农业环境科学学报 2009,28(11):2251-2254
Journal of Agro-Environment Science

镧对酸雨胁迫下大豆萌发种子糖代谢的动态影响

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摘　要:为了探索 La(Ⅲ)对酸雨胁迫下大豆萌发种子糖代谢的动态影响,以 pH 2.5、4.5 模拟酸雨和 La(Ⅲ)(25 mg·L^{-1})处理大豆种子,测定 La(Ⅲ)对不同酸雨强度胁迫下大豆萌发种子可溶性糖、还原性糖、蔗糖、淀粉及 α,β-淀粉酶的影响。结果表明,与 CK 相比,各糖代谢指标对酸雨胁迫的响应随胁强增加呈增加趋势;pH2.5 胁迫 5 d 后 α-淀粉酶,6 d 后 β-淀粉酶酶活性无法向 CK 趋近,酸雨胁迫对 α,β-淀粉酶活性造成致命性伤害;La(Ⅲ)浸种在低酸雨胁迫下(pH>2.5)可缓解酸雨胁迫对大豆萌发种子各糖代谢指标的影响,而在高胁强胁时(pH=2.5)下则无法缓解。说明 La(Ⅲ)可通过增强糖代谢来缓解酸雨,尤其是低强度酸雨(pH>2.5)对大豆萌发种子的伤害,增强种子抗酸雨能力,并且其缓解能力与酸雨胁迫强度呈负相关。

关键词:大豆;酸雨胁迫;镧;种子萌发;糖代谢

中图分类号:X503.231　　**文献标志码**:A　　**文章编号**:1672-2043(2009)11-2251-04

Dynamic Effects of Lanthanum on Sugar Metabolism of Soybean Seed Germination Under Acid Rain Stress

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Abstract: In order to explore the dynamic effects of La(Ⅲ) on the sugar metabolism during the seed germination of soybean, the effect of La(Ⅲ) on the contents of soluble sugar, reducing sugar, sucrose, starch and α,β-amylase activity were investigated during seed germination under acid rain stress. The two simulated acid rain(SAR) solutions with pH2.5 and 4.5, and the neutral solution(pH7.0) as control were used in the study. Seeds were pretreated with $LaCl_3$ at the optimum concentration (25 mg·L^{-1}) and placed in culture dishes with filter sheets to germinate in a culture container keeping at a constant temperature of 20 ℃. Each treatment group involved three dishes and each dish received 50 seeds. During germination, seeds were exposed to SAR with pH2.5 and 4.5, that was replaced everyday, for 7 d until the germination was over. The results showed that compared with the control (CK), the contents of soluble sugar, reducing sugar, sucrose and starch increased with increasing the stress strength of SAR. The activity of α-amylase could not approach that of CK when the soybean seeds were treated with SAR of pH2.5 for 5 d. The same phenomenon was observed in the activity of β-amylase of soybean seeds treated with SAR of pH2.5 for 6 d. Therefore, SAR stress could lead to the lethality damage to the activities of α, β-amylase. When soybean seeds were pretreated with La(Ⅲ), the contents of soluble sugar, reducing sugar, sucrose, starch and α, β-amylase activity in soybean seeds under acid rain stress increased comparing with that of the treatment without La(Ⅲ). The result indicated that the pretreatment of La(Ⅲ) could alleviate the damage of acid rain with the high pH (pH>2.5) to the indexes of sugar metabolism during seed germination. However, the effect of La(Ⅲ) mentioned above was not observed in the soybean seeds treated with pH=2.5. Theses results indicated that La(Ⅲ) could alleviate the damage of acid rain to seed germination of soybean through increasing the sugar metabolism, especially the low strength of acid rain(pH>2.5), and then increase the ability of seeds to resist acid rain. Moreover, the alleviation effect of La(Ⅲ) was negatively correlated with the stress strength of acid rain.

Keywords: soybean; acid rain stress; lanthanum; seed germination; sugar metabolism

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收稿日期:2009-04-27

基金项目:江南大学质量工程建设项目(20081218);江苏省教育厅"高等学校大学生实践创新训练"(20070730)

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种子萌发是作物生活史的起点及生物量形成之关键,酸雨(Acid rain,AR)可抑制作物种子萌发,而稀土元素在作物种子萌发、幼苗生长、植株对营养元素的吸收能力、植物抗逆性等方面的作用已有研究报道[1-3]。种子萌发是极复杂的生命过程,系统深入考察该过程中各种代谢对 AR 胁迫和稀土的响应是阐释其机制的有效路径。鉴于大豆种子萌发时,胚细胞分裂增殖旺盛及新组织与器官形成需要消耗大量物质和能量,研究糖代谢这一萌发过程中物质转化合成与能量代谢的基础过程实有必要。本实验在前期研究基础上[4-5],以大豆为研究对象,研究镧[La(Ⅲ)]对 AR 胁迫下大豆种子萌发糖代谢的影响,进一步揭示 AR 对作物的伤害效应和稀土增强大豆抗御 AR 能力的作用机理,为寻求减轻 AR 伤害的措施提供借鉴。

1 材料与方法

1.1 材料处理

AR 配制参考文献[4],将配好母液分别调配成 pH2.5、4.5 的 AR 溶液,并经 pHS-29A 酸度计(上海精密科学仪器有限公司)校准。选取大豆(台湾 292)种子用 0.1%$HgCl_2$ 溶液消毒 15 min, 自来水清洗数次,去离子水冲洗 3 次,自然晾干至恒重,称每份种子重量。常温下,将 AR+La(Ⅲ)组大豆于浓度为 25 mg·L^{-1} $LaCl_3$ 溶液中浸种,CK 和 AR 组于蒸馏水中浸种,浸种时间均为 24 h。浸种结束后,每处理大豆种子均匀排列在直径 12 cm 垫有两层滤纸的培养皿中,每皿 50 粒,进行 AR 胁迫处理,(20±1)℃恒温培养箱培养,每天更换相应强度的溶液(20 mL),萌发 7 d 结束,从萌发 1 d 开始测定可溶性糖、还原性糖、蔗糖、淀粉含量及 α,β-淀粉酶活性。对实验数据进行统计处理。本实验设置 La(Ⅲ)、AR2.5、AR2.5+La(Ⅲ)、AR4.5、AR4.5+La(Ⅲ)共 5 个处理组和一个对照组(CK,pH 7.0),每处理 3 皿(即每处理梯度有 3 个平行样)。

1.2 指标测定

还原糖测定采用 DNS 试剂法,可溶性糖、淀粉及蔗糖测定采用硫酸蒽酮法及间苯二酚法,α,β 淀粉酶活性测定采用 DNS 试剂法[5-6]。

2 实验结果

2.1 La(Ⅲ)对 AR 胁迫下大豆萌发种子糖和淀粉含量的动态影响

图 1 显示,与 CK 相比,La(Ⅲ)+AR 组和 AR 组处理后,大豆萌发种子可溶性糖(图 1A)含量 1~6 d 随胁迫时间(简称胁时)走势呈降-升-降-升-降(除 AR2.5+La(Ⅲ)/AR2.5 组),7 d 时,除 AR4.5+La(Ⅲ)组和 CK 组外,各组呈下降走势。蔗糖(图 1B)含量 1~6 d 随胁时走势呈升-降-升-降-升(除 AR2.5+La(Ⅲ)/AR2.5 组),7 d 各组走势:AR4.5+La(Ⅲ)/AR4.5 组和 AR2.5 组上升,其他下降。还原性糖(图 1C)1~7 d 含量随胁时走势趋异,AR2.5+La(Ⅲ)/AR2.5 组:降-升-降-升;AR4.5+La(Ⅲ)/AR4.5 组和 La(Ⅲ)组:升-降-升-降。淀粉(图 1D)含量 1~7 d 走势:升-降-升-降-升。4指标显示,同一胁迫强度处理组均有 AR+La(Ⅲ)组>AR 组,且各组呈正相关性,相关显著性随胁强增加而降低(各组相关系数均有 P>0.900 0)。AR2.5 胁迫下,5 d 后 4 指标均已低于 CK,7 d 时 La(Ⅲ)的缓解作用已不明显。

2.2 La(Ⅲ)对 AR 胁迫下大豆萌发种子 α,β-淀粉酶活性的动态影响

图 2 显示,La(Ⅲ)+AR 组和 AR 组处理后,大豆萌发种子 α-淀粉酶活性(图 2A)随胁时走势:升-降-升;β-淀粉酶活性(图 2B)随胁时走势略有差异:AR4.5+La(Ⅲ)和 La(Ⅲ)组:降-升-降-升-降;其他为升-降-升-降。自 4 d 起不同胁迫强度 α,β-淀粉酶活性走势趋异,AR2.5+La(Ⅲ)/AR2.5 组 α-淀粉酶活性迅速下降至低于 CK,β-淀粉酶活性先升,5 d 起迅速下降至低于 CK;AR4.5+La(Ⅲ)/AR4.5 组走势与 La(Ⅲ)组相似(P>0.900 0),酶活性均高于 La(Ⅲ)组,这可能与此 pH 值已经接近 α,β-淀粉酶最适 pH 值 5~6 有关。同一胁强处理组间呈显著正相关性(均有 P>0.980 0),且均有 La(Ⅲ)+AR 组>AR 组(除 β-淀粉酶2 d)。

图 1、图 2 分析表明:①AR 胁迫强度的增加,对大豆萌发种子糖代谢影响增大;②La(Ⅲ)预处理可缓解 AR 对大豆萌发种子糖代谢影响;③La(Ⅲ)无法缓解 AR 的长时间深度伤害。

3 讨论

可溶性糖是种子萌发至光合自养前的主要呼吸底物,也是种子萌发时的一种重要信号分子,糖浓度水平控制了胚胎从发育到衰老的整个过程[7]。1 d 时 AR2.5 胁迫下可溶性糖含量最高, 其原因是胁强增强,使种皮迅速软化,膜通透性改变,促进吸收水分和氧气及淀粉水解,且胁迫初期保护酶应激启动,使得 AR 对种子的伤害未显现;2 d 时酶保护机制启动(图 2),此时可溶性糖下降,可能与此前 1 d 种子大量消耗可溶性糖用于萌发和缓解胁迫,且可溶性糖生成相

对滞后有关；5 d时可溶性糖含量突然下降，可能与4 d时种子逐渐发霉腐烂、部分种子细胞破坏，导致淀粉水解无法进行，可溶性糖难以生成相关。

蔗糖含量3 d各组均呈下降走势，而还原糖含量最高（图1C），推测此阶段还原糖抑制了子叶中蔗糖合酶活性[8]。在种子早期发育过程中，蔗糖水解加强，

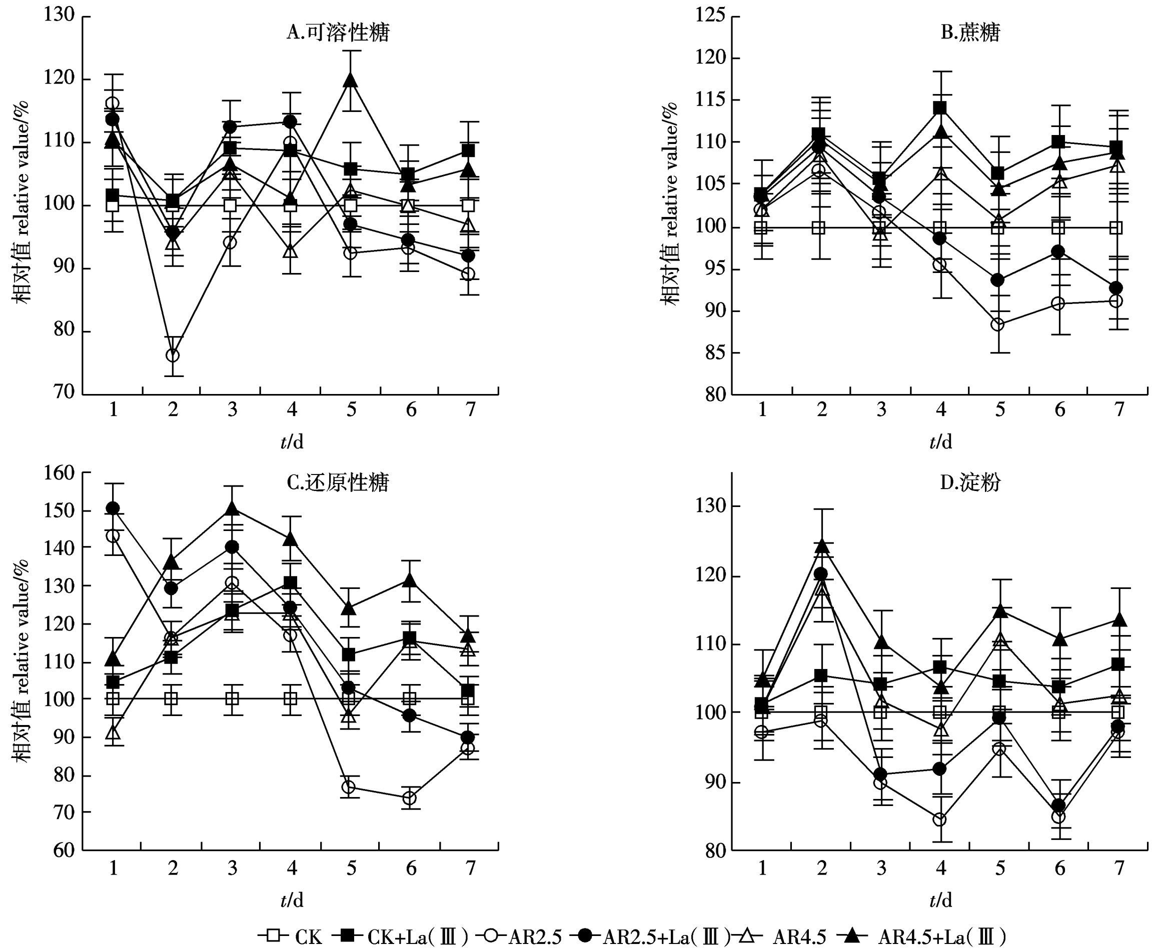

图1 镧对酸雨胁迫下大豆萌发种子糖和淀粉的动态影响

Figure 1 Dynamic effects of La(Ⅲ) on sugars and starch of soybean seed germination under acid rain stress

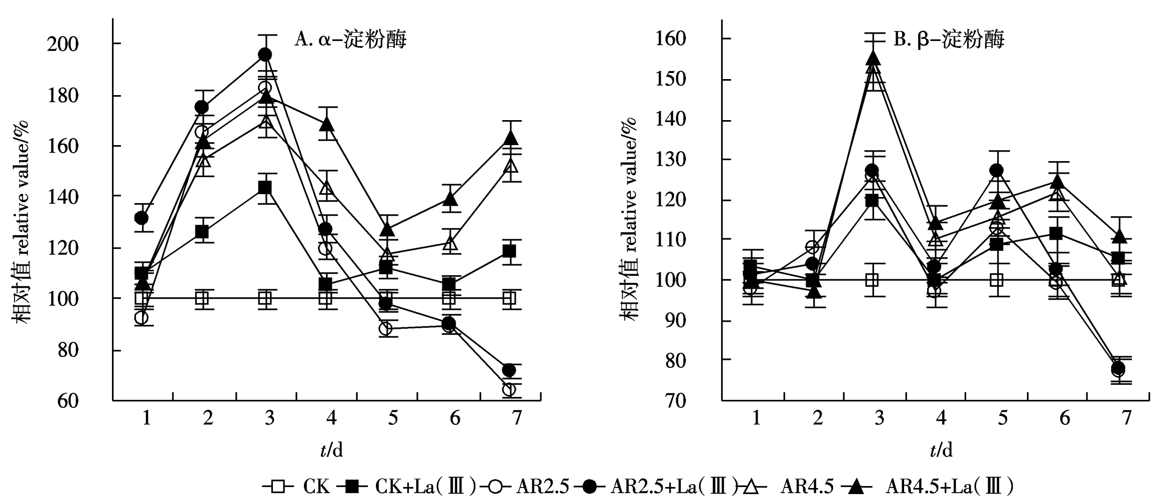

图2 镧对酸雨胁迫下大豆萌发种子α,β-淀粉酶活性的动态影响

Figure 2 Dynamic effects of La(Ⅲ) on α, β-amylase activity of soybean seed germination under acid rain stress

转化酶活性高,益于细胞分裂加速[9];pH2.5 胁迫下还原糖含量 1~3 d 维持较高水平,5 d 含量突然下降;3~5 d 各组还原糖含量均下降,这可能是由于:①在此期间种子萌发速度较快,高浓度的还原糖与子叶发育中有丝分裂活动的增强密切相关[10],因此需要大量还原糖供应萌发;②胁时增加使得伤害加深,种子只有消耗还原糖完善保护机制。淀粉含量 4 d 时最低(除 AR2.5+La(Ⅲ)),这与 3 d 时酶活最高(图 2)和此时萌发种子需大量能量有关。

La(Ⅲ)对 AR 胁迫糖和淀粉含量的影响机制可能与提高淀粉酶活性和降低膜透性有关。作为淀粉水解的起始酶,α-淀粉酶活性决定淀粉的水解强度,进而影响种子萌发,推测 AR 抑制 α,β-淀粉酶的机理可能是 AR 引发细胞内自由基积累或 Ca 流失,引发 α,β-淀粉酶分子氨基酸侧链交连,影响酶合成、分泌及酶结构稳定[11]。α,β-淀粉酶在 3 d 时对 AR 和 La(Ⅲ)均最敏感,酶活性均达到最高值。由图 1D 知,此时萌发种子淀粉水解加快,这可能与为种子生长提供所需碳架结构、能量物质及萌发过程加强有关。La(Ⅲ)能够缓解 AR 对 α,β-淀粉酶的抑制,其机理可能是增强 POD、CAT、SOD 等保护酶活性[12],提高清除自由基能力,维持细胞内环境 pH 条件,防止酶构象改变或者以超级钙的形式补偿 Ca 流失。

此外,或许 La(Ⅲ)能够缓解酸雨胁迫对 β-淀粉酶的影响,推测机理可能是 La(Ⅲ)作为活化剂直接作用于酶,或者提高线粒体活性,从而加大了可溶性糖消耗,减小了产物反馈抑制作用。

4 结论

(1)与 CK 相比,各糖代谢指标对 AR 胁迫的响应随胁强增加呈增加趋势。

(2)La(Ⅲ)浸种在低 AR 胁迫下(pH>2.5)可缓解 AR 胁迫对大豆萌发种子各糖代谢指标影响;La(Ⅲ)浸种后,α,β-淀粉酶活性较各自 AR 组升高,通过促进萌发种子淀粉水解,提高抗 AR 能力。

(3)在高胁强胁时(pH=2.5)下,La(Ⅲ)无法缓解 AR 对种子造成不可逆的伤害。

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