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ORIGINAL PAPER

# Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 3. QTL detection and coincidences

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**Abstract** The objective of this study was to map and characterize QTLs for traits related to nitrogen utilization efficiency (NUE), grain N yield, N-remobilization and post-silking N-uptake. Furthermore, to examine whether QTLs detected with recombinant inbred lines (RILs) crossed to a tester are common to those detected with line per se evaluation, both types of evaluations were developed from the same set of RILs. The material was studied over two years at high N-input, and one year at low N-input. We used $^{15}$N-labelling to evaluate with accuracy the proportion of N remobilized from stover to kernels and the proportion of postsilking N-uptake allocated to kernels. With 59 traits studied in three environments, 608 QTLs were detected. Using a method of QTL clustering, 72 clusters were identified, with few QTLs being specific to one environment or to the type of plant material (lines or testcross families). However, considering each trait separately, few QTLs were common to both line per se and testcross evaluation. This shows that genetic variability is expressed differently according to the type of progeny. Studies of coincidences among QTLs within the clusters showed an antagonism between N-remobilization and N-uptake in several QTL-clusters. QTLs for N-uptake, root system architecture and leaf greenness coincided positively in eight clusters. QTLs for remobilization mainly coincided in clusters with QTLs for leaf senescence. On the whole, sign of coincidences between QTLs underlined the role of a "stay-green" phenotype in favouring N-uptake capacity, and thus grain yield and N grain yield.

**Abbreviations**

| | |
|---|---|
| ASI | Anthesis-silking interval |
| GDH | Glutamate dehydrogenase |
| GS | Glutamine synthetase |
| N | Nitrogen |
| NHI | Nitrogen harvest index |
| NNI | Nitrogen nutrition index |
| NUE | Nitrogen utilization efficiency |
| NUtE | Nitrogen utilization efficiency |
| QTL | Quantitative trait locus |
| RIL | Recombinant inbred lines |

## Introduction

In maize, grain protein yield is the result of two nitrogen (N) fluxes: N-remobilization from the stover to the kernels and N allocation to the kernels coming directly from post-silking N-uptake. Since the proportion of N remobilized may vary from 30 to 70% according to the environment and the genotype, both N fluxes need to be considered to improve grain protein yield (see Gallais and Coque 2005 for a review). Although N-remobilization and post-silking

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N-uptake allocated to kernels are two major processes to consider at the whole plant N economy level, they are not easy to evaluate without bias. Gallais et al. (2006, 2007) and Coque and Gallais (2007) showed that $^{15}N$ labelling can be used to evaluate accurately N-remobilization and post-silking N-uptake. In these two studies it has been shown that, with testcross performance, N grain yield was mainly related to post-silking N-uptake and that there was an antagonism between N-remobilization and post-silking N-uptake. The physiological basis of such an antagonism was shown to be related to the counteractive effect of senescence on photosynthetic activity of the leaf. Unfortunately, traits related to N-remobilization and post-silking N-uptake are difficult to measure. Therefore, finding simple traits that are easy to measure will certainly be helpful for the breeder. For example, to improve N-uptake, leaf area duration and chlorophyll content could be used to select new varieties exhibiting a "stay-green" phenotype (Borrell and Hammer 2000; Borrell et al. 2001). Anthesis-silking interval (ASI), sterility and leaf senescence, which appeared to be correlated to N-remobilization and N-uptake, are also phenotypic traits easy to evaluate (Bertin and Gallais 2000; Coque and Gallais 2007, 2008). On the whole, correlations among traits were about the same at the level of line per se evaluation and testcross evaluation. However, the negative correlation between N-remobilization and post-silking N-uptake was lower in lines per se compared to testcross, whereas the contribution of N-remobilization in providing N to the kernels was greater in lines (Coque and Gallais 2008). The low correlation already observed between line per se value and testcross performance means that genetic variability is expressed differently in lines and hybrids, as already observed for grain yield or silage yield (Hallauer and Miranda 1981; Sampoux et al. 1989; Presterl et al. 2002). Therefore, selection at the level of lines is expected to be inefficient for traits related to N-utilization.

The identification of QTLs for N-remobilization and N-uptake and the study of their coincidences with QTLs for various traits related to N metabolism could allow a better understanding of the physiological and genetic bases of N utilization. Consequently, this approach could contribute to the development of more efficient breeding methods for selecting such traits. With evaluation of testcross performance of a set of RILs, Bertin and Gallais (2001) have shown that QTLs detected at low N-input were a subset of those detected at high N-input and did not explain the same type of variation. At high N-input, QTLs for traits related to N-uptake were detected, whereas at low N-input it was QTLs mainly related to N utilization. Such results were not observed by Agrama et al. (1999), probably because in their study they used lines evaluated for their per se value, whereas Bertin and Gallais (2001) used testcross progenies. Coincidences between QTLs and genes can lead to the identification of candidate genes involved in the control of genetic variation for NUE. Bertin and Gallais (2001) and Gallais and Hirel (2004) have already shown that there were coincidences between QTLs of traits related to NUE and several genes encoding enzymes involved in C and N primary metabolism. This approach allowed the identification of some members of the glutamine synthetase (GS) multigene family and possibly the enzyme glutamate dehydrogenase (GDH) as putative candidate genes involved in the control of NUE (Hirel et al. 2001; Gallais and Hirel 2004). The function of GS during the grain filling process was further validated using mutants and transgenic plants (Martin et al. 2006). Taken all together, QTL detection, QTL coincidences and identification of candidate genes have the most promise for the development of a marker-assisted selection, notably for traits difficult to measure.

To detect QTL for traits difficult to measure it is important to know whether QTLs must be detected at the level of line per se performance or at the level of testcross performance. The advantage of working with line per se performance, particularly for physiological traits difficult to measure with accuracy on a large number of plants, is that genetic variation is higher than with testcross performance. However, since the correlation between lines and testcross progenies is poor, QTLs detected for per se value are expected to be different from the QTLs detected for testcross performance. In the presence of dominance, and in the absence of epistasis, QTLs for testcross performance are expected to be a subset of those detected with line per se evaluation. However, as epistasis plays a different role in the expression of genetic variability for line and testcross performance for traits related to NUE (Coque and Gallais 2008), there could be a deviation from this expected result. Although QTLs for testcross performance are partially dependent on the tester, the problem for both maize geneticists and breeders is to know which type of material, that is lines or testcross progenies, is the most appropriate for the identification of QTLs having a significant impact on the variation of NUE at hybrid level, since hybrid varieties are used by farmers.

The objectives of the present study were thus (1) to identify, with a population of RILs evaluated for their per se and testcross performance in different environments (N fertilization and years), QTLs for NUE and related traits, especially remobilization and post-silking N-uptake, and (2) to study coincidences of QTLs for such traits by a clustering approach in order to study common and specific QTLs to both types of progenies and to examine the genetic and physiological meaning of the QTL clusters.

## Materials and methods

### Plant material and experimental design

The plant material used in the present study was described previously (Coque and Gallais 2007, 2008). It corresponds to a set of recombinant inbred lines (RILs) derived from the same population already studied by Bertin and Gallais (2000), that is from the cross between the flint F2 line and the dent line Io. Due to the variable amount of available seeds, the size of the population studied varied according to the year of study and type of progenies. In 2003, a set of 114 RILs was studied for per se value and 98 lines for their testcross value with inbred line tester F252. In 2004, 218 RILs were studied for per se value and 155 for testcross value, 155 RILs being common to both sets of lines. Due to the logistics of $^{15}$N-labelling experimentation, the material was only evaluated at the Station Le Moulon (Gif/Yvette, France) over two years, 2003 and 2004. Furthermore, in 2003, two N fertilization levels were applied: for testcross progenies, a high level (N1) with 154 kg ha$^{-1}$ of N fertilizer and a low level (N0) with 70 kg ha$^{-1}$. For lines which, under favourable conditions, have a grain yield 60–70% lower than testcross progenies, N1 was 60 kg ha$^{-1}$ and N0 corresponded to no application of N fertilizer. Separate trials were developed for each N fertilization level. In 2004, only one level of N fertilization was used with 145 kg/ha for testcross progeny evaluation and 60 kg ha$^{-1}$ for line per se evaluation. In both years, soil analyses showed that the soil provided 50–60 kg ha$^{-1}$. Therefore there were three test environments for lines and for testcross progenies. Three replicates were evaluated for each trial, with two-row plots, 5 m long and 80 cm between rows. The plant density was between 90,000 and 100,000 plant ha$^{-1}$. As low N fertilization was only studied in 2003, this experiment is mainly considered as a replication of the experiment in a different environment.

### Traits studied

The traits studied were described in detail by Coque and Gallais (2007, 2008). A total of 59 traits were studied. Table 1 gives in alphabetic order the abbreviations used in the tables or figures. They are summarized as follows according to the stage of observations:

*Traits evaluated at maturity*

- grain yield (*GY*) and its components, kernel number (*KN*), thousand kernel weight (*TKW*), and grain moisture (*GMoist*);
- stover dry-matter per plant (*StDM /pl*) and whole-plant dry-matter per plant (*WpDM/pl*);
- N content for grain (*GNC*), stover (*StNC*) and whole-plant (*WpNC*);
- grain dry-matter per plant (*GDM/pl*), N-yield (*GNY*), grain N-amount per plant (*GN*), stover N-amount per plant (*StN/pl*) and whole-plant N-yield (*WpNY*);
- N originating from post-silking N-uptake accumulated in the grain (*NupG*) and percentage of N originating from post-silking N-uptake accumulated in the grain (*%NupG*),
- percentage of plants without ear (*Sterile*);
- harvest index (*HI* = grain yield/whole-plant yield), N harvest index (*NHI* = N grain yield/whole-plant N yield), and N utilization efficiency (*NUtE* = (grain dry-matter per plant/whole-plant N-amount per plant);

*Traits evaluated at silking*

- anthesis date (*AD*) and silking date (*SD*), and anthesis-silking interval (*ASI* = silking date − anthesis date);
- whole-plant dry matter per plant (*DMsilk/pl*);
- N content (*NCsilk*) and whole-plant N yield per plant (*SilkNup/pl*);
- N nutrition index (*NNI*) computed as the ratio of the observed N-content to a critical N content corresponding to the minimum N-content allowing the maximum dry-matter yield (Lemaire and Gastal, 1997);
- N content of the ear leaf at silking + 25 days (*Pro*);

*Traits derived by comparison of dry-matter and amounts of N at maturity and at silking (N balance method, Coque and Gallais 2007, 2008)*

- amount and proportion of remobilized N from stover (*NremB* and *tremB*, respectively);
- amount of post-silking N-uptake (*psNup*) from which % of post-silking N-uptake (*%psNup*);
- amount of dry-matter accumulated between silking and maturity (*psWpDM/pl*);

*Traits derived from $^{15}$N labellings (Coque and Gallais 2007, 2008)*

- amount (*Nrem*) and proportion of remobilized N (*trem* and *tremC*) determined by $^{15}$N-labelling during the vegetative phase, for all types of material, except for the material evaluated at low N-input (N0) for which postsilking $^{15}$N-uptake was not evaluated. It was thus not possible to derive *tremC* corresponding to the corrected estimates of the proportion of remobilized N (Coque and Gallais 2007);
- proportion (*tG*) of post-silking N-uptake allocated to kernels determined by $^{15}$N-labelling just after silking; this type of labelling was developed only for testcross progeny evaluation (Coque and Gallais 2007);

**Table 1** Abbreviations in alphabetic order of the 59 traits studied

| Traits | Abbreviations |
|---|---|
| % $^{15}$N-uptake for labelling at stem elongation | %$^{15}$Nup1 |
| % $^{15}$N-uptake for labelling at silking | %$^{15}$Nup2 |
| % N from N-uptake within grain | %NupG |
| % postsilking N-uptake | %psNup |
| Dualex on abaxial surface | ABM |
| Anthesis date | AD |
| Dualex on adaxial surface | ADM |
| Anthesis-silking interval (SD-AD) | ASI |
| Ratio CCMlag/Dualex | CCM/Dualex |
| Chlorophyll meter at silking + 30 days | CCM30D |
| Chlorophyll meter at silking + 45 days | CCM45D |
| Chlorophyll meter at silking + 15 days | CCMlag |
| Whole-plant dry-matter/plant at silking | DMsilk/pl |
| Dualex at silking + 10 days (ADM + ABM)/2 | Dualex |
| Change between *CCMlag* and *CCM30D* | EVOCCM |
| Ear leaf GDH activity at silking + 25 days at pH8 (/Dry-matter) | GDH8DM |
| Ear leaf GDH activity at silking + 25 days at pH8 (/Protein) | GDH8Pro |
| Ear leaf GDH activity at silking + 25 days at pH9 (/Dry-matter) | GDH9DM |
| Ear leaf GDH activity at silking + 25 days at pH9 (/Protein) | GDH9Pro |
| Grain dry-matter/plant | GDM/pl |
| Grain moisture | GMoist |
| Grain nitrogen/plant | GN |
| Grain nitrogen content | GNC |
| Grain nitrogen yield | GNY |
| Ear leaf GS activity at silking + 25 days (/Dry-matter) | GSDM |
| Ear leaf GS activity at silking + 25 days (/Protein) | GSPro |
| Grain yield/m$^2$ | GY |
| Harvest index | HI |
| Kernel number/plant | KN |
| Nitrogen content at silking | NCsilk |
| Nitrogen harvest index | NHI |
| Nitrogen nutrition index | NNI |
| N remobilized | Nrem |
| N remobilized (balance method) | NremB |
| N from N uptake within grain | NupG |
| N utilisation efficiency | NUtE |
| Ear leaf protein content at silking + 25 days | Prot |
| Postsilking N-uptake | psNup |
| Postsilking stover dry-matter accumulation/plant | psStDM/pl |
| Whole-plant dry-matter/plant accumulated after silking | psWpDM/pl |
| Silking date | SD |
| Visual notation at silking + 10 days | Sen |
| Visual notation at silking + 45 days | Sen1 |
| Visual notation at maturity | Sen2 |
| Silking N-uptake/plant | SilkNup/pl |
| Stover dry-matter yield/plant at maturity | StDM/pl |
| Stover dry-matter content at maturity | StDMC |
| Stover N content at maturity | StNC |
| % of sterile plants | Sterile |
| Stover nitrogen per plant at maturity | StN/pl |
| Proportion of postsilking N-uptake allocated to kernels | tG |
| Thousand Kernel Weight | TKW |
| Proportion of N remobilized | trem |
| Proportion of remobilized N (balance method) | tremB |
| trem corrected by residual postsilking $^{15}$N-uptake | tremC |
| Whole-plant dry-matter/plant at maturity | WpDM/pl |
| Whole-plant N content at maturity | WpNC |
| Whole-plant N uptake at maturity | WpNup |
| Whole-plant N yield at maturity | WpNY |

To this list it must be added N responsiveness traits in absolute value (*resp*) or in relative value (*resp2*)

- proportion of $^{15}$N uptake for $^{15}$N labelling during vegetative phase (*%$^{15}$Nup1*) and just after silking (*%$^{15}$Nup2*);

*Traits related to leaf senescence*

- visual notation of leaf senescence at three developmental stages, just after silking (*Sen*), around the silage stage (*Sen1*) and at maturity (*Sen2*), with a visual notation ranging from 1 to 5 (1 green, 5 completely dried);
- chlorophyll content in 2003, at two stages of development at low N-input (20 and 35 days after silking), and in 2004, at three stages of development: 15 days (*CCMlag*), 30 days (*CCM30D*), and 45 days after silking (*CCM45D*). For this measurement the CCM 200 chlorophyll meter (Opti-Sciences, Hudson, USA) was used. Changes in CCM value were also studied between 15 and 30 days (*EVOCCM*);
- leaf senescence evaluated with a Dualex sensor developed by Cerovic et al. (2002) that allows the estimation of the polyphenol content which reflects the level of chlorophyll degradation. Polyphenol-content was measured 30 days after silking in 2003 at low N input and in 2004 on the leaf below the ear on its abaxial surface (*ABM*) and on its adaxial surface (*ADM*), on five plants per plot. The final Dualex measurement corresponded to the average of *ABM* and *ADM* measurement. The ratio *CCM/Dualex* at 30 days after silking was also derived from these measurements.

*Enzyme activities*

In 2004, from ear leaf at 25 days after silking, GS and GDH activities (expressed as nmol $min^{-1}$ $mg^{-1}$ of dry matter for *GSDM* and as $mg^{-1}$ of protein for *GSPro*) were determined according to the methods described by Hirel et al. (2005b). As already shown by these authors, the two enzyme activities represent good biochemical markers of the plant N status, particularly during the leaf protein remobilization process. GDH aminating activity was measured at pH 8 (*GDH8*) and deaminating activity was measured at pH 9 (*GDH9*) (expressed as nmol $min^{-1}$ $mg^{-1}$ of dry matter for *GDH8DM* or *GDH9DM* and $mg^{-1}$ of protein for *GDH8Pro* or *GDH9Pro*).

*N responsiveness*

In the 2003 experiment, for each trait, N responsiveness was evaluated by calculating the difference between the values of the trait at high N-input (N1) to its value at low N-input (N0). These responsiveness traits are identified by the abbreviations *resp* for responsiveness in absolute value and *resp2* for relative responsiveness [(N1 − N0)/N0] followed by the abbreviation of the trait.

Genetic map

The genetic map of the population Io × F2 published by Causse et al. (1996) was used. This genetic map is based on 152 markers, mainly RFLP loci, covering 1,813 cM. It was extended by mapping new enzymatic and RFLP loci, leading to a map containing 243 loci and covering 2,178 cM. Furthermore, to establish correspondence with other public maps 167 SSR loci were mapped using MAPMAKER/EXP v 3.0 (Lincoln et al. 1993) to finally obtain a reference map containing 410 loci covering 2,147 cM, with a mean interval between loci of 5 cM. A subset of 203 markers, well distributed along the chromosomes was used for QTL detection (Fig. 1).

QTL detection

Due to the use of different set of RILs grown under different environmental conditions (year and N input), QTLs were separately detected for each of the six experimental conditions in order to maximize the exploitation of the data. In addition, QTLs were detected from pooled data of the 2003 and 2004 experiments performed under high N-input using both testcross and line per se evaluations. QTLs were detected by composite interval mapping using the

| Chr1 | | Chr2 | | Chr3 | | Chr4 | | Chr5 | | Chr6 | | Chr7 | | Chr8 | | Chr9 | | Chr10 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | bnlg109 | 0.0 | mmc0111 | 0.0 | umc92 | 0.0 | gsy474_a_H | 0.0 | nc007 | 0.0 | gsy406b_6PG | 0.0 | mmc0171 | 0.0 | bnl1305a | 0.0 | phi068 | 0.0 | bnl304 |
| 13.6 | dupssr2 | 22.1 | umc1165 | 49.4 | umc1012 | 6.7 | So53_ChilR | 24.9 | bnlg565 | 8.0 | y1ssr | 17.7 | phi112 | 13.3 | bnlg1194 | 14.4 | umc113 | 4.6 | phi117 |
| 24.5 | phi001 | 30.8 | bnlg125 | 66.6 | umc1504 | 32.1 | gsy4a_ZPL1 | 44.8 | gsy168_GTP | 11.6 | bnlg249 | 28.6 | csu11 | 23.6 | umc1304 | 20.5 | phi028 | 21.5 | phi063 |
| 25.0 | bnlg2204 | 37.1 | bnlg2277 | 76.8 | bnlg1601 | 57.1 | phi021 | 58.2 | gsy154_MDH | 16.3 | bnlg426 | 33.0 | phi034 | 27.1 | bnlg2235 | 26.7 | dupssr6 | 30.6 | umc1576 |
| 34.6 | gsy297a_EM | 50.2 | umc44a | 79.9 | mmc0022 | 57.1 | nc004 | 70.2 | umc43 | 26.0 | umc1143 | 34.3 | bnlg1792 | 53.6 | bnlg2082 | 36.0 | bnlg1401 | 44.5 | phi059 |
| 50.4 | bnlg2238 | 62.7 | gsy348c_Ivr1 | 92.6 | gsy224a_SPS | 63.4 | gsy431_PDSI | 84.6 | gsy249b_B7 | 30.4 | bnlg161 | 53.4 | umc116 | 59.8 | bnlg1863 | 38.1 | umc1037 | 56.3 | gsy329_PPD |
| 65.0 | dupssr26 | 66.9 | bnlg2248 | 99.1 | dupssr23 | 83.3 | gsy4b_ZPL1 | 91.7 | umc1060 | 59.9 | umc39a | 55.7 | mmc0411 | 61.8 | phi115 | 45.9 | phi061 | 68.8 | bnlg1712 |
| 67.9 | bnlg2295 | 71.9 | bnlg381 | 102.7 | umc1730 | 90.6 | umc1088 | 94.3 | dupssr10 | 86.9 | umc65 | 63.4 | bnlg1070 | 76.3 | gsy60d_BT2 | 45.9 | phi022 | 70.3 | bnlg1655 |
| 91.8 | gsy351_CS | 91.0 | umc1580 | 107.9 | bnlg1063 | 90.6 | phi026 | 102.8 | umc1221 | 97.7 | gsy244_PL1 | 74.4 | umc110 | 88.8 | umc1343 | 46.4 | phi027 | 87.6 | gsy348a_Ivr1 |
| 99.5 | umc1590 | 100.7 | phi083 | 123.2 | gsy164_PROI | 90.6 | nc005 | 105.9 | phi330507 | 108.1 | gsy325a_BT1 | 78.3 | bnlg155 | 91.9 | umc1562 | 49.5 | dupssr19 | 96.1 | gsy64_RS |
| 106.9 | bnlg1057 | 103.6 | bnlg1175 | 131.1 | bnlg197 | 90.8 | phi079 | 123.3 | mmc0481 | 126.3 | mmc0241 | 80.9 | umc1134 | 96.6 | bnlg666 | 52.3 | bnlg430 | 112.5 | gsy54a_RPL |
| 111.8 | umc1335 | 113.6 | umc16a | 131.1 | dupssr17 | 107.2 | mmc0371 | 125.3 | bnlg278 | 132.6 | nc013 | 85.2 | phi114 | 96.6 | bnlg162 | 63.7 | bnlg1714 | 128.5 | umc1084 |
| 123.9 | umc1035 | 119.1 | phi092 | 135.6 | umc1135 | 113.4 | bnlg1621 | 133.6 | bnlg609 | 147.6 | umc38 | 89.2 | bnlg1666 | 99.2 | umc12a | 77.6 | gsy67_SUS2 | 139.5 | bnlg1450 |
| 149.4 | bnlg615 | 123.6 | umc1079 | 151.7 | mmc0251 | 123.9 | gsy274_gln5 | 143.7 | gsy258a_ANT | 160.7 | phi364545 | 91.1 | umc1029 | 115.1 | csu131 | 87.0 | bnlg1270 | | |
| 149.4 | umc1278 | 124.1 | bnlg371 | 159.8 | umc1320 | 125.5 | bnlg2291 | 167.9 | umc68 | 177.9 | umc1248 | 100.6 | dupssr13 | 122.2 | umc1287 | 88.0 | umc1387 | | |
| 157.2 | gsy60b_BT2 | 130.0 | bnlg1138 | 165.8 | gsy58_SH2 | 139.1 | umc1194 | 202.8 | bnlg118 | 179.2 | umc1897 | 108.3 | gsy425_RIP3 | 126.9 | bnlg240 | 106.6 | gsy515_c_AS | | |
| 179.6 | mmc0041 | 139.0 | gsy423_Tran | 187.5 | bnlg1754 | 152.1 | bnlg1444 | 235.0 | umc104a | 202.0 | umc1490 | 125.9 | umc1125 | 135.5 | mmc0181 | 121.2 | bnlg1191 | | |
| 185.2 | bnlg1643 | 144.5 | bnlg1329 | 193.3 | bnlg1496 | 165.5 | umc1051 | 248.1 | bnlg389 | 209.4 | umc1653 | 143.7 | umc1154 | 150.0 | gsy224b_SPS | 123.3 | umc1733 | | |
| 214.8 | gsy291_gln2 | 152.2 | phi127 | | | 180.1 | bnlg572 | 248.8 | bnlg1695 | 219.3 | gsy325c_BT1 | 146.2 | phi082 | 189.7 | umc36a | 123.6 | umc1366 | | |
| 220.0 | gsy52_ROOT | 162.5 | bnlg1633 | | | 188.3 | gsy34a_PEP | 250.6 | umc1153 | | | 151.7 | phi116 | 194.8 | umc32a | 132.6 | bnl1428 | | |
| 243.1 | umc161 | 165.2 | bnlg1335 | | | 213.5 | umc1101 | | | | | | | 208.3 | gsy111a_TRF | 149.2 | bnlg279 | | |
| 268.0 | bnlg131 | 170.6 | umc4 | | | | | | | | | | | | | 166.8 | gsy330_TDR3 | | |
| 273.7 | phi064 | 198.8 | phi090 | | | | | | | | | | | | | 172.4 | umc94 | | |

**Fig. 1** Markers used for QTL detection. On the chromosome (*Chr*) *left* is given the distance in cM, whereas on the chromosome *right* is given the name of the marker

Plab-QTL software (Utz and Melchinger 1995) with a LOD = 2.15. Using a permutation test (Churchill and Doerge 1994) with 1,000 runs according to the experiment, a LOD value between 2.15 and 2.35 corresponded to a global risk I of 25%, a value between 2.50 and 2.92 corresponded to a global risk of 10% and a value between 3.06 and 3.54 corresponded to a global risk of 5%. However, we will only consider clusters involving at least one QTL with a LOD > 2.5. For each QTL, a one-LOD score support interval was used. Epistasis between detected QTLs was studied by stepwise regression using the Plab-QTL software. Using the Biomercator software (Arcade et al. 2004), other QTLs detected with the same RIL population were projected on the genetic map (1) QTLs for physiological traits detected by Hirel et al. (2001) and Dubois et al. (2003) and (2) QTLs for the root system detected by Guingo et al. (1998). Therefore, it was possible to study their coincidence with the QTLs from our study.

### Study of QTL coincidences

To study coincidences of QTLs for a large number of traits, we have used QTL meta-analysis, according to the procedure proposed by Goffinet and Gerber (2000) and extended by Veyrieras et al. (2007). This method was developed to synthesize QTL information obtained from different independent populations and for a given trait. In a given genomic region, several models for the presence from one to *n* QTL are tested on the basis of a Gaussian mixture model with known variance. Veyrieras et al. (2007) showed that the optimal number of clusters can be chosen by mean of usual information based criteria (like the well-known Akaike criterion). The Gaussian mixture framework underlying the method of Goffinet and Gerber (2000) and Veyrieras et al. (2007) has several compelling aspects, like its robustness to non-independence of experiments, but also its flexibility and usefulness to investigate QTL coincidences, like a standard univariate cluster analysis. In particular, Goffinet and Gerber (2000) suggested that their approach could be extended to the situation corresponding to our present study in which only one population was evaluated but for many traits. In this situation, there is non independence of the QTLs coinciding in a given chromosome region. However, if confidence interval of the meta-QTL is not considered, the simulations of Goffinet and Gerber showed that the method is robust to the non independence of QTLs. Their method can thus be used to study coincidence of several QTLs for many traits in a given population. In that peculiar case, the original QTL meta-analysis purpose moves from the question "where are the actual QTLs located?" to the question "do the observed QTLs tend to cluster along the genome?", that is "Are there hot points or are the QTLs distributed at random?". We used the software MetaQTL developed by Veyrieras et al. (2007) to address this later question. Using simulations, it has been shown that the model selected by the Akaike criterion is a model with fewer clusters than expected with a random distribution of QTLs and that this model has a very low probability to happen by chance (Fig 2). The software MetaQTL gives for each QTL the probability that it belongs to a given cluster (Table 3 shows an example for chromosome 1). A QTL was assigned to a given cluster when its probability of belonging to this cluster was higher than 0.75.

The software MetaQTL was applied to three sets of QTLs: (1) all detected QTLs, (2) QTLs detected with line per se evaluation and (3) QTL detected for testcross evaluation. The clustering with all QTLs was also used to study, for a given trait, coincidence between QTLs detected under different environmental conditions (level of N fertilization) and with the two types of progenies. In the following, QTL clusters identified with all QTLs are identified by the chromosome number and their rank order on the chromosome. For example, cluster 2.3 means third cluster on chromosome two. In a cluster, coincidence of QTLs for two traits is said to be positive when the allele effect from one parent has the same sign for both QTLs, whereas coincidence is said to be negative if the two signs are different. As difference in flowering and grain maturity earliness can greatly affect N-uptake, remobilization, and senescence, mainly with line per se evaluation (Coque and Gallais 2007, 2008)

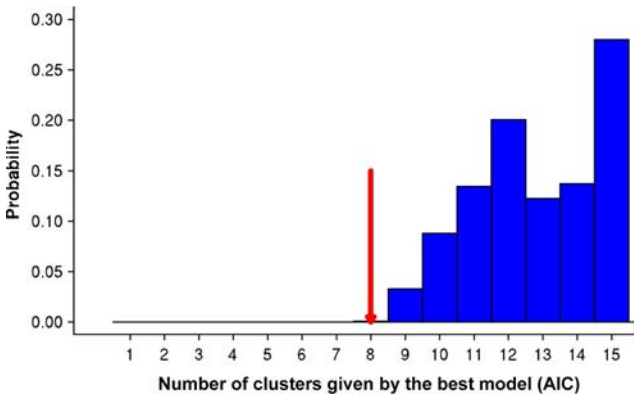


**Fig. 2** Distribution of the number of clusters given by the best model when the QTLs are randomly distributed along the chromosome. Case of chromosome 6 with 78 QTLs. Using simulations, we investigate whether the observed QTL clustering can be due to chance. QTL positions were randomly simulated along the chromosome assuming an uniform distribution. Furthermore, for each QTL the confidence interval was the same as in the data. The histogram shows the probability that the Akaike criterion determines a given model (i.e. a number of clusters) as the best one from 100 simulated configurations. The last class corresponds to 15 clusters or more. The arrow points the value (8) corresponding to the number of clusters found by applying MetaQTL to the data (see Fig. 4 and ESM S6). The probability that it appears by chance is very low

we have not considered QTL clusters which involved QTL for silking and anthesis date and kernel moisture.

Then, our approach consisted after QTLs detection for all traits, (1) in using clustering to study coincidences between QTLs for different traits, or for the same traits in different environment and (2) in examining whether QTL coincidences were consistent with already studied correlations among traits and our physiological knowledge of N metabolism (Coque and Gallais 2007, 2008).

## Results

### Clustering with all QTLs

With a LOD > 2.15 a total of 608 QTLs were detected by separate evaluation of the 59 traits under three environmental conditions (2003 N0, 2003 N1, 2004 N1) and with the two genetic backgrounds, line and testcross progenies (Table 2). The study of coincidences between all detected QTLs for a LOD > 2.15 showed the occurrence of 72 distinct QTL clusters, with an average of 8.4 QTLs per cluster (with a variation between one and 28 for cluster 6 on chromosome 8) (Figs. 3, 4; Table 4). Among all detected QTLs, 68.4% were affected nearly unambiguously to the clusters, that is with a probability higher than 0.98, 86% were affected to the clusters with a probability higher than 0.89 and 93.8% were affected to the clusters with a probability higher than 0.74 (see Table 3 for clusters on chromosome 1). Therefore, with this last probability, few QTLs were common to two or more clusters (6.2%, 38/608). Only seven QTLs were isolated. This led to 65 clusters grouping at least two QTLs. Twenty-nine clusters were involved in flowering or maturity earliness. The consideration of a LOD > 2.5 led to the detection of 367 QTLs. However, there results the disappearance of only six clusters either because LODs of the grouped QTLs were lower than 2.5 or because some clusters were grouped together (see ESM S11–S20). Furthermore, it appeared that for clusters grouping more than three QTLs, at least one was with a LOD higher than 2.5. In what follows we consider clusters determined with LOD > 2.15.

### QTLs and clusters for line per se and testcross performance

The number of QTLs detected for lines and testcross progenies was slightly higher for lines (328, i.e. 53.9%) than for testcross progenies (280, i.e. 46.1%). In 2003, at low N-input there were significantly more QTLs detected in lines than in testcross progenies (62.3 vs. 37.6%), whereas at high N-input their proportions were 56.5 and 43.4%, respectively. In 2004, the numbers of QTLs detected with lines and with hybrids were very similar. Many QTLs were detected only for a given material and a given environmental condition (year and N level). With line per se evaluation, 24.3% (38/156) of detected QTLs were common to N0 and N1, with more QTLs being specific for low N-input (46.1%) compared to high N-input (29.5%). For testcross progenies only 13.3% of the detected QTLs were common to low and high N-input and about the same number of QTLs appeared to be specific for low and high N-input conditions. Out of 47 QTLs detected for N responsive traits, 28 QTLs were detected for testcross evaluation and 19 for line per se evaluation. For all traits studied, very few QTLs were common to line and testcross evaluation (7.6%), that is, for a given trait, QTLs for lines were generally distinct from QTLs for testcross progenies. More QTLs were common to high and low N input than to lines and testcross progenies. Epistasis among detected QTLs was not significant. Indeed, considering only grain yield, grain N-yield, N-remobilization, N-uptake, GS and GDH activities, representing 16 traits and 202 QTLs, only six significant cases of QTL x QTL epistasis out of 223 tests were detected (data not shown). With a LOD > 2.5, the conclusions about the distribution of QTLs among lines or testcross progenies were nearly the same: 54.8% of the detected QTLs for line per se evaluation and 45.2% for testcross evaluation. With this LOD threshold, only 11 QTLs were common to line and testcross evaluation, that is 3% of the detected QTLs.

**Table 2** Number of QTLs with LOD > 2.15 detected according to the year and N-condition

| | 2003 | | | 2004 | 2003 + 2004 | Total |
|---|---|---|---|---|---|---|
| | Low N-input | High N-input | Responsiveness | High N-input | High N-input | |
| Lines | 91 | 65 | 19 | 92[a] | 61 | 328 (53.9%) |
| Testcross progenies | 55 | 50 | 28 | 99[a] | 48 | 280 (46%) |
| Total | 146 | 115 | 47 | 191 | 109 | 608 |
| Common QTLs[b] | 8 | 6 | 4 | 20 | 8 | 46 (7.6%) |

[a] Including physiological traits

[b] For the same traits

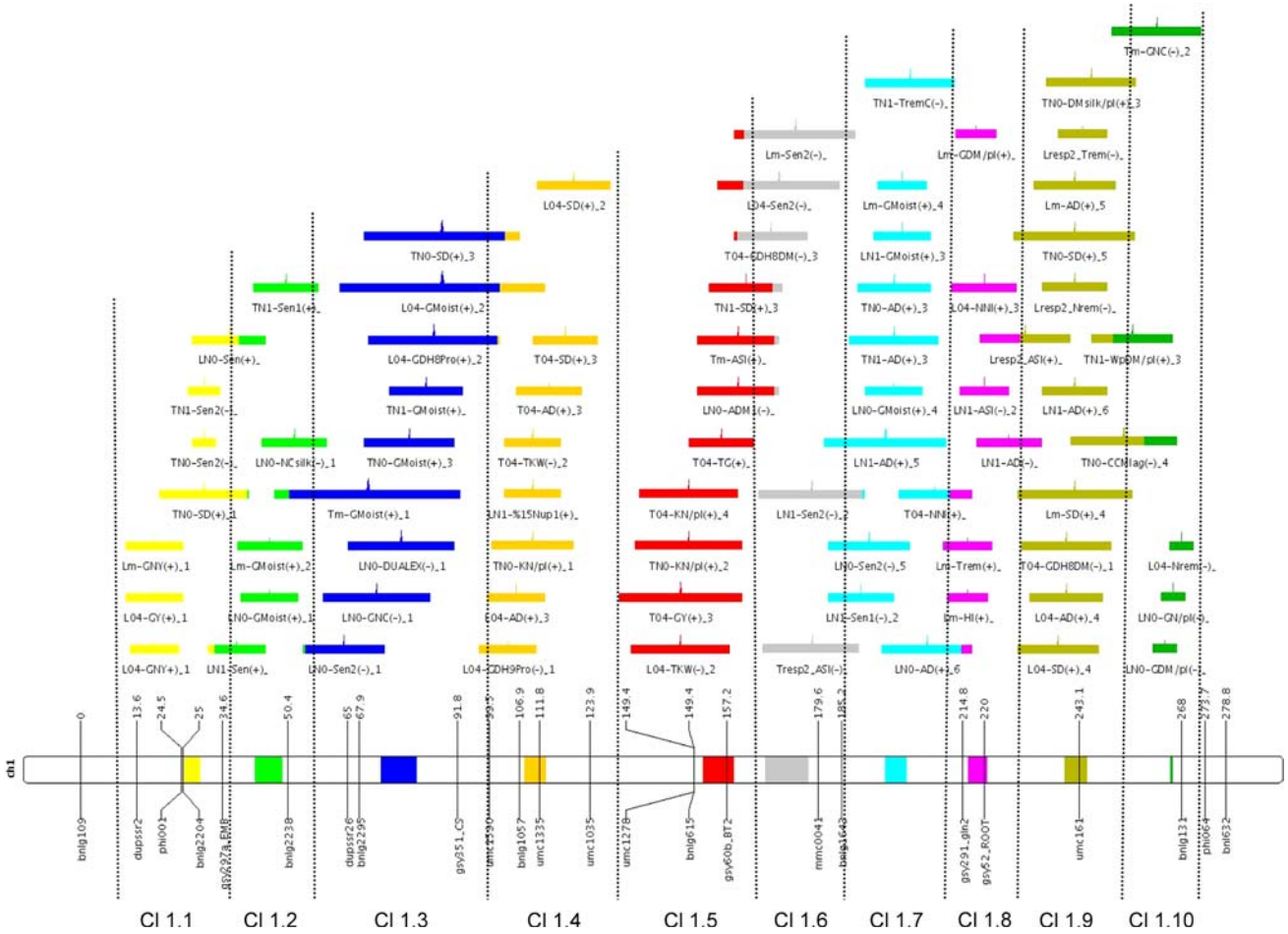


**Fig. 3** An example (for chromosome 1) of the result of QTL clustering by the use of MetaQTL software developed by Veyrieras et al. (2007). Each QTL is represented by its confidence interval. The name of the QTL is composed of two parts, (1) the name of the experiment: *L* for line per se evaluation, *T* for testcross evaluation, *N0* low N-input 2003, *N1* high N-input 2003, *04* high N-input 2004, *m* mean for high N-input 2003 and 2004, and (2) the trait acromnym followed between brackets by the sign of the allele effect, with reference to the allele from parent Io. *resp* and *resp2* refer to N-responsiveness for the trait whose the abbreviation follows. See Table 1 for the meaning of trait acronyms. Results for all chromosomes are given in ESM S1–S10 for LOD > 2.15 and ESM S11–S20 for LOD > 2.50. QTLs belonging to the same cluster have the same colour. QTLs common to two clusters are represented with the colour of each cluster, the length of one colour segment being proportional to the probability for the QTL of belonging to the cluster of the same colour. Note that for chromosome 3 clusters 3, 4 and 5 are in fact overlapping, and as shown when LOD > 2.5, they can be grouped in only one cluster. The QTL clusters for the other chromosomes are given in the electronic supplementary material, S1 to S10 for LOD > 2.15 and S11 to S20 for LOD > 2.49)

When considering separately QTL clustering from line per se and testcross evaluation, it appears that the corresponding clusters were subsets of the set of clusters detected with all QTL considered simultaneously (Table 4). Among the 65 clusters with at least two QTLs, 48 (73.8%) were common to both lines and testcross progenies, nine (13.8%) were specific to lines and six (9.2%) were specific to testcross progenies.

## QTLs and clusters for N-uptake and N-remobilization

Considering only post-silking N-remobilization traits, that is amount (*Nrem, NremB*) and proportion (*trem, tremB, tremC*) of N remobilized, and N-uptake traits, that is total N-uptake (*WpNup*), postsilking N-uptake (*psNup*, *%psNup*), *tG*, *%NupG*, *%$^{15}$Nup1*, and *%$^{15}$Nup2*, 77 QTLs were detected (Table 5). Individual QTL explained between 3.5 and 23.1% of phenotypic variation. A total of 43 QTLs were detected for N-uptake traits whereas 34 QTLs were detected for N-remobilization traits. However, taking into account coincidences between the QTLs, 21 clusters were detected for traits related to N-uptake and 19 for traits related to N-remobilization. Six clusters were common to both types of traits; three showed a negative coincidence, whereas two showed a positive coincidence. On average, the percentage of variance explained by each type of QTL was the same and between 10 and 12% of the phenotypic variance of the trait.

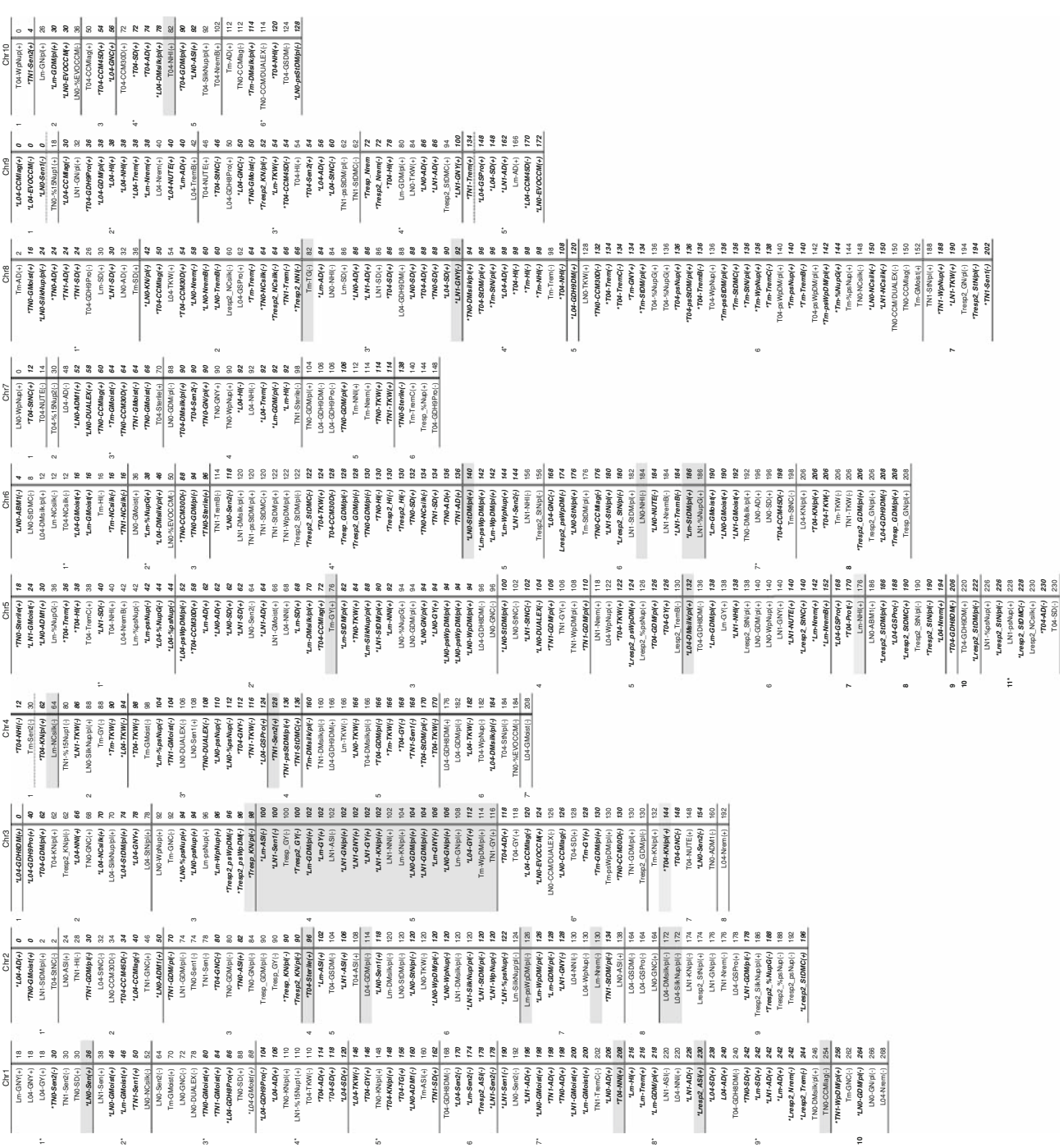

**Fig. 4** Location of the QTLs detected and result of the QTL clustering. Name of the QTL is given on the chromosome (*Chr*) left whereas its position in cM is given on chromosome right. The name of the QTL is composed of two parts (1) the name of the experiment, *L* line per se evaluation, *T* testcross evaluation, *N0* low N-input 2003, *N1* high N-input 2003, *04* high N-input 2004, *m* mean for high N-input 2003 and 2004, and (2) the acronym of the trait followed between brackets by the sign of the allele effect, with reference to the allele from parent Io. *resp* and *resp2* refer to N-responsiveness for the trait whose the abbreviation follows. Names in bold italics and an asterisk correspond to QTLs with a LOD higher than 2.49. *Horizontal lines* separates the clusters which are numbered per chromosome. Their *number* is given in the first column for each chromosome. *Cluster number with an asterisk* corresponds to a cluster involved in flowering date (silking or anthesis) and/or in kernel moisture content. *Shaded* QTL names correspond to QTLs common to two clusters

Several clusters involving QTLs for N-uptake also contained QTLs for grain yield, with positive coincidences for the two traits. They include clusters 2.6, 3.3–4–5, 4.2, 4.6, 5.3, 5.5, 5.6, 7.4 and 8.7. Cluster 2.6 corresponded to positive coincidences between QTLs for total N-uptake, N-remobilization, grain yields and kernel number with line

**Table 3** Results of QTL assignment to the clusters for chromosome 1 by the use of MetaQTL (associated with Fig 3)

| QTL name | Observed position | Predicted position | Contribution of the QTLs to the cluster | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cl 1 | Cl 2 | Cl 3 | Cl 4 | Cl 5 | Cl 6 | Cl 7 | Cl 8 | Cl 9 | Cl 10 |
| Lm-GNY(+)_1 | 18 | 27.09 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN0-Sen2(−)_ | 30 | 27.09 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-GNY +)_1 | 18 | 27.09 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN1-Sen2(−)_ | 30 | 27.09 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-GY(+)_1 | 18 | 27.09 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN0-SD(+)_1 | 30 | 27.46 | 0.98 | 0.02 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN0-Sen(+)_ | 36 | 33.79 | 0.64 | 0.36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN1-Sen(+)_ | 38 | 43.66 | 0.11 | 0.89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN0-NCsilk(−)_1 | 52 | 45.71 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN1-Sen1(+)_ | 50 | 45.71 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN0-GMoist(+)_1 | 46 | 45.71 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lm-GMoist(+)_2 | 46 | 45.71 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tm-GMoist(+)_1 | 70 | 74.17 | 0 | 0.08 | 0.91 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN0-Sen2(−)_1 | 64 | 76.54 | 0 | 0.03 | 0.97 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN1-GMoist(+)_ | 84 | 77.49 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN0-GMoist(+)_3 | 80 | 77.49 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN0-DUALEX(−)_1 | 78 | 77.49 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN0-GNC(−)_1 | 72 | 77.49 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-GDH8Pro(+)_2 | 86 | 78.15 | 0 | 0 | 0.98 | 0.02 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN0-SD(+)_3 | 88 | 80.81 | 0 | 0 | 0.9 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-GMoist(+)_2 | 88 | 84.79 | 0 | 0 | 0.78 | 0.22 | 0 | 0 | 0 | 0 | 0 | 0 |
| T04-AD(+)_3 | 114 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| TN0-KN/pl(+)_1 | 110 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| T04-SD(+)_3 | 118 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-AD(+)_3 | 106 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-SD(+)_2 | 120 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN1-%15Nup1(+)_ | 110 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| T04-TKW(−)_2 | 110 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-GDH9Pro(−)_1 | 104 | 110.67 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| L04-TKW(−)_2 | 146 | 155.32 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| TN0-KN/pl(+)_2 | 148 | 155.32 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| T04-KN/pl(+)_4 | 148 | 155.32 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| T04-TG(+)_ | 156 | 155.32 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| T04-GY(+)_3 | 146 | 155.32 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| LN0-ADM1(−)_ | 160 | 156.32 | 0 | 0 | 0 | 0 | 0.94 | 0.06 | 0 | 0 | 0 | 0 |
| Tm-ASI(+)_ | 160 | 156.32 | 0 | 0 | 0 | 0 | 0.94 | 0.06 | 0 | 0 | 0 | 0 |
| TN1-SD(+)_3 | 162 | 157.65 | 0 | 0 | 0 | 0 | 0.86 | 0.14 | 0 | 0 | 0 | 0 |
| L04-Sen2(−)_ | 170 | 166.74 | 0 | 0 | 0 | 0 | 0.21 | 0.78 | 0 | 0 | 0 | 0 |
| Tresp2_ASI(−)_ | 178 | 170.5 | 0 | 0 | 0 | 0 | 0 | 0.98 | 0.01 | 0 | 0 | 0 |
| Lm-Sen2(−)_ | 174 | 170.89 | 0 | 0 | 0 | 0 | 0.08 | 0.91 | 0.01 | 0 | 0 | 0 |
| T04-GDH8DM(−)_3 | 168 | 171.12 | 0 | 0 | 0 | 0 | 0.05 | 0.95 | 0 | 0 | 0 | 0 |
| LN1-Sen2(−)_2 | 178 | 174.3 | 0 | 0 | 0 | 0 | 0.01 | 0.97 | 0.03 | 0 | 0 | 0 |
| LN0-GMoist(+)_4 | 198 | 198.51 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| LN1-GMoist(+)_3 | 200 | 198.51 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| TN1-AD(+)_3 | 198 | 198.51 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| TN0-AD(+)_3 | 198 | 198.51 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

**Table 3** continued

| QTL name | Observed position | Predicted position | Contribution of the QTLs to the cluster | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cl 1 | Cl 2 | Cl 3 | Cl 4 | Cl 5 | Cl 6 | Cl 7 | Cl 8 | Cl 9 | Cl 10 |
| Lm-GMoist(+)_4 | 200 | 198.51 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| LN1-Sen1(−)_2 | 190 | 198.51 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| LN0-Sen2(−)_5 | 192 | 198.51 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| TN1-TremC(−)_ | 202 | 198.71 | 0 | 0 | 0 | 0 | 0 | 0 | 0.99 | 0.01 | 0 | 0 |
| LN1-AD(+)_5 | 196 | 198.71 | 0 | 0 | 0 | 0 | 0 | 0 | 0.99 | 0.01 | 0 | 0 |
| LN0-AD(+)_6 | 206 | 200.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0.88 | 0.12 | 0 | 0 |
| T04-NNI(+)_ | 208 | 204.47 | 0 | 0 | 0 | 0 | 0 | 0 | 0.7 | 0.3 | 0 | 0 |
| Lm-HI(+)_ | 216 | 218.39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| LN1-AD(−)_ | 226 | 218.39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| LN1-ASI(−)_2 | 220 | 218.39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| L04-NNI(+)_3 | 220 | 218.39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Lm-Trem(+)_ | 216 | 218.39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Lm-GDM/pl(+)_ | 218 | 218.39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Lresp2_ASI(+)_ | 230 | 231.78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.44 | 0.56 | 0 |
| TN0-SD(+)_5 | 242 | 242.06 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0.99 | 0 |
| L04-AD(+)_4 | 240 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| L04-SD(+)_4 | 238 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Lresp2_Trem(−)_ | 244 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Lm-SD(+)_4 | 242 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LN1-AD(+)_6 | 242 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Lm-AD(+)_5 | 242 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| TN0-DMsilk/pl(+)_3 | 246 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Lresp2_Nrem(−)_ | 242 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| T04-GDH8DM(−)_1 | 240 | 242.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| TN0-CCMlag(−)_4 | 254 | 249.53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.69 | 0.31 |
| TN1-WpDM/pl(+)_3 | 256 | 259.33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.27 | 0.73 |
| Tm-GNC(−)_2 | 262 | 265.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0.99 |
| LN0-GDM/pl(−)_ | 264 | 265.63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| LN0-GN/pl(−)_ | 266 | 265.63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| L04-Nrem(−)_ | 268 | 265.63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

The name of the QTL is composed of two parts (1) the name of the experiment; *L* line per se evaluation, *T* testcross evaluation, *N0* low N-input 2003, *N1* high N-input 2003, *04* high N-input 2004, *m* mean for high N-input 2003 and 2004, and (2) the acronym of the trait followed between brackets by the sign of the allele effect, with reference to the allele from parent Io. The QTL contribution to the cluster is the probability for a QTL of belonging to a given cluster

per se evaluation. One coinciding QTL explained 16% of the phenotypic variation for grain yield at high N-input while another explained 12.5% of the variation for the amount of N remobilized. Clusters 3.3, 3.4 and 3.5 (which were very close to each other) were involved in controlling the amount of N taken up after silking, total N-uptake (with a QTL showing a $r^2 = 23\%$), grain yield, grain N-yield and kernel number with line per se evaluation, with the favourable allele coming from parent Io for all traits. Cluster 4.2 was involved in the control of genetic variation for $^{15}$N-uptake *(%$^{15}$Nup1)*, grain yield and kernel weight with a favourable effect of the allele coming from parent F2. Cluster 4.6 was involved in the control of genetic variation for total N-uptake, grain yield and kernel weight, with the favourable allele coming from parent F2. Cluster 5.3 was involved in the control of % N in the kernels originating from N-uptake, grain yield and kernel weight (with line per se evaluation) with the favourable allele coming from parent Io. Cluster 5.5 corresponded to QTLs for N-uptake, grain yield, and kernel weight. Cluster 5.6 corresponds to QTLs for total N-uptake, grain yield, *NUtE*, and the amount of N remobilized, the allele from parent Io being favourable. Cluster 7.4 corresponded to QTLs involved in total N-uptake, proportion of N remobilized, senescence and grain yield, with the F2 allele favouring N-remobilization, *HI* and *NHI*, but being unfavourable for total N-uptake and grain

**Table 4** Clusters identified by MetaQTL by using (1) all detected QTLs, (2) QTLs detected only with lines (lines), and (3) QTLs detected only with testcross progenies (tc)

| Chromosome 1 | | | | Chromosome 2 | | | | Chromosome 3 | | | | Chromosome 4 | | | | Chromosome 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc |
| CI 1.1 | 27.1 | 18 | 30 | CI 2.1 | 0.5 | 0.6 | 0.4 | CI 3.1 | *0* | *0* | X | CI 4.1 | 21 | X | 18 | CI 5.1 | 36.7 | 37.5 | 34.2 |
| CI 1.2 | 45.7 | 43.7 | 50.2 | CI 2.2 | 34.8 | 36.4 | 32.8 | CI 3.2 | 71.3 | 74.8 | 64.2 | CI 4.2 | 85.8 | 86.7 | 78.5 | CI 5.2 | 62.9 | 62.7 | 71.9 |
| CI 1.3 | 77.5 | 72.9 | 82.1 | CI 2.3 | 83.6 | *74* | 81 | CI 3.3 | 96 | 95.1 | 97.6 | CI 4.3 | 107.9 | 109.8 | 106.6 | CI 5.3 | 93.1 | 93.5 | X |
| CI 1.4 | 110.7 | 109 | 113 | CI 2.4 | *96* | X | *96* | CI 3.4 | 102 | 102.3 | 100 | CI 4.4 | 133.6 | X | 135.8 | CI 5.4 | 103.4 | 102.2 | 106.9 |
| CI 1.5 | 155.3 | 147 | 153 | CI 2.5 | 104 | 100.4 | 105.8 | CI 3.5 | 102 | 102.3 | 100 | CI 4.5 | 166 | 166.6 | 165.4 | CI 5.5 | 125.6 | 125.6 | 126 |
| CI 1.6 | 170 | 169 | 173 | CI 2.6 | 120.7 | 120.6 | X | CI 3.6 | 126.7 | 129.8 | 127.9 | CI 4.6 | 180.5 | 180.5 | 182.6 | CI 5.6 | 137.5 | 139.2 | X |
| CI 1.7 | 198 | 197 | 201.6 | CI 2.7 | 128.5 | 127.6 | 134 | CI 3.7 | 153.9 | *153* | 153.9 | CI 4.7 | *208* | *208* | X | CI 5.7 | 168.6 | 168.3 | 170 |
| CI 1.8 | 218 | 219 | X | CI 2.8 | 164.3 | 164 | X | CI 3.8 | 192 | *192* | X | | | | | CI 5.8 | 188.6 | 188.1 | 190 |
| CI 1.9 | 242 | 241 | 250 | CI 2.9 | 182 | 175 | 185.9 | | | | | | | | | CI 5.9 | *206* | X | *206* |
| CI 1.10 | 269 | 266 | X | | | | | | | | | | | | | CI 5.10 | *221.7* | X | *220* |
| | | | | | | | | | | | | | | | | CI 5.11 | 229 | 229.6 | 230 |

| Chromosome 6 | | | | Chromosome 7 | | | | Chromosome 8 | | | | Chromosome 9 | | | | Chromosome 10 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc | Cluster | All QTLs | lines | tc |
| CI 6.1 | 13.1 | 12 | 14.8 | CI 7.1 | 1.1 | *0* | 0 | CI 8.1 | 21.9 | 27.3 | 20.2 | CI 9.1 | 2.8 | 0 | *18* | CI 10.1 | 0.5 | X | 0.5 |
| CI 6.2 | 39.5 | 40.1 | X | CI 7.2 | *26* | X | *30* | CI 8.2 | 59.9 | 59.5 | 61.5 | CI 9.2 | 38 | 38.6 | *36* | CI 10.2 | 30 | 30 | X |
| CI 6.3 | 92.8 | X | 92.8 | CI 7.3 | 63.3 | 54 | 64.9 | CI 8.3 | 88.2 | 88.6 | X | CI 9.3 | 50.3 | 51.1 | 50 | CI 10.3 | 53.8 | *56* | 52.5 |
| CI 6.4 | 126.4 | 119 | 127.3 | CI 7.4 | 90.6 | 91.9 | 90.1 | CI 8.4 | 95 | X | 93.2 | CI 9.4 | 84 | 86.7 | 79.8 | CI 10.4 | 74.3 | X | 73.4 |
| CI 6.5 | 145.4 | 144.6 | 156 | CI 7.5 | 107.5 | 106 | 108.7 | CI 8.5 | *119* | *120.4* | X | CI 9.5 | 146.3 | 157 | *134* | CI 10.5 | 91.6 | 86.5 | 91.2 |
| CI 6.6 | 177 | 175.3 | 176.4 | CI 7.6 | 147.2 | X | 147.2 | CI 8.6 | 136.8 | 150 | 136.4 | | | | | CI 10.6 | 118.1 | *128* | 115.6 |
| CI 6.7 | 194 | 187.7 | X | | | | | CI 8.7 | 194.2 | *190* | 194.6 | | | | | | | | |
| CI 6.8 | 206.1 | 206.6 | 205 | | | | | | | | | | | | | | | | |

For each cluster the estimated average position is given in cM for each set of QTLs. The *shaded clusters* correspond to largely overlapping clusters which can be considered as only one cluster. *Numbers in italics* correspond to only one QTL. X means that there was no QTLs in this cluster for the type of material considered (lines or testcross progenies)

N-yield in testcross progenies. Cluster 8.7 was involved in controlling the variation in total N-uptake, leaf greenness and kernel weight with the favourable allele (high N-uptake, stay-green phenotype and high kernel weight) coming from parent Io.

As far as QTLs for N-uptake and remobilization evaluated by $^{15}$N-labelling are considered, only two QTLs were detected by testcross evaluation for the proportion of $^{15}$N taken up after silking allocated to the kernels. One was located on chromosome 1 at 156 cM with a LOD of 2.8 (2004 data), and the other on chromosome 8 at 82 cM with a LOD of 2.4 (2003 + 2004 data). For the proportion of N remobilized, 20 QTLs were detected, leading to the detection of 12 chromosome regions or clusters (Table 3). Among these 12 regions only one was detected both by the N balance method and by the $^{15}$N-labelling method. Three regions were specific for the N balance method, whereas 8 regions were specific for the $^{15}$N-labelling method. As a consequence, among the 13 regions detected for the amount of N remobilized, only two of them were found with both methods.

## Clusters involving QTLs for root architecture

Several clusters involved QTLs for the root architecture detected by Guingo et al. (1998) with the same population. In cluster 2.6 there was a QTL for a deep and thin root system coinciding positively with QTLs for total N-uptake and N-remobilization. Cluster 3.7 showed coincidence between a QTL for the number of secondary roots and QTLs for *NUtE*, kernel number (positive coincidence), and senescence (*SEN2* and *ADM*, with a negative coincidence), the favourable allele (high *NUtE*, high kernel number and "stay-green" phenotype) coming from parent Io. In cluster 4.2 there was a coincidence between a QTL for the structure of the root system and QTLs for $^{15}$N-uptake (*%$^{15}$Nup1*), with a favourable effect of the allele coming from parent F2. Cluster 4.6 involved a QTL for the density of the root system in the topmost layer of the soil surface with a QTL for root traits exhibiting a strong effect ($r^2 = 20\%$), the favourable allele coming from F2 (Guingo et al. 1998); this cluster also included QTLs for total N-uptake, grain yield and kernel weight, with the favourable allele also coming from parent F2. In cluster 5.1 there was a coincidence between a QTL for root diameter and superficial root system coinciding negatively with a QTL for N-uptake, and positively with a QTL for N-remobilization. Cluster 5.4 was one the main regions identified by Guingo et al. (1998) as being involved in the control of variation of traits related to the structure of the root system. It corresponded to a QTL for grain yield ($r^2 = 13.8\%$) detected with testcross evaluation at high N-input. Cluster 5.5 grouped QTLs for the diameter of the roots, with the favourable allele coming from parent Io (large root diameter and superficial roots) and QTLs for grain yield, kernel weight, N-uptake and remobilization. In cluster 5.6 detected only with line per se evaluation, there was a QTL for root diameter coinciding with QTLs for total N-uptake,

**Table 5** QTLs detected for grain N-yield (*GNY*), N-utilization efficiency (*NUtE*), N-remobilization (*trem, Nrem*) and N-uptake (*Nup, %Nup, psNup, tG, %NupG, $^{15}$Nup*)

| Chr | pos | pos1 | pos2 | LOD | $r^2$ | Coincidences between QTL | | | | | Cluster |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ch1 | 18 | 6 | 20 | 2.3 | 12.93 | Lm-GNY(+) | L04-GNY(+) | | | | 1.1 |
| ch1 | 110 | 102 | 116 | 2.47 | 9.58 | LN1-15Nup1(+) | | | | | 1.4 |
| *ch1* | *156* | *152* | *168* | *2.77* | *6.52* | *T04-tG(+)* | | | | | *1.5* |
| ch1 | 202 | 190 | 212 | 2.18 | 9.91 | TN1-tremC(−) | | | | | 1.7 |
| *ch1* | *216* | *210* | *222* | *2.5* | *11.34* | *Lm-trem(+)* | | | | | *1.8* |
| *ch2* | *120* | *112* | *124* | *3.13* | *11.28* | *LN0-WpNup(+)* | *LN1-WpNup(+)* | *LN1-%psNup(+)* | | | *2.5* |
| *ch2* | *128* | *122* | *130* | *2.6* | *15.43* | *LN1-GNY(−)* | *Lm-Nrem(−)* | | | | *2.6* |
| ch2 | 176 | 166 | 192 | 2.27 | 8.97 | TN1-Nrem(−) | | | | | 2.8 |
| *ch3* | *78* | *70* | *80* | *3* | *8.41* | *L04-GNY(+)* | | | | | *3.2* |
| *ch3* | *96* | *92* | *104* | *3.07* | *23.12* | *Lm-WpNup(+)* | *Lm-psNup(+)* | *LN0-WpNup(+)* | *LN0-%psNup(+)* | *LN0-psNup(+)* | *3.3* |
| *ch3* | *106* | *102* | *116* | *2.84* | *17.69* | *Lm-GNY(+)* | *LN1-GNY(+)* | | | | *3.5* |
| ch3 | 148 | 136 | 158 | 2.45 | 5.78 | T04-NUtE(+) | | | | | 3.7 |
| ch3 | 192 | 186 | 192 | 2.16 | 6.88 | L04-Nrem(+) | | | | | 3.8 |
| ch4 | 80 | 72 | 84 | 2.35 | 11.06 | TN1-15Nup1(−) | | | | | 4.2 |
| *ch4* | *110* | *106* | *118* | *3.28* | *12.47* | *LN0-psNup(−)* | *Lm-%psNup* | *LN0-%psNup(−)* | *T04-GNY(−)* | | *4.3* |
| ch4 | 182 | 176 | 202 | 2.42 | 3.53 | T04-WpNup(−) | | | | | 4.6 |
| *ch5* | *44* | *32* | *56* | *4.75* | *16.64* | *L04-%psNup(−)* | *T04-tremC(+)* | *Lm-psNup(−)* | *Lm-%psNup(−)* | | *5.1* |
| | | | | | | L04-%psNup(−) | L04-NremB(+) | Lm-%NupG(−) | L04-%NupG | | |
| ch5 | 94 | 90 | 100 | 2.37 | 11.78 | LN0-%NupG(+) | | | | | 5.3 |
| ch5 | 118 | 106 | 126 | 2.34 | 9.32 | LN1-Nrem(+) | L04-WpNup(+) | | | | 5.5 |
| *ch5* | *142* | *134* | *158* | *3.22* | *19.49* | *Lm-Nrem(+)* | *Lm-NremB(+)* | *LN1-NUtE(+)* | *LN1-GNY(+)* | *LN0-WpNup(+)* | *5.7* |
| *ch5* | *194* | *186* | *204* | *2.64* | *7.9* | *L04-Nrem(+)* | | | | | *5.8* |
| ch5 | 228 | 216 | 230 | 2.25 | 9.01 | LN1-psNup(+) | LN1-%psNup(+) | | | | 5.11 |
| *ch6* | *38* | *30* | *50* | *2.85* | *8.4* | *Lm-%NupG(+)* | | | | | *6.2* |
| ch6 | 114 | 102 | 128 | 2.16 | 9.66 | TN1-tremB(−) | | | | | 6.4 |
| ch6 | 144 | 134 | 154 | 2.49 | 11.94 | Lm-WpNup(+) | | | | | 6.5 |
| *ch6* | *184* | *174* | *194* | *3.62* | *13.82* | *LN1-tremB(−)* | *LN0-NUtE(−)* | *LN1-NremB(−)* | *LN1-%NupG(+)* | | *6.6* |
| ch7 | 0 | 0 | 6 | 2.29 | 8.23 | LN0-WpNup(+) | | | | | 7.1 |
| ch7 | 14 | 2 | 28 | 2.18 | 6.89 | T04-NUtE(−) | T04-15Nup2(−) | | | | 7.1 |
| *ch7* | *92* | *88* | *98* | *2.54* | *10.33* | *L04-trem(−)* | *TN0-GNY(+)* | *TN0-WpNup(+)* | | | *7.2* |
| ch7 | 114 | 100 | 126 | 2.43 | 15.37 | Tm-Nrem(+) | | | | | 7.5 |
| ch7 | 140 | 128 | 148 | 2.3 | 17.5 | Tm-tremC(+) | | | | | 7.6 |
| *ch8* | *60* | *52* | *62* | *3.28* | *12.4* | *LN0-NremB(−)* | *LN0-Nrem(−)* | *Tm-trem(−)* | *LN0-tremB(−)* | *TN1-trem(−)* | *8.2* |
| ch8 | 82 | 68 | 114 | 2.41 | 15.28 | Tm-tG(−) | | | | | 8.3 |
| *ch8* | *92* | *88* | *96* | *2.5* | *10.86* | *LN1-GNY(−)* | *Tm-trem(−)* | | | | *8.4* |
| *ch8* | *134* | *128* | *136* | *4.37* | *11.5* | *Tm-trem(−)* | *T04-WpNup(+)* | *T04-psNup(+)* | *Tm-psNup(+)* | *Tm-GNY(+)* | *8.6* |
| | | | | | | *Tm-Nup(+)* | *T04-tremC(−)* | *T04-tremB(−)* | *Tm-tremC(−)* | *Tm-tremB(−)* | |
| *ch8* | *144* | *128* | *164* | *2.76* | *17.29* | *Tm-%NupG(+)* | *T04-%NupG(+)* | *Tm-%Nup(+)* | | | *8.6* |
| *ch8* | *188* | *172* | *196* | *2.67* | *11.11* | *T1-Nup(+)* | | | | | *8.7* |
| ch9 | 18 | 14 | 24 | 2.17 | 11.35 | TN0-15Nup1(+) | | | | | 9.1 |
| *ch9* | *38* | *36* | *44* | *2.78* | *17.83* | *Lm-Nrem(+)* | *L04-Nrem(+)* | *L04-tremB(+)* | *L04-trem* | | *9.2* |
| ch9 | 46 | 44 | 50 | 2.4 | 7.33 | T04-NUtE(+) | | | | | 9.3 |
| *ch9* | *100* | *88* | *112* | *2.94* | *15.85* | *LN1-GNY(+)* | | | | | *9.4* |
| *ch9* | *134* | *132* | *138* | *4.61* | *6.82* | *TN1-trem(+)* | | | | | *9.5* |
| ch10 | 0 | 0 | 4 | 2.21 | 4.75 | T04-Nup(+) | | | | | 10.1 |
| ch10 | 102 | 86 | 114 | 2.48 | 7.31 | T04-NremB(+) | | | | | 10.5 |

Values given for QTL position (*pos, pos1* and *pos2* defining the confidence interval), LOD and $r^2$ corresponds to the QTL given in the first column of coincidences. The name of the QTL is composed of two parts (1) the name of the experiment; *L* line per se evaluation, *T* testcross evaluation, *N0* low N-input 2003, *N1* high N-input 2003, *04* high N-input 2004, *m* mean for high N-input 2003 and 2004, and (2) the acronym of the trait followed between brackets by the sign of the allele effect, with reference to the allele from parent Io. QTL with a LOD ≥ 2.5 are in *italics*

amount of N remobilized, grain yield, and N-utilization efficiency, the allele from parent Io being favourable for all five traits in plants having a superficial root system.

### Clusters with QTLs for ASI and NNI

Both ASI and NNI correspond to traits related to the physiological state of the plant at silking. ASI is increased under stress conditions whereas NNI is related both to the N content and the N status of the chlorophyll apparatus. Among the nine clusters involved in the control of ASI (1.6, 1.8, 2.2, 2.3, 2.4, 2.6, 3.4, 3.5, 10.5), three showed a coincidence with QTLs for grain N yield, a short ASI being beneficial to an increase in grain N yield. The same number of coincidences with the same sign was observed with grain yield. However, for grain yield, two clusters showed a coincidence with an opposite trend. There were no clear coincidences between QTLs for ASI and QTLs for N-remobilization and N-uptake.

Among the nine clusters involving QTLs for *NNI* at silking (1.7, 1.8, 2.6, 3.2, 3.5, 5.2, 5.3, 8.2, 8.5), clear coincidences were observed: six positive coincidences with QTLs for grain yield, five positive coincidences with QTLs for grain N-yield, four positive coincidences with QTLs for thousand kernel weight, and four positive coincidences for QTLs for N-remobilization.

### Clusters with QTLs for senescence and related traits (CCM and Dualex measurements)

Among 20 clusters involving QTLs for senescence evaluated visually, nine showed a negative coincidence between QTLs for senescence and QTLs for N-uptake or a positive coincidence of QTLs for N-uptake and QTLs for leaf greenness (2.5, 3.3–3.4, 3.7, 4.3, 6.2, 6.5, 7.4 and 8.7). Measurements with the chlorophyll meter (CCM) did not show clear coincidences (positive and negative) among the 14 clusters where QTLs for *CCM* measurements were found. Four clusters showed a positive coincidence between QTLs for senescence and QTLs for grain yield, but three clusters showed a negative coincidence. For Dualex measurements, which provided information on the level of chlorophyll degradation, among nine clusters involving QTLs for Dualex measurements, three showed a negative coincidence with QTLs for grain yield, whereas three others showed a positive coincidence with QTLs for grain N-content.

### Clusters with QTL for GS and GDH activity

At cluster 2.4 a QTL for total leaf GS activity at the beginning of the grain filling period showed a coincidence between a QTL for *ASI* with a favourable effect of the allele coming from F2 (short ASI and high GS activity). Cluster 4.2 grouped QTLs for GS activity in the leaves of young vegetative plants, $^{15}$N-uptake (*%$^{15}$Nup1*), root thinness, grain yield and kernel weight with a favourable effect of the allele coming from parent F2. Cluster 4.3 grouped QTLs for leaf GS activity after silking, root GS activity in young vegetative plants (Hirel et al. 2001), kernel weight (with a QTL showing a $r^2 = 18\%$ for testcross evaluation) and senescence with the favourable allele (high-kernel weight and stay-green) coming from parent F2. At cluster 5.4 coincided a QTL for leaf GS activity in young vegetative plants, a QTL for root system and a QTL for grain yield ($r^2 = 13.8\%$) detected with testcross evaluation at high N-input. The allele from parent Io was simultaneously favourable to grain yield, leaf GS activity, and for the development of a deep and thin root system. Cluster 8.2 corresponded to a QTL for leaf GS activity grouped with QTLs mainly involved in controlling the variability in the amount and proportion of N remobilized, senescence (CCM) and kernel weight. This cluster also corresponded to QTLs for N responsiveness of NNI ($r^2 = 12.5\%$). The allele from parent F2 was favourable for N-remobilization but unfavourable for kernel weight, CCM and GS activity.

For GDH aminating and deaminating activities, 15 QTLs corresponding to 15 clusters were detected. However, there were no clear coincidences with other QTLs. Among six clusters, QTLs for GDH activity coincided negatively with QTLs for grain yield, although the correlations between the two traits were not significant (data not shown). Similarly, there were negative coincidences of QTLs for GDH activity in four clusters with QTLs for N-uptake and in three clusters with QTLs for kernel number. Therefore, high GDH activity appears to be rather unfavourable for these two traits.

## Discussion

### Meaning of the clustering

The low frequency of "mix" QTLs, that are belonging to two or three clusters, tends to show a low background noise in the clustering analysis due to the presence of false QTLs. Indeed, these "mix" QTLs could correspond to false QTLs. False QTLs can also be distributed within clusters or correspond to isolated QTLs which were also not very frequent. Indeed results from simulation (Fig. 2) show that even with random distribution of the QTLs there is still clustering. However, the model selected by the Akaike criterion, used in the software MetaQTL, is a model with fewer clusters than expected with a random distribution of QTLs and this model has a very low probability to happen by chance. An increase in the threshold LOD values to 2.5 led to the detec-

tion of nearly the same clusters or types of coincidences (Fig. 4, and ESM S11 to S20). With the two LOD levels, the non ambiguity of the clustering, that is the assignment of QTLs to clusters with a high probability, gives some confidence in the clustering. As a consequence, as discussed further, coincidences between two QTLs in a cluster can thus be due to chance but it can also have a genetic and physiological meaning. When consistent with known physiological relationship between the two involved traits it can mean pleiotropy. However, it could also be the result of linkage between loci.

## Expression of genetic variation for line per se and testcross performance

The high proportion (73.8%) of common clusters for the two types of progenies (lines and testcross progenies) contrasts with the low proportion of QTLs found to be common to both line per se evaluation and testcross evaluation for a given trait (7.6%). It is also worth noting that, in 2003, more QTLs were common to both levels of N fertilization than to lines and testcross progenies. The lack of power of the experiments appears to be insufficient to explain such results, because it will rather introduce noise in the clustering. In the absence of epistasis, due to the masking effect of the dominant genes from the tester, QTLs detected with testcross evaluation are expected to be a subset of those detected with line per se evaluation. However, in this study, we did not observe such a phenomenon. In several studies, QTLs for grain yield (Beavis et al. 1994; Austin et al. 2000) and for flowering time (Szalma et al. 2007) detected in inbred lines were also different from those detected in hybrids. In addition, different QTLs for heterosis and per se value were identified for plant height (Tang et al. 2007). If we consider the results from these previous studies and those presented here, we can conclude that genetic variation is expressed differently in lines and in hybrids.

To explain our results, with a large proportion of common clusters between lines and testcross progenies in spite of different QTLs for a given trait, we formulate the hypothesis that there are pleiotropic and epistatic QTLs underlying the clusters identified in the present study. For a given trait, the genetic variation could be expressed differently according to the heterozygosity of the genome due to an interaction between QTLs and the genetic background, that is epistasis. However, epistasis between detected QTLs was not significant both for lines and testcross progenies. This could be due to a lack of power of the experiment. A greater epistasis would be expected at the level of line per se evaluation due to the presence of a genetic load at some loci that limits the expression of favourable genes at other loci. However, while epistasis tested by a comparison of the RIL population mean to the parental mean affected different traits at the level of lines and of testcross progenies, epistatic interactions were not more frequent for lines (Coque and Gallais 2008). The study of Hua et al. (2003) on rice which showed that, probably due to epistasis, QTLs for heterosis were different from QTLs detected for the line per se value, further supports our hypothesis that genetic variation is expressed differently in lines than in testcross progenies. Similarly, genetic variation could also be expressed differently at low and high N-input mainly for testcross evaluation as already shown by Bertin and Gallais (2001). Tuberosa et al. (2002, 2007) also put forward the same hypothesis to explain the finding that QTLs detected under drought stress conditions were not the same as QTLs detected under water sufficient conditions. Bouchez et al. (2002) have also speculated that in the presence of genotype x environment interaction, pleiotropy could lead to the detection of QTLs for different related traits according to the environment. From a functional genomic point of view, this means that genes could be regulated differently according to their environment (physical or genetic). This is supported by several studies showing a differential gene expression between lines and hybrids (Sun et al. 2004; Stupar and Springer 2006; Swanson-Wagner et al. 2006).

## QTL clusters with QTLs for N utilization efficiency, N-remobilization and N-uptake

QTLs for N-uptake coincided positively either with QTLs for kernel number or with QTL for kernel weight. This could be explained by the role of the putative genes controlling these two QTLs that may be expressed differentially at different stages of plant development (Dubois et al. 2003; Limami et al. 2002). Clusters (e.g. 3.2, 3.5, 4.1, 8.2) involved in kernel number and N-uptake could correspond to genes controlling allocation of N to the developing embryos, just after fertilization. Indeed, these clusters also grouped QTLs for N content or amount at silking. QTLs involved in controlling kernel weight (e.g. 4.2, 4.6, 5.5) are likely playing a role during the grain filling period. Some clusters showed a positive coincidence between QTLs for grain yield and QTLs for N-remobilization (7.5, 8.2, 9.2). Again the coincidence can be positive with kernel number and kernel weight, showing the role of N remobilization just after fertilization in first determining kernel number and then kernel size during the grain filling period. Several clusters (5.1, 7.4, 8.6) exhibited a negative coincidence of QTLs for N-remobilization and postsilking N-uptake. This is consistent with the negative correlations between these two traits already observed by Coque and Gallais (2007, 2008) which has a physiological basis through the opposite effect of senescence on N-uptake and N-remobilization (see below). This could be the result of pleiotropy or linkage between loci affecting N-uptake and N-remobilization in an

opposite way. However, some clusters grouped QTLs only involved in the control of N-remobilization or N-uptake and some others (2.5, 2.6, 5.5, 5.6 and 6.6) showed some kind of break-down for the negative coincidence as they exhibited a positive coincidence between QTLs for N-uptake and QTLs for N-remobilization. This could correspond to two linked QTLs affecting N-uptake and N-remobilization in the same direction.

One of the objectives of this work was to detect QTLs for N-remobilization and postsilking N-uptake, utilizing the $^{15}$N-labelling technique developed by Coque and Gallais (2007, 2008). The low number of QTLs detected for postsilking N-uptake is consistent with the low genetic variation previously observed for this trait (Coque and Gallais, 2007). More QTLs were detected for N-remobilization with the labelling method because $^{15}$N-translocation was estimated more accurately, as already demonstrated by Coque and Gallais (2007, 2008), thus allowing a better detection of the genetic variability existing for this trait. Fewer QTLs for N-remobilization were detected using the $^{15}$N-method compared to the balance method. As predicted by the correlation studies, they in fact do not evaluate the same physiological processes. The balance method leads to more biased results than the $^{15}$N-method (Gallais et al. 2006, 2007).

### Clusters with QTLs for root architecture

Eight clusters showed a coincidence between root architecture and traits related to N-uptake, N-remobilization and grain yield (2.6, 3.7, 4.2, 4.6, 5.1, 5.4, 5.5, 5.6). Considering the role of root system in N-uptake, the coincidence between QTLs for root architecture and N-uptake was expected. The favourable allele of three clusters (2.6, 4.2, 5.4) corresponded to a deep and thin root system, able to catch N in deep soil horizons. Three clusters correspond to a positive association between root diameter and superficial root development, which can be efficient for N uptake before silking. In the case of cluster 4.6, a superficial root system, allowing the uptake of N mostly before flowering, could explain the association found between this trait and the onset of leaf senescence (evaluated by the relative change in the CCM values). Senescence is generally induced as the result of a shortage in soil N availability. Moreover, the overall efficiency of the root system in taking up N depends not only on root architecture, but also on because the availability of carbohydrates provided by photosynthesis that are necessary to maintain root activity (Tolley-Henry and Raper 1991).

### Clusters with QTLs for ASI and NNI

In maize, ASI is an indicator of stress (water, mineral deficiency…) suffered by the plant (Gallais and Coque 2005). The positive coincidence between QTLs for ASI and QTLs for grain yield or grain N yield is consistent with the positive correlation observed between ASI and both grain yield and grain N yield (Gallais and Coque 2005; Coque and Gallais 2007, 2008). The coincidence between a QTL for ASI and a QTL for GS activity (cluster 2.4) is consistent with the assumption that a short ASI could be associated with the ability of the plant to transfer carbon (C) and N compounds to young developing embryos (Pan et al. 1984; Andrade et al. 2002; Echarte et al. 2004; Gallais and Coque 2005). NNI evaluated at silking is an indicator of both the amount of N that can be remobilized and of the integrity of the photosynthetic apparatus (Lemaire and Gastal 1997). The positive coincidences between QTL for NNI and QTLs for grain yield, kernel weight and N remobilization illustrates the pertinence of this trait. However, NNI is difficult to determine, and cannot be replaced with enough accuracy by chlorophyll meter measurements at silking when studying genetic variation of the trait. Therefore, for a fast and reliable determination of the plant N status, the development of simple and affordable monitoring tools is required to help the breeder (Hirel et al. 2007).

### Clusters with QTLs for senescence

Several clusters showed positive coincidence between leaf greenness and N-uptake (2.6, 4.3, 6.5, 8.7, 9.1) and reciprocally between N-remobilization and senescence (6.4, 7.4). This type of coincidences was expected on the basis of (1) the physiological relationship existing between the efficiency of photosynthesis (involving a prolonged greenness of the leaves) and N-uptake (Coque and Gallais 2007, 2008), and (2) the relationship between senescence and remobilization. In some clusters (3.4, 3.7, 4.1) the coincidence was negative with QTLs for kernel number, which suggests that leaf greenness plays an important role just after ovule fertilization in limiting embryo abortion. During this period the plant must be able to transfer N and C compounds to young embryos, through efficient N-remobilization and active photosynthesis, both processes being less favoured in early senescing genotypes. The efficiency of these two processes also affects grain N yield, since four clusters showed a negative coincidence between QTLs for senescence and QTLs for grain N yield. However, QTLs for kernel weight and grain yield coincided negatively and positively with QTLs for senescence in almost the same number of clusters. This could mean that under some environmental conditions, senescence associated with N-remobilization is favourable to kernel weight and thus to grain yield. In other situations when there is not enough N accumulated in vegetative tissues, premature senescence will lead to a decrease in N-uptake and thus in grain yield.

Finally, it appears that under our experimental conditions the visual notation of senescence gives more reliable results as compared to the measurements performed with a chlorophyll meter or a Dualex apparatus.

Clusters with QTLs for GS and GDH activity

If we consider the coincidences between QTLs for NUE-related traits, and QTLs for GS activity, it seems that the enzyme could play two distinct roles. First, the coincidence of a QTL for leaf GS activity with a QTL for N-uptake and root architecture (cluster 4.2, 5.4) is consistent with the anabolic role of the enzyme for N assimilation (Hirel et al. 2001). GS may also be involved during N remobilization (cluster 4.3, 8.2). For example, on chromosome 4, coincidence of QTLs for GS activity, kernel weight, and senescence, at the GS locus encoding the GS isoform GS1.4 suggests that this isoform is specifically involved in controlling both kernel weight and senescence through the process of leaf protein remobilization (Vincent et al. 1997; Martin et al. 2006). Signs of coincidences also showed that high root GS activity in young vegetative plants and high leaf GS activity at the beginning of the grain filling period would be unfavourable to kernel weight as most of the N channelled through this pathway will be used for kernel set. However, the combination of the two GS activities could be favourable for setting kernel number just after fertilization, kernel weight and kernel number being traits negatively correlated.

Although GDH is induced during leaf senescence in several plant species (Masclaux et al. 2000; Dubois et al. 2003), we did not find any clear coincidence between QTLs for the enzyme activities and QTLs for senescence. This finding is not surprising if we consider that at least in maize, the enzyme is apparently not directly involved either in leaf senescence or in leaf N remobilization (Tercé-Laforgue et al. 2004; Hirel et al. 2005a, b) but rather in response to a stress condition (Dubois et al. 2003; Skopelitis et al. 2006).

## Conclusions

Our results show that genetic variability, for the set of traits evaluated, is expressed differently at the level of lines and at the level of hybrids. This has two consequences, one methodological for QTL detection and the other more applied for plant breeding. From the point of view of QTL detection, if the aim of the study is to detect QTLs that influence agronomic performance, then the detection must be performed at the hybrid level using testcross progenies. From a breeding point of view, the low number of QTLs common to line per se and testcross evaluation is consistent with the poor correlation already observed for most agronomic traits or traits related to N utilization when both types of plant material were evaluated. This means that the selection, if possible at the level of lines must be applied only at a low intensity (Coque and Gallais 2008).

At the level of identified clusters, it is impossible to separate pleiotropy or linkage between close loci. The resolution of any experimental design for the QTL detection is generally too low to distinguish between the two situations. If the coincidences have no physiological meaning, they can be due either to hazard or to the linkage between QTLs controlling variation for independent traits. If the coincidences of the QTLs for different traits are physiologically relevant, as found in several cases in our study, this, added to the non ambiguity of the clustering, gives more meaning and confidence to the clusters. It is worth mentioning that in the same population ten of the detected QTL clusters (2.3, 2.5, 3.2, 3.4–3.5, 4.3, 5.3, 5.5, 6.8, 8.2 and 9.2) have already been identified following a selection experiment for grain yield at low and high N-input based on the change in the frequency of markers (Coque and Gallais 2006).

One major result which emerges from our study, and that confirms previous correlation studies (Coque and Gallais 2007, 2008) is the role of both leaf greenness and the structure of the root system in controlling N-uptake. The study of coincidences between QTLs confirms the unfavourable effect of leaf senescence on N-uptake and kernel number. It appears possible to produce genetic material in which N-remobilization associated with a limited degradation of proteins from the photosynthetic apparatus will not occur at the expense of N-uptake. N-remobilization can be maximized if large amount of N is accumulated before silking. If the accumulation of N before silking is not sufficient, it is likely that N-remobilization will occur at the expense of a functional photosynthetic apparatus. Consequently, breeding for "stay-green" genotypes may favour N-uptake before and after silking and thus may limit the negative effect of remobilization on photosynthesis and N-uptake (Borrell and Hammer 2000, 2001). As shown by several positive coincidences between QTLs for N-uptake and QTLs for root system traits, the other way to increase N-uptake is to breed for a root system more efficient in terms of N-uptake (Lea and Azevedo 2006). However, evaluating the contribution of the root system to the plant N utilization in the field is not easy due to the difficulty of studying the functionality of the roots directly in the soil (Hirel et al. 2007). Therefore, breeding for "stay-green" genotypes could be an indirect way to improve the efficiency of the root system in capturing soil N under agronomic conditions.

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## References

Agrama H, Zakaria A, Said F, Tuinstra M (1999) Identification of quantitative trait loci for nitrogen use efficiency in maize. Mol Breeding 5:187–195

Andrade FH, Echarte L, Rizzalli R, Della Maggiora A, Casanovas M (2002) Kernel number prediction in maize under nitrogen or water stress. Crop Sci 42:1173–1179

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

Austin DF, Lee M, Veldboom LR, Hallauer AR (2000) Genetic mapping in maize with hybrid progeny across testers and generations grain yield and grain moisture. Crop Sci 40:30–39

Beavis WD, Smith OS, Grant D, Fincher R (1994) Identification of quantitative trait loci using a small sample of topcrossed and F4 progeny in maize. Crop Sci 34:882–886

Bertin P, Gallais A (2000) Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agrophysiological results. Maydica 45:53–66

Bertin P, Gallais A (2001) Physiological and genetic basis of nitrogen use efficiency. II. QTL detection and coincidences. Maydica 46:53–68

Borrell A, Hammer G (2000) Nitrogen dynamics and the physiological basis of stay-green in sorghum. Crop Sci 40:1295–1307

Borrell A, Hammer G, van Oosterom E (2001) Stay-green: a consequence of the balance between supply and demand for nitrogen during grain filling? Ann Appl Biol 138:91–95

Bouchez A, Hospital F, Causse M, Gallais A, Charcosset A (2002) Marker-assisted introgression of favourable alleles at quantitative trait loci between maize elite lines. Genetics 162:945–1959

Causse M, Santoni S, Damerval C, Maurice A, Charcosset A, Deatrick J, de Vienne D (1996) A composite map of expressed sequences in maize. Genome 39:418–432

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

Cerovic ZG, Ounis A, Cartelat A, Latouche G, Goulas Y, Meyer S, Moya I (2002) The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves. Plant Cell Environ 25:1663–1676

Coque M, Gallais A (2006) Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize traits in maize. Theor Appl Genet 112:1205–1220

Coque M, Gallais A (2007) Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 1. Evaluation by $^{15}$N-labeling, heritabilities and correlations among traits for testcross performance. Crop Sci 47:1787–1796

Coque M, Gallais A (2008) Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 2. With line per se evaluation, comparison with testcross performance. Maydica (in press)

Dubois F, Terce-Laforgue T, Gonzalez-Moro M-B, Estavillo J-M, Sangwan R, Gallais A, Hirel B (2003) Glutamate dehydrogenase in plants: is there a new story for an old enzyme? Plant Physiol Biochem 41:565–576

Echarte L, Andrade FH, Vega CRC, Tollenaar M (2004) Kernel number determination in Argentinean maize hybrids released between 1965 and 1993. Crop Sci 44:1654–1661

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

Gallais A, Coque M (2005) Genetic variation for nitrogen use efficiency in maize: a synthesis. Maydica 50:531–547

Gallais A, Coque M, Quilleré I, Prioul JL, Hirel B (2006) Modelling post-silking N-fluxes in maize using $^{15}$N-labeling-field experiments. New Phytol 172:696–607

Gallais A, Coque M, Quilleré I, Le Gouis J, Prioul JL, Hirel B (2007) Estimating proportions of N-remobilization and of post-silking N-uptake allocated to maize kernels by $^{15}$N labeling. Crop Sci 47:685–691

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

Guingo E, Hébert Y, Charcosset A (1998) Genetic analysis of root traits in maize. Agronomie 18:225–235

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, 468p

Hirel B, Bertin P, Quillere I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, 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

Hirel B, Martin A, Tercé-Laforgue T, Gonzalez-Moro MB, Estavillo JM (2005a) Physiology of maize I: A comprehensive and integrated view of nitrogen metabolism in a $C_4$ plant. Physiol Plant 124:167–177

Hirel B, Andrieu B, Valadier MH, Renard S, Quillere I, Chelle M, Pommel B, Fournier C, Drouet JL (2005b) Physiology of maize. II: Identification of physiological markers representative of the nitrogen status of maize (*Zea mays*) leaves during grain filling. Physiol Plant 124:178–188

Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369–2387

Hua J, Xing Y, Wu W, Xu C, Sun X, Yu S, Zhang Q (2003) Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. PNAS 100:2574–2579

Lea PJ, Azevedo RA (2006) Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. Ann Appl Biol 149:243–247

Lemaire G, Gastal F (1997) N-uptake and distribution in plant canopies. In: Lemaire G (ed) Diagnosis of the nitrogen status in crops. Verlag, Berlin, pp 3–43

Limami AM, Rouillon C, Glevarec G, Gallais A, Hirel B (2002) Genetic and physiological analysis of germination efficiency in maize in relation to N metabolism reveals the importance of cytosolic glutamine synthetase. Plant Physiol 130:1860–1870

Lincoln S, Daly A, Lander E (1993) Constructing genetic linkage maps with MAPMAKER/EXP Version 3.0. Withehead Institute

Martin A, Lee J, Kichey T, Gerentes D, Zivy M, Tatou C, Balliau T, Valot B, Davanture M, Dubois F, Terce-Laforgue T, Coque M, Gallais A, Gonzalez-Moro MB, Bethencourt L, Quillere I, Habash DZ, Lea PJ, Charcosset A, Perez P, Murigneux A, Sakakibara H, Edwards KJ, Hirel B (2006) Two cytosolic glutamine synthetase isoforms of maize (*Zea mays* L.) are specifically involved in the control of grain production. Plant Cell 18:3252–3274

Masclaux C, Valadier MH, Brugiere N, Morot-Gaudry JF, Hirel B (2000) Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. Planta 211:510–518

Pan WL, Kamprath EJ, Moll RH, Jackson WA (1984) Prolificacy in corn: its effects on nitrate and ammonium uptake and utilization. Soil Sci Soc Am 48:1101–1106

Presterl T, Groh S, Landbeck M, Seitz G, Schmidt W, Geiger HH (2002) Nitrogen uptake and utilization efficiency of European

maize hybrids developed underconditions of low and high nitrogen input. Plant Breeding 121:480–486

Sampoux JP, Gallais A, Lefort-Buson M (1989) Intérêt de la valeur propre des descendances S1 associée à la valeur en croisement avec un testeur pour la sélection du maïs fourrage. Agronomie 9:511–520

Skopelitis DS, Paranychiankis NV, Paschalidis KA, Plianokis ED, Delis ID, Yakoumakis DI, Kouvarakis A, Papadakis A, Stephanou EG, Roubelakis-Angelakis KA (2006) Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenase to form glutamate for proline synthesis in tobacco and grapevine. Plant Cell 18:2767–2781

Stupar RM, Springer NM (2006) Cis-transcriptional variation in maize inbred lines B73 and Mo17 lead to additive expression patterns in the F1 hybrid. Genetics 173:2199–2210

Sun Q, Wu LM NIZF, Meng FR, Wang ZK, Lin Z (2004) Differential gene expression patterns in leaves between hybrids and their parental inbreds are correlated with heterosis in a wheat diallel cross. Plant Sci 166:651–657

Swanson-Wagner RA, Jia Y, DeCook R, Borsuk LA, Nettleton D, Snable PS (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F1 hybrid and its inbre parents. Proc Natl Acad Sci USA 103:6805–6810

Szalma SJ, Hostert BM, LeDeaux JR, Stuber CW, Holland JB (2007) QTL mapping with near-isogenic lines in maize. Theor Appl Genet 114:1211–1228

Tang JH, Ma XQ, Teng WT, Yan JB, Wu WR, Dai JR, Li JS (2007) Detection of quantitative trait loci and heterotic loci for plant height using an immortalized F2 population in maize. Chin Sci Bull 52:477–483

Tercé-Laforgue T, Mäck G, Hirel B (2004) New insights towards the function of glutamate dehydrogenase revealed during source-sink transition of tobacco (*Nicotiana tabacum* L.) plants grown under different nitrogen regimes. Physiol Plant 120:220–228

Tolley-Henry L, Raper CD Jr (1991) Soluble carbohydrate allocation to roots, photosynthetic rate of leaves, and nitrate assimilation as affected by nitrogen stress and irradiance. Bot Gaz 152:23–33

Tuberosa R, Salvi S, Sanguineti MC, Landi P, MacCaferri M, Conti S (2002) Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives in drought-stressed maize. Ann Bot 89:941–963

Tuberosa R, Giuliani S, Parry MAJ, Araus JL (2007) Improving water use efficiency in Mediterranean agriculture: what limits the adoption of new technologies? Ann Appl Biol 150(2):157–162

Utz HF, Melchinger AE (1995) PLABQTL: a computer program to map QTL. J QTL. 1

Veyrieras JB, Goffinet B, Charcosset A (2007) MetaQTL: a package of new computational methods for the meta-analysis of QTL mapping experiments. BMC Bioinf 8:49

Vincent R, Fraisier V, Chaillou S, Limami MA, Deléens E, Phillipson B, Douat C, Boutin JP, Hirel B (1997) Overexpression of a soybean gene encoding cytosolic glutamine synthetase in shoots of transgenic *Lotus corniculatus* L. plants triggers changes in ammonium assimilation and plant development. Planta 201:424–433