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外源亚精胺及其抑制剂MGBG对硝酸盐胁迫下番茄幼苗生长和生理特性的影响

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摘　要:[目的]本研究旨在探讨硝酸盐胁迫下亚精胺(Spd)及其抑制剂甲基乙二醛双脒基脲(MGBG)对番茄幼苗生长和生理特性的影响,进而解析内外源Spd如何影响植物响应硝酸盐胁迫的生理过程。[方法]本试验以番茄品种'M82'为试材,研究在1/2倍日本山崎番茄配方营养液(CK)下叶面单施Spd(T1)、单施MGBG(T3)、配施Spd和MGBG(T2)以及在125 mmol•L^{-1} Ca$(NO_3)_2$模拟的硝酸盐胁迫(CS)下叶面单施Spd(T4)、单施MGBG(T6)、配施Spd和MGBG(T5)对番茄幼苗生长、光合作用、抗氧化能力和渗透调节特性的影响。[结果]与CK相比,CS番茄幼苗鲜重、干重、净光合速率、抗氧化酶活性及渗透势显著下降,丙二醛、超氧阴离子、脯氨酸含量和瞬时水分利用率显著上升。与CS相比,T4番茄幼苗干鲜重、净光合速率、蒸腾速率、抗氧化酶活性和可溶性糖含量显著上升,瞬时水分利用率、丙二醛、过氧化氢和渗透势显著降低;T5及T6提高了番茄幼苗的丙二醛、超氧阴离子和过氧化氢含量。[结论]单施Spd可促进番茄幼苗生长,提高光合能力,有效缓解由Ca$(NO_3)_2$介导的氧化胁迫和渗透胁迫。单施MGBG及配施Spd和MGBG均可加重硝酸盐胁迫对番茄幼苗的不利影响,表现为活性氧过量积累,干鲜重、净光合速率和抗氧化酶活性降低。

关键词:Spd; MGBG; 硝酸盐胁迫; 番茄; 生理特性
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植物对氮的需求量比任何其它矿物元素都大,因此蔬菜生产中往往需要施加大量的氮肥,而氮肥滥用会导致设施内土壤次生盐渍化[1-2]。发生次生盐渍化的土壤具有特殊的组成特点,阴离子以NO_3^-为主,约占阴离子总量的67%~76%;阳离子则以Ca^{2+}为主,占土壤盐分阳离子总量的60%以上[3]。研究表明硝酸盐胁迫下蔬菜会出现生长发育减缓、光合能力降低、活性氧积累和渗透压失衡等生理生化变化[4-6]。

多胺(PAs)是植物体内广泛存在的一种多元脂肪胺,常见的PAs包括精胺(Spm)、亚精胺(Spd)和腐胺(Put),其中Spd在胁迫信号转导中作为信号分子参与植物胁迫抗性机制的构建[7]。Hu等[8]的研究发现提高多胺合成能够增强甜瓜对硝酸盐胁迫的耐性。Du等[9]通过蛋白质组学分析发现,喷施Spd能够促进黄瓜的抗氧化酶、非酶促抗氧化剂和多胺积累,减少过氧化氢和丙二醛的积累。甲基乙二醛双脒基脲(MGBG)属于Spm和Spd的生物合成抑制剂,在小麦上喷施MGBG能够降低Spm和Spd含量,提高Put含量[10]。Liu等[11]的研究发现喷施MGBG降低了小麦叶片中游离态Spm和Spd含量,加重了逆境胁迫对植物的伤害。

番茄(*Solanum lycopersicum* L.)是我国设施农业广泛种植的蔬菜作物之一。目前已有大量研究表明Spd在植物抵御逆境胁迫中的积极作用,但

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关于$Ca(NO_3)_2$胁迫下单独喷施MGBG及组合喷施Spd和MGBG相关的研究报告较少,对于其如何诱导番茄生理特性变化的相关机理也不十分清楚。通过喷施MGBG来抑制Spd的生物合成,可以进一步明确内源Spd、外源Spd在植物抵御逆境胁迫中是否具有不同的作用。因此,本试验以番茄'M82'为试材,旨在探究硝酸盐胁迫下单施Spd、MGBG和配施Spd及MGBG如何影响番茄幼苗的生长发育、光合能力、抗氧化能力和渗透调节能力,为基于外源Spd施用的高效抗逆栽培提供理论依据。

1　材料和方法

1.1　试材及处理

试验于2021年3月至2022年6月在山西农业大学园艺站人工气候室中进行。以番茄品种'M82'为试材。选取籽粒饱满,大小一致的种子,经55 ℃温汤杀菌15 min后,置于28 ℃恒温培养箱浸种6 h,在铺有双层滤纸的培养皿中催芽,期间保持湿润。出芽后置于铺有无纺布的水培槽中进行水培育苗,待番茄幼苗长至两叶一心时定植于5 L水培槽(6孔,每孔定植1株),以1/2倍日本山崎番茄配方营养液缓苗,每处理24株重复(4槽)。缓苗6 d后,按表1进行相应的预喷施处理。缓苗7 d按表1更换不同配方营养液进入处理,处理期间每2 d喷施1次相应处理,于处理9 d时进行取样。试验所用$Ca(NO_3)_2·4H_2O$购自恒兴试剂(质量分数≥99%),Spd和MGBG均购自北京索莱宝生物有限公司(纯度98%)。

表1　不同处理的营养液配方和喷施类型

Table 1　Nutrient solution formulations and spray types of different treatments

处理 Treatments	营养液配方 Nutrient solution formulations	喷施处理 Spray treatments
CK	1/2倍营养液	蒸馏水
T1	1/2倍营养液	0.25 mmol·L^{-1} Spd
T2	1/2倍营养液	0.25 mmol·L^{-1} Spd+0.1 mmol·L^{-1} MGBG
T3	1/2倍营养液	0.1 mmol·L^{-1} MGBG
CS	1/2倍营养液+125 mmol·L^{-1} $Ca(NO_3)_2$	蒸馏水
T4	1/2倍营养液+125 mmol·L^{-1} $Ca(NO_3)_2$	0.25 mmol·L^{-1} Spd
T5	1/2倍营养液+125 mmol·L^{-1} $Ca(NO_3)_2$	0.25 mmol·L^{-1} Spd + 0.1 mmol·L^{-1} MGBG
T6	1/2倍营养液+125 mmol·L^{-1} $Ca(NO_3)_2$	0.1 mmol·L^{-1} MGBG

注:CK:1/2倍营养液(对照);T1:1/2倍营养液下喷施Spd;T2:1/2倍营养液下喷施Spd和MGBG;T3:1/2倍营养液下喷施MGBG;CS:$Ca(NO_3)_2$胁迫;T4:$Ca(NO_3)_2$胁迫下喷施Spd;T5:$Ca(NO_3)_2$胁迫下喷施Spd和MGBG;T6:$Ca(NO_3)_2$胁迫下喷施MGBG

Note:CK:1/2 times nutrient solution (control); T1:1/2 times nutrient solution spraying Spd; T2:Spd and MGBG were sprayed in 1/2 nutrient solution. T3:MGBG was sprayed in 1/2 times nutrient solution. CS:$Ca(NO_3)_2$ stress; T4:Spraying Spd under $Ca(NO_3)_2$ stress; T5:Spraying Spd and MGBG under Ca(NO3)2 stress; T6:Spraying MGBG under $Ca(NO_3)_2$ stress

1.2　测定指标及方法

1.2.1　生长指标测定

每处理取5株番茄幼苗,蒸馏水洗净擦干后从子叶处分为地上部和地下部,分别称取鲜重;随后在烘箱中105 ℃杀青15 min,60 ℃烘至恒重后称取干重。株高使用卷尺测量,茎粗使用游标卡尺测量。

1.2.2　光合参数测定

光合色素含量采用酒精浸提法测定。处理9 d的上午8:00~10:00,利用Li-6800便携式光合仪(LI-Cor Inc美国)测定净光合速率(P_n)、蒸腾速率(T_r)、气孔导度(G_s)、环境CO_2浓度(C_a)和胞间CO_2浓度(C_i)等。通过计算得到瞬时水分利用效率(WUE)和气孔限制值(L_S),其中$WUE=P_n/T_r$,$L_S=(C_a-C_i)/C_a\times100\%$。

1.2.3　丙二醛、活性氧含量及抗氧化酶活性的测定

每处理取8株番茄幼苗,用蒸馏水洗净擦干,置于-80 ℃超低温冰箱冻存。丙二醛含量采用2-硫代巴比妥酸法测定[12];超氧阴离子含量采用羟胺氧化法测定[13];过氧化氢含量采用试剂盒测定,购自北京索莱宝生物有限公司。酶液提取参照Gong等[14]的方法。取冻存样品0.5 g,加入5 mL

预冷的磷酸钠缓冲液(PBS,0.05 mol·L^{-1},pH 7.8),匀浆后移入10 mL离心管中,4℃ 9661×g下离心20 min;上清液用于超氧化物歧化酶(SOD)、过氧化物酶(POD)和过氧化氢酶(CAT)活性的测定。其中SOD活性采用NBT光还原法[15],POD活性采用愈创木酚法[16],CAT活性采用紫外吸收法[17]。

1.2.4 渗透势及渗调物质含量的测定

利用VAPR05600露点渗透压仪(Wescor,USA)测定组织液渗透势。可溶性蛋白含量采用考马斯亮蓝G-250比色法测定[18]。可溶性糖采用蒽酮-硫酸法测定,游离氨基酸含量采用茚三酮显色法,脯氨酸含量采用磺基水杨酸法测定[19]。

1.3 数据处理

使用Excel 2021软件统计数据,所有数据采用3次以上重复试验的平均值和标准差;使用SPSS 26.0分析数据,数据处理间差异显著性检验采用Duncan's法,以$P<0.05$为显著性检验水平;使用GraphPad Prism 8.4.0作图。

2 结果与分析

2.1 硝酸盐胁迫下不同喷施处理对番茄幼苗生长的影响

如图1和表2所示,与CK相比,T1的地上部和地下部的干鲜重和株高分别提高32.19%、22.22%和24.50%;T2的地下部鲜重、地下部干重和株高提高;T3的地上部鲜重和株高降低,地下部鲜重提高;CS的总鲜重、总干重、株高和茎粗分别降低80.14%、58.33%、66.14%和57.25%。与CS相比,T4的各项生长指标全面提高,其中地上部与地下部的干鲜重、株高和茎粗分别提高59.68%、62.22%、43.25%和37.06%。



CK:1/2倍营养液(对照);T1:1/2倍营养液下喷施Spd;T2:1/2倍营养液下喷施Spd和MGBG;T3:1/2倍营养液下喷施MGBG;CS:$Ca(NO_3)_2$胁迫;T4:$Ca(NO_3)_2$胁迫下喷施Spd;T5:$Ca(NO_3)_2$胁迫下喷施Spd和MGBG;T6:$Ca(NO_3)_2$胁迫下喷施MGBG

CK:1/2 times nutrient solution (control); T1:1/2 times nutrient solution spraying Spd; T2:Spd and MGBG were sprayed in 1/2 nutrient solution. T3:MGBG was sprayed in 1/2 times nutrient solution. CS:$Ca(NO_3)_2$ stress; T4:Spraying Spd under $Ca(NO_3)_2$ stress; T5:Spraying Spd and MGBG under Ca(NO3)2 stress; T6:Spraying MGBG under $Ca(NO_3)_2$ stress

图1 硝酸盐胁迫下不同喷施处理对番茄幼苗表型的影响

Fig.1 Effects of different spraying treatments on phenotype of tomato seedlings under nitrate stress

2.2 硝酸盐胁迫下不同喷施处理对番茄幼苗光合色素的影响

由表3可知,与CK相比,T1的类胡萝卜素含量提高41.66%;T3的类胡萝卜素含量提高25.00%。与CK相比,CS的番茄幼苗类胡萝卜素含量提高。与CS相比,T4的叶绿素a含量、叶绿素b含量和类胡萝卜素含量分别提高30.94%、23.46%和66.67%,T5的叶绿素a含量提高10.96%。

表2　硝酸盐胁迫下不同喷施处理对番茄幼苗生长的影响

Table 2　Effects of different spraying treatments on tomato seedling growth under nitrate stress

处理 Treatmenst	地上部鲜重/g Shoot fresh weight	地下部鲜重/g Root fresh weight	地上部干重/g Shoot dry weight	地下部干重/g Root dry weight	株高/cm Plant height	茎粗/mm Stem thickness
CK	13.53±1.12b	5.45±0.35c	0.88±0.02c	0.20±0.01b	23.63±0.64c	6.35±0.15b
T1	16.97±0.40a	8.12±0.25a	1.04±0.01a	0.28±0.02a	29.42±0.28a	6.82±0.06a
T2	12.87±1.24bc	6.64±0.57b	0.97±0.01b	0.21±0.02b	25.10±1.01b	6.35±0.06b
T3	12.02±1.03c	6.56±0.23b	0.88±0.06c	0.22±0.02b	22.09±1.41d	6.32±0.31b
CS	2.83±0.10e	0.94±0.05e	0.36±0.02e	0.09±0.01d	8.00±0.82f	2.86±0.17d
T4	4.45±0.44d	1.57±0.01d	0.60±0.03d	0.13±0.01c	11.46±0.68e	3.92±0.15c
T5	2.82±0.16e	0.96±0.05e	0.34±0.01e	0.08±0.01d	8.80±0.32f	2.94±0.08d
T6	2.61±0.01e	0.84±0.02e	0.38±0.02e	0.08±0.02d	8.96±0.89f	3.07±0.11d

注:同列不同小写字母表示处理间差异显著($P<0.05$),表3～表5同

Note:Different lowercase letters indicated significant differences in the same column($P<0.05$),same as table3～table5

表3　硝酸盐胁迫下不同喷施处理对番茄幼苗光合色素的影响

Table 3　Effects of different spraying treatments on Photosynthetic pigment of tomato seedlings under nitrate stress

处理 Treatments	叶绿素a/(mg•g^{-1}FW) Chlorophyll a	叶绿素b/(mg•g^{-1}FW) Chlorophyll b	类胡萝卜素/(mg•g^{-1}FW) Carotenoids	叶绿素a/b Ratio of chlorophyll a to chlorophyll b	总叶绿素/(mg•g^{-1}FW) Total chlorophyll
CK	1.88±0.34de	0.66±0.16cd	0.24±0.04d	2.87±0.18ab	2.54±0.49de
T1	2.12±0.06cd	0.72±0.04cd	0.34±0.00b	2.94±0.06a	2.84±0.10cd
T2	1.69±0.08e	0.57±0.02d	0.28±0.00cd	2.95±0.03a	2.26±0.10e
T3	1.91±0.03de	0.66±0.04cd	0.30±0.01bc	2.89±0.15ab	2.57±0.07de
CS	2.23±0.22cd	0.81±0.10bc	0.32±0.03bc	2.77±0.07ab	3.04±0.32cd
T4	2.92±0.09a	1.00±0.03a	0.45±0.01a	2.92±0.04a	3.91±0.12a
T5	2.60±0.17ab	0.95±0.05ab	0.35±0.03b	2.73±0.04b	3.55±0.22ab
T6	2.45±0.29bc	0.87±0.11ab	0.35±0.04b	2.81±0.05ab	3.32±0.40bc

2.3　硝酸盐胁迫下不同喷施处理对番茄幼苗光合参数的影响

由表4可知,与CK相比,T1的P_n和L_S提高;T2的P_n、T_r和G_s降低,L_S提高了33.33%;T3的P_n、T_r、C_i和G_s降低,其中G_s降低70.20%,L_S提高73.33%。与CK相比,CS的净光合速率(P_n)、蒸腾速率(T_r)、胞间CO_2浓度(C_i)和气孔导度(G_s)分别降低40.36%、85.58%、70.37%和84.75%,瞬时水分利用效率(WUE)和气孔限制值(L_S)分别提高3.14倍和4.07倍。与CS相比,T4的P_n、T_r、C_i和G_s提高,其中C_i提高1.31倍,WUE和L_S分别降低23.60%和43.42%;T5的P_n、WUE和L_S降低,C_i提高1.09倍;T6的P_n和L_S降低,C_i提高1.15倍。

2.4　硝酸盐胁迫下不同喷施处理对番茄幼苗丙二醛和活性氧含量的影响

由图2可知,在番茄幼苗叶片中,与CK相比,T2和T3的丙二醛含量提高;CS的丙二醛含量提高27.89%。与CS相比,T4的丙二醛含量降低;T5和T6的丙二醛含量提高。在番茄幼苗根系中,与CK相比,CS的丙二醛含量提高5.86倍。与CS相比,T4的丙二醛含量降低26.44%;T5和T6的丙二醛含量提高,其中T6提高34.08%,且T6的丙二醛含量显著高于T5。

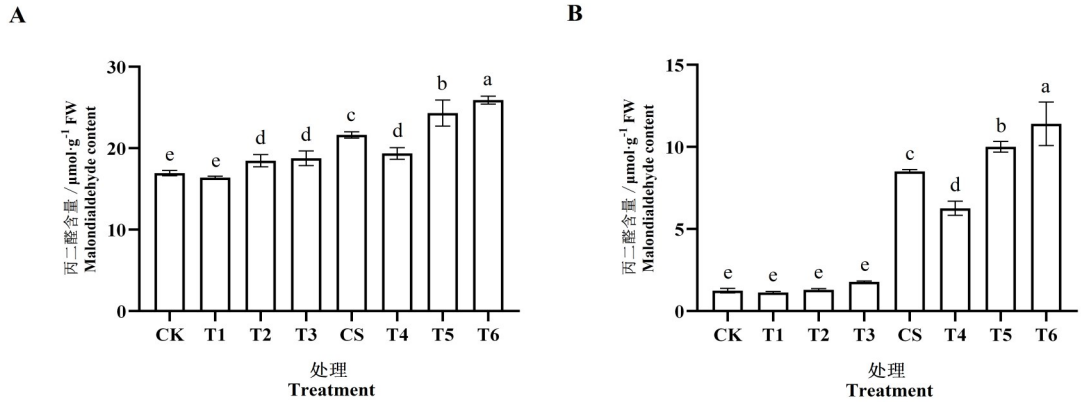
由图3可知,在番茄幼苗叶片中,与CK相比,CS的超氧阴离子含量提高1.40倍。与CS相比,T4的超氧阴离子含量降低30.73%;T5和T6的超氧阴离子含量提高。在番茄幼苗根系中,与CK相比,T1的超氧阴离子含量降低46.08%;CS的超氧阴离子含量提高49.62%。与CS相比,T4的超氧阴离子含量降低46.84%;T5和T6的超氧阴离子含量提高。

由图4可知,在番茄幼苗叶片中,与CK相比,T1的过氧化氢含量降低;CS的过氧化氢含量提高

表4 硝酸盐胁迫下不同喷施处理对番茄幼苗光合参数的影响

Table 4 Effects of different spraying treatments on photosynthetic parameters of tomato seedlings under nitrate stress

处理 Treatments	净光合速率/($\mu mol \cdot m^{-2} \cdot s^{-1}$) Net photosynthetic rate	蒸腾速率/($mmol \cdot m^{-2} \cdot s^{-1}$) Transpiration rate	胞间CO_2浓度/($\mu mol \cdot mol^{-1}$) Intercellular CO_2 concentration	气孔导度/($mmol \cdot m^{-2} \cdot s^{-1}$) Stomatal conductance	瞬时水分利用效率/($\mu mol\ CO_2 \cdot mmol\ H_2O$) Water use efficiency	气孔限制值/% Stomatal limitation
CK	13.28±0.30b	9.85±0.68a	378.96±1.38a	345.14±11.73a	1.35±0.07c	0.15±0.00f
T1	15.56±0.14a	9.61±0.23a	375.24±4.06a	347.68±14.63a	1.62±0.04c	0.18±0.01e
T2	10.49±0.26c	7.49±0.45b	369.02±2.42a	322.03±4.77b	1.40±0.06c	0.20±0.01e
T3	9.75±0.55d	7.52±0.47b	342.90±3.08b	112.92±15.02d	1.30±0.12c	0.26±0.01d
CS	7.92±0.04e	1.42±0.05d	112.30±10.47e	59.70±2.40e	5.59±0.21a	0.76±0.02a
T4	10.73±0.18c	2.61±0.10c	259.49±14.24c	184.30±11.58c	4.12±0.21b	0.43±0.03c
T5	6.42±0.37f	1.46±0.11d	235.45±9.25d	60.87±7.13e	4.39±0.15b	0.48±0.02b
T6	6.10±0.18f	1.21±0.25d	241.20±9.73d	43.38±4.67e	5.17±0.91a	0.47±0.02b



A:叶片;B:根系;不同小写字母表示差异显著($P<0.05$);图3~图9同

A: leaf; B:root;Different lowercase letters indicate significant differences($P<0.05$);same as fig. 3~fig. 9

图2 硝酸盐胁迫下不同喷施处理对番茄幼苗丙二醛含量的影响

Fig.2 Effects of different spraying treatments on MDA content of tomato seedlings under nitrate stress

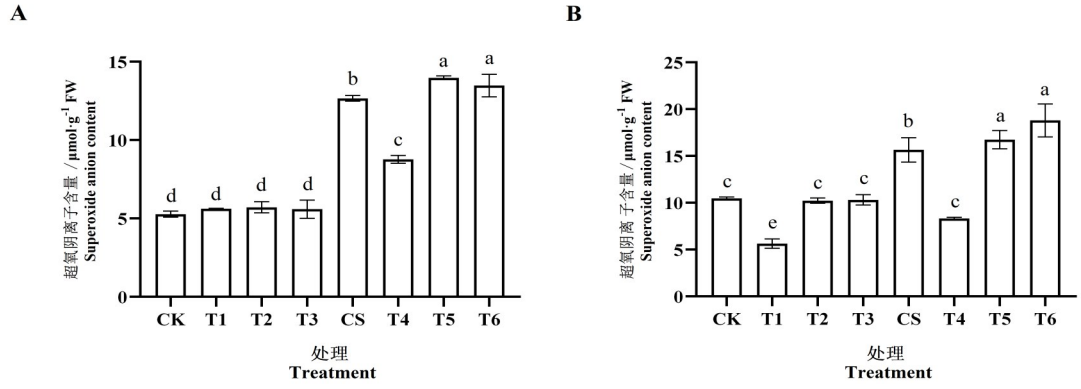


图3 硝酸盐胁迫下不同喷施处理对番茄幼苗超氧阴离子含量的影响

Fig.3 Effects of different spraying treatments on superoxide anion content of tomato seedlings under nitrate stress

1.38倍。与CS相比,T4的过氧化氢含量降低20.49%;T5和T6的过氧化氢含量提高。在番茄幼苗根系中,与CK相比,T1的过氧化氢含量降低29.00%。与CK相比,CS的过氧化氢含量提高34.57%。与CS相比,T4的过氧化氢含量降低46.88%,T5和T6的过氧化氢含量分别提高

84.52%和88.53%。

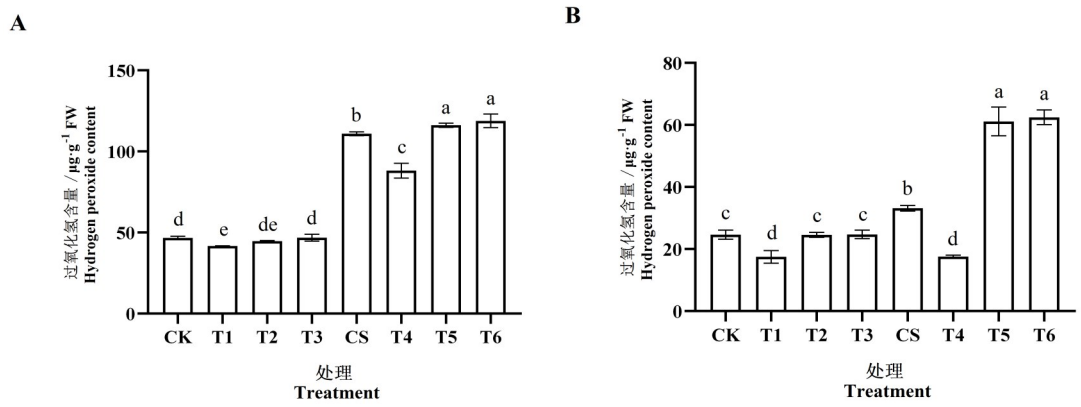


图4　硝酸盐胁迫下不同喷施处理对番茄幼苗过氧化氢含量的影响

Fig.4　Effects of different spraying treatments on H_2O_2 content of tomato seedlings under nitrate stress

2.5　硝酸盐胁迫下不同喷施处理对番茄幼苗抗氧化酶活性的影响

由表5可知,在番茄幼苗叶片中,与CK相比,T1的SOD、POD和CAT活性提高;T2的POD活性降低;T3的POD活性提高,CAT活性降低;CS的SOD和CAT活性分别提高36.90%和23.31%,POD活性降低63.99%。与CS相比,T4的SOD、POD和CAT活性提高;T6的POD活性提高,CAT活性降低。在番茄幼苗根系中,与CK相比,T1的SOD、POD和CAT活性提高,其中POD活性提高61.66%;T2和T3的CAT活性降低;CS的SOD、POD和CAT活性提高。与CS相比,T4的SOD、POD和CAT活性提高。

表5　硝酸盐胁迫下不同喷施处理对番茄幼苗抗氧化酶活性的影响

Table 5　Effects of different spraying treatments on antioxidant enzyme activities of tomato seedlings under nitrate stress

处理 Treatments	SOD活性/(U·g⁻¹ FW·min⁻¹) SOD activity		POD活性/(U·g⁻¹ FW·min⁻¹) POD activity		CAT活性/(U·g⁻¹ FW·min⁻¹) CAT activity	
	叶片 Leaf	根系 Root	叶片 Leaf	根系 Root	叶片 Leaf	根系 Root
CK	3.93±0.10d	3.10±0.03de	232.79±17.1b	165.86±14.64d	120.74±4.52de	1 034.72±25.03c
T1	4.68±0.13c	3.64±0.20c	297.00±8.46a	268.13±6.15a	142.88±4.72bc	1 084.99±13.50bc
T2	4.05±0.04d	2.71±0.25e	215.41±10.68c	190.52±17.12cd	118.30±2.28de	862.80±8.31d
T3	4.02±0.19d	2.85±0.19e	201.40±3.85c	193.94±27.87cd	111.99±3.87f	877.93±27.01d
CS	5.38±0.10b	4.32±0.33b	83.83±1.72f	183.17±21.77cd	148.89±5.22b	1 155.29±8.95b
T4	5.61±0.05a	4.87±0.09a	127.32±10.73d	232.07±5.76b	169.65±8.00a	1 624.91±72.19a
T5	5.36±0.04b	3.44±0.40cd	90.72±4.44ef	202.98±7.26c	140.31±18.47bc	1 095.43±64.50bc
T6	5.24±0.13b	3.59±0.38c	102.07±6.53e	202.03±0.78c	132.35±3.67cd	1 115.77±53.34b

2.6　硝酸盐胁迫下不同喷施处理对番茄幼苗渗透势及渗透调节物质含量的影响

由图5可知,在番茄幼苗叶片中,与CK相比,CS的渗透势降低53.95%。与CS相比,T4、T5和T6的渗透势降低,其中T4的渗透势低于T5和T6。在番茄幼苗根系中,与CK相比,T1的渗透势降低;CS的渗透势降低23.88%。与CS相比,T4的渗透势降低。

由图6可知,在番茄幼苗叶片中,与CK相比,CS的可溶性糖含量提高70.05%。与CS相比,T4的可溶性糖含量提高28.29%。在番茄幼苗根系中,与CK相比,T1的可溶性糖含量降低。

由图7可知,在番茄幼苗叶片中,与CK相比,T1的可溶性蛋白含量提高。与CS相比,T4的可溶性蛋白含量提高35.89%;CS的可溶性蛋白含量降低43.47%。在番茄幼苗根系中,与CK相比,

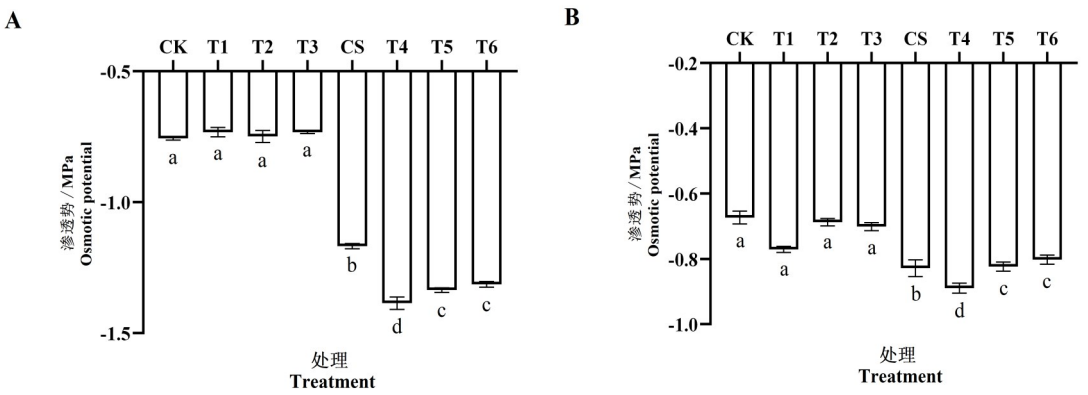


图5 硝酸盐胁迫下不同喷施处理对番茄幼苗渗透势的影响

Fig.5 Effects of different spraying treatments on osmotic potential of tomato seedlings under nitrate stress

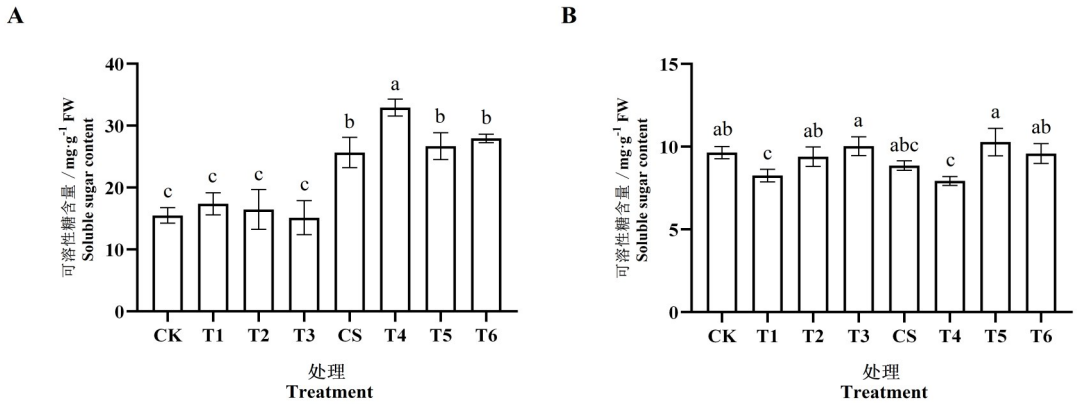


图6 硝酸盐胁迫下不同喷施处理对番茄幼苗可溶性糖含量的影响

Fig.6 Effects of different spraying treatments on soluble sugar content of tomato seedlings under nitrate stress

T1的可溶性蛋白含量提高。与CS相比,T4、T5和T6的可溶性蛋白含量均显著提高,其中T4提高了38.22%,并且T4显著高于T5和T6。

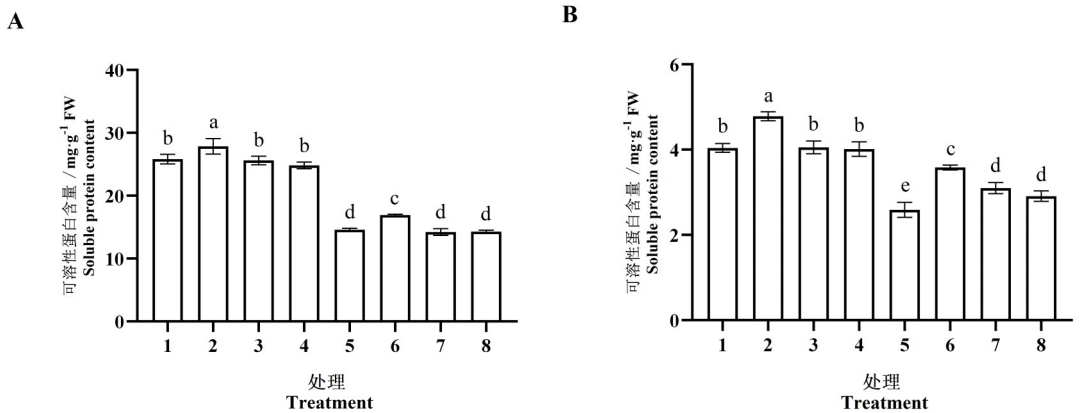


图7 硝酸盐胁迫下不同喷施处理对番茄幼苗可溶性蛋白含量的影响

Fig.7 Effects of different spraying treatments on soluble protein content of tomato seedlings under nitrate stress

由图8可知,在番茄幼苗叶片中,与CK相比,CS的游离氨基酸含量提高3.05倍。与CS相比,T4的游离氨基酸含量提高24.53%;T5和T6的游离氨基酸含量降低。在番茄幼苗根系中,与CK相

比,与CK相比,T1的游离氨基酸含量提高;CS的游离氨基酸含量提高96.56%。与CS相比,T4的游离氨基酸含量提高34.89%。

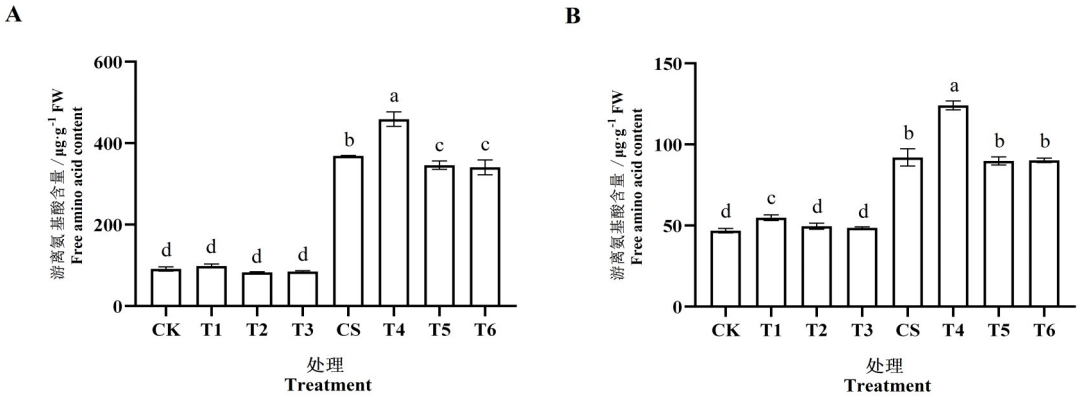


图8　硝酸盐胁迫下不同喷施处理对番茄幼苗游离氨基酸含量的影响

Fig.8　Effects of different spraying treatments on free amino acid content of tomato seedlings under nitrate stress

由图9可知,在番茄幼苗叶片中,与CK相比,T1的脯氨酸含量提高41.26%;T2的脯氨酸含量分别降低41.26%和26.21%;CS的脯氨酸含量提高2.55倍。与CS相比,T4、T5和T6的脯氨酸含量降低,其中T4降低45.14%且低于T5和T6。在番茄幼苗根系中,与CK相比,CS的脯氨酸含量提高7.42倍。与CS相比,T4、T5和T6的脯氨酸含量分别降低74.46%、48.12%和48.71%,其中T4低于T5和T6。

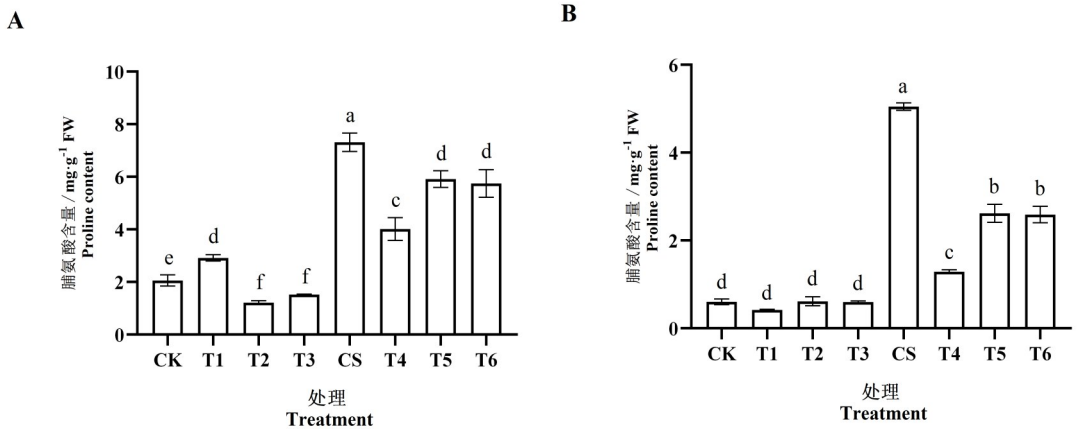


图9　硝酸盐胁迫下不同喷施处理对番茄幼苗脯氨酸含量的影响

Fig.9　Effects of different spraying treatments on proline content of tomato seedlings under nitrate stress

3　讨论

$Ca(NO_3)_2$胁迫下不同种类及不同形态PAs的相互转化影响着植物适应硝酸盐胁迫的能力。El-Shintinawy的试验结果表明,盐胁迫提高了小麦内源性Spm和Spd的积累,降低了Put含量[20]。Hu等[8]的研究表明,硝酸盐胁迫下甜瓜幼苗可通过增加Put向共轭态或结合态的Spm和Spd的转化来减轻硝酸盐胁迫的伤害。由此可知,逆境胁迫下Put向Spm和Spd的转化是植物面对胁迫的一种生理响应,且高水平的Spm和Spd能够提高植物抗逆性。植物内源性Spm和Spd的合成主要受S-腺苷甲硫氨酸脱羧酶(SAMDC)水平的调节[21],MGBG主要通过降低SAMDC水平来调控PAs水平[22]。Liu等[11]的研究发现渗透胁迫下喷施MGBG能够降低小麦游离态Spm和Spd含量,提高Put含量,并加重了胁迫对小麦生长的抑制。

为了明确喷施Spd和MGBG对番茄幼苗硝酸盐胁迫耐受性的调控效应,我们通过观察表型和测定生物量对其生长特性进行研究,发现过量的$Ca(NO_3)_2$会严重抑制番茄生长发育,而周晨楠等[23]发现在番茄幼苗上外源喷施Spd能够缓解这种生长抑制作用。本试验中,125 mmol·L^{-1} $Ca(NO_3)_2$处理后番茄幼苗鲜重、干重、株高和茎粗均降低(表2)。单施Spd后,地上部和地下部的干鲜重及株高、茎粗均有不同程度的提高;配施Spd和MGBG后,地上部和地下部的干鲜重以及株高、茎粗与单纯盐胁迫并无显著差异(表2)。因此,番茄幼苗喷施Spd对$Ca(NO_3)_2$介导的生长抑制具有缓解作用,喷施MGBG会抑制这种作用。

为了探究Spd促进番茄幼苗生物量积累的内在机理,我们测定了光合参数,以期从光合作用角度找到其加快干物质形成的根源。我们发现$Ca(NO_3)_2$胁迫能够降低植物的光合能力,主要表现为P_n下降、T_r下降、C_i下降、G_s下降[24],而WUE和Ls提高(表4)。因此,本试验中番茄幼苗光合能力的降低主要是受气孔因素限制。$Ca(NO_3)_2$胁迫下单施Spd后,P_n、T_r、C_i和G_s均有不同程度的提高(表4),说明Spd能够缓解$Ca(NO_3)_2$胁迫对叶片气孔的限制。与CS相比,T5和T6的P_n降低是由于MGBG的存在抑制了Spd起效,光合电子传递链受到活性氧积累的阻碍。WUE在$Ca(NO_3)_2$胁迫下显著提高,这是由于$Ca(NO_3)_2$胁迫能够减少植物的水分吸收而降低蒸腾速率[25]。杨妮等[26]的试验结果表明,喷施Spd能够缓解盐胁迫对叶绿素合成基因的抑制,提高光合色素含量。此外,$Ca(NO_3)_2$胁迫下喷施Spd能够提高番茄叶片叶绿素和类胡萝卜素含量(表3)。其中类胡萝卜素能够通过吸收三线态叶绿素的多余能量和猝灭单线态氧来保护细胞免受光氧化损伤[27]。

我们通过测定抗氧化系统及渗透调节系统的相关指标用于表征Spd和MGBG对番茄幼苗抗逆性的影响,发现$Ca(NO_3)_2$胁迫能够诱导氧化胁迫,提高超氧阴离子和过氧化氢积累量,进而增大细胞的氧化压力[28]。本试验中,$Ca(NO_3)_2$胁迫下番茄幼苗抗氧化酶活性下降(表5),说明酶促抗氧化酶系统受损,使细胞承受更大的氧化压力。金春燕等[29]的试验表明,$Ca(NO_3)_2$胁迫下黄瓜喷施Spd后抗氧化酶活性提高,活性氧和丙二醛含量降低。本试验中,单施Spd后番茄幼苗丙二醛和活性氧含量下降,抗氧化酶活性提高,即Spd能够加强活性氧代谢速率,从而降低质膜的氧化压力。Spd还能够直接与SOD和CAT结合形成一种渗透性更强的共价复合物,更容易渗入活性氧过量积累的细胞器,提高清除效率[30]。植物的生理代谢进程处于一定的渗透势环境下,$Ca(NO_3)_2$模拟的胁迫对植物造成的不利影响主要源于渗透胁迫[31]。渗透调节可以是由脱水引起的简单被动溶质浓度梯度,也可以是由净溶质积累引起的水势梯度,其中后者被视为主动渗透调节[32]。本试验中,$Ca(NO_3)_2$胁迫下渗透势降低,这是由于添加$Ca(NO_3)_2$模拟胁迫降低了营养液中的渗透势,番茄幼苗因脱水而形成了胞内外溶质浓度梯度;在$Ca(NO_3)_2$胁迫下喷施Spd后,提高了胞内净溶质积累,使渗透势进一步降低(图4)。喷施Spd可通过提高光合能力,调控碳代谢过程,加强可溶性蛋白的合成和积累[33]。本试验中,番茄幼苗可溶性糖(叶片)和游离氨基酸含量在喷施Spd后显著提高(图7),说明Spd通过积累可溶性糖(叶片)和游离氨基酸来调节渗透势,且该机制受被动渗透调节和主动渗透调节的共同影响。

4 结论

$Ca(NO_3)_2$胁迫能够抑制番茄幼苗生长并降低光合和代谢能力,喷施Spd可通过提高番茄幼苗的生长能力、光合能力、抗氧化能力和渗透能力来缓解$Ca(NO_3)_2$胁迫的不利影响,喷施MGBG能够抑制由内源、外源Spd带来的缓解效应并加重$Ca(NO_3)_2$胁迫介导的氧化伤害。

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Effects of exogenous Spd and MGBG on the growth and physiological characteristics of tomato seedlings under nitrate stress

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Abstract: [Objective] This study aimed to investigate the effects of spermidine (Spd) and its inhibitor methylglyoxal bis (guanylhydrazone) (MGBG) on the growth and physiological characteristics of tomato seedlings under nitrate stress, to further explore how endogenous and exogenous Spd affect the physiological responses of plants to nitrate stress. [Methods] In this study, the tomato variety 'M82' was used as the test material. Treatments under standard conditions with ½-strength Yasaki tomato formula nutrient solution (CK) included the foliar application of Spd alone (T1), MGBG alone (T3), and a combination of Spd and MGBG (T2). Under simulated nitrate stress with 125 mmol·L^{-1} $Ca(NO_3)_2$ (CS), treatments included Spd alone (T4), MGBG alone (T6), and a combination of Spd and MGBG (T5). The effects of these treatments on tomato seedling growth, photosynthetic, antioxidant capacity, and osmotic regulation were evaluated. [Results] The results indicated that compared with CK, CS-treated tomato seedlings showed significantly decrease in fresh weight, dry weight, net photosynthetic rate, antioxidant enzyme activity, and osmotic potential, along with significant increases in MDA, superoxide anion, proline content, and instantaneous water use efficiency. Compared with CS, T4 significantly increased dry weight, net photosynthetic rate, transpiration rate, antioxidant enzyme activity, and soluble sugar content, while significantly reducing instantaneous water use efficiency, MDA, hydrogen peroxide, and osmotic potential. In contrast, T5 and T6 treatments increased the accumulation of MDA, superoxide anion, and hydrogen peroxide in tomato seedlings. [Conclusion] In summary, the application of Spd alone promoted seedling growth, enhanced photosynthesis, and effectively alleviated oxidative and osmotic stress induced by $Ca(NO_3)_2$. However, the use of MGBG alone or in combination of Spd and MGBG exacerbated the adverse effects of nitrate stress, leading to excessive accumulation of reactive oxygen species, reduced dry weight, net photosynthetic rate, and antioxidant enzyme activity.

Keywords: Spd, MGBG, Nitrate stress, Tomato, Physiological characteristics

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