RESEARCH PAPER

Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply

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Abstract

Eighteen accessions of *Arabidopsis thaliana* were grown with low (N–) and high (N+) nitrogen supply. N uptake was monitored by feeding plants with 15N-enriched nutritive solution over 24 h. Biomass [fresh matter (FM) and dry matter (DM)], N concentration (N%), and 15N content were monitored and computed to determine the nitrogen use efficiency (NUE) and nitrogen uptake efficiency (NupE). NUE has been estimated as the ratio between biomass and N concentration (DM/N%) and NupE as the concentration of 15N in plants [μg (g⁻¹ DM)]. Accession traits were analysed to detect common and individual genotype features. The genetic variation in NUE at high N input was mainly explained by variation in N uptake. Even though plants managed N uptake and N metabolism differently under N+ and N–, NUE was similar in these two conditions, showing that NUE was exclusively genetically determined. Hierarchical classification revealed that the physiological classes arising were similar under N– and N+. Both wasteful and efficient genotypes were detected. Three extreme genotypes, Col-0, Bur-0, and Tsu-0, were noted. Bur-0 and Tsu-0 exhibited high NUE and large biomass. Col-0 showed the reverse: low NUE and low biomass. Bur-0 appeared poorly tolerant of a high N supply. The present data will facilitate the choice of *Arabidopsis* accessions as parents of recombinant inbred line populations suitable for the mapping of quantitative trait loci related to NUE, NupE, and N storage capacity.

Key words: 15N, natural variation, nitrogen, nitrogen use efficiency, NUE, NupE, QTL, uptake.

Introduction

Plants have a fundamental dependence on inorganic nitrogen (N), and millions of metric tons of nitrogenous fertilizers are added to the soil worldwide annually (Good et al., 2004). N is one of the most expensive nutrients to supply, and commercial fertilizers represent the major cost in crop production (Singh, 2005). Furthermore, there is serious concern regarding N loss in fields that pollutes soil and water. The ability of a plant to capture N from soil depends on the soil type, the environment, and the plant species. It has been estimated that 50–70% of the N provided to the soil is lost (Good et al., 2004). The possibility of lowering fertilizer input and breeding plants with better nitrogen use efficiency (NUE) has to be considered (Hirel et al., 2007; Lea and Azevedo, 2007). Improving NUE might help to limit use of N fertilizers and to preserve the environment and public health.

The effect of low N availability on plant biomass, nitrate uptake, and root architecture has already been widely studied (Brouwer, 1962; Drew and Saker, 1976; Van der Werf and Nagel, 1996; Lea and Azevedo, 2007; Lemaitre et al., 2008). It is known that plants modify their root architecture, changing their lateral/primary root ratio and decreasing the shoot/root ratio at the same time, to forage the soil for nutrients. The influence of N limitation on the...
fine-tuning of plant metabolism is less well documented. Attention has been paid mainly to plants’ response to N starvation and not to the response to chronic N limitation. Recently, the adaptation of Arabidopsis to chronic low nutrient availability was documented (Lemaître et al., 2008). The physiology of the Wassilewskija (WS) accession of Arabidopsis was investigated with low nitrate (2 mM) and high nitrate (10 mM) nutrition. N limitation reduced rosette biomass production and yield. Rosette leaves contained more sugars, fewer free amino acids, and higher cytosolic glutamine synthetase activity when grown in N-limiting conditions. Compared with high N nutrition, the concentrations of proline, asparagine, and glutamine in rosettes and seeds were decreased, thus suggesting profound modifications in the metabolite pathways.

The use of N by plants involves several steps including uptake, assimilation, translocation, and, when the plant is ageing, recycling and remobilization (Masclaux-Daubresse et al., 2008). Nitrogen use efficiency (NUE) has been defined for crops as the grain yield per unit of N available from the soil, including N fertilizer. For other plants used for biomass production, NUE is expressed as the fresh matter (FM) or dry matter (DM) produced per N content (Good et al., 2004). In both cases, NUE is the product of nitrogen uptake efficiency (NupE) and nitrogen utilization efficiency (NUtE), which is the optimal combination between nitrogen assimilation efficiency (NAE) and nitrogen remobilization efficiency (NRE). NupE is defined by the nutrient uptake efficiency from soil and depends on nitrate and ammonium transport at the root level. When considered at the whole-plant level, including the roots and shoots, NupE also depends on the plant’s storage capacity in its cells and on transport from the root to the shoot where assimilation takes place. The enzymes involved in the synthesis of organic N from inorganic N control NAE, together with the enzymes involved in pathways supplying the carbon skeletons needed for N assimilation. Nitrate, ammonium, and amino acid transporters control NupE at the whole-plant level (Masclaux-Daubresse et al., 2010).

Until now, most crop varieties have been selected under non-limiting N conditions (Presterl et al., 2003). To reduce the excessive input of fertilizers without affecting productivity it is important to improve N uptake and assimilation of plants under low N supply. Plant responses to chronic N-limiting conditions have been recently documented at the transcriptional level by Bi et al. (2007). In their report, the authors identify putative regulatory elements as possible new components of the regulatory network for plant adaptation to low N. It will be useful to test the candidate genes from that study by genomic and reverse genetic strategies and improve NUE.

Due to the large genetic variability available in Arabidopsis populations, the adaptive responses to low N availability can also be investigated by a quantitative genetic approach. In maize and Arabidopsis it has already been shown that plant responsiveness to N availability depends on both genotype and genotype×N fertilization level interaction (Loudet et al., 2003; Gallais and Hirel, 2004). The quantity and the quality of the root system that might partly explain differences in N uptake show a large variability in Arabidopsis (Loudet et al., 2005).

The present study investigates the natural variation of NUE- and NupE-related traits using Arabidopsis accessions grown under ample and chronic limiting nitrate supply. Traits were measured taking into account shoot biomass and 15NO3 uptake in rosettes. The objectives were (i) to set up reproducible experimental conditions to use 15N labelling to evaluate N uptake in rosettes; (ii) to determine NupE together with agronomical and NUE-related traits on a core collection of 18 accessions of Arabidopsis; (iii) to study correlations between those traits; (iv) to estimate trait variations under two types of N supply (limiting and ample); and (v) to identify good parents of recombinant inbred line (RIL) populations suitable for further NupE into the rosette and NUE quantitative trait locus (QTL) mapping.

Materials and methods

Plant material and growth conditions

Seeds of the Arabidopsis thaliana accessions Akita, Bl-1, Bur-0, Col-0, Cvi-1, Cvi-0, Ge-0, Gn-0, Mf-1, Mt-0, M13, Oy-0, Sakata, Shahdana, Sf-0, Sw-0, and Tsu-0 were provided by the Versailles Genetics and Plant Breeding Laboratory Arabidopsis thaliana Resource Centre (INRA Versailles France, http://dbsgap-versailles.inra.fr/mtat/; Supplementary Table S1 available at JXB online). Sixteen of these accessions are part of the core collection of 24 accessions selected by Mckhanh et al. (2004) on the basis of genetic variability. The 16 accessions retained from the 24 line core collection have been selected on the basis of their close flowering time in short days. Accessions that presented very early flowering or very late flowering times have been eliminated. Col-0, which is a parental line for most of the RIL populations available at the Versailles Resource Center, was also included, as was Mr-0.

Seeds were stratified for 48 h in 0.1% (w/v) agar solution (in water) at 4 °C in the dark. They were sown as described by Loudet et al. (2003) at the central position in a small pot (L=60 mm, l=65 mm, h=60 mm) filled with medium particle size sand topped with a thin layer of small particle size sand. One week after sowing, a single seedling was retained in each pot, and black sand was poured in to avoid proliferation of algae. The experiment was carried out in two consecutive culture cycles (repeats R1 and R2) performed in the same growth chamber at two different times. R1 and R2 represented two independent biological repeats. Each biological repeat included four accession repetitions (four plants per genotype). Plants were grown in short days (8 h light) with 21 °C day and 17 °C night temperatures. The photon flux density was 160 μmol m−2 s−1. Plants were cultivated under low N nutrition (N−, 2 mM nitrate) or under high N nutrition (N+, 10 mM nitrate). Phosphate (0.25 mM), sulphate (0.25 mM), magnesium (0.25 mM), and sodium (0.20 mM) were present in both solutions at the same concentration. The difference between N− and N+ solutions affects only potassium (5.25 mM and 2.75 mM in N+ and N− solutions, respectively), calcium (2.50 mM and 0.50 mM, respectively), and chloride ions (0.25 mM and 0.70 mM, respectively). Pots were watered three times per week over 2 h by immersion of the base of the pots.

Determination of nitrate uptake into the shoots using 15N labelling

The 15N uptake time point occurred 40 days after sowing (DAS) when plants were still vegetative. The unlabelled watering solution...
was replaced by an $^{15}$N-containing solution that had the same nutrient composition ($N$– or $N+$) except that $^{14}$NO$_3$ was replaced by $^{15}$NO$_3$ 10% enrichment (w/w). All the pots were watered during 24 h, using an equal volume of labelled solutions. Cutting the rosettes stopped $^{15}$N uptake. The fresh weight (FW) of each rosette was measured. Rosettes were then dried, and their dry weight (DW) was determined.

**Determination of total nitrogen content and $^{15}$N abundance**

After drying and weighing each plant, the material was ground to obtain a homogenous fine powder. A subsample of 1000–2000 µg was carefully weighed in tin capsules to determine the total N content and $^{15}$N abundance using an elemental analyser (roborprep CN, PDZ Europa Scientific Ltd, Crewe, UK) coupled to an isotope ratio mass spectrometer (Twen-to-twenty, PDZ Europa Scientific Ltd, Crewe, UK) calibrated using natural abundance. The $^{15}$N abundance of samples was calculated as atom percent and defined as $A\% = 100 \times (^{15}N / (^{15}N + ^{14}N))$ for labelled plant samples, and for unlabelled plant controls $A_{\text{control}}\%$ was $\sim 0.3660$. The $^{15}$N enrichment (E\%) of the plant material was then defined as $(A_{\text{sample}}\% - A_{\text{control}}\%)$. The absolute quantity of $^{15}$N contained in the i sample was defined as $Q_i = DM_i \times E_i \times N_i\%$, with N\% the concentration of nitrogen in the i sample (as mg of nitrogen per 100 mg DW).

**Statistical analysis**

Statistical analysis of phenotypic data was carried out by analysis of variance (ANOVA), using the GLM procedure of SAS. Adjusted means for each accession were estimated with the LSMEANS option. Genetic variances within $N$– or $N+$ were then estimated with the VARCOMP option of SAS. Heritability was estimated as $h^2 = \sigma^2_g / (\sigma^2_e + \sigma^2_r)$, with $\sigma^2_g$ being the genetic variance, $\sigma^2_e$ the residual variance, and $r$ the number of replicates. Correlations between traits within N conditions were computed using the Proc CORR procedure of SAS. Hierarchical classification of accessions was carried out by using XLSTAT software according to the Ward method. Differences between accessions were determined using XLSTAT ANOVA comparisons according to the Newman–Keuls (SNK) method.

**Results**

**Statistical analysis and calculation of key indicators for nitrogen uptake and use efficiencies**

ANOVA was performed using all the data measured under $N+$ and $N$– for weight of FM, weight of DM, N concentration (N\%, mg 100 mg$^{-1}$ DW), and $^{15}$N enrichment (E\%; $^{15}$N as % of total N), (Supplementary Table S2 at *JXB* online; Fig. 1). Following computed sums of square, ANOVA revealed that the variation between the measured traits under $N+$ and $N$– was mainly due to nutrition treatment. Variations were also explained by the genotype but to a lower extent, and a significant effect of the genotype x nutrition interaction was also observed. This indicated that the effect of nitrate nutrition on plant growth was partly genotype dependent, and that dissociation of the two nutritive conditions should reveal a larger part of the genetic variation of measured traits. For DM, N\%, and E\%, a minor but significant effect of the biological repeat was detected. Therefore, data were normalized according to the mean of repeats R1 and R2, before being used for further analysis and correlation analysis (Supplementary Table S3 at *JXB* online).

The correlations between the measured traits showed that under both N– and N+, DM was significantly correlated to FM (Table 1). N concentration [N\% as mg of nitrogen (100 mg DW)$^{-1}$] was positively correlated with FM under N+ and negatively to DM under N–. E\% was correlated with N\% under N+. As a consequence, under both N+ and N–, the total quantity of $^{15}$NO$_3$ absorbed by plants (estimated as µg of $^{15}$N per plant) was correlated with FM, DM, and E\%. The lack of correlation between the quantity of $^{15}$N absorbed by plants and N\% in the N– condition could be due to a strong genetic x nutrition interaction effect in the absorption efficiency or due to a large effect of repetition as suggested by the ANOVA results (Fig. 1). Correlation between $^{15}$N quantity per plant and biomass suggested that the biggest plants have absorbed a higher amount of N than smaller plants and indicated that to compare the efficiency of N uptake in shoots (NupE) between accessions, independently of plant biomass, the ratio $^{15}$N/DM (µg g$^{-1}$) has to be calculated and used as the indicator.

The definition of NUE is different depending on whether vegetative biomass productivity or grain production are important traits to be considered. In this study, plants that combined large shoot biomass and low N\% were considered as plants with a good NUE. The indicator used to estimate NUE was thus the ratio of the rosette biomass to the N concentration in the rosette (DM/N\%). NupE and NUE indicators were calculated for each individual plant. NupE and NUE values were then normalized and expressed as a percentage of the mean value under N+ or N–.

Before ANOVA, the normal distribution of NUE and NupE percentage values was verified using the

![Fig. 1. Schematic representation of ANOVA of fresh weight (FM, g), dry weight (DM, g), N concentration (N\%, mg 100 mg$^{-1}$ DW), and $^{15}$N enrichment (E\%; $^{15}$N/N as %), $^{15}$N (µg plant$^{-1}$), NupE (µg g$^{-1}$DW), and NUE (DM/N\%) traits in the 18 *Arabidopsis* accessions grown under N+ and N–. Histograms show the effects due to nutrition, genotype, repeat, and interactions as a percentage of the variation explained.](https://academic.oup.com/jxb/article-abstract/61/9/2293/527305)
was the highest under N+ and N– (Fig. 2a, b). In general, accessions under both N+ and N–, whereas Bur-0 biomass was higher (0.70) under N+ than under N– (0.56) (Table 2). The natural variation of measured and calculated traits was mainly due to a genetic effect (Figure 1).

The adaptation of plants to higher N condition was estimated calculating the biomass gain \([\left(DM_{10\text{mM}}-DM_{2\text{mM}}\right)\times(\text{DM}_{1\text{mM}})]\) and the N storage efficiency as the N% gain \([\left(N\%_{1\text{mM}}-N\%_{2\text{mM}}\right)\times(\text{N}\%_{1\text{mM}})]\).

**Natural variation of biomass, N concentration, NupE, and NUE**

The natural variation of measured and calculated traits was analysed by comparing individual accession values with the mean value calculated on the whole collection.

For the weight of the DM, significant variations were observed under both N+ and N– conditions (Fig. 2). The range of DM variations was smaller under N– than under N+. Together with higher plant growth heterogeneity in limited conditions, this explained that DM heritability was similar in the two conditions, with equally high heritabilities under both N+ and N–. The genetic variation of NUE was significantly higher under N– than under N+, and the heritability of NupE was also higher under N– than under N+, whereas Bur-0 had a significantly higher N%. Bur-0, showing one of the highest N% scores under N+ and one of the lowest N% under N–, was characterized by its high N% gain (Fig. 3c).

As expected, N% was also higher under N+ when compared with N– for most of the genotypes (Fig. 3a, b). However the difference of N% between N– and N+ was not as high as described in Loudet et al. (2003). Under N+, the range of N% variation was lower when compared with N–. The heritability of N% was thus higher under N– than under N+ (\(h^2=0.51\) and \(h^2=0.34\) respectively, Table 2). For a few accessions such as Col-0 the N% was similar under N+ and N–, showing that N availability did not influence N storage. As a consequence, N% gain was very low for Col-0. Col-0, N13, Shahdara, and Akita had the highest N% at N–. Bur-0 and Tsu-0 had the lowest N% at N–. At N+, Tsu-0 had a significantly lower N% than the other accessions, whereas N13, Shahdara, Oy-0, Akita, and Bur-0 showed that N availability did not influence N storage. The most efficient genotype for biomass and N storage gains was Tsu-0.

For most of the accessions, NUE (DM/N%) was not significantly different between the biological repeats and N conditions (Fig. 1, Supplementary Table S2 at JXB online). In order to facilitate comparisons between the genotypes, the relative values were normalized and expressed as a percentage of the mean value of the core collection. Means and standard deviations were then calculated for each accession and growth condition (Fig. 4). Significant differences between accessions were detected under both N+ and N–. The genetic variation of NUE was similar in the two conditions, with equally high heritabilities (\(h^2=0.67\) for both, Table 2). Col-0, N13, Shahdara, Akita, and Mt-0 presented significantly lower NUE than other accessions under both N– and N+ (Fig. 4a, b). Under N+, the NUE of Edi-0, Stw-0, Kn-0, Mt-0, Bur-0, and Tsu-0 had significantly higher N%. Bur-0, showing one of the highest N% scores under N+ and one of the lowest N% under N–, was characterized by its high N% gain (Fig. 3c).

The comparison between biomass gain (Fig. 2c) and N% gain (Fig. 3c) allowed the identification of accessions that favoured a biomass increase over N storage when cultivated in the higher N condition (N+ versus N–). The Col-0 accession was very unusual since both biomass and N% gains were minimal; Col-0 thus appeared to be insensitive to N nutrition. N13 and Ct-1 were poorly responsive lines showing limited biomass and N% gains. Mr-0, Bl-1, and Bur-0 favoured N storage rather than biomass, whereas Akita, Ge-0, Kn-0, and Mt-0 favoured biomass rather than N storage. The most efficient genotype for biomass and N storage gains was Tsu-0.
Shahdara, and N13 had the highest NupE, whereas Tsu-0, Oy-0, Bl-1, Edi-0, and Mh-1 had the lowest (Fig. 5a). Under N+, only Col-0 and Shahdara showed significantly higher NupE, while Tsu-0 and St-0 were the only accessions to have a significantly lower relative NupE value (Fig. 5b).

Correlations between NUE, NupE, DM, and N% traits determined at N+ or N–

The correlations between all the traits are presented in Table 1. NUE was positively correlated with DM and FM, and negatively with N% and NupE, under both N+ and N–. The correlation between NUE and DM was the highest found ($r=0.89$ and $r=0.85$, under N+ and N–, respectively), showing that DM is the main factor explaining NUE variations whatever the N supply. N% was positively correlated to NupE under N+ and N– ($r=−0.32$ and $r=−0.60$, respectively), showing that the N concentration was determined by N uptake. Surprisingly, the negative correlation between N% and DM was only found under N– ($r=−0.36$). This suggests that when nitrate supply is not plentiful, N storage and plant growth are opposite strategies. In contrast, under N–, plant growth depends on both N acquisition and N storage.

**Fig. 2.** Natural variation in dry matter weight (DM, a and b) and biomass gain (c). Arabidopsis accessions were grown on sand with a low (a) or high (b) nitrogen supply for 40 DAS. Data are the adjusted means from two biological repeats comprising four plants each, ± SD. The horizontal black lines represent the mean of the core collection. The different letters indicate values significantly different at $P<0.05$ as determined using XLSTAT ANOVA Newman–Keuls (SNK) comparisons.

**Table 2.** Heritability of the fresh weight (FM, g), dry weight (DM, g), N concentration (N%, mg 100 mg$^{-1}$ DW), $^{15}$N enrichment (E%; $^{15}$N/N as %), $^{15}$N (µg plant$^{-1}$), NupE (µg g$^{-1}$ DW), and NUE (DM/N%) under N– or N+. Two independent biological repeats comprising four plant replicates were carried out.

Biomass gain variation was mainly influenced by the variation of DM and NUE traits under N+. The variation of N% gain was also dependent on the variation of DM and NUE under N+, as was the variation of DM, NUE, N%, and NupE under N−. All these results show that while the biomass gain was mainly dependent on the plant features under N+, the N% gain was highly dependent on the plant features under N−. It has to be noted that $^{15}$N (µg plant$^{-1}$) was highly correlated with NUE under N+ but not under N−. This suggests that, as reported before by Gallais and Hirel (2004) in maize, the genetic variation in NUE is explained by variation in N uptake at high N input, whereas at low N input, NUE variability is mainly due to differences in the efficiency of N utilization.

Characterization of contrasted lines
A classification was carried out using the mean of relative values of NUE, NupE, and N% under N+ and N−. Ascendant hierarchical clustering was performed using the Ward method, and five distinct classes were defined (Fig. 6).

In Fig. 6, NUE, NupE, and N% data used for clustering are presented together with the biomass gain and N% gain. Cells containing data are represented with colours to indicate values that are significantly higher than the core collection mean (pink), significantly lower (green), or not significantly different compared with the mean (black).

Class 1 (Shahdara, Col-0, and N13) is characterized by high N%, high NupE, and low NUE under both N− and N+. Class 1 exhibits low N% gain. It is noticeable that Col-0 has the lowest NUE under N+ and N−, the highest NupE under N−, and the lowest N% gain and biomass gain.

Class 2 (St-0, Ct-1, Ge-0, Stw-0, and Mt-0) is characterized by low N% and NupE under both N+ and N−. The accessions belonging to class 2, except St-0, presented a good NUE under N+ and N−. Class 2 N% gain is low.

Class 3 (Sakata, Mr-0, Oy-0, Mh-1, Bl-1, Edi-0, Kn-0, and Akita) is mainly characterized by low NupE under N− and N+ and by high N% gain. NUE scores were in the medium range and rather close to the mean.

The only member of class 4 is Bur-0. This accession is characterized by a very high NUE under N+ and especially...
under N– (twice the mean). Under N–, the NupE and N% of Bur-0 were low, thus explaining that this accession presented the highest N% gain.

Class 5 has Tsu-0 as the only member and is differentiated from class 4 Bur-0 due to their large differences in NupE and N% under N+. Tsu-0 is an extreme genotype that exhibited the lowest NupE and N% under both N+ and N–, and the highest NUE under N+. NUE of Tsu-0 was also one of the highest under N–, just less than that of Bur-0. Tsu-0 presented high biomass and N% gains.

It is noticeable that the characteristics found for Tsu-0 under N+ and N– were the opposite of those found for Col-0, N13, and Shahdara. The characteristics found for Bur-0 were the opposite to those of Col-0, N13, and Shahdara, but only under N–. It is also noticeable that Tsu-0 and Col-0 are the two extreme genotypes of the core collection.

Discussion

In this report, traits related to biomass, N uptake, and rosette N concentration have been measured using a core collection of 18 accessions of Arabidopsis. The data collected allowed the determination of the natural variation of traits depending on N availability and observation of the global features of Arabidopsis under high and low nitrate supply.

Among the global features revealed by this study, it can be noted that nutrition influenced all the traits measured except NUE. Correlations between traits were thus mainly considered under the same nutritive conditions. Under N–, the negative relationship between plant biomass and N concentration was consistent with the ‘N dilution’ process described by Greenwood et al. (1990). Such a correlation was not found under N+, suggesting that the ‘N dilution’ process can only be observed under nutrient-limiting conditions for plant growth. The significant correlation between 15N (µg plant⁻¹) and NUE observed under N+ but not under N– was in good agreement with the previous findings of Bertin and Gallais (2001) and Coque et al. (2008) on maize, and showed that in Arabidopsis, as in maize, the genetic variation in NUE at high N input is mainly explained by variation in N uptake. At low N input, Gallais and Hirel, (2004) suggested that NUE variability might rather be due to differences in NAE. Albeit that this suggested that NUE variability in maize or Arabidopsis was not due to the same process under ample or limiting nitrate nutrition (Lemaître et al., 2008; this study), it was observed that the relative NUE was the same under N+ and N–. NUE thus appeared to be a robust trait, genetically determined and completely independent of the amount of

Fig. 4. Natural variation in nitrogen use efficiency (NUE). Arabidopsis accessions were grown on sand with a low (a) or high (b) nitrogen supply for 40 DAS. Data are the adjusted means from two biological repeats comprising four plants each, ±SD. The horizontal black lines represent the mean of the core collection.
nitrate supplied. This result is quite surprising considering that plants do not manage N uptake and N metabolism in a similar manner under high and low N supply.

Even though N availability greatly influenced the variation of all the measured traits and most of the computed traits (except NUE; Fig. 1), the analysis and comparisons of key indicators under N+ and N− facilitated the clustering of genotypes into five different classes. The physiological features, characterizing all the classes, appeared to be independent of whether N has been limiting or not, and can be described as follows.

Class 1 is mainly composed of ‘wasteful’ genotypes that are storing N in their rosettes regardless of N availability. While increasing nitrate nutrition might have favoured biomass and/or N storage in this plant class, Col-0, N13, Shahdara, and Akita presented the lowest N% gain. This was mainly due to the fact these accessions had the highest N% scores under N−. Since these accessions also had the lowest DM, it can be proposed that the growth rate of these plants was low and poorly dependent on nitrate availability. For Col-0 and N13, N was clearly not a limiting factor for plant growth. The poor growth rate potential of Col-0, N13, and Shahdara could explain the high N% found in their rosettes. Class 3 includes lines with high NUE together with low NupE under N+. Class 3 accessions are certainly well adapted to limiting N conditions since they increase N uptake and N storage strategies under N−. For that reason, class 3 had a low N% gain, in contrast to class 2, which was characterized by a high N% gain. Class 2 appeared indeed less efficient at N uptake and N storage under N− than class 3. Investigating the regulation of nitrate uptake in class 2 genotypes by comparison with class 3 genotypes would be informative.

Bur-0 and Tsu-0 are separate from the other lines. Both are characterized by large biomass under N− and N+ and they displayed the highest NUE detected in the whole core collection. NUE of Bur-0 under N− is particularly notable. Their low N% and large NUE under N− suggest that Bur-0 and Tsu-0 were highly efficient for carbon fixation and growth under N-limiting conditions. Bur-0 and Tsu-0 were, however, separated into two different classes due to their differences in biomass gain and N% under N+. Compared with other accessions and especially Tsu-0, Bur-0 appeared poorly efficient under high N nutrition. Indeed, while Bur-0 biomass under N− was twice as high as that of most of the other genotypes, Bur-0 biomass gain was one of the lowest. For Bur-0, increasing nitrate supply resulted mainly in N storage.

Fig. 5. Natural variation in nitrogen uptake efficiency (NupE). Arabidopsis accessions were grown on sand with a low (a) or high (b) nitrogen supply for 40 DAS. Uptake was performed during 24 h, prior to harvest. Data are the adjusted means from two biological repeats comprising four plants each, ± SD. The horizontal black lines represent the mean of the core collection. The different letters indicate values significantly different at P < 0.05 as determined using XLSTAT ANOVA Newman–Keuls (SNK) comparisons.
Col-0 exhibited the lowest NUE, N% gain, and biomass. This showed that Col-0 was poorly sensitive to an increase of nitrate supply. In a recent report, North et al. (2009) investigated the adaptation of Arabidopsis to growth under a low nitrate supply and measured natural variation of biomass and N concentration on a core collection of 10 accessions, some of which were common to the plant material used for the present study. Albeit that the growth conditions were clearly different in their study compared with the present study, since North et al. (2009) used vertical plates and hydroponics, it is remarkable that in some aspects the results are very similar, especially for Col-0. North et al. (2009) indeed observed that Col-0 was insensitive to the amount of nitrate supplied and exhibited the same biomass under N+ and N−. Because of the particular behaviour of Col-0, the judiciousness of using mutants in the Col-0 genetic background to investigate adaptation to nitrate limitation might be questionable.

In contrast, the present study suggests that it would be very interesting to use Col-0 together with Tsu-0 or Bur-0 as parent lines of RIL populations to perform QTL mapping of traits related to NUE, NupE, and N storage capacity. In addition, the high heritability reported here for most of the traits shows that the experimental set-up used in this study is suitable for QTL mapping.

Supplementary data

Supplementary data are available at JXB online. Table S1 lists the Arabidopsis thaliana accessions used in this work and presents details about the geographic location and climate where accessions are originating.

Table S2 presents the numeric data for ANOVA of FM, DM, N E% (15N/N as %), 15N (µg plant−1), NupE, and NUE.

Fig. 6. Hierarchical classification of accessions performed by computing the N%, NupE, and NUE data obtained from plants grown under low (2 mM) and high (10 mM) nitrogen supply. Data from the biomass gain (Bio Gain) and the N% gain (N% Gain) have been added to the table represented in this figure but were not used to perform clustering. Ascendant hierarchical classification was carried out using XLSTAT software according to the Ward method.

Table S3 is an Excel file containing the whole data set used for this study.

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