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外源NO对Ca(NO₃)₂胁迫下番茄叶片活性氧损伤的缓解效应*

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摘要 为探讨外源一氧化氮(NO)对次生盐渍胁迫下植物抗氧化系统的调节作用,以番茄品种‘秦丰保冠’为试材,在营养液栽培条件下研究叶面喷施外源NO供体硝普钠(SNP)对80 mmol·L⁻¹ Ca(NO₃)₂胁迫下番茄幼苗生长、叶片光合、活性氧物质、抗氧化酶活性和抗坏血酸-谷胱甘肽(AsA-GSH)循环的影响。结果表明,Ca(NO₃)₂胁迫下喷施SNP处理的番茄幼苗叶片超氧阴离子(O₂⁻)的产生速率以及过氧化氢(H₂O₂)、丙二醛(MDA)、脱氢抗坏血酸(DHA)、氧化型谷胱甘肽(GSSG)的含量和电解质渗漏率显著降低,超氧化物歧化酶(SOD)、过氧化物酶(POD)、过氧化氢酶(CAT)、抗坏血酸过氧化物酶(APX)、谷胱甘肽还原酶(GR)、脱氢抗坏血酸还原酶(DHAR)和单脱氢抗坏血酸还原酶(MDHAR)的活性显著升高或得以维持,同时叶片抗坏血酸(AsA)、谷胱甘肽(GSH)含量及其还原力(AsA/DHA、GSH/GSSG值)显著升高,叶片活性氧损伤得到有效缓解,叶绿素降解和光合速率的下降得到有效抑制,进而促进了植株的生长发育,提高了番茄幼苗的耐盐能力。

关键词 硝酸钙胁迫;硝普钠(SNP);番茄;膜脂过氧化;AsA-GSH循环

中图分类号 Q945.78 **文献标识码** A

在季节性或常年性覆盖的高温设施内,由于土壤长期得不到雨水淋洗,随水分蒸发运移至土壤表层的盐分逐渐积累,加之连作、高复种指数和过量施肥等多种原因导致的土壤次生盐渍化问题日趋严重^[1-2]。设施土壤次生盐渍化已经成为当前设施栽培的主要限制性因素和设施农业生产可持续发展的严重障碍^[2]。

与滨海、内陆盐土的组分不同,设施次生盐渍土壤的主要盐分组成是:阳离子有Na⁺、K⁺、

Ca²⁺、Mg²⁺等,以Ca²⁺为主,约占阳离子总量的60%以上;阴离子有NO₃⁻、Cl⁻、SO₄²⁻、HCO₃⁻等,以NO₃⁻为主,约占阴离子总量的67%~76%。可见,Ca(NO₃)₂的过量积累是设施次生盐渍土壤的主要特征^[1-2]。番茄(*Lycopersicon esculentum* Mill.)是一种世界性蔬菜,具有产量高、营养丰富和生产效益好等特点,在国内外蔬菜设施栽培生产中具有举足轻重的地位^[3]。因此,研究番茄在Ca(NO₃)₂胁迫下的生理生态变化及其机制,对其高

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产、优质栽培及耐盐品种选育鉴定具有极其重要的意义。

一氧化氮 (Nitric oxide, NO) 是植物体内普遍存在的一种兼具水溶性和脂溶性、能够自由穿梭于细胞膜之间的气态活性物质。作为活跃的小分子信号, NO介导了植物诸多生长发育和生理代谢过程。如种子萌发、根和花粉管的生长、气孔关闭、开花、细胞铁稳态的维持和程序性细胞死亡等^[4]。研究发现, NO还可通过S-亚硝基化过程参与调控几乎所有信号通路, 并与活性氧 (ROS) 相互依赖、相互影响, 共同参与植物对高温^[5]、低温^[6]、水分^[7]、UV-B辐射^[8]、重金属^[9]和盐渍^[10]等多种非生物逆境胁迫应答的防御调控过程。目前, NO调节植物耐盐的研究大多集中于对NaCl胁迫的探讨, 有关NO能否缓解Ca(NO₃)₂胁迫对蔬菜、特别是对番茄植株危害的研究鲜见报道。本课题组前期试验中发现, Ca(NO₃)₂胁迫下, 经NO喷施处理的番茄幼苗叶片光系统II (PS II) 光化学活性显著改善, 过剩光能通过反应中心耗散份额的增加并未加剧光合机构的氧化损伤, 这暗示叶肉组织内强化了ROS清除系统的运转^[2]。鉴于此, 本研究进一步探讨外源NO处理对Ca(NO₃)₂胁迫下番茄幼苗叶片ROS损伤、抗氧化酶活性变化及抗坏血酸-谷胱甘肽 (AsA-GSH) 循环的影响, 旨在揭示NO缓解番茄叶片Ca(NO₃)₂胁迫伤害的机理, 为利用化学诱抗剂缓解设施土壤次生盐渍障碍提供理论和技术依据。

1 材料与方法

1.1 试验材料与处理

供试番茄品种为‘秦丰保冠’, 种子由西安秦丰蔬菜研究所提供。

将籽粒饱满、大小一致的种子进行浸种催芽 (55 °C温水中浸泡3~4 h后放在铺有湿润纱布的培养皿内, 置于29 °C恒温箱中) 处理后, 播种于装有蛭石的塑料营养钵 (直径×高: 10 cm×10 cm) 中, 每钵1株。在日光温室内培育, 待真叶展开后每2天浇1/8浓度霍格兰 (Hoagland) 营养液1次, 每钵浇50 mL, 当幼苗普遍长至四叶一心时, 挑选长势较好且一致的植株栽植于水培箱 (长×宽×高: 60 cm×40 cm×20 cm) 内, 每箱定植6

株, 株行距均为15 cm, 定植前用去离子水洗净根部的育苗基质。恢复生长10 d后开始进行试验处理。用1/4浓度Hoagland营养液栽培, 利用充气泵24 h不间断补充营养液中氧气, 每3天更换1次营养液。

试验设4个处理: (1) 1/4浓度Hoagland营养液栽培 (CK); (2) 1/4浓度Hoagland营养液+100 μmol·L⁻¹硝普钠 (SNP) (CS); (3) 含80 mmol·L⁻¹ Ca(NO₃)₂的1/4浓度Hoagland营养液 (N); (4) 含80 mmol·L⁻¹ Ca(NO₃)₂的1/4浓度Hoagland营养液+100 μmol·L⁻¹ SNP (NS), 每处理重复3次。开始试验后, CS和NS处理每天6:00和18:00进行叶面喷施SNP, 喷液量以叶片正反两面完全湿润且无液体下滴为准; CK和N处理喷施等量的去离子水。Ca(NO₃)₂胁迫浓度、SNP喷叶浓度均根据预备试验筛选确定: 依据是Ca(NO₃)₂达到80 mmol·L⁻¹时, 番茄幼苗生长抑制程度在70%左右, 同时各项形态指标数值的降低几乎均达到了显著水平; SNP处理预设了0、50、100、200、400、800 μmol·L⁻¹ 6个浓度, 100 μmol·L⁻¹ SNP喷叶处理对幼苗的各项形态指标均有显著提高。为防止盐激, 先将栽培营养液中含有的Ca(NO₃)₂浓度增加至40 mmol·L⁻¹, 1天后再将盐浓度补充增至80 mmol·L⁻¹ (将固体Ca(NO₃)₂直接溶于1/4浓度Hoagland营养液中), 此时定为Ca(NO₃)₂胁迫处理开始时间。在胁迫后的第6和12天, 各处理选取6株分别测定上数第2片完全展开叶的净光合速率, 然后随机拔出3株, 摘下叶片, 用液氮速冻后于-80 °C冰箱中保存, 用于其他各项生理生化指标的测定。

1.2 测定项目与方法

生长指标测定: 第15天结束处理后, 各处理取样6株, 用游标卡尺测量茎粗、用直尺测量株高, 分离植株根、茎、叶后分别洗净, 用吸水纸吸干, 并烘干至恒重, 按‘(茎粗/株高)×全株干重100’计算壮苗指数。

叶绿素和光合速率测定: 采用80%丙酮浸提法^[11]测定叶绿素含量; 采用便携式光合仪 (LI-6400XT, LI-COR公司, 美国) 测定光合速率, 仪器使用开放式气路、6400-02B LED-红蓝光源, 光强设置为800 μmol·m⁻²·s⁻¹, 叶室温度为(28±2) °C、CO₂浓度为(360±20) μL·L⁻¹。

活性氧和质膜过氧化水平测定: 按照王爱国和

罗广华^[12]方法测定O₂的产生速率; H₂O₂含量参照林植芳等^[13]方法测定; 丙二醛(MDA)含量测定采用硫代巴比妥酸(TBA)法^[14]; 电解质渗出率测定采用张宪政^[15]的方法。

抗氧化酶活性测定: 超氧化物歧化酶(SOD)活性测定采用氮蓝四唑(NBT)还原法^[14]; 过氧化物酶(POD)活性测定采用愈创木酚比色法^[11]; 过氧化氢酶(CAT)活性测定采用紫外吸收法^[14]; 抗坏血酸过氧化物酶(APX)和脱氢抗坏血酸还原酶(DHAR)活性参照Nakano和Asada^[16]方法测定; 谷胱甘肽还原酶(GR)活性按照Foyer和Halliwell^[17]方法测定; 单脱氢抗坏血酸还原酶(MDHAR)活性采用Hossain等^[18]的方法测定。

非酶抗氧化物质含量测定: 还原型抗坏血酸(AsA)和脱氢抗坏血酸(DHA)含量参照Arakawa等^[19]方法测定; 还原型谷胱甘肽(GSH)和氧化型谷胱甘肽(GSSG)含量采用Griffith^[20]方法测定。

1.3 数据处理

用Excel 2003和Origin Pro 8.5软件整理试验数

据和作图, 用SPSS 19.0统计软件进行单因素方差分析, 采用最小显著差数(LSD)法进行差异显著性检验($P < 0.05$)。

2 结果

2.1 Ca(NO₃)₂胁迫下SNP对番茄生长的影响

生长受抑制、生物量降低是盐胁迫下植物最敏感的生理响应。由表1可以看出, CK处理下, 叶面喷施SNP对番茄幼苗生长无显著影响。Ca(NO₃)₂胁迫15 d后, 植株各生长参数(株高、茎粗、茎叶及根系生物量和壮苗指数)均显著降低。与Ca(NO₃)₂胁迫处理相比, 胁迫条件下叶面喷施SNP处理不同程度提高了番茄幼苗的上述生长指标, 其中, 茎叶和根系生物量及壮苗指数增幅显著, 3指标依次较Ca(NO₃)₂胁迫处理分别增加了17.16%、9.23%和23.35%。说明喷施外源NO能够有效缓解Ca(NO₃)₂胁迫对番茄幼苗生长的抑制, 促进幼苗生长。

表1 不同处理下番茄幼苗生长状况

Table 1 Growth of tomato seedlings under different treatment

处理 Treatment	株高 Plant height/cm	茎粗 Stem diameter/cm	生物量 Biomass/(g·plant ⁻¹)		壮苗指数 Sound seedling index
			茎叶 Shoots	根 Roots	
CK	42.40 ± 1.83ab	0.97 ± 0.05a	2.67 ± 0.08a	0.73 ± 0.01ab	7.80 ± 0.17ab
CS	43.29 ± 2.91a	0.99 ± 0.08a	2.68 ± 0.09a	0.74 ± 0.01a	7.86 ± 0.94a
N	36.48 ± 0.85c	0.74 ± 0.02b	2.04 ± 0.10c	0.65 ± 0.02c	5.44 ± 0.20c
NS	39.27 ± 1.00bc	0.85 ± 0.05b	2.39 ± 0.06b	0.71 ± 0.01b	6.71 ± 0.42b

注: CK、CS、N、NS分别表示对照(1/4浓度霍格兰营养液栽培)、对照条件下叶面喷施100 μmol·L⁻¹硝普钠处理、硝酸钙胁迫处理(含80 mmol·L⁻¹硝酸钙的1/4浓度霍格兰营养液)、胁迫条件下叶面喷施100 μmol·L⁻¹硝普钠处理。同列不同小写字母表示差异显著($P < 0.05$)。下同 Note: CK, CS, N and NS stand for Control (1/4 concentration of Hoagland nutrient solution cultivation), Foliar application of 100 μmol·L⁻¹ sodium nitroprusside under control conditions, Calcium nitrate stress treatment (1/4 concentration of Hoagland nutrient solution medium containing 80 mmol·L⁻¹ calcium nitrate) and Foliar application of 100 μmol·L⁻¹ sodium nitroprusside under stress conditions, respectively. Different lowercase letters in the same column mean significant difference at 0.05 level. The same below

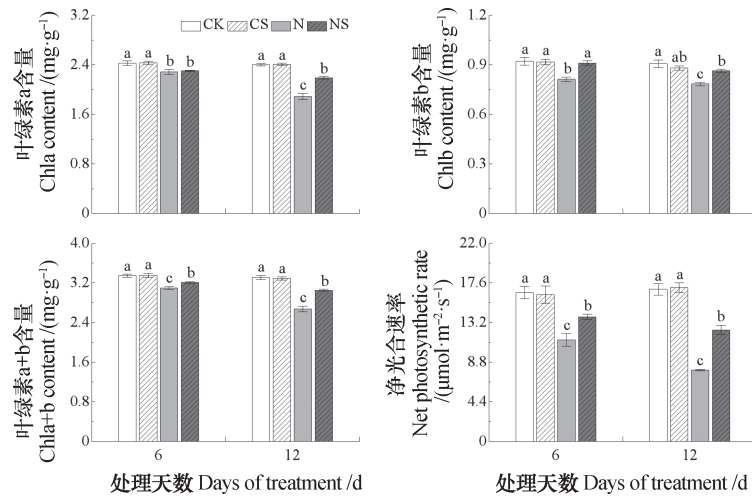
2.2 Ca(NO₃)₂胁迫下SNP对番茄叶片叶绿素含量和净光合速率的影响

如图1所示, 与CK相比, Ca(NO₃)₂胁迫处理显著降低了番茄幼苗叶片的叶绿素a、叶绿素b、叶绿素a+b含量和净光合速率, 且处理时间越长,

降幅越大。Ca(NO₃)₂胁迫下, 叶面喷施SNP处理不同程度缓解了番茄幼苗叶片上述光合生理指标的降低, 处理6 d, 各参数分别较Ca(NO₃)₂胁迫处理高出了0.79%、11.92%、3.74%和22.61%; 处理12 d, 各参数分别较Ca(NO₃)₂胁迫处理显著

高出了15.69%、10.19%、14.08%和56.06%。
CK和CK条件下叶面喷施SNP处理(CS)的

上述各指标变化不明显,且两处理间无显著差异。



注: 不同小写字母表示相同时间不同处理间差异显著 ($P < 0.05$)。下同 Note: Different lowercase letters mean significant difference at 0.05 level between different treatments in the same time. The same below

图1 不同处理下番茄幼苗叶片叶绿素含量和净光合速率

Fig. 1 Chlorophyll content and net photosynthetic rate of tomato seedlings under different treatments

2.3 Ca(NO₃)₂胁迫下SNP对番茄叶片活性氧和质膜过氧化水平的影响

如图2所示,番茄幼苗在Ca(NO₃)₂胁迫下,叶片O₂⁻产生速率以及H₂O₂、MDA含量和电解质渗出率均显著升高,且处理时间越长,升幅越大。与Ca(NO₃)₂胁迫处理相比,胁迫条件下叶面喷施SNP处理显著抑制了幼苗叶片上述

各指标的显著升高,处理6 d,各指标分别较Ca(NO₃)₂胁迫处理降低20.40%、20.58%、29.39%和26.25% ($P < 0.05$);处理12 d,各指标分别较Ca(NO₃)₂胁迫处理降低18.82%、20.15%、26.17%和29.49% ($P < 0.05$)。CK条件下,叶面喷施SNP对番茄幼苗叶片的上述各指标均无显著影响。

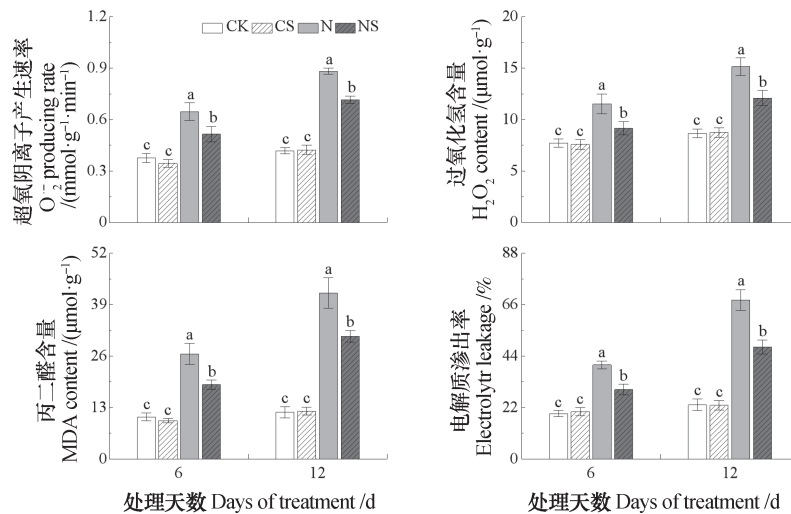


图2 Ca(NO₃)₂胁迫下硝普钠对番茄幼苗叶片活性氧、丙二醛含量及电解质渗出率的影响

Fig. 2 Effects of sodium nitroprusside on active oxygen, malondialdehyde (MDA) content and electrolyte leakage in tomato seedling leaves under Ca(NO₃)₂ stress

2.4 $\text{Ca}(\text{NO}_3)_2$ 胁迫下SNP对番茄幼苗叶片抗氧化系统相关酶活性的影响

番茄幼苗在 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理6 d时, 叶片SOD、POD和CAT活性均较CK显著升高; 在胁迫处理12 d时, 与CK相比, SOD活性显著升高, POD活性变化不显著, CAT活性显著降低(图3)。与 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理相比, 胁迫条件

下叶面喷施SNP处理显著提高了番茄幼苗叶片SOD、POD和CAT活性, 处理6 d, 3指标依次较 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理高出了9.19%、10.28%和14.51% ($P<0.05$); 处理12 d, 各指标依次较 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理高出了48.96%、12.57%和35.33% ($P<0.05$)。CK条件下, 叶面喷施SNP对番茄幼苗叶片SOD、POD和CAT的活性无显著影响。

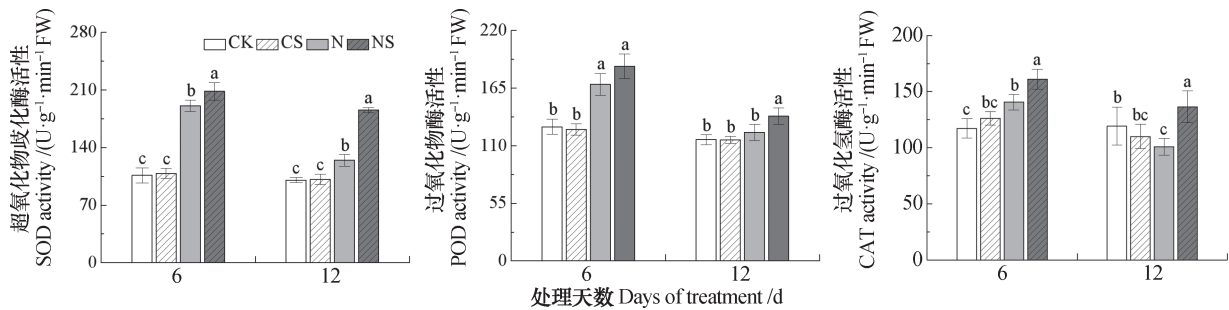


图3 $\text{Ca}(\text{NO}_3)_2$ 胁迫下硝普钠对番茄幼苗叶片抗氧化酶活性的影响

Fig. 3 Effects of sodium nitroprusside on superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities in tomato seedling leaves under $\text{Ca}(\text{NO}_3)_2$ stress

番茄幼苗在 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理6 d时, 与CK相比, 叶片APX活性显著升高, GR活性升幅不显著; 在胁迫处理12 d时, 与CK相比, APX活性升幅不显著, 而GR活性却显著升高(图4)。与 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理相比, 胁迫条件下叶面喷施SNP处理显著提高了番茄幼苗叶片APX和GR的

活性, 处理6 d, 依次较 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理增加了27.73%和46.94% ($P<0.05$); 处理12 d, 依次较 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理增加了43.14%和24.24% ($P<0.05$)。CK和CK条件下叶面喷施SNP处理的APX、GR活性变化不明显, 且两处理间无显著差异。

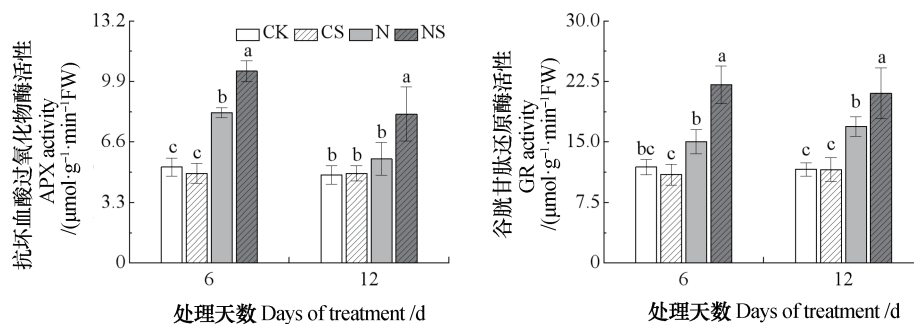


图4 $\text{Ca}(\text{NO}_3)_2$ 胁迫下硝普钠对番茄幼苗叶片抗坏血酸过氧化物酶和谷胱甘肽还原酶活性的影响

Fig. 4 Effects of sodium nitroprusside on activities of ascorbate peroxidase (APX) and glutathione reductase (GR) in leaves of tomato seedlings under $\text{Ca}(\text{NO}_3)_2$ stress

如图5所示, 番茄幼苗在 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理的6 d和12 d, 与CK相比, 叶片DHAR活性显著升高, 且处理时间越长, 升幅越大; MDHAR活性在6 d变化不显著, 在12 d显著提高。 $\text{Ca}(\text{NO}_3)_2$ 胁迫下, 叶面喷施SNP显著提高了番茄幼苗叶片的DHAR活性; 对MDHAR活性的提高在6 d时不显著, 在12 d时达

显著水平, 处理6 d, DHAR和MDHAR活性分别较 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理高出了43.32%和6.61%; 处理12 d, DHAR和MDHAR活性分别较 $\text{Ca}(\text{NO}_3)_2$ 胁迫处理提高了12.83%和25.32% ($P<0.05$)。CK和CK条件下叶面喷施SNP处理的DHAR、MDHAR活性变化不明显, 且两处理间无显著差异。

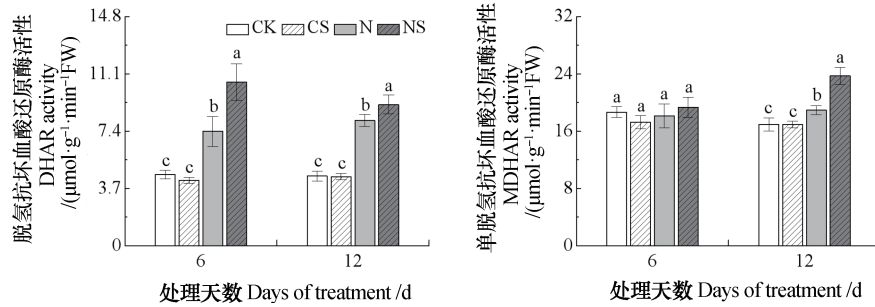


图5 Ca(NO₃)₂胁迫下硝普钠对番茄幼苗叶片脱氢抗坏血酸还原酶和单脱氢抗坏血酸还原酶活性的影响

Fig. 5 Effects of sodium nitroprusside on activities of dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) in leaves of tomato seedlings under Ca(NO₃)₂ stress

2.5 Ca(NO₃)₂胁迫下SNP对番茄幼苗叶片非酶抗氧化物质的影响

番茄幼苗在Ca(NO₃)₂胁迫处理6 d时，叶片AsA含量较CK无显著变化；在胁迫处理12 d时，与CK相比，AsA含量显著降低；同样是在Ca(NO₃)₂胁迫处理6 d和12 d，DHA含量显著升高，AsA/DHA值却显著降低，且处理时间越长，变幅越大（图6）。Ca(NO₃)₂胁迫下，叶面喷施SNP显著提高了番茄幼苗叶

片AsA含量和AsA/DHA值，同时降低了DHA含量，处理6 d，AsA含量和AsA/DHA值分别升高了11.03%和23.88%（*P*<0.05），DHA含量降低了10.53%（*P*<0.05）；处理12 d，AsA含量和AsA/DHA值分别升高了40.73%和61.73%（*P*<0.05），DHA含量降低了13.19%（*P*<0.05）。CK条件下，叶面喷施SNP对番茄幼苗叶片AsA、DHA含量和AsA/DHA值无显著影响。

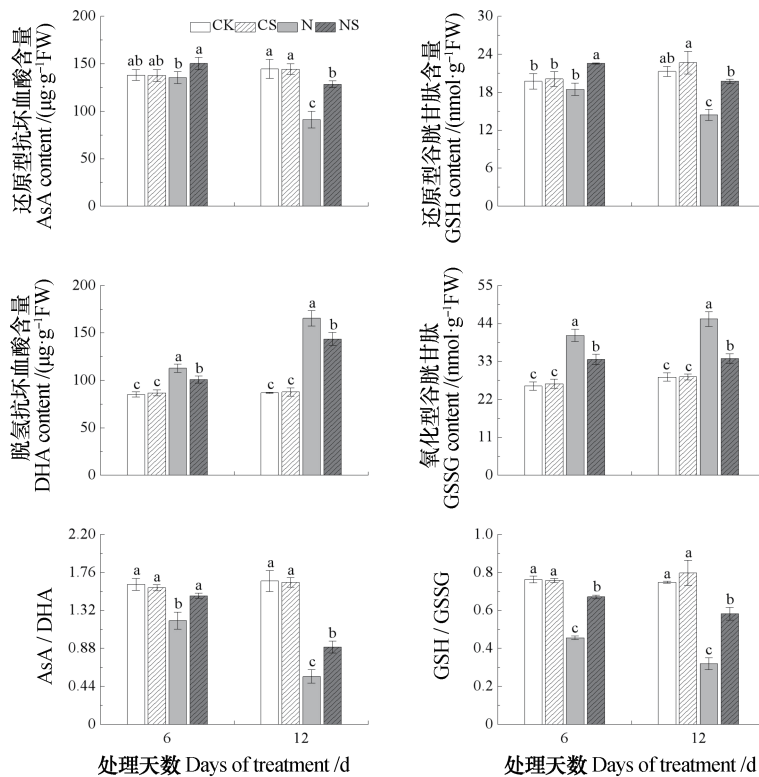


图6 Ca(NO₃)₂胁迫下硝普钠对番茄幼苗叶片抗坏血酸、谷胱甘肽含量及其还原力的影响

Fig. 6 Effects of sodium nitroprusside on contents of ascorbic acid (AsA, DHA) and glutathione (GSH, GSSG) and their reducing power (AsA/DHA, GSH/GSSG) in leaves of tomato seedlings under Ca(NO₃)₂ stress

如图6所示,与CK相比,Ca(NO₃)₂胁迫处理的番茄幼苗叶片GSH含量在6 d时无显著变化,在12 d时显著降低。同样是在Ca(NO₃)₂胁迫处理的6 d和12 d,GSSG含量显著升高,GSH/GSSG值却显著降低,且处理时间越长,变幅越大。Ca(NO₃)₂胁迫下,叶面喷施SNP显著提高了番茄幼苗叶片GSH含量和GSH/GSSG值,降低了GSSG含量,处理6 d,GSH含量和GSH/GSSG值分别升高了22.22%和47.37% ($P<0.05$),GSH含量降低了17.13% ($P<0.05$);处理12 d,GSH含量和GSH/GSSG值分别升高了36.78%和82.76% ($P<0.05$),GSSG含量降低了25.25% ($P<0.05$)。CK条件下,叶面喷施SNP对番茄幼苗叶片GSH、GSSG含量和GSH/GSSG值无显著影响。

3 讨论

尽管Ca²⁺和NO₃⁻是植物吸收钙和氮素营养的主要形式,但生长介质中过量Ca²⁺和NO₃⁻的存在也会干扰植物正常的生理代谢,造成ROS过量积累,引发膜脂过氧化,并导致包括光合膜在内的整个生物膜系统遭到破坏,致使叶片光合作用受阻,植株生物积累量下降^[1-2, 21-23]。本试验中,Ca(NO₃)₂胁迫处理的番茄幼苗叶片MDA含量和电解质渗透率显著升高,叶绿素含量和净光合速率显著降低,进而阻碍了植株生长发育和壮苗的形成。Ca(NO₃)₂胁迫下,叶面喷施SNP处理的幼苗叶片MDA含量和电解质渗透率显著降低、叶绿素含量和净光合速率显著升高,同时植株生物量和壮苗指数亦显著升高,表明外源NO对Ca(NO₃)₂胁迫下番茄幼苗叶片膜脂过氧化和生长的抑制作用均具有缓解效应。

植物在长期适应环境的进化中,发展出了一整套酶促和非酶促抗氧化系统以抵御逆境胁迫产生氧化伤害^[24]。SOD是这一系统内ROS清除的第一道防线,它能将O₂⁻歧化为O₂和毒性相对较弱的H₂O₂,而H₂O₂则可在POD、CAT、AsA-GSH循环及其他抗氧化物质的协同作用下获得清除^[7, 9, 25-26]。Ca(NO₃)₂胁迫下,番茄^[22]、黄瓜^[27]和茄子^[23]的嫁接苗之所以较自根苗具有更强的ROS清除能力,是因为嫁接苗具有更高的抗氧化酶活性水平。Li等^[28]研究认为,在Ca(NO₃)₂胁迫下,应用南瓜砧木嫁接对黄瓜幼苗叶片SOD、POD、CAT、APX

活性提高的促进与这一措施激发了叶中依赖H₂O₂的NO的积累、诱导了上述抗氧化酶基因的表达有关。焦娟等^[29]以0.1 mmol·L⁻¹ SNP处理受硝酸盐胁迫的黄瓜幼苗,结果发现,植株叶片SOD、CAT和APX活性显著升高,POD活性虽有所降低,但表征细胞膜脂过氧化程度的MDA含量却显著下降,相似的研究结果也出现在杨全勇等^[30]对相同硝酸盐及其与缺镁复合胁迫的研究报道中。本试验结果显示,对Ca(NO₃)₂胁迫处理的番茄幼苗进行叶面喷施SNP处理后,叶片SOD、POD、CAT、APX活性均可获得不同程度的提高,而O₂⁻的产生速率和H₂O₂含量却显著下降,表明外源NO处理可以通过提高抗氧化酶活性减少ROS的产生,进而增强了植株的抗(耐)盐能力。这与杜长霞等^[21]对黄瓜幼苗研究的结果一致。以往已有报道指出,通过诱导内源NO的合成,外源NO介导的植物ROS清除途径有二:其一是促进内源NO直接对ROS的清除;其二是利用内源NO信号调控网络增强编码抗氧化酶基因的表达,通过酶促抗氧化反应强化对ROS的清除^[10, 31]。Ca(NO₃)₂胁迫下,外源NO对番茄幼苗叶片ROS清除能力提高的机制是否同时兼具上述两种途径尚待进一步验证。

在APX催化下,AsA将H₂O₂还原成H₂O后,最终被转化成DHA。AsA含量及其还原力(AsA/DHA)与植物抗(耐)逆能力正相关^[25]。GSH是一种能够稳定细胞膜结构的重要抗氧化剂,在AsA-GSH循环中,它可作为电子供体将DHA还原成AsA,而自身被氧化成GSSG^[26, 32]。GSH含量及其还原力(GSH/GSSG)是激活植物抗性基因的信号之一^[26]。有研究指出,相比AsA、GSH的绝对含量,AsA/DHA和GSH/GSSG值的变化更能反映出植物对逆境胁迫的适应^[33]。本研究中,番茄幼苗在Ca(NO₃)₂胁迫第6 d,叶片AsA和GSH含量变化不显著;在胁迫第12 d,AsA、GSH含量显著降低,其原因可能与植株对Ca(NO₃)₂的应激适应有关。胁迫过程中DHA和GSSG含量持续显著升高,AsA/DHA和GSH/GSSG值显著降低,这可能与AsA、GSH参与了ROS的清除而被氧化成DHA和GSSG有关。Ca(NO₃)₂胁迫下,叶面喷施SNP处理在提高幼苗叶片AsA、GSH含量和AsA/DHA、GSH/GSSG值的同时,使DHA、GSSG含量降低,表明外源NO可通过提高AsA-GSH循环中抗氧化剂

的含量及维持其较高的还原状态来降低Ca(NO₃)₂胁迫对番茄幼苗叶片质膜造成的危害,从而起到了保护膜结构稳定的作用。

单脱氢抗坏血酸(MDHA)是AsA的初级氧化产物,它很不稳定,在MDHAR的作用下迅速还原成AsA,作为MDHA的歧化产物,DHA能在DHAR催化下重新生成AsA,而在此过程中,被氧化了的底物GSSG又可在GR的催化下重新还原成GSH^[26,32]。可见,较高的MDHAR、DHAR、GR活性水平是确保AsA、GSH再生和维持AsA-GSH循环高效运转的关键。Ahmad等^[9]研究指出,通过对MDHAR、DHAR、GR等与AsA和GSH再生及AsA-GSH循环运转相关的酶活性的保护,外源NO缓解了重金属镉胁迫对番茄幼苗造成氧化损伤,类似的研究结果也出现于Hasanuzzaman等^[5]对小麦抵御高温胁迫的报道中。在NaCl胁迫下,经SNP处理的黄瓜^[34]和黑麦草^[32]幼苗叶片MDHAR活性无显著变化,对DHAR和GR活性的提升才是外源NO促进AsA、GSH再生及高效运转AsA-GSH循环的真正原因。本研究结果显示,Ca(NO₃)₂胁迫下经叶面喷施SNP处理,幼苗叶片DHAR、GR活性均可获得不同程度的提升;MDHAR活性在第6天变化不显著,在第12天显著提升,表明外源NO可通过适时调控相关酶活性的提高而起到促进或维持AsA和GSH循环再生的作用。需要说明是,MDHAR催化MDHA向AsA再生的同时也关系到光合膜上光系统I(PS I)处O₂⁻的产生(当MDHA缺乏时,MDHAR在PS I处被光还原时产生电子并传递给O₂,而产生O₂⁻)^[35]。本研究中,外源NO调节的MDHAR活性在第6天未发生显著变化的原因可能与NO介导了PS I的光保护有关,此推论的合理与否尚不明确,对此尚需深入研究。

4 结 论

通过对SOD、POD、CAT、APX、GR、MDHAR和DHAR活性升高的维持与促进,以及对AsA、GSH含量及其还原力(AsA/DHA、GSH/GSSG比值)的提高,喷施外源NO处理显著减轻了Ca(NO₃)₂胁迫对番茄幼苗造成的氧化损伤,使叶片膜系统稳定性和光合功能得以显著改善,植株生长抑制得到显著缓解,进而增强番茄幼苗的耐盐

能力。

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Alleviating Effects of Exogenous Nitric Oxide on Oxidative Damage in Tomato Seedling Leaves under Ca(NO₃)₂ Stress

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Abstract 【Objective】In relatively enclosed greenhouses, soil salts tend to accumulate gradually in topsoil duo to the special greenhouse environment of no rain water leaching, high temperature and resultant high evaporation, thus leading to serious secondary salinization of the greenhouse soil. According to relevant reports, the high level of Ca(NO₃)₂ accumulation was one of the main causes of high soil salinity in greenhouses. Nitric oxide (NO) is a kind of micromolecule active material that generally exists inside plants. As a plant hormone and signaling molecule, NO extensively participates in regulating of plants' responses to various adversity stress. The aim of this paper is to investigate roles of exogenous NO in regulating the antioxidant system of plants under Ca(NO₃)₂ stress. 【Method】A hydroponic experiment was conducted to investigate effects of foliar spray of sodium nitroprusside (SNP) as exogenous NO donor on growth of tomato (‘Qin Feng Bao Guan’) seedlings, and photosynthetic, reactive oxygen, anti-oxidase activities,

and ascorbate-glutathione cycle in their leaves under the stress of $80 \text{ mmol}\cdot\text{L}^{-1} \text{ Ca}(\text{NO}_3)_2$. 【 Result 】 In the leaves of the tomato seedlings under $\text{Ca}(\text{NO}_3)_2$ stress, foliar spray SNP significantly lowered O_2^- production rate, H_2O_2 , malondialdehyde, dehydroascorbic acid and oxidized glutathione in content and electrolyte leakage rate, but heightened or maintained superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase and monodehydroascorbate reductase in activity; besides, it significantly increased the contents of ascorbic acid and glutathione and their reducing power. As a result, active oxygen damage of the leaves was effectively alleviated, and chlorophyll degradation and photosynthetic rate declining trend were effectively suppressed. 【 Conclusion 】 Exogenous NO treatment plays an important role in maintaining or improving the activity of antioxidant enzymes and promoting the operation of ascorbate-glutathione (AsA-GSH) cycle in tomato under the stress of $\text{Ca}(\text{NO}_3)_2$, thus relieving the plants of oxidative damage significantly, and improving their leaf membrane system in stability, their photosynthetic function, their tolerance to salt and eventually their growth.

Key words $\text{Ca}(\text{NO}_3)_2$ stress; Sodium nitroprusside; Tomato; Membrane lipid peroxidation; AsA-GSH cycle

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