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ORIGINAL PAPER

A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency

A. Laperche · F. Devienne-Barret · O. Maury · J. Le Gouis · B. Ney

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Abstract Breeding new varieties adapted to low-input agricultural practices is of particular interest in light of current economical and environmental concerns. Improving nitrogen (N) uptake and N utilization efficiency (NUE) are two ways of producing varieties tolerant to low N input. To offer new possibilities to breeders, it is necessary to acquire more knowledge about these two processes. Knowing C and N metabolisms are linked and knowing N uptake is partly explained by root characteristics, we carried out a QTL analysis for traits associated with N uptake and NUE by using both a conceptual model of C/N plant functioning and a root architecture description. A total of 120 lines were selected according to their genotype among 241 doubled haploids derived from two varieties, one N stress tolerant and the other N stress sensitive. They were grown in hydroponic rhizotrons under N-limited nutritional conditions. Initial conditions varied among genotypes; therefore, total root length on day 1 was used to correct traits. Heritabilities ranged from 13 to 84%. Thirty-two QTL were located: 6 associated with root architecture (on chromosomes 4B, 5A, 5D and 7B), 6 associated with model efficiencies (1B, 2B, 6A, 6B, 7A, 7B and 7D) and 20 associated with state variables (1A, 1B, 2B, 4B, 5A, 5B and 6B). The effects of the dwarfing gene *Rht-B1* on root traits are discussed, as well as the features of a conceptual plant functioning model, as a useful tool to assess pertinent traits for QTL detection. It is suggested that further studies that couple QTL with a functioning model and a root architecture description could serve in the search for ideotypes.

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A. Laperche (✉) · F. Devienne-Barret · O. Maury · B. Ney
UMR INRA-INA PG Environnement et Grandes Cultures,
78850 Thiverval Grignon, France
e-mail: Anne.Laperche@agrocampus-rennes.fr

J. Le Gouis · A. Laperche
INRA, Unité de Génétique et d'Amélioration des Plantes,
BP 50136, 80203 Péronne Cedex, France

Abbreviations

ADM	Aerial dry matter
DH	Doubled haploids
h^2	Heritability
LA	Leaf area
LG	Linkage group
LRL	Lateral root length
LRN	Lateral root number
N	Nitrogen
Nc_{AP}	Aerial parts nitrogen content
Nc_R	Root nitrogen content
NTOT	Amount of total nitrogen
NUR	Nitrogen-specific uptake rate
PAR	Photosynthetically active radiation
PRL	Primary root length
QTL	Quantitative trait locus
RDM	Root dry matter
SRL	Specific root length
SSR	Simple sequence repeats
TDM	Total dry matter
TRL	Total root length
RUE	Radiation use efficiency

Introduction

Throughout Europe, low crop prices and growing environmental concerns are pushing farmers to lower their inputs. Profit margin can be maintained, even if yield is lower, when adapted varieties are combined with low-input practices (such as decreases in seed density, nitrogen and pesticides) (Rolland et al. 2004). These varieties need to be both disease resistant and N stress tolerant. Much effort has been devoted to disease resistance (Rolland et al. 2004). Few genetic studies have been devoted to N tolerance and have involved only barley (Mickelson et al. 2003), maize (Agrama et al. 1999; Bertin and Gallais 2001; Hirel et al. 2001; Gallais and Hirel 2004), rice (Lian et al. 2005) and *Arabidopsis thaliana* (Loudet et al. 2003a, b).

There are difficulties inherent in screening traits linked to N-limited nutritional adaptation such as root characteristics, N uptake rate and N utilization efficiency (NUE), information hardly accessible in plants grown in the field. Hydroponic rhizotron conditions provide needed access. Indeed, there are multiple advantages to this method. First, it is possible to evaluate a large number of genotypes in a limited space. Second, it is possible to partially control the environment. Third, radically destructive uprooting is replaced by direct non-destructive observations. Even if hydroponic characterization is only possible for the early stages of plant development, this technique has shown to be reliable for evidencing genotypic variability correlated to the variability existing in the field at maturity (Price et al. 1997). It also allowed us to locate the QTL involved in genetic control of traits. These QTL can be used in marker-assisted selection (MAS) programs that offer breeders new possibilities of easily screening the large number of genotypes such a breeding approach requires.

Another difficulty in QTL detection lies in first pinpointing the traits to seek out. Knowing N and carbon metabolisms are linked, we were interested in investigating traits that take this link into account. Therefore, we propose, in this paper, a general and simple conceptual model of the carbon and N relationship as the framework for QTL detection. This framework combines state variables (biomasses or total N accumulated) and exchange surfaces (leaf area for carbon as well as root length and architecture for N), as well as efficiencies (radiation conversion into biomass for carbon and N-specific uptake). The genetic variations of each of these variables were analyzed, using a hydroponic screen, in a doubled haploid subpopulation of winter wheat derived from the cross between two varieties, Arche and Récital, chosen for their contrasting reactions to N deficiency conditions (Le Gouis et al. 2000) and their differential root protein expression pattern under N deficiency (Bahrman et al. 2005). To remain representative of the whole 241-lines population and to optimize QTL detection, a subpopulation of 120 genotypes was chosen based on genotype data to perform selective phenotyping (Vales et al. 2005).

Materials and methods

Genetic mapping

The ARE population (Laperche et al. 2006) is composed of 241 DH lines derived from the cross between the cultivars Arche and Récital. The 241 DH lines were genotyped for 183 wheat SSR markers, 2 glutenin markers (*GLU-1A*, *GLU-1D)*, the specific markers of the storage protein transcription factor *SPA* (Guillaumie et al. 2004), the dwarfing gene *Rht-B1* (Ellis et al. 2002) and the glutamate synthetase gene *Fdgogat-D1* (Boisson et al. 2005); phenotypic observations were also carried out for awning and gibberelin response. The SSR were supplied by the collections of IPK Gatersleben (gwm, gdm), Agrogene-Consortium (wmc), ''Génoplante'' (gpw), INRA Clermont-Ferrand (cf) and USDA-ARS Beltsville Agriculture Research Station (barc). The mapping was carried out within the framework of a French ''Génoplante'' program and the population genotyping was performed at INRA Clermont-Ferrand, INRA Rennes and Biogemma Aubière.

The linkage map was developed using Mapmaker version 3.0 (Lander et al. 1987) with a minimum LOD score of 3.0 and a recombination fraction of 0.35 assuming Haldane mapping function (cM). Using the convert command of the software PLABQTL (Utz and Melchinger 1996), any missing marker value was replaced by its expected marker type, depending on the marker type of its flanking markers and the map distances of those markers. The linkage map covered 2,614 cM and markers were divided into 30 linkage groups (LG). When two LG corresponded to the same chromosome, they were labeled with the chromosome name plus a number: for example, 5A1 and 5A2 represent two LG that corresponded to 5A chromosome.

Plant material

The experiment was carried out on a subpopulation of 120 doubled haploid (DH) lines, sampled from the

ARE population that was derived from the cross between parents chosen for their contrasting reactions to nitrogen deficiency: Arche is N stress tolerant and Recital is N stress sensitive (Le Gouis et al. 2000). The subpopulation was generated to perform a selective phenotyping method (Vales et al. 2005): the 120 DH lines were chosen according to their genotypic information, using MapPop software (Vision et al. 2000). This method relied in selecting the genotypes bearing complementary recombinational breakpoints.

Conceptual framework

A simple conceptual model, based on biologically meaningful state variables and their relationships to each other, called efficiencies, was used as a framework for analysis (Fig. 1). The final aim was to assess whether or not the efficiencies depended on genotype. In this model, it is given that leaf area is the carbon source and that the plant elaborates total dry matter (TDM) according to radiation use efficiency (RUE). To determine root dry matter (RDM), TDM is multiplied by the ratio of RDM over TDM. RDM is converted into root length (TRL) according to specific root length (TRL/RDM), and total root length is taken to be the nitrate uptake surface. Total amount of N (NTOT) is calculated according to a N-specific uptake rate (which is the N uptake rate per cm of root length). To complete the cycle, NTOT is converted into leaf area (LA) by multiplying NTOT by the ratio of LA over NTOT, termed N conversion into leaf area efficiency. For our purposes, the model time-wise was 1 day.

Experimental method: the rhizotron

Wheat lines were grown in a growth chamber, in rhizotrons (Pagès 1992). Each rhizotron was 60 cm wide and 1 m high. Vertically, the typical rhizotron is assembled with a PVC back sheet, an ''Aquanap®'' sheet, a sheet made of fine meshed cloth (bolt sheet), a transparent polycarbonate sheet and a sheet of transparent tracing paper. All sheets are kept together with clamps (Fig. 2). The plants grew in 2D conditions between the polycarbonate sheet and the bolt sheet. Aquanap® (Puteaux SA, 78344 Les Clayes-sous-Bois, France) is made of shredded finely recycled cloth and pressed into a 5 mm thick layer and is able to retain ten times its weight of water. It is the means by which the nutrient solution is supplied to the plants. Micro-irrigation, on the upper edge of the Aquanap®, assures a constant supply. The sheet made of fine meshed cloth prevents root development within the Aquanap®. The sheet of transparent tracing paper allows for the tracing of the root system. In our case, a different color was used every time a measurement was made. Another white opaque plastic sheet was placed over the tracing paper to keep roots in darkness and to prevent any temperature increase. Twenty-two rhizotrons were placed in the growth chamber. Twenty lines as well as the two parents were grown at the same time. To test all 120 lines, six replications were run.

All grains were vernalized in darkness at 4°C for 4 weeks. Three seedlings per genotype were planted in a given rhizotron 10 cm apart. Plants were grown for

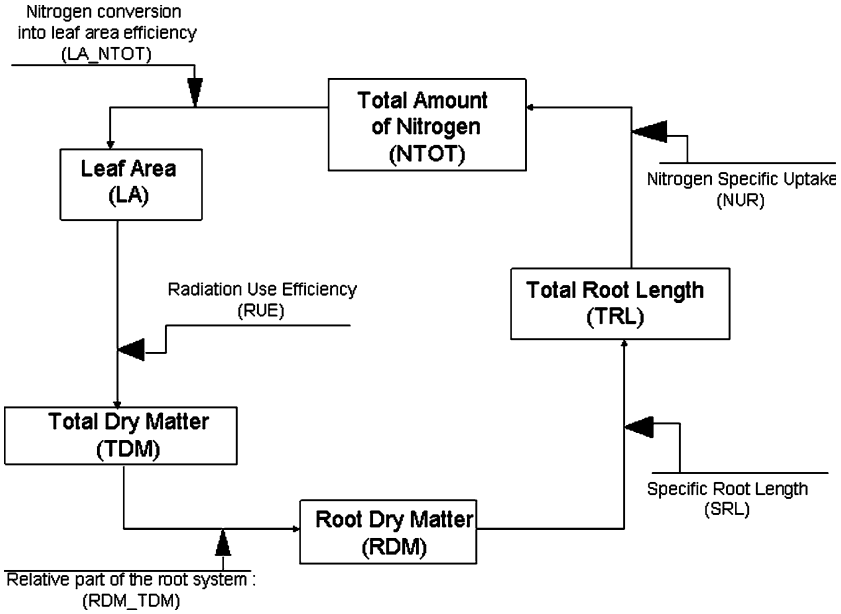

Fig. 1 Schematic representation of the simple conceptual model of plant functioning. State variables are represented *within rectangles* and efficiencies are represented by *arrows*. We considered leaf area (LA) as a carbon source. The carbon is then distributed to the root according to the relative part of the root biomass (RDM_TDM). The root biomass is involved in the elaboration of root length (according to specific root length: SRL) that we consider as a nitrate uptake surface. The total root length pilots the nitrogen entrance into the plant. Total nitrogen allows the elaboration of leaf area

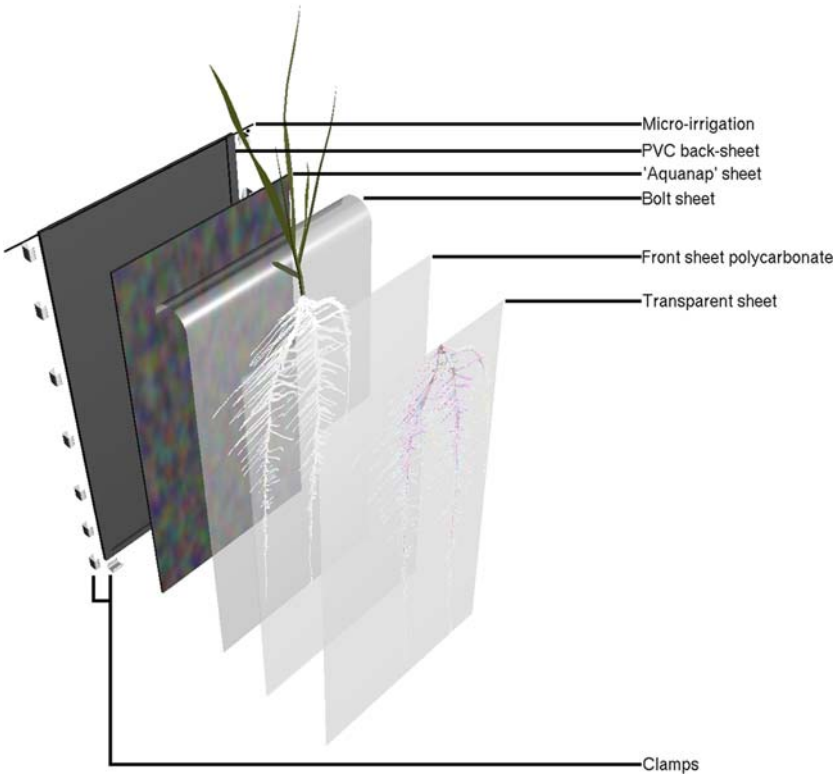

Fig. 2 Schematic representation of a rhizotron. Plants grow between a transparent polycarbonate sheet and a sheet made of fine meshed cloth (bolt sheet). They access the nutrient solution contained in the Aquanap® through the sheet made of fine meshed cloth. Nutrient solution is supplied to the Aquanap® sheet by micro-irrigation. Tracing paper placed on the transparent polycarbonate sheet allows for the tracing of the root system

22 days. The photoperiod was 16 h day and 8 h night and photosynthetically active radiation (PAR) was on average 330 μmol/m^2/s. Lighting was assured by two ceiling light fixtures, each one containing nine thermally regulated sodium light bulbs. The temperature was maintained at 20°C during the day and at 18°C at night. The average humidity was 46%. PAR was recorded for each plant. Six thermocouples were placed in the chamber to keep track of temperature at all times. Micro-irrigation supplied plants a nutrient solution with a nitrate concentration of 0.5 mM. The solution was composed of tap water (31 mg NO_3^-/l) to which was added 0.70 mM of KH_2PO_4, 0.90 mM of $Ca(H_2PO_4)_2{\cdot}H_2O$, 0.80 mM of $MgSO_4{\cdot}7H_2O$, 0.20 mM of NaCl. Oligo-elements were added to the solution as follows (concentration in mg/l): H_3BO_3 (1.5), $MnSO_4{\cdot}H_2O$ (2.38), $ZnSO_4{\cdot}7H_2O$ (1.78), $(NH4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.053) and 0.04 g/l of a solution of EDDHA (iron chelate) at a concentration of 6%. Plants were irrigated once an hour during the day and once every 2 h at night. Each irrigation lasted 1 min. On average, 317.5 l of solution was supplied to the 22 rhizotrons over the 22 days. We assessed the mean aerial nitrogen content of each genotype to check that the environmental conditions were N-limiting. Aerial nitrogen content ranged from 1.01 to 5.22% and was on average of 2.97% (standard deviation of 0.73%). Based on results obtained in a previous experiment, we considered that below a nitrogen content of 4.5% plants are under N stress conditions.

Measurements and calculations

Root number and root lengths

For every run, measurements were taken every day during the first 5 days of the first week and then twice a week for the remaining 2 weeks. A final measurement was taken on the last day. Root growth was recorded for each rhizotron on the tracing paper using different colors. We used GIMP software (http://www.gimp.org) to separate the root systems of the three plants grown in the same rhizotron, therefore providing a clear drawing of each root system. The WINRHIZO PRO software (WinRhizo, Regent Instruments, Canada) was then used to estimate primary and lateral root length according to color.

For each plant p of genotype i at day d, lateral root number ($LRN_{d,i,p}$) as well as primary ($PRL_{d,i,p}$) and lateral root length ($LRL_{d,i,p}$) were estimated. Total root length $TRL_{d,i,p}$ was calculated as:

$$TRL_{d,i,p} = PRL_{d,i,p} + LRL_{d,i,p}. \quad (1)$$

Then we calculated variables that characterized the root system organization:

- The number of lateral roots per cm of primary root $LRN\_PRL_{d,i,p}$:

$$LRN\_PRL_{d,i,p} = \frac{LRN_{d,i,p}}{PRL_{d,i,p}}. \quad (2)$$

- The ratio between lateral root length and primary root length $LRL\_PRL_{d,i,p}$:

$$LRL\_PRL_{d,i,p} = \frac{LRL_{d,i,p}}{PRL_{d,i,p}}. \quad (3)$$

- The ratio between lateral root length and total root length $LRL\_TRL_{d,i,p}$:

$$LRL\_TRL_{d,i,p} = \frac{LRL_{d,i,p}}{TRL_{d,i,p}}. \quad (4)$$

- The mean length of a lateral root $LRL\_LRN_{d,i,p}$:

$$LRL\_LRN_{d,i,p} = \frac{LRL_{d,i,p}}{LRN_{d,i,p}}. \quad (5)$$

Dry biomass, leaf area and total nitrogen amount at 22 days

Each plant was harvested on day 22. Roots and aerial parts were separated. Total leaf area $LA_{22,i,p}$ for plant p of genotype i was measured by scanning all the leaves of a given plant. Roots and aerial parts were then separately oven-dried for 48 h at 80°C and finally weighed to obtain root dry matter ($RDM_{22,i,p}$) and aerial dry matter

($ADM_{22,i,p}$). Root dry matter and aerial dry matter were ground into a fine powder, 6–9 mg of each sample aliquoted to measure N content using the Dumas (1831) method. Total N amount $NTOT_{22,i,p}$ was then calculated as

$$NTOT_{22,i,p} = Nc_{AP} \times ADM_{22,i,p} + Nc_R \times RDM_{22,i,p}, \quad (6)$$

where Nc_{AP} and Nc_R are, respectively, the measured N contents of aerial parts and roots.

Efficiencies calculations

For each plant, relative root biomass $(RDM\_TDM)_{22,i,p}$ was calculated as

$$(RDM\_TDM)_{22,i,p} = \frac{RDM_{22,i,p}}{TDM_{22,i,p}}. \quad (7)$$

We assumed that as for many C_3 species there is a strong relationship between total nitrogen accumulation and leaf area developed, we would calculate the following efficiency:

$$(LA\_NTOT)_{22,i,p} = \frac{LA_{22,i,p}}{NTOT_{22,i,p}}. \quad (8)$$

The specific root length $SRL_{22,i,p}$ was determined using the following equation:

$$SRL_{22,i,p} = \frac{TRL_{22,i,p}}{RDM_{22,i,p}}. \quad (9)$$

We took specific nitrogen uptake rate $NUR_{i,p}$ (g of N by cm of root) to be constant over time and specific to each genotype i and each plant p. $NUR_{i,p}$ was calculated as

$$NUR_{i,p} = \frac{NTOT_{22,i,p}}{\sum_{d=1}^{22} TRL_{d,i,p}}. \quad (10)$$

Missing points were estimated by intrapolations.

Radiation use efficiency $RUE_{i,p}$ was calculated as follows:

$$RUE_{i,p} = \frac{TDM_{22,i,p}}{\sum_{d=1}^{22} (LA_{d,i,p} \times PAR_{i,p})}. \quad (11)$$

Given the experimental conditions, we assumed that the total leaf area intercepted the radiation regardless of the leaf angle. Therefore, we estimated the intercepted PAR as the product of $LA_{d,i,p}$ by $PAR_{i,p}$. $LA_{d,i,p}$ was calculated as follows:

$$LA_{d,i,p} = NUR_{i,p} \times TRL_{d,i,p} \times LA\_NTOT_{22,i,p}, \quad (12)$$

assuming the $(LA\_NTOT)_{d,i,p}$ was constant over the 22 days.

For our purposes, we distinguished three types of traits: first of all integrative trait that regroups the model state variables (LA, NTOT, TDM, RDM, TRL) and ADM; second, the model efficiencies (LA_NTOT, RUE, RDM_TDM, SRL as well as NUR) and finally root architecture traits (LRL, PRL, LRN, LRL_PRL, LRL_TRL, LRL_LRN and LRN_PRL).

Statistical analysis

All statistical analyses were carried out using SAS statistical package (SAS Institute Inc. 1999). The first variance analysis tested the genotypic effect on the different traits. Each trait was calculated as the mean value of the three plants representing the given line in the given rhizotron. The mean value for each parent was calculated for each run as the mean value of the three parent plants in a given rhizotron. The statistical model given below was used following the GLM procedure of SAS:

$$Y_{i,j} = \mu + g_i + t_j + e_{i,j}, \quad (13)$$

where $Y_{i,j}$ is the value of the trait for genotype i in run j, μ the general mean, g_i the genotypic effect, t_j the ''run'' effect and $e_{i,j}$ the residual. The presence of the two parents in each run allowed testing for a ''run'' effect. To assess the heritability, the same statistical model was used following the proc MIXED, taking the genotypic effect to be random. Heritability was defined for each trait as a ratio between genotypic (σ_g^2) and residual (σ_e^2) variances:

$$h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2). \quad (14)$$

To test the influence of the initial conditions, we performed a covariance analysis. The initial conditions were represented by the total root length on the first day ($TRL_{1,i,j}$). The covariance model was the following:

$$Y_{i,j} = \mu + TRL_{1,i,j} + g_i + t_j + e_{i,j}, \quad (15)$$

with $Y_{i,j}$ the value of the given trait for genotype i in run j, μ the general mean, $TRL_{1,i,j}$ the total root length on day 1 for genotype i in run j, g_i the genotypic effect, t_j the run effect and $e_{i,j}$ the residual.

This model was also used to assess heritability as in Eq. 14.

Because our population was in segregation for the dwarfing gene *Rht-B1* (located on 4B chromosome), awnedness (phenotypic marker placed on 5A chromosome), vernalization (*Vrn-A1*, 5A) and photoperiod sensitivity (*Ppd-D1*, 2D), we tested the effect of these loci on our variables using the following model:

$$Y_{i,j} = \mu + TRL_{1,i,j} + gene_i + g_i + t_j + e_{i,j}, \quad (16)$$

with $Y_{i,j}$ the value of the given trait, μ the general mean, $TRL_{1,i,j}$ the total root length on day 1 for genotype i in run j, $gene_i$ the allele of genotype i at the closest marker of the gene, g_i the genotypic effect, t_j the ''run'' effect and $e_{i,j}$ the residual. This calculation was carried out four times with $gene_i$ as either *Rht-B1*, *Ppd-D1*, *B1*, or *Vrn-A1*.

For QTL detection, we used the traits corrected for the effect of the initial conditions by using the following model and taking the corrected trait to be the residual of the model:

$$Y_{i,j} = \mu + TRL_{1,i,j} + t_{i,j} + e_{i,j}. \quad (17)$$

QTL detection

Each trait and parameter was estimated for each plant and the mean of each line was then considered for QTL detection. QTL analyses were carried out using the Unix version of QTL cartographer 1.17d (Basten et al. 1994, 2002). Model 6 was used to carry out composite interval mapping (CIM). The maximum cofactor number involved in model 6 was set at 5, and the window size was 10 cM. We used the 'experiment-wise' threshold defined at the 10% error level. It was estimated from the 1,000 permutation test analyses (Churchill and Doerge 1994). The perl script Permute.pl (Basten et al. 2002) allowed the re-estimation of the cofactors for each permutation. In our case, LOD threshold corresponded to 2.60. Confidence intervals were defined by a LOD drop-off of one unit.

Results

Using a C/N functioning model to determine pertinent traits to study genotypic variation

Close correlations were found between NTOT and LA, intercepted PAR and TDM, as well as between TDM and RDM (Table 1). The correlation was weaker between RDM and TRL. The largest genetic variability was found for specific N uptake rate (NUR) as shown in the relationship between cumulative root length and total N amount (Fig. 3). Despite the close relationships, we analyzed genotypic variation in relation to radiation use efficiency ($RUE_{i,p}$), root/total dry matter ratio ($RDM\_TDM_{22,i,p}$), specific root length ($SRL_{22,i,p}$), N-specific uptake rate ($NUR_{i,p}$) as well as the ratio between leaf area and total N amount ($LA\_NTOT_{22,i,p}$). We also analyzed $RDM_{22,i,p}$, $LA_{22,i,p}$, $TRL_{22,i,p}$ and $NTOT_{22,i,p}$.

Phenotypic variation was normally distributed for the following traits: LRN_PRL, LRL_PRL, LRL, PRL, LRN, TRL and RUE (Table 2). Other traits

Table 1 Correlations between traits involved in the plant functioning model

Investigated traits (ordinate/abscissa)	Equation	R^2
Leaf area (cm^2/plant)/total N amount (cg/plant)	LA = 36.65NTOT + 1.34	0.92
Total dry matter (g/plant)/intercepted PAR (MJ/plant)	TDM = 2.90PAR – 0.004	0.92
Root dry matter (g/plant)/total dry matter (g/plant)	RDM = 0.54TDM + 0.01	0.94
Total root length (cm/plant)/root dry matter (g/plant)	TRL = 6,207.3RDM + 411	0.87
Total N amount at 22 days (mg/plant)/cumulated root length (cm/plant)	NTOT = 0.0008cumTRL – 1.64	0.60

A linear model was assumed to explain the relation between traits

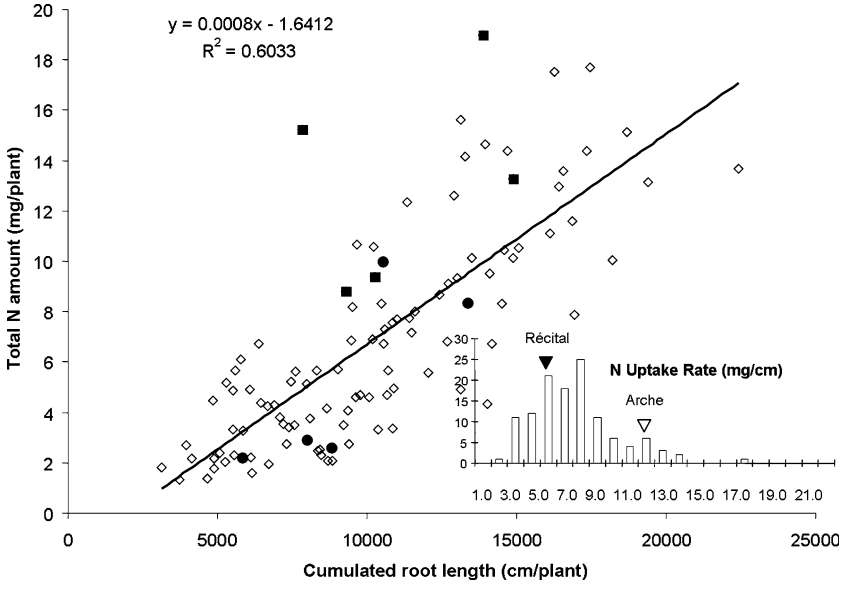

Fig. 3 Relationship between total nitrogen amount (NTOT) and the cumulated root length for Arche (*filled square*), Récital (*filled circle*) and 120 DH lines (*open diamond*). The linear regression between NTOT and the cumulated root length explained 60% of the total variation. The *inset* graph in the *bottom right corner* represents the repartition of nitrogen-specific uptake rate (NUR) for the whole population. The two parents, Arche and Récital, are identified by *white* and *black arrows*, respectively

distributions were close to a normal distribution and therefore could be kept for variance analyses or QTL detection because these statistical methods are robust. We also computed the following new variables: ln(ADM), ln(RDM), ln(NTOT) and ln(LA) that were thus rendered normally distributed (Table 2), as well as ln(SRL). To avoid confusion, we labeled these new variables tADM, tRDM and tSRL. LA_NTOT distribution was bi-modal. Transgressions were observed for all traits in both directions meaning that both parents carried favorable and unfavorable alleles. Transgression for NUR is shown by the inset graph of Fig. 3 that represents the distribution of the 120 DH lines population for this trait.

Genotypic variation was significant for all traits (Table 3). Heritabilities (h^2) ranged from 12.8 to 53.2%, therefore more than half of the phenotypic variation was due to environmental effect. The lowest h^2 (<20%) were found for LRN, SRL, LRN_PRL and LRL_PRL, all relative to root architecture. The highest h^2 were found for TRL, ADM, LA, TDM, NTOT, RDM, PRL, LRL, RDM_TDM, RUE and NUR, traits included in the model (except for PRL, TDM and LRL). SRL h^2 was inferior to 15% and h^2 for LA_NTOT was 18.3%.

Traits variations were influenced by the initial conditions

Models are usually sensitive to initial conditions. In our study, this was illustrated by the exceptionally large range of variations observed for the integrative traits: TDM [0.019:0.977] g, TRL [71.50:3,430.45] cm and LA [1.53:173.22] cm^2. We therefore tested the effect of the initial conditions on the traits using the plant total root length measured on the first day as covariate in the variance analysis (Table 4). TRL on day 1 ranged from 0 to 37.6 cm and its mean value was 11.5 cm. The effect of initial conditions was significant at the 5% level for LRN, TRL, RDM, LRL, LA, TDM and NTOT, while the genotype effect remained significant for SRL, LRL, TRL, LA, NTOT, TDM and RDM. Initial conditions did not influence ratio variables. For variables displaying a significant effect of initial conditions, h^2 calculated according to Eq. 15, which includes a covariate, were much higher than h^2 calculated

Table 2 General mean, parent values and normality tests for all traits

	Trait	Kurtosis	Skewness	Mean	Arche	Récital
Root architecture traits	LRN_PRL (cm^{-1})	0.036	–0.074	1.09	1.14	1.34
	LRL_TRL	*2.97*	*–1.407*	0.71	0.75	0.71
	LRL_PRL	0.043	0.088	2.83	3.12	2.97
	LRL (cm)	–0.4	0.4	1,009.0	1,173.03	878.61
	PRL (cm)	–0.54	0.38	353.3	379.62	278.68
	LRN	–0.24	0.33	373.3	419.8	383.7
	LRL_LRN (cm)	0.36	*0.77*	2.79	2.84	2.20
Integrative traits	RDM (g)	0.22	*0.81*	0.156	0.180	0.098
	ln(RDM)	–0.74	–0.16	–	–	–
	ADM (g)	0.71	*1.02*	0.113	0.185	0.070
	ln(ADM)	–0.896	0.052	–	–	–
	LA (cm^2)	*1.95*	*1.46*	32.67	45.94	17.27
	ln(LA)	–0.72	0.03	–	–	–
	TDM (g)	–0.30	*0.75*	0.269	0.366	0.168
	NTOT (mg)	*1.98*	*1.27*	6.73	11.54	4.51
	ln(NTOT)	–0.49	–0.20	–	–	–
	TRL (cm)	–0.51	0.35	1,362.3	1,552.7	1,157.3
Efficiencies	SRL (cm/g)	*33.48*	*4.498*	9,991.6	8,848.0	11,877.6
	ln(SRL)	*5.25*	*1.29*	–	–	–
	RDM_TDM	*22.86*	*3.58*	0.54	0.50	0.63
	ln(RDM_TDM)	*2.83*	*0.86*	–	–	–
	NUR (mg/cm)	*1.73*	*1.07*	6.20	10.74	4.51
	LA_NTOT (cm^2/cg)	*3*	*2.07*	56.75	40.28	51.83
	RUE (g/MJ)	–0.28	–0.44	2.80	2.51	2.43

Distributions were considered as normal when the kurtosis value was within the interval [–0.89; 0.89] and when skewness value was within the interval [–0.44; 0.44]. Those intervals were assessed as two times the kurtosis or skewness standard error. Mean represents the general mean of the investigated trait in the population. Arche and Récital columns represent the mean values of Arche and Récital genotypes, respectively. Distributions of the variables in italics were not normal

LRN_PRL lateral root number/primary root length, *LRL_TRL* lateral root length/total root length, *LRL_PRL* lateral root length/primary root length, *LRL* lateral root length, *PRL* primary root length, *LRN* lateral root number, *LRL_LRN* mean length of a lateral root, *RDM* root dry matter, *ADM* aerial dry matter, *LA* leaf area, *TDM* total dry matter, *NTOT* total nitrogen amount, *SRL* specific root length, *RDM_TDM* root dry matter/total dry matter, *NUR* nitrogen-specific uptake rate, *LA_NTOT* leaf area/total nitrogen amount, *RUE* radiation use efficiency

according to Eq. 13 (Tables 3, 4). For example, TRL h^2 was 41.4% without the covariate and 84.2% with the covariate, suggesting that the corrections involving initial conditions allowed us to better estimate genetic variability. h^2 did not change for traits that were not sensitive to the covariate effect.

Dwarfing gene *Rht-B1* mainly influenced integrative traits and, to a lesser extent, efficiencies and root architectural traits

The Arche × Récital (ARE) population was in segregation for the dwarfing gene *Rht-B1* (located on 4B chromosome), the gene of photoperiod sensitivity *Ppd-D1* (2D), the gene for awnedness *B1* (5A) and the vernalization gene *Vrn-A1* (5A). A variance analysis was conducted to assess the effects of these genes, or genes close to them, on the given traits (Table 5). LRN and LRL_TRL were not influenced by any of these genes. The effects of *Vrn-A1* were only significant at a level of 5% for LA_NTOT, RUE and RDM_TRM. No effect of *Vrn-A1* was expected because grains were vernalized. The display of effect, although weak, may be due to the fact that the grains were vernalized for only 4 weeks, a time span that may be too short for lines carrying the Récital allele. Photoperiod (*Ppd-D1*) had an effect only on four traits (ADM, TDM, NTOT and NUR). As the photoperiod was not limiting (16 h daylight), other genes located in the vicinity of *Ppd-D1* may be involved. Awnedness showed significant effects on two efficiencies LA_NTOT and NUR and on the architectural trait LRL_PRL. The *Rht-B1* gene had the most significant effect on traits (Table 5). However, three architectural traits (LRN, SRL and LRL_PRL) and two efficiencies (LA_NTOT and RUE) were not influenced by *Rht-B1*.

Thirty-two QTL were detected: 21 for integrative traits, 6 for model efficiencies and 5 for root architectural traits

To evaluate the genetic determinants involved in plant adaptation to N-limited nutritional conditions and root

Table 3 Results of variance analysis

	Variable	Heritability (%)	Genotypic effect	Experiment effect
Root architecture traits	LRL (cm)	38.4	53.59[a]	2.15[c]
	PRL (cm)	42.4	57.97[a]	0.65[ns]
	LRN	14.3	36.88[b]	0.00[ns]
	LRL_PRL	14.5	40.44[b]	1.88[ns]
	LRL_TRL	24.6	46.64[a]	2.36[c]
	LRL_LRN (cm)	46.3	54.22[a]	1.95[b]
	LRN_PRL (cm^{-1})	17.2	39.63[a]	1.19[ns]
Integrative traits	LA (cm^2)	49.6	61.06[a]	0.88[c]
	TDM (g)	53.2	65.96[a]	1.29[b]
	ADM (g)	46.1	60.99[a]	1.55[ns]
	RDM (g)	47.0	59.21[a]	1.08[ns]
	TRL (cm)	41.4	55.73[a]	1.92[c]
	NTOT (mg)	52.6	67.00[a]	1.53[b]
Efficiencies	RUE (g/MJ)	20.9	45.69[b]	1.35[ns]
	RDM_TDM	28.4	49.81[a]	0.70[ns]
	SRL (cm/g)	12.8	38.23[c]	0.10[ns]
	NUR (mg/cm)	42.2	60.97[a]	3.89[b]
	LA_NTOT (cm^2/cg)	18.3	8.55[a]	2.28[a]

Values represented in the genotypic effect and experiment effect columns represent the percentage of variation explained by the considered effect (% of total sum of squares).a: highly significant, b: significant at 1%, c: significant at 5%, ns: not significant

LRL lateral root length, *PRL* primary root length, *LRN* number of lateral roots, *LRL_PRL* LRL/PRL, *LRL_TRL* lateral root length/total root length, *LRL_LRN* mean length of a lateral root, *LRN_PRL* LRN/PRL, *LA* leaf area, *TDM* total dry matter, *ADM* aerial dry matter, *RDM* root dry matter, *TRL* total root length, *NTOT* total nitrogen amount, *RUE* radiation use efficiency, *RDM_TDM* root dry matter/total dry matter, *SRL* specific root length (TRL/RDM), *NUR* nitrogen-specific uptake rate, *LA_NTOT* leaf area/nitrogen total amount

architecture, we carried out QTL detection. When the initial condition effect was significant (Table 4), we assessed a corrected trait as the residual of the model described in Eq. 17. New traits were labeled as the one they came from, adding the prefix ''c'' to it. When variable distribution was not normal, we transformed the variable before QTL detection using $\ln(x)$ and in that case, the label of the transformed traits begins with t.

A total of 32 QTL were detected on chromosomes 1A, 1B, 2B, 4B, 5A, 5B, 5D, 6A, 6B, 7A, 7B and 7D (Table 6). Six QTL were detected for root architectural traits, six for C/N model efficiencies and 20 QTL for integrative traits (ADM, RDM, TDM, LA, NTOT,

Table 4 Results of covariance analysis, using total root length on first day as covariate

	Trait	Heritability (%)	TRL on day 1	Genotypic effect
Root architecture traits	LRL (cm)	41.6	5.11[c]	72.86[c]
	PRL (cm)	41.7	10.59[b]	69.23[ns]
	LRN	60.9	9.17[b]	65.22[ns]
	LRL_PRL	15.4	0.01[ns]	59.72[ns]
	LRL_TRL	25.6	0.00[ns]	60.51[ns]
	LRL_LRN (cm)	46.0	0.1[ns]	75.35[c]
	LRN_PRL	17.2	0.12[ns]	48.86[ns]
Integrative traits	LA (cm^2)	52.2	2.91[a]	60.23[a]
	TDM (g)	54.9	1.83[b]	65.46[a]
	ADM (g)	47.7	2.91[ns]	58.86[ns]
	RDM (g)	48.4	7.687[b]	74.2[b]
	TRL (cm)	84.2	6.417[b]	73.42[c]
	NTOT (mg)	54.4	1.69[b]	67.51[a]

Values represented in the genotypic effect and TRL on day 1 columns represent the percentage of variation explained by the considered effect (% of total sum of squares) .a: highly significant, b: significant at 1%, c: significant at 5%, ns: not significant

LRL lateral root length, *PRL* primary root length, *LRN* number of lateral roots, *LRL_PRL* LRL/PRL, *LRL_TRL* lateral root length/total root length, *LRL_LRN* mean length of a lateral root, *LRN_PRL* LRN/PRL, *LA* leaf area, *TDM* total dry matter, *ADM* aerial dry matter, *RDM* root dry matter, *TRL* total root length, *NTOT* total nitrogen amount

Table 5 Significance of four genes [dwarfing (*Rht-B1*), awnedness (*B1*), vernalization (*Vrn-A1*) and photoperiod sensitivity (*Ppd-D1*)] effect on root architecture traits and plant functioning traits tested by covariance analysis

	Trait	*Rht-b1*	*Vrn-A1*	*B1*	*Ppd-D1*
Root architecture traits	LRL (cm)	2.619^{b}	0.489^{ns}	0.079^{ns}	0.002^{ns}
	PRL (cm)	1.718^{b}	0.109^{ns}	0.651^{ns}	0^{ns}
	LRN	0.155^{ns}	0.207^{ns}	0.029^{ns}	0.166^{ns}
	LRL_PRL	2.08^{ns}	0.701^{ns}	2.08^{c}	0.026^{ns}
	LRL_TRL	1.21^{c}	0.751^{ns}	0.401^{ns}	0.002^{ns}
	LRL_LRN (cm)	1.295^{a}	0.316^{ns}	0.17^{ns}	0.152^{ns}
	LRN_PRL	5.796^{b}	0.218^{ns}	5.796^{ns}	0.32^{ns}
Integrative traits	LA (cm^2)	4.29^{a}	0.01^{ns}	0.10^{ns}	0.68^{c}
	TDM (g)	6.49^{a}	0.21^{ns}	0.15^{ns}	1.45^{b}
	ADM (g)	5.776^{a}	0.002^{ns}	0.337^{ns}	0.865^{c}
	RDM (g)	2.015^{b}	0.056^{ns}	0.324^{ns}	0.193^{ns}
	TRL (cm)	2.645^{a}	0.428^{ns}	0.0016^{ns}	0.004^{ns}
	NTOT (mg)	3.80^{a}	0.002^{ns}	0.21^{ns}	1.30^{b}
Efficiencies	RUE (g/MJ)	0.020^{ns}	4.768^{a}	0.027^{ns}	0.002^{ns}
	RDM_TDM	2.581^{b}	1.42^{c}	0.0052^{ns}	0.512^{ns}
	SRL (cm/g)	0.075^{ns}	0.035^{ns}	0.135^{ns}	0.729^{ns}
	NUR (mg/cm)	2.674^{b}	0.667^{ns}	1.183^{b}	2.096^{b}
	LA_NTOT (cm^2/cg)	8.009^{ns}	0.526^{b}	2.015^{a}	0.073^{ns}

a: highly significant, b: significant at 1%, c: significant at 5%, ns: not significant

LRL lateral root length, *PRL* primary root length, *LRN* number of lateral roots, *LRL_PRL* LRL/PRL, *LRL_TRL* lateral root length/total root length, *LRL_LRN* mean length of a lateral root, *LRN_PRL* LRN/PRL, *LA* leaf area, *TDM* total dry matter, *ADM* aerial dry matter, *RDM* root dry matter, *TRL* total root length, *NTOT* total nitrogen amount, *RUE* radiation use efficiency, *RDM_TDM* root dry matter/total dry matter, *SRL* specific root length (TRL/RDM), *NUR* nitrogen-specific uptake, *LA_NTOT* leaf area/nitrogen total amount

TRL and LRL). Out of these 32 QTL, 14 were detected for traits corrected for the initial conditions and 25 were on the B genome. Only one QTL was detected for SRL and it was for a transformed variable.

QTL detection for integrative traits

Twenty QTL were detected for integrative traits. They are located on LG 1A1, 1B, 2B2, 4B, 5A1, 5B and 6B. R^2 ranged from 8.6 to 39% and were higher for corrected traits than for uncorrected traits, as shown by TRL and cTRL QTL (Table 6). Two loci gathered several QTL on LG 2B2: a locus at position 0 cM gathered QTL for tADM, cADM, LA, NTOT and cTDM, and a locus at position 70 cM gathered QTL for TRL, cTRL, tRDM and TDM. Arche allele was the favorable one for all the QTL detected on this LG. Arche allele also increased all TDM, ADM and NTOT QTL, and Récital allele increased QTL located on LG 1A1, 1B, 5A1 and 6B (Table 6). Four of the five QTL (ADM and RDM) detected on 4B chromosome coincided with *Rht-B1* and the tall allele (Arche) was associated with a dry matter increase either for root or aerial part.

QTL detection for efficiencies

Two QTL for NUR were detected on LG 7A2 and 7B1. They explained, respectively, 11.8 and 14.9% of the total variation. Arche allele increased NUR when present at the QTL located on LG 7A2 and Récital allele increased NUR when present at the QTL located on LG 7B1. Two QTL were observed for tRDM_TDM (root dry matter/total dry matter) on LG 6A and 7D3, respectively, and Récital provided the favorable allele for both QTL. A QTL for tSRL was detected on chromosome 6B explaining 10.6% of the total variation. Arche allele was the favorable one for SRL. A QTL for RUE, explaining 15% of the total variation, was detected on LG 2B2 and coincided with a QTL for TRL. Arche allele increased both TRL and RUE. No efficiency-QTL was detected on LG 2D1, 4B or 5A where *Ppd-D1*, *Rht-B1*, *Vrn-A1* and the awnedness genes are located.

QTL detection for root architecture traits

QTL for architectural traits were detected on chromosomes 4B, 5A and 7B. A QTL linked to *Rht-B1* was detected for the mean lateral root length. The QTL for the number of lateral roots per cm of primary root, that is to say the branching rate, was located on 7B2 and Récital allele increased this trait. LG 5A2 grouped two QTL for the ratio between lateral root length and primary root length. Récital alleles increased this ratio. A QTL for the ratio between lateral root length and total root length was detected on 5D chromosome: the

Table 6 Results of QTL detection

	Trait	Linkage group	Marker	Position (cM)	Confidence interval	LOD	R^2 (%)	Fav. allele
Root architecture traits	cLRL	4B	gwm540b	102	82–131	2.71	16.0	A
	LRL_PRL	5A2	cfa2149	11	0–24	2.80	12.5	R
	cLRL_PRL	5A2	cfa2149	10	0–21	3.33	14.5	R
	cLRL_TRL	5D	gwm190	54	39–71	2.77	11.2	R
	LRL_LRN	4B	Rht-B1	41	34–58	3.28	9.6	A
	cLRN_PRL	7B2	gwm146	45	35–59	2.63	10.5	R
Integrative traits	LA	2B2	cfa2086	0	0–18	3.67	16.0	A
	cLA	1A1	cfa2153	2	0–2	3.06	13.0	R
	TDM	2B2	gwm2046	70	52–98	3.19	24.8	A
	cTDM	2B2	cfa2086	0	0–19	2.84	12.2	A
	tADM	2B2	cfa2086	9	0–32	3.02	13.9	A
	cADM	2B2	cfa2086	0	0–13	3.54	12.5	A
	cADM	4B	Rht-B1	47	34–60	2.87	11.4	A
	tADM	4B	Rht-B1	43	33–58	2.61	9.2	A
	ADM	4B	gwm540b	87	61–110	3.60	18.1	A
	tRDM	1B	gwm131a	38	21–49	3.08	10.3	R
	tRDM	2B2	gwm526	85	60–99	5.13	25.5	A
	cRDM	4B	Rht-B1	49	34–89	2.80	12.6	A
	RDM	4B	Rht-B1	48	33–61	2.71	11.2	A
	RDM	5A1	gpw2249a	0	0–12	2.70	8.6	R
	cTRL	1B	gpw2067d	0	0–10	2.63	10.4	R
	TRL	2B2	gwm526	70	51–101	3.05	19.7	A
	cTRL	2B2	gpw2046	52	38–68	4.52	39.0	A
	cTRL	6B	gdm113	12	1–45	3.74	17.9	R
	NTOT	2B2	cfa2086	0	0–21	3.01	14.2	A
	tNTOT	5B	gwm297	118	97–118	3.10	16.9	A
Efficiencies	RUE	2B2	gwm526	81	63–103	2.83	15.1	A
	tRDM_TDM	6A	wmc201	100	88–115	2.85	9.1	R
	tRDM_TDM	7D3	gdm150	17	0–38	3.00	14.4	R
	tSRL	6B	cfd13a	0	0–6	3.13	10.6	A
	NUR	7A2	cfa2028	15	1–36	3.64	11.8	A
	NUR	7B1	gwm063	5	0–23	3.36	14.9	R

Marker column contains the name of the QTL closest marker; LOD stands for the LOD score value; R^2 represents the part of the phenotypic variation explained by the QTL; position is most probable QTL position on the linkage group; and Fav. allele represents the parent whose allele increased the trait when present at the given QTL

ratio was increased by the presence of the Récital allele.

No QTL were detected on LG 2D1 where *Ppd-D1* was located. No QTL were detected for the uncorrected traits LRN, PRL, LRL, LA_NTOT, SRL, LRN_PRL, LRL_TRL and RDM_TDM or for the transformed traits tLA_NTOT, tLA and tTDM and the corrected traits cLRN, cPRL and cLRL_LRN.

Discussion

Doubled haploid lines grown in hydroponic rhizotron under N-limited nutritional conditions presented genotypic variability at an early developmental stage for both root architecture traits and those related to C and N relationships. Thirty-two QTL were detected on chromosomes 1A, 1B, 2B, 4B, 5A, 5D, 6A, 6B, 7A, 7B and 7D. The effect of the dwarfing gene *Rht-B1*, located on 4B chromosome, had a significant influence on almost all traits. QTL detection is discussed in relation to comparable studies, highlighting the importance of the dwarfing gene *Rht-B1*. Pertinence of experimental choices (population size and growing conditions), the features of a simple model to assess genetic variability and further developments of this model are discussed as well.

Validation of detected QTL by coincidence analysis

Our objective was to seek out any root or N uptake related variation that could explain differences in N use efficiency observed in the field. Therefore, we compared our QTL with QTL for grain yield or N-related traits already pinpointed in the literature. For wheat, most QTL related to N have been detected for bread-making quality, such as grain protein composition or grain protein content. Studies have evidenced QTL for grain protein content on chromosomes where we evidenced root QTL: 2B, 6B, 7A, 7D

(Prasad et al. 2003), 6B (Olmos et al. 2003), 2B, 5A (Charmet et al. 2005), but the lack of common markers did not allow for a more detailed comparison. Of greater interest to us was the study carried out by Charmet et al. (2005). First of all, they detected QTL on 6A and 7D chromosomes of a Récital × Renan population for 1,000 kernel weight, grain protein content, maximum rate of N or biomass accumulation. Their results coincided with our QTL for RDM_TDM (root/total dry matter) ratio under N-limited nutritional conditions. In their study, the rate of N or biomass accumulation was assessed between 200–250 and 500–550 degree-days after anthesis. The authors reported that the Récital allele decreased both the rate of N accumulation and the quantity of total protein. We found that Récital allele increased RDM_TDM for QTL evidenced on 6A and 7D3. Further investigation using the same population could reveal the role of the Récital allele in the genetic control of the two traits. Second of all, Charmet et al. (2005) detected on 7A chromosome a QTL for grain protein content, N accumulation rate and grain protein composition. When we projected our genetic map on the ITMI map (Leroy, personal communication), we could situate our ''NUR'' QTL of linkage group 7A2 between markers cfa2049 and gwm282, indicating a possible coincidence with the QTL detected by Charmet et al. (2005). This eventual coincidence and the fact that Récital allele decreased N accumulation rate in both studies suggests that experimental work conducted for early developmental stages under controlled conditions can be a good predictor of crop characteristics at pertinent phenologic stages under field conditions.

The dwarfing gene *Rht-B1* is involved in grain yield genetic control, as evidenced by its coincidence with grain yield QTL (Quarrie et al. 2005). It has been widely used in breeding programs since the green revolution and has resulted in an increase in the harvest index. As this gene has been widely used, it is interesting, for our purposes, to characterize its effects on N use efficiency, and thus on root system and on N uptake rate. Root/total dry matter ratio at anthesis was increased for near-isogenic lines incorporating the dwarf allele when compared with the near-isogenic line incorporating the tall allele (McCaig and Morgan 1993). Bush and Evans (1988) suggested that the dwarfing gene effect on root system changed along the growth cycle: at the beginning, tall lines would develop a more important root system, in terms of biomass, whereas dwarf lines would present a larger root system later on. Moreover, Miralles et al. (1997) have shown that at anthesis, dwarf lines presented longer, heavier and thicker roots than tall lines only in the 0–30 cm soil profile, whereas no significant differences between dwarf and tall lines were reported at the terminal spikelet elongation stage. They proposed the following explanation: during stem elongation, the dwarfing gene inhibits stem sink activity and assimilates would be used to only thicken existing organs (stem and roots). According to them, the dwarfing allele would not provide an advantage for the exploration of the soil profile, explaining why dwarf lines have not been shown to tolerate drought better than tall lines. Our covariance results (Table 4) are in agreement with an effect of the dwarfing gene on root architecture, root N uptake rate and therefore N use efficiency. We also showed that *Rht-B1* coincided with QTL for mean lateral root length and with QTL for aerial dry matter and root dry matter. In each case, the tall allele (provided by Arche) increased the traits, indicating that dwarf lines would present smaller lateral roots. The use of near-isogenic lines, grown under the same experimental conditions, would be a way to draw more conclusions about the use of *Rht-B1* for improving NUE.

More evidence between root growth and plant response to varying N sources has been provided for *A. thaliana*. Root length and root and aerial biomasses were assessed under low N conditions and three varying sources of N (Raugh et al. 2002). First, the QTL detected for root and aerial parts appeared to be specific to a given N treatment because few common QTL were detected. Secondly, they did coincide with candidate genes involved in N pathways such as glutamine synthetase, glutamate synthetase and glutamate dehydrogenase, as well as a transporter that could be involved in ammonia uptake. Furthermore, QTL coincidences for root characteristics assessed in hydroponics and grain yield assessed in field trials, both under low nitrogen and high N supplies, are reported in maize (Tuberosa et al. 2003).

The choice of specific experimental and conceptual methods to study the response of the entire plant (including roots) to N deficiency

Experimental conditions involved two main factors: the selection of the parents and the choice of growing conditions. The selection of Arche and Récital was based on the fact that they have markedly different responses to low N input (Le Gouis et al. 2000). This population was well adapted to study the genetic variability of N use efficiency in our specific conditions as illustrated by the transgression observed for all traits within the population. Because of experi-

mental constraints, we decided to phenotype only 120 genotypes from the ARE population. Reducing the population size is known to decrease the number of detected QTL and to overestimate the total amount of phenotypic variation explained. Small populations can also lead to the detection of spurious QTL (Vales et al. 2005). However, populations recording around 120 genotypes have already been used to produce reliable data for QTL detections: Tuberosa et al. (2002) as well as Landi et al. (2002) used 118 F3 families to identify QTL for root characteristics in maize grown on hydroponics, and those detected QTL were validated by overlapping with QTL for grain yield detected under field conditions. Li et al. (2005) also mapped QTL for root traits in rice using a 116 DH lines population. In this paper, our aim was to detect the main genetic determinants of nitrogen use efficiency in bread wheat, as no other QTL detection has already been performed for this trait on wheat. Our size-limited population already allowed the identification and characterization of wheat genome regions involved in the plant response to nitrogen deficiency. As the genotypic information was available for the whole ARE population, the 120 DH lines were chosen to perform selective phenotyping. This sampling method improves in theory the accuracy of QTL detection (Jannink 2005) and has been shown to be more efficient than random sampling when the effective is superior to 100 genotypes (Vales et al. 2005).

In our study, plants were experimented in rhizotrons as it gave us access to the traits involved in plant response to nitrogen-limited nutrition, such as root architecture or model efficiencies. Moreover, several studies reported that traits variations reported for seedling roots grown under hydroponics or greenhouse conditions were positively associated with root characteristics of adult plants grown in the field (Mian et al. 1994; Landi et al. 1998; Sanguineti et al. 1998). Rhizotron growing conditions were chosen because they are non-destructive. Moreover, most root description studies performed for mapping populations limit measurement to root biomass and/or root length (Landi et al. 2002), whereas we could assess the real branching rate (defined by LRN_PRL) and the ratio between lateral roots and primary roots. Because measurements were recorded each day, we could estimate nitrogen-specific uptake rate and study the influence of initial conditions on our traits. Conscious that we also needed to take into account root hair traits such as root hair length and root hair distribution, as reported for the study of tolerance to phosphorus deficiency (Wang et al. 2004; Zhu et al. 2005), we based our approach on two hypothesis: one, that nitrate uptake was uniformly distributed along the root system and two, that for a given genotype, nitrate uptake rate only depended on total root length.

Because studies based on root description architecture record significant genotype × environment interactions (Raugh et al. 2002), we decided to do our experimental work directly under N-limited nutritional conditions. Studies have found that direct selection for low N environments was more efficient than indirect selection, even if heritabilities were lower (Bänziger et al. 1997; Brancourt-Hulmel et al. 2005). Therefore, we expected to evidence more pertinent genetic determinants under low N supply. We are aware of the fact that we were not able to distinguish constitutive QTL (more probably involved in plant development) from adaptive QTL (more specific in terms of plant adaptation to its environment). Complementary studies under optimal N fertilization and the comparison of QTL detections would allow us to distinguish between the two types of QTL.

The use of an ecophysiological model was equally important. Because the model allowed us to well define the adaptive traits (the efficiencies) to investigate, we had access to adaptive QTL and, therefore, we limited the confusion between constitutive and adaptive QTL. The model we relied on helped us to take into account developmental aspects and relationships between carbon and N pathways, by considering efficiencies and not directly biomass variation, knowing that, for instance, root biomass was largely correlated to total biomass and then indirectly to leaf area. That also explained why QTL for efficiencies were mainly evidenced on LG 6A, 6B, 7A2, 7B1 and 7D3, whereas QTL for total root length or dry matter were located on LG 1B, 2B2 and 4B, possibly explained by genes such as *Rht-B1*.

When we compared QTL detected for the model efficiencies and QTL detected for the state variables of the model (LA, NTOT, TDM and RDM) we observed few coincidences between the QTL of the two types of traits. Only QTL for RUE coincided with QTL for TRL or TDM, suggesting that carbon metabolism was also involved in plant response to N-limited nutritional conditions. Model efficiencies gave access to information that was not contained in integrative traits. Efficiency QTL were confirmed by coincidences with QTL reported in literature and thus were not artifacts. Therefore, we can conclude that a simple conceptual model can be used as a convenient tool for QTL analysis.

Towards a more integrative model of plant functioning under N stress

Ecophysiological models have already been used for understanding genotypic variability in peach total sugar content (Quilot et al. 2002, 2004) and genetic variability of the response of maize leaf growth to temperature and water deficit (Reymond et al. 2003). Models have led to the identification of breeding criterion not subject to genotype × environment interactions. We found that the use of a model allowed us to evidence QTL that would not have been detected using only state variables, reinforcing our stance that modeling is a good means for choosing pertinent traits. Our conceptual model was kept simple and integrative. For instance, we assumed total N amount controlled the elaboration of leaf area. But the functioning of this relationship has not been clearly established. It can equally be assumed that leaf area controls total N amount. In this case, the dwarfing gene *Rht-B1*, which also results in leaf area differences, would partly explain total N amount differences. To test this hypothesis, the same experiment needs to be carried out under non-limiting conditions to express the full range of leaf area variation due to *Rht-B1* and to study coincidences between *Rht-B1* and QTL for NTOT. Under our experimental conditions, as regards the LA/NTOT relationship, we did not evidence clear-cut differences between the two *Rht-B1* allele classes (data not shown). The integrative relation we observed might very well be the result of leaf area regulated by N amount combined with N amount regulated by leaf area.

Yin et al. (1999) showed for barley that the use of ecophysiological models in QTL detection could lead to a better estimation of genetic parameters involved in specific leaf area control. Their model allowed them to compare genotypes at the same developmental stage. QTL mostly involved in duration of developmental stages were then distinguished from QTL really involved in the control of the traits under study. We took a first step in that direction by using initial conditions as covariate. To enhance our model, the taking into account of developmental stages is foreseen.

In this study, we used an ecophysiological model as a tool for screening genetic variability. Another application of this work would be to use the detected QTL for improving the ecophysiological model so that it can predict the C/N plant functioning of different genotypes. For each tested genotype, model efficiencies values would depend on the allelic combination of the genotype at the QTL involved in the genetic control of efficiencies. Such an approach has been used for barley (Yin et al. 2000) and maize, as regards response to temperature and water deficit (Reymond et al. 2003). It allowed them to calculate the response of a precise genotype, knowing its allelic composition. An even more far-reaching possibility would be its use to pinpoint allele combinations that optimize nitrogen use efficiency, combinations that could serve as ideotypes for breeders.

Conclusion

We investigated QTL involved in root architecture and in the relation between N and C functioning under N-limited nutritional conditions to characterize the mechanisms involved in plant tolerance to N-limited nutritional conditions. The use of a simplified plant functioning model allowed us to determine pertinent traits that were studied to detect associated QTL. We completed our data set with root system architectural traits. A coincidence study of the QTL we detected for N traits with QTL reported in literature validated the pertinence of our approach. The next step would be to compare these newly acquired QTL with QTL detected, on the whole ARE population, for grain yield and N traits in the field, and especially those for plant adaptation to low N input. MAS appears to be a very suitable way to improve root characteristics of varieties.

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