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内生真菌感染对黑麦草抗盐性的影响

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摘要: 以感染内生真菌的多年生黑麦草(*Lolium perenne* L.) (SR4000)为实验材料, 建植内生真菌感染(EI)和不感染(EF)的黑麦草种群, 并对其进行盐胁迫实验, 通过观察生长和生理生态指标的变化, 分析内生真菌对宿主植物抗盐性的影响。结果表明, 内生真菌感染对宿主黑麦草的营养生长没有增益效应, 相反在高盐浓度下, EI 种群的分蘖能力和地上部分生物量均低于 EF 种群; 但内生真菌能够改变宿主种群生物量的分配格局, 将更大比例的生物量分配于根系。在高盐浓度下, 内生真菌感染可导致黑麦草叶内的脯氨酸含量显著增加、可溶性糖含量显著降低, 但对 PSⅡ光化学效率 F_v/F_m 值的变化没有影响。总体来看, 内生真菌感染并未改善宿主黑麦草的抗盐性。

关键词: 黑麦草; 内生真菌; 盐胁迫

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Effect of endophyte infection on salt resistance of ryegrass

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Abstract: In their evolution, grasses have developed symbiotic associations with fungi including mycorrhizal fungi that grow in or on the roots, and endophytes that live their entire life cycle within the aerial portion of the host grass, typically forming nonpathogenic, systemic and usually intercellular associations. Currently, seven genera have been identified, including *Atkinsonella*, *Balansia*, *Balansiopsis*, *Echinodothis*, *Epichloë*, *Myriogenospora* and *Parepichloë*. Among them, the anamorphic (imperfect) stage of *Epichloë* spp., i.e. *Neotyphodium* spp., is most closely related to cultivated grasses. The most widely known *Neotyphodium* endophytes are *N. lolii* and *N. coenophialum*, which colonize perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinaceae* Schreb.) respectively.

Endophytes may protect the hosts from biotic and abiotic stresses. In the past twenty years, much research has focused on the beneficial effect of endophyte infection on the biotic stress resistance of host grasses such as mammalian, insect and nematode herbivores. When abiotic stress was regarded, many studies showed that endophyte infection could improve drought resistance ability of host grasses. Similar to drought stress, salt stress also led plants to physiological drought. But up to now, no related research results were reported. Thus, in this paper *Lolium perenne* cv SR4000 infected by *Neotyphodium lolii* was chosen as experimental material. Vegetative growth and several physiological indexes (such as free proline content, soluble sugar content and photochemical efficiency of PSII) of endophyte-infected (EI) and endophyte-free (EF) populations under normal and salt stress conditions were compared in order to discuss the effect of endophyte infection on the salt resistant ability of the host ryegrass.

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The experiment was performed under greenhouse condition. EI and EF plants were transplanted into culture pots filled with 1L 1/2 Hoagland solution. One week later, salt (NaCl) was added to impose salt stress (control, low and high stress). Salt content for the above stresses was 0, 0.3% and 0.6%, respectively. Each treatment was replicated 5 times. From the beginning, tiller number and free proline content of each population were recorded every 5 days. Photochemical efficiency of PSII of experimental populations was surveyed at the middle and late period separately. At the end of the experiment, shoots and roots were harvested and weighed separately. At the same time, soluble sugar content of both shoots and roots was measured. The whole experiment lasted 35 days. During the period, distilled water was supplied whenever the solution level in the pot dropped due to transpiration and evaporation.

The results showed that endophyte infection did not improve shoot growth of ryegrass. Under high salt stress, tillering ability and shoot biomass of EI populations were all lower than those of EF populations. However, endophyte infection was beneficial to roots of ryegrass, which was demonstrated by the fact that root biomass of EI populations, under salt stresses, decreased more slowly than that of EF populations. When it came to the ratio of root to shoot, no significant change was observed between under control and under high salt stress for EI populations, while for EF populations, it decreased significantly in comparison with control, and the decrease rate was up to 41.7%. Taking these factors in consideration, under high salt stress, EI populations may maintain root growth at the cost of shoot growth, which may be beneficial to their survival.

Salt stresses led to increase of free proline content of ryegrass leaves, and the degree of increase was heightened with extended stress time and advanced stress level. When compared with EF populations, proline content of EI populations was higher and especially significant under high stress. As for the content of soluble sugar, its value was similar under both control and low salt stress in EI and EF populations. Under high salt stress, however, the sugar content of EI populations was significantly lower than that of EF populations, which was considered to be unbeneficial to host grass' survival. Moreover, during the experimental period, photochemical efficiency of PSⅡ was measured three times, but no significant difference was observed between EI and EF populations. In conclusion, endophyte infection did not alleviate negative effect of salt stress on the host ryegrass.

Key words: *Lolium perenne* L; endophyte; salt stress

与植物共生的真菌按照共生部位的不同可分为两类: 一类是存在于植物根部的菌根真菌; 另一类是存在于健康植物茎叶中的内生真菌[1]。禾本科植物内生真菌到目前为止被划分成7属[2], 其中最受关注的是 *Neotyphodium lolii* 和 *N. coenophialum* 两个种, 其两者分别与黑麦草(*Lolium perenne* L.)和高羊茅(*Festuca arundinacea* Shreb.)构成共生关系。

内生真菌可以增强宿主植物对生物胁迫和非生物胁迫的抗性。关于内生真菌对禾本科植物抗逆性影响的研究, 目前的工作集中在对生物胁迫的抗性方面, 其中包括食草动物[3]和食草昆虫[4]的取食、线虫[5]和病原菌[6]的危害以及其它植物的竞争[7]等。至于对非生物胁迫的抗性, 以往的研究重点多放在对干旱胁迫的抗性上面, 大量的研究工作表明, 内生真菌可增强宿主植物的抗旱性[8~10]。与干旱胁迫相类似, 盐胁迫也会导致植物生理性干旱, 而关于内生真菌感染对植物的抗盐性有无效应目前还未见报道。本文以感染内生真菌的黑麦草为材料, 构建内生真菌感染(endophyte-infected, EI)和非感染(endophyte-free, EF)的黑麦草种群, 在温室环境中对黑麦草种群进行不同浓度的盐胁迫处理, 通过比较植株的营养生长、生物量积累以及脯氨酸含量、可溶性糖含量、叶绿素荧光等多项生理指标的变化, 探讨内生真菌在黑麦草抵抗盐胁迫中所起的作用。

1 材料和方法

1.1 实验材料的建植

实验材料为多年生黑麦草(*Lolium perenne* L.), 是优良的牧草和草坪草, 具有很高的经济价值。与内生真菌 *Neotyphodium lolii* 构成共生关系, 供实验的品种是SR4000。选取长势良好、大小一致的植株移入1L培养缸中, 每缸7孔, 每孔1株, EI和EF分别移栽15缸, 共30缸。当移入培养缸的植株恢复正常生长状态时, 实施盐胁迫处理, 胁迫实验在温室中进行。

1.2 盐胁迫处理

本实验同时考虑内生真菌、盐胁迫两个因素, EI 和 EF 种群都分别设置 1 个对照(1/2Hoagland 溶液培养)和 2 个处理(1/2Hoagland + 0.3%NaCl 溶液培养; 1/2Hoagland+ 0.6% NaCl 溶液培养), 各设 5 个重复。胁迫实验从 2003 年 5 月 28 日到 7 月 2 日共持续 35d。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 各项指标的测定

每 5d 计数总分蘖数、测定叶片脯氨酸含量; 在实验的中期和后期分别进行叶绿素荧光强度日变化的测定。胁迫周期结束后, 分别收获各培养缸植株的地上部分和地下部分, 测其鲜重, 于 80℃烘 48h 后称其干重。脯氨酸的测定采用张殿忠等[11]的磺基水杨酸法; 可溶性糖的测定采用蒽酮比色法[12]; 叶绿素荧光的测定利用 Handy PEA 荧光仪, 测量前叶片暗适应 10min。

2 结果与分析

2.1 总分蘖数

在所有的处理中, 黑麦草植株的分蘖数均随处理时间的延长而有所增加, 但是增加的程度随不同盐浓度及内生真菌感染与否而存在差异, 这个差异在胁迫后期尤为明显(图 1), 具体表现在随着盐浓度的升高, 黑麦草种群分蘖数显著下降; 内生真菌感染所导致的差异随不同处理条件而异, 在对照和低盐浓度处理下, 感染和未感染种群的分蘖数基本持平, 二者间没有显著差异; 但是在高盐浓度胁迫下, 在胁迫后期, 未感染种群的分蘖数显著高于感染种群, 这说明在低盐浓度下, 内生真菌的感染与否对黑麦草的分蘖能力没有影响, 而在高盐浓度下, 内生真菌感染使宿主黑麦草的分蘖能力下降。

2.2 总生物量分配

随着盐浓度的升高, 黑麦草植株各部分的生物量积累均显著降低(表 1)。对于地上部分生物量, 在对照条件下, EI 种群有低于 EF 种群的趋势, 在低盐浓度下, EI 种群生物量下降比 EF 种群慢, 使 EI 种群有高于 EF 种群的趋势, 但是两者均无显著差异, 当植株受到高盐胁迫时, EI 地上部分生物量下降迅速, 导致其数值显著低于 EF 种群; 对于地下部分, EI 和 EF 种群生物量在所有盐浓度下均未出现显著差异; 结果导致总生物量的变化规律与地上部分生物量相似。对于根冠比而言, EI 种群随盐浓度的升高变化不大, 而 EF 种群随着盐浓度的升高显著降低, 下降幅度达 41.7%, 这说明内生真菌的

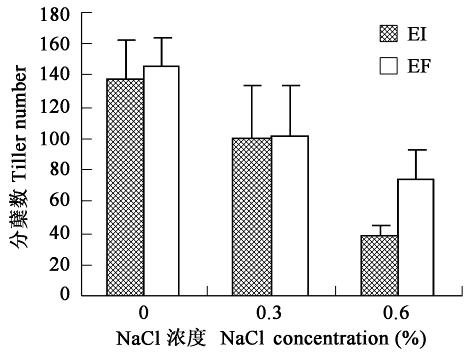

图 1 不同盐浓度下内生真菌感染对黑麦草分蘖能力的影响

Fig. 1 Effect of endophyte infection on tiller number of ryegrass under different salt concentrations

表 1 不同盐浓度下内生真菌感染对黑麦草生物量分配格局的影响

Table 1 Effect of endophyte infection on biomass pattern of ryegrass population under different salt concentrations

NaCl 浓度(%) NaCl concentration		重量 Weight(g)			
		地上部干重 Shoots (DW)	根干重 Roots (DW)	总生物量 Total biomass	地下/地上 Root/Shoot
0	EI	4.13±0.52ab	1.21±0.45ab	5.35±0.96ab	0.29±0.076ab
	EF	4.66±3.01a	1.72±0.61a	6.37±1.14a	0.36±0.0876a
0.3	EI	3.14±1.01bc(24.0%)	0.81±0.36bc(33.1%)	3.95±1.36bc(26.2%)	0.25±0.039b(13.8%)
	EF	3.01±1.26bc(35.4%)	0.94±0.63bc(45.3%)	3.95±1.87bc(38.0%)	0.30±0.0855ab(16.7%)
0.6	EI	1.28±0.31d(69.0%)	0.33±0.09c(72.7%)	1.61±0.35d(69.9%)	0.27±0.064ab(6.9%)
	EF	2.15±1.01cd(53.9%)	0.43±0.19c(75.0%)	2.57±1.19cd(59.7%)	0.21±0.053b(41.7%)

* 表中数据以平均值±标准偏差表示; 右上角字母相同差别不显著, 字母不同则差别显著(α= 0.05), 括号中的数据代表与对照相比下降的百分数 Data are presented in the format of mean±STDEV; same letter denotes non-significant difference while different letters denote a significant difference (α = 0.05); Data in brackets denote decrease percent compared with control

感染对黑麦草的营养生长没有增益效应, 但能够改变黑麦草种群生物量的分配格局, 使其将更大比例的生物量分配于根系。

2.3 游离脯氨酸含量的变化

不同浓度的盐胁迫均可使黑麦草叶片的脯氨酸含量增加, 并且增加程度随胁迫时间的延长和胁迫强度的增加而增加(图 2, 图中选取第 0、10、25 天为代表)。与未感染植株相比, 内生真菌感染可导致宿主植物叶内的脯氨酸含量增加, 且这一促进作用在高盐浓度下表现得尤为明显。

2.4 可溶性糖含量的变化

在对照和低盐浓度处理下, 感染和未感染黑麦草种群的地上部分和地下部分的可溶性糖含量均无显著差异(图 3), 随着盐浓度的升高, 感染种群的可溶性糖含量无论是地上部分还是地下部分, 仍无显著变化; 而未感染种群的地上部分和地下部分可溶性糖含量均显著增加。内生真菌的感染在无盐和低盐处理下, 对宿主植物的可溶性糖含量无显著影响, 但在高盐浓度下, 与未感染种群相比, 内生真菌感染显著降低了宿主黑麦草中可溶性糖的含量。

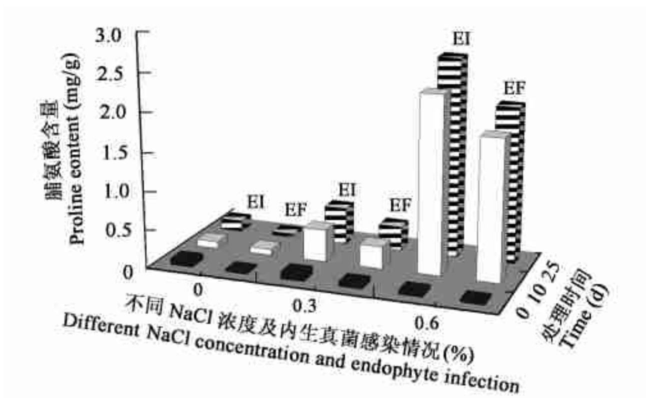

图 2　不同盐浓度及内生真菌感染情况对黑麦草脯氨酸含量的影响

Fig.2　Effect of different salt concentration and endophyte status on proline content of ryegrass leaves

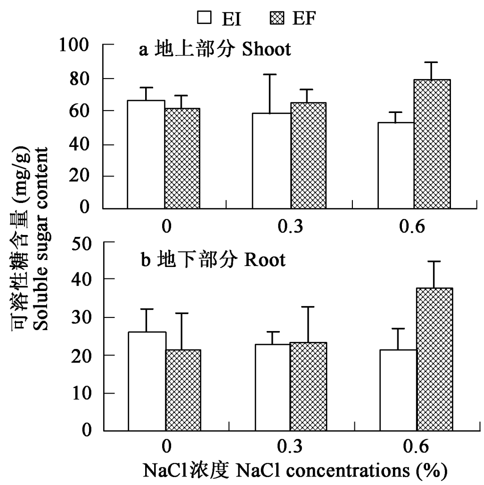

图 3　不同盐浓度下内生真菌感染对黑麦草可溶性糖含量的影响

Fig.3　Effect of endophyte infection on soluble sugar content of raegrass under different salt concentrations

2.5 叶绿素荧光特性

在胁迫的中期和后期对所有实验种群分别进行叶绿素荧光日进程的测定, 结果表明各实验种群的 PSⅡ光化学效率(F_v/F_m)日进程都呈现单谷曲线(图 4), 在 8:00 时 F_v/F_m 值稳定在 0.8 左右, 随着时间的推移, F_v/F_m 值逐渐减小, 并于 12:00 左右达到最小值, 随后逐渐上升, 至 18:00 再次稳定在 0.8 左右。盐胁迫对 F_v/F_m 值有影响, 但影响程度因不同盐浓度及不同的胁迫时间而不同, 在胁迫中期, 盐胁迫使 F_v/F_m 值有降低趋势, 但与对照组之间并无显著差异, 随着胁迫时间的延长, 盐胁迫的影响越来越明显, 到胁迫后期, 高浓度盐处理导致 F_v/F_m 值降低, 尤其在最小值附近出现了显著差异。至于内生真菌的影响, 在所有盐胁迫浓度及各个胁迫时期内, 内生真菌感染与未感染种群的 F_v/F_m 值均无显著差异。

3　讨论

3.1 盐胁迫下内生真菌感染对黑麦草营养生长的影响

许多研究发现内生真菌可提高宿主植物的分蘖能力, 促进宿主的生物量累积, 但不确定和相反的结论也有报道, Elbersen & West[13] 发现高羊茅和内生真菌构成 3 个不同的共生体, 在有的共生体中内生真菌可增加宿主植物的分蘖密度, 在有的共生体中减小其分蘖密度, 在另一些共生体中内生真菌对分蘖密度无影响; Assuero

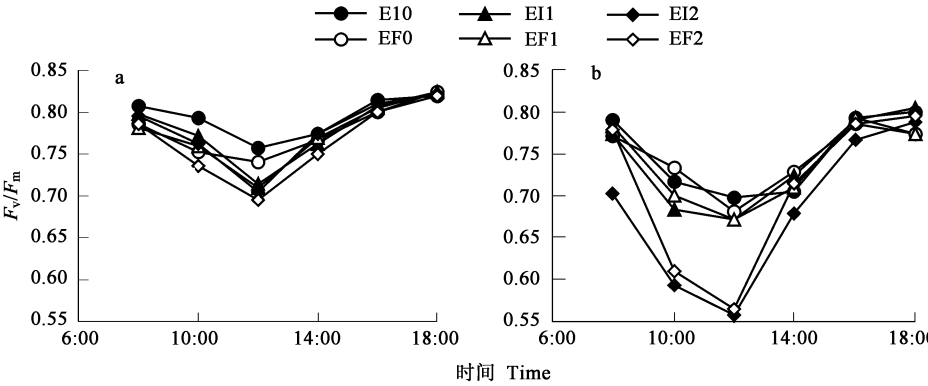

图4 盐胁迫中期和后期黑麦草感染和未感染种群PSⅡ光化学效率日进程

Fig. 4 Diurnal changes of F_v/F_m of EI and EF ryegrass populations in the middle and at the end of the stress periods

a. 中期 in the middle; b. 后期 at the end; 0, 对照 Treatment; 1, 0.3% NaCl; 2, 0.6% NaCl

等[14]采用人工接种的方法研究内生真菌感染和水分胁迫对高羊茅的影响, 结果发现在干旱条件下, EI种群的干重和分蘖数均低于EF种群; 任安芝等[15]报道渗透胁迫下内生真菌对宿主黑麦草种群分蘖数和生物量的变化没有促进作用, 本文对黑麦草实施盐胁迫也得出了同样的结论。Hill等[16]认为分蘖率被促进还是被抑制依赖于内生真菌产生的IAA的量, 故产生少量IAA的内生真菌对一些基因型起促进作用, 而对另一些基因型起抑制作用; 而产生大量IAA的内生真菌对所有的基因型均有抑制作用, 而且干旱等胁迫可能改变内生真菌生长调节物质的产量而影响分蘖的产生。

Eerens等[17]在对黑麦草的研究中发现, 干旱条件下感染植株的水分利用效率低于非感染植株, 内生真菌的作用在于协助宿主植物以降低生长为代价来维持生理生化过程, 直至复水后有利于宿主植物迅速恢复生长。本文中内生真菌的存在没有提高地上、地下部分的生物量, 但在高浓度盐胁迫下, 在未感染种群的根冠比显著下降时, 感染种群能维持固有的根冠比率, 这与感染种群的根系在盐胁迫下仍能维持一定的营养生长有关(在低浓度和高浓度盐胁迫下, 感染种群的根系干重分别下降33%和73%; 而未感染种群的根系干重分别下降45%和75%), 这一结果同样说明在盐胁迫下感染植株以降低地上部分的生长为代价以相对维持根系的生长, 一旦胁迫解除, 感染种群有可能迅速恢复生长。

3.2 盐胁迫下内生真菌感染对黑麦草生理功能的影响

在盐胁迫下, 许多植物都出现游离Pro大量积累的现象[18~20]。关于盐胁迫下植物积累Pro的生理意义目前仍存在分歧: 一种普遍的观点是, Pro积累可以增加对渗透胁迫的耐性, 例如, Kishor等[21]发现组成性表达外源P5CS (Δ1-pyrroline-5-carboxylate synthetase)的转基因烟草植株中Pro含量比对照株高10~18倍, 在干旱胁迫下转基因植株的根、花的发育明显比对照株好, Pro组成性超表达导致对渗透胁迫的耐性增加, 其后, Igarashi等[22]比较了盐敏感和耐盐水稻中P5CS转录及Pro积累的水平, 发现在盐处理10h内, 两种水稻的P5CS均开始转录, 但耐盐水稻中P5CS的转录及Pro积累的水平比盐敏感水稻的高, 说明P5CS表达及Pro积累可能与水稻的耐盐性有关。然而也有不少实验表明, Pro积累只是胁迫的结果, 与耐盐性之间缺少相关性, Liu和Zhu[23]在一种对盐敏感的拟南芥突变体(sosl)中发现, 在NaCl胁迫下P5CS在sosl中过量表达, 尽管sosl植株比野生型多积累2~3倍的Pro, 但耐盐性并没有随之增加; Lutts等[24,25]研究也发现在盐胁迫下对盐敏感的水稻品种比耐盐品种能积累更多的Pro, 盐胁迫下水稻幼苗中Pro积累的多少是一个胁迫伤害指标, 而不是一个抗性指标。本研究中内生真菌感染可导致宿主植物叶内的脯氨酸含量增加, 但同时其生物量却下降, 因此, 盐胁迫下感染植株中Pro的积累可能具有双重性。据Malinowski[26]报道, 生物碱产量增加是感染内生真菌的黑麦草对干旱胁迫的生理响应之一, 而脯氨酸又是麦角碱降解和波胺合成的产物, 故这些生物碱浓度的变化将直接影响到脯氨酸的代谢。总之, 盐胁迫和内生真菌的双重作用使得脯氨酸的变化更趋复杂, 要对脯氨酸的作用作

出综合的评价,还必须进行进一步的实验。

盐胁迫下植物生长受到抑制,糖利用减少,植物叶片内可溶性糖浓度增加[27,28]。关于内生真菌对可溶性糖的影响,目前的研究结果有所不同。Hardy[29]报道,在充足灌水的条件下,高羊茅(品种名为Kentucky-31)叶片内可溶性糖含量不受内生真菌感染的影响,Hill[30]在干旱胁迫条件下对相同品种高羊茅的研究中也得出相同的结论;Richardson[31]以基因型为CB1的高羊茅为材料,发现干旱胁迫下内生真菌感染植株比未感染植株在叶片和叶鞘中都积累更多的葡萄糖和果糖;而Hill等[16]以高羊茅的5个不同基因型为材料则发现,植物叶片中非结构碳水化合物的浓度在其中3个基因型中表现为EI< EF,1个基因型中为EI> EF,1个基因型中二者之间无差异,这一结果说明相同环境条件下被相同内生真菌感染的植物,其宿主的反应不仅随宿主种、品种而且随同一品种的不同基因型而各不相同。本实验中,在无盐和低盐处理下,内生真菌感染对宿主植物的可溶性糖含量无显著影响,但在高盐浓度下,与未感染种群相比,内生真菌感染显著降低了宿主黑麦草中可溶性糖的含量。

3.3 内生真菌感染对叶绿素荧光特性的影响

叶绿素a荧光与光合作用中各种反应紧密相关,任何逆境对光合作用某过程的影响都能通过体内叶绿素a的荧光动力学反应出来,叶绿素a荧光已经被用来描述和检测多元环境因子对植物的影响。在众多叶绿素a荧光参数中,F_v/F_m(PSⅡ光化学效率)大小被用来评价植物对环境的适应性,在无光抑制情况下,C_3植物的光化学效率$F_v/F_m=0.832$,$F_v/F_m<0.832$说明有光抑制产生[32]。强光能导致光抑制[33],高温[34]和水分胁迫也能加强光抑制[35]。至于盐胁迫的影响,Aro等[36]认为盐胁迫可改善PSⅡ功能,而Everard等[37]则认为盐胁迫能抑制PSⅡ的功能,这有可能与所采用的实验材料不同有关。本实验进行到胁迫后期,高浓度盐的处理导致了F_v/F_m值的降低,尤其在最小值的附近出现了显著差异,但内生真菌感染与否,对PSⅡ光化学效率影响并不显著。

总之在盐胁迫下,内生真菌感染与否对黑麦草的营养生长和生理功能均有一定的影响。就营养生长而言,内生真菌感染对宿主黑麦草的营养生长没有增益效应,相反在高盐浓度下,EI种群的分蘖能力和地上部分生物量均低于EF种群;但内生真菌能够改变宿主种群生物量的分配格局,将更大比例的生物量分配于根系,这一结果同样说明内生真菌在盐胁迫下以降低地上部分的生长为代价以相对维持根系的生长,一旦胁迫解除,感染种群有可能迅速恢复生长。

NaCl抑制光合作用的可能原因有三[38]:(1)渗透胁迫导致水势及气孔导度降低[39],限制CO_2到达光合结构,从而抑制光合作用;(2)糖积累造成的反馈抑制,盐胁迫下植物生长受到抑制,糖利用减少,植物叶片内可溶性糖浓度增加,从而反馈性地抑制光合作用[27,28];(3)离子伤害,包括离子积累[40]和离子亏缺[41,42]引起的伤害。本研究中,在高盐浓度下,内生真菌感染可导致宿主植物叶内的脯氨酸含量显著增加、可溶性糖含量显著降低,由此有理由推测内生真菌感染可能缓解盐胁迫对光合作用的抑制作用,但从PSⅡ光化学效率F_v/F_m值的变化及生物量的累积来看,内生真菌感染并未改善盐胁迫下宿主植物的光合作用。总体来看,内生真菌感染并未改善宿主黑麦草的抗盐性。

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