ELSEVIER

Natural variation of the root morphological response to nitrate supply in *Arabidopsis thaliana*

Jérôme De Pessemier [a,1], *Fabien Chardon* [b,1], *Michal Juraniec* [a], *Pierre Delaplace* [c], *Christian Hermans* [a,*]

[a] *Lab. of Plant Physiology and Molecular Genetics, Université Libre de Bruxelles, Campus Plaine CP 242, Bd du Triomphe, 1050 Brussels, Belgium*
[b] *Institut Jean-Pierre Bourgin (IJPB), UMR1318 INRA-AgroParisTech, Saclay Plant Sciences (SPS), Route de Saint Cyr, Versailles 78026 Cedex, France*
[c] *University of Liège, Gembloux Agro-Bio Tech, Plant Biology Unit, Passage des Déportés 2, 5030 Gembloux, Belgium*

ARTICLE INFO

ABSTRACT

Nitrogen fertilization increases crop yield but excessive nitrate use can be a major environmental problem due to soil leaching or greenhouse gas emission. Root traits have been seldom considered as selection criteria to improve Nitrogen Use Efficiency of crops, due to the difficulty of measuring root traits under field conditions. Nonetheless, learning about mechanisms of lateral root (LR) growth stimulation or repression by nitrate availability could help to redesign root system architecture (RSA), a strategy aimed at developing plants with a dense and profound root system and with higher N uptake efficiency. Here, we explored the genetic diversity provided by natural populations of the model species *Arabidopsis thaliana* to identify potentially adaptive differences in biomass production and root morphology in response to nitrate availability. A core collection of 24 accessions that maximizes the genetic diversity within the species and Col-0 (the reference accession) were grown vertically on agar medium at moderate (N+) nitrate level for 6 days and then transferred to the same condition or to low (N−) nitrate concentration for 7 days. There was a major nutritional effect on the shoot biomass and root to shoot biomass ratio. The variation of the root biomass and RSA traits (primary root length, LRs number, LR mean length, total LRs length and LR densities) was primarily genetically determined. Differences in RSA traits between accessions were somewhat more pronounced at N−. Some accessions produced almost no visible LRs (Pyl-1, N13) at N−, while other produced up to a dozen (Kn-0). Taken together our data illustrate that natural variation exists within *Arabidopsis* for the studied traits. The identification of RSA ideotypes in the N response will facilitate further analysis of quantitative traits for root morphology.

1. Introduction

Nitrogen fertilization has been used for decades to increase crop yield with a relatively low efficiency since a considerable fraction (up to two-thirds) of N input accumulates as runoffs (Frink et al., 1999). The resulting nitrous oxide emissions in the atmosphere and nitrate leaching from soil have detrimental consequences to the environment (Donner

and Kucharik, 2008). In order to reduce eutrophication and the costly component of crop production, there is an immediate need to reduce N fertilizer inputs. To compensate for that reduction, improved crop genotypes must be sought with higher Nitrogen Use Efficiency (NUE) and particularly its uptake component (Edgerton, 2009; Den Herder et al., 2010; Kant et al., 2011). Currently NUE is defined as the crop yield per unit of available N, estimated as the product of nitrogen uptake efficiency (NupE) and nitrogen utilization efficiency (NutE), which is itself the combination of the efficiencies of assimilation and remobilization (Good et al., 2004; Masclaux-Daubresse et al., 2010). Greater allocation of root biomass along with a branched and dense root system would increase NupE and limit the impact of nitrogen fertilization on the environment. However, the root organ, responsible for nutrient capture, has largely been out of crop breeders' mind due to the difficulty of measuring root traits underground (Robinson, 2004).

Because plants are sessile organisms and cannot migrate towards more prosperous habitats, they have evolved mechanisms to adapt to water and nutrient availability changes. Root organs respond to resource fluctuations by showing developmental plasticity in order to modulate the surface available for the uptake, where the lateral root (LR) branching pattern occupies an important role (Zolla et al., 2010). The formation of LR from pericycle tissue is characterized by several consecutive developmental steps from the initiation of the founder cell division to the meristem elongation out of the parent root; each of those steps being subjected to nutritional influence (López-Bucio et al., 2003; Hermans et al., 2006; Péret et al., 2009; Giehl et al., 2012). A dual effect of external nitrate, which is the main nitrogen mineral form taken up by roots, on LR development has been depicted in the model species *Arabidopsis thaliana*: (i) a systemic inhibition of uniformly high nitrate concentrations at a post-emergence stage of the lateral primordium and (ii) a localized stimulation on N-starved

Table 1 – Definition of biomass and root architecture parameters.

Fresh biomass parameters	
R	Root biomass (5 pooled organs)
S	Shoot biomass (5 pooled organs)
R:S	Root to shoot biomass ratio
Root architecture parameters	
L_{PR}	Length of primary root
N_{LR}	Number of lateral roots visibly emerged (>1 mm) from primary root
ΣL_{LR}	Sum of lateral root length per seedling
L_{LR}	Mean lateral root length
$D_{LR}–Z_1$	Lateral root density calculated as the number of emerged lateral roots divided by the total primary root length (zone 1)
$D_{LR}–Z_2$	Lateral root density calculated as the number of emerged lateral roots divided by the length of the primary root portion between the first and last visibly emerged lateral roots (zone 2)
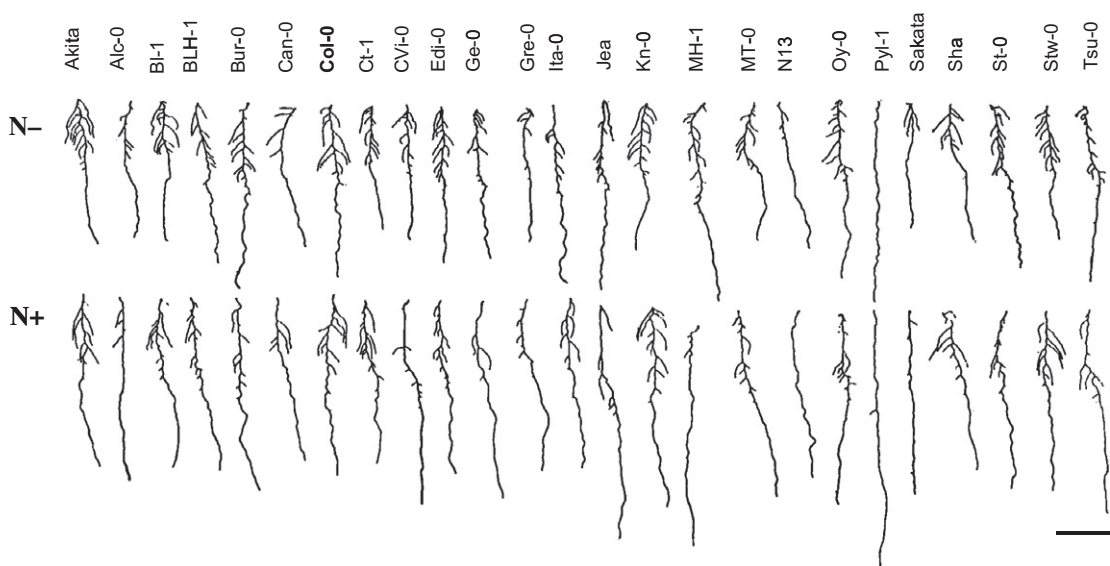
Fig. 1 – **Representative root phenotype of 25 *Arabidopsis* accessions in response to the nitrate supply. Seedlings of 24 accessions of the core collection selected by McKhann et al. (2004) and the reference accession Col-0 were grown on medium containing 10 mM NO$_3^-$ and transferred 6 days after germination on a medium of the same concentration (N+) or at 0.01 mM NO$_3^-$ (N−). The photos were taken 7 days after transfer. Scale bar: 5 cm.**
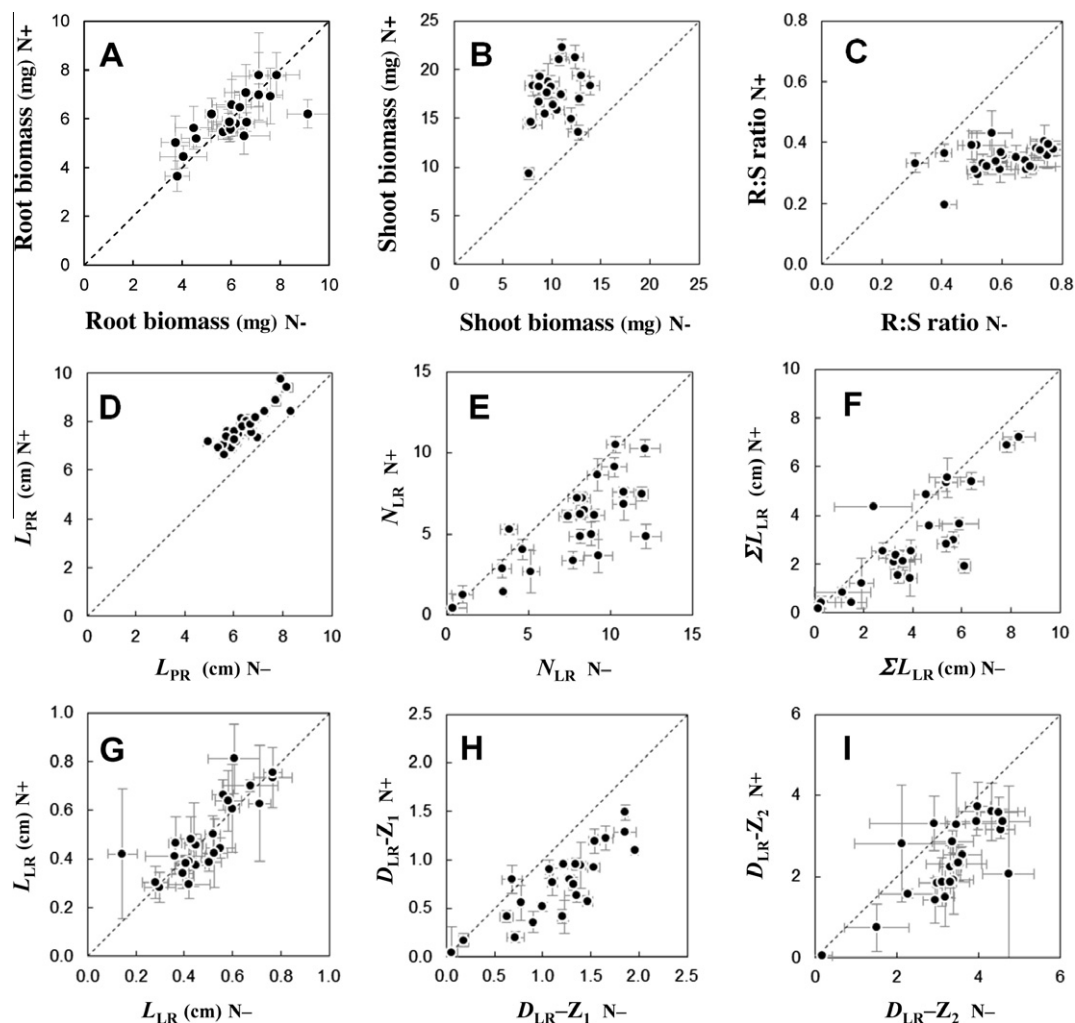
Fig. 2 – Biomass production and root architecture traits of 25 *Arabidopsis* accessions in response to the nitrate supply. Traits are defined in Table 1. Biomass traits: (A) production of fresh root biomass, (B) production of fresh shoot biomass and (C) ratio of the root biomass to the shoot biomass (R:S); root architecture traits: (D) Length of primary root (L_{PR}), (E) number of lateral roots (>1 mm) (N_{LR}), (F) sum of the length of lateral roots (ΣL_{LR}), (G) mean lateral root length (L_{LR}), (H) lateral root density calculated per primary root length ($D_{LR}–Z_1$), (I) lateral root density calculated per root portion between the first and last visibly emerged lateral root ($D_{LR}–Z_2$) at 0.01 mM (N–) and 10 mM (N+) NO_3^-. For biomass traits $n = 3–4 \times 5$ organs grouped, for root architecture traits $n = 15–20$. The standard deviations are indicated on the graphics. Growth conditions are described in legend of Fig. 1.

roots elongation at the contact with a nitrate rich supply, known as the foraging capacity (Zhang and Forde, 1998; Zhang et al., 1999; Linkohr et al., 2002; Ruffel et al., 2011). Despite few known pathways that involve transcription factors, micro-RNAs, hormonal signals and more recently nitrate transporters with dual affinity for nitrate and auxin (Little et al., 2005; Miller et al., 2007; Remans et al., 2006; Gifford et al., 2008; Krouk et al., 2010, 2011; Vidal et al., 2010; Castaings et al., 2011; Rubio-Somoza and Weigel, 2011; Ruffel et al., 2011), our understanding of sensing external nitrate conditions and of the signal transduction system that terminates in LR development is still poor.

Our applied ambition is to transfer the benefits from *A. thaliana* research to crops of the Brassicaceae family. Previously, we aimed at identifying key genes shaping root system architecture (RSA) by pursuing direct genetic approaches with the analysis of mutants that have altered morphological response to N (Hermans et al., 2010, 2011). Here, we developed a second approach by screening natural populations of *Arabidopsis* for the biomass production and the root morphology upon various nitrate supplies. Indeed, *Arabidopsis* has a broad geographical distribution and consequently is subject to varying nutritional environments which makes it a useful model for studying possible contrasting adaptation of the root architecture to nutrient availability (Chevalier et al., 2003; Chevalier and Rossignol, 2011). We want to know whether such natural variation exists in the model species in response to N supply before attempting to identify genetic determinants of a possible amelioration of root characteristics of Brassica crops.

2. Results

2.1. Influence of nitrate supply on biomass production and root architecture in A. thaliana accessions

A small number of accessions (24), which maximize the genetic diversity of *A. thaliana* (McKhann et al., 2004), and the reference Columbia 0 (Col-0) accession were analyzed to assess the variation of biomass production and the plasticity degree of the RSA in response to nitrate availability. Seedlings were vertically grown on agar medium with moderate N+(10 mM) nitrate concentration for six days and then transferred to a medium of the same N+ composition or low N− (0.01 mM) concentration. Biomass production and root architecture traits (as defined in Table 1) were measured after an additional 7 days growth period (Figs. 1 and 2 and Table S1). On average for the whole collection, the root fresh biomass (R) was 6.1 mg at N− versus 6.0 mg at N+ and the shoot fresh biomass (S) was 10.3 and 17.6 mg respectively in the same conditions (Fig. 2A and B). Because S biomass was stimulated further at N+, the root to shoot (R:S) biomass ratio was decreased in that case compared to N− condition (Fig. 2C). Accessions were quite contrasted for the biomass production . At N−, a variation factor of 2.4 was observed for the R biomass between Sha and Bur-0, and of 1.8 for the S biomass between Alc-0 and Sakata (Table S1). At N+, a factor of 2.4 was found for the R biomass between N13 and Jea, and of 3.1 for the S biomass between N13 and Sakata (Table S1). Root architecture traits were also measured (Table 1). For the whole collection, the average of primary root length was shorter at N− (L_{PR} = 6.5 cm) than at N+(L_{PR} = 7.7 cm) (Fig. 2D), the lateral root number was greater at N− (N_{LR} = 7.7) than at N+(N_{LR} = 5.4) (Fig. 2E), the sum of lateral root length was longer at N− (ΣL_{LR} = 4.0 cm) than at N+(ΣL_{LR} = 2.9 cm) (Fig. 2F), the mean lateral root length was equal at both N concentrations (L_{LR} = 0.5 cm) (Fig. 2G), the lateral root density calculated per primary root length was higher at N− (D_{LR}–Z_1 = 1.2) than at N+(D_{LR}–Z_1 = 0.7) (Fig. 2H) and the lateral root density calculated per primary root portion between the first and last visibly emerged lateral root was higher at N− (D_{LR}–Z_2 = 3.3) than at N+(D_{LR}–Z_2 = 2.4) (Fig. 2I). At N−, a variation factor of 1.7 was observed for L_{PR} between Sakata and Bur-0, of 30.7 for N_{LR} between Pyl-1 and Kn-0, of 86.0 for ΣL_{LR} length between Pyl-1 and Kn-0, of 5.5 for L_{LR} between Pyl-1 and Col-0, of 20.0 for D_{LR}–Z_1 between Pyl-1 and Edi-0 and of 23.5 for D_{LR}–Z_2 between Pyl-1 and Sakata and (Table S2). At N+, a variation factor of 1.4 was observed for L_{PR} between Sha and Pyl-1, of 23.5 for N_{LR} between Pyl-1 and Ct-1, and of 36.5 for ΣL_{LR} length between Pyl-1 and Kn-0, of 2.9 for L_{LR} between Gre-0 and Sha, of 32.4 for D_{LR}–Z_1 between Pyl-1 and Ct-1 and of 82.0 for D_{LR}–Z_2 between Pyl-1 and Akita (Table S2).

2.2. Genotype explains most of the root biomass and RSA variation

After an overall assessment of the accessions response to N supply, we looked more closely to each measured biomass and root morphological traits. A global ANOVA was carried in order to determine the effects of nutrition (N−/N+), genotype (accessions) and their interaction between genotype and

nutrition (genotype × nutrition) on those traits variation (Fig. 3). The nutritional effect on the observed variation for S biomass and R:S biomass ratio was clearly dominant (>50%) over the genotype, interaction term or residuals. The variation of the R biomass and all RSA parameters was mainly dependent on the genotype. Effect of nutrition was not significant for R biomass but significant for other RSA traits, showing that plants respond to N limitation mainly through plasticity in the root architecture. Effect of interaction (genotype x nutrition) was weak but significant for all traits, revealing that accessions responded differently to N limitation. Significant correlations between traits have been mapped at two N conditions in Fig. 4. Under N− condition, the R biomass was the key trait related to S biomass and almost all RSA traits (Fig. 4A, Table S3). It was significantly ($P < 0.05$) correlated with S biomass (r^2 = 0.43), L_{PR} (r^2 = 0.58), N_{LR} (r^2 = 0.78), ΣL_{LR} (r^2 = 0.68) and D_{LR}–Z_1 (r^2 = 0.64). Interestingly, upon N+ condition, the R biomass was less central (Fig. 4B, Table S3). It was still positively correlated with the S biomass (r^2 = 0.57), L_{PR} (r^2 = 0.43), N_{LR} (r^2 = 0.40) and ΣL_{LR} (r^2 = 0.40) but there was a lack of correlation with R:S biomass ratio and D_{LR}–Z_1.

### 2.3. Characterization of contrasted accessions phenotypes

A classification of the accessions was carried out using the mean values of the biomass production and root architecture traits (Tables S1, S2). Ascendant hierarchical clustering was performed using the Ward's method, and the dissimilarity cut-off was chosen to define five distinct groups (Fig. 5). Group 1 (Akita, Ct-1, Kn−0) was set apart by the highest number of lateral roots and the total lateral root length under both N− and N+ conditions. Group 2 (Bur-0, Cvi-0, Ge-0, Ita-0, Jea) was characterized by high S and R biomasses. Group 3 (Bl-1, Blh-1, Col-0, Edi-0, Oy-0, Mh-1, Mt-0, St-0, Stw-0, Tsu-0) had plants showing the most common characteristics with the average of the whole analyzed population. Group 4 (Alc-0, Can-0, Gre-0, N13, Pyl-1, Sha) was differentiated by the lowest R biomass and lateral root number at N− and N+. Pyl-1 and N13 produced the least number of lateral roots and N13 had the lowest S biomass at N− and N+. Sakata is the only member of group 5 and is set apart by a strong stimulation by nitrate supply on S biomass and almost no lateral root at N+.

3. Discussion

3.1. Natural populations of A. thaliana show ample variation of biomass production and root morphology

The natural variation of biomass production and root architecture in response to nitrate supply was here studied in a core collection of *Arabidopsis* accessions. That collection has already provided a valuable resource for studying factors as various as water limitation (Bouchabke et al., 2008) or acclimation to cold (McKhann et al., 2008). Regarding nitrogen nutrition, natural variability was previously described for plant growth, NUE, nitrate uptake and nitrogen remobilization for seed filling (North et al., 2009; Chardon et al., 2010; Masclaux-Daubresse and Chardon, 2011; Ikram et al., 2012).
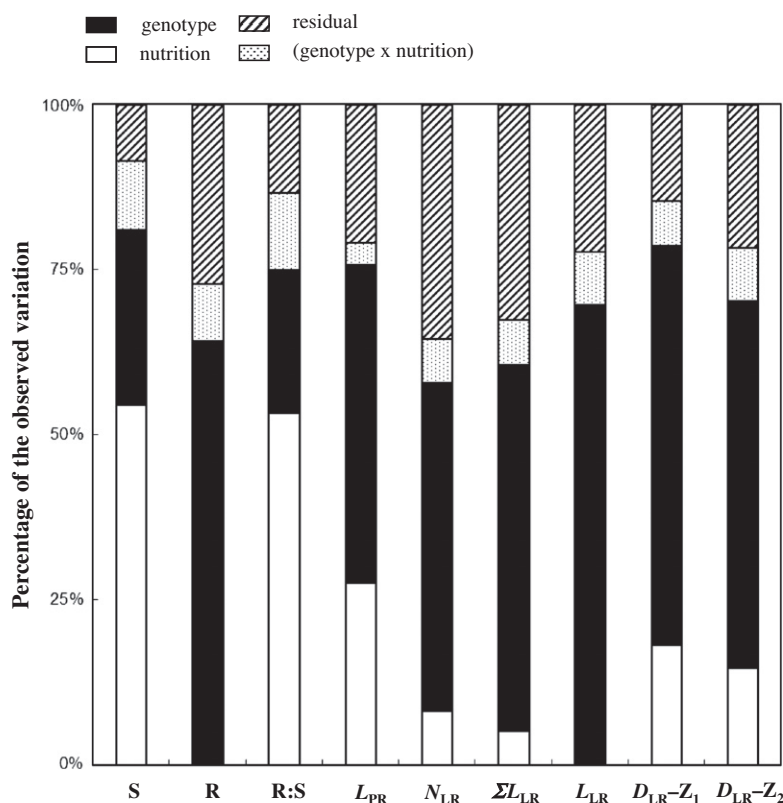
Fig. 3 – Global ANOVA of biomass and root architecture traits. Schematic representation of ANOVA of traits (as defined in Table 1) in 25 *Arabidopsis* accessions growing at 0.01 mM and 10 mM NO$_3^-$ supplies. Histograms show the significant effect due to genotype/accession, nutrition and interaction (genotype × nutrition) as a percentage of the observed variation (P < 0.05).

Earlier studies have established that nutrition treatments have major effects on biomass production and N uptake in *Arabidopsis* (Loudet et al., 2003; Chardon et al., 2010). However for some traits, such as NUE, harvest index and some root-related traits, most of observed variations were largely due to genotype effect with a (genotype × nutrition) interaction (Rauh et al., 2002; Masclaux-Daubresse and Chardon, 2011; Ikram et al., 2012), revealing that effect of nitrate nutrition on those traits is genotype dependent. Similarly, in our *in vitro* study, variance analysis showed that the nutrition treatment had a main effect on S biomass production but not on R (Fig. 3), for which the variation was mainly explained by genotype and (genotype × nutrition) effects. However, the N nutrition had a direct effect on the RSA traits, even if the genotype was the most important source of variation. The results revealed that in our conditions, the plant adjusted their root architecture in response to N limiting supply without extensively modifying their R biomass. The development of LRs was balanced by the limitation of the PR growth. It is noteworthy to emphasize that particular absence of nutritional effect on R biomass and the stimulatory effect of N+ on S biomass only. In most cases, nitrate depletion is reported to enhance the development of the root system whereas high supplies of N reported to promote the development of the shoot (Castaings et al., 2011). However, allocation of biomass between organs may also depend on developmental stage and plant size (Poorter et al., 2012). Nitrate acts not only as nutriment but also as a signal molecule (Crawford, 1995; Zhang and

Forde, 2000). Undeniably, that signal has a local and systemic morphological effect on LR elongation (Zhang et al., 1999; Ruffel et al., 2011). It is thus assumed that genetic variation of RSA observed in our experiment was due to variation in the management of the nitrate signal between the 25 accessions. Further investigations on natural allelic variation of genes involved in systemic responses, such as *High nitrogen insensitive 9* (*HIN9*) (Girin et al., 2010), will provide an exciting opportunity for unraveling a piece of the N signaling process.

3.2. Identification of attractive features to improve the nitrogen uptake efficiency

The root is the key to a new revolution in agriculture to develop crops producing high yields at lower fertilizer input (Den Herder et al., 2010; Gewin, 2010). From a fundamental point of view, the influence of nitrogen on root development is still poorly understood. Modifying root architecture is a strategy that aims at developing crops that capture nutrients more efficiently and are thus suitable for sustainable agriculture with fewer fertilizer inputs. Comparative genomics through a model-to-crop pipeline could allow the information gained in *Arabidopsis* to be exploited via Brassica crops with redesigned RSA for enhanced nutrient acquisition. Recently, Chardon et al. (2012) explored natural variation of *Arabidopsis* accessions to define ideotypes for yield and seed quality. In a similar manner, we can learn from genetic and environmental regulation of root growth and development of natural *Ara-*
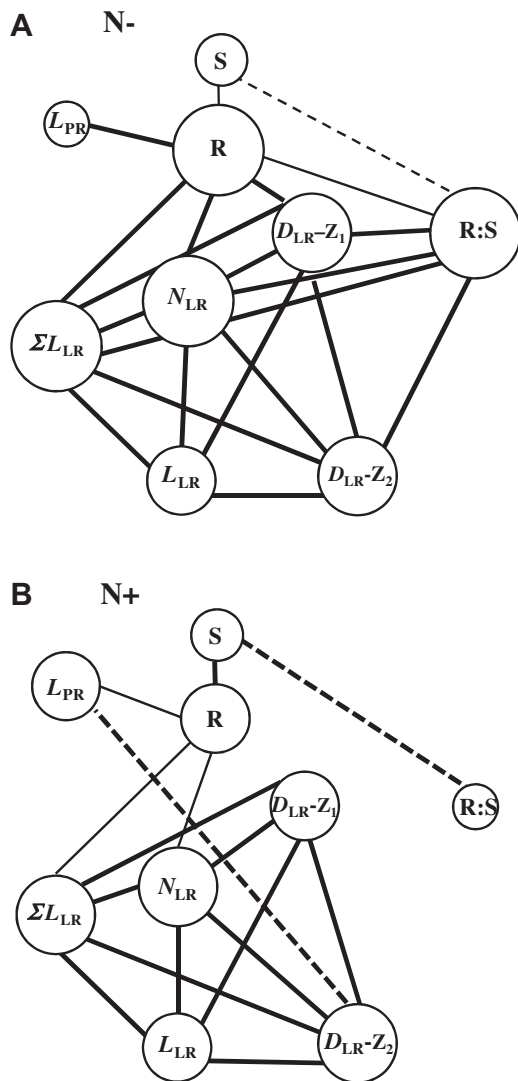
Fig. 4 – Correlations between biomass and root architecture traits. Network representation of correlations between traits defined in Table 1, at 0.01 mM (A) or 10 mM (B) NO_3^- in 25 Arabidopsis accessions. All traits correlations that were significant at $P < 0.05$ were indicated by diagonals. Solid (thin: $0 < r^2 \leqslant 0.5$; bold: $r^2 > 0.5$) and dashed (thin: $0 > r^2 \geqslant -0.5$; bold: $r^2 < -0.5$) lines represent positive and negative correlations, respectively. Size of circle indicates the number of connections starting from circle. Correlations were computed using all the means calculated for each accession (Table S3).

bidopsis populations. Class 1 plants (Akita, Ct-1, Kn-0) which produced high R biomass with a highly branched and long root system at low and moderate nitrate levels form an interesting ideotype. Some class 2 plants (Bur-0, Cvi-0) responded the strongest to the nitrate depletion by developing proportionally more LRs compared to moderate conditions. Class 4 plants, such as N13, are relatively poor performers, producing nearly the lowest root and shoot biomasses in regard to poor RSA traits at N– and N+. On the opposite end, the class 5 Sakata produces the highest shoot biomass at both concentrations but does not match our ideotypical root architecture.

That discrepancy illustrates that the integration of different NUE components (NupE, NutE) should be taken into account. Particular accessions such as Sha and Blh-1 do not respond to nitrate stimulatory effect and produce equal shoot biomass at N– and N+. They can be interesting case studies because they ensure a constant aerial biomass production regardless of the amount of nitrate supplied. A stimulation of PR length by nitrate is observed for Alc-0, Cvi-0 or Sakata. Accessions that have high lateral root number and sum of the lateral root length at N– are Bur-0, Edi-0 or Oy-0. Kn-0 and Ct-1 maintain a relatively high lateral root number at N– and N+. Other accessions like Pyl-1 and N13 have very few or no visible lateral roots whatever the nitrate levels. Nonetheless, it is difficult to establish if the RSA of those mentioned accessions actually reflect an adaptation to a particular soil environment where they have been collected. Soil structure and mineral analyses of the collection sites are generally lacking in seeds stock databases. Occasionally, attempts were made, for example, to correlate root skewing behavior of Cvi-0 accession as observed in vitro with growth over long spans of rocks in the wild (Vaughn and Masson, 2011).

4. Conclusion

After having identified Arabidopsis accessions with contrasted phenotypes, we will pursue the analysis of quantitative traits for root morphology. The identification of key developmental genes and those involved in a specific nitrogen pathway may represent a great potential for improving root characteristics. A first milestone would be to validate the observations made in vitro and to verify if genes identified at an early developmental stage are also actors in RSA shaping over time and in heterogeneous soil conditions.

5. Material and methods

5.1. Plant growth conditions

A. thaliana accessions were obtained from INRA Versailles Genomic Resource center; seeds were surface sterilized with ethanol 70% (v/v) during 10 min and in a 20% (v/v) HClO solution during 5 min. They were plated on 1x Murashige and Skoog medium modified with nitrate (KNO_3) as the sole source of nitrogen concentration (Hermans et al., 2010, 2011), 1% sucrose, 0.8% agar and pH = 5.7. Seeds were then stratified at 4 °C for 2 days in the dark, and then incubated vertically in a culture chamber at a temperature of 22 °C and a day light regime of 16 h (75 μmol photons $m^{-2} s^{-1}$)/8 h darkness. Six days after germination on moderate N+(10 mM KNO_3) nitrate medium, seedlings (∼2 cm PR length) were transferred to the same medium or low N– (0.01 mM KNO_3) nitrate medium. KCl (9.99 mM) was added to prevent K^+ depletion in the low nitrate medium. After 7 additional days, the root systems were scanned at 400 dpi and seedlings harvested.

5.2. Quantitative analysis of root system architecture

Root system architecture parameters such as the primary root length, the number and length of lateral roots, which
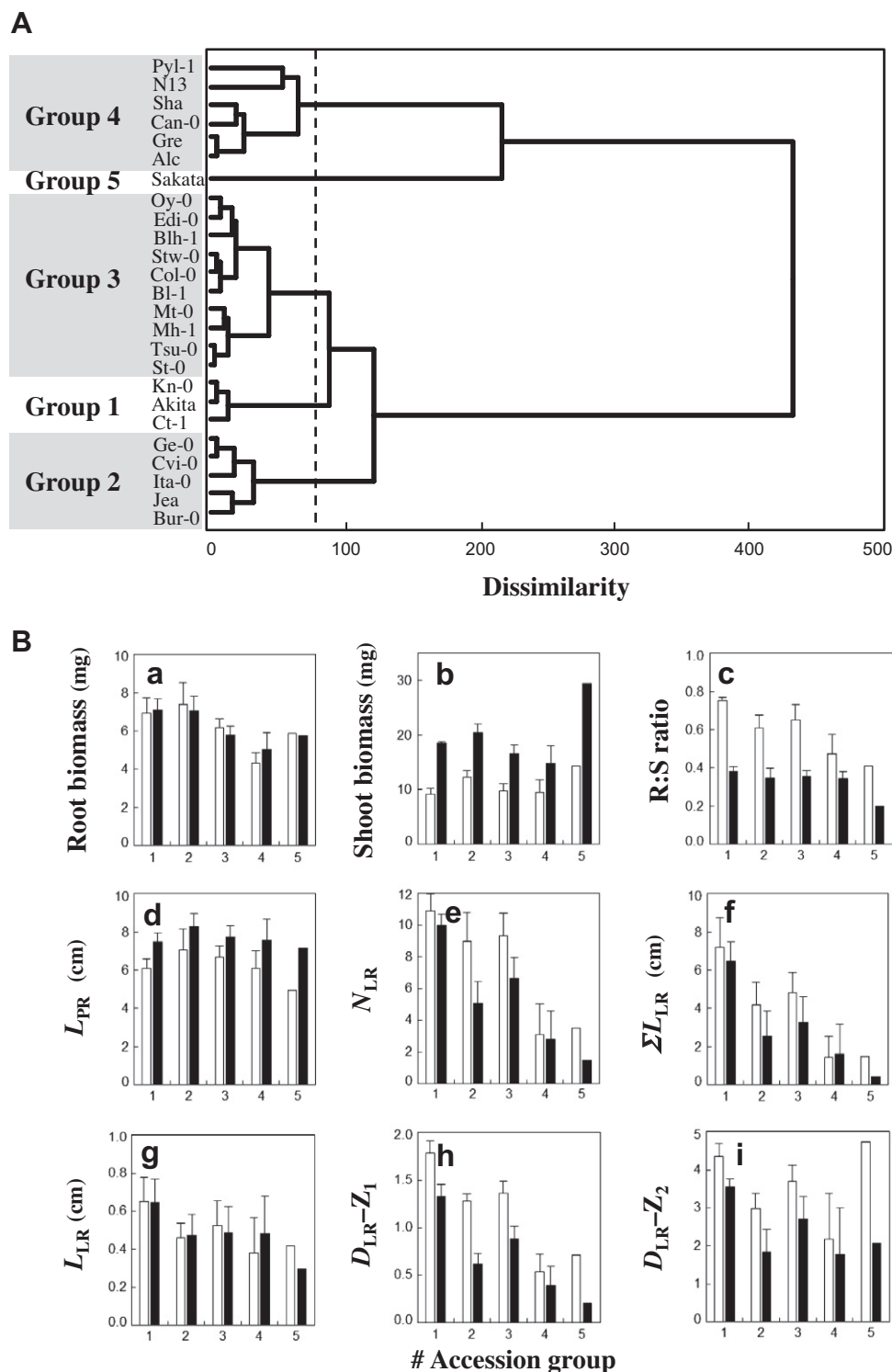
Fig. 5 – Ascendant hierarchical classification of 25 *Arabidopsis* accessions. (A) Dendogram of the classification of the whole accessions set was performed by computing the data obtained from plants grown under 0.01 mM (N−) and 10 mM (N+) NO_3^- supplies. Five groups were identified. (B) Performance of five groups of *Arabidopsis* accessions for traits as defined in Table 1: Biomass traits: (a) production of fresh root biomass, (b) production of fresh shoot biomass and (c) ratio of the root biomass to the shoot biomass (R:S); root architecture traits: (d) length of primary root (L_{PR}), (e) number of lateral roots (>1 mm) (N_{LR}), (f) sum of the length of lateral roots (ΣL_{LR}), (g) mean lateral root length (L_{LR}), (h) lateral root density calculated per primary root length ($D_{LR}-Z_1$), (i) lateral root density calculated per root portion between the first and last visibly emerged lateral root ($D_{LR}-Z_2$) Black columns: 0.01 mM; white columns: 10 mM NO_3^-. The standard deviations are indicated on the graphics.

were emerged (>1 mm) from the primary root, were quantified with the EZ-Rhizo® program (Armengaud et al., 2009).

5.3. Statistical treatment

Phenotypic correlations were calculated for all combinations of traits in each N environment by using XLSTAT software (http://www.xlstat.com). All significant trait pair correlations were visualized by using software Cytoscape (Shannon et al., 2003) (http://www.cytoscape.org/). ANOVA analysis was carried out using XLSTAT. Further genotypes comparisons were realized by using XLSTAT ANOVA comparisons according to the Fisher test. Hierarchical classification of accessions was carried out by using XLSTAT software according to the Ward's method.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mod.2012.05.010.

R E F E R E N C E S

Armengaud, P., Zambaux, K., Hills, A., Sulpice, R., Pattison, R.J., Blatt, M.R., Amtmann, A., 2009. EZ-Rhizo: integrated software for fast and accurate measurement of root system architecture. Plant J. 57, 945–956.

Bouchabke, O., Chang, F.Q., Simon, M., Voisin, R., Pelletier, G., Durand-Tardif, M., 2008. Natural variation in Arabidopsis thaliana as a tool for highlighting differential drought responses. PLoS ONE 3, e1705.

Castaings, L., Marchive, C., Meyer, C., Krapp, A., 2011. Nitrogen signaling in Arabidopsis: how to obtain insights into a complex signaling network. J. Exp. Bot. 62, 1391–1397.

Chardon, F., Barthélémy, J., Daniel-Vedele, F., Masclaux-Daubresse, C., 2010. Natural variation of nitrate uptake and nitrogen use efficiency in Arabidopsis thaliana cultivated with limiting and ample nitrogen supply. J. Exp. Bot. 61, 2293–2302.

Chardon, F., Noël, V., Masclaux-Daubresse, C., 2012. Exploring NUE in crops and in Arabidopsis ideotypes to improve yield and seed quality. J. Exp. Bot. 63, 3401–3412.

Chevalier, F., Pata, M., Nacry, P., Doumas, P., Rossignol, M., 2003. Effects of phosphate availability on the root system architecture: large-scale analysis of the natural variation between Arabidopsis accessions. Plant Cell Environ. 26, 1839–1850.

Chevalier, F., Rossignol, M., 2011. Proteomic analysis of Arabidopsis thaliana ecotypes with contrasted root architecture, in response to phosphate efficiency. J. Plant Physiol. 168, 1885–1890.

Crawford, N.M., 1995. Nitrate: nutrient and signal for plant growth. Plant Cell. 7, 859–868.

Den Herder, G., Van Isterdael, G., Beeckman, T., De Smet, I., 2010. The roots of a new green revolution. Trends Plant Sci. 15, 600–607.

Donner, S.D., Kucharik, C.J., 2008. Corn-based ethanol production compromises goal of reducing nitrogen export by the Mississippi River. Proc. Natl. Acad. Sci. USA 105, 4513–4518.

Edgerton, M.D., 2009. Increasing crop productivity to meet global needs for feed, food, and fuel. Plant Physiol. 149, 7–13.

Frink, C.R., Waggoner, P.E., Ausubel, J.H., 1999. Nitrogen fertilizer: retrospect and prospect. Proc. Natl. Acad. Sci. USA 96, 1175–1180.

Gewin, V., 2010. An underground revolution. Nature 466, 552–553.

Giehl, R.F.H., Lima, J.E., von Wirén, N., 2012. Localized iron supply triggers lateral root elongation in Arabidopsis by altering the AUX1-mediated auxin distribution. Plant Cell 24, 33–49.

Gifford, M.L., Dean, A., Gutierrez, R.A., Coruzzi, G.M., Birnbaum, K.D., 2008. Cell-specific nitrogen responses mediate developmental plasticity. Proc. Natl. Acad. Sci. USA 105, 803–808.

Girin, T., El-Kafafi, E.-S., Widiez, T., Erban, A., Hubberten, H.-M., Kopka, J., Hoefgen, R., Gojon, A., Lepetit, M., 2010. Identification of Arabidopsis mutants impaired in the systemic regulation of root nitrate uptake by the nitrogen status of the plant. Plant Physiol. 153, 1250–1260.

Good, A.G., Shrawat, A.K., Muench, D.G., 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends Plant Sci. 9, 597–605.

Hermans, C., Hammond, J.P., White, P.J., Verbruggen, N., 2006. How do deficiencies of essential mineral elements alter biomass allocation? Trends Plant Sci. 11, 610–617.

Hermans, C., Porco, S., Verbruggen, N., Bush, D.R., 2010. Chitinase-like protein CTL1 plays a role in altering root system architecture in response to multiple environmental conditions. Plant Physiol. 152, 904–917.

Hermans, C., Porco, S., Vandenbussche, F., Gille, S., De Pessemier, J., Van Der Straeten, D., Verbuggen, N., Bush, D.R., 2011. Dissecting the role of CTL1 in nitrate-dependent changes in root architecture. Plant Physiol. 157, 1313–1326.

Ikram, S., Bedu, M., Daniel-Vedele, F., Chaillou, S., Chardon, F., 2012. Natural variation of Arabidopsis response to nitrogen availability. J. Exp. Bot. 63, 91–105.

Kant, S., Bi, Y.M., Rothstein, S.J., 2011. Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. J. Exp. Bot. 62, 1499–1509.

Krouk, G., Lacombe, B., Bielach, A., Perrine-Walker, F., Malinska, K., Mounier, E., Hoyerova, K., Tillard, P., Leon, S., Ljung, K., Zazimalova, E., Benkova, E., Nacry, P., Gojon, A., 2010. Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. Develop. Cell 18, 927–937.

Krouk, G., Ruffel, S., Gutiérrez, R.A., Gojon, A., Crawford, N.M., Coruzzi, G.M., Lacombe, B., 2011. A framework integrating plant growth with hormones and nutrients. Trends Plant Sci. 16, 178–182.

Linkohr, B.I., Williamson, L.C., Fitter, A.H., Leyser, H.M.O., 2002. Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. Plant J. 29, 751–760.

Little, D.Y., Rao, H., Oliva, S., Daniel-Vedele, F., Krapp, A., Malamy, J.E., 2005. The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues. Proc. Natl. Acad. Sci. USA 102, 13693–13698.

Loudet, O., Chaillou, S., Merigout, P., Talbotec, J., Daniel-Vedele, F., 2003. Quantitative trait loci analysis of nitrogen use efficiency in Arabidopsis. Plant Physiol. 131, 345–358.

López-Bucio, J., Cruz-Ramirez, A., Herrera-Estrella, L., 2003. The role of nutrient availability in regulating root architecture. Curr. Opin. Plant Biol. 6, 280–287.

Masclaux-Daubresse, C., Chardon, F., 2011. Exploring nitrogen remobilization for seed filling using natural variation in Arabidopsis thaliana. J. Exp. Bot. 62, 2131–2142.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann. Bot. 105, 1141–1157.

McKhann, H., Camilleri, C., Berard, A., Bataillon, T., David, J., Reboud, X., Le Corre, V., Caloustian, C., Gut, I., Brunel, D., 2004. Nested core collections maximizing genetic diversity in *Arabidopsis thaliana*. Plant J. 38, 193–202.

McKhann, H., Gery, C., Berard, A., Leveque, S., Zuther, E., Hincha, D., De Mita, S., Brunel, D., Teoule, E., 2008. Natural variation in CBF gene sequence, gene expression and freezing tolerance in the Versailles core collection of *Arabidopsis thaliana*. BMC Plant Biol. 8, 105.

Miller, A., Smith, S., Fan, X., Shen, Q., 2007. Expression and functional analysis of rice NRT2 nitrate transporters. Comp. Biochem. Physiol. 146, S241.

North, K.A., Ehlting, B., Koprivova, A., Rennenberg, H., Kopriva, S., 2009. Natural variation in *Arabidopsis* adaptation to growth at low nitrogen conditions. Plant Physiol. Biochem. 47, 912–918.

Péret, B., De Rybel, B., Casimiro, I., Benkova, E., Swarup, R., Laplaze, L., Beeckman, T., Bennett, M.J., 2009. *Arabidopsis* lateral root development: an emerging story. Trends Plant Sci. 14, 399–408.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193, 30–50.

Rauh, B.L., Basten, C., Buckler, E.S., 2002. Quantitative trait loci analysis of growth response to varying nitrogen sources in *Arabidopsis thaliana*. Theor. Appl. Genet. 104, 743–750.

Remans, T., Nacry, P., Pervent, M., Filleur, S., Diatloff, E., Mounier, E., Tillard, P., Forde, B.G., Gojon, A., 2006. The *Arabidopsis* NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc. Natl. Acad. Sci. USA 103, 19206–19211.

Robinson, D., 2004. Scaling the depths: below-ground allocation in plants, forests and biomes. Funct. Ecol. 18, 290–295.

Rubio-Somoza, I., Weigel, D., 2011. Micro RNA networks and development plasticity in plants. Trends Plant Sci. 16, 258–264.

Ruffel, S., Krouk, G., Ristova, D., Shasha, D., Birnbaum, K.D., Coruzzi, G.M., 2011. Nitrogen economics of root foraging: transitive closure of the nitrate–cytokinin relay and distinct systemic signaling for N supply vs. demand. Proc. Natl. Acad. Sci. USA 108, 18524–18529.

Shannon, P., Markiel, A., Ozier, O., Baliga, N., Wang, J., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504.

Vaughn, L.M., Masson, P.H., 2011. A QTL study for regions contributing to *Arabidopsis thaliana* root skewing on tilted surfaces. G3: Genes, Genomes, Genetics 1, 105–115.

Vidal, E.A., Tamayo, K.P., Gutiérrez, R.A., 2010. Gene networks for nitrogen sensing, signaling, and response in *Arabidopsis thaliana*. Wiley Interdiscip. Rev. Syst. Biol. Med. 2, 683–693.

Zhang, H., Forde, B.G., 1998. An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. Science 279, 407–409.

Zhang, H., Jennings, A., Barlow, P.W., Forde, B.G., 1999. Dual pathways for regulation of root branching by nitrate. Proc. Natl. Acad. Sci. USA 96, 6529–6534.

Zhang, H., Forde, B.G., 2000. Regulation of *Arabidopsis* root development by nitrate availability. J. Exp. Bot. 51, 51–59.

Zolla, G., Heimer, Y.M., Barak, S., 2010. Mild salinity stimulates a stress-induced morphogenic response in *Arabidopsis thaliana* roots. J. Exp. Bot. 61, 211–224.