Genetic analysis of wheat nitrogen use efficiency: coincidence between QTL for agronomical and physiological traits

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ABSTRACT

Nitrogen (N) fertilizer represents one of the main inputs of the winter wheat crop in terms of operational and energetic costs. Our objective is then to identify chromosomal regions that control N use efficiency (NUE). We developed a QTL approach using a wheat mapping population grown in the field over three years. QTL were detected for the activities of two marker enzymes of N metabolism: glutamine synthetase (GS) and glutamate dehydrogenase (NADH-GDH). Analyses of variance showed significant genotypic effects for the two enzyme activities and broad sense heritabilities were 0.41 for GDH and 0.53 for GS. QTL for GDH activity were detected on 18 regions located on 13 chromosomes. QTL for GS activity were detected on 15 regions spread over 12 chromosomes. Several QTL regions were detected only in one year, but four for GDH and three for GS were detected on at least two years. QTL for GS and GDH activities overlapped in four regions located on chromosomes 2A, 2D, 5A and 7A. The QTL regions detected in the present study were compared to QTL previously detected for agronomical traits using the same population. Fourteen of the 18 regions containing a QTL for NADH-GDH activity were common to regions previously detected for yield and its components. This was also the case for seven regions containing a QTL for GS activity. Out of the regions containing both physiological and agronomical QTL, three were known to carry major genes acting on photoperiod sensitivity (*Ppd-D1* on 2D), plant height (*Rht-B1* on 4B) and awnedness (*B1* on 5A). The present study enabled better characterization of the other regions and the identification of possible candidate functions involved in the control of yield in relation to NUE.

INTRODUCTION

Nitrogen (N) fertilizers have been largely used to increase grain yield and grain protein content in bread wheat. However, farmers must optimise their use to avoid pollution risks and lower their operational costs. Nitrogen has a negative environmental impact by causing pollution to groundwater and rivers, and by contributing to green-house gas emissions. Nitrogen also represents a substantial cost for the farmers that tends increasing as the energetic cost for their production is very high. Therefore, N use efficiency (NUE) has become a trait of major importance for plant research and breeding (Hirel et al. 2007).

NUE is a complex polygenic trait that has been subjected to quantitative trait locus (QTL) analyses. The first QTL studies were focused on integrative traits such as NUE itself or its components on different crop species (eg Bertin and Gallais 2001 on maize; An et al. 2006 on wheat) The combination of agronomical analyses and physiological studies on N metabolism enabled the identification of candidate genes putatively involved in both the control of NUE and yield (Obara et al 2001, Hirel et al. 2001, Habash et al. 2007). It was shown in maize that different QTL related to yield were coincident to QTL for cytosolic glutamine synthetase (GS1, EC 6.3.1.2) activity and to GS structural genes (Hirel et al 2001). Glutamine synthetase is a key enzyme involved in ammonia assimilation and recycling in plants. There is also evidence that the glutamate dehydrogenase enzyme (NAD(H)-GDH, EC 1.4.1.2) enzyme may also be implicated in the control of crop productivity at least in maize as demonstrated by using a quantitative genetics approach (Dubois et al. 2003).

The objective of this work was to investigate the potential role of the two enzymes GS and GDH in the genetic determinism of N use for grain production and protein content.

MATERIALS AND METHODS

A population of di-haploid lines (DHL) was obtained from the cross between two bread wheat (*Triticum aestivum* L.) cultivars. Arche is a cultivar tolerant to N deficiency, while Récital is susceptible (Le Gouis et al. 2000). We used in the present study subsets of the original 241 DHL already studied by Laperche et al. (2006; 2007). Three experiments were carried out in 2004, 2006 and 2007 at Estrées-Mons INRA experimental station (Somme, Northern France). Depending on the year, the crop received between 160 and 180 kg N.ha^{-1} according to the current agricultural practices. Fungicide, herbicide, and insecticide treatments were applied each year to minimise non-treatment yield effects. At each sampling date, two samples of five main shoots or first-order tillers were considered for each parental line and for each DHL. In

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2004, the flag leaf lamina was harvested 14 days after flowering (DAF) and 28 DAF. In 2006, the flag leaf lamina was collected at flowering (FL), 14 DAF and 28 DAF. In 2007, the flag leaf lamina, the flag leaf sheath and the peduncle were harvested at 14 DAF. GS and NADH-GDH activities were measured as described by Kichey et al. (2007). The mean of each line for each year was considered for QTL detection. QTL analyses were carried out using the Unix version of QTL cartographer 1.17d (Basten et al. 2002). Model 6 was used to carry out composite interval mapping (CIM). We used the 'experimentwise' threshold defined at the 10% error level estimated from 1,000 permutations. In our case, the LOD threshold corresponded to 2.50. Confidence intervals were defined by a LOD drop-off of one unit. Two QTL were considered to co-localized in the same region when their confidence interval overlapped.

Table 1: Significance of the genotype effect, coefficient of variation (CV), broad sense heritability and means in each environment (combination of year, organ and sampling date) for glutamine synthetase (GS) and glutamate dehydrogenase (NADH-GDH) activities measured in a wheat DH lines population. For a given trait, two means followed by the same letter were not significantly different according to the standard Newman-Keuls multirange test (p<0.05).

	GS μmol min^{-1} gDW^{-1}	NADH-GDH μmol min^{-1} gDW^{-1}
Genotype effect	0.0001	0.0001
CV (%)	18.7	23.0
Heritability (%)	0.53±0.05	0.41±0.07
2004-Lamina-14DAF	23.1c	1.87d
2004-Lamina-28DAF	13.9d	2.02c
2006-Lamina-F	28.2a	3.02a
2006-Lamina-14DAF	22.8c	2.09c
2006-Lamina-28DAF	8.7e	2.43b
2007-Lamina-14DAF	25.9b	1.55e
2007-Sheath-14DAF	10.6	0.72f
2007-Peduncle-14DAF	5.3f	0.79f

RESULTS AND DISCUSSION

Analyses of variance showed highly significant genotypic effects for both NADH-GDH and GS activities (Table 1). Broad sense heritabilities were 0.41 for GDH and 0.53 for GS. The changes observed in the physiological traits between the sampling dates were consistent over the three years of experimentation (Table 1). In 2004 and 2006, we observed a decrease in GS activity between 14DAF and 28DAF. GDH activity increased during the same period. In 2006, GS and NADH-GDH activities were higher at FL compared to 14DAF and 28DAF. In 2007, both enzyme activities were higher in the flag leaf lamina than in the flag leaf sheath or peduncle. These results are consistent with

those previously published on the two parents of the population by Kichey et al. (2007) in different experiments. The whole population was highly variable for the two enzyme activities. For example, on average for the 108 DH lines that were common to the three experimental years, GS activity ranged from 16.5 to 29.5 μmol min^{-1} gDW^{-1} and NADH-GDH activity ranged from 1.34 to 2.68 μmol min^{-1} gDW^{-1}. In both cases, the frequency distribution of DHL means followed a normal distribution.

QTL were detected for a combination of year, stage and organ. QTL for NADH-GDH activity were detected on 18 chromosomal regions (Table 2) located on 13 chromosomes: 1A, 1D, 2A, 2B, 2D, 3B, 4B, 5A, 5B, 5D, 6A, 7A and 7B. The percentage of phenotypic variance explained by the QTL (R²) ranged from 5 to 18,2 %. The allele coming from the parental line Arche was favourable for 12 QTL regions and the allele coming from Récital for the 6 other regions. Most of the QTL were detected only on one experimental year. However, three QTL regions were detected over two years and one region (2D) over the three years. Yang et al. (2007) on barley also reported such a genotype x year interaction for protease activities in barley. However they indicated the predominance of interactions associated with differences in the magnitude of effects between years. In our study, the finding that many QTL were only detected on one year probably arose from the relatively low heritability often associated with physiological traits (Yang et al. 2007).

Table 2: Number of QTL detected for glutamine synthetase (GS) and glutamate dehydrogenase (NADH-GDH) activities and co-localisation with QTL regions detected by Laperche et al (2007) on the same wheat population.

	NADH-GDH	GS
Number of QTL regions	18	15
% variance explained	5.0 / 18.2	5.4 / 20.4
Detected one year	14	12
Detected two years	3	3
Detected three years	1	0
Favourable allele Arche	12	4
Favourable allele Récital	6	13
Colocalisation with Laperche et al (2007)	14	7

QTL for GS activity were detected on 15 chromosomal regions (Table 2) spread on 12 chromosomes: 1D, 2A, 2B, 2D, 3B, 3D, 4A, 4B, 5A, 5B, 5D and 7A. The R² ranged from 5.4 to 20.4%. The allele from Arche was favourable for this trait in four regions. The allele from Récital was favourable for this trait in 13 regions. For two regions, the favourable allele was either coming from Arche or Récital depending either on the year of experiment (chromosome 2D) or the organ examined (chromosome 2B). In the latter case, Arche allele increased GS activity in the flag leaf sheath and Récital

allele increased GS activity in the flag leaf lamina. Like for NADH-GDH, most of the QTL regions were detected only on one year. Three QTL regions were detected over two years on chromosome 2B, 2D, 5D.

QTL for GS and NADH-GDH activities overlapped in five regions (chromosomes 2A, 2D, 4B, 5A, 7A). For four regions, the same parental line carried the favourable allele for both enzyme activities. For one region, located on chromosome 2A, the favourable allele came from Récital for NADH-GDH activity and from Arche for GS activity.

The QTL regions detected in the present study were compared to the QTL detected by Laperche et al. (2007). In their study, the same DHL population was used to detect QTL in 14 different environments that were combinations of four locations, two years and two N treatments. Agronomical traits such as grain yield and its components, grain protein content and aerial dry mass at maturity were measured. Fourteen of the 18 regions containing a QTL for NADH-GDH activity (Table 2) corresponded to QTL regions detected by Laperche et al. (2007). This was also the case for seven regions containing a QTL for GS activity. Among these regions common to the two studies, we found that three of them co-localized with known major genes controlling photoperiod sensitivity (*Ppd-D1* on 2D), plant height (*Rht-B1* on 4B) and awnedness (*B1* on 5A). No obvious co-localization with candidate genes was found for the other chromosomal regions.

CONCLUSIONS

Using a bread wheat mapping population exhibiting a contrasting response to N deficiency we identified several chromosomal regions that explained variations for NADH-GDH and GS activities, two enzymes involved in the process of N assimilation and recycling. Several of these chromosomal regions were detected only on one year but most of the regions coincided with QTL regions identified for agronomical traits in a previous investigation. By associating physiological traits to integrative agronomical traits, we have identified QTL regions in which the expression of both types of traits may be controlled by either structural or regulatory candidate genes. It remains now to identify the nature and the function of these genes with a view to improving NUE in wheat while maintaining high grain quality and productivity.

REFERENCES

An DG, Su JY, Liu QY, Zhu YG, Tong YP, Li JM, Jing RL, Li B, Li ZS (2006) Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). Plant and Soil 284:73-84

Basten C, Weir B, Zeng Z (2002) QTL cartographer version 1.16.

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

Dubois F, Tercé-Laforgue T, Gonzalee-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

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

Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retaillau 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, 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. Journal of Experimental Botany 58:2369-2387

Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J (2007) In wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlate with agronomic traits and nitrogen physiological markers. Field Crops Research 102:22-32

Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret F, Le Gouis J (2007) Using genotype x nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. Theoretical and Applied Genetics 115:399-415

Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B (2006) A simplified conceptual model of carbon and nitrogen functionning for QTL analysis of winter wheat adaptation to nitrogen deficiency. Theoretical and Applied Genetics 113:1131-1146

Le Gouis J, Béghin D, Heumez E, Pluchard P (2000) Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. European Journal of Agronomy 12:163-173

Obara M, Kajiura M, Fukuta Y, Yano M, Hayashi M, Yamaya T, Sato T (2001) Mapping of QTLs associated with cytosolic glutamine synthetase and NADH-glutamate synthase in rice (*Oryza sativa* L.). Journal of Experimental Botany 52:1209-1217

Yang LT, Mickelson S, See D, Blake TK, Fischer AM (2004) Genetic analysis of the function of major leaf proteases in barley (*Hordeum vulgare* L.) nitrogen remobilization. Journal of Experimental Botany 55:2607-2616