

水分胁迫下内生真菌感染对黑麦草叶内游离脯氨酸和脱落酸含量的影响

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摘要:以黑麦草为实验对象,研究了干旱胁迫条件下内生真菌感染对植株叶片含水量和叶内游离脯氨酸含量的影响,同时对渗透胁迫条件下植株叶内 ABA 含量的变化进行了分析。结果表明:①内生真菌的感染有助于使叶片保持较高的含水量;②在两种形式的水分胁迫下,前期至中期高感染种群的叶片游离脯氨酸含量低于低感染种群,而在末期则有高出低感染种群的趋势;③内生真菌感染对黑麦草叶内 ABA 累积的正效应只发生在轻度渗透胁迫下的较短时间范围内。

关键词:水分胁迫;内生真菌;黑麦草;游离脯氨酸;脱落酸

Effect of endophyte infection on the concentrations of free proline and ABA of leaves of *Lolium perenne* L. under water stress

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Abstract: Endophytes or Endophytic fungi are a kind of fungi that live inside plant. They widely exist in higher plants, particularly in grasses. The fungi are members of class Ascomycetes, family Clavicipitaceae, tribe Balansiae, including some imperfect fungi that are in close relationship with tribe Balansiae, such as fungi of *Acremonium*. It has been well documented that endophytes and their host plants are mutually symbiotic. On one hand, plants provide photosynthates for the fungi; on the other hand, endophytes may enhance the host's growth and resistance to abiotic stresses (such as drought, high temperature) and biotic stresses (such as herbivore graze). Although the mechanisms of how endophytes enhance the drought resistance of host plant are still unknown, it is clear that endophytes benefit their host through affecting its substance metabolism, or through producing physiologically activated substances (i. e. alkaloid, hormone). Some researchers have found that the infected plants have greater ability of osmotic regulation, so as to maintain a higher turgor pressure and thus keep growing under water stress. Most of these studies, however, were focused on polyols. Two important molecules in osmotic regulation, proline and ABA, have seldom been mentioned. A comparative study of HI (highly-infected) and LI (low-infected) populations were conducted under two different water stress conditions. Physiological characteristics related to drought resistance of plants, including leaf water content (LWC), free proline content and abscisic acid (ABA) content, were determined.

Seeds of *Lolium perenne* cv Pinnacle (from Oregon Seed Company, USA) were treated in two different ways: some were placed on moist filter paper and germinated under room temperature to get HI plants, others were exposed to heat treatment in bath (43 C 15min, then 57 C 35min) and then placed on moist filter paper to get LI plants. The experiment was conducted on the campus of Nankai University.

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Tianjin, China. There were 18 plots, 40cm×50cm in size each, and 15cm wide interspaces between plots. HI and LI seedlings were transplanted into each of the nine plots, respectively. The drought stress (without watering) was imposed to the plots from September 18 to September 30, 1999, and the plots were fully watered a week before the experiment started. HI and LI plants were transplanted into culture pot, which was filled with 500ml 1/2 Hoagland solution. One week later, osmoticum (PEG6000) was added to impose osmotic stress (control, mild stress and severe stress). PEG content for the above stresses is 0%, 10%, 20%, respectively. Newly expanded leaves were taken for the determination of water content, free proline and ABA concentration. In field experiment, leaf samples were taken between 2:30 and 3:00 pm on daily basis or at 3~4 day intervals. In PEG osmotic stress experiment, the sampling work was carried out between 9:00 and 9:30 am at 1~3 day intervals. Immediately after leaf blade was cut off the plant, the sample was taken to laboratory and fresh weight was measured. The sample was then placed in an oven at 105°C for a quick termination of enzyme activity before it was kept at 60°C for 24h, and then the dry weight was measured. Proline was determined following the procedure by Zhang DZ *et al.* (1990). ABA concentration of leaf was determined with ELISA and the kits were purchased from Nanjing Agricultural University, China.

The soil water content (SWC) and leaf water content (LWC) decreased progressively during drought stress. The LWC of both HI and LI plants changed in similar way, but the LWC of HI plants was higher in the middle and later stages of stress, which indicated that HI plants could maintain higher LWC under prolonged water stress. The SWC and LWC of plants were positively correlated (correlation equations are as follows: HI $y_1 = 0.6509x_1 + 0.6796$, $r_1 = 0.78$, $P < 0.01$; LI $y_2 = 0.9493x_2 + 0.6254$, $r_2 = 0.79$, $P < 0.01$). The changes of proline concentration of HI and LI plants were similar and both showed a wave-like increase. After two waves, however, the increasing trend of HI curve exceeded that of LI. There was significant difference in LWC of HI and LI plants between different stress treatments. When no stress was imposed, the LWC of HI and LI plants kept a high level during the experiment period; under mild stress, the LWC of HI and LI plants decreased slowly and demonstrated a relatively stable stage in the middle of stress; under severe stress, however, the LWC of HI and LI plants decreased sharply. Both HI and LI plants showed an ability of osmotic adjustment and their proline content increased under osmotic stress. Under mild stress, HI plants had a substantial increase in proline content while LI plants did not. Under severe stress, the proline curves of HI and LI plants had a "peak" in the early stage of water stress and proline content of LI plants was higher than that of HI plants. In the middle and later stages of stress, however, proline content of HI was higher than that of LI. Because proline acts as osmoticum, the accumulation of proline may be a protective reaction to water stress. Under no stress conditions, ABA content of HI and LI plants kept low and shifted slightly; under mild stress, the change of ABA content of HI and LI plants exhibited "single-peak" curves, but the "peak" of HI plants appeared in the early stage of stress, while that of LI plants appeared in the later stage; under severe stress, the change of ABA content of both HI and LI exhibited "two-peak" curves and the timing of "crest" and "trough" was same for HI and LI plants. Under severe stress, the accumulation of ABA occurred earlier than that of proline and there existed a 2~3 day time-lag between these events. This result indicated that the increase of ABA induced the accumulation of proline. Under mild stress, for HI plants, the time-lag between the changes of ABA and proline still existed, but it was shortened to one day long; for LI plants, the accumulation of proline occurred before the accumulation of ABA, which implied that the accumulation of proline might have other mechanisms. Under severe stress, proline accumulation of HI and LI plants was mainly ABA-dependent. Under mild stress, proline accumulation of HI plants was ABA-dependent while that of LI plants might be

ABA-independent. Endophyte may affect the signal transfer path of proline accumulation.

Under different stress conditions, especially in severe stress, HI plants demonstrated greater ability to keep a high LWC to sustain growth than LI plants. The proline concentration of HI plants was lower than that of LI plants under mild or short time water stress, higher under severe or prolonged stress. During greenhouse osmotic stress, ABA content of HI and LI plants increased and the dynamic characteristics depended on stress levels and stress duration. There was no significant difference in ABA content between HI and LI plants and the positive effect of endophyte infection on ABA accumulation could only occur within a short period of mild stress. There was a time-lag between the increases of ABA and that of proline, which indicated that accumulation of ABA induced the accumulation of proline. This cause-effect relationship between ABA and proline was expressed more markedly in HI plants.

Key words: water stress; free proline; *Lolium perenne* L.; ABA

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内生真菌(Endophytic fungi)是一种生长在植物体内的真菌。现已在许多高等植物中发现了内生真菌的存在,并以禾本科植物中尤为常见^[1]。它们包括子囊菌纲(Ascomycetes)麦角菌科(Clavicipitaceae)瘤座菌族(Balansieae)的许多种真菌,也包括与瘤座菌族关系密切的一些半知菌类如顶孢霉属(*Acremonium*)的真菌^[2]。

大量实验表明顶孢霉-植物之间的共生关系是互惠性的^[3]。植物为真菌提供光合产物并通过种子帮助其传播,而真菌产生的生物碱可以阻抑昆虫和其他植食性动物的采食^[4-6]。内生真菌还可以促进植物的生长^[7,8],并增强其对于旱、高温等环境胁迫的抗性或持久性^[9,10]。从目前的研究结果来看,内生真菌主要是通过影响受感染植物的物质代谢、产生生理活性物质(如生物碱、激素等)来改变植株的生理特性,提高植株的抗逆性,刺激植株生长。已有研究发现感染植株表现出更大的渗透调节能力,从而在干旱条件下能维持较高膨压,以维持其生长。但这些研究多集中在多元醇(polyols),而关于水分胁迫条件下感染植株体内脯氨酸含量和脱落酸含量的变化还未见报道。

本文对两种不同水分胁迫条件下,高感染种群和低感染种群植株叶片含水量(LWC)及叶内游离脯氨酸含量进行了比较研究,以期探明水分胁迫下内生真菌感染对植物体内脯氨酸含量的变化是否产生影响;同时对温室渗透胁迫条件下高感染种群和低感染种群植株叶内 ABA 含量变化及 ABA 与脯氨酸之间的关系进行了初步分析。

1 材料与方法

1.1 实验材料

实验材料为黑麦草(*Lolium perenne* L.),是优良的牧草和草坪植物,具有很高的经济价值,与内生真菌 *Acremonium lolii* 构成共生关系。供实验的品种为 Pinnacle,种子自美国俄勒冈种子子公司购进,其内生真菌感染率不低于 85%。

1.2 高感染(HI)种群和低感染(LI)种群的建植与水分胁迫处理^①

本实验通过种子加热处理使真菌灭活的方法获得 HI 和 LI 种群。

1.2.1 固定样地干旱胁迫实验 选取均匀、饱满的种子,一部分先经过加热处理(43℃温浴 15min 后,升温至 57℃温浴 35min)后,再摆放于湿滤纸上,室温萌发(用以产生 LI 种群);另一部分则直接摆放于湿滤纸上,室温萌发(用以产生 HI 种群)。一周后(1999 年 4 月 16 日),将已萌发的种子植入固定样地中。样地面积为 4×2.5m²,将其分成 18 个 40×50 cm² 大小的样方,样方之间间隔为 15cm。将热处理和未经热处理的已萌发种子分别植入 9 个样方,即 LI 种群和 HI 种群分别占据 9 个样方。移栽 5 周后进行内生真菌感染

^① 为方便起见,下文将高感染率的(highly-infected)种群简称为 HI 种群,将低感染率的(low-infected)种群简称为 LI 种群

率的检测,采用的方法是苯胺蓝染色光镜观察法^[11]。检测结果是 HI 样方的平均感染率为 67%;LI 样方的平均感染率为 14%。

实验开始前一周,将样地充分灌水,使各样方水分状况一致。一周后开始干旱胁迫实验,实验从 1999 年 9 月 18 日至 9 月 30 日持续 13d,胁迫期间停止浇水,使样方内土壤水分含量自然下降。

1.2.2 温室环境 PEG 渗透胁迫实验 HI 和 LI 实验种群的建植方法同上,它们的内生真菌感染率分别为 60% 和 0%。挑选长势良好、大小一致的 HI 和 LI 植株移入温室培养缸中(缸内盛有 500ml 1/2 Hoagland 培养液),缸盖上开有 7 个栽植孔,每孔植 2 株。HI 和 LI 种群分别移入 27 缸,每 3 缸为一组,算作一个取样单元,这样 HI 和 LI 各有 9 个单元。当移入培养缸的植株恢复正常生长状态时实施渗透胁迫处理。

渗透胁迫从 2000 年 1 月 3 日至 1 月 16 日共持续 14d。本实验采用两因素随机区组设计,即考虑内生真菌和渗透胁迫两个因素的作用。采用聚乙二醇(PEG6000)作为渗透剂。HI 和 LI 种群都分别设置 1 个对照(1/2 Hoagland 溶液培养)和 2 个处理(1/2 Hoagland 溶液+PEG 培养)。2 个处理 PEG 浓度分别为 10% (轻度胁迫)和 20% (重度胁迫),对照及每个处理设 3 个重复。胁迫过程中注意及时补充由于叶片蒸腾损失的水分,以保持缸内培养液一定的浓度。

1.3 植物样品的采集与叶片含水量、游离脯氨酸含量和 ABA 含量的测定

固定样地干旱胁迫实验期间,以 1d 为间隔,于每天下午 2:30~3:00 随机剪取植株的自心叶起第 2 叶和第 3 叶,测定叶片含水量和游离脯氨酸含量;温室渗透胁迫实验期间,以 1~3 d 为间隔,于每天上午 9:00~9:30 随机剪取植株的自心叶起第 2 叶和第 3 叶,测定叶片含水量、游离脯氨酸含量和 ABA 含量。

叶片含水量(leaf water content, *LWC*):按上述规则取 1~2 个叶,称其鲜重(fresh weight, *FW*),于 60℃ 烘干 24h,称其干重(dry weight, *DW*),由下式计算叶片含水量:

$$LWC(\%) = [(FW - DW) / DW] \times 100\%$$

游离脯氨酸含量的测定采用磺基水杨酸法^[12]。

脱落酸(ABA)含量采用固相抗体酶联免疫测定法(ELISA),ELISA 试剂盒购自南京农业大学,具体操作按吴颂如等人的方法^[13]进行。

2 结果与分析

2.1 固定样地干旱胁迫实验

2.1.1 干旱胁迫期间土壤含水量(SWC)和叶片含水量(LWC)的变化 在整个胁迫期内 SWC 逐渐下降, *LWC* 也呈同样的下降趋势(图 1)。HI 和 LI 植株的 *LWC* 变化趋势基本一致,而在胁迫中、后期,HI 植株的 *LWC* 高于 LI 植株,并且后者的波动幅度更大些,这表明随着胁迫时间的延长,HI 植株具有保持较高 *LWC* 的能力。SWC 和 *LWC* 之间的相关分析结果表明,HI 和 LI 植株的 SWC 和 *LWC* 均为极显著的线性正相关,即随着 SWC 的降低, *LWC* 呈线性降低。二者的拟合方程分别为:HI $y_1 = 0.6509x_1 + 0.6796$, $r_1 = 0.78$, $P < 0.01$; LI $y_2 = 0.9493x_2 + 0.6254$, $r_2 = 0.79$, $P < 0.01$ 。LI 植株 *LWC* 降低的速率比 HI 植株快,说明 LI 植株的 *LWC* 对 SWC 的变化更敏感。

2.1.2 干旱胁迫期间 HI 和 LI 植株叶片游离脯氨酸(Pro)含量的变化 随着干旱胁迫的进行和持续,HI 和 LI 植株叶片中 Pro 含量均表现为波动式上升,变化趋势基本一致;但经过两个波峰与波谷的交替变化后,HI 曲线的上扬趋势超过 LI 曲线(图 2)。

比较 Pro 和 *LWC* 的变化曲线可以看出二者的变化趋势恰好相反, *LWC* 表现为波动型下降,而 Pro 含量则呈现出波动型上升。对于 HI 和 LI 植株的 Pro 含量和 *LWC* 之间的相关分析均证实了二者之间存在显著的负线性相关关系,拟合方程分别为 $y_3 = -96193x_3 + 75948$, $r_3 = 0.86$, $P < 0.01$ (HI); $y_4 = -55324x_4 + 44624$, $r_4 = 0.82$, $P < 0.01$ (LI)。

2.2 PEG 渗透胁迫实验

2.2.1 渗透胁迫期间 HI 和 LI 植株 LWC 的变化 渗透胁迫实验中的对照、轻度和重度三个处理之间的 *LWC* 表现出明显的差别。从图 3 可以看出,对照条件下,HI 和 LI 植株 *LWC* 一直维持稳定的高水平;轻度胁迫下,HI 和 LI 植株 *LWC* 缓慢下降,且在胁迫中期出现一小段相对稳定的时期;重度胁迫下,HI 和 LI 植

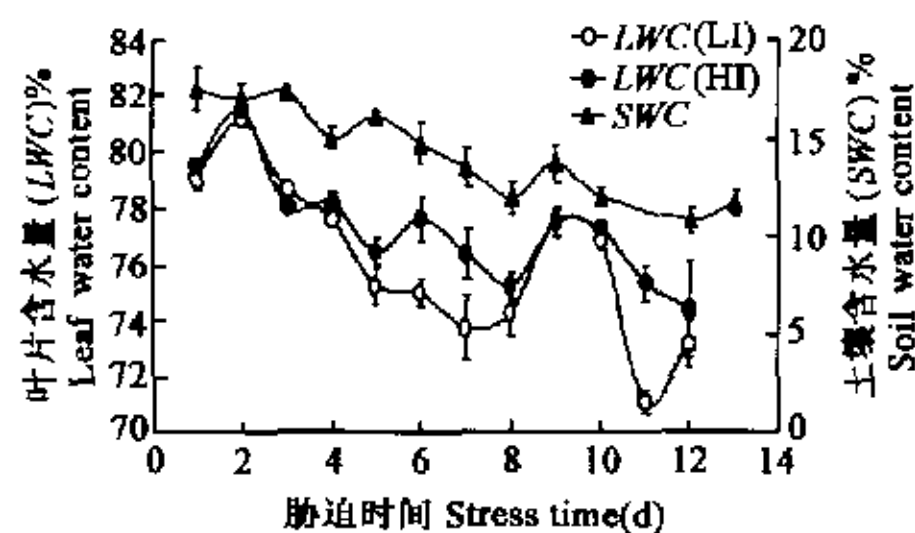


图1 固定样地干旱胁迫下土壤含水量(SWC)及植物叶片含水量(LWC)的变化

Fig. 1 Changes of SWC and LWC of plants under drought stress in field plots

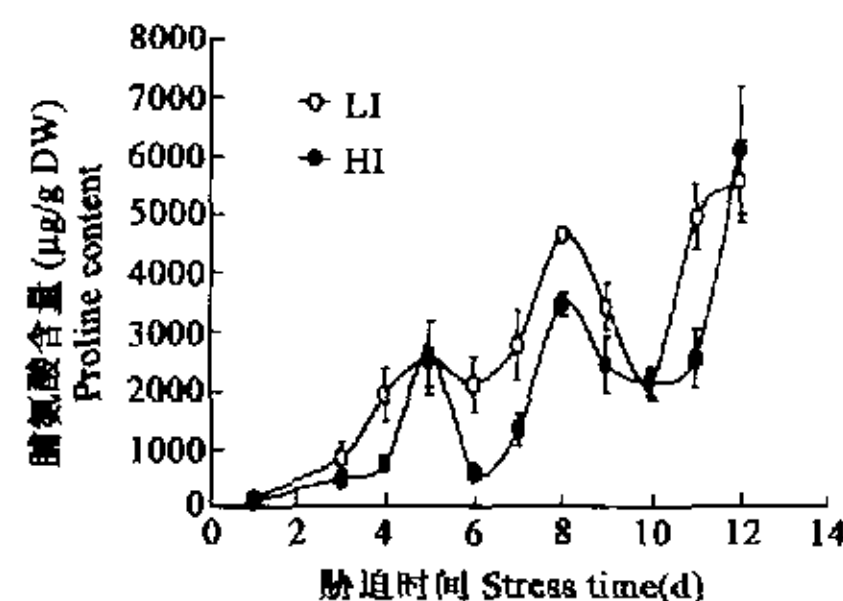


图2 固定样地干旱胁迫下HI和LI植株叶内游离脯氨酸含量的变化

Fig. 2 Changes of free proline content of leaves of HI and LI plants under drought stress in field plots

株LWC表现为迅速地、大幅度地降低,只是在中期下降速率略有降低。在整个胁迫期内,对照条件下,HI和LI植株LWC基本相同;轻度胁迫下,有几个测定日(5d, 7d, 13d)HI植株LWC大于LI植株;而在重度胁迫下大部分时间LI植株与HI植株的LWC无显著差异。

2.2.2 渗透胁迫期间HI和LI植株叶片游离脯氨酸含量的变化 整个胁迫期内,在对照条件下,HI和LI植株叶片中Pro含量均未发生明显变化;在渗透胁迫处理下,HI和LI植株叶片中Pro含量均发生了明显的累积(图4)。轻度胁迫下,HI和LI植株叶片中Pro含量都从胁迫第3天开始迅速上升,但LI植株叶片中Pro含量在胁迫第7天达到最大值,随后迅速降低,后期又略有回升;而HI植株叶片中Pro含量在第5天即达到最大值,后期Pro基本保持稳定直至胁迫结束。重度胁迫条件下HI和LI植株也都从胁迫后第3天开始Pro含量大量增加,并且增加的幅度明显大于轻度胁迫,其中LI的累积峰值高于HI植株。但在胁迫末期,当LI植株Pro持续缓慢下降时,HI植株Pro却在明显回升。

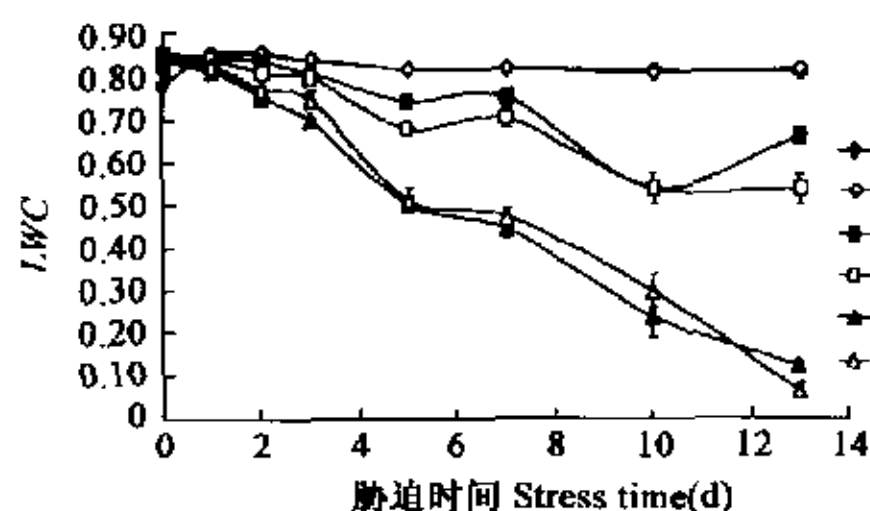


图3 温室渗透胁迫下HI和LI植株叶片含水量(LWC)的变化:0,1,2分别代表对照、轻度和重度胁迫

Fig. 3 Changes of LWC of HI and LI plants under osmotic stress in greenhouse; 0, 1, 2 represents control, mild stress and severe stress, respectively

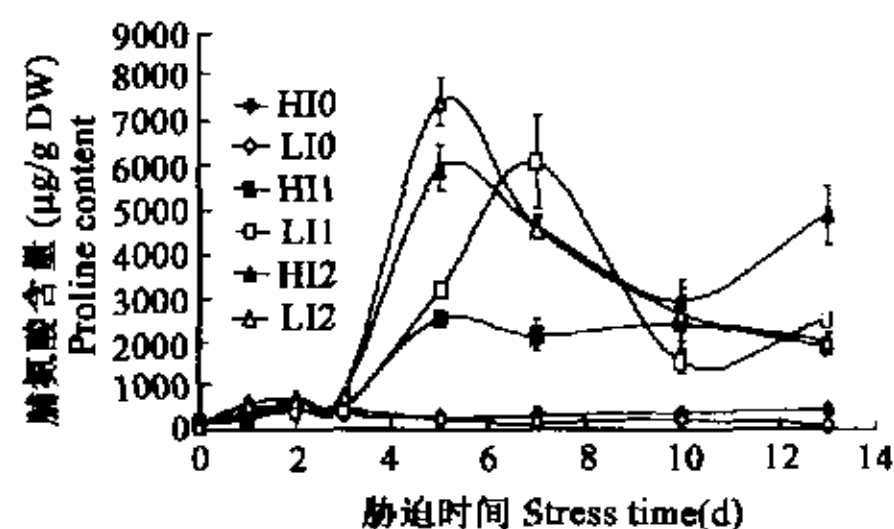


图4 温室渗透胁迫下HI和LI植株叶内游离脯氨酸含量的变化:0,1,2分别代表对照、轻度和重度胁迫

Fig. 4 Changes of free proline content of leaves of HI and LI plants under osmotic stress in greenhouse; 0, 1, 2 represents control, mild stress and severe stress, respectively

相关分析表明,在轻度和重度胁迫下,HI和LI植株脯氨酸含量与LWC之间均表现出显著的二次相关。轻度胁迫下的拟合方程为: $y_1 = -27074x_1^2 + 33810x_1 - 9222.8$, $r_1 = 0.90$, $P < 0.01$ (HI); $y_2 = -72753x_2^2 + 96636x_2 - 29976$, $r_2 = 0.84$, $P < 0.05$ (LI)。重度胁迫下拟合方程为: $y_3 = -10135x_3^2 + 6537.2x_3 + 1363.5$, $r_3 = 0.88$, $P < 0.01$ (HI); $y_4 = -14433x_4^2 + 12016x_4 - 7.1682$, $r_4 = 0.83$, $P < 0.05$ (LI)。

2.2.3 渗透胁迫期间 HI 和 LI 植株叶片中 ABA 含量的变化 如图 5 所示,在整个胁迫期间,对照条件下,HI 和 LI 植株叶片中 ABA 均保持较低水平,只在较小范围内发生轻微的变化。在轻度胁迫下,HI 和 LI 植株叶片中 ABA 含量的变化曲线均呈“单峰型”,只是 HI 植株的“单峰”出现在胁迫的前期而 LI 植株的“单峰”出现在胁迫的后期,二者的 ABA 峰值相差不大。在重度胁迫下,HI 和 LI 植株叶片中 ABA 含量的变化曲线均表现为“双峰型”(后一个峰值比前一个峰值高),且“峰”和“谷”出现的时间同步;当第一个峰出现时,LI 峰值较 HI 高,而当第二个峰出现时,二者的峰值无显著差异。

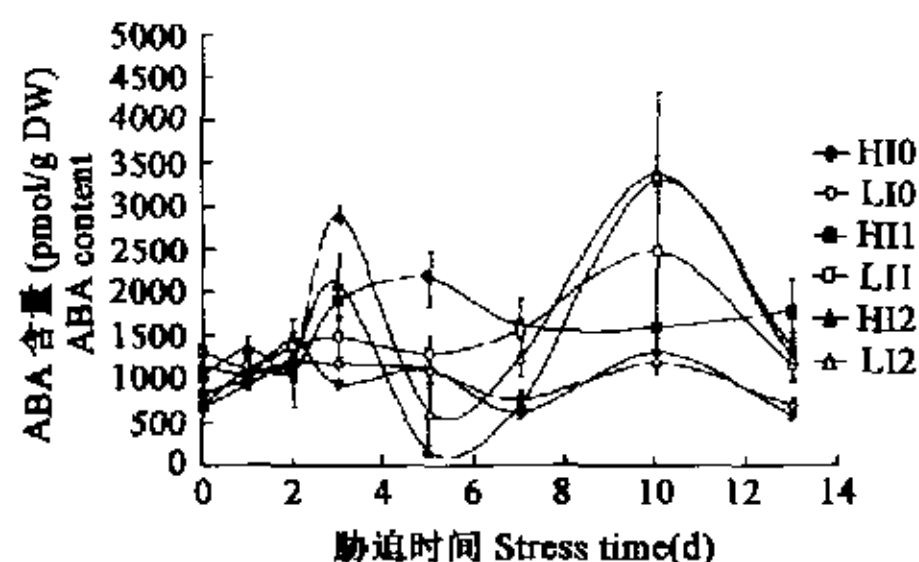


图 5 温室渗透胁迫下 HI 和 LI 植株叶内 ABA 含量的变化;0、1、2 分别代表对照、轻度和重度胁迫

Fig. 5 Changes of ABA content of leaves of HI and LI plants under osmotic stress in greenhouse; 0, 1, 2 represents control, mild stress and severe stress, respectively

2.2.4 渗透胁迫期间 HI 和 LI 植株叶片中 ABA 含量变化与 Pro 含量变化之间的关系 在重度胁迫下,HI 和 LI 植株叶片中 ABA 的升高先于 Pro 的累积(图 6A),ABA 含量变化与 Pro 含量变化之间存在一个时间差,据此可以推测 ABA 的增加引起了 Pro 的累积。HI 和 LI 植株都在 ABA 第 1 个峰出现后的第 2 天发生 Pro 大量累积,ABA 第 2 个峰出现后 LI 植株 Pro 含量继续降低。虽然 HI 植株 Pro 含量在其后 3d 出现较大幅度回升,但此时植株已呈现严重的萎蔫枯死状态,不可能具有重新合成 Pro 进行渗透调节的功能了。此时 Pro 含量的增加可能是叶片失水引起叶细胞死亡、蛋白质快速分解造成的,是一种伤害症状而不是植物对 ABA 第 2 个峰值的响应。

轻度胁迫下,对 HI 植株来说,ABA 的变化与 Pro 的变化之间仍然存在时间差,只是缩短至 1d,峰值过后二者均保持较稳定状态,表现出植物对轻度胁迫的适应性调节(图 6B);而对 LI 植株来说,其 Pro 的累积却发生在 ABA 含量升高之前,这与 ABA 诱导 Pro 累积的推论相反,表明 Pro 的累积不只与 ABA 的含量有关,很可能受到其它因素的影响。

3 讨论

3.1 水分胁迫条件下 HI 和 LI 植株叶片含水量的变化

在两种不同形式的水分胁迫实验中,HI 和 LI 植株 LWC 均随胁迫时间的延长而不断下降,且下降的程度与胁迫强度密切相关。Arachevaleta 等^[14]和 Hill 等^[15]认为内生真菌感染可造成植物水分利用上的差异,通常来说感染植株能维持一个较高的水势,避免萎蔫。在本实验设计的两种不同的水分胁迫条件下,HI 植株 LWC 均高于 LI 植株(除重度渗透胁迫下二者无显著差异外),且随着胁迫时间的延长和胁迫强度的增加这种效应表现得愈明显。这表明 HI 植株在胁迫条件下具有保持较高 LWC 的能力,即内生真菌的存在

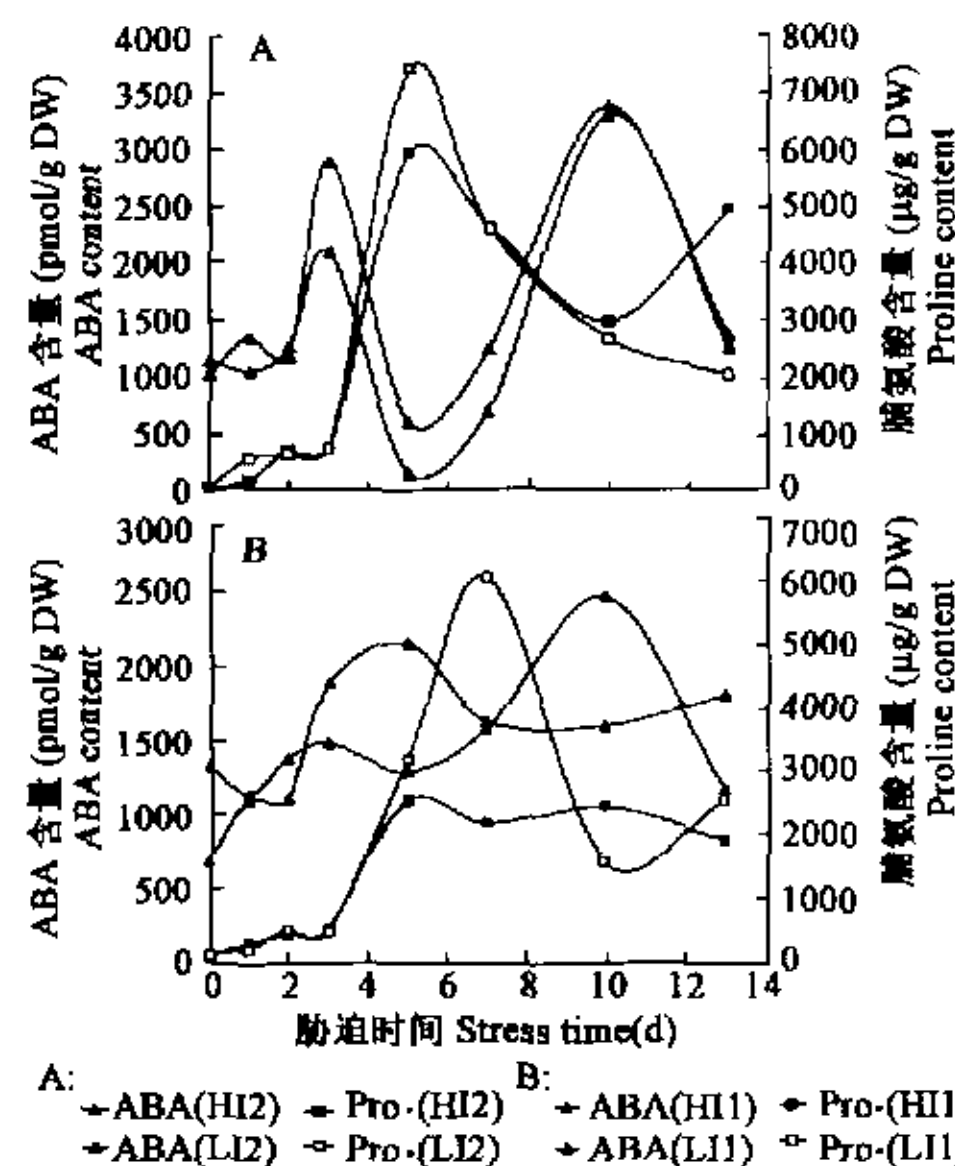


图 6 温室渗透胁迫下 HI 和 LI 植株叶内 ABA 含量变化与游离脯氨酸含量变化之间的关系
A 重度渗透胁迫,B 轻度渗透胁迫

Fig. 6 Relationship between ABA content and free proline content of leaves of HI and LI plants under osmotic stress in greenhouse
A, severe stress; B, mild stress

有助于植物在干旱胁迫条件下保持较好的水分状况。

3.2 水分胁迫条件下 HI 和 LI 植株叶片内游离脯氨酸含量的变化

水分胁迫下植物体内游离脯氨酸的积累是个普遍现象^[16]。本实验室在过去几年对黑麦草的抗旱生理生态机制的研究中发现,叶片内游离脯氨酸的累积是其在各种不同形式水分胁迫的一种普遍反应,且反应敏感、变化幅度大,因此认为游离脯氨酸含量可作为黑麦草对于旱逆境反应的一个较灵敏的参数^[17, 18]。然而到目前为止人们尚没有获得足够的证据以便在植物体内游离脯氨酸累积同其抗旱性之间建立起必然的联系。一些研究表明,干旱胁迫下植物体内发生脯氨酸的累积是一种适应性反应^[19~21],因此可将其作为抗旱性指标用于植物育种工作中;而另一些研究却表明脯氨酸累积仅仅是一种损伤性表征^[22, 23],本文的结果表明这种影响的确存在,但其生理生态学意义值得深入思考。

在固定样地干旱胁迫情形下,植物叶片含水量随土壤含水量的下降而呈波动式下降,而与此同时叶片游离脯氨酸含量在波动中上升。在脯氨酸含量曲线的前两个波峰与波谷区间内,HI 植株低于 LI 植株,这可以用 HI 植株与 LI 植株相比具有较高的叶片含水量来解释(图 1),因为本实验的结果和以前的报道^[24]都证实黑麦草叶内游离脯氨酸含量与叶片含水量之间存在着显著的负相关关系。III 植株脯氨酸曲线在胁迫期末上升速度加快并超出 LI 植株(此时 HI 植株水分状况仍优于 LI),可能具有生态适应上的意义,前者可能因此获得相对较强的渗透调节能力。

与前面的情形不同,在温室渗透胁迫条件下,植物含水量曲线在胁迫开始后不久就随胁迫强度不同而产生分化,但 HI 和 LI 植株之间却基本上没有出现明显的分异。二者的脯氨酸含量曲线变化可能反映了它们对渗透胁迫的反应特点。轻度胁迫下 LI 曲线有一个明显的峰值而 HI 却没有,表明前者对渗透胁迫更敏感。重度胁迫下 LI 和 HI 植株先是出现一个峰值,且有 $LI > HI$,说明在胁迫前期 LI 植株对胁迫较为敏感;而到了胁迫后期 HI 植株的脯氨酸含量却出现超过 LI 植株的趋势,有可能是一种保护性反应,脯氨酸在体内的积累作为一种渗透调节物质而起作用。

3.3 温室渗透胁迫下, HI 和 LI 植株叶片中 ABA 含量的变化及其与脯氨酸含量变化的关系

ABA 作为一种“逆境激素”,在植物受到干旱、盐渍或寒冷引起的渗透胁迫时,其含量会急剧上升^[25]。目前已经发现许多植物在水分胁迫下体内 ABA 含量升高。Hanson 等发现 16 种不同基因型的珍珠粟 (*Pennisetum americanum* (L.) Leeke) 在水分胁迫下叶片中均发生不同程度的 ABA 累积^[26]。de Bruxelles 等发现在渗透胁迫下拟南芥 (*Arabidopsis thaliana*) 根和叶片中的 ABA 水平显著提高^[27]。本实验结果表明,在渗透胁迫下,HI 和 LI 植株 ABA 含量与对照相比均明显增加,且增加的速度和幅度与胁迫强度和胁迫时间有关,在重度胁迫下 ABA 增加的速度最快、幅度最大。内生真菌感染对植物体内 ABA 水平的影响与胁迫强度和有关:在轻度胁迫下,前期 HI 的 ABA 含量高于 LI,而后期则相反;在重度胁迫下,前期 LI 的 ABA 含量高于 HI,而后期二者趋于一致。由此可以看出内生真菌对 ABA 累积的正效应只发生在轻度胁迫下的较短时间范围内。

在水分胁迫下,植物体内 ABA 和脯氨酸的含量都出现累积现象,但在时间上有先后之分,如在干旱胁迫期间,大麦中 ABA 的增加发生在脯氨酸增加之前^[28]。研究还表明外施 ABA 可以促进小麦、大麦、水稻等植物体内脯氨酸的积累^[29~31],因此人们认为 ABA 可能激发了脯氨酸的增加^[30, 32]。Stewart & Voetberg 发现大麦叶片经萎蔫处理后,ABA 和脯氨酸含量均明显增加且脯氨酸的增加发生在 ABA 增加之后,并且发现只有 ABA 含量达到 0.3 nmol/g FW 时,脯氨酸才发生累积;外施 ABA 得到了同样的结果^[33]。Stewart 认为 ABA 诱导的脯氨酸累积的代谢原因是刺激了谷氨酸合成脯氨酸的过程^[30]。目前的研究表明 ABA 和脯氨酸的累积之间的因果关系存在于一些植物中,但并不存在于所有的植物中^[32, 34, 35];而且在胁迫条件下,植物体内 ABA 水平经常是在胁迫施加后不久出现累积峰,而后即使胁迫继续 ABA 含量仍下降至非胁迫水平。因此 Hare 等认为胁迫条件下脯氨酸的累积受 ABA 依赖型 (ABA dependent) 和 ABA 非依赖型 (ABA independent) 两种信号传递途径的调节^[36]。

本实验结果指出,在轻度和重度渗透胁迫下 III 植株叶片内 ABA 与脯氨酸的累积之间均保持较为一致的“时间差”,即在 ABA 累积最大值出现 1~3d 后,脯氨酸的累积最大值才出现;而 LI 植株只在重度胁迫

迫下才与 HI 植株一样表现出“时间差”,而在轻度胁迫下,其脯氨酸的累积却发生在 ABA 累积之前。以上的结果可以表明,在渗透胁迫下 ABA 作为感受胁迫的信号物质首先发生累积,而后诱导渗透调节物质发生积累,提高植物的抗旱性。在重度胁迫下,HI 和 LI 植株脯氨酸累积的信号传递途径以 ABA 依赖型为主,ABA 与脯氨酸之间表现出较好的诱导关系;而在轻度胁迫下,LI 植株脯氨酸的累积发生在 ABA 累积之前,其脯氨酸累积的信号传递可能以 ABA 非依赖型为主。据此推断,胁迫条件下内生真菌可能会影响植物体内脯氨酸累积的信号传递途径。

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