were 40.85% and 28.19% higher than those of the control, POD activity of the treatment inoculated with *T. koningiopsis* was 26.03% higher than that of the control, there were significant differences with the control. In addition, the colonization rate of *T. gamsii* in the root system of seedlings was 58.87% and the colonization rate of *T. koningiopsis* was 52.16%. The correlation analysis showed that the stem diameter, the number of haustorium, the fresh weight of the whole plant, the chlorophyll content and the PDA activity of leaves were significantly or extremely significantly negatively correlated with the maximum diameter of haustorium, and also significantly or extremely significantly positively correlated with other indicators. [Conclusion] *T. gamsii* and *T. koningiopsis* had positive effects on the seedlings growth, and the increase of biomass, chlorophyll content and antioxidant enzyme activities of *M. oleifera* seedling. Since

T. gamsii had a more significant growth promoting effect, it was an ideal growth promoting fungus for *M. oleifera* seedlings.

Keywords: *Malania oleifera*; endophytic *Trichoderma*; species identification; growth promoting effect

【研究意义】蒜头果(*Malania oleifera*)属铁青树科蒜头果属,主要分布于我国云南东南部广南、富宁两县以及广西西部的部分石灰岩地区,为国家Ⅱ级珍稀濒危保护植物。蒜头果种仁油中富含神经酸,该物质能促进受损神经组织的修复与再生,故被誉为植物油中的“液体黄金”^[1-2]。随着蒜头果市场前景和应用价值的不断凸显,云南省广南、富宁两县积极开展蒜头果人工种植,然而蒜头果幼苗造林存活率及保存率较低,且易受多种病害侵染^[3-4],因此研究蒜头果促进生长、提高抗性的方法是实现其种植产业持续健康发展的技术保障。【前人研究进展】在与植物长期的协同进化过程中,植物内生真菌成为决定植物健康和产量的关键因素之一,不仅与植物的营养摄取、生长发育及存活率紧密相关,也对植物免疫系统调节起着重要作用^[5]。前期研究表明,木霉为蒜头果内生真菌的优势属^[6]。据报道,内生木霉可代谢产生吲哚乙酸(IAA)、细胞分裂素和类植物生长素等多种植物生长调节剂刺激植物生长,同时降低高浓度植物外源激素对寄主生长产生的抑制作用^[7-9]。而且,内生木霉通过增加不溶性化合物的溶解性以及微量营养物质的可利用性,提高植物对矿质元素的摄取率^[10-11]。大量研究表明,内生木霉可显著促进植物的种子萌发、组织分化、根系生长以及生物量和产量的增加^[12-14]。此外,内生木霉可通过增强植物抗氧化系统能力,有效清除活性氧或阻止活性氧的产生,提高宿主对生物和非生物胁迫的耐受力^[15-16]。【本研究切入点】目前国内外未见蒜头果内生木霉对其幼苗生长发育和抗性水平有何影响的报道。【拟解决的关键问题】本研究将对前期分离获得的两株蒜头果内生木霉进行物种鉴定,揭示其产IAA及解钾能力,并通过接种幼苗的生长性状、生物量、叶绿素含量及抗氧化酶活性测定,综合评价供试木霉的促生作用,为蒜头果微生物资源利用及优质苗木培育提供参考数据。

1 材料与方法

1.1 试验材料

1.1.1 供试菌株 SF151和SF231于2019年4月分别采集自云南省文山州广南县健康蒜头果植株的根和茎,从表面消毒后的样品中分离获得^[6]。

1.1.2 供试苗木 2019年10月收集当年生大小一致且无病害症状的蒜头果种子,用50%多菌灵可湿性粉剂浸泡30 min后无菌水漂洗自然风干,河沙经高压灭菌后添加无菌水保持湿度,将浸泡后的种子置于河沙中沙藏4个月待萌发。营养钵使用75%乙醇溶液表面消毒,将萌发的蒜头果种子用灭菌混合基质(红土:腐殖土:珍珠岩=3:2:1)单株种植于营养钵,定期浇无菌水保持营养钵中土壤湿润,待蒜头果长至6~8片叶时进行接种试验。

1.2 供试菌株的种类鉴定

1.2.1 分子学鉴定 通过尿素提取法提取上述两株真菌的DNA^[17],分别对其DNA原液进行核糖体内转录间隔区(ITS)、转录延长因子(TEF)和RNA聚合酶大亚基(RPB2)3个基因片段的PCR扩增,相关引物和PCR反应条件参照Zhu等^[18]和Chaverri等^[19]。PCR产物经凝胶电泳检测送昆明硕擎生物科技有限公司进行双向测序。

测序结果在CExpress中手动拼接校正后经NCBI比对,选出相似度较高且已发表的菌株作为参考,查阅文献并下载参考序列基于最大似然法(ML)和贝叶斯法(BI)构建系统发育树。使用jModelTest 2.1.4构建最佳模型,系统发育树在MrBayes 3.1.2中采用Markov Chain Monte Carlo(MCMC)法构建,去除25%的最初结果,后验概率经最终的贝叶斯系统树计算^[20]。最大似然法使用RAxML分析,靴带值重复计算1 000次^[21]。

1.2.2 形态学鉴定 将活化后的菌株转接至玉米葡萄糖培养基(CMD)、马铃薯葡萄糖培养基(PDA)和低营养琼脂培养基(SNA)平板,25℃条件下黑暗培养5~20 d后观察菌落形态、质地以及颜色等特征。并

用灭菌解剖针挑取SNA培养基上的气生菌丝或疱状结构制备水玻片,显微镜下观察菌株的分生孢子梗、瓶梗、分生孢子形态及着生方式,每类结构测量30次。

1.3 促生功能测定

1.3.1 产IAA能力测定 供试菌株活化后接种于PDB液体培养基,经180 r/min,28℃,12 d培养后抽滤菌丝收集发酵液。发酵液与Salkowski比色液等比例混合后静置30 min,根据显色结果对菌株的产IAA能力进行定性检测。混合液颜色越深,表明其产IAA能力越强。将显色的发酵液与Salkowski比色液等比例混合后采用紫外分光光度计测定其OD₅₃₀,并通过绘制标准曲线计算供试菌株的产IAA活性。

1.3.2 解钾能力测定 将木霉菌株接种于PDA培养基中28℃暗培养7 d,用灭菌打孔器取直径5 mm的菌饼接种于解钾培养基培养20 d,若菌饼周围出现透明圈,表明该菌具有解钾活性,测量透明圈直径,并通过四苯硼酸钠重量法测定菌株的解钾能力^[22]。

1.4 幼苗接种效应研究

取上述7个菌饼接种于600 mL PDB液体培养基,置于恒温振荡培养箱中180 r/min,28℃培养96 h,经无菌滤纸过滤并通过血球计数板统计将菌液浓度稀释至 1×10^6 个/mL。

试验设2个处理组和1个对照组,处理组每月通过灌根的方式定期施用50 mL供试菌液,对照组施用等量无菌水,共处理6次,每处理20个重复。每处理随机取10株幼苗测量施菌前和培养6个月后的幼苗株高、茎粗和叶片数,其中株高用钢卷尺测量植株茎基部到茎尖的距离,叶片数为整株幼苗所有叶片的数量,茎粗用游标卡尺测定茎基部直径。随后将植株取出清水洗净根部后自然风干,测定其鲜重,105℃杀青30 min,再65℃烘干至恒重后测定其干重。每处理另取10株幼苗,采集第2~4片功能叶测定其叶绿素含量、SOD和POD活性,其中叶片叶绿素含量采用乙醇浸提比色法进行测定^[23],叶片的SOD和POD活性参照罗阳兰^[13]进行测定,再将植株取出洗净根部后统计单株吸器数量和最大吸器直径。最后每株2段总长6 cm的侧根,剪成1 cm的根段通过KOH-台盼蓝染色法来观察接种真菌在蒜头果幼苗根部定殖情况并计算定殖率^[24]。

1.5 数据统计

使用SPSS25.0对不同处理间幼苗的生长性状、生物量、叶绿素含量及抗氧化酶活性进行单因素方差分析,并对供试菌株接种幼苗后的各项测定指标进行相关性分析。

2 结果与分析

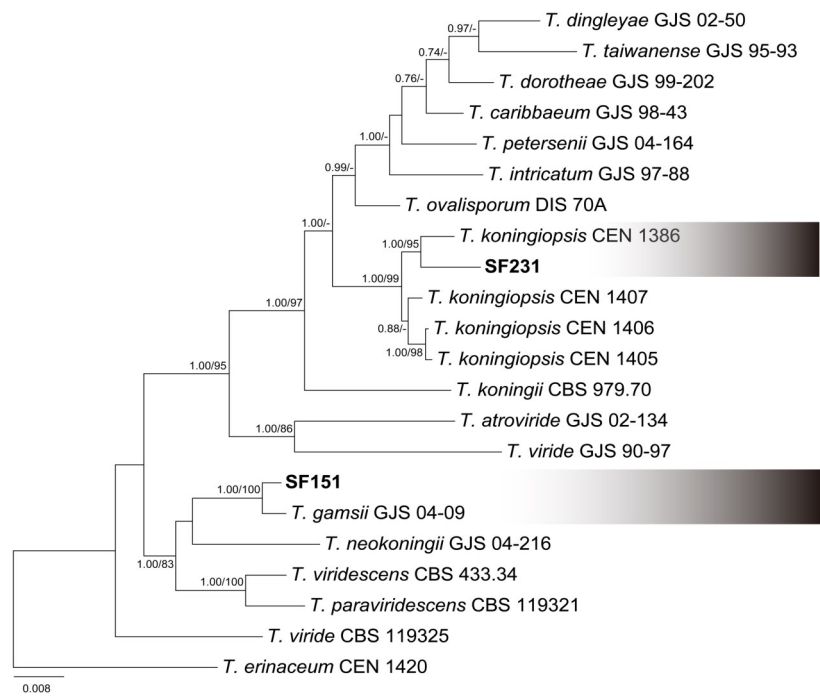
2.1 两株蒜头果内生木霉的物种鉴定

如图1所示,经ITS、TEF和RPB2 3个片段构建系统发育树,SF231与SF151两株菌分别位于两个不同的分类单元。其中SF231与*T. koningiopsis*聚为一支,与*T. koningiopsis* CEN1386的贝叶斯和最大似然后验概率分别达到1.00和95。SF151与*T. gamsii* GJS04-09位于相同支系,且贝叶斯和最大似然分别1.00和100。

如图2所示,SF151在PDA培养基上25℃培养7 d后菌落呈白色,菌丝致密。在SNA培养基中25℃菌落白色,在CMD培养基上25℃培养,菌丝颜色较浅呈黄绿色。分生孢子梗树状,瓶梗中部略膨大,成对出现于单个或末端分枝,也会多个聚集,大小为(6.1-)7.1-10.5(-12.4)×(1.4-)1.8-2.3(-2.8)μm,长宽比(2.5-)3.7-4.9(-7.4)。分生孢子光滑,卵圆、椭圆或不规则椭圆形,大小(2.4-)2.7-3.9(-4.2)×(2.2-)2.3-2.9(-3.3)μm,长宽比(1.1-)1.2-1.4(-1.5)。

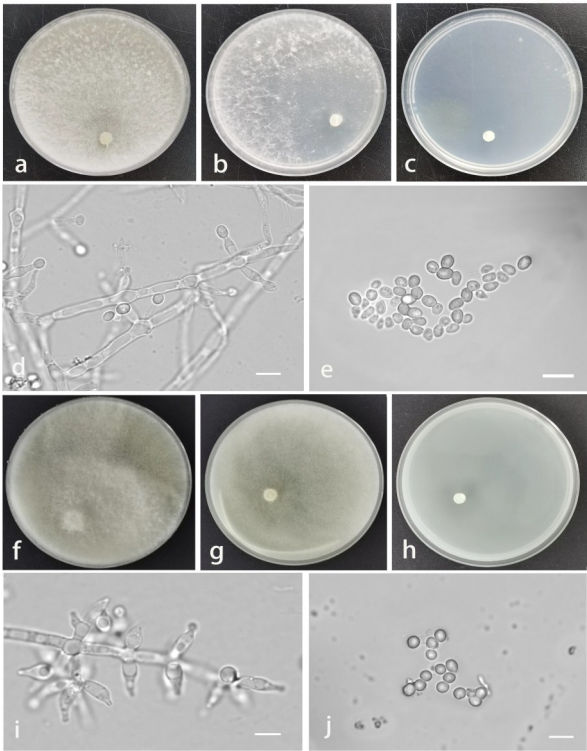
SF231在PDA培养基上25℃培养7 d菌落呈浅灰至深灰,菌丝致密。在SNA培养基中25℃培养菌落浅灰色,在CMD培养基上25℃培养,菌落透明状,边缘疱状结构聚合。分生孢子梗呈树状,瓶梗基部较窄,大小为(4.3-)5.4-9.6(-10.6)×(1.9-)2.0-2.4(-2.6)μm,长宽比(2.0-)2.2-4.7(-5.0),分生孢子光滑,椭圆形,大小为(2.8-)2.9-3.8(-4.2)×(2.2-)2.3-3.0(-3.2)μm,长宽比(1.0-)1.2-1.6(-1.7)。结合两株内生木霉的系统

发育分析和形态学特征,将SF151和SF231分别鉴定为*T. gamsii*和*T. koningiopsis*。



支持度在分支的节点标注,贝叶斯后验概率<0.75和最大似然法的靴带值<75用-标识,粗体字表示本研究中产生的序列。
Support values were indicated at nodes, Bayesian posterior probabilities and Maximum Likelihood bootstrap <75 were recorded by -, bold type indicated sequences generated from this study.

图1 基于ITS、TEF和RPB2联合的贝叶斯系统发生树
Fig.1 Bayesian consensus tree generated from the DNA sequences of ITS, TEF and RPB2



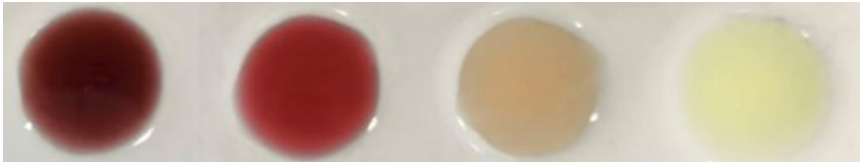
a~c 15-1: 菌株在3种培养基上的菌落形态(25 °C)(a: PDA, 7 d; b: SNA, 7 d; c: CMD 7 d), d: 主梗、分枝和瓶梗; e: 分生孢子; f~h 23-1: 菌株在3种培养基上的菌落形态(25 °C)(f: PDA, 7 d; g: SNA, 7 d; h: CMD 7 d), i: 主梗、分枝和瓶梗; j: 分生孢子; 标尺: 5 μm。
a~c 15-1: Colony morphology of the strain on three media (25 °C) (a on PDA, 7 d; b on SNA, 7 d; c on CMD, 7 d), d: Conidiophores, branches and phialides e: Conidia ; f~h 23-1: Colony morphology of the strain on three media (25 °C) (f on PDA, 7 d; g on SNA, 7 d; h on CMD, 7 d), i: Conidiophores, branches and phialides j: Conidia; Scale bars: 5 μm.

图2 两株蒜头果内生木霉在不同培养基上的菌落形态和显微特征
Fig.2 Colony morphology and microscopic characteristics of two endophytic *Trichoderma* spp. on different media

2.2 两株蒜头果内生木霉的促生功能

2.2.1 蒜头果内生木霉的产 IAA 能力 如表 1 所示,IAA 标准品与 Salkowski 比色液混合后显色反应为深红,产 IAA 活性为 1 000 mg/L,*T. gamsii* 发酵液与 Salkowski 比色液混合后显示为红色,活性为 89.46 μg/mL,*T. koningiopsis* 发酵液的显色反应颜色较浅,活性为 6.69 μg/mL,结果表明,两株菌均具有 IAA 分泌能力,其中 *T. gamsii* 的产 IAA 能力较强。

表 1 两株蒜头果内生木霉的产 IAA 能力

Tab.1 IAA producing ability of two endophytic <i>Trichoderma</i> strains from <i>M. oleifera</i>				
测定项目 Measurement items	CK(IAA)	<i>T. gamsii</i>	<i>T. koningiopsis</i>	CK(无菌水) CK(Sterile water)
定性显色 Qualitative color rendering				
定量测定/(μg·mL ⁻¹) Quantitative determination IAA	1 000	89.46±0.095	6.69±0.036	0

数据表示为平均值±标准误。
The data were expressed as mean±SE.

2.2.2 蒜头果内生木霉的解钾能力 *T. gamsii* 和 *T. koningiopsis* 接种于解钾培养基后出现大小不同的透明圈,其中接种 *T. koningiopsis* 的透明圈直径为 36.22 mm,较接种 *T. gamsii*(直径 25.91 mm)的透明圈大。经定量检测,*T. koningiopsis* 和 *T. gamsii* 的解钾活性分别为 29.26 mg/L 和 11.55 mg/L(表 2),由此表明两株菌均具有解钾活性,且 *T. koningiopsis* 的活性较高。

表 2 两株蒜头果内生木霉的解钾能力

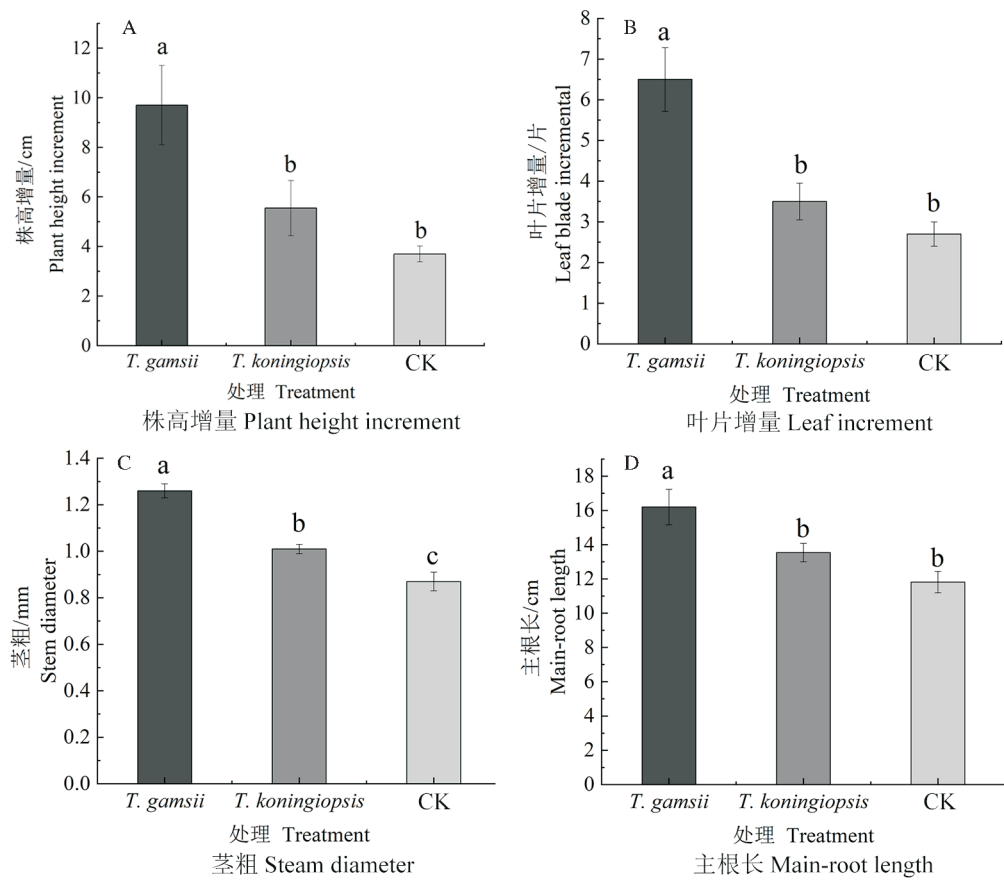
Tab.2 Potassium dissolving ability of two endophytic <i>Trichoderma</i> strains from <i>M. oleifera</i>				
测定项目 Measurement items		CK	<i>T. gamsii</i>	<i>T. koningiopsis</i>
定性测定 Qualitative determination				
透明圈直径/mm	Diameter of transparent ring	—	25.91±0.42	36.22±0.13
定量测定/(mg·L ⁻¹)	Quantitative determination	—	11.55±0.10	29.26±0.81

数据表示为平均值±标准误。
The data were expressed as mean±SE.

2.3 两株蒜头果内生木霉对幼苗的促生作用研究

2.3.1 接种蒜头果内生木霉对其幼苗的生长性状的影响 如图 3 所示,接种 *T. gamsii* 6 个月后蒜头果幼苗的株高增量、叶片增量、茎粗和主根长分别为 9.7 cm、6.50 片、1.26 cm 和 16.20 cm,较对照增加了 162.16%、140.74%、44.83% 和 37.06%,且与接种 *T. koningiopsis* 的处理和对照差异显著($P<0.05$)。经 *T. koningiopsis* 接种处理的幼苗茎粗较对照增加 16.09%,且与对照差异显著($P<0.05$)。结果表明,接种两株内生木霉均能促进蒜头果幼苗的生长,其中 *T. gamsii* 的促生效果较为显著。

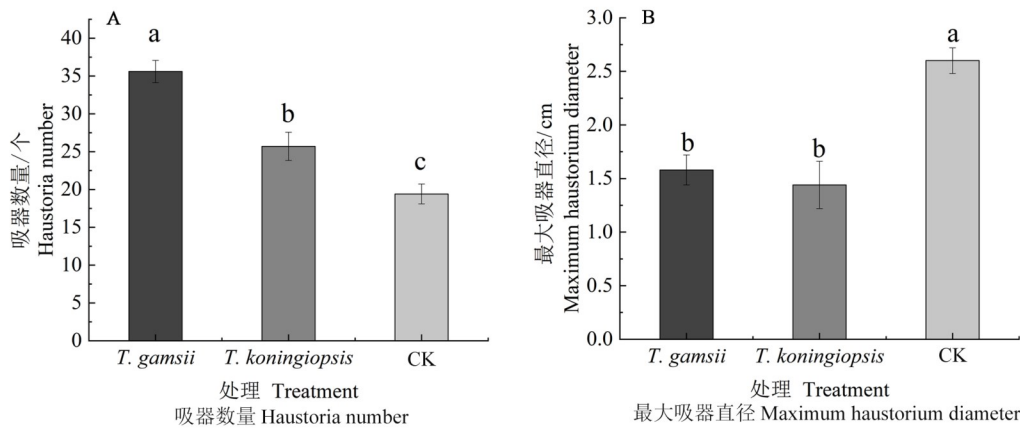
2.3.2 接种蒜头果内生木霉对其幼苗吸器数量和最大吸器直径的影响 如图 4 所示,接种 *T. gamsii* 的处理组蒜头果幼苗根部吸器数量最多,为 35.60 个,与接种 *T. koningiopsis* 的处理组和对照差异显著($P<0.05$);接种 *T. koningiopsis* 的蒜头果幼苗根部吸器数量为 25.70 个,与对照差异显著($P<0.05$)。而对照组的蒜头果幼苗根部吸器最大直径为 1.58cm,显著高于接种 *T. gamsii* 和 *T. koningiopsis* 的处理组($P<0.05$),两组接菌处理的根部吸器最大直径则无显著差异($P>0.05$)。



不同小写字母表示不同处理间差异显著($P<0.05$)。

Different lowercase letters indicate significant differences between different treatments ($P<0.05$).

图3 接种两株蒜头果内生木霉的幼苗株高增量(A)、叶片增量(B)、茎粗(C)和主根长(D) of *M. oleifera* seedlings inoculated with two strains of endophytic *Trichoderma*



不同小写字母表示不同处理间差异显著($P<0.05$)。

Different lowercase letters indicate significant differences between different treatments ($P<0.05$).

图4 接种两株蒜头果内生木霉的幼苗根部吸器数量及最大吸器直径

Fig.4 The number of haustoria and the maximum haustorium diameter in the root of *M. oleifera* seedlings inoculated with two strains of endophytic *Trichoderma*

2.3.3 接种蒜头果内生木霉对其幼苗生物量的影响 如表3所示,接种 *T. gamsii* 的地上、地下和全株鲜重以及地上、地下和全株干重分别较对照增加 59.71%、33.06%、364.76%、59.47%、33.04% 和 41.07%, 其中地上、全株鲜重以及地上干重与接种 *T. koningiopsis* 的处理组和对照差异显著($P<0.05$),地下鲜重、地下干重以及全株干重与对照差异显著($P<0.05$)。接种 *T. koningiopsis* 的处理全株鲜重显著高于对

照 ($P<0.05$)。结果表明,接种两株内生木霉均能一定程度促进蒜头果幼苗生物量的提高,其中 *T. gamsii* 的促进效果更为显著。

表 3 接种两株蒜头果内生木霉对蒜头果幼苗生物量的影响

Tab.3 Effect of inoculating two strains of endophytic *Trichoderma* on the biomass of *M. oleifera* seedlings

处理 Treatment	地上鲜重 The ground fresh weight	地下鲜重 Underground fresh weight	全株鲜重 Whole plant fresh weight	地上干重 The ground dry weight	地下干重 Underground dry weight	全株干重 Whole plant dry weight
<i>T. gamsii</i>	12.01±1.61 ^a	22.94±1.33 ^a	34.95±2.68 ^a	4.80±0.64 ^a	9.18±0.53 ^a	13.98±1.07 ^a
<i>T. koningiopsis</i>	8.73±0.52 ^b	20.60±1.46 ^{ab}	29.33±1.78 ^b	3.49±0.21 ^b	8.24±0.58 ^{ab}	11.73±0.71 ^{ab}
CK	7.52±0.50 ^b	17.24±1.58 ^b	7.52±0.50 ^c	3.01±0.20 ^b	6.90±0.63 ^b	9.91±0.81 ^b

数据表示为平均值±标准误,不同小写字母代表不同基质处理间的差异显著性 ($P<0.05$)。

Data are expressed as mean±standard error, and different lowercase letters represent the significance of differences among different substrate treatments ($P<0.05$).

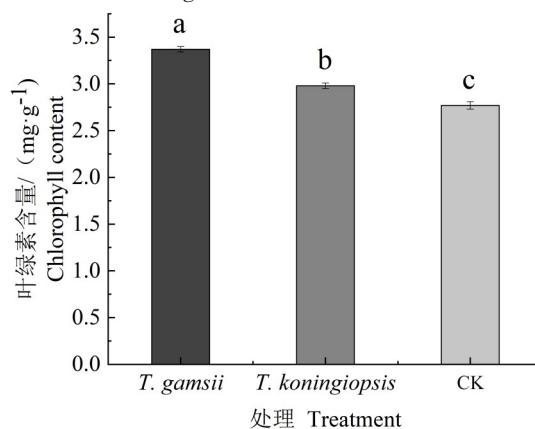
2.3.4 接种蒜头果内生木霉对其幼苗叶绿素含量的影响

接种 *T. gamsii* 的蒜头果幼苗叶绿素含量最高,为 3.37 mg/g,其次是接种 *T. koningiopsis* 的处理,二者分别较对照高出 21.66% 和 7.58% (图 5),且均与对照差异显著 ($P<0.05$)。由此表明,接种两种蒜头内生木霉均能促进幼苗叶绿素含量的升高。

2.3.5 接种蒜头果内生木霉对其幼苗抗性相关指标的影响

如图 6 所示,接种 *T. gamsii* 的幼苗 POD 和 SOD 活性最高,分别较对照高出 40.85% 和 28.19%,其中 POD 活性与接种 *T. koningiopsis* 的处理和对照差异显著 ($P<0.05$),SOD 活性与对照差异显著 ($P<0.05$)。接种 *T. koningiopsis* 的处理,其 POD 活性较对照高出 26.03% 且与对照差异显著 ($P<0.05$)。由此表明,接种两种内生木霉能一定程度提高蒜头果幼苗的抗性水平。

接种 *T. gamsii* 的蒜头果幼苗叶绿素含量最

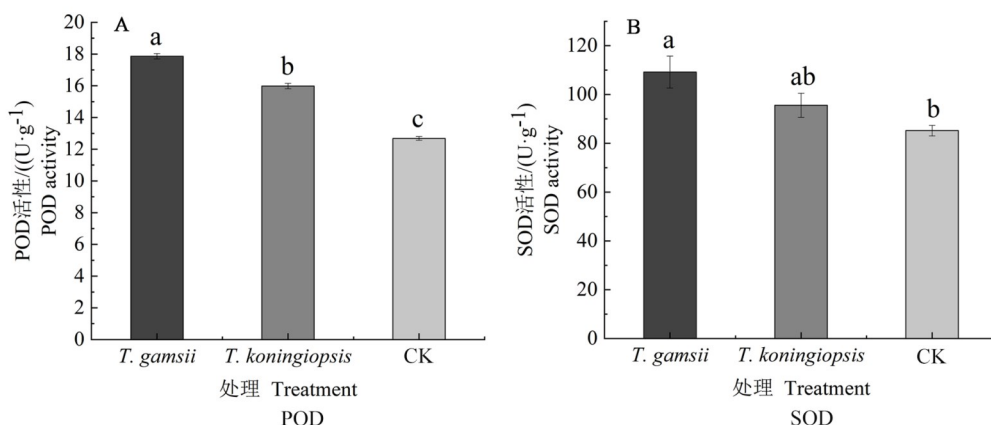


不同小写字母表示不同处理间差异显著 ($P<0.05$)。

Different lowercase letters indicate significant differences between different treatments ($P<0.05$).

图 5 接种两株蒜头果内生木霉的幼苗叶绿素含量

Fig.5 The chlorophyll content of *M. oleifera* seedlings inoculated with two strains of *Trichoderma*



不同小写字母表示不同处理间差异显著 ($P<0.05$)。

Different lowercase letters indicate significant differences between different treatments ($P<0.05$).

图 6 接种两株蒜头果内生木霉的幼苗叶片 POD 和 SOD 活性

Fig.6 The contents of Pod, SOD activities in leaves of *M. oleifera* seedlings inoculated with two strains of endophytic *Trichoderma*

2.3.6 接种蒜头果内生木霉对幼苗根部定殖率的影响

定殖率为58.87%,*T. koningiopsis*的定殖率为52.16%,表明两种木霉对蒜头果幼苗根系有较好的定殖能力。

2.3.7 接种幼苗不同测定指标的相关性分析

经不同处理的蒜头果幼苗各项测定指标的相关性分析(表4),茎粗、吸器数量、全株鲜重、叶绿素含量和叶片PDA活性除与最大吸器直径呈显著或极显著负相关外,与其余指标均呈显著或极显著正相关。株高增量、叶片SOD含量除与主根长、最大吸器直径和全株干重无显著相关性外,与其余指标均呈显著或极显著相关。叶片增量除与最大吸器直径、全株干重外,与其余指标显著或极显著正相关。由此表明,不同接种处理的蒜头果幼苗生长性状、生物量、叶绿素或抗性相关生理指标间密切相关。

如图7所示,其中*T. gamsii*在蒜头果幼苗根系的

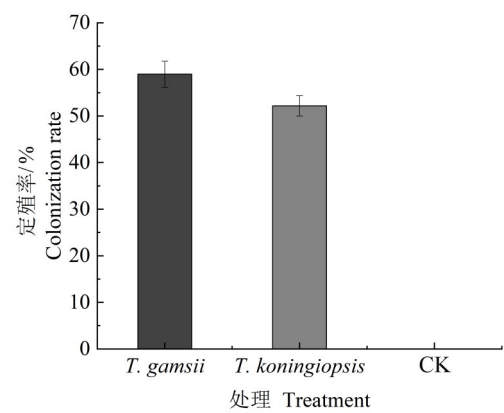


图7 两株蒜头果内生木霉接种幼苗后在其根系的定殖率
Fig.7 The colonization rate of two strains of endophytic *Trichoderma* in *M. oleifera* root

表 4 蒜头果幼苗各指标相关性分析
Tab.4 Correlation analysis of various indexes of *M. oleifera* seedlings

	株高增量 Height increm-ent	叶片增量 Leaf increm-ent	茎粗 Stem diameter	主根长 Main-root length	吸器数量 Haustoria number	最大吸器 直径 Maximum haustorium diameter	全株鲜重 Whole plant fresh weight	全株干重 Whole plant dry weight	叶绿素含量 The chlorophyll content	POD 活性 POD Activi-ty	SOD 活性 SOD Activi-ty
株高增量 Height increment	1.000										
叶片增量 Leaf incremental	0.678**	1.000									
茎粗 Stem diameter	0.423*	0.566**	1.000								
主根长 Main-root length	0.166	0.449*	0.579**	1.000							
吸器数量 Haustoria number	0.417*	0.586**	0.808**	0.546**	1.000						
最大吸器直径 Maximum haustorium diameter	-0.041	-0.126	-0.395*	-0.205	-0.484**	1.000					
全株鲜重 Whole plant fresh weight	0.432*	0.453*	0.709**	0.432*	0.657**	-0.560**	1.000				
全株干重 Whole plant dry weight	0.285	0.287	0.600**	0.212	0.508**	-0.208	0.778**	1.000			
叶绿素含量 The chlorophyll content	0.578**	0.714**	0.770**	0.591**	0.730**	-0.452*	0.727**	0.472**	1.000		
POD 活性 POD activity	0.532**	0.594**	0.815**	0.580**	0.797**	-0.625**	0.862**	0.504**	0.826**	1.000	
SOD 活性 SOD activity	0.504**	0.372*	0.531**	0.221	0.548**	-0.187	0.466**	0.297	0.499**	0.542**	1.000

*表示显著相关($P<0.05$),**表示极显著相关($P<0.01$)。
* indicates significant correlation($P<0.05$),** indicates highly significant correlation($P<0.01$).

3 讨 论

本研究中,通过ITS、RPB2 和TEF 三片段联合构建系统发育树表明,SF151 和SF231 分别与*T. gamsii* 和*T. koningiopsis* 的已知序列位于同一分支,且贝叶斯和最大似然的后验概率较高;同时,两株菌的菌落和微观结构与*T. gamsii* 和*T. koningiopsis* 有关形态学的描述基本一致^[25-26],虽然分生孢子的形态较文献报道略小,但其长宽比较为接近。通过分子系统学分析和形态学比较可鉴定两株内生木霉。

木霉的次生代谢产物中含有多种生长激素类物质,通过植物根系吸收能有效促进寄主的生长发育。相关研究表明,野生型拟南芥幼苗接种 *T. virens* 和 *T. atroviride* 后能促进其生物量增加及侧根发育。同时检测到 *T. virens* 能产生吲哚-3-乙酸、吲哚-3-乙醛和吲哚-3-乙醇3种生长素相关化合物^[8]。类似地,You等^[27]发现 *T. koningiopsis* 也能产生吲哚-3-乙酸。本研究 *T. gamsii* 和 *T. koningiopsis* 均具有产 IAA 活性,其中 *T. gamsii* 产 IAA 活性较强,接种蒜头果后二者均能促进幼苗生长及生物量的增加,其中 *T. gamsii* 的促生效果尤为显著,由此表明供试木霉产 IAA 能力对寄主植物的生长发育有重要影响,下一步还有待对其产 IAA 活性的促生机制进行研究探讨。此外,Lilliana等^[28]研究表明接种木霉能增加豆科植物的叶面 K 浓度,另有研究表明喷施木霉可提高大豆对钾元素 20% 的吸收^[29]。本研究中 *T. koningiopsis* 具有较高的解钾活性,推测对植株钾元素的吸收有积极的促进作用。

一些木霉种类接种寄主植物后能显著提高植株的生长性状和生物量。哈茨木霉 T23 能有效促进花生分枝、增加结果数、有籽果率及百粒籽重^[30];棘孢木霉 M45 浸种处理的水稻株高、地上干重以及根际土壤总氮含量显著上升^[31]。本研究中, *T. gamsii* 和 *T. koningiopsis* 接种蒜头果幼苗后对其生长性状及生物量均有提升,其中 *T. gamsii* 能显著增加植株的株高、叶片数、茎粗、主根长以及鲜重及干重。这与接种 *T. gamsii* 显著促进玉米的生长^[32],接种 *T. koningiopsis* 明显提高马尾松幼苗的株高、湿重以及根长的研究报道相符^[33]。此外,蒜头果为根部半寄生植物,即使没有其他寄主植物,根部也会通过吸器产生自寄生现象^[34]。本研究中,接种 *T. gamsii* 和 *T. koningiopsis* 的蒜头果幼苗根部自吸器数量显著多于对照,而最大自吸器直径则显著小于对照,由此推测自吸器数量多更有利于充分吸收根部的营养物质,这与杨贵钗等的研究报道相符^[35]。

叶绿素作为植物生长过程中必不可少的因素之一,不仅可以促进植物的生长,还能提高其抗逆境胁迫能力。本研究中经 *T. gamsii* 和 *T. koningiopsis* 接种处理的幼苗叶绿素含量显著高于对照,这之前木霉促进寄主植物叶绿素含量升高的研究报道相一致^[36-37]。而且,木霉能诱导寄主植物的系统抗性,SOD 酶通过催化超氧自由基歧化成 O_2 和 H_2O_2 ,有效抵抗有害物质对植物的伤害^[38]。POD 酶除参与植物氧自由基的清除,还与软木脂及木质素的合成相关^[39]。本研究中, *T. gamsii* 和 *T. koningiopsis* 接种处理能显著提高蒜头果幼苗叶片的 POD 及 SOD 活性,这与接种深绿木霉和长枝木霉后显著提高寄主植物抗氧化酶活性的结论相符^[15,40]。

此外,研究表明木霉菌具有较强的定殖能力,能有效利用根系里复杂的碳水化合物加快其在根系表面的生长,同时溶解可溶性或微溶性物质,通过整合或降解作用溶解根系里的金属氧化物,增加根系对矿质元素的吸收^[41]。这与本研究中 *T. gamsii* 和 *T. koningiopsis* 在蒜头果幼苗根部的定殖率较高且明显促进植株生长的结果相一致。相关性分析表明,幼苗的生长形状、生物量与抗氧化酶活性等指标多呈显著正相关,由此表明,植物作为协调统一的有机体,各项指标间相互促进、互为影响,综合反映植株的品质与健康状况。

4 结 论

本研究结合分子系统学和形态学鉴定了两株蒜头果内生木霉分别为 *T. gamsii* 和 *T. koningiopsis*,同时确定了二者均具有产 IAA 能力和解钾活性。接种试验表明两株菌对蒜头果幼苗生长、生物量增加、叶绿素含量提升及抗氧化酶活性增强有积极的促进作用,其中 *T. gamsii* 的促生效果更为显著,为蒜头果理想的促生菌。

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