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ORIGINAL PAPER

Using genotype × nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints

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Abstract Lower market prices and environmental concerns now orientate wheat (*Triticum aestivum* L.) breeding programs towards low input agricultural practices, and more particularly low nitrogen (N) input management. Such programs require knowledge of the genetic determination of plant reaction to N deficiency. Our aim was to characterize the genetic basis of N use efficiency and genotype × N interactions. The detection of QTL for grain yield, grain protein yield and their components was performed on a mapping population of 222 doubled haploid lines (DH), obtained from the cross between an N stress tolerant variety and an N stress sensitive variety. Experiments on the population were carried out in seven different environments, and in each case under high (N^+) and low (N^-) N supplies. In total, 233 QTL were detected for traits measured in each combination of environment and N supply, for ''global'' interaction variables (N^+–N^- and N^-/N^+), for sensitivity to N stress and for performance under N-limited conditions which were assessed using factorial regression parameters. The 233 QTL were detected on the whole genome and clustered into 82 genome regions. The dwarfing gene (*Rht-B1*), the photoperiod sensitivity gene (*Ppd-D1*) and the awns inhibitor gene (*B1*) coincided with regions that contained the highest numbers of QTL. Non-interactive QTL were detected on linkage groups 3D, 4B, 5A1 and 7B2. Interactive QTL were revealed by interaction or factorial regression variables (2D2, 3D, 5A1, 5D, 6A, 6B, 7B2) or by both variables (1B, 2A1, 2A2, 2D1, 4B, 5A2, 5B). The usefulness of QTL meta-analysis and factorial regression to study QTL × N interactions and the impact of *Rht-B1*, *Ppd-D1* and *B1*, are discussed.

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Introduction

To reduce production costs and groundwater pollution by nitrate leaching, there is currently considerable interest in breeding winter wheat varieties adapted to low input management systems. Studies have shown that such wheat varieties grown with low input levels (including reductions in seed density, N and pesticides) can sustain profit margins even if yields are lower (Félix et al. 2002, 2003). Much effort has been devoted to disease resistance, but wheat varieties that are specifically N stress tolerant still need to be developed. These varieties will have to maintain yield and grain protein content under moderate N deficiency as well as in the event of the intense N stress which occasionally occurs under low input cropping systems.

To breed such varieties, genetic variation for adaptation traits to N deficiency is required. In wheat, studies have reported genetic variability for N use efficiency (NUE) and its components: N uptake efficiency and N utilization efficiency (Dhugga and Waines 1989; Le Gouis and Pluchard 1996; Le Gouis et al. 1998, 2000). It was thus concluded that selection for NUE, and particularly N uptake efficiency, was possible. Direct selection for yield under a low N supply would also be more efficient than indirect selection conducted under high N levels (Brancourt-Hulmel et al. 2005).

As well as breeding strategies, it is also important to understand the genetic control of plant adaptation to N deficiency. QTL analyses have been performed under field conditions using different fertilization levels for barley (Kjaer and Jensen 1995), maize (Agrama et al. 1999; Bertin and Gallais 2001; Hirel et al. 2001; Gallais and Hirel 2004) and rice (Lian et al. 2005) and in growth chambers for *Arabidopsis thaliana* (Raugh et al. 2002; Loudet et al. 2003). These authors identified specific QTL for N supply. In wheat, few QTL detections have been performed for adaptation to N deficiency (Habash et al. 2007) as most QTL studies have concerned grain quality (Perretant et al. 2000; Groos et al. 2003; Prasad et al. 2003; Charmet et al. 2005), yield components (Börner et al. 2002), earliness (Hanocq et al. 2004) and disease resistance (Liu and Anderson 2003; Mallard et al. 2005; Mardi et al. 2005; Yang et al. 2005). As for abiotic stress, a wheat QTL study was performed on senescence traits during grain-filling in relation to drought tolerance (Verma et al. 2004).

Most QTL studies involving adaptation to abiotic stresses have revealed the existence of QTL × environment interactions (Campbell et al. 2004). Leflon et al. (2005) reported two approaches for the analysis of QTL environment interactions. The first approach deduced interactions by comparing QTL detected separately in different environments: in many cases, an interaction was merely detected and no estimate made of the interaction itself. In other cases, QTL × environment interactions were assessed by colocalisation between QTL detected for the main effect and QTL detected for stability statistics (Emebiri and Moody 2005). The second approach takes interaction effects into account in the analysis of multi-environment trials by introducing QTL main effects and QTL × environment interactions effects. Interaction effects can be modeled by means of environmental covariates and factorial regressions, like studies of genotype × environment interactions (see, for instance, Crossa et al. 1999; Campbell et al. 2003, 2004; Groos et al. 2003). These methods are powerful but a large set of environmental measurements is necessary for their application.

The first objective of our study was to characterize the genetic basis for NUE in bread wheat. We also aimed to characterize the QTL involved in a plant-specific response to N deficiency and then in QTL × N interactions. For this purpose, our experiments utilized a DH line population in a broad range of environments differing in N status. The parents were chosen for their contrasting response to N deficient conditions (Le Gouis et al. 2000; Laperche et al. 2006a). Three methodologies were compared to reveal QTL × N interactions: first, we examined QTL detected separately under both types of N supply, second we detected QTL for ''global'' interaction variables assessed as N^+–N^- and N^-/N^+, and finally we considered QTL for factorial regression slope and ordinate, parameters which represent plant sensitivity to N stress and plant performance under a limited N supply, respectively.

Materials and methods

Field experiment and variables

A population of 241 doubled-haploid (DH) lines was produced from the F1 of a cross between the cultivars Arche and Récital which differ in reaction to N deficiency (Le Gouis et al. 2000). The experiments utilized 222 lines in 1999/2000 and 216 in 2000/2001. The two parents and two other cultivars (Soissons and Ritmo) were used as controls. The experiment was conducted at Nickerson Chartainvilliers (48°35′N, 1°35′E) in 2000 and at three different INRA sites: Clermont-Ferrand (45°47′N Lat., 3°05′E Long.), Le Moulon (48°42′N, 2°08′E) and Mons (49°53′N, 3°00′E) in 2000 and 2001. Two levels of N supply were tested at each location: a high N supply (N^+) corresponding to the prevailing agricultural practice at each site (ranging from 116 to 215 kg N/ha), and a low N supply (N^-) where the level of N applied was between 60 and 144 kg N ha^{-1} less than the high N supply, depending on the site. The two N levels differed by approximately 100 kg N/ha. Two replications were grown at each site. More details on the experimental design are reported on Table 1. On all sites except Chartainvilliers, the Nitrogen Nutrition Index (NNI), computed as the ratio between the N content in vegetative parts and a critical N content (Justes et al. 1994), was assessed at flowering for each control. Grain yield (GY), grain number per m^2 (GPA), thousand kernel weight (TKW), aerial dry matter (ADM), straw N amount per unit area (NSA), straw N content (NS%), grain protein yield (GPY), grain protein content (GPC) and N harvest index (NHI) were determined on each plot.

Genetic map

The genetic map covers 2,164 cM distributed into 30 linkage groups (LG). When two linkage groups corresponded

Table 1 Plot size, row spacing and sowing density, soil N and N applied for each year and location and N treatment

Location	Harvest year	Plot size (m^2)	Row spacing (m)	Sowing density (per m^2)	Soil N (kg/ha)	N applied (kg/ha) N^+/N^-	N treatments
Chartainvilliers	2000	7.0	0.18	280	59	**184**/40	Separate adjacent blocks
Clermont	2000	5.4	0.16	275	80	**116**/40	Separate adjacent blocks
Clermont	2001	5.4	0.16	275	70	**130**/30	Separate adjacent blocks
Le Moulon	2000	5.2	0.18	250	35	**160**/100	Separate adjacent blocks
Le Moulon	2001	5.2	0.18	250	35	**215**/115	Separate adjacent blocks
Mons	2000	6.5	0.18	240	52	**160**/50	Split-plot
Mons	2001	6.5	0.18	240	40	**180**/50	Split-plot

As N treatment effect was controlled in a randomized complete block with two replications, the N treatments column indicates the experimental design chosen to control the N treatment effect

to the same chromosome, a number was added at the end of the chromosome name. For instance, linkage groups 2D1 and 2D2 corresponded both to the same chromosome 2D. The map contains 183 markers, mostly wheat SSR markers except two glutenin markers (GLU-1A and GLU-1D), a specific marker for the storage protein transcription factor SPA (Guillaumie et al. 2004), the dwarfing gene Rht-B1 (Ellis et al. 2002), the glutamate synthase gene Fdgogat-D1 (Boisson et al. 2005), as well as phenotypic markers that characterized the gibberelin response and the presence/absence of awns. Mapping was carried out within the framework of a French Génoplante program and five SSR markers were reserved for use by Genoplante members and are not freely available; they are labeled GENO-1 to GENO-5. For more details on map construction refer to (Laperche et al. 2006b).

QTL detection

QTL analyses were performed using the Unix version of QTL cartographer 1.17d (Basten et al. 1994, 2002). Composite Interval Mapping (CIM) was performed using Model 6. The maximum number of cofactors involved in model 6 was set at five and the window size was 10 cM. We used the ''experiment-wise'' threshold defined at the 5% error level. This was estimated from 1,000 permutation test analyses (Churchill and Doerge 1994) and corresponded to a LOD score of 2.93. The perl script ''Permute.pl'' allowed re-estimation of the cofactors for each permutation (Basten et al. 2002). Confidence intervals were defined by a LOD decrease of one unit.

Meta-analyses were performed using the Biomercator software system (Arcade et al. 2004) to summarize QTL detection results. The method used by Biomercator is robust and can be applied to non-independent experiments where QTL are detected for different traits on the same population grown in different environments (Goffinet and Gerber 2000). We therefore performed four different meta-analyses: the first on QTL detected under N^+, the second on QTL detected under N^- and the last two on QTL detected for ''global'' interaction variables and QTL detected for factorial regression variables, respectively. These two types of variables are described below.

Statistical analyses and assessment of traits for QTL detection

Broad-sense heritability of yield and N traits were assessed for each combination of year, location and N supply as $\sigma^2_G/(\sigma^2_G + \sigma^2_e/J)$. σ^2_G stands for the genetic variance, σ^2_e for the residual variance and J for the number of replications. These variances were evaluated using the following model:

$$Y_{ij} = m + G_i + \mathrm{Rep}_j + e_{ij},$$

with Y_{ij} the value of trait Y for the line i in the replication j, G_i the genotypic effect, Rep_j the replication effect and e_{ij} the residual.

QTL detection was performed for each trait measured in each combination of year, location and N supply and with respect to both ''global'' interaction variables and factorial regression variables. ''Global'' interaction variables, the plant response to N deficiency, were calculated for each trait in each combination of location and year as the difference between the value under N^+ and the value under N^- (defined as N^+–N^-, and abbreviated as G × N), and as the ratio between N^- and N^+ (N^-/N^+). The difference is an estimate of the G × N interaction term, whereas the ratio approximates the deviation of the linear relationship between the N^- and N^+ values.

Laperche et al. (2006a) showed that 1-NNI of Récital at flowering was the best indicator of the N stress level. Therefore, to evaluate the sensitivity to N stress of each DH line, as well as its performance under N-limited conditions, we performed a factorial regression (Denis 1988) for each trait, using NNI at flowering of Récital as an

environmental covariate. For each DH line *i*, the model was the following:

$$Y_{ij} = \alpha_i + \beta_i \, \mathrm{NNI}_j + e_{ij},$$

where Y_{ij} stands for the value of trait *Y* for the DH line *i* in the environment *j*, α_i is the regression ordinate, NNI_j is the nitrogen nutrition index recorded for Récital at flowering in environment *j* and e_{ij} is the residual. The slope β_i of the regression represented the sensitivity of the DH line *i* to the environmental covariate (Van Eeuwijk 1995) (i.e., in this case, the N condition) and the ordinate value for an abscissa equal to NNI = 0.5 represented its performance under a low N supply. The ordinate enabled a comparison of performance at an equal level of N deficiency. R^2 was also examined for each regression because it is indicative of the quality of interaction modeling, representing the proportion of variation explained by the model. Because the model describes the reaction to N, the non-explained variation was related to other limiting environmental factors.

Results

A variance analysis was carried out to determine the significance of genotype, N level and genotype × N level interaction in each combination of year and location. Results were reported in Laperche et al. (2006a) and showed that genotype and N level effects were significant for all traits in all environments and that genotype × nitrogen effect was significant except (a) in Mons in 2001 for grain protein yield, straw N amount, total N amount, grain protein content and straw N content, (b) in Mons in 2000 for straw N content, (c) in Chartainvilliers in 2000 for aerial dry matter, straw N amount and grain protein content, (d) in Clermont-Ferrand in 2001 for straw N content and (e) in Clermont-Ferrand in 2000 for grain protein yield. A preliminary data description is reported in Table 2 for Mons in 2000, and in Tables S1, S2, S3, S4, S5, and S6 for the other environments. Transgression was reported for all traits. The trial conducted at Chartainvilliers in 2000 under N^- showed low heritabilities for all traits except thousand kernel weight and grain protein content (Table S6). This might indicate some problems during the experimentation. Higher heritabilities were found under N^+ (from 0.42 to 0.97) than under N^- (from 0.20 to 0.97) and lower heritabilities were recorded for straw N content and straw N amount than for other traits.

Two hundred and thirty-three QTL were detected on the whole genome, mostly distributed in the vicinity of the dwarfing gene *Rht-B1* and the *Ppd-D1* gene

QTL detection was carried out for each of the 14 environments. Sixty-seven QTL were detected under N^+, and 51 QTL under N^-. Eighty-five QTL were detected for the two ''global'' interaction variables, N^+–N^- and N^-/N^+. Thirty QTL were detected, either for a factorial regression slope, r^2 or ordinate when the NNI of Récital was 0.5. A total of 233 QTL were detected on all homoeology groups (Table 3). QTL r^2 values ranged from 3.0 to 33.0%. The highest r^2 values were observed on linkage group (LG) 4B and 2D1.

LG 2D1 and 4B contained most of the QTL. These QTL clusters could be explained by the vicinity of the dwarfing gene *Rht-B1* on chromosome 4B, and of the *Ppd-D1* gene involved in sensitivity to photoperiod on linkage group 2D1. Thirteen QTL were also located on linkage group

Table 2 Mean, standard error of the difference between two means (SED), minimal and maximal values as well as heritabilities observed for the DH line population in Mons in 2000 compared between the two parents Arche and Récital

Traits	Mean	SED	Minimal value	Maximal value	Arche mean value	Récital mean value	Heritabilities
GY	**629.2**/480.3	0.71	**402.0**/246.1	**764.2**/575.7	**693.8**/529.3	**682.4**/506.8	**0.88**/0.78
GPA	**18,092**/13,396	27.3	**10,630**/7,377	**25,401**/16,888	**174,993**/13,207	**19,885**/14,917	**0.91**/0.86
TKW	**36.2**/34.9	0.047	**22.27**/25.53	**46.47**/46.3	**39.70**/40.12	**34.33**/34.02	**0.92**/0.96
ADM	**1,296**/1,000	1.25	**862**/552	**1,570**/1,258	**1,450**/1,070	**1,270**/995	**0.81**/0.62
NTOT	**16.24**/8.84	0.016	**11.41**/5.78	**20.15**/11.74	**17.38**/9.37	**16.38**/8.49	**0.69**/0.53
NS%	**0.64**/0.37	0.001	**0.46**/0.26	**0.85**/0.55	**0.65**/0.40	**0.61**/0.34	**0.61**/0.55
NSA	**1.94**/4.26	0.008	**2.68**/1.17	**8.06**/3.55	**4.91**/2.17	**3.56**/1.65	**0.65**/0.58
GPC	**10.83**/8.21	0.006	**9.23**/6.73	**12.43**/10.49	**10.26**/7.75	**10.72**/7.70	**0.79**/0.84
GPY	**68.40**/39.33	0.071	**44.0**/26.28	**84.30**/52.10	**52.10**/41.04	**72.96**/38.93	**0.80**/0.58
NHI	**0.74**/0.78	0.0002	**0.58**/0.68	**0.83**/0.86	**0.72**/0.77	**0.78**/0.81	**0.77**/0.67

Bold values correspond to N^+ and regular values to N^-

GY, Grain yield (g/m^2); *GPA*, grain per area (m^2); *TKW*, thousand kernel weight (g); *ADM*, aerial dry matter (g/m^2); *NTOT*, T total N amount (g/m^2); *NS%*, straw N content (%); *NSA*, straw N amount (g/m^2); *GPC*, grain protein content (%); *GPY*, grain protein yield (g/m^2); *NHI*, nitrogen harvest index

Table 3 Number of QTL detected per linkage group and for four different kind of variables: (1) trait evaluation under N^+; (2) trait evaluation under N^-; (3) global interaction variables: N^+–N^- values and N^-/N^+ values, (4) variables related to factorial regression using NNI of Récital at flowering as the regressor (factorial regression slope, r^2 and ordinate when the abscissa equaled an NNI of 0.5)

Linkage group	N^+ supply	N^- supply	Interaction	Factorial regression	Total of QTL	R^2 min	R^2 max
1A2	1				1	7.5	7.5
1B		4	3	2	9	6.0	10.7
2A1	2	3	1	1	7	5.3	10.4
2A2	4	3	1	1	9	6.5	10.7
2B1		1			1	7	7
2B2		1			1	6.9	6.9
2D1	13	11	17	4	45	6	27.2
2D2				1	1	5.3	5.3
3A	1				1	5.3	5.3
3B	2		3	2	7	5.3	9.1
3D	6	4	2		12	5.4	10.4
4B	26	15	38	12	91	4.9	33
5A1	5	2	1	4	12	4.6	11.7
5A2	2		9	1	12	3.0	9.0
5B	1	4	2	1	8	3.2	12.3
5D	1	1	2		4	5.9	7.6
6A	1		2		3	9.4	11.8
6B			1		1	9.0	9.0
7A1	1				1	9.2	9.2
7B1		1			1	6.8	6.8
7B2	1	1	2	1	5	5.2	15.7
7D3			1		1	13.3	13.3
Total of QTL	67	51	85	30	233		
R^2min	3.3	3.2	3.0	3.8			
R^2max	33.0	28.1	27.1	26.1			

5A2, coinciding with the phenotypic marker for awnedness (Ari) located at position 18.6 cM. *Rht-B1* was located on the genetic map at position 41.1 cM of chromosome 4B, using a specific PCR marker (Ellis et al. 2002). The *Ppd-D1* gene is located on chromosome 2D (Worland 1996 near genetic marker gwm484, as revealed by QTL analysis for photoperiod sensitivity with the Renan × Récital genetic map (Hanocq et al. 2004). The gwm484 marker was also located on the Arche × Récital genetic map at position 40.5 cM on LG 2D1. This locus was involved in earliness control for the Arche × Récital population, as revealed by the relation to the QTL for earliness (data not shown).

To summarize QTL information, QTL meta-analyses were carried out. The highlighted meta-QTL (MQTL) were regrouped into genomic regions (Fig. 1; Tables 4, 5, 6 and 7). These regions were labeled according to the name of the linkage group to which they belong, extended by a number when two regions were located on the same linkage group. To distinguish two MQTL located in the same region, but that differed for their peak position, a letter was added to the genome region name.

Comparison of QTL detected under N^+ and N^- levels: thirteen non specific loci, eight N^+ specific loci and seven N^- specific loci were detected

The 67 QTL detected under an N^+ level were clustered in 22 genomic regions (MQTL) (Table 4). The 51 QTL detected under an N^- level clustered in 22 MQTL (Table 5). When the two MQTL sets were compared, eight loci were shown to be N^+ specific and seven to be N^- specific.

Thirteen loci were detected under both N^+ and N^-, located on LG 2A1, 2A2, 2D1, 3D, 4B, 5A1, 5B, 5D and 7B2 (Tables 4 and 5; Fig. 1). We considered that two QTL belonged to the same region when their confidence intervals overlapped. Straw N content QTL detected under N^+ as well as grain protein content and grain protein yield QTL detected under N^- colocalised on genome region 2A1 (Fig. 1), Arche allele increased grain protein content and grain protein yield under N^+, but decreased straw N content under N^-. The influence of this locus was moderate as the QTL were either N^- or N^+ specific and detected different traits. On genome region 2A2 (Fig. 1), seven QTL for grain protein content, grain yield and straw N content were identified. Grain protein content QTL were detected under both types of N supply and in each case, the favorable allele came from Arche. The genome region 2D1-1 grouped seven QTL for thousand kernel weight, straw N content and grain number. The Récital allele was favorable for thousand kernel weight under both N^- and N^+. Seventeen QTL were recorded on genome region 2D1-2 for six traits: N harvest index, straw N amount, grain yield, total N amount, grain number and grain protein content. The Récital allele improved grain protein content and N harvest index, and the Arche allele increased the others traits. The gwm484 marker was the closest on region 2D1-2b and was located near the *Ppd-D1* gene controlling photoperiod sensitivity. We can therefore suppose that *Ppd-D1* was located within the confidence interval of genome region 2D1-2. Genome region 3D-1 contained three QTL for grain number, detected under either N^+ or N^- and a QTL for grain protein content under N^+ Genome region 3D-2 contained thousand kernel weight and grain number QTL detected under N^+ and N^-, each being improved by the presence of the Arche allele. Three regions were detected on chromosome 4B: 4B-1, 4B-2 and 4B-4. Region 4B-1 controlled grain number and thousand kernel weight under both N levels. The Arche allele improved thousand kernel weight and decreased grain number. The highest number of

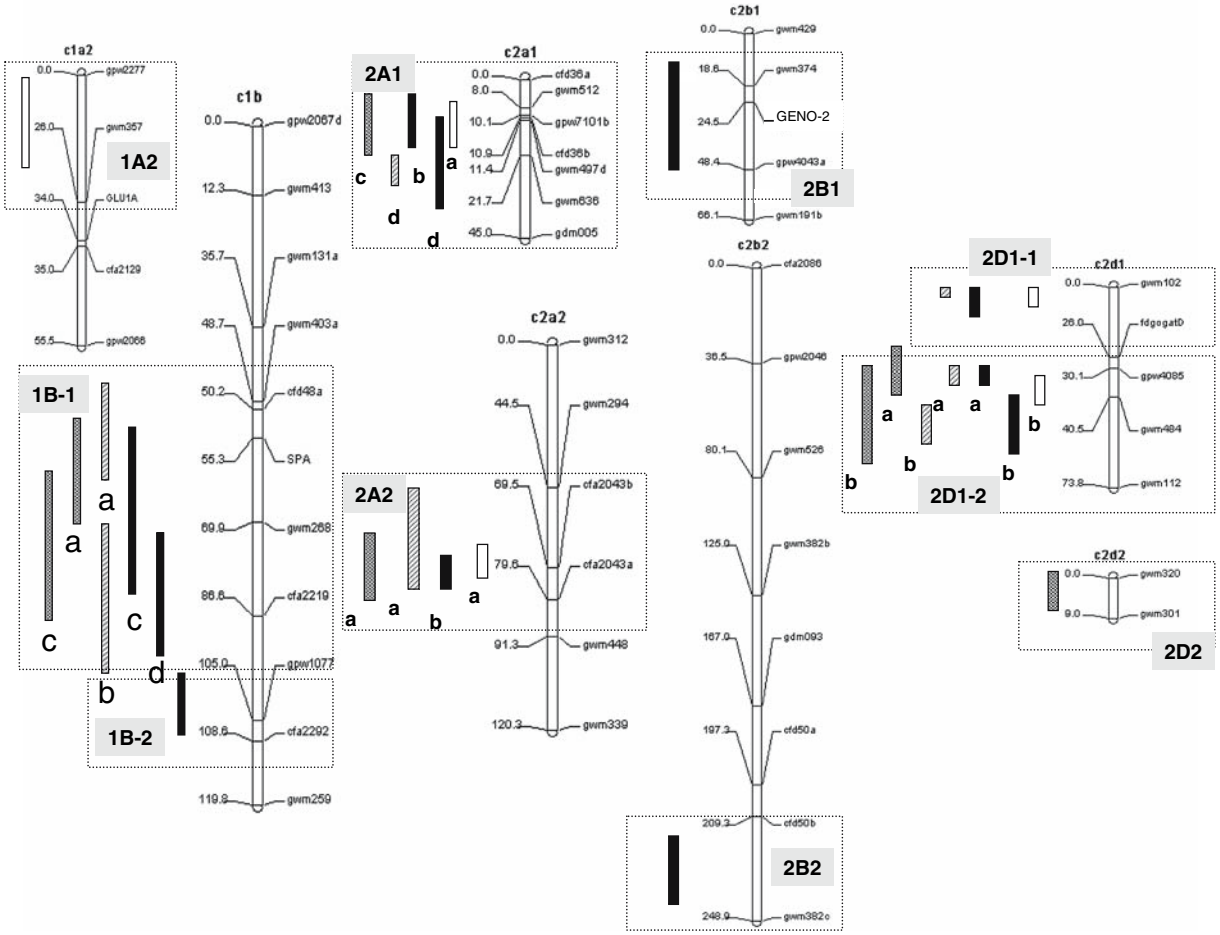

Fig. 1 Results of the four QTL meta-analyses. Only linkage groups on which QTL were detected are shown. All linkage groups are not represented at the same scale. QTL are located to the left of the linkage group or chromosome to which they belong. *White* QTL were detected under N^+, *black* QTL under N^-, *striped* for interactions and *grey* recorded for factorial regressions. Lengths of the rectangles refer to confidence intervals. Genome regions in Tables 4, 5, 6 and 7, are represented by *discontinuous rectangles* on which the names are reported

QTL was detected in region 4B-2. This might be in the vicinity of the major gene *Rht-B1*. QTL were detected for all investigated traits except grain protein content. The third region 4B-4 was detected under both N levels and contained three QTL: two for grain protein content (N^+ and N^-) and one for grain number (N^+). Six QTL were detected on region 5A1-3 for grain yield, straw N amount and straw N content, where the Récital allele improved grain yield. Three other regions recorded a smaller number of QTL: three QTL were reported on region 5B-2, two QTL on region 5D-2 and two thousand kernel weight QTL on region 7B2-2.

N^+ specific MQTL were recorded on genome regions 1A2, 3A, 3B-2, 5A1-2, 5A2-1, 6A-1 and 7A1 (Table 4). Each contained one or two QTL, for a total of nine. The Récital allele improved traits at genome regions 1A2 (grain protein content), 3A (grain number), 3B-2 (total N amount and grain protein content), 5A2-1 (thousand kernel weight), 6A (straw N amount) and 7A1 (straw N content). The Arche allele improved traits at regions 5A1-2 and 5A2-1 (grain yield and straw N amount).

N^- specific MQTL were identified on genome regions 1B-1, 1B-2, 2B1, 2B2, 5B-1 and 7B1(Table 5). Each contained one or two QTL. The Arche allele was favorable at regions 1B-1c (grain protein yield), 1B-1d (thousand kernel weight) and 1B-2 (straw N content). The Récital allele was favorable regions 1B-2 (grain protein yield), 2B1 (straw N content), 5B-1a (grain protein content) and 7B1 (straw N content).

QTL for ''global'' interaction variables: four ''adaptative'' loci were validated, eight ''constitutive'' loci were also involved in G × N interactions and seven new loci were detected

Plant response to N deficiency was first evaluated using two ''global'' interaction variables: N^+–N^- and N^-/N^+.

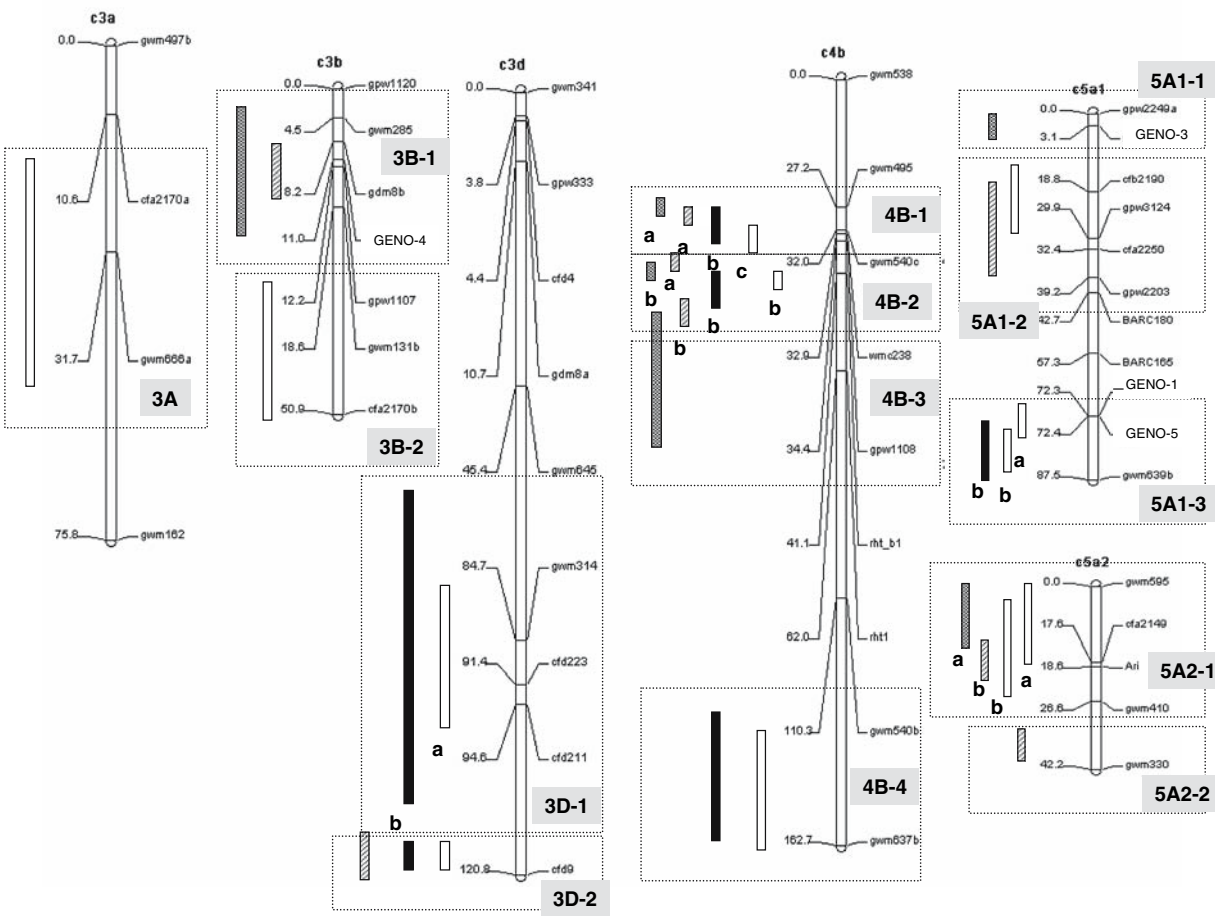

Fig. 1 continued

QTL detections were performed for these two variables with respect to all traits. The 85 QTL detected (Table 3) were grouped into 23 genomic regions (Table 6). Again (Tables 4, 5), most of the QTL were located near the three major genes that segregated in the population: *Ppd-D1* (2D1-2), *Rht-B1* (4B-2) and awnedness (5A2-1).

When these results were compared with the separate analysis at N^+ and N^-, three observations could be made: (a) loci that were characterized as adaptative or interactive were also detected with this new data set, (b) some loci that were characterized as constitutive, or non-interactive, were involved in the plant reaction to N deficiency, and (c) new loci were detected using this new data set.

Five loci characterized as N^+ or N^- colocalised with QTL for ''global'' interaction traits: with N^+ regions and two QTL with N^- regions (Fig. 1).

N^+ QTL and ''global'' interaction QTL were identified on genome regions 5A1-2, 5A2-1 and 6A-1. Region 5A1-2 contained a N^+ QTL for grain yield and an interaction QTL for total N amount. Region 5A2-1 contained eight interaction QTL for thousand kernel weight (5 QTL), grain yield and straw N amount, as well as two N^+ QTL for straw N amount and thousand kernel weight. The awnedness marker ''Ari'' is 1 cM away from 5A2-1b peak for thousand kernel weight under increasing N stress. The allelic effect was the difference between the means of the two allele classes (Arche–Récital). N stress intensity was characterized by the 1-NNI value for Récital at flowering (Fig. 2). When the N stress was low (1-NNI close to 0), the Récital allele was favorable to thousand kernel weight, whereas the Arche allele was favorable when N stress was high (1-NNI close to 1). Region 6A-1 contained N^+ and ''global'' interaction QTL for straw N amount.

N^- QTL and ''global'' interaction QTL were identified on genome regions 1B-1 and 5B-1. Region 1B-1 contained N^- (Table 5) and ''global'' interaction QTL (Table 6) both for thousand kernel weight and grain protein yield. Récital allele increased the G × N interaction term for thousand kernel weight. The Arche allele increased the interaction term for grain protein yield. Confidence intervals of MQTL on region 5B-1 (Tables 5, 6) only overlapped by 1 cM.

Eight regions identified as constitutive were also involved in G × N interactions: 2A1, 2A2, 2D1-1, 2D1-2, 3D-2, 4B-2, 5B-1 and 5D-2. Region 2A1 contained QTL

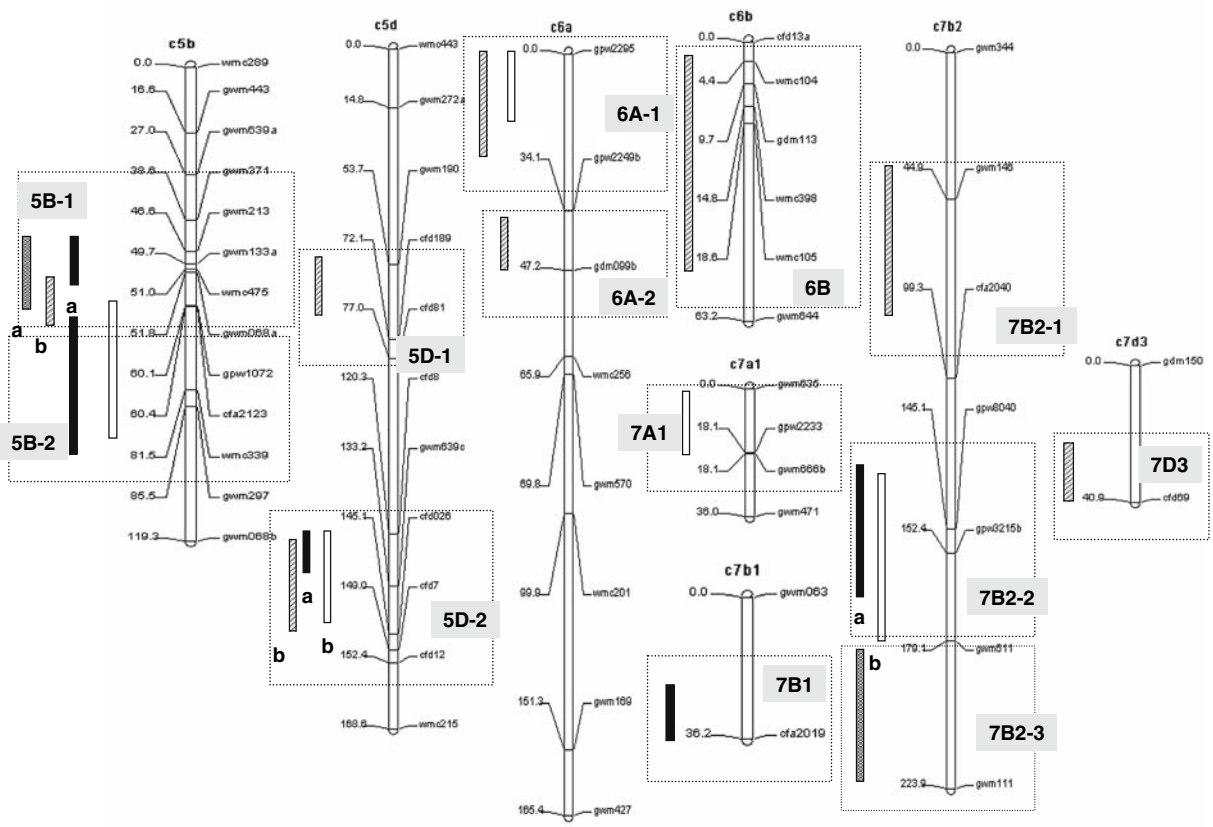

Fig. 1 continued

detected under both N^+ and N^- (grain number, grain protein content), but only one QTL, straw N content, was N^+ specific. This straw N content QTL was difficult to interpret as the high straw N content value may have been due to several factors: a high N absorption capacity, a low remobilization capacity or low biomass production associated with a nitrogen dilution effect (Justes et al. 1994). Region 2A2 contained ''global'' interaction QTL (straw N content) and QTL detected under N^+ and N^- (grain protein content, grain yield, straw N content). ''Global'' interaction QTL (grain yield, grain protein content, total N amount) and N^- and N^+ QTL (thousand kernel weight, straw N content, grain number) were identified on genome region 2D1-1. On region 2D1-2 13 QTL for ''global'' interaction variables and 17 QTL were detected under either N^+ or N^-. QTL were identified on region 3D-2 for N^+ and N^- for grain number, grain protein yield, grain yield, grain protein content and for G × N interaction for grain yield and straw N content. A large number of QTL detected under N^+ and N^- as well as for interaction variables on region 4B-2 were located in the vicinity of *Rht-B1*. On genome region 5B-1, constitutive and ''global'' interaction QTL, grain number and grain yield, were clustered. A G × N interaction QTL for straw N content was identified on region 5D-2.

QTL analysis for interaction variables enabled detection of seven chromosome regions not revealed by analyses of N^+ and N^-. Grain number stability, grain protein yield and total N amount G × N interaction QTL were identified on 3B-1. Region 4B-2 also contains marker *Rht-B1* and region 5A2-2 controlled the grain protein content interaction. The other regions were 5D-1 (grain protein yield), 6A-2 (thousand kernel weight), 6B (straw N amount), 7B2-1 (straw N amount and straw N content) and 7D3 (grain protein content).

Nine interactive loci were validated and three new loci detected using factorial regression variables

Factorial regression variables were assessed to characterize G × N interactions. Laperche et al. (2006a) showed that a factorial regression using the NNI of Récital at flowering as an environmental covariate was a good method to characterize plant sensitivity to N stress and its performance under N-limited conditions. N stress intensity was not the same in all environments (Table 8). For instance, the N stress intensity observed in Le Moulon in 2001 (1-NNI = 0.09) under an N^- supply was less than those observed at other sites under an N^+ supply: Mons 2000 (0.23) and 2001 (0.29), Clermont-Ferrand, 2001 (0.17). We performed QTL

Table 4 QTL identified for N^+

Genome region	Closest marker	Position (cM)	Confidence interval (cM)	No. of QTL	Lod (mean/ median)	Traits	R^2 max	Favorable allele
1A2	gpw2277	0	0–19	1	3.6	GPC(Moul00)	7.5	R
2A1a	*gwm497d*	*12*	*6–19*	*2*	*3.5/3.5*	*NS%(Moul00, Cler01)*	*7*	*R*
2A2a	*cfa2043b*	*69*	*63–74*	*4*	*3.6/3.5*	*GPC(Cler01, Moul01), GY(Cler01), NS%(Mons00)*	*10*	*A(GPC) R(GY, NS%)*
2D1–1	*gwm102*	*2*	*0–8*	*3*	*4.7/4.7*	*TKW(Moul01, Cler01, Mons01)*	*10.2*	*R*
2D1–2b	*gwm484*	*38*	*34–43*	*10*	*4.4/3.9*	*NHI(Moul01), NSA(Mons00, Moul00, Moul01, Mons01), GY(Cler01), NTOT(Mons00), GPA(Cler01, Moul01), GPC(Cler01)*	*13.6*	*A(NSA, NTOT, GPA) R(NHI, GPC)*
3A	gwm666a	32	17–50	1	3.4	GPA(Cler01)	5.3	R
3B-2	cfa2170b	42	30–50	2	3.3/3.3	NTOT(Nick00), GPC(Moul01)	8.1	R
3D-1a	*cfd223*	*91*	*81–100*	*4*	*4.2/4.1*	*GPC(Mons01), GPA(Mons01, Moul00)*	*9.9*	*A(GPA) R(GPC)*
3D-2	*cfd9*	*121*	*117–121*	*2*	*4.0/4.0*	*GPA(Cler01), GY(Cler01)*	*7.9*	*A*
4B-1c	*wmc238*	*33*	*30–37*	*6*	*5.8/5.1*	*NS%(Moul01), GPA(Mons01, Moul01), GY(Mons00), GPY(Mons01), TKW(Nick00)*	*19.8*	*A(TKW, GPY) R(NS%, GPA, GY)*
4B-2b	*rht-B1*	*42*	*41–43*	*19*	*9.4/8.4*	*NTOT(Moul00), GPA(Mons00, Nick00, Moul00, Mons00, Cler01, Cler00, Moul01, Cler01), GY(Moul01), TKW(Cler01, Moul01, Moul00, Mons00, Cler00, Mons01), NS%(Nick00, Moul00), NSA(Cler01)*	*33*	*A(TKW, NSA) R(GPA, GY, NTOT)*
4B-4	*gwm367b*	*162*	*136–162*	*1*	*3.2*	*GPC(Moul01)*	*9.5*	*A*
5A1-2	gpw3124	25	11–30	1	3.9	GY(Nick00)	8.8	A
5A1-3a	*GENO-1*	*73*	*68–78*	*3*	*4.6/5.3*	*NSA(Cler01), NS%(Cler01, Mons00)*	*11.7*	*A*
5A1-3b	*gwm639b*	*86*	*75–86*	*1*	*2.9*	*GY(Cler00)*	*5.7*	*R*
5A2-1a	gwm595	6	0-18	1	3.3	NSA(Mons01)	3.33	A
5A2-1b	cfa2149	18	4–25	1	3.9	TKW(Nick00)	17.6	R
5B-2	*wmc339*	*72*	*60–95*	*1*	*3.3*	*TKW(Cler01)*	*3.29*	*A*
5D-2b	*gwm639c*	*130*	*122–143*	*1*	*3.9*	*NS%(Moul00)*	*7.5*	*A*
6A-1	gpw2295	0	0–16	1	4.3	NSA(Nick00)	11.8	R
7A1	gwm635	8	0–18	1	3.1	NS%(Moul01)	9.2	R
7B2-2b	*gpw3215b*	*149*	*127–178*	*1*	*3.6*	*TKW(Cler00)*	*5.7*	*A*

No. QTL is the number of QTL contained in the considered QTL, and traits are the traits for which those QTL were detected. R^2max represents the maximum r^2 of all QTL in the QTL, and fav allele indicates the parent that provided the favorable allele at the considered QTL for the trait(s) indicated in brackets. R represents Récital and A represents Arche. Genome regions are also reported on Fig. 1. QTL common to N^+ and N^- are represented in italics

Mons00, Mons in 2000; *Mons01*, Mons in 2001; *Moul00*, Le Moulon in 2000; *Moul01*, Le Moulon in 2001; *Cler00*, Clermont-Ferrand in 2000; *Cler01*, Clermont-Ferrand in 2001; *Nick00*, Nickerson site in 2000; *GPC*, grain protein content; *NS%*, straw nitrogen content; *TKW*, thousand Kernel weight; *NSA*, nitrogen amount in the straw; *GY*, grain yield, *NTOT*, total amount of nitrogen; *GPA*, number of grain per area (m^2); *GPY*, grain protein yield; *ADM*, aerial dry matter; *NHI*, nitrogen harvest index

detection for factorial regression parameters (r^2, slope and ordinate for an abscissa corresponding to a Récital NNI value of 0.5). Thirty QTL were detected (Table 3) and clustered into 14 genomic regions (Table 7).

Regions 1B-1 and 5A2-1, which were shown to be interactive both by comparing N^- and N^+ and by using ''global'' interaction variable, were also interactive with QTL for factorial regression variables. On region 1B-1 QTL related to plant performance under an N^- supply for total N amount and grain protein yield coincided with a N^- QTL for grain protein yield. Arche allele improved the grain protein yield value under N^-. QTL for grain yield factorial regression r^2 and ''global'' interaction were identified on region 5A2-1. At this locus, the Récital allele increased the factorial regression r^2.

Genome regions 2A1, 2A2, 2D1-2, 4B-1, 4B-2 and 5B-1 were initially considered as constitutive, and also involved in the G × N interaction (Table 7). They also coincided with MQTL for factorial regression variables. On region 2A1, the overlap between factorial regression and the other

Table 5 QTL identified under N^-

Genome region	Closest marker	Position (cM)	Confidence Interval (cM)	No. of QTL	Lod (mean/ median)	Traits	r^2max	Favorable allele
1B-1c	gwm268	66	54–84	1	4.3	GPY (Cler01)	8.5	A
1B-1d	cfa2219	84	73–94	2	3.1/3.1	TKW (Mons01, Moul00)	6.4	A
1B-2	cfa2292	107	95–109	1	3.1	GPY(Cler00)	10.73	R
2A1b	*gpw5122*	*11*	*4–18*	*2*	*3.1/3.1*	*GPC(Mons00, Mons01)*	*5.5*	A
2A1d	*gwm636*	*22*	*11–34*	*1*	*3.0*	*GPY(Moul00)*	*7.2*	A
2A2b	*cfa2043*	*64*	*64–76*	*3*	*3.7/3.4*	*GPC(Nick00, Cler01, Moul01)*	*10.7*	A
2B1	GENO-2	35	10–48	1	3.2	NS%(Moul01)	7	R
2B2	gwm382c	247	233–247	1	3.0	NS%(Moul01)	6.9	A
2D1-1	*gwm102*	*7*	*2–11*	*4*	*8.2/8.6*	*TKW(Moul01, Cler01), NS%(Moul01), GPA(Cler01)*	*27.2*	*A(GPA) R(TKW, NS%)*
2D1-2a	*gpw4085*	*32*	*27–37*	*4*	*6.7/5.0*	*GPC(Moul01), NSA(Mons00), GPA(Cler01, Moul01)*	*24.7*	*A(NSA, GPA) R(GPC)*
2D1-2b	*gwm484*	*51*	*41–62*	*3*	*6.7/7.3*	*NSA(Moul01), GY(Cler01), GPC(Cler01)*	*22*	*A(NSA, GY) R(GPC)*
3D-1b	*gwm314*	*85*	*67–113*	*1*	*3.0*	*GPA(Moul01)*	*5.4*	A
3D-2	*cfd9*	*121*	*117–121*	*3*	*4.2/4.3*	*GY(Cler01), GPA(Cler01), GPC(Moul01)*	*8.5*	*A(GPA, GY) R(GPC)*
4B-1b	*gwm540c*	*30*	*26–34*	*2*	*4.8/4.8*	*GPA(Moul00), TKW(Cler01)*	*10.2*	*A(TKW) R(GPA)*
4B-2b	*rht-B1*	*43*	*41–46*	*11*	*6.2/5.4*	*NS%(Moul01, Mons00), TKW(Moul01, Mons00, Cler00, Moul00, Nick00), GPA(Moul01, Moul00, Mons00), GY(Cler00)*	*28.1*	*A(TKW, GY) R(NS%, GPA)*
4B-4	*gwm367b*	*148*	*134–162*	*2*	*3.5/3.5*	*GPA(Cler01), GPC(Moul01)*	*11.3*	*A(GPC) R(GPA)*
5A1-3b	*gwm639b*	*81*	*75–87*	*2*	*3.5/3.5*	*NS%(Mons00), GY(Cler01)*	*7*	*A(NS%) R(GY)*
5B-1a	gwm133a	50	44–55	2	4.0/4.0	GPC(Mons00, Cler01)	9.4	R
5B-2	*wmc339*	*80*	*64–97*	*2*	*3.7/3.7*	*TKW(Cler01), GPC(Mons00)*	*4.18*	*A(TKW) R(GPC)*
5D-2a	*cfd8*	*120*	*119–132*	*1*	*3.57*	*GPY(Mons01)*	*7.6*	R
7B1	cfa2019	36	22–36	1	3.64	NS%(Moul01)	6.8	R
7B2-2a	*gpw8040*	*147*	*125–166*	*1*	*3.33*	*TKW(Moul01)*	*5.2*	A

For a more detailed description, see Table 4. QTL common to N^+ and N^- are represented in italics

QTL covered only 1 cM. The factorial regression region contained a QTL for grain protein content values under a low N (INN = 0.5). This QTL value was improved by the ''Arche'' parental allele. The 2A1 region also contained N^- specific QTL for grain protein content that were improved by the Arche allele. On region 2A2, the factorial regression QTL (grain protein content value under a low N supply) coincided with QTL for straw N content, grain protein content and grain yield. Region 2D1-2 recorded two factorial regression QTL, grain number and aerial dry matter. On region 4B-1, a factorial regression QTL was detected for total N amount, grain number, grain protein yield and grain protein content. Six QTL controlling total N amount, grain number, grain protein yield and aerial dry matter, were identified on region 4B-2. On region 5B-1, factorial regression QTL for grain protein content partly overlapped with ''global'' interaction QTL (grain number, grain yield) and N^- QTL (thousand kernel weight, grain protein content).

Region 3B-1 was only highlighted by factorial regression, grain number and aerial dry matter, and ''global'' interaction QTL, grain number, grain protein yield, total N amount.

Finally, this method highlighted three new interactive regions that had not been detected using previous variables: 2D2 (grain protein yield), 5A1-1 (total N amount, grain protein content, grain number) and 7B2-3 (grain number).

The 82 MQTL detected (Tables 4, 5, 6, 7) were distributed into 37 genome regions. We considered that two QTL corresponded to the same genome region when their confidence intervals overlapped by more than 1 cM. Regions 1B-1, 2A1, 2D1-2, 4B-1, 4B-2 and 4B-3, as well as 5B-1 and 5B-2, were difficult to determine because of the high density of QTL and differences in the lengths of confidence intervals. Regions that only comprised one QTL had longer confidence intervals than the others. The reduction in confidence interval length was a consequence

Table 6 QTL (N^+–N^- also named G × N, and N^-/N^+)

Genome region	Closest marker	Position (cM)	Confidence interval (cM)	No. of QTL	Lod (mean/median)	Traits	r^2max	Fav allele
1B-1a	*SPA*	*54*	*46–62*	*2*	*3.2/3.2*	*TKW(G × N Moul01), GPY(G × N Mons00)*	*6.85*	*A(GPY) R(TKW)*
1B-1b	*cfa2292*	*86*	*70–97*	*1*	*3.1*	*GPC(N–/N+ Moul01)*	*6.34*	A
2A1d	gwm636	22	21–28	1	3.9	NTOT(N–/N+ Moul00)	10.35	A
2A2a	cfa2043b	64	46–78	1	3.4	NS%(G × N Mons00)	9.97	R
2D1–1	gwm102	1	0–5	4	3.9/4.1	GY(G × N Moul01, N–/N+ Moul01), GPY(G × N Moul01), NTOT(G × N Moul01)	9.79	A(GY-N–/N+) R(GPY, NTOT, GY-G × N)
2D1-2a	gwm4085	33	30–36	7	4.1/3.9	GY(G × N Cler01, N–/N+Cler01), GPC(G × N Mons00, N–/N+ Mons01), NSA(G × N Moul00), TKW(N–/N+Moul01), NS%(N–/N+ Nick00)	11.2	A(NSA, NS%, GY-N–/N+) R(TKW, GPC, GY-G × N)
2D1-2b	gwm484	51	45–57	6	5.6/4.6	GPA(G × N Cler01, N–/N+Cler01), TKW(G × N Cler01, N–/N+ Cler01), GPY(G × N Nick00), NTOT(G × N Nick00)	25.1	A(TKW, GPA-N–/N+) R(GPY, NTOT, GPA-G × N)
3B-1	gpw1107	13	8–17	3	3.3/3.4	GPA(N–/N+ Cler01), GPY(G × N Nick00), NTOT(G × N Nick00)	7.9	A(GPA) R(GPY)
3D-2	cfd9	121	117–125	2	4.1/4.1	GY(G × N Mons01), NS%(N–/N+Mons01)	10.4	A(GY) R(NS%)
4B-1a	gwm495	28	27–30	9	5.9/5.1	NSA(N–/N+Moul01, G × N Cler01), GPA(G × N Mons01, Cler00), GY(G × N Cler00), GPC(G × N Moul00), TKW(N–/N+ Moul00, Moul01, Mons01)	17.48	A(NSA, TKW) R(GPA, GY, GPC)
4B-2a	gpw1108	37	36–39	18	6.8/5.1	GPA(G × N Moul00, Cler00, Moul01, Mons00, Mons01, N–/N+Moul00, Moul01, Cler00), NTOT(N–/N+Moul01, G × N Moul00 Moul01), GY(G × N Cler00, N–/N+ Moul01 Cler00), NSA(G × N Moul00), GPY(G × N Moul01), TKW (N–/N+ Mons01)	27.1	A(GPA-N–/N+, NTOT-N–/N+, GY-N–/N+, TKW) R(GPA-G × N, NTOT-G × N, GY-G × N, NSA, GPY)
4B-2b	rht-B1	49	46–53	11	6.3/6.3	NS%(G × N Nick00, Moul00), GPC(G × N Mons01, Mons00, N–/N+ Mons01), NTOT(G × N Mons01), GPY(N–/N+ Mons01, G × N Mons01), GPA(G × N Mons00, Nick00, Moul01)	26.06	A(GPC, NTOT, GPY-G × N) R(NS%, GPY-N–/N+)
5A1-2	*gpw3124*	*25*	*17–39*	*1*	*2.96*	*NTOT (G × N Mons01)*	*6.56*	A
5A2-1b	*cfa2149*	*18*	*13–22*	*8*	*4.5/4.2*	*TKW(N–/N+Mons00, Moul01, Moul00, Nick00), GY(N–/N+Nick00, Cler01, G × N Nick00), NS%(G × N Mons01)*	*8.99*	*A(GY-N–/N+, NS%) R(TKW, GY-G × N)*
5A2-2	gwm330	41	33–41	1	3.3	GPC(G × N Cler01)	6.85	A
5B-1b	*cfa2123*	*60*	*54–66*	*2*	*3.7/3.7*	*GPA(G × N Mons01), GY(N–/N+Mons01)*	*7.6*	*A(GY) R(GPA)*
5D-1	gwm190	54	53–66	1	3.16	GPY(G × N Cler01)	7.6	R
5D-2b	gwm639c	133	122–145	1	3.57	NS%(G × N Moul00)	5.9	A
6A-1	*gpw2295*	*6*	*0–22*	*1*	*3.06*	*NSA(G × N Nick00)*	*9.8*	R
6A-2	gdm099b	46	36–47	1	4.45	TKW(N–/N+ Nick00)	9.4	A
6B	wmc398	14	4–51	1	3.51	NSA(G × N Mons00)	9.01	R
7B2-1	gwm146	59	37–81	2	3.0/3.0	NSA (N–/N+ Nick00), NS%(N–/N+ Nick00)	15.7	A(NSA) R(NS%)
7D3	cfd69	40	32–40	1	3.5	GPC(G × N Cler00)	13.3	A

The type of interaction is in brackets in the ''Traits'' column. For a more detailed description, see Table 4. The QTL that colocalised with N^+ or N^- QTL are represented in italics. The QTL detected using global interaction variables that did not colocalised with N^+ or N^- QTL are underlined. For example region 1B-1 (in italic) was identified as an interactive region as it gathered (a) no QTL detected under N^+ (Table 4), (b) two QTL detected under N^- (1B-1c and 1B-1d, Table 5), and two QTL for interaction variables (1B-1a and 1B-1b). Region 3B-1 (underlined) gathered no QTL detected either under N^+ or N^- (Tables 4, 5) and a QTL for interaction variable

Table 7 QTL for factorial regression

Genome region	Closest marker	Position (cM)	Confidence interval (cM)	No. of QTL	Lod (mean/ median)	Traits	r^2max	Fav allele
1B-1a	SPA	57	51–70	1	3.1	GPY(ordinate NNI = 0.5)	6	A
1B-1c	gwm268	78	59–87	1	3.4	NTOT(ordinate NNI = 0.5)	7.2	A
2A1c	gwm497d	11	4–22	1	3.3	GPC(ordinate NNI = 0.5)	5.4	A
2A2a	cfa2043b	72	61–78	1	3.5	GPC(ordinate NNI = 0.5)	8.3	A
2D1-2a	gpw4085	31	21-40	3	5.1/3.7	ADM(r^2), GPA(ordinate NNI = 0.5), GPA(r^2)	17.9	
2D1-2b	gwm484	53	30–65	1	5.5	ADM(ordinate NNI = 0.5)	18.1	A
2D2	gwm320	0	0–6	1	3.4	GPY(r^2)	5.3	R
3B-1	gpw1107	13	3–23	2	3.4/3.4	GPA(r^2), ADM(r^2)	9.1	R
4B-1a	gwm495	27	25–29	5	4.4/4.2	NTOT(slope), GPY(slope), GPA(r^2), GPC(r^2, slope)	11.3	R
4B-2b	rht-B1	40	38–42	6	8.6/7.6	GPA(slope), GPA(ordinate NNI = 0.5), GPA(r^2), GPY(r^2), NTOT(r^2), ADM(r^2)	26.1	R
4B-3	Rht1	61	47–84	1	3.3	NTOT(ordinate NNI = 0.5)	6.4	A
5A1-1	GENO-3	3	0–6	4	3.6/3.6	NTOT(ordinate NNI = 0.5), GPC(slope), GPC (r^2), GPA(slope)	8	R except NTOT
5A2-1a	gwm595	0	0–14	1	3.8	GY(r^2)	3.8	R
5B-1a	gwm133a	50	43–60	1	6.9	GPC(ordinate NNI = 0.5)	12.3	R
7B2-3	gwm111	20	181–223	1	3.1	GPA(slope)	9.3	R

The type of factorial regression variable related to each detected QTL is indicated in brackets in the ''Traits'' column. For a more detailed description, see Table 4. QTL detected using only factorial regression variables are underlined

of QTL meta-analysis. We therefore grouped these QTL into regions, even if sometimes one QTL-MQTL overlapped two regions (4B-2, 4B-3, 5B-1 and 5B-2). In other cases, it was difficult to distribute the QTL into different groups, and all QTL were grouped in one region (1B-1 and 2A1). This clustering revealed some adaptive regions detected using the three methods (comparison between N^+ and N^- QTL, ''global'' interaction QTL, and factorial regression QTL): 1B-1, 5A-2 and 5B-1 and constitutive regions, 3D-1, 4B-4, 5A1-3, 5B-2 and 7B2-2, that were detected under both N^+ and N^- supplies and not by any interaction variable. We identified regions that were first considered as constitutive, by comparing N^+ and N^- QTL, and that were shown to be involved in G × N interactions by one type of interaction variable: 2A1, 2A2, 2D1-1, 2D1-2, 3D-2, 4B-1, 4B-2 and 5D-2. Some N^- QTL were not involved in interactions: 1A2, 1B-2, 2B1, 2B2, 3A, 3B-2, 7A1 and 7B1. And finally, some adaptative regions were only detected using interactive variables: 2D2, 3B-1, 4B-3, 5A1-1, 5A2-2, 5D-1, 6A-2, 7B2-1 and 7D3.

Discussion

In this study, we aimed at distinguishing genomic regions specifically involved in the control of nitrogen use efficiency at a specific N level from those expressed under all nitrogen conditions. Regions detected specifically under a N condition are more probably involved in plant adaptation to nitrogen constraint. The others may be involved in constitutive processes, for which polymorphism exists between the two parents Arche and Récital, but that may not be involved in plant response to N stress. Therefore, we used a mapping population obtained from the cross between two contrasted cultivars for nitrogen use efficiency (Le Gouis et al. 2000; Laperche et al. 2006a). This population allowed to detect a large set of QTL, gathered into 37 genomic regions. However, more than half of all QTL detected were regrouped on two chromosomes (90 on 4B and 45 on 2D1), in the region of the dwarfing gene *rht-B1* (chromosome 4B) and the photoperiod sensitivity gene *Ppd-D1* (2D1). These genes might have impacted the QTL detection and hidden other genomic regions potentially interesting. Their effects were however minimized by the use of composite interval mapping instead of simple interval mapping for QTL detection. We initially separated the population into two sub-populations using genotyping data at *Rht-B1* locus as selection criterion. However, we observed, in those cases, that the sub-population size was too small for QTL detection as few QTL were conserved between the two populations (independently of chromosomes 4B and 2D1). Therefore, we decided to consider the whole population. In the future, we would check the parental allelic composition for major genes to choose a suitable population. The same approach could be conducted for *Ppd-D1*.

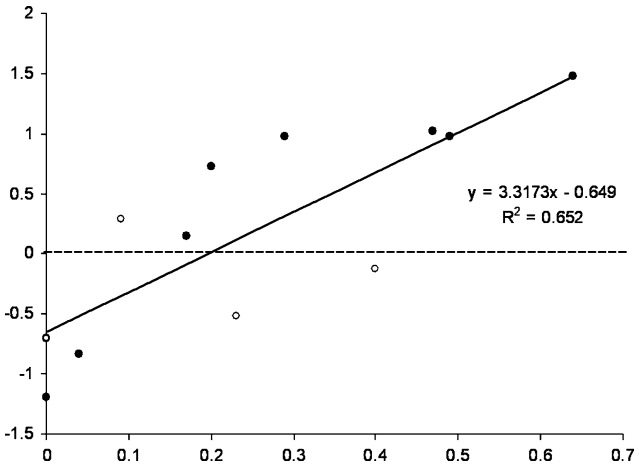

Fig. 2 Dynamics of the allele effect regarding thousand kernel weight, at the awnedness marker on the 5A2 linkage group, in line with N stress intensity. Differences between Arche and Récital allele classes mean values are represented on the ordinate. N stress intensity, assessed by 1-NNI of Récital at flowering, is reported on the abscissa. *Filled circle* represents environments where the difference between Arche and Récital classes were significant at the 5% level using ANOVA. *Open circles* represent environments where the differences were not significant

QTL-meta-analysis: a tool to summarize large QTL datasets

In this study, QTL meta-analysis was shown to be a useful tool to summarize a large set of QTL (233) into 82 MQTL corresponding to only 37 genome regions (Fig. 1). Because we separated the meta-analysis between interaction variables (''global'' interaction variables and factorial regression variables) and variables estimated in each environment, we were able to distinguish those regions involved in plant reaction to N stress. Initially, QTL meta-analysis had been developed to enable a more accurate estimate of QTL positions using independent datasets obtained for a single trait or related traits (Goffinet and Gerber 2000). Moreover, two QTL detected during the same experiment for the same trait and on the same chromosome were not to be considered in the analysis. However, Goffinet and Gerber (2000) showed that meta-analysis was robust enough to be used for non-independent datasets; we therefore used this software for data obtained on the same population in different environments, and for traits that were not directly related. In the case of two QTL detected by the same experiment, on the same linkage group but at different positions, we checked that they were not grouped in the same MQTL. Only MQTL on region 3D-1a did not comply with the latter rule, as it was composed of two QTL detected in the same environment for the same trait (grain protein content). To conclude, we used the QTL meta-analysis method in a way other than that for which it was constructed, but still complied with the conditions for running this analysis, in view of its robustness. Because the MQTL were obtained with a larger number of QTL than what is usually used, and because the QTL were not independent, careful consideration was given to MQTL confidence interval values. An increase in the number of QTL within an MQTL would reduce its length. Moreover, grouping the QTL into MQTL was based on the modified Akaike criterion proposed by (Goffinet and Gerber 2000), and in some cases the differences between n and $n + 1$ MQTL models were weak, suggesting that another QTL extending into the MQTL could have been proposed (especially on 2D1 and 4B that contained a large number of QTL), and this would have changed the MQTL position and confidence intervals.

Combining interaction and factorial regression variables: a method to assess QTL × N interactions

During this study, we compared three approaches to studying QTL × N interactions. The first was to compare two QTL sets detected under the two N levels. The second was to consider variables such as ''N^+–N^-'' and N^-/N^+, computed for each combination of location and year. This is an initial, simple method to consider environmental specificities and has already been used to characterize plant response or sensitivity to stress (Yan et al. 1999; Yadav et al. 2003; Lian et al. 2005). Factorial regression constituted the third approach. This method was relevant to the study of QTL × N interactions insofar as interactive regions revealed by the first approach were validated (1B-1, 2A2, 2D1-2, etc.), and new interactive loci were detected (2D2, 5A1-1 and 7B2-3). We chose to apply factorial regression rather than the more widely employed joint linear regression method (Finlay and Wilkinson 1963; Campbell et al. 2003; Quarrie et al. 2005), because it has

Table 8 [1-NNI of Récital at flowering] values observed in each combination of a year, a location and an N supply, as an indicator of N stress

	Mons 2000	Mons 2001	Moulon 2000	Moulon 2001	Clermont 2000	Clermont 2001	Nickerson 2000
1-NNI (N^+)	0.23	0.29	0.00	0.00	0.04	0.17	–
1-NNI (N^-)	0.49	0.64	0.47	0.09	0.20	0.40	–

A null value indicates an absence of N stress in the tested environment. A value of 1 indicates the highest intensity of N stress. In our study, a value of 0.5 was linked to a 30% yield reduction

been shown to be more efficient in ranking environments as a function of stress intensity (Laperche et al. 2006a). We therefore considered N status as the principal varying environmental parameter, and confounded QTL × environment interactions with QTL × N interactions. There is a clear need for more precise analysis of QTL × environment interactions, for example using factorial regression and environmental covariates as was first proposed for maize (Crossa et al. 1999), and then more widely applied (Groos et al. 2003; Campbell et al. 2004; Malosetti et al. 2004). QTL × environment interactions could also be analyzed further through the detection of QTL for the slope of factorial regressions, using other environmental covariates as regressor. Relevant environmental covariates, which are yield limiting, would be chosen for example after an analysis of probe genotype yield variation (Brancourt-Hulmel et al. 2001), as was achieved during the present study.

Impact of the candidate genes *Rht-B1*, *Ppd-D1* and *B1* on plant tolerance to N constraints

Three regions, on chromosome 4B-2 (47 QTL located in the vicinity of *Rht-B1* marker), 2D1-2 (20 QTL located near marker gwm484, in the vicinity of the *Ppd-D1* gene), and 5A2-1 (9 QTL located near marker cfa2149, in the vicinity of the phenotypic marker for awnedness), contained a large number of QTL.

At the dwarfing gene locus, the tall allele increased straw N amount as well as grain protein content and thousand kernel weight. The dwarf allele increased grain number and straw N content, and therefore grain yield and grain protein yield. This illustrated the negative correlation between grain yield and grain protein content and the competition to assimilate between grain number formation (grain number) and grain-filling (thousand kernel weight). The impact of *Rht-B1* on grain yield and grain number has already been demonstrated (Rebetzke and Richards 2000). However, competition for assimilates is not the only reason for differences in thousand kernel weight between dwarf and tall lines, as was shown by Miralles et al. (1998), according to whom, dwarf lines produce a larger number of grains per spikelet than tall lines. Grains with lower potential aborted in tall lines and survived in dwarf lines, due to a more favorable assimilate distribution. And because tall lines only filled high potential grains, this resulted in higher thousand kernel weight. The increased straw N content in dwarf lines may be related to an N dilution effect (Justes et al. 1994) linked to the reduction in internode length and straw height. Moreover, Lewicki and Chery (1992) found that N remobilization was less efficient in a barley dwarf compared to tall lines. Such differences could also explain an increase in straw N content in dwarf lines. The authors did not give any explanation for this observation, which needs to be confirmed in wheat near-isogenic lines, as differences in remobilization efficiency may also be due to different genetic backgrounds. *Rht-B1* was also involved in plant adaptation to N constraint, as this locus contained QTL for global interaction variables and factorial regression variables. The dwarf allele (Récital) was shown to increase G × N for grain protein yield and sensitivity to N constraint (slope of factorial regression) with respect to grain number, grain protein yield and total N amount, and the tall allele increased the total N amount value under a low N supply (QTL for factorial regression ordinate). We could therefore propose that tall plants are better adapted to a low N input. However, the dwarf allele also increased grain number under a low N supply (ordinate of factorial regression, QTL detected under N^-). The dwarfing gene, associated with the dwarf allele, has already been shown to play a role in plant adaptation to abiotic stresses, such as drought in wheat (Quarrie 2005) or nitrogen constraint in rice (Fang and Wu 2001).

Ppd-D1 coincided with seven QTL for G × N (G × N, ratio and slope of factorial regression) and thirteen other QTL detected under either N^- or N^+. Grain yield, grain number and straw N amount were increased by the Arche allele (delaying flowering), whereas grain protein content was increased by the Récital allele. Later growing plants may have had more time to benefit from late-stage N mineralization in the soil (Lecomte, personal communication). Significant correlations between earliness and grain yield, grain protein yield and grain protein content have also been reported in a QTL study of barley (Mickelson et al. 2003) but had different effects: late types exhibited higher grain protein content but lower grain yield and grain protein yield. In this case, lines with a longer grain-filling period could achieve more complete N remobilization. Moreover, in late-flowering lines, there was a more marked reduction in carbohydrate than in protein yield, leading to fewer or smaller grains but with a higher protein concentration (Mickelson et al. 2003).

Several QTL for grain yield and thousand kernel weight involved in G × N interactions were localized on chromosome 5A. The most probable candidate gene is the awn inhibitor *B1* gene. It has been shown that the presence of awns increases photosynthetic rate and allows the development of more assimilates that can be used for grain-filling (Motzo and Giunta 2002). We can hypothesize that at low N levels, where the number of grains is markedly reduced, grain weight is mostly limited by the assimilate availability during grain-filling. Awned genotypes would then be favored because awns are effective photosynthetic organs. It seems less clear why awnless genotypes have higher thousand kernel weight under high N levels but in the absence of QTL for grain number. One hypothesis

would be that awn growth may interact with the development of the flower or the grain at an early stage, thus limiting potential grain size.

Some QTL × environment interactions were not explained by QTL × N interactions

When considering each trait independently, genomic regions 4B-4 (Table S7), 5A1-3 (Table S8), 3D-1, 3D-2 (Table S10), 5B-2 and 7B2-2 (Table S13) were identified as stable. They are detected under N^+ and N^- and do not correspond to QTL for interaction variable or factorial regression. On genomic region 4B-4 (Grain Protein Content), 3D-1 (Grain number), and 7B2-2 (Thousand Kernel Weight), N^-–QTL were only detected at Le Moulon in 2001. However, we showed (Table 8) that N stress intensity in Le Moulon in 2001 under N^- was less intense than for instance in Clermont-Ferrand in 2001 under N^+. Therefore, those regions could be considered as N^+ specific. Region 5B-2 (Thousand Kernel Weight, Table S13), 5A1-3 (Straw N content, Table S8) and 3D-2 (Grain number, Table S10) showed stable QTL detected under N^+ and N^-, but those regions were specific of the environment (location and year): regions 5B-2 and 3D-2 were specific of Clermont-Ferrand in 2001 and region 5A1-3 was specific of Mons in 2000. We can hypothesize that these regions were also affected by QTL × environment interactions, but that nitrogen may not have been the main environmental limiting factor inducing the interaction. The stability of region 3D-2 when considering Grain number (Table S10) should also be taken with caution as this region was identified as ''interactive'' when considering GY (Table S9). Three other regions highlighted as ''interactive'' were also environment specific: regions 2A2, 5D-2 (straw N content, Table S8), and 6A-1 (Straw N amount, Table S12). Therefore, it would be interesting to use more intensively the environment description provided by the analysis of the probes genotypes, in order to characterize those environment specific QTL and the corresponding QTL × environment interactions.

Conclusion

It can be concluded that varieties exhibiting the tall allele at locus *Rht-B1*, the late allele at locus *Ppd-D1* and awns may be favored under the N-limited conditions we tested. These loci, to which considerable attention has been paid during breeding trials, have already been shown to be involved in crop adaptation to abiotic stresses such as N stress, salinity or drought (Quarrie et al. 2005). However, it is also important to take account of other genome regions detected by QTL analyses. Low N supply specific QTL were detected that corresponded either to QTL which were only detected under an N^- supply, or to QTL detected for factorial regression ordinate for a 0.5 NNI of Récital. For instance, regions 1B-1 and 1B-2 (thousand kernel weight and grain protein yield) and 2A1 for grain protein content and grain protein yield under N^- and straw N content under N^+. Improving grain protein content under N-limited conditions, but without decreasing grain yield, may be possible because these QTL did not colocalise with other negatively correlated QTL.

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References

Agrama HAS, Zakaria AG, Said FB, Tuinstra M (1999) Identification of quantitative trait loci for nitrogen use efficiency in maize. Mol Breed 5:187–195

Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004) Biomercator: integrating genetic maps and QTL towards discovery of candidate genes. Bioinformatics 20:2324–2326

Basten CJ, Weir BS, Zeng ZB (1994) Zmap-a QTL cartographer. Quel J 22:65–66

Basten CJ, Weir BS, Zeng ZB (2002) QTL Cartographer version 1.16

Bertin P, Gallais A (2001) Genetic variation for nitrogen use efficiency in a set of recombinant inbred lines. II-QTL detection and coincidences. Maydica 46:53–68

Boisson M, Mondon K, Torney V, Nicot N, Lainé A-L, Bahrman N, Gouy A, Daniel-Vedele F, Hirel B, Sourdille P, Dardevet M, Ravel C, Le Gouis J (2005) Partial sequences of nitrogen metabolism genes in hexaploid wheat. Theor Appl Genet 110:932–940

Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105:921–936

Brancourt-Hulmel M, Lecomte C, Denis JB (2001) Choosing probe genotypes for the analysis of genotype–environment interaction in winter wheat trials. Theor Appl Genet 103:371–382

Brancourt-Hulmel M, Heumez E, Pluchard P, Beghin D, Depatureaux C, Giraud A, Le Gouis J (2005) Indirect versus direct selection of winter wheat for low input or high input levels. Crop Sci 45:1427–1431

Campbell BT, Baezinger PS, Gill KS, Eskridge KM, Budak H, Erayman M, Dweikat I, Yen Y (2003) Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. Crop Sci 43:1493–1505

Campbell BT, Baenziger PS, Eskridge KM, Budak H, Streck NA, Weiss A, Gill KS, Erayman M (2004) Using environmental

covariates to explain genotype × environment and QTL × environment interactions for agronomic traits on chromosomes 3A of wheat. Crop Sci 44:620–627

Charmet G, Robert N, Branlard G, Linossier L, Martre P, Triboï E (2005) Genetic analysis of dry matter and nitrogen accumulation and protein composition in wheat kernels. Theor Appl Genet 111:540–550

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Crossa J, Vargas M, Van Eeuwijk FA, Jiang C, Edmeades GO, Hoisington D (1999) Interpreting genotype × environment interaction in tropical maize using linked molecular markers and environmental covariables. Theor Appl Genet 99:611–625

Denis JB (1988) Two ways analysis using covariates. Stat 19:123–132

Dhugga KS, Waines JG (1989) Analysis of nitrogen accumulation and use in bread and durum wheat. Crop Sci 29:1232–1239

Ellis M, Spielmeyer W, Gale K, Rebetzke G, Richards R (2002) ''Perfect'' markers for the Rht-B1b and Rht-D1b dwarfing genes in wheat. Theor Appl Genet 105:1038–1042. Initiales des auteurs

Emebiri LC, Moody DB (2005) Heritable basis for some genotype–environment stability statistics: inferences from QTL analysis of heading date in two-rowed barley. Field Crops Res (in press), Corrected Proof. Réf plus complètes

Fang P, Wu P (2001) QTL × N-level interaction for plant height in rice (*Oriza Sativa* L.). Plant Soil 236:237–242

Félix I, Loyce C, Bouchard C, Meynard J-M, Bernicot MH, Rolland B, Haslé H (2002) Associer des variétés rustiques à des niveaux d'intrants réduits. Intérêts économiques et perspectives agronomiques. Persp Agric 279:30–35

Félix I, Loyce C, Bouchard C, Meynard J-M, Rolland B, Bernicot MH, Haslé H (2003) Une voie pour s'adapter aux baisses du prix du blé: Des variétés rustiques conduites à faible coût. Persp Agric 290:22–29

Finlay KW, Wilkinson GN (1963) The analysis of adaptation in plant-breeding programme. Aust J Agric Res 14:742–754

Gallais A, Hirel B (2004) An approach to the genetics of nitrogen use efficiency in maize. J Exp Bot 55:295–306

Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis. Genetics 155:463–473

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032–1040

Guillaumie S, Charmet G, Linossier L, Torney V, Robert N, Ravel C (2004) Colocation between a gene encoding thebZip factor SPA and a eQTL for a high-molecular-weight glutenin subunit in wheat (*Triticum aestivum*). Genome 47:705–713

Habash D, Bernard S, Schondelmaier J, Weyen J, Quarrie SA (2007) The genetics of nitrogen use in hexaploid wheat: N utilisation devolpment and yield. Theor Appl Genet 114:403–419

Hanocq E, Niarquin M, Heumez E, Rousset M, Le Gouis J (2004) Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. Theor Appl Genet 110:106–115

Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Retailliau C, Falque M, Gallais A (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. Plant Physiol 125:1258–1270

Justes E, Mary B, Meynard J-M, Machet J-M, Thelier-Huche L (1994) Determination of a critical nitrogen dilution curve for winter wheat crops. Ann Bot 74:397–407

Kjaer B, Jensen J (1995) The inheritance of nitrogen and phosphorus content in barley analysed by genetic markers. Hereditas 123:109–119

Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Le Gouis J (2006a) Estimation of genetic parameters of a DH wheat population grown at different N stress levels characterized by probe genotypes. Theor Appl Genet 112:797–807

Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B (2006b) A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. Theor Appl Genet 113:1131–1146

Le Gouis J, Pluchard P (1996) Genetic variation for nitrogen use efficiency in winter wheat (*Triticum aestivum* L.). Euphytica 92:221–224

Le Gouis J, Jeuffroy M-H, Heumez E, Pluchard P (1998) Différences variétales pour le fonctionnement du blé tendre en conditions de nutrition azotée suboptimales. In: Maillard P, Bonhomme R (eds) Fonctionnement des peuplements végétaux sous contraintes environnementales, vol 93. INRA Editions (les colloques n 93), Paris, pp 531–538

Le Gouis J, Béghin D, Heumez E, Pluchard P (2000) Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. Eur J Agron 12:163–173

Leflon M, Lecomte C, Barbottin A, Jeuffroy M-H, Robert N, Brancourt-Hulmel M (2005) Characterization of environments and genotypes for analyzing genotype × environment interaction. Some recent advances in winter wheat and prospects for QTL detection. J Crop Imp 14:249–298

Lewicki S, Chery J (1992) Eude de l'accumulation et de la remobilisation de l'azote chez l'orge (Hordeum vulgare L.): comparaisonde variétés possédant ou non le gène de seminanisme. Agronomie 12:235–245

Lian X, Xing Y, Yan H, Xu C, Li X, Zhang Q (2005) QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid. Theor Appl Genet 112:85–96

Liu SX, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for *Fusarium* head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

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

Mallard S, Gaudet D, Aldeia A, Abelard C, Besnard AL, Sourdille P, Dedryver F (2005) Genetic analysis of durable resistance to yellow rust in bread wheat. Theor Appl Genet 110:1401–1409

Malosetti M, Voltas J, Romagosa I, Ullrich SE, van Eeuwijk FA (2004) Mixed models including environmental covariables for studying QTL by environment interaction. Euphytica 137:139–145

Mardi M, Buerstmayer H, Ghareyazie B, Lemmens M, Mohammadi SA, Nolz R, Ruckenbauer P (2005) QTL analysis of resistance to *Fusarium* head blight in wheat using a 'Wangshuibai'-derived population. Plant Breed 124:329–333

Mickelson S, See D, Meyer FD, Garner JP, Foster CR, Blake TK, Fischer AM (2003) Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves. J Exp Bot 54:801–812

Miralles DJ, Katz SD, Colloca A, Slafer GA (1998) Floret development in near isogenic wheat lines differing in plant height. Field Crop Res 59:21–30

Motzo R, Giunta F (2002) Awnedness affects grain yield and kernel weight in near-isogenic lines of durum wheat. Aust J Agric Res 53:1285–1293

Perretant MR, Cadalen T, Charmet G, Sourdille P, Nicolas P, Boeuf C, Tixier MH, Branlard G, Bernard S (2000) QTL analysis of bread-making quality in wheat using a doubled haploid population. Theor Appl Genet 100:1167–1175

Prasad M, Kumar N, Kulwal PL, Röder MS, Balyan HS, Dhaliwal HS, Gupta PK (2003) QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. Theor Appl Genet 106:659–667

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, YessimbekovaM, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti M-C, Hollington PA, Aragués R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880

Raugh BL, Basten C, Buckler ES (2002) Quantitative trait loci analysis of growth response to varying nitrogen sources in *Arabidopsis thaliana*. Theor Appl Genet 104:743–750

Rebetzke GJ, Richards RA (2000) Gibberellic acid-sensitive dwarfing genes reduce plant height to increase kernel number and grain yield of wheat. Aust J Agric Res 51:235–245

Van Eeuwijk FA (1995) Linear and bilinear models for the analysis of multi-environment trials.1. An inventory of models. Euphytica 84:1–7

Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter weat under optimal and drought-stressed environments. Euphytica 135:255–263

Worland AJ (1996) The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89:49–57

Yadav R, Bidinger RF, Hash C, Yadav Y, Yadav O, Bhatnagar S, Howarth C (2003) Mapping and characterisation of QTL × E interactions for traits determining grain and stover yield in pearl millet. Theor Appl Genet 106:512–520

Yan J, Zhu J, He C, Benmoussa M, Wu P (1999) Molecular marker-assisted dissection of genotype × environment interaction for plant type traits in rice (*Oryza sativa* L.). Crop Sci 39:538–544

Yang ZP, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to *Fusarium* head blight in a doubled-haploid spring wheat population. Genome 48:187–196