

菠萝硝态氮的吸收积累及其转运 蛋白基因表达模式研究

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摘要:【目的】了解菠萝硝态氮转运蛋白基因在菠萝硝态氮吸收与同化中的作用。【方法】以金菠萝为材料, 通过2个试验, 分别研究了施氮对菠萝各组织硝态氮含量和40个硝态氮转运蛋白基因表达日变化的影响以及不同施氮处理对菠萝植株生长、叶片硝态氮含量和其转运蛋白基因表达的影响。【结果】菠萝根、茎、叶硝态氮含量均在10:00和16:00具有最高值; 施氮后2 h, 根系硝态氮含量显著增加; 施氮增加了叶中18:00的硝态氮含量, 降低了茎中14:00和18:00时及根中16:00的硝态氮含量。根中高表达的硝态氮转运蛋白基因最多, 峰值多在12:00和14:00; 叶中高表达基因的峰值多在18:00; 茎中高表达基因的峰值多在12:00; 施氮后, 多数基因的表达峰值被延后, 有些基因的表达峰值升高、降低或提前。不同施氮处理对菠萝植株的生长效应不同, MS营养液处理的植株质量增加幅度最大, 其次为纯氮处理, 无氮MS营养液和清水处理的植株质量增加幅度最小, 但前者促进了根系生长; 处理后3 d, 叶片硝态氮含量增加; 处理后6 d, 叶片硝态氮同化加强; 处理后26 d, 叶片进入缺氮状态; 绝大多数基因在清水和无氮MS营养液处理后的6 d达到表达峰值, 但两者仅共有3个基因; 处理后26 d, 高表达基因主要分布在30 mmol·L⁻¹氮、无氮MS和清水处理中, 三者无共有高表达基因。【结论】硝态氮转运蛋白基因表达受植株氮营养状态调控, *AcNRT 1.13*、*AcNRT 2.1*和*AcNRT 1.12*基因可能与叶片氮吸收有关, *AcNRT 1.14*、*AcNRT 1.21*和*AcNRT 1.22*基因可能与叶片氮再分配有关。

关键词:菠萝; 硝态氮转运蛋白; 基因表达; 氮营养

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Study on the absorption and accumulation of nitrate and the expression of nitrate transporter genes in pineapple

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Abstract:【Objective】Reducing nitrogen use and improving nitrogen use efficiency are two of the central concerns in pineapple cultivation. Nitrate transporters (NRT) play important roles in the absorption, transportation, redistribution and signaling of nitrate and other nutrients, but their roles in nitrate absorption and redistribution in pineapple remain unclear.【Methods】Two experiments were carried out. The first was application of 30 mmol·L⁻¹ NH₄NO₃ to pineapple plantlet, and changes of nitrate contents and expressions of 40 nitrate nitrogen transporter genes in pineapple roots, stems and leaves were analyzed every 2 h from 10:00 to 18:00; The second was application of six treatments (30 mmol·L⁻¹ NH₄NO₃, 60 mmol·L⁻¹ NH₄NO₃, MS nutrient solution containing 60 mmol·L⁻¹ NH₄NO₃, nitrogen-deficient MS nutrient solution, MS nutrient solution and water) to pineapple plantlet, and changes of nitrate contents and

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expressions of 40 nitrogen transporter genes in leaves were analyzed in 3rd d, 6th d, 9th d and 26th d after treatment, and root, stem and leaf weights were analyzed 60th d after treatment. 【Results】 The nitrate contents of pineapple roots, stems and leaves all showed higher values at both 10:00 and 16:00; 2 h after nitrate application ($30 \text{ mmol} \cdot \text{L}^{-1} \text{ NH}_4\text{NO}_3$), the nitrate content in roots increased significantly; nitrate application also increased the nitrate content in leaves at 18:00, while it decreased the nitrate content in stems at 14:00 and 18:00 as well as that in roots at 16:00. Twenty-five NRT genes were highly expressed in roots. Among them, the highest expression levels across all sampling time points appeared at 12:00 for 2 genes that were postponed (*AcNRT1.14*) to 14:00 or weakened (*AcNRT1.12*) after nitrate application; expression levels of 13 genes peaked at 14:00 that were delayed to 14:00 (*AcNRT1.25*, *AcNRT1.17*, *AcNRT1.20*, *AcNRT1.8*, *AcNRT1.19*, *AcNRT1.21*, *AcNRT1.32*, *AcNRT1.24*, *AcNRT1.23*, *AcNRT1.35*, *AcNRT1.22*, *AcNRT3.1*) or to 16:00 (*AcNRT1.42*) after nitrate application; expression levels of *AcNRT1.31* peaked at 18:00, which were advanced to 14:00 after nitrate application; for other genes, the times when the expression peaked were not altered but the expression level was enhanced (*AcNRT1.9*, *AcNRT1.17*, *AcNRT1.18*, *AcNRT1.11*, *AcNRT1.33*) or weakened (*AcNRT2.3*, *AcNRT1.35*, *AcNRT1.38*, *AcNRT1.12*) by nitrate application. Four NRT genes were highly expressed in stems. Among them, expression levels of *AcNRT1.7* peaked at 14:00 and were enhanced till 18:00 by nitrate application; expression levels of *AcNRT1.17* peaked at 12:00 and 18:00, and both peaks were enhanced by nitrate application; expression levels of *AcNRT1.5* and *AcNRT1.37* peaked at 18:00 and were weakened by nitrate application. Twelve NRT genes were highly expressed in leaves. After nitrate application, *AcNRT1.31*, *AcNRT1.41*, *AcNRT1.2*, *AcNRT2.1* and *AcNRT1.36* were up-regulated at 12:00; *AcNRT1.34* was up-regulated at 14:00; *AcNRT1.27* and *AcNRT1.10* were up-regulated at 18:00; *AcNRT1.44* were up-regulated at both 12:00 and 18:00; *AcNRT1.38* were up-regulated at both 12:00 and 14:00; *AcNRT1.26* were up-regulated at 12:00, 16:00 and 18:00; *AcNRT1.4* was down-regulated at 18:00. The six different nitrogen fertilization treatments had different effects on the growth of pineapple plantlet. The MS nutrient solution treatment had the largest weight gain, followed by the pure nitrogen treatment (30 and $60 \text{ mmol} \cdot \text{L}^{-1} \text{ NH}_4\text{NO}_3$). The plant weight gain of treatment with nitrogen-deficient MS nutrient solution and water was the least, but the former promoted root growth. At 3rd d after treatments, except for the treatment of water (control), the nitrate content in leaves of pineapple plantlet with all other treatments increased. At 6th d after treatments, except for the treatment of MS nutrient solution containing $60 \text{ mmol} \cdot \text{L}^{-1} \text{ NH}_4\text{NO}_3$, the nitrate content in leaves of plants with all other treatments decreased, indicating that the assimilation of nitrate was enhanced. At 9th d after treatment, except for the treatments of pure nitrogen (30 and $60 \text{ mmol} \cdot \text{L}^{-1} \text{ NH}_4\text{NO}_3$), the nitrate content in leaves of plants with all other treatments decreased, indicating that plants treated with pure nitrogen-initiated accumulation of nitrate. At 26th d after the treatment, the nitrate content of leaves with all treatments decreased significantly, indicating that the leaves were undergoing a nitrogen-deficient status. Three days after treatment, only a few genes were up-regulated by the treatments (e.g. *AcNRT1.2*, *AcNRT1.43* and *AcNRT1.3* by treatment of nitrogen-deficient MS nutrient solution; *AcNRT1.18*, *AcNRT1.5* and *AcNRT1.37* by water control), and there was no common up-regulated gene among all nitrogen-containing treatments; most genes reached their peak expression at 6th day in leaves with water control (*AcNRT1.17*, *AcNRT1.42*, *AcNRT1.20*, *AcNRT1.34*, *AcNRT1.41*, *AcNRT1.44*, *AcNRT1.13*, *AcNRT1.30*, *AcNRT1.38*, *AcNRT2.1*, *AcNRT1.43*, *AcNRT3.1*, *AcNRT1.24*, *AcNRT2.3*, *AcNRT1.25*, *AcNRT1.23*, *AcNRT1.31*, *AcNRT1.37*, *AcNRT1.8*, *AcNRT1.9* and *AcNRT1.12*) and nitrogen-deficient MS nutrient solution treatment (*AcNRT1.7*, *AcNRT2.1*, *AcNRT1.33*, *AcNRT1.36*, *AcNRT1.12*, *AcNRT1.2*), but only 3 genes were shared by both; 6 days

after treatment, 8 genes (*AcNRT 1.11*, *AcNRT 1.19*, *AcNRT 1.24*, *AcNRT 2.3*, *AcNRT 1.25*, *AcNRT 1.37*, *AcNRT 1.8* and *AcNRT 1.32*) were highly expressed in leaves of plants treated with 30 mmol·L⁻¹ NH₄NO₃ but not in those treated with 60 mmol·L⁻¹ NH₄NO₃; Except for *AcNRT 1.11*, *AcNRT 1.19* and *AcNRT 1.32*, these genes were also highly expressed in those with water control. 26th day after treatment, the highly expressed genes were mainly found with 30 mmol·L⁻¹ NH₄NO₃ treatment (*AcNRT 1.5*, *AcNRT 1.11*, *AcNRT 1.19* and *AcNRT 1.35*), nitrogen-deficient MS treatment (*AcNRT 1.14*, *AcNRT 1.21* and *AcNRT 1.22*) and water control (*AcNRT 1.6* and *AcNRT 1.10*), but no genes were shared by these three treatments. 【Conclusion】The contents of nitrate and the expressions of nitrate transporter genes in various tissues of pineapple plantlet showed diurnal variation patterns, and nitrate application could change the time when the peaks of these two events occurred; the expression of nitrate transporter genes was affected by the nitrogen status of the plant. *AcNRT 1.13*, *AcNRT 2.1* and *AcNRT 1.12* genes may be related to leaf nitrogen uptake, and *AcNRT 1.14*, *AcNRT 1.21* and *AcNRT 1.22* genes may be related to leaf nitrogen redistribution.

Key words: Pineapple; Nitrate transporter; Gene expression; Nitrogen

菠萝 [*Ananas comosus* (L.) Merr.] 为凤梨科凤梨属多年生草本植物, 是我国重要的热带果树, 在中国福建、广东、广西、云南、海南、台湾等地均有大面积栽培^[1]。据联合国粮农组织发布的统计数据, 2020 年我国菠萝种植面积 9.96 万 hm², 总产量 263.93 万 t, 位居世界第三(FAO, 2022)。广东是我国最大的菠萝种植区域^[1], 2020 年种植面积 3.90 万 hm², 总产量 121.02 万 t。

氮是植物生长发育的必需元素, 也是作物产量的限制因子。为保证作物产量, 全球每年使用的氮肥超过 1.1 亿 t^[2]; 然而, 作物对氮肥的吸收利用率较低, 仅为施用量的 30%~50%, 剩余氮肥流失到大气、深层土壤或水体中^[3-4], 造成能源浪费和环境污染^[5]。菠萝也是需氮较多的作物, 每形成 1 kg 的菠萝果实需要吸收 7.22 kg 的氮^[6], 但氮肥施用过多也会降低菠萝产量和果实品质^[7-9]。

硝酸盐是大多数作物的主要氮源^[10], 其利用过程包括吸收、转运、同化和再转运, 硝酸盐转运载体在其中起着重要作用^[11]。植物中的硝酸盐转运载体包括 3 个基因家族, 分别为 NRT1/PTR(Nitrate transporters 1/Peptide transporters) 基因家族、NRT2(Nitrate transporters 2) 基因家族和 NRT3/NAR2(Nitrate transporters 3/ Nitrate Assimilation Related protein 2) 基因家族^[12]。菠萝全基因组测序结果表明, 菠萝中有 44 个 NRT1/PTR 基因家族成员, 3 个 NRT2 基因家族成员和 1 个 NRT3/NAR1 基因家族成员^[13], 但它们在菠萝生长发育中的作用尚不清楚, 这些基因家族成员对菠萝施氮后的响应变化及其在菠萝氮稳态维

持中的作用还鲜有报道。对基因家族成员进行鉴定和表达分析, 有助于明确在相应生理过程中发挥关键作用的基因成员^[14]。笔者在本研究中结合氮肥施用和基因的荧光定量表达, 对这些基因在菠萝氮肥吸收与利用中的作用进行了深入分析, 以期为菠萝栽培氮精准施用和氮高效育种提供参考。

1 材料和方法

1.1 植物材料

试验材料为移栽至育苗基质中生长 3 个月后的菠萝组培苗, 品种为金菠萝, 每盆 1 株种植于 25 cm×25 cm 的塑料花盆中。

1.2 试验设计

试验设 5 个处理和 1 个清水(蒸馏水)对照。处理前 1 个月停止施肥。5 个处理分别为 30 mmol·L⁻¹ 的 NH₄NO₃(Nn), 60 mmol·L⁻¹ 的 NH₄NO₃(Nhn), 含 60 mmol·L⁻¹ NH₄NO₃ 的 MS 营养液(Nhm), 不含氮源的 MS 营养液(Nd), MS 营养液(Nm)。选择生长基本一致的盆栽菠萝苗, 每株施用 50 mL 处理溶液或清水(Nw)。每个处理 5 株, 3 次重复。处理后的 0、3、6、9 和 26 d 的 10:00 采集菠萝植株的 D 叶用于 RNA 提取及硝态氮含量测定; 60 d 后取全株, 分别测定根、茎和叶的鲜质量及干质量。

为揭示硝酸盐吸收和相关基因表达的日变化模式, 试验还设计了 30 mmol·L⁻¹ 的 NH₄NO₃ 处理。选晴朗天气, 10:00 开始处理, 选择生长基本一致的盆栽菠萝苗, 每株施用 50 mL 处理溶液或清水(蒸馏水), 每个处理 5 株, 3 次重复。处理后的 0、2、4、6 和

8 h 分别采集菠萝植株的叶、茎及根系用于 RNA 提取及硝态氮含量测定。

1.3 方法

1.3.1 干质量和鲜质量的测定 使用万分之一天平测定植株各组织的鲜质量;将各组织烘干至恒重后,用万分之一天平测定其干质量。

1.3.2 硝态氮含量的测定 硝态氮含量的测定采用水杨酸比色法^[15]。精确称取 2 g 左右鲜样,加 10 mL 去离子水研磨后,沸水浴 30 min。冷却后,过滤,取上清液定容后,使用 5% 水杨酸-浓硫酸试剂进行比色测定。

1.3.3 总 RNA 提取及基因表达分析 RNA 的提取按照试剂盒(RNAiso plus, 大连宝生物)的操作手册进行。采用 PrimeScript(大连宝生物)试剂盒进行反转录反应,TB Green(大连宝生物)试剂盒进行基因表达水平的荧光法检测,反应程序为:95 °C 30 s; 95 °C 5 s, 40 cycles; 60 °C 34 s; 95 °C 15 s。基因表达引物参考 Li 等^[13]的方法设计,各引物的扩增产物经回收、测序验证后再使用。

1.4 数据处理

基因的相对表达水平按照 $2^{-\Delta\Delta CT}$ 法计算,参考 Jin 等^[16]的方法选用 *Alpha-tubulin* 和 *Ubiquitin* 为内参基因。使用 Microsoft excel 2010 计算平均值和标准误,使用 SPSS statistics 24 软件进行差异显著性分析,使用 R 软件绘制热图。

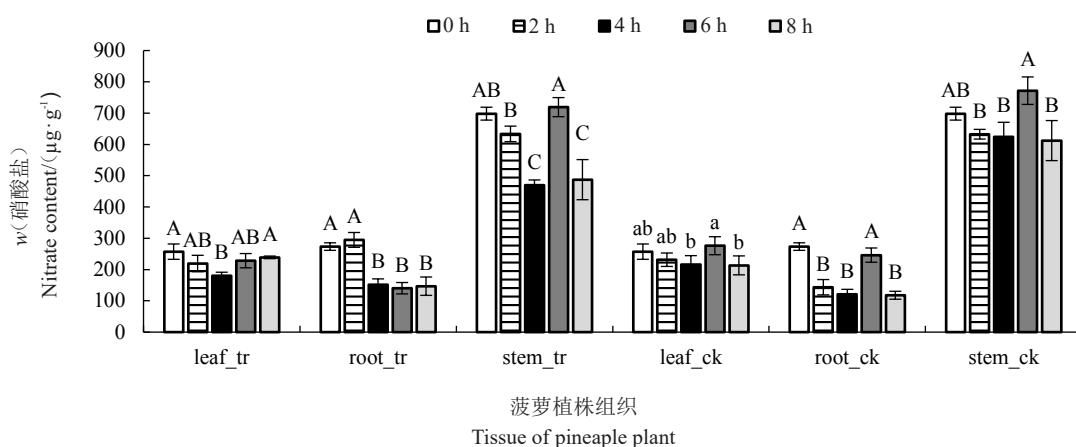
2 结果与分析

2.1 施氮对菠萝植株硝酸盐吸收与积累日变化的影响

施用 30 mmol·L⁻¹ NH₄NO₃ 后,菠萝植株硝酸盐积累的日变化见图 1。从图 1 中可看出,菠萝植株的茎中含量较高的硝酸盐,可能是菠萝植株硝酸盐的储存库。未施氮时,菠萝植株的叶片、根和茎呈现类似的日变化模式,均在 10:00 和 16:00 有个峰值。施入硝态氮后,叶片硝酸盐含量在施氮后的 6 h(16:00) 低于对照,施氮后 8 h(18:00) 高于对照;根系硝酸盐含量在施氮后 2 h(12:00) 较对照有显著增加($p < 0.01$),施氮后 6 h(16:00) 根系硝酸盐含量较对照有显著减少($p=0.0194$);茎中硝酸盐含量在施氮后 4 h(14:00) 和 8 h(18:00) 显著低于对照($p < 0.01$)。

2.2 不同施氮处理对菠萝植株各组织质量的影响

从表 1 中可看出,菠萝植株各组织鲜质量与干质量的变化模式相似。清水处理的叶片质量、根质量和茎质量均最低,MS 处理的叶片质量、根质量和茎质量最高。叶片鲜质量,MS 处理最高,其次为 60 mmol·L⁻¹ 氮和含 60 mmol·L⁻¹ 氮的 MS 处理,均与清水处理差异达到极显著水平;30 mmol·L⁻¹ 氮和含 0 mmol·L⁻¹ 氮的 MS 处理与清水差异未达到极显著水平。各处理间的根鲜质量和根干质量差异未达到显著水平。MS 处理的茎鲜质量极显著高于其他处



leaf_tr, root_tr 和 stem_tr 分别表示处理植株的叶、根和茎;leaf_ck, root_ck 和 stem_ck 分别表示未处理植株的叶、根和茎。不同大写和小写字母分别表示在 $p < 0.01$ 和 $p < 0.05$ 水平上差异显著(LSD 法)。下同。

leaf_tr, root_tr 和 stem_tr indicated respectively leaf, root and stem of treated plantlet; leaf_ck, root_ck and stem_ck indicated respectively leaf, root and stem of untreated plantlet. Different uppercase and lowercase letters indicated respectively significant levels at $p < 0.01$ and $p < 0.05$ (LSD test). The same below.

图 1 30 mmol·L⁻¹ NH₄NO₃ 处理与清水对照植株各组织硝酸盐含量的日变化

Fig. 1 Diurnal changes of nitrate content in various tissues of plants treated with 30 mmol·L⁻¹ NH₄NO₃ and water

表 1 6 种施氮处理对菠萝植株各组织质量的影响

Table 1 Effects of six nitrogen treatments on the weight of various tissues of pineapple plantlet

处理 Treatment	叶鲜质量 Leaf fresh weight/g	叶干质量 Leaf dry weight/g	根鲜质量 Root fresh weight/g	根干质量 Root dry weight/g	茎鲜质量 Stem fresh weight/g	茎干质量 Stem dry weight/g
Nn	112.748 3±12.918 3 AB	17.475 4±1.906 0 ab	8.843 2±4.122 1	2.277 2±0.523 2	6.769 0±0.731 7 AB	0.828 4±0.095 2 A
Nhn	123.186 5±24.382 4 A	19.145 9±4.167 3 a	9.437 0±2.195 4	2.296 6±0.549 8	6.933 2±0.926 8 AB	0.880 1±0.144 8 A
Nhm	121.398 6±18.298 2 A	18.685 4±3.769 8 ab	8.088 2±3.006 7	1.922 2±0.645 9	6.907 6±1.208 0 AB	0.858 0±0.178 4 A
Nd	101.456 2±13.622 1 AB	15.583 3±2.954 2 ab	9.189 2±3.668 4	2.114 1±0.901 4	5.740 7±0.931 4 AB	0.678 6±0.145 5 AB
Nm	141.640 0±26.462 3 A	19.349 9±3.279 4 a	10.671 0±3.743 5	2.642 8±0.830 3	7.674 4±1.025 0 A	0.916 1±0.084 9 A
Nw	72.191 0±5.261 5 B	12.626 2±3.058 8 b	6.139 0±2.232 5	1.643 9±0.492 9	5.147 0±1.176 6 B	0.386 9±0.078 6 B

理,MS、60 mmol·L⁻¹氮、含 60 mmol·L⁻¹氮的 MS 和 30 mmol·L⁻¹氮处理的茎干质量均与清水处理的差异达到极显著水平,含 0 mmol·L⁻¹氮的 MS 处理与清水处理间无显著差异。

2.3 不同施氮处理对菠萝植株硝酸盐吸收与积累的长期影响

不同施氮处理下菠萝植株叶片硝酸盐含量的长期动态变化见图 2。从图 2 中可看出,施氮处理后第 3 天,除清水处理的叶片硝酸盐含量下降外,其余各处理的叶片硝酸盐含量均较对照有大幅度增加,30 mmol·L⁻¹氮、60 mmol·L⁻¹氮处理的叶片硝酸盐含量最高,其次为含 60 mmol·L⁻¹氮的 MS、MS 和含 0 mmol·L⁻¹氮的 MS。单纯氮处理可能促进了叶片硝态氮的积累,含氮 MS 处理中的氮可能被吸收转化,无氮 MS 处理中的氮可能来自氮库(如茎)的转运。

施氮处理 6 d 后,除含 60 mmol·L⁻¹氮的 MS 处理外,其余处理的叶片硝酸盐含量均较 3 d 时下降。30 mmol·L⁻¹氮、60 mmol·L⁻¹氮处理的叶片硝酸盐含量低于清水处理,其中 30 mmol·L⁻¹氮处理的叶片硝

酸盐含量与清水处理的差异达到显著水平($p<0.05$);含 60 mmol·L⁻¹氮的 MS 处理的叶片硝酸盐含量显著高于清水处理($p<0.05$)。此时的硝酸盐含量下降表明,施氮促进了叶片硝态氮的同化。

施氮处理 9 d 后,30 mmol·L⁻¹氮、60 mmol·L⁻¹氮处理的叶片硝态氮含量上升,高于清水处理;其余处理的叶片硝酸盐含量下降,低于清水处理。结合施氮处理对菠萝各组织质量影响的结果来看,单纯氮处理促进植株生长的作用不及 MS 营养液基础上的施氮,因此硝态氮在叶片中开始积累;MS 营养液基础上施用的氮则继续被同化以供植株生长。

施氮处理 26 d 后,所有处理的叶片硝酸盐含量均大幅下降,30 mmol·L⁻¹氮和清水处理的下降幅度最大,其次为含 0 mmol·L⁻¹氮的 MS 处理,60 mmol·L⁻¹氮处理、含 60 mmol·L⁻¹氮的 MS 和 MS 处理的叶片硝酸盐含量下降幅度近似,表明此时大多数处理的植株处于缺氮状态。结合施氮处理对菠萝各组织质量影响的结果来看,低氮处理和清水的缺氮应当源自较低的施氮量,含 0 mmol·L⁻¹氮的 MS 处理的低氮可能源自营养元素不均衡导致的生理性缺氮。

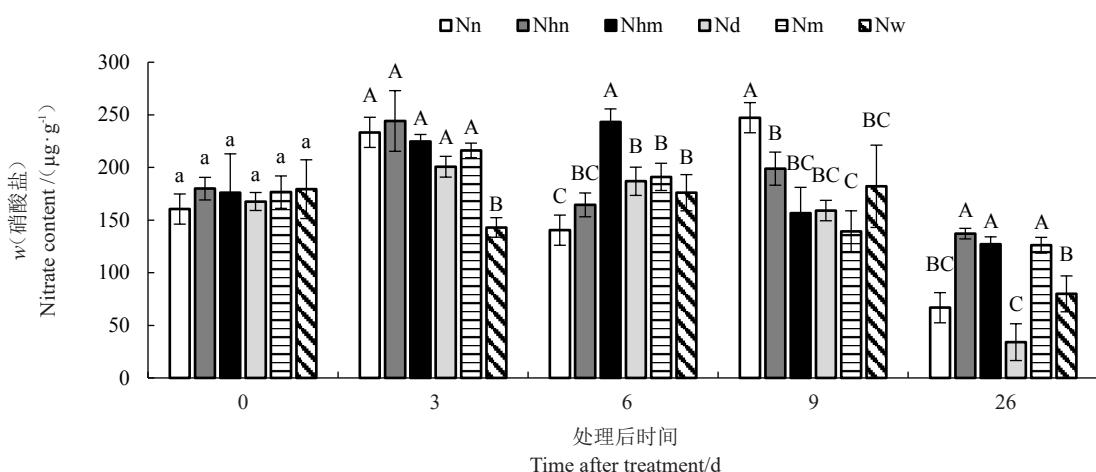


图 2 6 种施氮处理下菠萝植株叶片硝酸盐含量的动态变化

Fig. 2 Dynamic changes in leaf nitrate content of pineapple plantlet under six nitrogen treatments

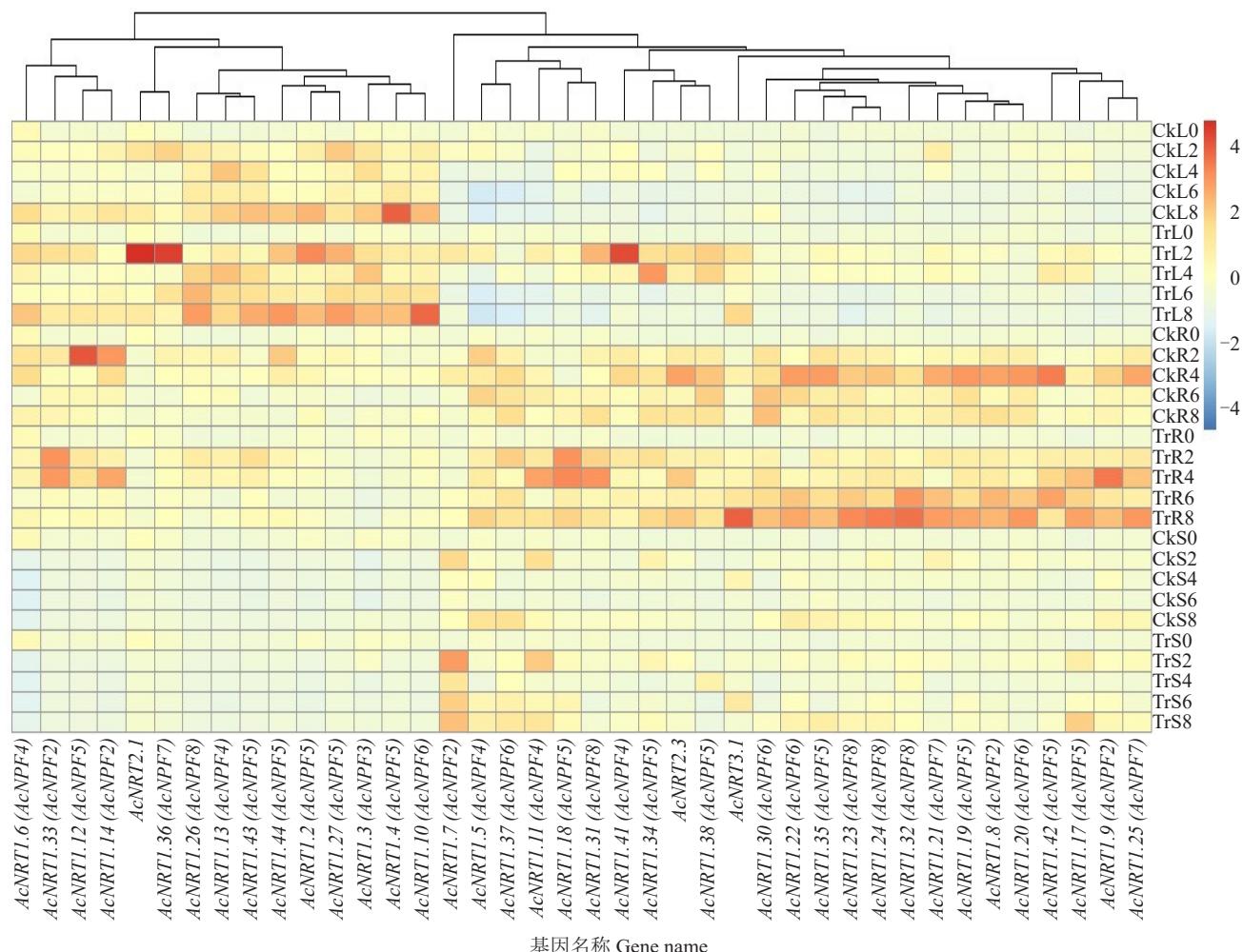
2.4 施氮处理对菠萝各组织硝酸盐转运相关基因表达日变化的影响

40个菠萝氮转运相关基因在处理与对照的菠萝根、茎和叶中的日变化见图3。从图3中可看出,不同基因在根、茎和叶中的表达量不同,具有组织特异性。各基因在对照根、茎和叶组织中具有日变化的表达模式。

菠萝叶片中,各基因的日表达模式大致可分为3类,12:00和14:00具有表达峰值(如 $AcNRTI.38$),12:00和18:00具有表达峰值(如 $AcNRT2.1$),12:00至18:00均具有较高表达量(如 $AcNRTI.26$)。与对照相比,施氮植株中,12:00上调表达的基因有 $Ac-$

$NRTI.31$ 、 $AcNRTI.41$ 、 $AcNRTI.2$ 、 $AcNRT2.1$ 和 $AcNRTI.36$,14:00上调表达的基因有 $AcNRTI.34$,18:00上调表达的基因有 $AcNRTI.27$ 和 $AcNRTI.10$,12:00和18:00上调表达的基因有 $AcNRTI.44$,12:00和14:00上调表达的基因有 $AcNRTI.38$; $AcNRTI.26$ 基因的表达在14:00、16:00和18:00均增强;与其他基因表达量上调不同, $AcNRTI.4$ 基因的表达量在处理后下调。

菠萝根中受施氮影响的基因较多。大多数基因在14:00出现峰值,施氮后,该峰值延后到18:00,这些基因有 $AcNRTI.25$ 、 $AcNRTI.17$ 、 $AcNRTI.20$ 、 $AcNRTI.8$ 、 $AcNRTI.19$ 、 $AcNRTI.21$ 、 $AcNRTI.32$ 、 $Ac-$



CkL. 对照叶;TrL. 处理叶;CkR. 对照根;TrR. 处理根;CkS. 对照茎;TrS. 处理茎;处理名称后的数字表示处理(或对照)后0 h、2 h、4 h、6 h、8 h。颜色深度表示归一化后各基因的相对表达量。下同。

CkL. control leaf; TrL. treated leaf; CkR. control root; TrR. treated root; CkS. control stem; TrS. treated stem; numbers after the treatment name indicates the relative gene expression after 0 h, 2 h, 4 h, 6 h, 8 h of treatment or control. The color depth indicates the normalized relative expression levels of each gene. The same below.

图3 硝态氮转运蛋白基因在处理与对照菠萝植株的根、茎和叶中的日表达变化

Fig. 3 Heat map of the expression changes of nitrate nitrogen transporter gene in the roots, stems, and leaves of treated and control pineapple plantlet

NRT1.24、*AcNRT1.23*、*AcNRT1.35*、*AcNRT1.22*、*AcNRT3.1*；*AcNRT1.42*基因在14:00的表达高峰延后到16:00；*AcNRT1.14*基因在12:00的表达高峰被延后到14:00。除峰值延后外，*AcNRT1.31*基因的表达高峰从18:00提前至14:00。有些基因的表达峰值因施氮而加强，如*AcNRT1.9*、*AcNRT1.17*、*AcNRT1.18*、*AcNRT1.11*和*AcNRT1.33*基因；相反，部分基因在施氮后，峰值减弱，如*AcNRT2.3*、*AcNRT1.35*、*AcNRT1.38*和*AcNRT1.12*基因。

菠萝茎中受施氮影响的硝酸盐转运载体基因较少。其中，*AcNRT1.7*基因在茎中的表达量较高，12:00为其峰值；施氮后峰值被强化，并持续到18:00。*AcNRT1.17*基因的峰值在施氮后得到增强；*AcNRT1.5*和*AcNRT1.37*基因的峰值在施氮后被弱化。

2.5 不同施氮处理对菠萝叶片硝酸盐转运相关基因长期表达变化的影响

菠萝硝酸盐转运相关基因在6种施氮处理后菠萝叶片中的表达变化见图4。从图4中可看出，处理后第3天，*AcNRT1.18*基因在所有处理中均有较高

表达量，清水处理的表达水平最高；在不含氮源MS处理中上调表达的基因有*AcNRT1.2*、*AcNRT1.43*和*AcNRT1.3*，而在清水处理中上调表达的基因有*AcNRT1.18*、*AcNRT1.5*和*AcNRT1.37*，两者无共有上调表达的基因。在所有含氮处理中，也未找到特异的共有上调表达基因。这一结果表明，与木质部硝酸盐卸载有关的基因此时未受到调控。

处理后第6天，多数处理的叶片硝酸盐含量开始下降，此时被上调表达的基因也最多，且多在清水处理和不含氮处理（不含氮的MS营养液处理）中具有最大表达量。所研究的40个基因，21个基因在清水处理中具有表达峰值，如*AcNRT1.17*、*AcNRT1.42*、*AcNRT1.20*、*AcNRT1.34*、*AcNRT1.41*、*AcNRT1.44*、*AcNRT1.38*、*AcNRT1.44*、*AcNRT1.30*、*AcNRT1.36*、*AcNRT1.39*、*AcNRT1.33*、*AcNRT1.35*、*AcNRT1.36*、*AcNRT1.37*、*AcNRT1.38*、*AcNRT1.39*、*AcNRT1.40*、*AcNRT1.41*、*AcNRT1.42*、*AcNRT1.43*、*AcNRT1.44*、*AcNRT1.45*、*AcNRT1.46*、*AcNRT1.47*、*AcNRT1.48*、*AcNRT1.49*、*AcNRT1.50*、*AcNRT1.51*、*AcNRT1.52*、*AcNRT1.53*、*AcNRT1.54*、*AcNRT1.55*、*AcNRT1.56*、*AcNRT1.57*、*AcNRT1.58*、*AcNRT1.59*、*AcNRT1.60*、*AcNRT1.61*、*AcNRT1.62*、*AcNRT1.63*、*AcNRT1.64*、*AcNRT1.65*、*AcNRT1.66*、*AcNRT1.67*、*AcNRT1.68*、*AcNRT1.69*、*AcNRT1.70*、*AcNRT1.71*、*AcNRT1.72*、*AcNRT1.73*、*AcNRT1.74*、*AcNRT1.75*、*AcNRT1.76*、*AcNRT1.77*、*AcNRT1.78*、*AcNRT1.79*、*AcNRT1.80*、*AcNRT1.81*、*AcNRT1.82*、*AcNRT1.83*、*AcNRT1.84*、*AcNRT1.85*、*AcNRT1.86*、*AcNRT1.87*、*AcNRT1.88*、*AcNRT1.89*、*AcNRT1.90*、*AcNRT1.91*、*AcNRT1.92*、*AcNRT1.93*、*AcNRT1.94*、*AcNRT1.95*、*AcNRT1.96*、*AcNRT1.97*、*AcNRT1.98*、*AcNRT1.99*、*AcNRT1.100*、*AcNRT1.101*、*AcNRT1.102*、*AcNRT1.103*、*AcNRT1.104*、*AcNRT1.105*、*AcNRT1.106*、*AcNRT1.107*、*AcNRT1.108*、*AcNRT1.109*、*AcNRT1.110*、*AcNRT1.111*、*AcNRT1.112*、*AcNRT1.113*、*AcNRT1.114*、*AcNRT1.115*、*AcNRT1.116*、*AcNRT1.117*、*AcNRT1.118*、*AcNRT1.119*、*AcNRT1.120*、*AcNRT1.121*、*AcNRT1.122*、*AcNRT1.123*、*AcNRT1.124*、*AcNRT1.125*、*AcNRT1.126*、*AcNRT1.127*、*AcNRT1.128*、*AcNRT1.129*、*AcNRT1.130*、*AcNRT1.131*、*AcNRT1.132*、*AcNRT1.133*、*AcNRT1.134*、*AcNRT1.135*、*AcNRT1.136*、*AcNRT1.137*、*AcNRT1.138*、*AcNRT1.139*、*AcNRT1.140*、*AcNRT1.141*、*AcNRT1.142*、*AcNRT1.143*、*AcNRT1.144*、*AcNRT1.145*、*AcNRT1.146*、*AcNRT1.147*、*AcNRT1.148*、*AcNRT1.149*、*AcNRT1.150*、*AcNRT1.151*、*AcNRT1.152*、*AcNRT1.153*、*AcNRT1.154*、*AcNRT1.155*、*AcNRT1.156*、*AcNRT1.157*、*AcNRT1.158*、*AcNRT1.159*、*AcNRT1.160*、*AcNRT1.161*、*AcNRT1.162*、*AcNRT1.163*、*AcNRT1.164*、*AcNRT1.165*、*AcNRT1.166*、*AcNRT1.167*、*AcNRT1.168*、*AcNRT1.169*、*AcNRT1.170*、*AcNRT1.171*、*AcNRT1.172*、*AcNRT1.173*、*AcNRT1.174*、*AcNRT1.175*、*AcNRT1.176*、*AcNRT1.177*、*AcNRT1.178*、*AcNRT1.179*、*AcNRT1.180*、*AcNRT1.181*、*AcNRT1.182*、*AcNRT1.183*、*AcNRT1.184*、*AcNRT1.185*、*AcNRT1.186*、*AcNRT1.187*、*AcNRT1.188*、*AcNRT1.189*、*AcNRT1.190*、*AcNRT1.191*、*AcNRT1.192*、*AcNRT1.193*、*AcNRT1.194*、*AcNRT1.195*、*AcNRT1.196*、*AcNRT1.197*、*AcNRT1.198*、*AcNRT1.199*、*AcNRT1.200*、*AcNRT1.201*、*AcNRT1.202*、*AcNRT1.203*、*AcNRT1.204*、*AcNRT1.205*、*AcNRT1.206*、*AcNRT1.207*、*AcNRT1.208*、*AcNRT1.209*、*AcNRT1.210*、*AcNRT1.211*、*AcNRT1.212*、*AcNRT1.213*、*AcNRT1.214*、*AcNRT1.215*、*AcNRT1.216*、*AcNRT1.217*、*AcNRT1.218*、*AcNRT1.219*、*AcNRT1.220*、*AcNRT1.221*、*AcNRT1.222*、*AcNRT1.223*、*AcNRT1.224*、*AcNRT1.225*、*AcNRT1.226*、*AcNRT1.227*、*AcNRT1.228*、*AcNRT1.229*、*AcNRT1.230*、*AcNRT1.231*、*AcNRT1.232*、*AcNRT1.233*、*AcNRT1.234*、*AcNRT1.235*、*AcNRT1.236*、*AcNRT1.237*、*AcNRT1.238*、*AcNRT1.239*、*AcNRT1.240*、*AcNRT1.241*、*AcNRT1.242*、*AcNRT1.243*、*AcNRT1.244*、*AcNRT1.245*、*AcNRT1.246*、*AcNRT1.247*、*AcNRT1.248*、*AcNRT1.249*、*AcNRT1.250*、*AcNRT1.251*、*AcNRT1.252*、*AcNRT1.253*、*AcNRT1.254*、*AcNRT1.255*、*AcNRT1.256*、*AcNRT1.257*、*AcNRT1.258*、*AcNRT1.259*、*AcNRT1.260*、*AcNRT1.261*、*AcNRT1.262*、*AcNRT1.263*、*AcNRT1.264*、*AcNRT1.265*、*AcNRT1.266*、*AcNRT1.267*、*AcNRT1.268*、*AcNRT1.269*、*AcNRT1.270*、*AcNRT1.271*、*AcNRT1.272*、*AcNRT1.273*、*AcNRT1.274*、*AcNRT1.275*、*AcNRT1.276*、*AcNRT1.277*、*AcNRT1.278*、*AcNRT1.279*、*AcNRT1.280*、*AcNRT1.281*、*AcNRT1.282*、*AcNRT1.283*、*AcNRT1.284*、*AcNRT1.285*、*AcNRT1.286*、*AcNRT1.287*、*AcNRT1.288*、*AcNRT1.289*、*AcNRT1.290*、*AcNRT1.291*、*AcNRT1.292*、*AcNRT1.293*、*AcNRT1.294*、*AcNRT1.295*、*AcNRT1.296*、*AcNRT1.297*、*AcNRT1.298*、*AcNRT1.299*、*AcNRT1.300*、*AcNRT1.301*、*AcNRT1.302*、*AcNRT1.303*、*AcNRT1.304*、*AcNRT1.305*、*AcNRT1.306*、*AcNRT1.307*、*AcNRT1.308*、*AcNRT1.309*、*AcNRT1.310*、*AcNRT1.311*、*AcNRT1.312*、*AcNRT1.313*、*AcNRT1.314*、*AcNRT1.315*、*AcNRT1.316*、*AcNRT1.317*、*AcNRT1.318*、*AcNRT1.319*、*AcNRT1.320*、*AcNRT1.321*、*AcNRT1.322*、*AcNRT1.323*、*AcNRT1.324*、*AcNRT1.325*、*AcNRT1.326*、*AcNRT1.327*、*AcNRT1.328*、*AcNRT1.329*、*AcNRT1.330*、*AcNRT1.331*、*AcNRT1.332*、*AcNRT1.333*、*AcNRT1.334*、*AcNRT1.335*、*AcNRT1.336*、*AcNRT1.337*、*AcNRT1.338*、*AcNRT1.339*、*AcNRT1.340*、*AcNRT1.341*、*AcNRT1.342*、*AcNRT1.343*、*AcNRT1.344*、*AcNRT1.345*、*AcNRT1.346*、*AcNRT1.347*、*AcNRT1.348*、*AcNRT1.349*、*AcNRT1.350*、*AcNRT1.351*、*AcNRT1.352*、*AcNRT1.353*、*AcNRT1.354*、*AcNRT1.355*、*AcNRT1.356*、*AcNRT1.357*、*AcNRT1.358*、*AcNRT1.359*、*AcNRT1.360*、*AcNRT1.361*、*AcNRT1.362*、*AcNRT1.363*、*AcNRT1.364*、*AcNRT1.365*、*AcNRT1.366*、*AcNRT1.367*、*AcNRT1.368*、*AcNRT1.369*、*AcNRT1.370*、*AcNRT1.371*、*AcNRT1.372*、*AcNRT1.373*、*AcNRT1.374*、*AcNRT1.375*、*AcNRT1.376*、*AcNRT1.377*、*AcNRT1.378*、*AcNRT1.379*、*AcNRT1.380*、*AcNRT1.381*、*AcNRT1.382*、*AcNRT1.383*、*AcNRT1.384*、*AcNRT1.385*、*AcNRT1.386*、*AcNRT1.387*、*AcNRT1.388*、*AcNRT1.389*、*AcNRT1.390*、*AcNRT1.391*、*AcNRT1.392*、*AcNRT1.393*、*AcNRT1.394*、*AcNRT1.395*、*AcNRT1.396*、*AcNRT1.397*、*AcNRT1.398*、*AcNRT1.399*、*AcNRT1.400*、*AcNRT1.401*、*AcNRT1.402*、*AcNRT1.403*、*AcNRT1.404*、*AcNRT1.405*、*AcNRT1.406*、*AcNRT1.407*、*AcNRT1.408*、*AcNRT1.409*、*AcNRT1.410*、*AcNRT1.411*、*AcNRT1.412*、*AcNRT1.413*、*AcNRT1.414*、*AcNRT1.415*、*AcNRT1.416*、*AcNRT1.417*、*AcNRT1.418*、*AcNRT1.419*、*AcNRT1.420*、*AcNRT1.421*、*AcNRT1.422*、*AcNRT1.423*、*AcNRT1.424*、*AcNRT1.425*、*AcNRT1.426*、*AcNRT1.427*、*AcNRT1.428*、*AcNRT1.429*、*AcNRT1.430*、*AcNRT1.431*、*AcNRT1.432*、*AcNRT1.433*、*AcNRT1.434*、*AcNRT1.435*、*AcNRT1.436*、*AcNRT1.437*、*AcNRT1.438*、*AcNRT1.439*、*AcNRT1.440*、*AcNRT1.441*、*AcNRT1.442*、*AcNRT1.443*、*AcNRT1.444*、*AcNRT1.445*、*AcNRT1.446*、*AcNRT1.447*、*AcNRT1.448*、*AcNRT1.449*、*AcNRT1.450*、*AcNRT1.451*、*AcNRT1.452*、*AcNRT1.453*、*AcNRT1.454*、*AcNRT1.455*、*AcNRT1.456*、*AcNRT1.457*、*AcNRT1.458*、*AcNRT1.459*、*AcNRT1.460*、*AcNRT1.461*、*AcNRT1.462*、*AcNRT1.463*、*AcNRT1.464*、*AcNRT1.465*、*AcNRT1.466*、*AcNRT1.467*、*AcNRT1.468*、*AcNRT1.469*、*AcNRT1.470*、*AcNRT1.471*、*AcNRT1.472*、*AcNRT1.473*、*AcNRT1.474*、*AcNRT1.475*、*AcNRT1.476*、*AcNRT1.477*、*AcNRT1.478*、*AcNRT1.479*、*AcNRT1.480*、*AcNRT1.481*、*AcNRT1.482*、*AcNRT1.483*、*AcNRT1.484*、*AcNRT1.485*、*AcNRT1.486*、*AcNRT1.487*、*AcNRT1.488*、*AcNRT1.489*、*AcNRT1.490*、*AcNRT1.491*、*AcNRT1.492*、*AcNRT1.493*、*AcNRT1.494*、*AcNRT1.495*、*AcNRT1.496*、*AcNRT1.497*、*AcNRT1.498*、*AcNRT1.499*、*AcNRT1.500*、*AcNRT1.501*、*AcNRT1.502*、*AcNRT1.503*、*AcNRT1.504*、*AcNRT1.505*、*AcNRT1.506*、*AcNRT1.507*、*AcNRT1.508*、*AcNRT1.509*、*AcNRT1.510*、*AcNRT1.511*、*AcNRT1.512*、*AcNRT1.513*、*AcNRT1.514*、*AcNRT1.515*、*AcNRT1.516*、*AcNRT1.517*、*AcNRT1.518*、*AcNRT1.519*、*AcNRT1.520*、*AcNRT1.521*、*AcNRT1.522*、*AcNRT1.523*、*AcNRT1.524*、*AcNRT1.525*、*AcNRT1.526*、*AcNRT1.527*、*AcNRT1.528*、*AcNRT1.529*、*AcNRT1.530*、*AcNRT1.531*、*AcNRT1.532*、*AcNRT1.533*、*AcNRT1.534*、*AcNRT1.535*、*AcNRT1.536*、*AcNRT1.537*、*AcNRT1.538*、*AcNRT1.539*、*AcNRT1.540*、*AcNRT1.541*、*AcNRT1.542*、*AcNRT1.543*、*AcNRT1.544*、*AcNRT1.545*、*AcNRT1.546*、*AcNRT1.547*、*AcNRT1.548*、*AcNRT1.549*、*AcNRT1.550*、*AcNRT1.551*、*AcNRT1.552*、*AcNRT1.553*、*AcNRT1.554*、*AcNRT1.555*、*AcNRT1.556*、*AcNRT1.557*、*AcNRT1.558*、*AcNRT1.559*、*AcNRT1.560*、*AcNRT1.561*、*AcNRT1.562*、*AcNRT1.563*、*AcNRT1.564*、*AcNRT1.565*、*AcNRT1.566*、*AcNRT1.567*、*AcNRT1.568*、*AcNRT1.569*、*AcNRT1.570*、*AcNRT1.571*、*AcNRT1.572*、*AcNRT1.573*、*AcNRT1.574*、*AcNRT1.575*、*AcNRT1.576*、*AcNRT1.577*、*AcNRT1.578*、*AcNRT1.579*、*AcNRT1.580*、*AcNRT1.581*、*AcNRT1.582*、*AcNRT1.583*、*AcNRT1.584*、*AcNRT1.585*、*AcNRT1.586*、*AcNRT1.587*、*AcNRT1.588*、*AcNRT1.589*、*AcNRT1.590*、*AcNRT1.591*、*AcNRT1.592*、*AcNRT1.593*、*AcNRT1.594*、*AcNRT1.595*、*AcNRT1.596*、*AcNRT1.597*、*AcNRT1.598*、*AcNRT1.599*、*AcNRT1.600*、*AcNRT1.601*、*AcNRT1.602*、*AcNRT1.603*、*AcNRT1.604*、*AcNRT1.605*、*AcNRT1.606*、*AcNRT1.607*、*AcNRT1.608*、*AcNRT1.609*、*AcNRT1.610*、*AcNRT1.611*、*AcNRT1.612*、*AcNRT1.613*、*AcNRT1.614*、*AcNRT1.615*、*AcNRT1.616*、*AcNRT1.617*、*AcNRT1.618*、*AcNRT1.619*、*AcNRT1.620*、*AcNRT1.621*、*AcNRT1.622*、*AcNRT1.623*、*AcNRT1.624*、*AcNRT1.625*、*AcNRT1.626*、*AcNRT1.627*、*AcNRT1.628*、*AcNRT1.629*、*AcNRT1.630*、*AcNRT1.631*、*AcNRT1.632*、*AcNRT1.633*、*AcNRT1.634*、*AcNRT1.635*、*AcNRT1.636*、*AcNRT1.637*、*AcNRT1.638*、*AcNRT1.639*、*AcNRT1.640*、*AcNRT1.641*、*AcNRT1.642*、*AcNRT1.643*、*AcNRT1.644*、*AcNRT1.645*、*AcNRT1.646*、*AcNRT1.647*、*AcNRT1.648*、*AcNRT1.649*、*AcNRT1.650*、*AcNRT1.651*、*AcNRT1.652*、*AcNRT1.653*、*AcNRT1.654*、*AcNRT1.655*、*AcNRT1.656*、*AcNRT1.657*、*AcNRT1.658*、*AcNRT1.659*、*AcNRT1.660*、*AcNRT1.661*、*AcNRT1.662*、*AcNRT1.663*、*AcNRT1.664*、*AcNRT1.665*、*AcNRT1.666*、*AcNRT1.667*、*AcNRT1.668*、*AcNRT1.669*、*AcNRT1.670*、*AcNRT1.671*、*AcNRT1.672*、*AcNRT1.673*、*AcNRT1.674*、*AcNRT1.675*、*AcNRT1.676*、*AcNRT1.677*、*AcNRT1.678*、*AcNRT1.679*、*AcNRT1.680*、*AcNRT1.681*、*AcNRT1.682*、*AcNRT1.683*、*AcNRT1.684*、*AcNRT1.685*、*AcNRT1.686*、*AcNRT1.687*、*AcNRT1.688*、*AcNRT1.689*、*AcNRT1.690*、*AcNRT1*

NRT 1.13、*AcNRT 2.1* 和 *AcNRT 1.12* 在这两个无氮处理中共同表达,可能与植株的缺氮信号有关。

处理后第9天,单氮处理的叶片硝酸盐含量上升,其他处理的叶片硝酸盐含量下降。从基因表达水平上看,*AcNRT 1.11*、*AcNRT 1.19*、*AcNRT 1.24*、*AcNRT 2.3*、*AcNRT 1.25*、*AcNRT 1.37*、*AcNRT 1.8*、*AcNRT 1.32*在30 mmol·L⁻¹氮处理中具有表达峰值,但这些基因未在60 mmol·L⁻¹氮处理中上调表达。这些基因中,除*AcNRT 1.11*、*AcNRT 1.19*和*AcNRT 1.32*外,其他基因也是清水处理6 d后上调表达的基因。

处理后第26天出现表达高峰的基因较少。在30 mmol·L⁻¹氮处理中具有表达峰值的基因有*AcNRT 1.5*、*AcNRT 1.11*、*AcNRT 1.19*和*AcNRT 1.35*。无氮MS处理中具有表达峰值的基因有*AcNRT 1.14*、*AcNRT 1.21*和*AcNRT 1.22*。在清水处理中具有表达峰值的基因有*AcNRT 1.6*和*AcNRT 1.10*。

3 讨 论

3.1 菠萝植株硝态氮积累及基因表达的日变化

土壤中可供作物利用的氮源包括硝态氮和铵态氮等无机氮以及氨基酸等有机氮,其中硝态氮是富氧土壤中植物吸收的主要无机氮^[17]。植物对硝态氮的吸收除受到土壤中硝态氮浓度的影响外,还受到植株的光合作用、营养状态、蒸腾作用、环境条件等多种因素的影响^[18]。在很多作物中,植株对硝态氮的吸收和同化具有日变化周期^[19]。在本研究中,未施氮植株的叶片、茎和根系的硝态氮含量呈现出特定的日变化模式,在10:00和16:00具有最高峰,其他时间段含量较低,可能与光合作用促进的硝态氮同化有关^[20]。施氮处理后,改变了该变化模式,降低了根系和叶中的16:00的峰值,提高了根系12:00的硝态氮含量,降低了茎中14:00和18:00的硝态氮含量,说明施氮后,根系增强了对硝态氮的吸收,促进了植株各部位对硝态氮的同化。

与此相应,菠萝植株各组织中硝态氮吸收和转运相关基因的表达变化也呈现出日变化模式。拟南芥中也有多个硝酸盐转运相关基因具有日变化模式^[21-23]。在本研究中,40个菠萝硝态氮转运与吸收基因在菠萝茎、根和叶3个组织中的表达模式并不完全相同,说明各基因可能具有各自特异的生理功能。

根系中受施氮处理影响的硝态氮吸收与转运相

关基因最多。施氮后,14个基因的表达峰值后延,1个基因的表达峰值提前,5个基因的表达峰值增强,4个基因的表达峰值减弱。根系中的硝态氮吸收与转运相关基因除直接参与根系自土壤中吸收和转运硝态氮外,还可作为信号分子感知和传递环境中的硝态氮^[24]。拟南芥中就有多个硝态氮转运相关基因参与根系中的硝态氮积累与分配,除了直接负责根系硝态氮吸收外,根系中表达的*AtNRT1.8*可自木质部中卸载硝态氮^[25],*AtNRT1.5*参与根系木质部硝态氮的负载^[26],*AtNRT1.9*则参与根系韧皮部硝态氮的运输^[22]。菠萝根系中的硝态氮含量分别在10:00和16:00有峰值,施氮后2 h,即12:00出现峰值,而16:00的峰值消失;根系中受施氮影响的大多数基因的表达峰值则从14:00延后到18:00;因此,这些基因在14:00的表达可能与随后16:00硝态氮含量增加有关,施氮2 h后,根系硝态氮含量迅速增加,可能导致这些基因表达减弱,峰值延后。施氮2 h后,*AcNRT 1.33*和*AcNRT 1.18*基因即上调表达,*AcNRT1.12*和*AcNRT1.14*基因下调表达,这些基因可能与根系16:00硝态氮的峰值消失有关,可能促进了根系硝态氮向地上部的转运。

叶片中也有不少基因受施氮处理的影响。施氮处理对叶片硝态氮的主要影响是增加了叶片18:00的硝态氮含量;主要在叶片中基因的表达峰值也是出现在18:00,如*AcNRT1.4*等。施氮处理对这些基因的影响主要是进一步上调了其在12:00(*AcNRT1.6*、*AcNRT1.44*、*AcNRT1.2*)、14:00(*AcNRT1.26*、*AcNRT1.13*、*AcNRT1.3*)和16:00(*AcNRT1.26*、*AcNRT1.27*)的表达,但*AcNRT1.4*基因在18:00的表达高峰被减弱。叶片中的硝态氮转运载体可能参与叶片中硝态氮的卸载和重新分配^[11, 24-25]。这些基因在菠萝叶片中的提前表达可能与随后叶片中的硝态氮含量下降有关。

本研究结果表明菠萝茎中含有较多的硝态氮,可能为菠萝硝态氮的贮藏库。茎中受施氮影响的基因非常少,施氮对茎硝态氮含量变化模式的影响也较小。施氮降低了茎中12:00和18:00的硝态氮含量。*AcNRT1.7*基因是少数在茎中具有高表达水平的基因,该基因在12:00有表达峰值,施氮后其较高的表达量可持续至18:00。该基因可能与茎中的硝态氮积累有关。在拟南芥中,有多个NRT基因家族的基因在茎中表达,主要参与硝酸盐的转运^[27]。

3.2 不同施氮处理对菠萝植株硝态氮积累及基因表达的影响

为揭示硝态氮转运蛋白基因与菠萝叶片营养状态的关系,本研究通过5个施氮处理,创造了分别处于高氮、氮均衡和缺氮等不同氮营养状态的菠萝植株。从结果来看,处理后3 d,除清水处理外,各处理的菠萝叶片都有显著的硝态氮积累。处理后6 d,除含 $60 \text{ mmol} \cdot \text{L}^{-1}$ 氮的MS处理外,其他处理的叶片硝态氮含量开始下降,说明此时硝态氮的同化得到激活,同化的速度超过吸收的速度。处理后9 d,除2个纯氮处理外,其他处理的叶片硝态氮含量均呈下降趋势,说明含有MS的全营养液最有利于植物硝态氮同化。对植株生长情况的分析进一步验证了该结果。不含氮的MS和清水处理的植株质量无明显增加,植株几乎没有生长,前者可能是由于营养元素供应不均衡,后者则可能是由于无营养元素供应;不含氮的MS处理的根系鲜质量高于 $60 \text{ mmol} \cdot \text{L}^{-1}$ 氮+MS处理和清水处理,也说明不含氮的MS处理促进了根系生长以利于营养元素的吸收。MS处理植株的鲜质量增加幅度最大,营养元素供应均衡,最有利于植株的生长。

从叶片中硝态氮转运相关基因的表达变化来看,处理3 d后,未找到施氮与未施氮处理特异的共有上调表达基因;清水处理叶片中硝态氮的增加可能与*AcNRT 1.18*基因的高表达有关,不施氮情况下叶片硝态氮的增加可能源自氮库的重新分配。处理6 d后,含氮处理的叶片硝态氮含量因被同化而降低,不含氮处理的植株叶片处于氮饥饿状态,大量基因被上调表达,其中包括高亲和力的硝态氮转运载体基因*AcNRT 2.1*、*AcNRT 2.3*和*AcNRT 3.1*;不含氮MS处理和清水处理间上调表达的基因并不完全相同,表明有些NRTs基因响应了其他矿质元素缺乏的信号,比如钾元素的运载^[28]。

施氮处理9 d后,受元素供应不均衡的影响,纯氮处理植株的叶片氮同化减弱、开始积累氮。此时,有8个清水处理6 d后上调表达的基因在 $30 \text{ mmol} \cdot \text{L}^{-1}$ 氮处理中具有表达峰值,进一步表明这些基因可能参与了其他矿质元素的调运或信号传递。这些基因在 $60 \text{ mmol} \cdot \text{L}^{-1}$ 氮处理中并未上调表达,一种可能就是这些基因的表达峰值在9 d前出现,此时已经检测不到。

施氮处理26 d后,大多数处理的叶片硝态氮含

量均有较大幅度下降,以无氮MS处理的下降幅度最大。此时叶片中硝态氮含量的下降可能是由于硝态氮向新叶或其他库的转移;因此,有生长量但无氮供应的无氮MS处理的下降幅度最大,无氮供应但也无生长量的清水处理下降幅度较小,MS营养液处理的生长量虽大,但元素供应均衡,硝态氮含量下降幅度也不大。从基因表达上看,清水处理和无氮MS处理无共有高表达基因;*AcNRT 1.14*、*AcNRT 1.21*和*AcNRT 1.22*基因在无氮MS处理中具有表达峰值,这些基因可能与叶片硝态氮的重新分配有关^[29]。拟南芥中,这一功能主要由*AtNRT1.7*基因完成^[12],可将硝态氮自老叶转运至幼叶。促进硝态氮自老叶向新叶的转运可提高氮的利用效率,进而促进烟草和水稻的生长或提高其产量^[30]。这些基因在菠萝育种中能否实现类似应用值得尝试。

4 结 论

菠萝根、茎、叶硝酸盐含量和硝酸盐转运蛋白基因表达均呈现日动态变化,施氮后其变化模式发生改变;硝酸盐转运蛋白基因表达受植株氮营养状态调控,*AcNRT 1.13*、*AcNRT 2.1*和*AcNRT 1.12*基因可能与叶片氮吸收有关,*AcNRT 1.14*、*AcNRT 1.21*和*AcNRT 1.22*基因可能与叶片氮再分配有关。

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