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硝普钠及其光解产物对日本晴水稻幼苗生长和 5 种激素标记基因表达的影响

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摘　要:以粳稻品种日本晴水稻为材料,检测硝普钠及其光解产物 KNO_2、$K_4Fe(CN)_6$ 对幼苗生长的影响,并采用 qRT－PCR 技术检测了 5 种植物激素标记基因在经上述处理后在幼苗根中的表达水平.结果表明 SNP 能够显著抑制水稻幼苗的根长和株高;SNP 对根生长的抑制主要是通过其光解产物 $K_4Fe(CN)_6$ 实现的.SNP 和 $K_4Fe(CN)_6$ 处理都能够抑制生长素、细胞分裂素、脱落酸和赤霉素 4 种激素标志基因在水稻根中的表达,但是 SNP 处理可以抑制一氧化氮标志基因 *OsNOA1* 的表达,而 $K_4Fe(CN)_6$ 则上调该基因的表达.

关键词:硝普钠;水稻;幼苗;植物激素;基因表达

中图分类号:Q945.78　**文献标志码**:A

在高等植物中,根是重要的营养器官之一,具有吸收、储藏和运输营养物质的功能.更重要的是,根部产生的植物激素影响着地上部分的生长和发育.以往的研究表明,多种植物激素参与调控根的生长发育,其中生长素、细胞分裂素及赤霉素均能促进根的生长[1].近期的研究发现,气体信号分子一氧化氮(nitric oxide,NO)也参与植物根的发育调节过程[2~3],其作用方式涉及植物激素信号传导网络,但是机制尚不清楚.因此,对植物根生长发育与 NO 等激素信号关系的研究有待深入.

水稻是重要的粮食作物和模式植物,已有的研究表明 NO 影响水稻根的形态建成,包括水稻侧根的形成[4].硝普钠(sodium nitroprusside ,SNP)作为研究常用的 NO 供体,经过光解后会产生 3 种产物即 NO,KNO_2 和 $K_4Fe(CN)_6$,尽管已有 SNP 对水稻生长发育影响的检测[5~7],但是 SNP 及其光解产物对水稻生长发育的系统研究尚未见报道.为此本文以粳稻品种日本晴为材料,以外源 NO 供体 SNP 以及光解产物 KNO_2,$K_4Fe(CN)_6$ 处理幼苗,并采用 qRT－PCR 检测在上述处理条件下水稻植物激素标记基因的表达水平,旨在检测其对水稻幼苗生长的影响,并明确 SNP 处理对生长素、细胞分裂素等植物激素水平的影响,为进一步鉴定 NO 在水稻生长发育中的功能奠定基础.

1　材料和方法

1.1　供试材料

水稻粳稻品种日本晴(*Oryza sativa L. japonica. cv.* Nipponbare)由本实验室保存.

1.2　供试试剂

硝普钠,$K_4Fe(CN)_6$,KNO_2 购于北京鼎国生物有限公司;总 RNA 提取试剂 TRNzol,DNaseI 酶,PrimeScript　RT Master Mix 购于大连宝生物有限公司;SYBR　Green Master Mix 购于南京诺唯赞生物科技有限公司.

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1.3 方　法

1.3.1 水稻幼苗的培养和处理

取水稻种子适量用 75% 的酒精消毒，无菌水冲洗，室温浸泡 1 h 后，置于人工气候箱 28 ℃ 黑暗培养催芽，待种子发芽后转移至 Yoshida 完全营养液[8] 进行水培，培养温度 28 ℃，光照强度为 16 000 lx，光照时间为 12 h/d. 外源 NO 处理采用 SNP 添加到 Yoshida 完全营养液中，终浓度分别为 0、0.02、0.04、0.06、0.08 mM，每 4 d 更换一次营养液，培养 12 d 取样，测量不同处理组水稻的根长和株高，每组样品重复 3 次.

1.3.2 总 RNA 的提取

取生长至 12 d 的水稻幼苗根，液氮研磨后，按照 TRNzol 说明书的要求提取总 RNA，并采用超微分光光度计检测 RNA 样品浓度及质量.

1.3.3 反转录

按照宝生物反转录试剂盒说明书的要求进行反转录，制备 cDNA 反应体系如下：5×PrimeScript RT Master Mix(2 μL)、Total RNA(≤500 ng)、RNase Free H_2O(补至 10 μL). 将上述反应体系短暂离心混匀后，在 37 ℃ 保温 15 min，然后 85 ℃ 5 s 终止反应.

1.3.4 实时定量 PCR

用以上制备的 cDNA 为模板，内参基因选用 *OsAct*1，其他检测基因引物见表 1. 按照 SYBR Green Master Mix 说明书进行配制 20 μL 体系：2×SYBR Green Master Mix(10 μL)、上游引物(0.4 μL)、下游引物(0.4 μL)、ROX Reference Dye Ⅱ(50×)(0.4 μL)、cDNA(2 μL)和 RNase Free H_2O(6.8 μL). 采用两步法进行 PCR 扩增：95 ℃ 预变性，5 min；PCR 反应 95 ℃，10 s；然后 60 ℃，30 s，并收集数据. 设置未处理水稻为对照组，每个样品平行重复 3 次.

表 1　实时定量 PCR 引物一览表

引物序号	引物序列 (5′—3′)	基因名称	基因特征
P1	TCTTCCAGCCTTCCTTCA	*OsAct*1	肌动蛋白基因[9]
P2	ATCCACGTCGCACTTCAT		
P3	ATAATCCTCGCCACAGGCTACA	*OsYUCCA*1	IAA 标记基因[10]
P4	GCAGTGCGGACAGAAAGAAAA		
P5	TACGAGTGCTGCTTCCTCTGGG	*OsIPT*	CTK 标记基因[11]
P6	AGATGCCCCTGGAGTAGTCGGT		
P7	ATCGGCTGGAGATGAAGAGGG	*OsGA20ox*1	GA 标记基因[12]
P8	GCGGCTCATCTCGTGGCAGT		
P9	ACTGTTGTCGGAGCCTGTG	*OsNCED*2	ABA 标记基因[13]
P10	GGAAGGTCAGATTCGCATAG		
P11	TGAAGGATGGGTTGGTCTG	*OsNOA*1	NO 标记基因[14]
P12	ACCCACCCGAGACCAGAAA		

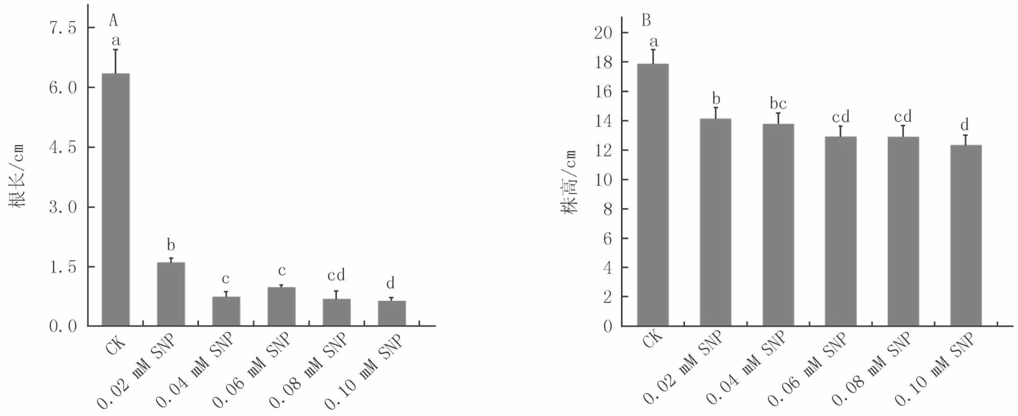
1.4 数据分析

采用 DPS 数据处理软件对水稻根长和株高数据进行方差分析，实时定量 PCR 的数据采用 $2^{-\Delta\Delta C_t}$ 法进行处理分析. 数据分析结果用 Origin 8.5 软件绘制图表.

2 结果与分析

2.1 SNP 对水稻幼苗生长的影响

采用不同浓度的 SNP 处理水稻幼苗，统计根长和株高，发现与未处理的水稻相比，不同浓度的 SNP 对幼苗的根和地上部分的生长均有抑制作用(图 1). 结果显示，随着 SNP 处理浓度的升高，水稻的根长和株高都明显的降低，统计学分析显示 SNP 对地上部分生长的抑制程度不如对根的抑制显著. 与对照相比，随着 SNP 浓度的增加，根长降低幅度为 88%～90%.



"a, b, c, d" 代表$P<0.05$显著分析

图1 不同浓度SNP对水稻幼苗根长(A)和株高(B)的影响

2.2 SNP 及其光解产物对水稻幼苗生长的抑制效应分析

为了解 SNP 抑制根生长的作用机制,进一步采用 SNP 及其光解产物 KNO_2 和 $K_4Fe(CN)_6$ 分别处理幼苗,与对照根长进行比较,结果显示,SNP 和 $K_4Fe(CN)_6$ 处理后,根的长度与对照相比显著降低,SNP 处理根长略微短于 $K_4Fe(CN)_6$ 处理,而 KNO_2 处理后,水稻幼根长度与对照差别不大,甚至略有增加(图 2,图 3A).此外,对株高的统计也显示出类似的结果(图 3B),表明 SNP 对根生长的抑制效应中,$K_4Fe(CN)_6$ 发挥主要作用.

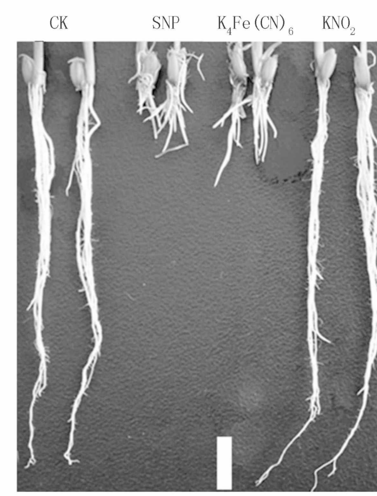


图2 SNP及其光解产物对水稻幼苗根长的影响
处理浓度为0.06 mM,Bar=1 cm

2.3 SNP 及其光解产物对激素标记基因在水稻幼苗根中表达的影响

分别选择 5 种植物激素生长素(IAA)、细胞分裂素(CTK)、赤霉素(GA)、脱落酸(ABA)和 NO 的标记基因 *OsYUCCA*1,*OsIPT*,*OsGA*20*ox*1,*OsNCED*2 和 *OsNOA*1,采用 qRT-PCR 检测上述激素标记基因在经 SNP 及其光解产物处理后的水稻根中表达特征,结果显示,SNP 处理后,5 种基因表达都下调;而 $K_4Fe(CN)_6$ 处理后,除 *OsNOA*1 基因表达上调外(图 4E),其余 4 种基因表达都下调;另外 KNO_2 处理后,除了 *OsIPT*(图 4B)和 *OsNCED*2(图 4C)这两种基因表达与对照组没有显著差异外,其他 3 种标记基因表达水平都显著上调.

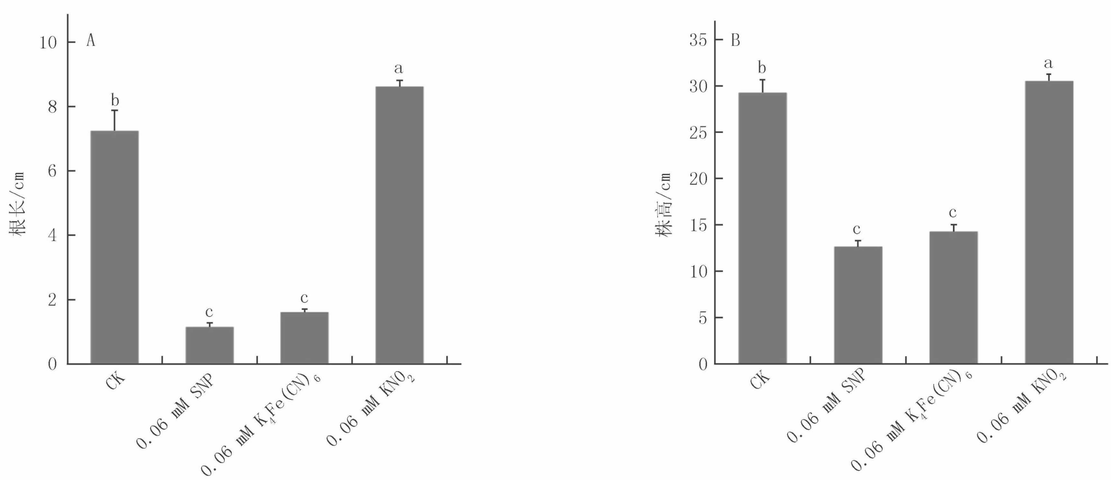
3 讨 论

NO 已被证明是普遍存在于动植物中的气体信号分子.已有的报道表明,NO 参与植物多种生长发育过程的调节,例如植物根的形态建成[15],植物细胞的程序性死亡[16],气孔的关闭[17]等.本研究用 SNP 处理水稻幼苗,结果显示水稻幼苗根系伸长生长被抑制,根的形态与处理前相比也发生明显的改变,侧根呈现短粗特征.SNP 处理后,水稻株高也明显的降低,可能是由于 SNP 对根生长的抑制,影响了根对营养的吸收,可能还引起植物激素水平降低,导致水稻株高的降低.

由于 SNP 光解时生成 KNO_2,$K_4Fe(CN)_6$ 和 NO 3 种物质,为此,本研究采用 SNP 及其光解产物分别处理水稻进一步研究 SNP 的作用机制.结果显示,SNP 与 $K_4Fe(CN)_6$ 都能够抑制水稻根的生长,且两种处理水稻幼根的表型相似,提示在上述条件下 SNP 对根生长的抑制效应是 NO 和 $K_4Fe(CN)_6$ 共同作用的结果,且以 $K_4Fe(CN)_6$ 为主.

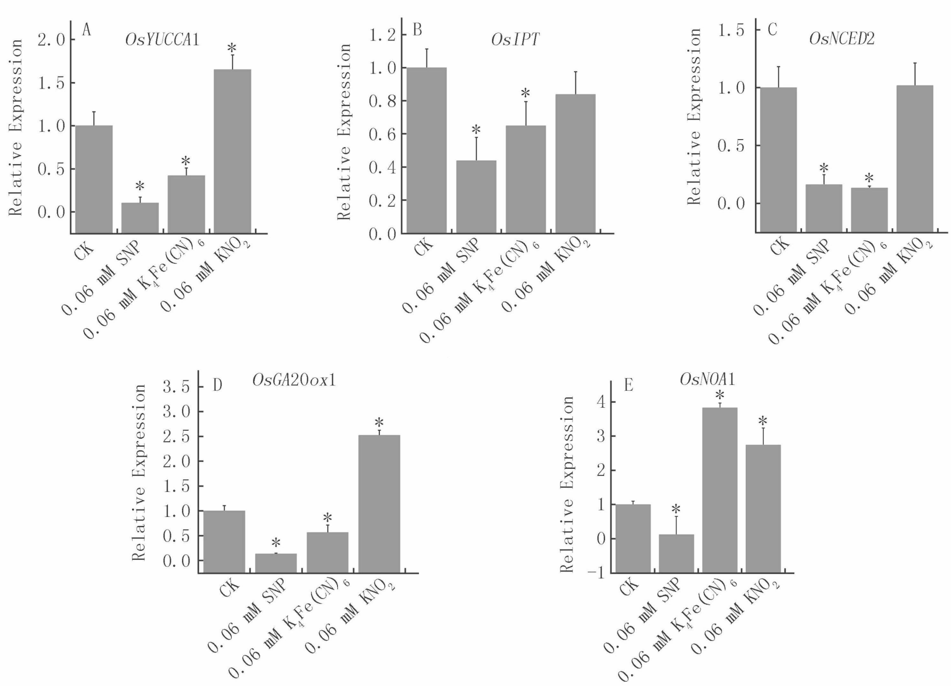
已有研究表明 NO 与多种植物激素信号途径相关,比如通过参与 IAA[18-19],CTK[20],ABA[21],乙

烯[22],水杨酸[23]等植物激素之间的信号转导网络来调节植物生理过程.因此在 SNP 及其光解产物处理的条件下,水稻幼苗生长受抑制的过程中,其他植物激素水平是否发生变化值得研究.为此,本研究选取了 5 种水稻植物激素的标记基因,进行 qRT－PCR 检测,发现 SNP 和 $K_4Fe(CN)_6$ 处理都能下调 IAA,CTK 和 GA 标记基因在根中的表达水平,提示可能会因此导致上述激素含量的降低,说明外源 NO 和 $K_4Fe(CN)_6$ 可能通过降低水稻幼苗根中 IAA,CTK,GA 的水平,达到对根生长的抑制效应,在功能上与上述激素发生拮抗作用.SNP 处理的效应显著于 $K_4Fe(CN)_6$ 处理,也进一步证明 SNP 的复合效应.另外,值得注意的是,KNO_2 和 $K_4Fe(CN)_6$ 处理都明显提高了 *OsNOA*1 基因的表达水平,推测由此导致水稻内源 NO 水平的提高,提示 SNP 不仅提供外源 NO,也影响水稻内源 NO 水平.本研究为探究 NO 在抑制水稻根生长中的作用机制以及与 IAA,CTK 等 5 种植物激素的关系提供了线索,为揭示水稻幼苗根发育过程中复杂的信号网络奠定理论基础.



“a, b, c”代表P<0.05显著分析

图3　SNP及其光解产物对水稻幼苗根长(A)和株高(B)的统计学分析



*代表$P<0.05$显著分析

图4　SNP及其光解产物对水稻5种植物激素标志基因在水稻根中表达的影响

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Extraction and Identification of Host-Plant Volatiles of *Acer mono* and EAG Responses of *Anoplophora nobilis* to the Primary Compounds of *A. mono* Volatiles

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Abstract: To explore the most appropriate hosts of nutrient supplement and oviposition for *Anoplophora nobilis* adults in Yili Prefecture, the volatiles composition of *Acer mono* were analyzed with GC-MS, and the electrophysiological activities of adults towards main volatiles were tested by EAG responses. The results showed that *A. nobilis* preferred fresh twigs of *A. mono* for diet supplement and carve twigs to oviposit in mixed plant area. Thirty-four volatiles were identified from shoots and leaves of *A. mono*, and terpenoids and esters were the major components, with the relative concentration up to 70.49% and 23.94%, respectively. (Z)-β-ocimene, sabinene, β-caryophyllene, α-phellandrene and cis-3-Hexenyl acetate represented a higher proportion in all components, with the relative concentration of 21.07%, 19.59%, 7.35%, 13.58% and 12.99%, respectively. With the concentration of 10 $mg \cdot mL^{-1}$, the EAG responses of female adults to β-caryophyllene was significantly higher than the other volatile compounds, and the male showed the most intensively EAG responses to ethyl acetate and α-pinene. The EAG responses of male adults to ethyl acetate and α-pinene were significantly stronger than female, while the female adults had more strongly EAG responses to β-caryophyllene and cis-3-Hexenyl-2-methy butyrate compared to males. This study will contribute to further research of chemical fingerprinting relating to host location of *A. nonilis*, therefore have a certain practical significance to development plant source attractants.

Keywords: *Anoplophora nobilis*; host selection; plant volatiles; electroantennogram responses; olfactory communication

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Effects of SNP and Its Photolysis Products on the Seedlings Growth of Rice and Expression of Marker Genes for Five Plant Hormones

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Abstract: Taking japonica cultivar Nipponbare rice as materials, the effects of sodium nitroprusside and its photolysis products KNO_2、$K_4Fe(CN)_6$ on the rice seedlings growth were analyzed, and marker genes for five different plant hormones in rice seedlings roots under the above treatments were detected by qRT－PCR in the study. The results showed that SNP can obviously inhibit the root length and plant height of the two rice seedlings, which implement mainly through its photolysis products $K_4Fe(CN)_6$. Moreover, the expression of the marker genes of auxin, cytokinins, abscisic acid and gibberellic acid were inhibited after the treatment of SNP and $K_4Fe(CN)_6$ in rice roots, but the marker gene of nitric oxide was inhibited by SNP, induced by $K_4Fe(CN)_6$.

Keywords: sodium nitroprusside; rice; seedling; plant hormone; gene expression

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