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硅对自毒胁迫下黄瓜种子萌发和生理特性的影响

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[摘 要] 【目的】研究外源硅对肉桂酸(trans-cinnamic acid,CA)模拟自毒胁迫下黄瓜种子萌发和生理特性的影响,为黄瓜自毒作用的缓解提供参考。【方法】以'新春四号'黄瓜为试材,添加 2 mmol/L 肉桂酸模拟黄瓜种子自毒胁迫,再施用外源硅($Na_2SiO_3 \cdot 9H_2O$,AR),通过人工气候箱培养,以不添加外源硅为对照(CK),测定不同处理黄瓜种子的发芽率、胚根长、鲜质量、丙二醛、可溶性糖和淀粉含量以及淀粉酶、超氧化物歧化酶(SOD)、过氧化物酶(POD)、过氧化氢酶(CAT)活性。【结果】与 CK 相比,2 mmol/L CA 处理显著降低了黄瓜种子发芽率、胚根长和鲜质量,较 CK 分别减少了 92.00%,91.46%和 36.77%,显著增加了黄瓜种子中可溶性糖、淀粉和丙二醛(MDA)含量,降低了黄瓜种子中 α-淀粉酶、β-淀粉酶和总淀粉酶活性以及 SOD、POD 和 CAT 的活性,从而严重抑制了黄瓜种子的萌发。在 2 mmol/L CA 胁迫下外源添加 3 mmol/L 硅酸钠能显著增加黄瓜种子的发芽率、胚根长及鲜质量,分别较 CA 处理增加了 91.48%,84.19%和 30.31%,降低了黄瓜种子中可溶性糖、淀粉和 MDA 含量,提高了 α-淀粉酶、β-淀粉酶和总淀粉酶以及 SOD、POD 和 CAT 的活性,进而减轻了活性氧对细胞膜的损伤,促进了碳水化合物的转化速率。【结论】添加外源硅对肉桂酸模拟自毒胁迫的黄瓜种子有明显的缓解作用。

[关键词] 黄瓜;肉桂酸;自毒胁迫;种子萌发;生理特性;硅

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Effects of silicon on physiological characteristics of cucumber seed germination under self-toxic stress

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Abstract:【Objective】This study investigated the effect of exogenous silicon on physiological characteristics of cucumber seed germination under simulated self-toxic stress using cinnamic acid (trans-cinnamic acid, CA) to provide reference for alleviating the self-toxic effect.【Method】'Xinchun 4' seeds were treated with 2 mmol/L cinnamic acid to simulate self-toxic stress, and exogenous silicon ($Na_2SiO_3 \cdot 9H_2O$, AR) was applied under artificial climate box culturing taking no exogenous silicon treatment as the control (CK). The germination rate, radicle length, fresh quality, malondialdehyde and available nitrogen of cucumber seeds, contents of soluble sugar and starch, and activities of amylase, superoxide dismutase, peroxidase and catalase under different treatments were measured.【Result】Compared with CK, 2 mmol/L CA

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significantly reduced germination rate, radicle length and fresh weight of cucumber seeds by 92.00%, 91.46% and 36.77%, and significantly increased contents of soluble sugar and starch. It also decreased total activities of amylase, β-amylase and α-amylase, and significantly increased accumulation of malondialdehyde (MDA). Under the stress of 2 mmol/L CA, germination rate, radicle length and fresh weight of cucumber seeds were significantly increased by adding 3 mmol/L sodium silicate, and they were 91.48%, 84.19% and 30.31% higher than that of CA. Contents of soluble sugar and starch were decreased and activities of total amylase, β-amylase and α-amylase were increased. Activities of SOD, POD and CAT were significantly increased, MDA content was decreased, damage of active oxygen on cell membrane was reduced, and conversion rate of carbohydrate was promoted.【Conclusion】The addition of exogenous silicon had a significant alleviating effect on cucumber seeds under cinnamic acid simulated self-toxic stress.

Key words: cucumber; cinnamic acid; self-toxic stress; seed germination; physiological characteristics; silicon

黄瓜(*Cucumis sativus* L.)是最重要的蔬菜种类之一，其设施栽培产量高、效益好，但高复种指数造成的连作障碍已成为设施黄瓜栽培的关键制约因素。许多作物的连作障碍与自毒物质的含量和种类有关[1-3]。肉桂酸(CA)是造成黄瓜自毒胁迫的主要物质[4]。已有研究表明，肉桂酸对黄瓜生长具有显著抑制作用，且随着肉桂酸浓度的增加其抑制作用增强[5]；此外，肉桂酸还可显著促进尖孢镰刀菌的萌发，导致甜瓜病害发生[6]。

外源硅可以通过减轻生物和非生物胁迫促进植物的生长和发育[7]，提高作物的抗逆性[8-11]。研究表明，硅可以减少盐胁迫下黄瓜幼苗 Na^+ 的吸收，提高叶绿体的活性氧清除能力，从而减轻盐胁迫下黄瓜叶绿体膜的损伤[12]；硅还可以提高黄瓜叶片超氧化物歧化酶(SOD)、过氧化物酶(POD)、过氧化氢酶(CAT)及抗坏血酸过氧化物酶等抗氧化酶的活性，增强清除活性氧的能力，抑制黄瓜叶片中 O^{2-} 和 H_2O_2 的积累，从而减轻活性氧对细胞膜的破坏，使黄瓜叶片电解质渗透率及丙二醛(MDA)含量降低，以减轻铵态氮胁迫[13]。但目前有关硅缓解黄瓜种子自毒胁迫的生理机制研究较少。卜瑞方[14]研究认为，苯丙酸自毒条件下，硅显著提高黄瓜种子萌发活力及其淀粉酶和同工酶的表达水平，并诱导淀粉酶基因的表达。本研究以肉桂酸模拟自毒胁迫，研究外源硅对黄瓜自毒胁迫的缓解机制，旨在为克服连作障碍、提高黄瓜产量和品质的关键技术研发提供理论依据。

1　材料与方法

1.1　试验材料

'新春四号'黄瓜种子，购自甘肃省农业科学院；肉桂酸(trans-cinnamic acid，CA，99%)，Alfa Aesar (China) Chemicals；外源硅(硅酸钠，$Na_2SiO_3 \cdot 9H_2O$，AR)，购自源叶生物科技有限公司。

1.2　试验设计与处理

将均匀饱满的黄瓜种子置于放有 2 张滤纸的培养皿(直径 9 cm)中，每培养皿 20 粒，分别加入不同处理液 10 mL，每处理 3 次重复。在 25 ℃、黑暗的人工气候箱中萌发，每天统计发芽数，萌发 72 h 后测量其胚根长和鲜质量。

1.2.1　肉桂酸(CA)浓度筛选试验　设置 CA 浓度依次为 0，0.5，1，1.5，2，2.5，3，3.5，4 mmol/L 共 9 个处理，依次记录为 CK1、CA1、CA2、CA3、CA4、CA5、CA6、CA7、CA8 处理。根据黄瓜种子萌发条件，测定不同处理下黄瓜种子萌发特性，筛选肉桂酸自毒胁迫最佳浓度。

1.2.2　外源硅($Na_2SiO_3 \cdot 9H_2O$)浓度筛选试验　共设置 10 个外源硅处理，分别为：CK2(清水对照)，CA(2 mmol/L CA)以及 CA 基础上添加 0.5，1，1.5，2，2.5，3，3.5 和 4 mmol/L Si 处理(即 S1、S2、S3、S4、S5、S6、S7 和 S8 处理)。根据黄瓜种子萌发条件，测定不同处理下黄瓜种子萌发特性，筛选外源硅的最佳浓度。

1.2.3　硅对自毒胁迫下黄瓜种子萌发的影响　试验共设置 4 个处理，分别为 CK (清水对照)、CA(2 mmol/L CA)、CA＋Si(2 mmol/L CA＋3 mmol/L Si)和 Si(3 mmol/L Si)，根据黄瓜种子萌发条件，测定不同处理下黄瓜种子萌发的生理指标。

1.3　测定指标及方法

1.3.1　萌发特性　黄瓜种子萌发以胚根长度达到种子全长的 1/2 为发芽标准[14]。当黄瓜种子萌发 72 h 时，用直尺量取从胚根根尖至胚根与下胚轴连

接处(有凸起)的长度,即为胚根长。鲜质量测定用20个萌发的黄瓜芽苗为一组称量并记录,计算每个黄瓜芽苗的平均胚根长和鲜质量,每处理3组重复,每组重复20个芽苗。

1.3.2 生理指标 当黄瓜种子萌发48 h时,测定黄瓜种子的生理指标,其中可溶性糖和淀粉含量测定采用蒽酮-乙酸乙酯法[15],淀粉酶活性测定采用3,5-二硝基水杨酸法[16],MDA含量测定采用TBA法(硫代巴比妥酸法)[17],SOD活性测定采用NBT(氮蓝四唑)法[18],POD活性测定采用愈创木酚法[19],CAT活性测定采用紫外吸收法[20]。

1.4 数据处理

试验数据用"平均值±标准差"表示,采用Microsoft Excel 2010软件处理并作图,采用SPSS 17.0软件进行方差分析,采用单因素方差分析(ANOVA)和最小显著差异法(S-N-K)比较不同组间的差异显著性($P<0.05$)。

2 结果与分析

2.1 不同浓度CA对黄瓜种子萌发特性的影响

由表1可知,随着CA浓度增大,黄瓜种子发芽率呈先增加后降低的趋势,胚根长和鲜质量呈逐渐下降的趋势。其中,CA5处理黄瓜种子的胚根长为0,CA6、CA7及CA8处理黄瓜种子发芽率与胚根长均为0,说明2.5 mmol/L CA及以上属于重度胁迫浓度。与CK1处理相比,CA1处理黄瓜种子发芽率和鲜质量无显著差异,CA2、CA3和CA4处理黄瓜种子发芽率分别下降了14.28%,53.06%,91.83%,胚根长分别下降了57.82%,78.23%,89.11%,鲜质量分别下降了13.64%,30.30%,30.30%。因此,肉桂酸最佳胁迫浓度为2 mmol/L。

表1 不同浓度CA对黄瓜种子发芽率、胚根长和鲜质量的影响

Table 1 Effect of CA on germination rate, radicle long and fresh weight of cucumber bud seedlings

处理 Treatments	发芽率/% Germination rate	胚根长/cm Radicle long	鲜质量/g Fresh weight
CK1	81.67±0.02 a	1.47±0.21 a	0.066±0.005 a
CA1	83.33±0.02 a	1.23±0.18 b	0.066±0.007 a
CA2	70.00±0.05 b	0.62±0.11 c	0.057±0.003 a
CA3	38.33±0.02 c	0.32±0.11 cd	0.046±0.001 b
CA4	6.67±0.02 d	0.16±0.06 d	0.046±0.002 b
CA5	5.00±0.03 d	0.00±0.00 d	0.045±0.001 b
CA6	0.00±0.00 d	0.00±0.00 d	0.045±0.001 b
CA7	0.00±0.00 d	0.00±0.00 d	0.450±0.001 b
CA8	0.00±0.00 d	0.00±0.00 d	0.440±0.002 b

注:同列数据后标不同小写字母表示不同处理间差异显著($P<0.05$)。下表同。

Note: Different lowercase letters indicate significant difference among different treatments ($P<0.05$). The same below.

2.2 不同浓度硅对肉桂酸自毒胁迫下黄瓜种子萌发特性的影响

由表2可知,随着Si浓度的增大,黄瓜种子的发芽率、胚根长和鲜质量呈逐渐增加趋势。与CK2处理相比,S6、S7及S8处理黄瓜种子的发芽率、胚根长和鲜质量无显著差异。与CA处理相比,S6、S7及S8处理黄瓜种子的发芽率分别增加了11.24倍、11.74倍和11.79倍,胚根长分别增加了10.45,10.72和11.18倍,鲜质量分别增加了32.6%,32.6%和34.78%,但S6、S7及S8处理间黄瓜种子的发芽率、胚根长和鲜质量无显著差异。因此筛选缓解CA自毒胁迫的最佳外源硅浓度为3 mmol/L。

表2 不同浓度硅对黄瓜种子发芽率、胚根长和鲜质量的影响

Table 2 Effect of Si on germination rate, radicle long and fresh weight of cucumber bud seedlings

处理 Treatments	发芽率/% Germination rate	胚根长/cm Radicle long	鲜质量/g Fresh weight
CK2	83.33±1.67 a	1.45±0.21 a	0.066±0.005 a
CA	6.67±1.67 c	0.11±0.08 d	0.046±0.002 d
S1	10.00±2.89 c	0.14±0.03 d	0.051±0.002 cd
S2	70.00±1.15 b	0.75±0.02 c	0.051±0.002 cd
S3	80.00±2.89 a	0.92±0.09 bc	0.052±0.002 cd
S4	81.67±1.67 a	0.94±0.02 bc	0.055±0.001 bc
S5	81.67±1.67 a	1.05±0.07 b	0.055±0.001 bc
S6	81.67±1.67 a	1.26±0.06 a	0.061±0.001 ab
S7	85.00±2.89 a	1.29±0.03 a	0.061±0.001 ab
S8	85.33±0.33 a	1.34±0.14 a	0.062±0.001 ab

2.3　硅对自毒胁迫下黄瓜种子萌发特性的影响

由表 3 可知，与 CK 相比，CA 处理显著抑制了黄瓜种子萌发，其中发芽率、胚根长和鲜质量分别减少了 92.00%，91.46%和 36.77%；与 CA 处理相比，CA＋Si 处理显著缓解了 CA 处理对黄瓜种子萌发的抑制作用，CA＋Si 处理黄瓜种子的发芽率、胚根长和鲜质量分别是 CA 处理的 11.74，6.06 和 1.50倍；与 CK 相比，CA＋Si 处理黄瓜种子发芽率和鲜质量差异不显著；与 CK 相比，Si 处理黄瓜种子发芽率、胚根长和鲜质量均无显著性差异。

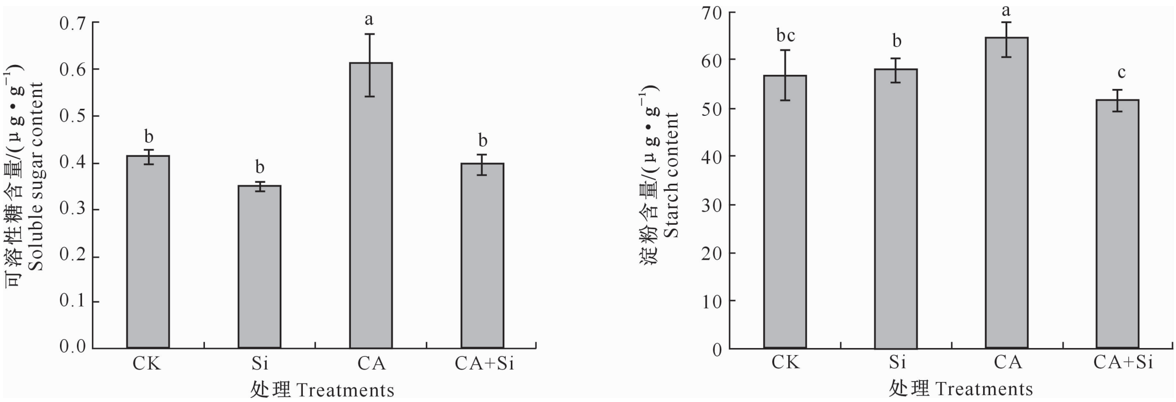
表 3　硅对自毒胁迫下黄瓜种子萌发特性的影响

Table 3　Effect of Si on germination characteristics of cucumber seeds under self-toxic stress

处理 Treatments	发芽率/% Germination rate	胚根长/cm Radicle long	鲜质量/g Fresh weight
CK	83.33±0.04 a	1.84±0.21 a	0.07±0.001 ab
Si	88.33±0.04 a	1.29±0.04 ab	0.08±0.007 a
CA	6.67±0.06 b	0.16±0.06 c	0.04±0.001 c
CA＋Si	78.33±0.17 a	0.97±0.02 b	0.06±0.004 b

2.4　硅对自毒胁迫下黄瓜种子萌发生理特性的影响

2.4.1　可溶性糖和淀粉含量　图 1 表明，与 CK 相比，CA 处理黄瓜种子萌发 48 h 时可溶性糖增加了 48.78% ，且差异显著。与 CA 相比，CA＋Si 处理黄瓜种子萌发 48 h 时可溶性糖含量显著降低了 34.42%，且与 CK 处理和 Si 处理均无显著差异。与 CK 相比，CA 处理黄瓜种子萌发 48 h 时淀粉含量显著增加了 13.32% ；与 CA 处理相比，CA＋Si 处理黄瓜种子萌发 48 h 时淀粉含量显著降低了 20.04%，但与 CK 处理无显著差异。



图柱上不同小写字母表示不同处理间差异显著($P<0.05$)。下同

Different lowercase letters indicate significant difference among different treatments ($P<0.05$). The same below

图 1　Si 对自毒胁迫下黄瓜种子萌发时可溶性糖和淀粉含量的影响

Fig. 1　Effects of Si on soluble sugar and starch contents of cucumber seeds after germination under self-toxic stress

2.4.2　淀粉酶活性　表 4 表明，与 CK 处理相比，CA 处理黄瓜种子萌发 48 h 时 α-淀粉酶、β-淀粉酶和总淀粉酶的活性显著降低，且分别较 CK 降低了 44.73%，44.16% 和 65.51%；与 CA 处理相比，CA＋Si 处理黄瓜种子萌发 48 h 时总淀粉酶、β-淀粉酶和 α-淀粉酶的活性分别显著提高了 43.52%，43.36%和 52.47%；与 CK 处理相比，CA＋Si 处理总淀粉酶、β-淀粉酶的活性无显著性变化，Si 处理也对总淀粉酶、β-淀粉酶的活性无显著影响。

表 4　Si 对自毒胁迫下黄瓜种子萌发时淀粉酶活性的影响

Table 4　Effects of Si on activities of in cucumber seeds after germination under self-toxic stress　　mg/(g·min)

处理 Treatments	α-淀粉酶活性 α-amylase activity	β-淀粉酶活性 β-amylase activity	总淀粉酶活性 Amylase activity
CK	0.94±0.04 a	34.86±0.64 a	35.80±0.74 a
Si	0.70±0.04 b	33.70±0.53 a	34.39±0.73 a
CA	0.33±0.05 c	19.46±1.00 b	19.79±1.60 b
CA＋Si	0.69±0.03 b	34.35±0.87 a	35.03±1.10 a

2.4.3　MDA 含量和抗氧化酶活性　由图 2 可知，与 CK 相比，CA 处理黄瓜种子萌发 48 h 时 MDA

含量显著增加了11.46%;与CA处理相比,CA+Si处理黄瓜种子MDA含量显著降低了13.76%;CK、CA+Si和Si处理间黄瓜种子MDA含量差异不显著。与CK相比,CA处理显著降低了SOD、POD和CAT的活性,降幅分别为29.85%,87.62%和35.97%;与CA处理相比,CA+Si处理显著增加了SOD和POD的活性,分别增加了35.79%和63.01%,但CAT活性差异不显著;与CK相比,CA+Si处理黄瓜种子中SOD和CAT活性差异不显著,Si处理黄瓜种子中SOD、POD和CAT的活性无显著性差异。

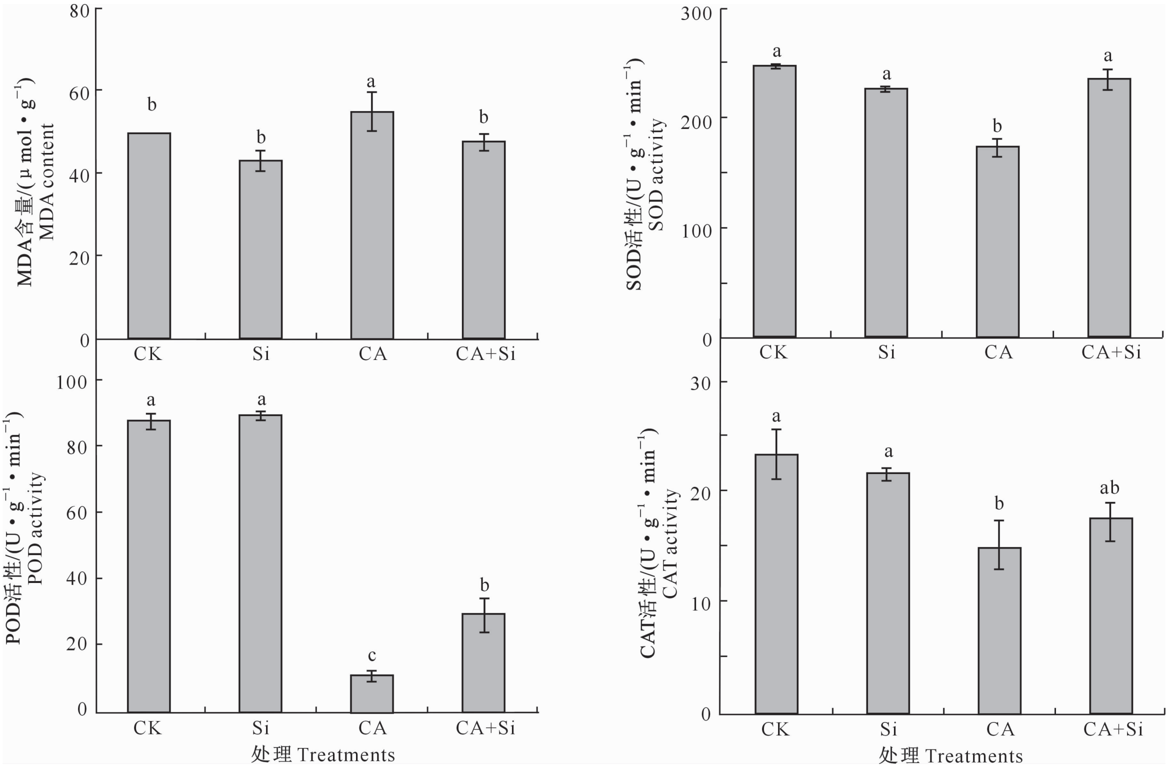


图2 Si对自毒胁迫下黄瓜种子萌发时MDA含量和抗氧化酶活性的影响

Fig.2 Effects of Si on MDA content and antioxidant enzymes activities of cucumber seeds after germination under self-toxic stress

3 结论与讨论

随着黄瓜种植年限的增加,日光温室土壤中肉桂酸含量明显积累[21],高浓度肉桂酸能够降低黄瓜幼苗根系组织中SOD、POD和CAT活性,增加根系组织中活性氧自由基含量,加速膜质过氧化,抑制离子吸收,增加离子外渗,降低叶片气孔导度,导致净光合速率和蒸腾作用减弱,进而导致黄瓜产量和品质下降[5,14,22]。本研究结果表明,2 mmol/L CA处理显著抑制了黄瓜种子的发芽率、胚根长和鲜质量,这与曹光球[23]对杉木种子的研究结果一致。2 mmol/L CA显著降低了黄瓜种子SOD、POD和CAT活性,从而降低了其清除活性氧的能力,增加了MDA含量,这与吴凤芝[5]对黄瓜幼苗的研究结果一致。已有研究表明,正常条件下Si能促进种子琉璃苣萌发[24],但对番茄种子萌发无促进作用[25]。本试验中,添加外源Si对自毒胁迫下黄瓜种子萌发和生理特性均有显著影响,这与石玉[25]的研究结果一致,说明Si在胁迫条件下对黄瓜种子萌发的促进作用更明显。

Si可以缓解生物和非生物胁迫[26-28]。已有研究表明,在铜胁迫下,添加硅能够使小麦幼苗根尖细胞结构保持完整,也可在一定程度上缓解小麦幼苗和细胞成分对铜胁迫的伤害[29];硅通过影响光合作用和提高抗氧化酶活性来增加玉米的抗旱能力[30];高浓度硅处理后,黄瓜叶片厚度、干质量、根质量等有明显的正向效应,显著降低了叶片衰老速度,提高了叶片叶绿素含量,光合作用关键物质(1,5-二磷酸核酮糖)含量提高了50%[31]。在干旱胁迫下,Si能够提高大豆幼苗的光合作用和抗氧化参数[32-33],促进种子萌发,减轻番茄幼苗期的氧化胁迫,进而提高番茄叶片的净光合速率[34-38]。叶根施硅防治黄瓜白

粉病后,黄瓜叶片中抗坏血酸(AsA)、谷胱甘肽(GSH)含量及SOD、CAT、POD活性增加[39]。本试验结果表明,在肉桂酸自毒胁迫下添加外源硅可显著增强黄瓜芽苗中的SOD、POD和CAT活性,提高了其清除活性氧的能力,降低了丙二醛含量,进而显著提高黄瓜种子的发芽率,促进胚根伸长并提高鲜质量。自毒作用是一种非生物胁迫,因此添加适宜浓度的外源硅能够有效缓解CA胁迫对黄瓜种子萌发的抑制作用。

种子萌发所需能量主要来源于种子自身储存干物质(淀粉、蛋白质等)的分解转化[40]。种子储藏物质淀粉的代谢主要由一系列水解酶来完成,淀粉酶作为其中的一种,是水解淀粉、糖原的酶类总称,一般作用于可溶性淀粉、直链淀粉、糖原等[41]。苯丙酸(PA)是黄瓜主要自毒物质之一,通过添加外源苯丙酸(PA)模拟黄瓜种子自毒条件,外源硅处理后,显著增加了PA自毒胁迫条件下黄瓜种子萌发过程中的淀粉酶活性,在种子萌发48 h时,对照和PA+Si处理黄瓜的可溶性糖积累量小于PA自毒胁迫处理,对照和PA+Si处理的淀粉含量也显著低于PA自毒胁迫处理[14]。本试验结果表明,在CA自毒条件下添加外源硅,可以显著增加黄瓜苗芽淀粉酶含量,降低了可溶性糖和淀粉含量。

综上所述,2 mmol/L CA处理显著降低了黄瓜种子淀粉酶活性,增加了可溶性糖和淀粉含量,降低了SOD、POD和CAT活性,显著增加了丙二醛(MDA)积累,严重抑制了黄瓜种子萌发;外源添加3 mmol/L Si能显著提高黄瓜种子淀粉酶活性,降低了黄瓜种子中可溶性糖和淀粉含量,显著提高SOD、POD和CAT活性,降低丙二醛(MDA)含量,减轻了活性氧对细胞膜的损伤。因此,适宜的外源硅对肉桂酸模拟的自毒胁迫有明显缓解作用。

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