

氮胁迫对枳和‘资阳香橙’砧木氮代谢及相关基因表达的影响

朱礼乾^{1a}, 沈鑫健^{1a}, 周上铃², 曾 瑶¹, 彭良志¹, 付行政¹, 凌丽俐¹, 淳长品^{1*}

(¹西南大学柑桔研究所·中国农业科学院柑桔研究所,重庆 400712; ²西南大学园艺园林学院,重庆 400712)

摘要:【目的】对枳 [*Poncirus trifoliata* (L.) Raf.] 与‘资阳香橙’ (*Citrus junos* Sieb. ex Tanaka) 在氮胁迫下的无机氮含量、氮同化酶活性与基因表达进行研究, 为深入揭示柑橘氮高效利用机制奠定基础。【方法】以采用水培培养的枳与‘资阳香橙’幼苗为试材, 进行正常氮水平 (10 mmol·L⁻¹, 对照)、缺氮 (0 mmol·L⁻¹) 和高氮 (50 mmol·L⁻¹) 胁迫处理。【结果】(1) 枳与‘资阳香橙’的氮累积量与对照相比, 缺氮分别降低了 46.6% 和 51.1%, 高氮分别升高了 50.4% 和 81.6%。(2) ‘资阳香橙’各处理的硝态氮含量均显著大于枳, 枳与‘资阳香橙’叶片中的硝态氮含量以及铵态氮含量各处理均呈高氮>对照>缺氮的趋势。(3) 缺氮和高氮处理均显著降低了枳与‘资阳香橙’叶片和根的硝酸还原酶、亚硝酸还原酶、谷氨酰脱氢酶和谷氨酸合成酶活性, 而高氮处理时枳叶片中的硝酸还原酶、亚硝酸还原酶、和谷氨酸合成酶的活性与‘资阳香橙’相比被抑制的程度更低。(4) 枳和‘资阳香橙’在氮胁迫下叶片中基因 *NR*、*NiR*、*GDH2* 的相对表达量比对照有明显下降; 根系中基因 *NR*、*NADH1*、*NADH3*、*NADP* 相对表达量也显著下降。【结论】氮胁迫显著抑制了枳与‘资阳香橙’的氮代谢相关酶活性和氮同化相关酶基因的相对表达量, 枳在高氮处理下表现出更高的耐受性, ‘资阳香橙’与枳相比有更高的氮吸收能力。

关键词: 枳; ‘资阳香橙’; 砧木; 氮代谢; 氮胁迫; 相关基因表达

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Effects of nitrogen stresses on the nitrogen metabolism and expression of related genes in *Poncirus trifoliata* and ‘Ziyang Xiangcheng’ (*Citrus junos*) rootstocks

ZHU Liqian^{1a}, SHEN Xinjian^{1a}, ZHOU Shangling², ZENG Yao¹, PENG Liangzhi¹, FU Xingzheng¹, LING Lili¹, CHUN Changpin^{1*}

(¹*Citrus Research Institute of Southwest University/Citrus Research Institute of Chinese Academy of Agricultural Sciences, Chongqing 400712, China*; ²*College of Horticulture and Landscape Architecture, Southwest University, Chongqing 400712, China*)

Abstract:【Objective】‘Ziyang Xiangcheng’ [*Citrus junos* Sieb. ex Tanaka] and trifoliate orange [*Poncirus trifoliata* (L.) Raf.] are widely used as citrus rootstocks in China. There is little information about the physiological and molecular basis of nitrogen utilization in the two citrus rootstocks. In this paper, the content of inorganic nitrogen, and the enzyme activity and gene expression of nitrogen metabolism were studied in the two rootstocks under nitrogen deficiency and excess stresses in order to reveal the mechanism of citrus nitrogen efficient utilization and lay a foundation for further study on the nitrogen transport function of citrus rootstocks.【Methods】The experiment was conducted in the Citrus Research Institute of the Chinese Academy of Agricultural Sciences, Beibei, Chongqing. The seed coats of ‘Ziyang Xiangcheng’ and trifoliate orange were removed, and then seeds germinated in relative humidity

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作者简介:朱礼乾,男,在读硕士研究生,主要从事果树栽培与生理研究。Tel:15520087835,E-mail:1172023107@qq.com。a 为共同第一作者。沈鑫健,男,在读硕士研究生,主要从事果树栽培与生理研究。Tel:13047310296,E-mail:1129971502@qq.com

*通信作者 Author for correspondence. Tel:13883396612,E-mail:chuncp@cric.cn

of 70% at 27 °C under darkness for 7 d. Subsequently, the germinated seedlings were transferred to a modified Hoagland solution ($\text{pH } 6.0 \pm 0.5$) at 25 °C under a 16 h photoperiod ($50 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 30 d. The nutrient solution was replaced every 5 d. Three nitrogen (N) levels including normal nitrogen level ($10 \text{ mmol} \cdot \text{L}^{-1}$, control), N deficiency stress ($0 \text{ mmol} \cdot \text{L}^{-1}$) and N excess stress ($50 \text{ mmol} \cdot \text{L}^{-1}$) were used in this experiment. The pots were distributed among a randomized complete block design with nine replicates. After 20 d of nitrogen stress treatments, the roots and leaves were collected and immediately frozen in liquid nitrogen, then stored at -80°C for analyzing the Physiological and biochemical parameters related to nitrogen metabolism. The harvested roots and leaves were rinsed with deionized water 3 times, the water was absorbed with quantitative filter paper, then the samples were dried in a forced draft oven at 80°C for 72 h to a constant weight. The dry weight was measured. Then they were stored in a desiccator for analyzing N content. The activities of NR, NiR, NADH-GDH, and GOGAT were determined according to the method of *Experimental guide of modern plant physiology*, and the expression level of nitrogen metabolism relevant genes were analyzed by qRT-PCR. **【Results】** Compared with the control, nitrogen deficiency significantly reduced the nitrogen accumulation in ‘Ziyang Xiangcheng’ and excess nitrogen significantly increased the nitrogen accumulation in the two citrus rootstocks. The total nitrogen accumulation of ‘Ziyang Xiangcheng’ was significantly higher than that of trifoliate orange. This result might be caused by the higher biomass and root hair density of ‘Ziyang Xiangcheng’. In the roots and the leaves, the nitrate content of ‘Ziyang Xiangcheng’ was significantly higher than that of trifoliate orange. The highest nitrate content in the leaves of trifoliate orange and ‘Ziyang Xiangcheng’, was found when they were treated with high nitrogen, followed by the control and low nitrogen treatment. Similar results were obtained for the ammonium content. We further measured the expression of nitrogen metabolism-related genes. The relative expression levels of genes such as *Fd-GOGAT*, *NR*, *NiR* and *GDH2* in the leaves of trifoliate orange under N deficiency condition were significantly lower than those of the control, and nitrogen stresses significantly increased the relative expression of genes such as *NADH-GOGAT1*, *NADH-GOGAT2* and *NADH-GOGAT3*. Meanwhile, the relative expression levels of genes such as *NR*, *NiR*, *GDH1*, *GS* and *GDH2* in ‘Ziyang Xiangcheng’ leaves under nitrogen stresses were significantly lower than those of the control. the relative expression levels of *NR*, *NADH-GOGAT1*, *NADH-GOGAT3* and *NADP-GDH* genes were significantly lower than those of the control, while there was almost no change in expression levels of genes such as *Fd-GOGAT* and *GDH1*. Similarly, the relative expression level of the genes *Fd-GOGAT*, *NR*, *NiR*, *NADH-GOGAT1*, *NADH-GOGAT2*, *NADH-GOGAT3* and *NADP-GDH* in the roots of ‘Ziyang Xiangcheng’ under nitrogen stresses were significantly lower than those of the control. The relative expression of the gene *GDH2* was not significantly different from that of the control. We also found that the nitrogen deficiency and high nitrogen treatment significantly reduced nitrate reductase, nitrite reductase, glutamyl dehydrogenase and glutamate synthase in the leaves and the roots of ‘Ziyang Xiangcheng’ and trifoliate orange. The trends in the activities of *NR*, *NiR*, *NADH-GDH*, and *GOGAT* were consistent with those of *NR*, *NiR*, *NADH-GDH* and *GOGAT* transcription, respectively. All above results showed that nitrogen stresses significantly reduced the activities of nitrogen-related enzymes in trifoliate orange and ‘Ziyang Xiangcheng’, resulting in the reduction of nitrate absorption and ammonium production. **【Conclusion】** Nitrogen deficiency significantly reduced the nitrogen content and the activity of nitrogen-related enzymes and the expression of nitrogen-related genes in the roots and the leaves of trifoliate orange and ‘Ziyang Xiangcheng’. Trifoliate orange showed higher tolerance under high nitrogen treatment compared with ‘Ziyang Xiangcheng’, and ‘Ziyang Xiangcheng’ had higher nitrogen absorption capacity.

compared with trifoliate orange.

Key words: Trifoliate orange; ‘Ziyang Xiangcheng’; Rootstock; Nitrogen metabolism; Nitrogen stress; Expression of related genes

中国是世界柑橘的起源中心之一^[1],有4 000多年的栽培历史,柑橘也是我国南方农村重要的经济作物,截至2017年我国柑橘栽培面积达259.85万hm²,产量为3 885.38万t,面积与产量稳居世界第一位^[2]。柑橘植株是由地下部分的砧木和地上部分嫁接的接穗构成,不同砧木对植株抗病、抗逆境、营养物质利用、果实产量与品质的影响差异很大^[3]。枳 [*Poncirus trifoliata* (L.) Raf.]因其适应范围广,抗逆性强,是我国广泛使用的优良砧木,更是作为目前中国柑橘产业应用的主导砧木^[4];‘资阳香橙’(*Citrus junos* Sieb. ex Tanaka)具有良好的耐碱能力,能适应pH值7.2~8.4的碱性土壤,能够很好地避免像枳一样在碱性土壤出现的黄化症状,已经成为柑橘苗木市场的新兴砧木^[5]。尤其以近年来火热的沃柑,在广西的种植面积超过了20万hm²,砧木都以枳和‘资阳香橙’为基础,同时在生产中,‘资阳香橙’砧的沃柑与枳砧的沃柑相比,生长势强,树冠高大,果实品质更好,可以说是沃柑的理想砧木^[6-7]。

氮是植物生长发育必须的营养元素之一,也是植物体内氨基酸、核苷酸、蛋白质和叶绿素等生物分子的组成部分^[8-9]。国内柑橘多以丘陵山地栽培为主,立地条件较差,土壤中氮含量不足;同时种植户为了追求高产过量施肥,导致氮肥施用过多。通过近些年的调查研究发现,柑橘园普遍存在氮肥施用缺乏和过量现象。朱攀攀等^[10]研究发现,云南玉溪柑橘园土壤碱解氮含量普遍不足;易晓瞳等^[11]对广西产区柑橘叶片大中量元素营养调查表明,叶片氮含量超量与不足并存,分别占到调查果园的13.53%和45.41%;苏婷婷等^[12]对重庆市柑橘主产区104个柑橘园土壤养分状况分析表明,土壤碱解氮处于极缺和缺乏级的果园分别占7.7%和70.2%;习建龙等^[13]在对三峡库区加工甜橙果园土壤养分含量分析表明,其中碱解氮含量不足和过量的果园总共占到38.5%;温明霞等^[14]在对浙江省78个柑橘园0~30 cm的土壤样品进行分析发现:39.7%果园碱解氮呈过量状态,10.3%的果园碱解氮含量不足。有关枳与‘资阳香橙’两种砧木,前人的研究多围绕二者的抗病、抗碱能力^[15-17],针对这两种砧木在氮胁迫条件下对氮的吸收和累积差异目前还不清楚。

植物的氮源主要是无机氮化物,而无机氮化物中又以铵盐和硝酸盐为主。植物从土壤中吸收铵盐后,可直接加以利用合成氨基酸,但吸收硝酸盐,则需经过代谢还原形成铵态氮后才能被利用。这其中主要涉及到硝酸盐的还原和氨的同化过程,参与还原和同化的关键酶有硝酸还原酶(NR)、亚硝酸还原酶(NiR)、谷氨酰胺合成酶(GS)、谷氨酸脱氢酶(GDH)、谷氨酸合成酶(GOGAT)等^[18],硝酸还原酶和亚硝酸还原酶是硝态氮还原为铵态氮的关键酶,前人研究表明,NR受硝酸根离子、光照、生长条件、激素、氮素含量以及磷酸化的调节^[19-20]。GDH主要在NH₄⁺合成的初始阶段起作用,同时也在谷氨酸合成循环中起补充作用^[21],以氨根离子或谷氨酰胺及天冬氨酸为底物催化天冬酰胺的生物合成。GOGAT位于叶片叶绿体中,参与铵同化作用谷氨酸形成是一种过程^[22-23],通过该过程,一元铵进入由GOGAT催化的含氮化合物。同化为谷氨酸的铵离子可通过氨基转移酶转移至合适的α-酮酸以形成α-氨基酸。氨基酸反过来被同化为蛋白质和其他含氮化合物,如核酸^[24]。这些酶将直接影响植物对氮素代谢的响应机制,决定其吸收和利用的效率。生产上发现同一果园枳和‘资阳香橙’两种不同柑橘砧木生长和结果差异很大。因此,笔者选取这两种砧木为材料,探讨其在氮胁迫下氮代谢的生理响应和相关基因表达量差异,从而探索二者对氮吸收和转运的差异,为柑橘生产上选取合适的砧木提供理论依据。

1 材料和方法

1.1 材料

试验在重庆市北碚区中国农业科学院柑桔研究所进行,供试作物为两种柑橘砧木,分别为枳 [*Poncirus trifoliata* (L.) Raf.]和‘资阳香橙’(*Citrus junos* Sieb. ex Tanaka)。采摘当年的果实取其种子,挑选其中籽粒饱满的,用80%代森锰锌WP(美国陶氏益农公司)2 000倍液浸泡种子24 h,然后在3%次氯酸钠30 ℃溶液中浸泡1 h消毒,随后用蒸馏水反复冲洗干净,剥去内外种皮后,放入漂浮板上再置于盛有蒸馏水塑料盒中,在光照培养箱27 ℃黑暗下催芽。生根后移栽至含有1 L蒸馏水的塑料水

培盒中,每盆定植10株,待有3~5枚真叶长出后,换用1/2改良霍格兰溶液培养。用1%NaOH溶液和1%HCl溶液调节营养液的pH至 6.0 ± 0.5 ,每天定时通气3 h,每5 d更换1次营养液。

1.2 方法

霍格兰溶液培养1个月后进行试验处理,试验设置3个N水平:N(常规氮水平,N浓度为 $10\text{ mmol}\cdot\text{L}^{-1}$,对照)、N-(缺N胁迫,N浓度为 $0\text{ mmol}\cdot\text{L}^{-1}$,缺氮)和N+(高N胁迫,N浓度为 $50\text{ mmol}\cdot\text{L}^{-1}$,高氮)。单盆(10株)为1个重复,每个处理9个重复,在处理的营养液中添加硝化抑制剂 $7\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 双氰胺($\text{C}_2\text{H}_4\text{N}_4$)起抑制硝化作用。处理20 d后取样,植株样品用液氮速冻后立刻保存至 -80°C 冰箱备用,然后对全部植株进行破坏性取样,样品按清水、洗涤剂、清水、1%盐酸、3次去离子水顺序冲洗后,在 105°C 下杀青30 min,随后在 80°C 下烘干至恒重,电磨粉碎后过60目($250\text{ }\mu\text{m}$)筛后装袋备用。

1.3 植株氮含量的测定

称取0.50 g植株样品,加入浓硫酸以及催化剂, 420°C 消煮2 h后用全自动凯氏定氮仪测定全氮含量。

1.4 硝态氮和铵态氮含量的测定

硝态氮含量采用紫外分光光度法(双波长法)测定;铵态氮含量采用靛酚蓝比色法测定。

1.5 氮代谢相关酶活性的测定

谷氨酸合成酶、硝酸还原酶、亚硝酸还原酶、谷氨酸脱氢酶活性参考《现代植物生理学实验指南》^[25]的方法测定。

1.6 氮代谢相关基因的表达

用Trizol试剂盒(Invitrogen,美国)提取两种柑橘砧木根部和叶片的RNA,用NanoDrop 2000(赛

默飞有限公司,美国)超微量分光光度计检测RNA的浓度与纯度,用1.0%的琼脂糖电泳检测总RNA的完整性。检测合格后,使用TaKaRa公司的反转录试剂盒(PrimeScriptTM RT reagent Kit)合成第一条cDNA链,氮代谢关键基因的引物序列参照曹雄军等^[26]的方法合成。氮代谢关键基因的表达情况利用实时荧光定量PCR(qRT-PCR)技术进行分析。qRT-PCR反应按照ChamQ SYBR Color qPCR Master Mix的试剂盒说明书进行。反应体系($10\text{ }\mu\text{L}$)为: $1\text{ }\mu\text{L}$ cDNA, $0.2\text{ }\mu\text{L}$ 前引物, $0.2\text{ }\mu\text{L}$ 后引物, $5\text{ }\mu\text{L}$ ChamQ SYBR Color qPCR Master Mix, $3.6\text{ }\mu\text{L}$ ddH₂O。在Bio-RAD IQ5荧光定量PCR仪上完成反应。 95°C 预变性30 s, 95°C 变性5 s, 58°C 退火15 s,40个循环。

1.7 数据统计分析

采用Excel 2010软件进行数据处理和Origin 2019作图,采用SPSS 20.0软件进行统计分析,采用Duncan法进行差异显著性检验($\alpha=0.05$),图表中数据均以平均值±标准差表示。

2 结果与分析

2.1 氮胁迫对枳与‘资阳香橙’氮累积量的影响

由表1可知,枳与‘资阳香橙’无论是在地上部还是根系中,氮累积量随着氮胁迫浓度增加而增加,均为高氮>对照>缺氮。与对照相比,缺氮胁迫下,枳与‘资阳香橙’的地上部与根系氮的累积量均下降,枳分别降低了31.4%和60.3%,‘资阳香橙’为44.1%和57.1%;而高氮胁迫时,枳与‘资阳香橙’地上部与根系氮的累积量均增加,枳分别增加了65.8%和36.1%,‘资阳香橙’为100.6%和66.8%。

常规氮水平下的枳与‘资阳香橙’的根系与地

表1 不同氮水平两种柑橘砧木的氮累积量

Table 1 N accumulation by citrus rootstock seedlings at different N levels

砧木 Rootstock	处理 Treatment	w(地上部氮) Shoot N uptake/(g·kg ⁻¹)	w(根系氮) Root N uptake/(g·kg ⁻¹)	w(总氮) Plant N uptake/(g·kg ⁻¹)
枳 Trifoliate orange	对照 CK	47.74±0.09 c	52.74±0.07 c	100.48±0.03 c
	N-	32.73±0.17 d	20.93±0.06 d	53.66±0.12 d
	N+	79.14±0.23 b	71.71±0.11 b	150.85±0.21 b
资阳香橙 Ziyang Xiangcheng	对照 CK	47.76±0.09 c	54.77±0.02 c	102.53±0.10 c
	N-	26.69±0.05 e	23.48±0.02 d	50.17±0.05 d
	N+	92.90±0.19 a	91.42±0.21 a	184.38±0.38 a

注:同列数值后不同小写字母表示差异显著($p < 0.05$)。

Note: Values followed by different small letters mean significant difference ($p < 0.05$) in same column.

上部氮的累积量差异不大,总氮累积量分别为 $(100.48\pm0.03)\text{ g}\cdot\text{kg}^{-1}$ 和 $(102.53\pm0.10)\text{ g}\cdot\text{kg}^{-1}$ 。在缺氮处理下,枳根系氮累积量低于‘资阳香橙’,而地上部高于‘资阳香橙’,总氮累积量分别为 $(53.66\pm0.12)\text{ g}\cdot\text{kg}^{-1}$ 和 $(50.17\pm0.05)\text{ g}\cdot\text{kg}^{-1}$,经方差分析表明,枳地上部氮累积量显著高于‘资阳香橙’。在高氮胁迫下,枳根系与地上部氮累积量与缺氮处理正好相反,均低于‘资阳香橙’的累积量,总氮累积量分别为 $(150.85\pm0.21)\text{ g}\cdot\text{kg}^{-1}$ 和 $(18.43\pm0.38)\text{ mg}\cdot\text{kg}^{-1}$,经方差分析表明,二者之间差异显著。

2.2 氮胁迫对枳与‘资阳香橙’硝态氮和铵态氮含量的影响

如图1所示,无论是叶片还是根系中,‘资阳香橙’各处理的硝态氮含量都显著大于枳,枳与‘资阳香橙’叶片中的硝态氮含量各处理均为高氮>对照>缺氮,经方差分析,三个处理相互间均呈显著差异。与对照相比,缺氮处理枳与资阳叶片的硝态氮含量

分别降低了13.5%和7.5%,而高氮处理分别升高了13.6%和113.6%。在根系中,高氮处理枳的硝态氮含量显著大于缺氮和氮适量处理,而缺氮与对照之间无显著差异,但‘资阳香橙’正常处理的硝态氮含量显著高于缺氮和高氮处理,缺氮和高氮二者之间无显著差异。较对照相比,枳与‘资阳香橙’根系在缺氮处理下分别降低了3.9%和32.6%,高氮处理枳提高了11.2%,但‘资阳香橙’降低了28.1%。

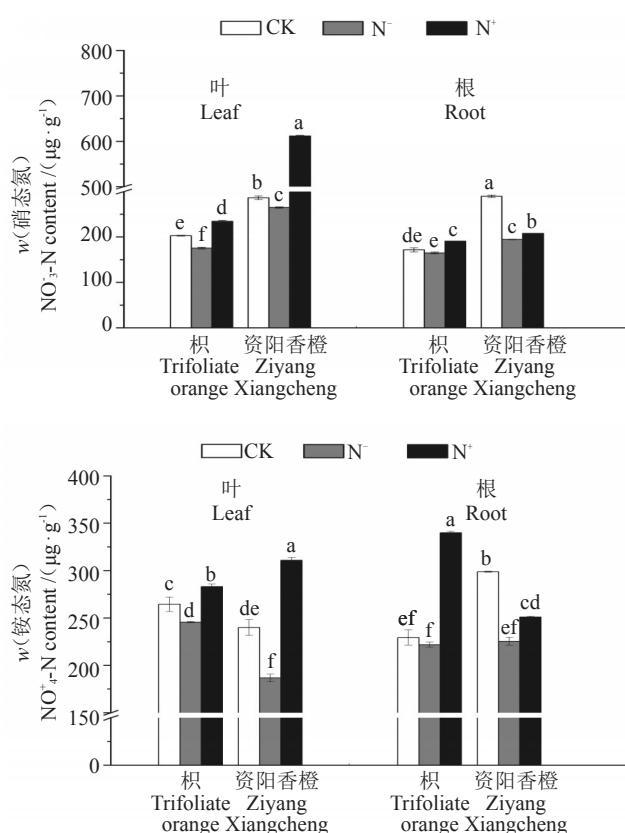
枳与‘资阳香橙’铵态氮含量与各处理的硝态氮基本一致,呈高氮>对照>缺氮(‘资阳香橙’根系除外)。在叶片中,‘资阳香橙’对照和缺氮处理铵态氮含量均显著低于枳,但高氮处理显著高于枳;与对照相比,枳与‘资阳香橙’缺氮处理铵态氮含量分别降低了7.2%和22.2%,而高氮处理分别升高了7.0%和29.5%。在根系中,枳高氮处理铵态氮含量显著高于对照和缺氮处理,而对照和缺氮处理二者没有显著差异,但‘资阳香橙’的对照显著高于缺氮和高氮处理,且三者相互之间均呈显著差异。

2.3 氮胁迫对枳与‘资阳香橙’氮代谢关键基因相对表达量的影响

如图2所示,枳的叶片中,在氮胁迫下基因 *Fd-GOGAT*、*NR*、*NiR*、*GDH2* 的相对表达量比对照有明显下降,其中基因 *NR* 和 *NiR* 在缺氮和高氮胁迫下相对表达量分别比对照下降了94.5%、92.4%和64.3%、65.9%。与对照相比,基因 *GDH1*、*GS*、*NADP-GDH* 的相对表达量差异不显著,氮胁迫明显提高了基因 *NADH-GOGAT1*、*NADH-GOGAT2* 和 *NADH-GOGAT3* 的相对表达量。

在‘资阳香橙’的叶片中,基因 *NR*、*NiR*、*GDH1*、*GDH2*、*GS* 在氮胁迫下的相对表达量与对照相比明显下降,其中 *NR*、*GDH1*、*GDH2* 在氮胁迫下的相对表达量与对照有显著差异。氮胁迫下基因 *Fd-GOGAT*、*NADH-GOGAT1*、*NADH-GOGAT3* 的相对表达量与对照没有显著差异,而 *NADH-GOGAT2* 和 *NADP-GDH* 的相对表达量在缺氮下显著高于对照,氮过量处理与对照没有显著差异。

氮胁迫下枳根系 *NR*、*NADH-GOGAT1*、*NADH-GOGAT3*、*NADP-GDH* 基因的相对表达量比对照显著下降,而基因 *Fd-GOGAT*、*GDH1* 的相对表达量与对照差异不显著。虽然 *NiR* 的相对表达量在缺氮下与对照没有显著差异,但在高氮处理下显著低于对照和缺氮处理。基因 *GS* 的相对表达量由大到小



不同小写字母表示差异显著($p < 0.05$)。下同。

Different small letters mean significant difference at 0.05 level. The same below.

图1 氮胁迫对两种柑橘砧木硝态氮和铵态氮含量的影响

Fig. 1 Effects of nitrogen stress on NO_3^- -N and NH_4^+ -N contents in two citrus rootstocks

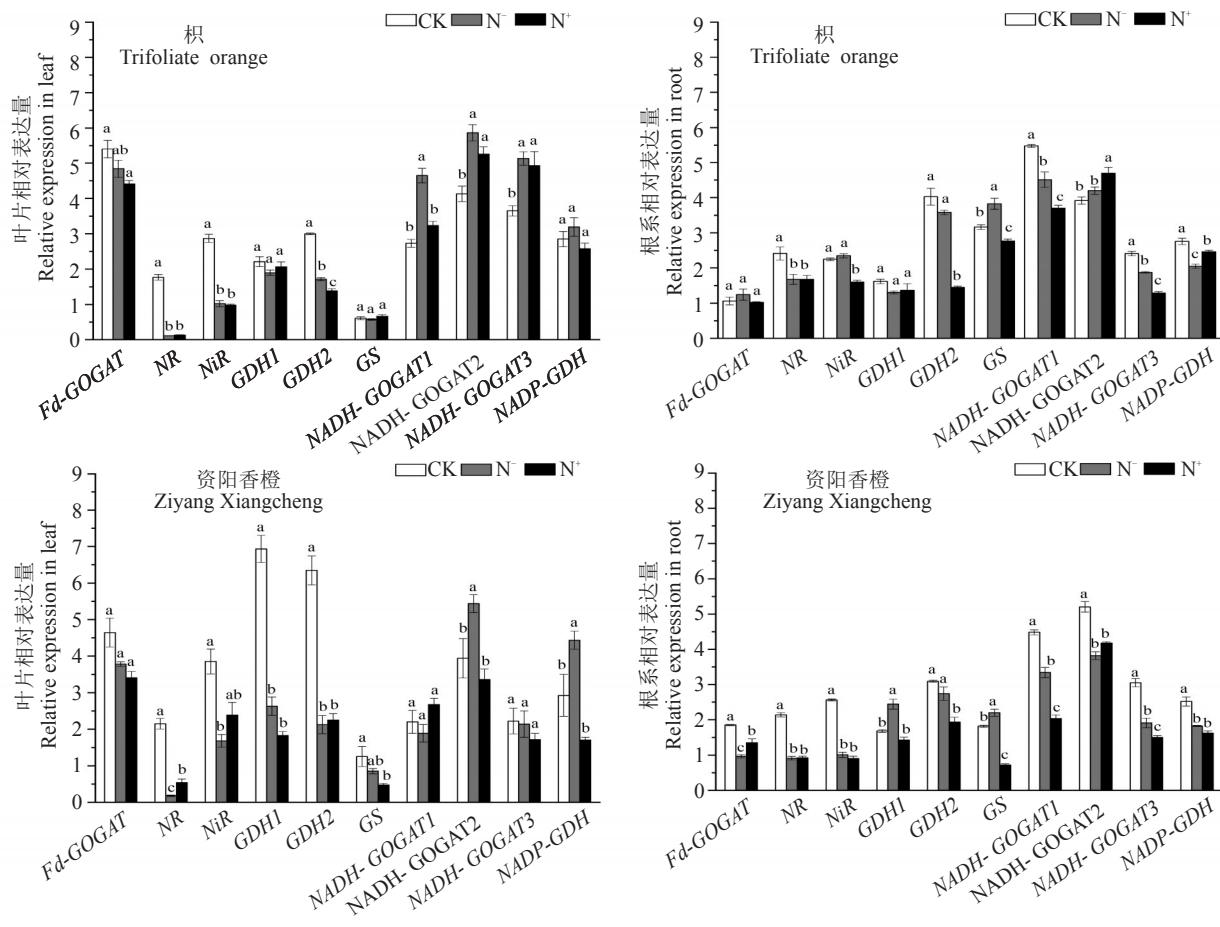


图2 氮胁迫对两种柑橘砧木氮同化相关酶基因表达量的影响

Fig. 2 Effects of nitrogen stress on expression level of genes involved in nitrogen assimilation of two citrus rootstocks

为缺氮>对照>高氮, *NADH-GOGAT2* 在高氮处理下的相对表达量显著高于对照与缺氮处理。

在‘资阳香橙’的根中, 基因 *Fd-GOGAT*、*NR*、*NiR*、*NADH-GOGAT1*、*NADH-GOGAT2*、*NADH-GOGAT3*、*NADP-GDH* 在氮胁迫下的相对表达量显著低于对照, 在缺氮处理时基因 *GDH2* 的相对表达量与对照没有显著差异, 但高氮处理显著低于对照和缺氮处理; 在缺氮处理下, 基因 *GDH1* 和 *GS* 的相对表达量都显著高于对照。

2.4 氮胁迫对枳与‘资阳香橙’氮代谢关键酶活性的影响

氮胁迫处理与对照相比, 显著降低了枳与‘资阳香橙’的硝酸还原酶活性, 在叶片中, 枳与‘资阳香橙’缺氮处理硝酸还原酶活性比对照分别降低了 31.1% 和 31.9%, 高氮处理比对照分别降低了 14.4% 和 38.9%(图 3); 在根系中, 枳与‘资阳香橙’缺氮处理硝酸还原酶活性比对照分别降低了 25.4% 和 6.1%, 高氮处理分别降低了 29.7% 和 4.1%。

无论是胁迫处理还是对照, 叶片的亚硝酸还原酶活性都大于根系。枳和‘资阳香橙’叶片的亚硝酸还原酶活性缺氮处理比对照分别降低了 15.0% 和 15.9%, 高氮处理也分别降低了 9.6% 和 22.7%。经方差分析, 枳与‘资阳香橙’缺氮与高氮处理叶片的亚硝酸还原酶活性显著低于对照; 枳与‘资阳香橙’缺氮处理根中的亚硝酸还原酶活性也显著低于对照, 而高氮处理与对照没有显著差异。

枳叶片中的谷氨酸脱氢酶以对照活性最高, 高氮与对照显著不差异, 缺氮显著低于对照, 而对照根系中的谷氨酸脱氢酶的活性显著大于高氮和缺氮。‘资阳香橙’叶片中的谷氨酸脱氢酶活性仍以对照最高, 高氮与缺氮两种处理显著低于对照, 缺氮又显著低于高氮; 缺氮根系中的谷氨酸脱氢酶活性显著低于对照, 而高氮虽较对照低, 但没有显著差异。

枳与‘资阳香橙’叶片和根中的谷氨酸合成酶活性在氮胁迫处理均比对照低。枳与‘资阳香橙’叶片的谷氨酸合成酶活性高氮处理显著低于对照,

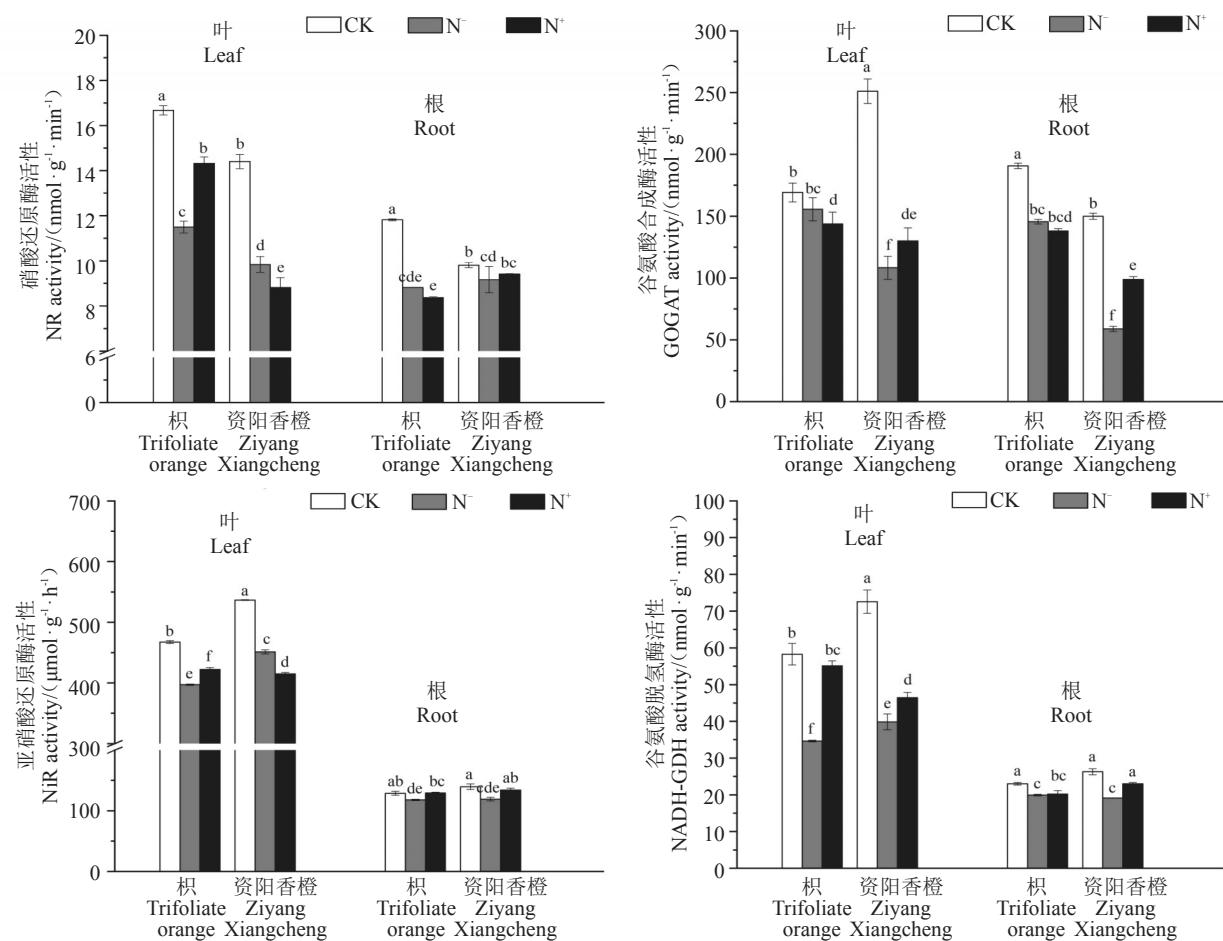


图3 氮胁迫对两种柑橘砧木氮代谢关键酶活性的影响

Fig. 3 Effects of nitrogen stress on key enzyme activities of nitrogen metabolism in two citrus rootstocks

分别降低了 15.0% 和 48.3%; 缺氮处理枳叶片的谷氨酸合成酶活性与对照无显著差异, 但‘资阳香橙’显著低于对照, 比对照降低了 56.9%。在根中, 枳的谷氨酸合成酶活性对照显著高于高氮与缺氮处理, 而高氮与缺氮间无显著差异, ‘资阳香橙’表现出对照>高氮>缺氮, 且三者之间有显著差异。

3 讨 论

前人研究表明, 氮胁迫对植物的生长有明显的抑制作用。低氮胁迫可降低植株地径、株高、叶片含氮量等^[27]。本研究也表明, 枳与‘资阳香橙’在缺氮处理下显著降低了地上部与地下部氮的累积量, 高氮处理下两种砧木的氮累积量显著高于其对照, 这与前人在茶、黄果柑上观察到的结果相似^[28-29]。‘资阳香橙’在高氮胁迫下总氮累积量显著高于枳, 这可能是由于‘资阳香橙’根系生物量和根毛密度大于枳^[30], 而氮吸收的器官主要是根系, 发达的根系有利于氮素的吸收。

硝态氮和铵态氮是植物的两大无机氮源^[31]。前人的研究显示, 植品种与种类的不同会导致其体内硝态氮与铵态氮含量的差异, 同时同一植株不同部位间含量也存在差异^[32-33]。在本研究中, ‘资阳香橙’体内的硝态氮含量显著高于枳的硝态氮含量, 缺氮处理显著降低了枳与‘资阳香橙’体内的硝态氮和铵态氮含量。黄海涛等^[34]也发现对两个基因型的品种进行缺氮处理, 油菜植株体内硝态氮和铵态氮含量显著降低。原因可能是硝态氮和铵态氮的利用受 NR、NiR 活性的影响, 氮胁迫显著降低了枳与‘资阳香橙’的氮代谢相关酶活性, 导致在低氮情况下植株对硝态氮吸收的减少, 同时硝态氮还原产生的铵态氮也减少。

在枳与‘资阳香橙’中, 很多氮代谢相关基因受到氮胁迫的影响, 比如基因 NR、NiR、GDH2 的相对表达量与对照相比都显著降低, 硝酸还原酶和亚硝酸还原酶作为氮代谢的关键酶, 其酶基因的表达受到硝酸根离子、氮代谢产物的影响^[35-36], 缺氮导致根

系周围硝酸根离子的减少,氮代谢相关酶基因表达下降,这与罗凤等^[37]在水稻中研究结果一致。

林郑和等^[28]发现茶树在缺氮时叶片中的 GDH 酶基因表达显著下降,而 GS 酶基因和 GOGAT 酶基因在高氮时表达受到抑制;Liao 等^[29]对黄果柑施用过量的氮肥发现 NR 和 NiR 基因的转录水平显著降低;在本研究中枳与‘资阳香橙’NR、NiR、GDH 酶基因同样在高氮时表达量受到抑制,而 NADH-GOGAT2 酶基因在枳中表达上调,结合前人的研究结果,这说明在枳与‘资阳香橙’中,部分氮代谢相关基因的表达并不是随着氮浓度的升高而上升,较高的氮浓度反而对氮代谢基因的表达有负面结果。同时不同氮处理下,氮代谢相关基因的转录水平与蛋白水平表达基本一致,如 NR、NiR、Fd-GO-GAT、NADH-GOGAT 基因表达结果与酶活结果一致,这也侧面反映了试验结果的可靠性。

在缺氮处理下,枳叶片中的硝酸还原酶、亚硝酸还原酶、谷氨酸合成酶的活性与对照相比分别降低了 31.1%、15.0% 和 8.0%,‘资阳香橙’这三种酶的活性与对照相比分别降低了 31.9%、15.9% 和 56.9%。与枳相比,‘资阳香橙’在缺氮时谷氨酸合成酶活性更容易受到影响。在高氮处理下,枳叶片中的硝酸还原酶、亚硝酸还原酶、谷氨酸合成酶的活性与对照相比分别降低了 14.4%、9.6% 和 15.0%,‘资阳香橙’这三种酶的活性与对照相比分别降低了 38.9%、22.7% 和 48.3%。这说明在施氮过量的情况下,与枳相比,‘资阳香橙’氮代谢相关酶活性更容易受到抑制。

高氮处理使得枳根系中的硝态氮含量与对照相比降低了 3.3%,铵态氮含量升高了 48.1%;而‘资阳香橙’根系中的硝态氮和铵态氮含量与对照相比分别降低了 24.6% 和 15.9%。高氮处理下‘资阳香橙’的根系同样受到抑制。但是即使是这样,在高氮处理时‘资阳香橙’依然积累了更多的氮,一方面笔者推测在‘资阳香橙’的根系上氮吸收转运蛋白发挥作用,此外发达的根系也可能是造成这种现象的原因。综合以上结果,可以得出枳在高氮时比‘资阳香橙’有更高的耐性,进一步得到‘资阳香橙’比枳对氮肥具有更好的吸收性。

4 结 论

缺氮显著降低了枳与‘资阳香橙’根与叶片中

的氮累积量以及氮代谢相关酶活性和氮代谢基因的表达,同时也降低了两者体内的硝态氮和铵态氮的含量。枳的氮代谢相关酶活与‘资阳香橙’相比耐受性更高,‘资阳香橙’氮吸收能力强于枳。基于以上结果,笔者建议在生产中针对土壤中氮含量处于中量及过量的水平的果园,可以优先发展以枳为砧木的柑橘,对于氮含量较缺乏的果园采用‘资阳香橙’砧的柑橘。同时建议施肥时做到少量多次,避免对柑橘树产生毒害。

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