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ORIGINAL ARTICLE

The effect of nitrate assimilation deficiency on the carbon and nitrogen status of *Arabidopsis thaliana* plants

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Abstract Carbon (C) and nitrogen (N) metabolism are integrated processes that modulate many aspects of plant growth, development, and defense. Although plants with deficient N metabolism have been largely used for the elucidation of the complex network that coordinates the C and N status in leaves, studies at the whole-plant level are still lacking. Here, the content of amino acids, organic acids, total soluble sugars, starch, and phenylpropanoids in the leaves, roots, and floral buds of a nitrate reductase (NR) double-deficient mutant of *Arabidopsis thaliana* (*nia1 nia2*) were compared to those of wild-type plants. Foliar C and N primary metabolism was affected by NR deficiency, as evidenced by decreased levels of most amino acids and organic acids and total soluble sugars and starch in the *nia1 nia2* leaves. However, no difference was detected in the content of the analyzed metabolites in the *nia1 nia2* roots and floral buds in comparison to wild type. Similarly, phenylpropanoid metabolism was affected in the *nia1 nia2* leaves; however, the high content of flavonol glycosides in the floral buds was not altered in the NR-deficient plants. Altogether, these results suggest that, even under conditions of deficient nitrate assimilation, *A. thaliana* plants are capable of remobilizing their metabolites from source leaves and maintaining the C–N status in roots and developing flowers.

Keywords Amino acid · Carbon–nitrogen status · Flavonol glycosides · Nitrate reductase · Source–sink interaction

Introduction

Nitrogen (N) is the most limiting nutrient for plant growth and productivity in various environments because it is the root-absorbed nutrient that is required in the highest amount for plant tissues and is a component of such essential molecules as amino acids, nucleotides, and chlorophylls (Kraiser et al. 2011; Kusano et al. 2011). N-derived compounds are also precursors for the synthesis of secondary metabolites, such as those derived from the phenylpropanoid pathway, which have important functions in growth, signaling, and defense (Dixon and Paiva 1995; Peer et al. 2001). The plant N status exerts great influence on a series of physiological processes, including photosynthesis (Foyer et al. 2001), and a positive correlation is observed between the leaf N content and CO_2 assimilation rate (Boussadia et al. 2010). Thus, N deficiency not only affects the levels of N compounds but also has consequences on carbon (C) metabolism (Fritz et al. 2006; Boussadia et al. 2010). Conversely, alterations in photosynthesis and sugar metabolism are known to greatly influence N assimilation, thereby demonstrating the integration of C and N metabolism in plants (Matt et al. 2002; Stitt et al. 2002; Kruse et al. 2003; Sicher and Bunce 2008).

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Plants are capable of obtaining N from the soil, mainly in the form of nitrate (NO_3^-) and ammonium (NH_4^+), though organic forms, such as amino acids, can also be utilized (Bloom 1997; Harrison et al. 2007; Näsholm et al. 2009). The first step in plant N assimilation is catalyzed by nitrate reductase (NR), which reduces NO_3^- to nitrite (NO_2^-) in a reaction that is tightly regulated at both the transcriptional and post-transcriptional levels (Meyer and Stitt 2001). NO_2^- is then reduced to NH_4^+ in plastids by nitrite reductase. The NH_4^+ resulting from this reaction, in addition to the NH_4^+ absorbed from the soil by the roots, is assimilated into amino acids by the sequential action of the glutamine synthetase and glutamine-2-oxoglutarate aminotransferase enzymes (Hirel and Lea 2001). Because NO_3^- can be translocated in the xylem sap in relatively high amounts, NO_3^- assimilation may occur in the leaves in addition to the roots, with the relative contribution of each organ varying according to each plant species (Engels and Marschner 1995). In contrast, the assimilation of newly absorbed NH_4^+ occurs mainly in the roots, and the resulting amino acids are translocated by the xylem (Oliveira et al. 2013a).

The N-use efficiency depends on its transport into a cell and its assimilation and translocation within the plant (Xu et al. 2012). Thus, the absorption and assimilation of inorganic N from the soil and the transport of N compounds from source (roots and mature leaves) to sink organs (developing leaves, flowers, and seeds) are integrated into the plant physiology and are essential for plant growth, development, and stress responses (Rentsch et al. 2007).

Previous studies from our group have shown that an NR double-deficient mutant of *Arabidopsis thaliana* (*nia1 nia2*) exhibits reduced growth, a yellowish aspect, and lower levels of leaf amino acids in comparison to wild-type plants (Modolo et al. 2006; Oliveira et al. 2009). The development of this phenotype can be prevented by irrigating the mutant plant with glutamine, a treatment that recovers the leaf amino acid levels (Oliveira et al. 2009). NR deficiency also leads to decreased nitric oxide (NO) production in *nia1 nia2* leaves, resulting in compromised resistance to bacterial infection (Modolo et al. 2006; Vitor et al. 2013). Secondary metabolism in *nia1 nia2* leaves was also recently reported to be altered, as suggested by the reduced content of sinapoylmalate, the main end product of the phenylpropanoid pathway in *A. thaliana* leaves (Santos-Filho et al. 2012). Although the reduced NO content was found to be involved in this alteration, the compromised sinapoylmalate synthesis in the *nia1 nia2* leaves was also related to low levels of malate, indicating that organic acid metabolism is also disrupted by NR deficiency in *A. thaliana* leaves (Santos-Filho et al. 2012). These results demonstrate that leaf metabolism is strongly affected by NR deficiency; however, the levels of metabolites in other organs of the *A. thaliana nia1 nia2* mutant have not been investigated to date.

In this study, we investigated the contribution of NR to the homeostasis of N and C metabolism in different organs of *A. thaliana* plants at the beginning of the reproductive phase. The levels of primary and secondary metabolites in the roots, leaves, and floral buds of the *nia1 nia2* mutant were determined and compared to those of the wild-type plants. The results are discussed with regard to source–sink interactions and the importance of foliar NO_3^- assimilation in *A. thaliana* for maintaining the C and N status in the whole plant.

Materials and methods

Plant material and growth conditions

Wild-type ecotype Columbia-0 and *nia1 nia2* mutant *A. thaliana* plants (Wilkinson and Crawford 1993) were cultivated in vermiculite:perlite (1:1) in a growth chamber at 24 °C, with a 12-h photoperiod and light intensity of 90 μmol photons m^{-2} s^{-1}. The wild-type plants were irrigated with a modified Wilkinson and Crawford (1991) medium containing 5 mM KH_2PO_4, 2.5 mM NH_4NO_3, 2.5 mM $(NH_4)_2SO_4$, 1 mM $MgSO_4$, 0.5 mM $CaCl_2$, and 10 mM KNO_3 and the micronutrients described by Wilkinson and Crawford (1991). For the cultivation of *nia1 nia2* plants, KNO_3 was replaced with 10 mM KCl. Under these growth conditions, the wild-type and *nia1 nia2* leaves presented no differences in their endogenous NO_3^- content (Online Resource 1), thereby excluding the possibility of a differential signaling effect of NO_3^- per se in our assays. As previously reported, NO_3^- acts as a signal to induce organic acid metabolism and repress starch and large sectors of phenylpropanoid metabolism in tobacco (Scheible et al. 1997; Fritz et al. 2006). The roots and fully expanded leaves were collected from 4- to 5-week-old plants at the bolting stage (before anthesis); floral buds at stage 11 of development (Smyth et al. 1990) were used for the analysis. The plant tissues were collected at 6 h after the beginning of the light period, immediately frozen in liquid N_2, and kept at −80 °C until extraction.

Determination of NR activity

NR activity was determined according to Su et al. (1996), with some modifications. Briefly, the leaves, roots, and floral buds (150 mg) were ground to a powder in liquid N_2 and then homogenized with 1 mL of chilled extraction buffer containing 50 mM HEPES (pH 7.5), 1 mM EDTA, 10 μM FAD, 7 mM cysteine, 0.5 mM PMSF, 25 μM

leupeptin, and 5 μM Na_2MoO_4. The mixture was centrifuged at 13,000×*g* for 10 min at 4 °C. An equal volume of assay buffer (50 mM HEPES, pH 7.5, 1 mM EDTA, 1 mM NADH, and 10 mM KNO_3) was added to the resulting supernatant and incubated in the dark at room temperature. After 60 min, the reaction was stopped by the addition of the Griess reagent, and nitrite production was quantified at 540 nm (Green et al. 1982).

Extraction of primary metabolites

Frozen tissue samples (200 mg) were ground in 2 mL of an MCW solution (methanol:chloroform:water, 12:5:3 v:v:v) and incubated at room temperature for 24 h. After this period, the samples were centrifuged for 30 min at 1,500×*g*, and the supernatant was mixed with chloroform and water (4:1:1.5 v:v:v). The aqueous phase was collected after 24 h and used for the analysis of amino acids, organic acids, NO_3^-, and soluble sugars. Starch was extracted from the pellet after centrifugation by incubating with 30 % PCA (McCready et al. 1950).

Analysis of primary metabolites

Free amino acids were analyzed by reverse-phase HPLC (Shimadzu Corporation, Kyoto, Japan) using a Waters Spherisorb ODS2 C-18 column (4.6 μm, 4.6 × 250 mm) after derivatization with *O*-phthaldialdehyde, according to the conditions described by Puiatti and Sodek (1999). For the determination of organic acids, the dried MCW extracts were derivatized with *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide (Sigma, St. Louis, MO, USA) in pyridine and then subjected to analysis using a Shimadzu QP2010plus system (Shimadzu Corporation, Tokyo, Japan) equipped with a DB-5 column (J & W Scientific, Folsom, CA, USA) (Oliveira et al. 2013b). The total soluble sugars and starch were determined colorimetrically after reaction with 0.15 % anthrone in H_2SO_4 (McCready et al. 1950; Graham and Smydzuk 1965). The NO_3^- content was analyzed by the absorbance at 410 nm after reaction with 5 % salicylic acid in H_2SO_4 (Cataldo et al. 1975).

Extraction and analysis of secondary metabolites

Secondary metabolites were extracted from frozen plant tissues with an aqueous solution of 50 % methanol (v/v), as previously described (Santos-Filho et al. 2012). The analysis was performed using a C-18 Shimpack VP-ODS column as a part of an HPLC system (Shimadzu Corporation, Kyoto, Japan) following the solvent and gradient conditions described by Santos-Filho et al. (2012). Flavonol and sinapic acid derivatives were detected at 345 nm with a photodiode array detector (SPD-M20A) and quantified using kaempferol and sinapic acid, respectively, as standards. For the further identification of phenylpropanoid compounds, mass spectrometry analyses were performed using the same HPLC system connected to a mass spectrometer Esquire 4000 (Bruker Daltonics, Bremen, Germany) with an electrospray ionization source and an ion-trap analyzer (Santos-Filho et al. 2012). The product peaks were identified by their fragmentation pattern in comparison to previously published data (Tohge et al. 2005; Stobiecki et al. 2006; Matsuda et al. 2009; Santos-Filho et al. 2012).

Results

Nitrogen metabolism

The leaves, roots, and floral buds of the *A. thaliana nia1 nia2* mutant presented no detectable NR activity by in vitro assays (Online Resource 2), which is in accordance with previous reports (Seligman et al. 2008). These results demonstrate that NR deficiency occurs in all organs of the mutant analyzed in the present study. Compared to the organs of wild-type plants, the leaves presented the highest NR activity, whereas a very low activity of this enzyme was detected in the floral buds (Online Resource 2; Seligman et al. 2008).

To evaluate the effect of NR deficiency on N metabolism, the levels of free amino acids in the *nia1 nia2* leaves, roots, and floral buds were compared to those of the wild-type plant (Fig. 1a). The total content of amino acids in the *nia1 nia2* leaves (1,088.30 ± 206.95 nmol g FW^{-1}) was much lower than that in the wild-type leaves (3,760.34 ± 317.58 nmol g FW^{-1}), in accordance with the results of Modolo et al. (2006) and Oliveira et al. (2009). In these previous studies, it was found that the individual levels of most amino acids were reduced in the *nia1 nia2* leaves. In contrast to the leaves, no significant difference was detected in the total amino acid content of the *nia1 nia2* and wild-type roots (1,879.36 ± 296.11 and 1,693.81 ± 332.65 nmol g FW^{-1}, respectively) or their individual amino acid levels (Fig. 1b). It is noteworthy that the *nia1 nia2* roots accumulated a higher total amount of amino acids than the leaves, whereas the opposite was found in the wild-type plants. For both genotypes, very high levels of amino acids were detected in the floral buds (Fig. 1a), with a prominent accumulation of glutamine, asparagine, serine, and aspartate (Fig. 1c). Similar to the roots, the total and individual contents of amino acids in the *nia1 nia2* floral buds (21,174.56 ± 5,149.65 nmol g FW^{-1}) did not differ significantly from that found in the wild-type buds (24,968.08 ± 2,577.99 nmol g FW^{-1}). These results show that, although the levels of

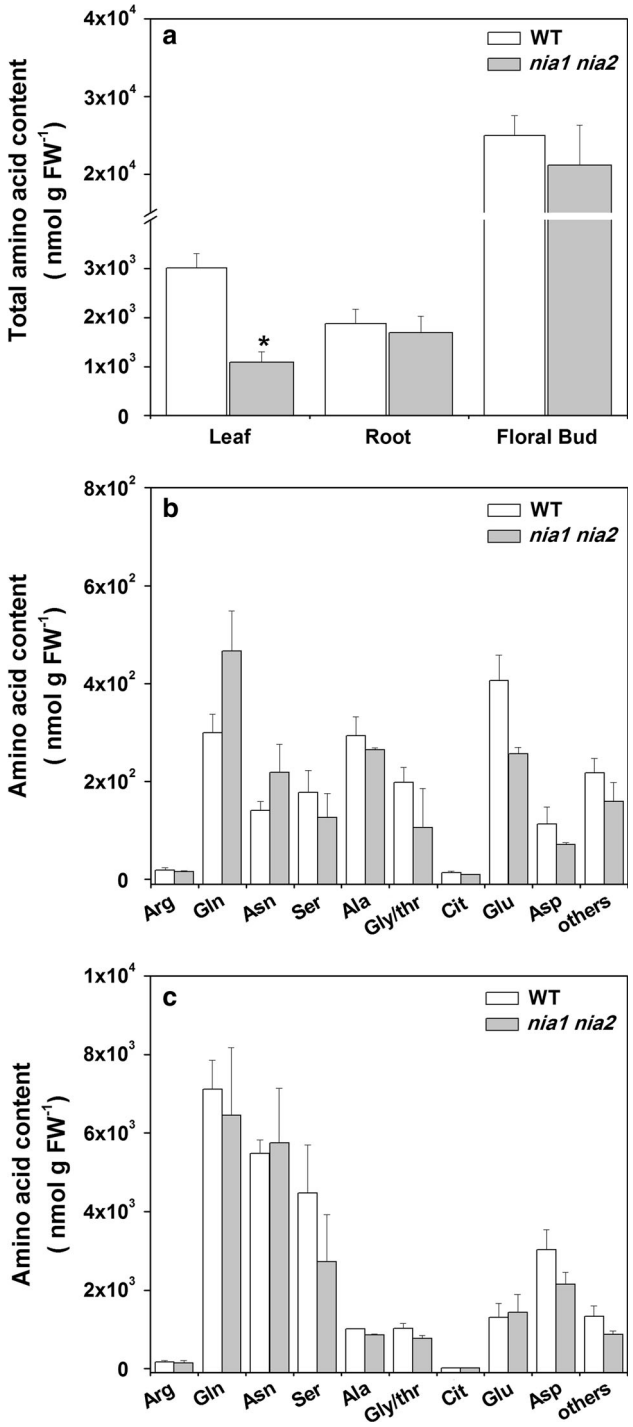


Fig. 1 Amino acid contents in different organs of wild-type (WT) and *nia1 nia2 A. thaliana* plants. **a** Total amino acid content in leaves, roots, and floral buds. **b** Individual amino acid contents in roots. **c** Individual amino acid contents in floral buds. Data represent the mean ± SE ($n = 3$). *$P < 0.05$ compared to the respective wild-type organ, according to Student's t test

amino acids in the leaves of *A. thaliana* are affected by NR deficiency, the contents of these metabolites are not altered in the roots and floral buds.

Carbon metabolism

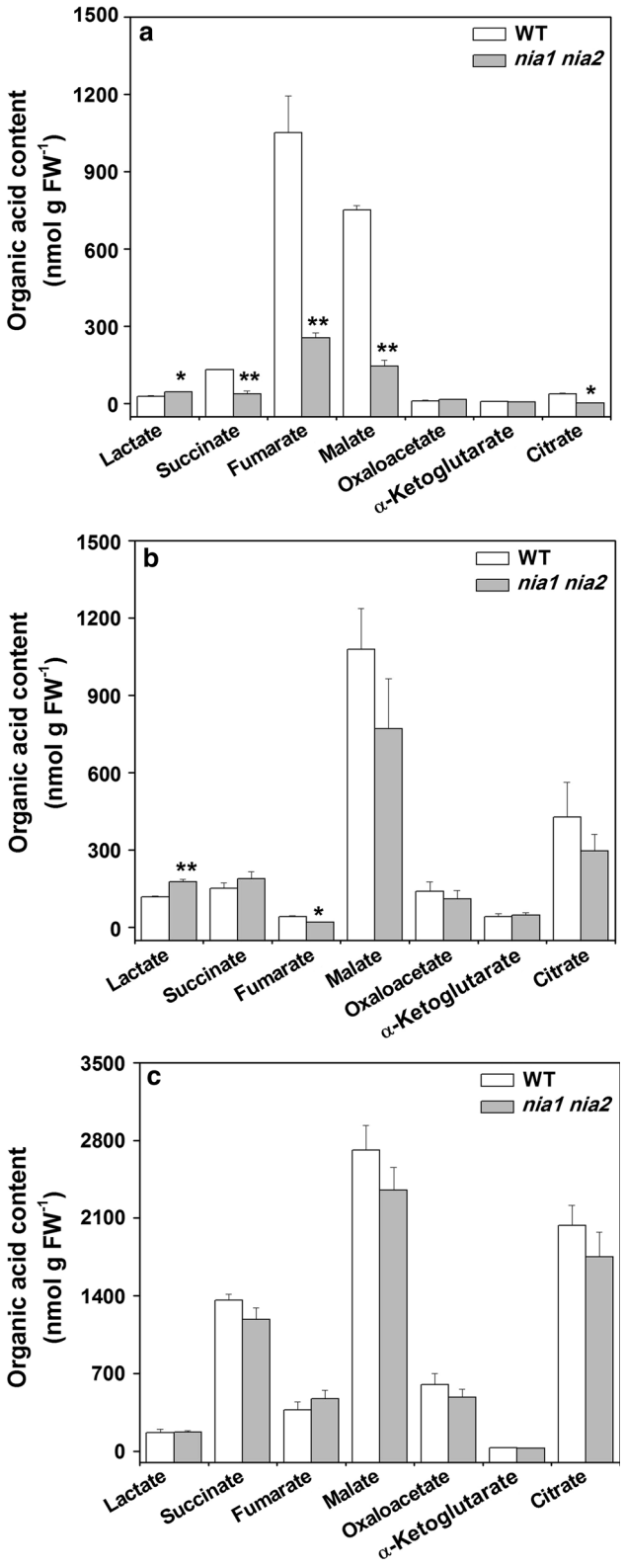
To analyze the impact of NR deficiency on C metabolism in the whole plant, the levels of organic acids and sugars in different organs of the *nia1 nia2* plants were compared to those of wild type (Figs. 2, 3). Fumarate and malate were the most abundant organic acids accumulated in the leaves (Fig. 2a), though the levels of both compounds, in addition to succinate and citrate, were markedly reduced in the *nia1 nia2* leaves in comparison to the wild-type leaves. Thus, the total content of the analyzed organic acids was of 2,017.1 ± 174.3 nmol g FW^{-1} in the wild-type leaves, whereas it decreased to 510.6 ± 62.1 nmol g FW^{-1} in the *nia1 nia2* leaves. In the roots, malate accounted for almost 50 % of the total organic acid content, and, differently from the leaves, NR deficiency did not markedly affect the levels of any of the analyzed organic acids (Fig. 2b). A similar scenario occurred in the floral buds, with no significant difference detected for the individual content of organic acids among the genotypes (Fig. 2c). In comparison to other organs, the floral buds accumulated higher amounts of malate, succinate, citrate, and oxaloacetate resulting in a higher total organic acid content in both the wild-type and *nia1 nia2* plants (7,273.5 ± 667.8 nmol g FW^{-1} and 6,446.6 ± 696.2 nmol g FW^{-1}, respectively).

For the analysis of sugar metabolism, the content of total soluble sugars (TSS) and starch was determined in the wild-type and *nia1 nia2* leaves (Fig. 3). The levels of both TSS and starch were higher in the wild-type leaves compared to the roots and floral buds, and TSS and starch were decreased in the *nia1 nia2* leaves compared to the wild-type leaves. However, the content of these metabolites was not affected by NR deficiency in the roots and floral buds, similar to the observations for organic acids (Fig. 2). Overall, these results suggest that, as observed for N metabolism, NR deficiency affected C metabolism in the leaves but not in the roots and floral buds.

Secondary metabolism

nia1 nia2 leaves were recently shown to present lower levels of phenylpropanoids when compared to the amounts accumulated in wild-type (Santos-Filho et al. 2012). In particular, *nia1 nia2* leaves had lower levels of sinapoylmalate, the major end product of the phenylpropanoid pathway in *A. thaliana* leaves, and accumulated its immediate precursor, sinapoylglucose. This decrease was associated with the reduced activity of 1-*O*-sinapoylglucose:malate sinapoyltransferase (SMT) in *nia1 nia2* leaves (Santos-Filho et al. 2012).

In the present study, the level of phenylpropanoids in the wild-type and *nia1 nia2* roots and floral buds were analyzed by HPLC and mass spectrometry to evaluate whether



◀**Fig. 2** Organic acid contents in the **a** leaves, **b** roots, and **c** floral buds of wild-type (WT) and *nia1 nia2* plants. The organic acids were quantified using gas chromatography–mass spectrometry after derivatization with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide in pyridine. Data represent the mean ± SE ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the respective wild-type organ, according to Student's *t* test

NR deficiency would affect the production of these secondary metabolites, as observed for leaf tissues. In both genotypes, no phenylpropanoids were detected in the root extracts, which is in accordance with a previous report showing that the roots of soil-grown *A. thaliana* plants contain almost no soluble phenylpropanoids because the accumulation of these metabolites is light dependent (Hemm et al. 2004).

However, high levels of phenylpropanoids were detected in the extracts of the wild-type and *nia1 nia2* floral buds. In particular, glycoside derivatives of the flavonols kaempferol, quercetin, and isorhamnetin were identified in the floral buds of both genotypes, as observed in the chromatographic profile shown in Fig. 4 and the mass spectrometry characterization (Online Resource 3). In contrast to leaves (Santos-Filho et al. 2012), sinapic acid esters were not detected in the floral buds. Of the metabolites observed in floral buds, only kaempferol derivatives were detected in the leaves, though at much lower amounts (Santos-Filho et al. 2012). A quantitative analysis demonstrated that the wild-type and *nia1 nia2* floral buds did not present any significant difference with regard to the levels of any of the flavonol glycosides (Fig. 5). These results show that phenylpropanoid metabolism is not affected in the floral buds of NR-deficient plants.

Discussion

NR-deficient plants of different species have been widely used as a model to demonstrate the importance of NO_3^- assimilation for diverse aspects of plant growth and metabolism (Foyer et al. 1994; Scheible et al. 1997; Fritz et al. 2006; Sicher and Bunce 2008; Kruse et al. 2010). In the present study, the *nia1 nia2* mutant was used to investigate the contribution of NO_3^- assimilation to the C and N status in *A. thaliana* plants undergoing the floral transition. In addition to leaves, the *nia1 nia2* mutant presents a strongly reduced NR activity in roots and floral buds (Online Resource 2; Seligman et al. 2008). Nevertheless, although leaf metabolism was drastically affected by NR deficiency, no difference was detected in the levels of the analyzed metabolites in the *nia1 nia2* roots and floral buds in comparison to wild type. These findings demonstrate source–sink interactions and the importance of leaf NO_3^- assimilation in *A. thaliana* plants.

Due to the very limited capacity of NO_3^- assimilation by the *nia1 nia2* mutant, the plants with this genotype rely mostly on NH_4^+ as an N source for the synthesis of amino acids and other N-derived compounds. When first characterized, a

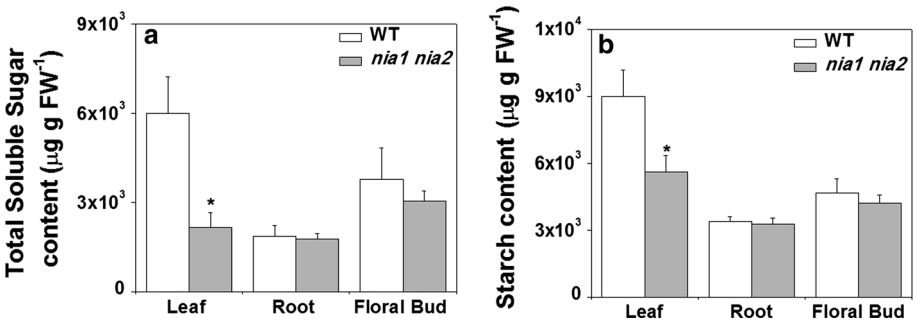


Fig. 3 Content of **a** total soluble sugars and **b** starch in the leaves, roots, and floral buds of wild-type (WT) and *nia1 nia2 A. thaliana* plants. The total soluble sugars and starch were determined colorimetrically after reaction with 0.15 % anthrone in H_2SO_4. Data represent the mean ± SE ($n = 3$). *$P < 0.05$ compared to the respective wild-type organ, according to Student's *t* test

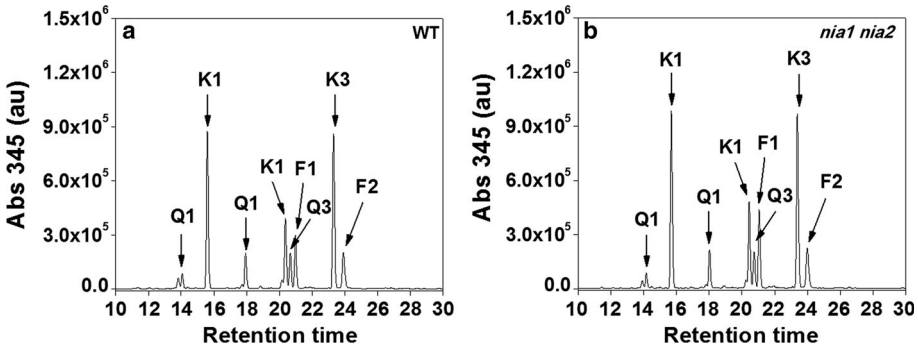


Fig. 4 Chromatographic profile of the methanolic extracts of the floral buds of wild-type (WT) and *nia1 nia2 A. thaliana* plants. The identification of the peaks is according to Online Resource 3

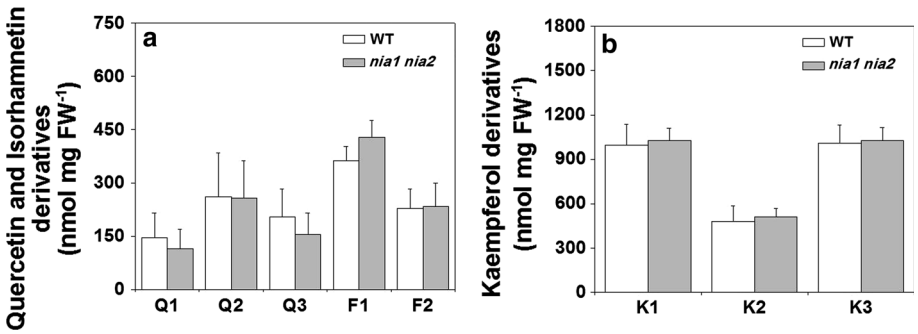


Fig. 5 Content of **a** quercetin, isorhamnetin, and **b** kaempferol derivatives in the floral buds of wild-type (WT) and *nia1 nia2 A. thaliana* plants. Data represent the mean ± SE ($n = 3$). The identification of the compounds is according to Online Resource 3

nutrient solution with a low NO_3^- concentration (2.5 mM) and a predominance of NH_4^+ (7.5 mM) was considered as most suitable for the growth of the *nia1 nia2* mutant (Wilkinson and Crawford 1993); indeed, these growth conditions were applied here for the *nia1 nia2* plants. As it is not translocated at high levels in the xylem sap, NH_4^+ is mainly assimilated into amino acids in root tissues (Oliveira et al. 2013a); therefore, these characteristics of NH_4^+ assimilation may explain why the levels of root amino acids are not affected in NR-deficient plants (Fig. 1a, b). In contrast, leaf N metabolism is more dependent on NO_3^- assimilation, relying on the NO_3^- that is translocated at relatively high amounts by the xylem as an important N source (Oliveira et al. 2013a). In *nia1 nia2* plants, amino acids would mainly be provided to the leaves by the roots via the xylem sap. The reduced amino acid levels in the *nia1 nia2* leaves compared to wild type (Fig. 1a; Modolo et al. 2006; Oliveira et al. 2009) suggest that NH_4^+ assimilation in roots is insufficient to supply the leaf demand for N, which is high due to the intensive synthesis of chlorophyll and photosynthetic proteins (Boussadia et al. 2010). Indeed, in wild-type, higher levels of amino acids were detected in the leaves in comparison to the roots, whereas the opposite was observed in the *nia1 nia2* mutant (Fig. 1a).

The development of foliar chlorosis and the reduced leaf amino acid levels in the *nia1 nia2* plants (Fig. 1a; Modolo et al. 2006; Oliveira et al. 2009) strongly suggest that a condition of N deficiency occurs in *nia1 nia2* leaves. It has been shown that N deficiency results in low contents of chlorophyll and Rubisco, leading to compromised CO_2 assimilation (Foyer et al. 1994). Alterations in photosynthetic metabolism

are most likely related to the decreased levels of total soluble sugars and starch, as well as organic acids, in the *nia1 nia2* leaves (Figs. 2a, 3). Additionally, the C skeletons produced by the source *nia1 nia2* leaves may be directed to the roots, which act as an important sink due to the C demand for NH_4^+ assimilation. In fact, the contents of soluble sugars, starch, and organic acids were not affected in the *nia1 nia2* roots (Figs. 2b, 3), indicating that the source leaves of this mutant are able to supply the C demand of the roots, thus maintaining NH_4^+ assimilation.

Developing floral buds present intense metabolic activity and also constitute important sinks for C and N compounds (Ludewig and Flügge 2013). In accordance with this, the developing flowers of *A. thaliana* presented very high levels of the metabolites analyzed in this study. In particular, the floral buds accumulated much higher levels of amino acids than the leaves and roots in both genotypes (Fig. 1). It is also noteworthy that the NR activity was very low, even in the wild-type floral buds (Online Resource 2; Seligman et al. 2008). Taken together, these results show that, rather than assimilating inorganic N, these reproductive structures import amino acids from source organs, as has been previously reported (Tegeder 2012). In NO_3^--cultivated *A. thaliana* plants, it was estimated that most of the N exported to the floral axis and reproductive organs is derived from rosette leaves, whereas N remobilization from roots to reproductive structures is very weak (Diaz et al. 2008). The amino acids synthesized in the roots are mainly exported through the xylem sap to photosynthetically active leaves, as transpiration is intense in these organs (Hirner et al. 2006). These amino acids can be metabolized, transiently stored, or loaded into the phloem for redistribution to flowers, fruits, and seeds (Hirner et al. 2006; Tegeder 2012), and the transfer of amino acids from the xylem directly to the phloem also contributes to the rapid provision of sink organs (Zhang et al. 2010). The sink strength of the floral buds appeared to be unaffected by NR deficiency, as the levels of amino acids, total soluble sugars, starch, and organic acids were not reduced in the *nia1 nia2* floral buds in comparison to the wild-type plants (Figs. 1, 2, 3). Thus, organic compounds from source leaves might also be directed to feed the demand of developing flower buds, which additionally may have contributed to the reduced content of the analyzed metabolites in the *nia1 nia2* leaves. Thus, it may be suggested that *nia1 nia2* plants address NR deficiency in a way that effectively maintains the remobilization of key compounds of C and N metabolism from source leaves to developing flowers and related structures to ensure reproductive success.

In addition to primary C and N metabolism, the phenylpropanoid content was shown to be affected in NR-deficient leaves (Santos-Filho et al. 2012). The limited conversion of sinapoylglucose to sinapoylmalate has been associated with low levels of malate and with the deficient production of NO by *nia1 nia2* leaves (Santos-Filho et al. 2012). In contrast to leaves, which predominantly accumulate sinapate esters (Santos-Filho et al. 2012), floral buds present high levels of flavonol glycosides (Figs. 4, 5), most of which have not previously been detected in leaves (Santos-Filho et al. 2012). Indeed, the presence of glycoside derivatives of kaempferol, quercetin, and isorhamnetin in the reproductive structures of *A. thaliana* has been reported (Peer et al. 2001; Jones et al. 2003; Routaboul et al. 2006). Similar to the observations of primary metabolites in floral buds, NR deficiency did not affect the production of phenylpropanoids in these organs, as no significant difference was detected in the levels of flavonols accumulated in the wild-type and *nia1 nia2* buds (Fig. 5). It is noteworthy that flavonol production in leaves, although much lower than that in floral buds, is also unaffected in *nia1 nia2* leaves (Santos-Filho et al. 2012).

The occurrence of high levels of flavonols in floral buds may be associated with full fertility and reproductive success, as suggested by studies in corn and petunia (Mo et al. 1992). More recently, Thompson et al. (2010) demonstrated that the absence of the flavonoid transporter FFT (At-DTX35), normally found at high levels in flowers, affects the content of flavonoids in *A. thaliana* plants and impairs pollen development and seed germination. Moreover, flavonoids have been reported to promote the reduction of nitrite to NO (Takahama et al. 2002), a signaling molecule that has been suggested to be involved in flower development (Seligman et al. 2008). Thus, it may be hypothesized as to whether the high levels of flavonoids accumulated in *A. thaliana* flower buds have a role in NO synthesis, even under conditions of limited nitrite, as occurs in the *nia1 nia2* mutant (Seligman et al. 2008). Consistent with this, NO production in floral buds is only slightly affected in NR-deficient plants, in contrast to the drastic effect observed in leaves and roots (Seligman et al. 2008).

A better understanding of the metabolic basis of N-use efficiency by plants is required to promote the growth of economically important crops under conditions of low N (see Tschoep et al. 2009). In addition to the efficiency of N uptake, the efficiency by which N is assimilated by the plant and transformed into biomass determines the efficient use of N (Hirel et al. 2007). The present results demonstrate the existence of N and C allocation and recycling in NR-deficient *A. thaliana* plants, suggesting the occurrence of a mechanism for plant acclimation to growth under conditions of low N assimilation.

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Conflict of interest The authors declare that they have no conflict of interest.

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