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ORIGINAL PAPER

Using probe genotypes to dissect QTL × environment interactions for grain yield components in winter wheat

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Abstract Yield is known to be a complex trait, the expression of which interacts strongly with environmental conditions. Understanding the genetic basis of these genotype × environment interactions, particularly under limited input levels, is a key objective when selecting wheat genotypes adapted to specific environments. Our principal objectives were thus: (1) to identify genomic regions [quantitative trait loci (QTL)] involving QTL × environment interactions (QEI) and (2) to develop a strategy to understand the specificity of these regions to certain environments. The two main components of yield were studied: kernel number (KN) and thousand-kernel weight (TKW). The Arche × Récital doubled-haploid population of 222 lines was grown in replicated field trials during 2000 and 2001 at three locations in France, under two nitrogen levels. The 12 environments were characterized in terms of water deficit, radiation, temperature and nitrogen stress based on measurements conducted on the four-probe genotypes: Arche, Récital, Ritmo and Soissons. A four-step strategy was developed to explain QTL specificity to some environments: (1) the detection of QTL for KN and TKW in each environment; (2) the estimation of genotypic sensitivities as the factorial regression slope of KN and TKW to environmental covariates and the detection of QTL for these genotypic sensitivities; (3) study of the co-locations of QTL for KN and TKW and of the QTL for sensitivities; in the event of a co-location partitioning the QEI, appropriate covariates were employed; (4) a description of the environments where QTL were detected for KN and TKW using the environmental covariates. A total of 131 QTL were found to be associated with KN, TKW and their sensitivity to environmental covariates across the 12 environments. Four of these QTL, for both KN and TKW, were located on linkage groups 1B, 2D1, 4B and 5A1, and displayed pleiotropic effects. Factorial regression explained from 15.1 to 83.2% of the QEI for KN and involved three major environmental covariates: cumulative radiation-days ±3 days at meiosis, cumulative degree-days >25°C ±3 days at meiosis and nitrogen stress at flowering. For TKW, 13.5–81.8% of the effect of the QEI was partitioned and involved three major environmental covariates: water deficit from flowering to the milk stage, cumulative degree-days >0°C from the milk stage to maturity and soil water deficit at maturity. A comparative analysis was then performed on the QTL detected during this and previous studies published on QEI and some interacting QTL may be common to different genetic backgrounds. Focusing on these QTL common to different genetic backgrounds would give some guidance to understand genotype × environment interaction.

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Introduction

The grain yield (GY) of winter wheat is known to be a complex trait, the expression of which is strongly dependent on environmental conditions. Some of these conditions can be controlled by the use of fungicides, pesticides or fertilizers, etc. Other conditions, such as climate or soil characteristics, etc., cannot be controlled in the field, which does not facilitate the study of genotype × environment interactions (GEI). Understanding the genetic basis of GEI for GY is thus an important objective when breeding wheat varieties with improved performance, particularly under low input levels.

Genotype × environment interactions are a common phenomenon in the multi-environment trials that are essential to identify genotypes adapted either to a broad range of environments or to specific environments. Numerous methods have been developed to characterize GEI (Crossa 1990; Vargas et al. 1999); these include analysis of variance, joint linear regression (Finlay and Wilkinson 1963), partial least squares regression (Aastveit and Martens 1986), factorial regression (Denis 1988; van Eeuwijk et al. 1996), additive main effects and multiplicative interaction (AMMI) models (Gauch 1988) or biadditive factorial regression, which generalizes both factorial regression and AMMI models (Denis 1991). Mixed models have become widely used to deal with heterogeneity effects across and within trials and to explain genetic correlations among environments (Smith et al. 2001; Mathews et al. 2008). Cooper et al. (2006) discussed the opportunities to apply mixed model methods to the study of GEI. Mathews et al. (2008) outlines a mixed model approach and showed that parsimonious factor analytic models best captured the variance–covariance structure, including genetic correlations, among environments. However, a major challenge for these statistical methods is how to deal with the complex interactions that occur within plants between genes and traits (Van Eeuwijk et al. 2005; Cooper et al. 2009).

Factorial regression model can split the interaction term when the covariates integrated into the model are significant. When the included covariates are environmental and describe the nitrogen stress by the nutrition index at flowering, for instance, the slopes correspond to the genotypic sensitivities to nitrogen stress. A variety adapted to nitrogen stress, i.e., low nitrogen availability, will maintain its yield under nitrogen stress and display a low slope (in absolute value). The retained covariates are those that explain the largest amount of GEI and those that are complementary. This selection leads to an efficient model because it reduces the unexplained interaction with a moderate "consumption" of degrees of freedom (Denis 1980; Brancourt-Hulmel et al. 1997). The factorial regression is quite easy to analyze when a few covariates are introduced. Furthermore, the effects of the different introduced covariates can be statistically tested. However, it has the drawback of generating too many parameters when numerous covariates are used, which decreases its parsimony and makes its interpretation more difficult (Leflon et al. 2005).

The expression of many quantitative traits, such as yield, yield components [such as kernel number (KN) per square meter or thousand-kernel weight (TKW)] and other agronomic traits, results from numerous quantitative trait loci (QTL) (e.g., Paterson et al. 1988; Leflon et al. 2005; Marza et al. 2006; Nalini et al. 2007). The identification of QTL is useful to explain the genetic regulation of phenotypes and may provide markers that can assist plant breeding. However, many QTL studies have produced inconsistent results regarding their detection in different environments (Leflon et al. 2005). The study of GEI using conventional biometrical procedures has also benefited from the development of molecular markers to measure individual genetic effects (Sari-Gorla et al. 1997; Emebiri and Moody 2006) and dissect GEI into QTL × environment interactions (QEI) (Beavis and Keim 1996).

QEI can be evidenced by testing for marker locus × environment interactions to determine the genetic factors responsible for GEI (Sari-Gorla et al. 1997). However, such approaches only provide information on the presence of QEI and do not reflect the environmental factors involved. To better quantify QEI, a variety of statistical methods and models have been developed (Leflon et al. 2005; Vargas et al. 2006; van Eeuwijk et al. 2007). For example, the AMMI model was used to analyze QEI with respect to the GY of barley (Romagosa et al. 1996) and rice (Hittalmani et al. 2003). The authors used synthetic values of the multiplicative terms as variables for QTL detection. QEI studies can also be performed by partitioning the total genetic variance into genotype and GEI effects (Yan et al. 1999; Yadav et al. 2003). QTL are then detected for each of these effects separately, and the QTL detected for GEI effects are significantly affected by changes to environmental conditions.

The expression of QTL for GY and its components can be largely influenced by edaphic and climatic features such as soil fertility, precipitation, temperature or solar radiation, which may result in such QEI. Therefore, descriptors of environmental conditions are candidate covariates to interpret QEI for GY and its components using either factorial regression (Denis 1980, 1988), the partial least squares regression (Crossa et al. 1999) or the biadditive factorial regression (Denis 1991; van Eeuwijk et al. 1996). Climatic data corresponding to particular environmental characteristics can then be introduced into the model.

The effects of environmental factors influencing QEI have been quantified in different species. Studies of QEI in

Arabidopsis thaliana (Jansen et al. 1995), barley (Korol et al. 1998; Malosetti et al. 2004), maize (Crossa et al. 1999), and wheat (Groos et al. 2003; Campbell et al. 2004; Laperche et al. 2007, 2008; Kuchel et al. 2007b) showed that photoperiod, temperature, nitrogen stress and the severity of stripe rust infection were major environmental covariates interacting with QTL for many traits such as GY, KN, TKW or above-ground biomass. Of these different authors, only Campbell et al. (2004), Malosetti et al. (2004) and Kuchel et al. (2007b) tried to explain the specificity of QTL detected in particular environments by using differences in the genotype sensitivities of the population to the environmental covariates.

The explanation of QEI could be improved by means of thorough environment characterization using different approaches. (1) Environments can be characterized in light of direct measurements of variables such as temperature, radiation and water deficit. (2) Environments can be characterized indirectly based on plant responses for the entire population under study, although this is problematic when large mapping populations are considered. (3) These indirect measurements of plant responses could be limited to a small set of probe genotypes to reduce the number of measurements. Probe genotypes are a specific set chosen for their known reactions to one or more environmental factors and can thus be used to identify yield-limiting factors (Cooper and Fox 1996; Brancourt-Hulmel et al. 1999).

The objectives of this study were thus to determine the regions involved in QEI for two components of GY, KN and TKW, and to develop a strategy to explain the environment specificity of QTL. This strategy was based on using four-probe genotypes and a doubled-haploid (DH) population evaluated in 12 environments. QEI were analyzed using factorial regression that introduced environmental covariates to assist with the biological interpretation of GEI. The QTL thus identified were then compared with the interactive QTL previously published on wheat.

Materials and methods

Materials and data

A population of 222 DH lines derived from crossing Arche and Récital parents was used to study the two GY components: KN per square meter and TKW. The two parents chosen from 20 winter wheat varieties (Le Gouis et al. 2000) were characterized by their different reactions to nitrogen stress: Arche is tolerant to nitrogen stress and Récital is sensitive to nitrogen stress. Moreover, Arche is a line with intermediate earliness and Récital is very early flowering (Hanocq et al. 2003).

Four genotypes (Arche, Récital, Ritmo and Soissons) were used as probe genotypes (Cooper and Fox 1996) to identify environmental limiting factors. Arche and Récital were the parents of the studied population; Ritmo is characterized by its late maturity, while Soissons displays intermediate precocity between Récital and Arche.

The DH lines and probe genotypes were studied in 12 environments, which were combinations of 3 locations in France: INRA Estrées-Mons (Ms, 03°00′N latitude 49°08′E longitude), INRA Le Moulon (Ml, 02°08′N 48°42′E) and INRA Clermont-Ferrand (Cl, 45°47′N latitude, 03°05′E longitude), during 2 consecutive years, 1999–2000 (0) and 2000–2001 (1), and under 2 nitrogen levels, a high N supply (+N) and a low N supply (−N). For 2 years, the DH lines were planted under nursery conditions to record height differences. The DH lines were classified into six plant heights. For this study, at each site the field was divided into six blocks and DH was divided up in the blocks according to the previous classification to limit any competition height difference would have brought about within a block. The six blocks were replicated two times. For each replicate, a block adjustment was computed for each line with the four-probe genotypes, which were included in each of the six blocks. This adjusted value for each trait was used in further analyses. Environments were given a code that combined year (0, 1), location (Cl, Ml, Ms) and N treatment (+N, −N).

GY (g m^{-2}) and TKW (g) were measured in all lines and probe genotypes. KN was calculated as (GY × 1,000)/TKW. Further analyses were conducted using the mean over the two replicates in each environment. Daily meteorological data were also measured: minimum, maximum and average temperatures, precipitation, potential evapotranspiration and global radiation. Lodging and leaf rust (*Puccinia triticina*) scores were recorded. The N nutrition index (NNI) at the 1-cm ear stage and at flowering was calculated according to the formula developed by Juste et al. (1994). Correlations between TKW and KN in each environment, between the means of each GY component and the environmental covariates measured with probe genotypes, were computed using the SAS CORR procedure (SAS Institute Inc 1999).

Characterization of environments

Probe genotypes

Probe genotypes were used to characterize the environments (Brancourt-Hulmel 1999). In this analysis, two variates, KN deviation and TKW reduction, computed as in Laperche et al. (2006), were used to identify the limiting factors of the environments: the KN deviation described

the environments during the period before flowering and the TKW deviation from flowering to maturity.

Selection of environmental indicators

To characterize the limiting factors of the environments, environmental indicators were calculated on the basis of daily meteorological data and the dates of different developmental stages. These indicators were transformed so that all indicators varied within the same range (1–10). A score of 1 represented an absence of environmental stress and a score of 10 represented maximum stress. Two formulae were used. For indicators, the high values of which corresponded to a limitation of GY (for example, water deficit at maturity), the value was transformed according to the formula (1): $Y = 1 + 9 \times (X_{obs} - X_{min})/(X_{max} - X_{min})$, where X_{obs} is the observed value, X_{min} is the minimum value and X_{max} is the maximum value; for those indicators, the high values of which corresponded to an increase in GY (for example, the sum of radiation at meiosis), the following formula (2) was used: $Y = 1 + 9 \times (X_{obs} - X_{max})/(X_{min} - X_{max})$.

NNI values higher than 1 represented luxury consumption by the plant and values equal to 1 represented optimum nitrogen nutrition (Juste et al. 1994). Therefore, values higher than or equal to 1 were transformed into 1 and values lower than 1 were transformed according to formula (2).

Indicators showing similar values in more than eight environments were discarded from the analysis, as we considered that a reliable factorial regression could not be performed with fewer than four values. Because many indicators were computed, a pre-selection using principal component analysis (PCA) was performed to constitute a subset of indicators that were not correlated and represented the principal environmental stresses present (heat, water deficit, biotic stresses, etc.). The selection was done using the correlation matrix between each indicator to each principal component. The indicators were selected according to their loadings with the two-first components of the PCA: they corresponded to those having the highest loadings in magnitude, whatever the sign.

A second round of selection was undertaken among these pre-selected indicators using multiple linear regression on KN deviations and TKW reductions of probe genotypes: the indicators, varying within the same range (1–10), were tested and added to the model by a backward and forward stepwise selection using the REG procedure under SAS (SAS Institute Inc 1999). Significant indicators were maintained at the threshold of 15% (Lecomte et al. 2002). At each step during selection, indicators with a negative coefficient of regression were eliminated. A negative coefficient of regression meant that the corresponding factor had not had the expected effect on our data set and it could not be introduced into the model: a limiting factor should increase and not decrease the KN deviation or TKW reduction. The selected indicators were used as covariates in factorial regression for further partitioning of the QEI for KN and TKW. Tables S1 and S2 provide detailed data on each covariate for each environment. Correlations between KN and TKW with environmental covariates were calculated using Pearson coefficient of linear correlation.

QTL detection

The broad-sense heritability of KN and TKW were calculated for each environment, as described in Laperche et al. (2006). QTL were detected using a genetic map that contained 182 markers (mainly microsatellites) positioned on 30 linkage groups (Laperche et al. 2007). When two linkage groups corresponded to the same chromosome, they were labeled with an additional number (e.g., 5A1 and 5A2). Simple interval mapping (SIM) and then composite interval mapping (CIM) QTL analyses were conducted using PlabQTL (Utz and Melchinger 2000). In the CIM analysis, an F-to-Enter value corresponding to the threshold of probability of 0.005 was used to select the cofactors, and a 2 cM interval scan was chosen for the detection of QTL. A 1,000 permutation tests were performed for CIM to establish a threshold of significance that could control a genome-wide Type I error. The LOD threshold of 2.2 was thus determined. QTL confidence intervals were estimated as the distance corresponding to a decrease of ±1 unit from the peak LOD. The positive or favorable allele was defined as that which increased the trait under consideration.

Partitioning of QTL × environment interactions and explaining QTL specificity to environments

Estimating genotype × environment interactions

Genotype × environment interactions were detected using ANOVA according to the following model:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \gamma_{ge} + R(\beta)_{er} + \varepsilon_{ger} \quad (1)$$

where Y_{ger} is the value of the trait (KN or TKW) for genotype g (=DH line) in environment e and replicate r, μ is the grand mean, α_g is the main effect of line g, β_e is the main effect of environment e, γ_{ge} is the interaction between genotype g and environment e, $R(\beta)_{er}$ is the replicate effect nested in the environment effect, and ε_{ger} is the residual error term.

After checking the significance of a GEI, it was partitioned into QEI. A four-step strategy was used to explain QTL specificity to an environment. (1) Detection of QTLs for KN and TKW on means estimated in each environment

and means estimated over the 12 environments. (2) Detection of QTLs for genotypic sensitivities to different environmental covariates estimated as the slope of the following factorial regression:

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_{k=1}^{K} \rho_{gk} \times C_{ek} + \gamma'_{ge} + \varepsilon_{ge} \quad (2)$$

where Y_{ge} is the value of the trait (KN or TKW) for genotype g (=DH line) in environment e, μ is the grand mean, α_g is the main effect of line g, β_e is the main effect of environment e, ρ_{gk} is the sensitivity of genotype g to covariate k, C_{ek} is the value of covariate k in environment e, γ'_{ge} is the remainder effect of the interaction between the genotype and the environment, ε_{ge} is the residual error term, and the other terms of the model are defined as in Eq. 1. (3) Study of the co-locations of QTLs for KN and TKW and QTLs for sensitivity and (in case of a co-location) partitioning of the QEI using "co-located" covariates. A co-location was defined as an overlap of the confidence intervals of two QTLs. (4) Partitioning of the genotype and QEI using environmental covariates for the environments where QTLs were detected for KN and TKW.

Partitioning of the genotype and QTL × environment interactions

For KN or TKW, measured for genotype g in environment e and replicate r (Y_{ger}), the genotype effect and the interaction between a given QTL (represented by the nearest marker m) and the environment were modeled using the following equation:

$$Y_{ger} = \mu + M_m + \alpha'_g + \beta_e + M_{me} + \gamma''_{ge} + R(\beta)_{er} + \varepsilon_{ger} \quad (3)$$

where M_m is the effect of allele m at the marker, α'_g is the remainder of the genotypic effect considered as random, M_{me} corresponds to the interaction between the marker and the environment e, γ''_{ge} is the remainder effect of the GEI considered as random, and the other effects are defined as in Eqs. 1 and 2.

When a co-location was found between a QTL for KN or TKW (as defined in Eq. 3) and a QTL for sensitivity of the corresponding component of GY (as defined in Eq. 2), the QEI was then partitioned as a QTL × environmental covariate interaction according to the following factorial regression:

$$Y_{ger} = \mu + M_m + \alpha'_g + \beta_e + \sum_{k=1}^{K} \rho'_{mk} \times C_{ek} + \gamma'''_{ge} + R(\beta)_{er} + \varepsilon_{ger} \quad (4)$$

where ρ'_{mk} is the sensitivity of allele m to covariate k, γ'''_{ge} is the remainder effect of the GEI considered as random. Other terms are as previously defined in Eqs. 1–3. Regression analysis was performed using the GLM procedure under SAS with the SS1 option for computing the sums of squares and the random statement for the genotype effect. This partitioning using the mixed model was undertaken for significant QEI whatever the significance of the corresponding marker's main effect.

Results

Highly significant GEI effects for KN and TKW

KN and TKW measured on the four-probe genotypes displayed variations across the 12 environments. KN ranged from 11,436 grains m^{-2} in 1Ms−N to 26,617 grains m^{-2} in 1Ml+N (Table 1), and TKW ranged from 33.9 g in 1Cl+N to 43.1 g in 1Ms+N (Table 1). When these values were compared with those of the parents (Arche and Récital), transgression was observed in the DH population for KN and TKW (Table 1). ANOVA indicated that the genotype, environment and GEI effects were highly significant for both KN and TKW (Tables 2, 3). However, the relative contributions of variance components to the total variation were different for the two components of GY. For KN, variances among environments accounted for 71.3% of the total phenotypic variation (Table 2), while for TKW, variances among environments accounted for 30.1% (Table 3). GEI variance represented 13.8 and 21.1% of total variance for KN and TKW, respectively. Broad-sense heritabilities for KN ranged from 0.54 (0Cl−N) to 0.91 (0Ms+N and 1Cl−N) (Table 1). For TKW, heritabilities ranged from 0.87 (1Cl+N) to 0.98 (1Ml+N). Only four environmental covariates, namely finnR, finnS, stmpflA and stmplmA (defined in supplementary Table S1) were significantly correlated to KN ($P < 0.05$). Six environmental covariates, sdflmR, sdflmS, setrmflR, setrmflS, spetpemS and sri1200bI (defined in supplementary Table S1), were significantly correlated to TKW. In addition, there was no significant correlation between KN and TKW across the 12 environments studied during this work.

QTL and QTL × environment interactions for KN

A total of 32 QTL for KN and 35 QTL for the sensitivity of KN to environmental covariates were detected and co-locations occurred on chromosomes 1B, 2D1, 3D and 4B

The KN QTL detected for each environment are listed in Table S3 and their chromosomal locations are shown in Fig. 1. A total of 32 QTL that significantly affected KN were detected and located on the nine chromosomes 1B, 2B1, 2D1, 3B, 3D, 4B, 5A1, 6A and 7D2, respectively

Table 1 Kernel numbers (kernels m^{-2}) and thousand-kernel weights (g) in the 12 environments, respectively, in (a) and (b): mean, standard deviation, minimum, maximum and heritabilities for the Arche × Récital DH population and mean values for the four-probe genotypes

Environment	DH population					Probe genotypes
	Mean	SD	Minimum	Maximum	h^2	Mean
(a)						
0Cl−N	14,124	1,749.2	7,381	18,311	0.54	14,252
0Cl+N	16,812	1,697.7	8,447	21,592	0.88	16,780
0Ms−N	13,387	1,724.1	7,377	16,888	0.84	13,594
0Ms+N	18,189	2,571.1	10,630	25,401	0.91	19,043
0Ml−N	13,546	1,957.1	4,694	19,142	0.71	13,363
0Ml+N	22,419	2,784.1	11,319	29,529	0.78	22,904
1Cl−N	17,778	3,838.0	7,213	27,024	0.91	19,383
1Cl+N	19,928	3,219.7	7,890	27,161	0.88	20,636
1Ms−N	11,859	1,501.7	6,824	16,733	0.75	11,436
1Ms+N	18,796	2,155.3	12,182	23,578	0.88	18,795
1Ml−N	22,437	3,012.5	12,838	31,043	0.85	22,992
1Ml+N	25,825	3,819.3	13,368	37,423	0.87	26,617
Mean	17,877	1,918.3	11,354	22,629	–	18,316
(b)						
0Cl−N	40.4	3.40	33.8	55.3	0.88	42.3
0Cl+N	40.3	4.17	29.7	54.4	0.96	41.7
0Ms−N	36.1	3.65	25.0	46.2	0.96	38.8
0Ms+N	34.9	3.63	23.2	46.2	0.93	37.6
0Ml−N	37.1	3.34	29.9	46.3	0.96	39.5
0Ml+N	35.6	3.84	24.2	45.4	0.96	37.7
1Cl−N	33.8	4.14	23.4	45.3	0.94	33.9
1Cl+N	33.4	3.45	25.2	44.4	0.87	34.4
1Ms−N	39.7	3.51	31.1	50.1	0.96	41.7
1Ms+N	40.3	3.64	31.4	52.3	0.97	43.1
1Ml−N	38.3	3.76	28.1	50.5	0.97	39.8
1Ml+N	37.4	4.19	25.1	51.8	0.98	38.6
Mean	37.3	3.12	29.0	46.7	–	39.1

Environments are coded combining year (0, 1), location (Cl, Ml, Ms) and N treatment (+N, −N)

(Fig. 1). No QTL was detected across all 12 environments. One QTL for KN (close to marker wmc238 on chromosome 4B) displayed the highest level of stability as it was detected in five environments. Two QTL on chromosomes 2D1 and 3D were detected in four environments. One QTL was detected in three environments (4B) and one (5A1) in two environments. The others were detected in only one environment. R^2 values were high for some QTL, such as the 4B-QTL close to wmc238 that explained 30.3% of KN variation with an additive effect of +1,441 kernels for the Arche allele in environment 0Ms+N (Table S3). The positive alleles at the QTL on chromosomes 1B, 2B1, 2D1, 3B and 3D came from Récital, while those at the QTL on chromosomes 4B, 5A1, 6A and 7D2 arose from Arche.

A total of 35 QTL were detected for the sensitivity of KN to environmental covariates (slope of factorial regression). They were located on the five chromosomes 1B, 2B1, 2D1, 3D and 4B (Table 4; Fig. 1). Based on confidence interval overlap, the QTL could be grouped into six regions. Between 1 (4B) and 11 (2D1) covariates were associated with each region. The most frequent environment covariate was "finn" (nine times). This corresponded to N stress at flowering (see Supplementary Table S1). The "sri1200b" and "stcmb" covariates linked to high radiation and high temperature around meiosis appeared six times, respectively. The "srglmb" covariate linked to cumulative radiation around meiosis appeared four times. This highlights the fact that nitrogen stress was the most limiting, but not the only, factor involved. Other environmental factors were identified, such as water deficit from half filling to meiosis and high temperature from heading to flowering. Arche was the most effective probe genotype as covariates based on Arche appeared 19 times, compared to 10 times for Ritmo, 4 times for Soissons and only twice for Récital.

A total of five co-locations between QTL for KN and KN sensitivity to environmental covariates were determined (Fig. 1): these QTL were positioned on chromosomes 1B (close to gwm268), 2D1 (gpw4085), 3D (cfd211) and 4B (wmc238 and gwm540b).

Table 2 Analysis of variance of KN observed on Arche × Récital DH lines grown in 12 environments (a), partition of the genotypic effect by the effect of allele m at the marker, partition of the GEI effect by the marker × environment effect and QEI by the sensitivity of allele m to the environmental covariates (b)

Effects	*df*	SS	*P* value	%SS of QEI	%SS of GEI	%SS of model
(a)						
G	219	18,095,750,027	<0.001			14.9
E	11	86,542,814,451	<0.001			71.3
GEI	2,388	16,685,978,546	<0.001			13.8
Pure residual	2,611	5,601,844,285				
Total	5,237	128,281,917,172				
(b)						
gwm268 (1B)	1	247,692,308	0.090			0.2
gwm268 × E	11	453,603,959	<0.001		2.7	0.4
gwm268 × srglmfA	1	174,298,248	<0.001	38.4	1.0	
gwm268 × srglmbA	1	148,966,319	<0.001	32.8	0.9	
gwm268 × finnA	1	32,588,075	0.007	7.2	0.2	
gpw4085 (2D1)	1	809,622,521	0.002			0.7
gpw4085 × E	11	630,761,234	<0.001		3.8	0.5
gpw4085 × stcmbA	1	280,609,676	<0.001	44.5	1.7	
gpw4085 × srglmfA	1	140,145,882	<0.001	22.2	0.8	
gpw4085 × srglmbA	1	101,256,345	<0.001	16.1	0.6	
gpw4085 × finnA	1	19,996,188	0.027	3.2	0.1	
cfd211 (3D)	1	694,294,917	0.003			0.6
cfd211 × E	11	135,359,741	0.002		0.8	0.1
cfd211 × st25efS	1	91,839,542	<0.001	67.8	0.6	
cfd211 × stcmbI	1	25,804,245	0.019	15.2	0.2	
wmc238 (4B)	1	1,517,172,581	<0.001			1.3
wmc238 × E	11	754,300,243	<0.001		4.5	0.6
wmc238 × finnR	1	236,579,073	<0.001	31.4	1.4	
wmc238 × finnI	1	261,342,217	<0.001	34.6	1.6	
wmc238 × finnA	1	77,031,926	<0.001	10.2	0.5	
wmc238 × finnS	1	31,943,459	0.009	4.2	0.2	
gpw1108 (4B)	1	2,560,526,853	<0.001			2.1
gpw1108 × E	11	1,031,387,101	<0.001		6.2	0.9
gpw1108 × st25efS	1	232,174,504	<0.001	22.5	1.4	
Rht-B1 (4B)	1	880,452,010	<0.001			0.7
Rht-B1 × E	11	770,389,037	<0.001		4.6	0.6
Rht-B1 × srglmbA	1	156,707,837	<0.001	20.3	0.9	
gwm540b (4B)	1	1,160,193,489	<0.001			1.0
gwm540b × E	11	289,974,836	<0.001		1.7	0.2
gwm540b × stcmbA	1	39,790,974	0.003	13.7	0.2	

QTL are indicated by the name of their nearest marker and their chromosome location (in brackets). Covariates "srglmfA, finnA…" are coded according to Table S1

A small percentage of genotype × environment interactions were explained by each individual marker × environment interaction (QEI), the QEI being themselves partitioned by marker × environmental covariate interactions ranging from 13.7 to 67.8%

In addition to GEI, marker × environmental covariate interactions for KN are summarized in Table 2. QEI were tested using the marker closest to the QTL peak. Among the five co-locations of QTL for KN and sensitivity to environmental covariates, only one lacked any significant marker × environmental covariate interaction; namely the locus on chromosome 4B close to marker gwm192. For the others, the variation in marker × environment interactions accounted for <1% of the total variation, ranging from 0.1% for marker cfd211 on chromosome 3D to 0.9% for

Table 3 Analysis of variance of TKW observed on Arche × Récital DH lines grown in 12 environments (a), and partition of the genotypic, GEI and QEI effects as in Table 2(b)

Effects	*df*	SS	Pvalue	%SS of QEI	%SS of GEI	%SS of model
(a)						
G	221	51,022.4	<0.001			48.8
E	11	31,438.6	<0.001			30.1
GEI	2,395	22,068.1	<0.001			21.1
Pure residual	2,626	4,070.6				
Total	5,253	108,599.8				
(b)						
SPA (1B)	1	639.0	0.011			0.6
SPA × E	11	226.4	<0.001		1.0	0.2
SPA × setrmflS	1	49.8	0.002	22.0	0.2	
gwm268 (1B)	1	688.3	0.087			0.7
gwm268 × E	11	366.5	<0.001		1.7	0.4
gwm268 × stmplmR	1	337.8	<0.001	92.2	1.5	
gwm268 × nj30lmI	1	0.5	0.762	0.1	0.0	
gwm102 (2D1)	1	1,392.6	0.012			1.3
gwm102 × E	11	991.3	<0.001		4.5	0.9
gwm102 × setrmflS	1	147.5	<0.001	14.9	0.7	
gpw4085 (2D1)	1	24.3	0.742			0.0
gpw4085 × E	11	675.6	<0.001		3.1	0.6
gpw4085 × setrmflA	1	374.1	<0.001	55.4	1.7	
gpw4085 × hlmtA	1	26.2	0.017	3.9	0.1	
gpw4085 × stmplmR	1	20.1	0.036	3.0	0.1	
Fdgo-D1 (2D1)	1	538.4	0.141			0.5
Fdgo-D1 × E	11	726.8	<0.001		3.3	0.7
Fdgo-D1 × hlmtS	1	440.7	<0.001	60.6	2.0	
Fdgo-D1 × stmpflR	1	64.6	<0.001	8.9	0.3	

Abbreviations of indicators are given in Table S2. QTLs are indicated by the name of their nearest marker and their chromosome location. Covariates are coded according to Table S1

marker gpw1108 on chromosome 4B. The marker × environment interaction explained a small percentage of the sum of squares of the GEI, ranging from 0.8% for marker cfd211 on chromosome 3D to 6.2% for marker gpw1108 on chromosome 4B. This small percentage was mainly due to the fact that only 11 degrees of freedom were used for marker × environment interactions, versus 2,388 for GEI. These results nonetheless confirmed that chromosomal regions where co-locations between KN QTL and sensitivity QTL were observed interacted significantly with different environmental conditions.

Marker × environmental covariate interactions explained between 13.7 and 86.0% of marker × environment interactions (Table 2). The smallest share was explained by marker gwm540b × environment interaction on chromosome 4B and the largest was explained by gpw4085 × environment interaction on chromosome 2D1. Partitioning of the marker × environment interaction showed that the individual marker × environmental covariate interaction ranged from 13.7 to 67.8% of QEI. The smallest share was explained by the marker gwm540b × stcmbA interaction on chromosome 4B and the single largest share was explained by cdf211 × st25efS on chromosome 3D.

Of all these marker × environmental covariate interactions, only one region on 4B was affected by the same covariate (NNI at flowering) measured on the four-probe genotypes (Table 2). The interaction between wmc238 on 4B and finn explained from 4.2 to 34.6% of the total marker × environment interaction, although this interaction only accounted for a small percentage of the total GEI sums of squares. Three QTL were affected by only one environmental covariate. The other three individual QTL interacted with more than two diverse environment stresses including high temperature, water deficit and radiation.

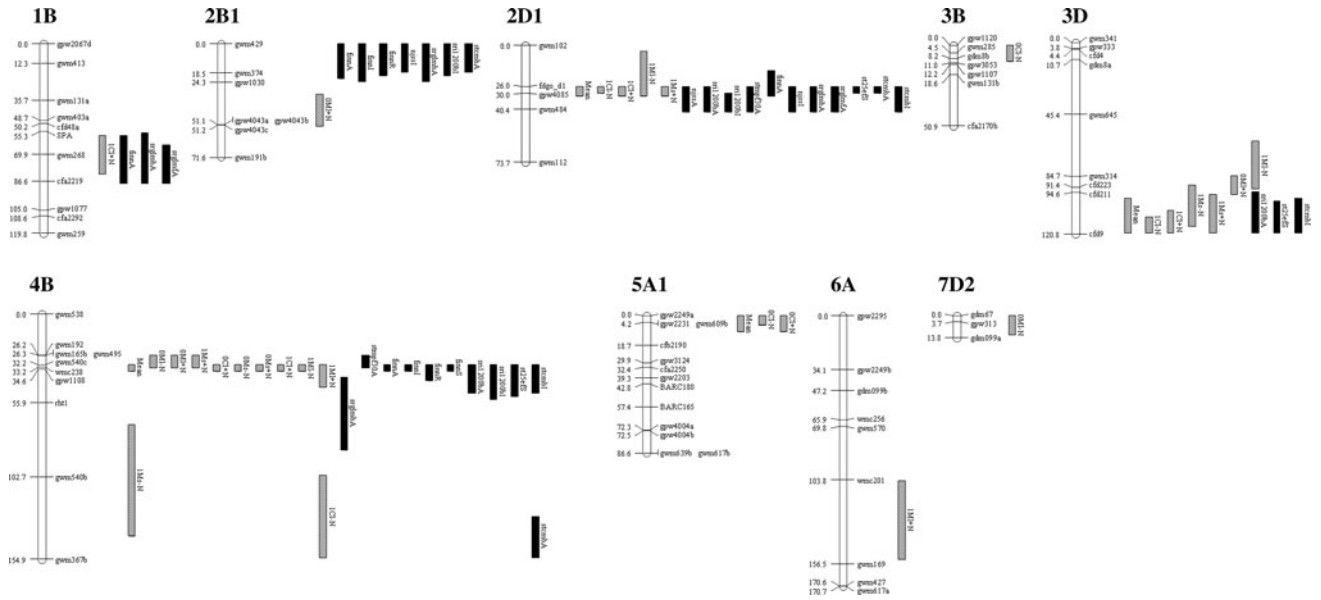

Fig. 1 QTL detected for KN (*gray symbols*) and its sensitivity to environmental covariates (*black symbols*) on the Arche × Récital DH population. Marker names are shown on the *right-hand side* of the chromosome, and the genetic distance between markers on the *left*. Environments are coded to combine the year (0, 1), location (Cl, Ml, Ms) and N treatment (+N, −N). Covariates are coded according to Table S1

QTL and QTL × environment interactions for TKW

Thirty-six QTL for TKW and 28 QTL for the sensitivity of TKW to environmental covariates were detected and co-locations occurred on chromosomes 1B, 2D1, and 4B

A total of 36 QTL were detected for TKW (Table S4; Fig. 2). One region close to marker gpw1108 on chromosome 4B was detected in all environments except for 0Ms+N. One region close to marker cfa2123 on chromosome 5B was detected in six environments. Five regions were detected in two environments, including one close to SPA on chromosome 1B, two close to cfa2043a and gwm448 on chromosome 2A2, one close to gwm102 on chromosome 2D1 and one close to gpw8040 on chromosome 7B2. The other regions were only detected in one environment. Individual QTL accounted for between 5.2 and 24.6% of phenotypic variations. The positive alleles were carried by Récital for all QTL except those on chromosomes 2A2 and 2D1.

A total of 28 QTL for TKW sensitivity to environmental covariates were detected and located on eight chromosomes: 1B, 2D1, 4A, 4B, 4D, 5A2, 6A and 7A1 (Table 5; Fig. 2). R^2 ranged from 4.6% (1B) to 16.2% (2D1). Based on confidence interval overlap, the QTL could be grouped into eight regions corresponding to the eight chromosomes mentioned above. Between 1 (4B, 7A1) and 11 (2D1) covariates were associated with each region. The most frequent environment covariate was "stmplm" (10 times), which represented cumulative degree-days >0°C from flowering to the milk stage. The "setrmfl" covariate, which represented water deficit from flowering to the milk stage, and covariate "hlmt", which represented soil water deficit at maturity, appeared six and four times, respectively. Soissons was the most effective probe genotype, as covariates based on Soissons appeared 12 times, compared to 7 times for both Arche and Récital and only twice for Ritmo. Thus, for TKW, water deficit at maturity and high temperatures from the milk stage to maturity were the two essential environmental factors, while nitrogen stress was less important.

A total of four co-locations were identified between QTL of sensitivity to environmental covariates and QTL for TKW (Fig. 2): one QTL on chromosome 1B close to gwm268; two QTL on chromosome 2D1 close to the three markers Fdgo-D1, gpw4085 and gwm102; and one QTL on chromosome 4B close to gwm192.

For KN, a small percentage of genotype × environment interactions was explained by each individual marker × environment interaction (QEI), the QEI being themselves partitioned by marker × environmental covariate interactions ranging from 14.9 to 92.2

In addition to significant GEI, genotype × environmental covariate interactions and marker × environmental covariate interactions for TKW are summarized in Table 3. Marker × environment interactions accounted for 1.0–4.5%

Table 4 QTL detected on the Arche × Récital DH population for the slope of factorial regression of KN on environmental covariates

Chromosome	Closest marker	Environment covariate	Position	Confidence interval (cM)	LOD	R^2 (%)	Add (grains m^{-2})	Favorable allele
1B	gwm268	finnA	74	58–88	2.7	5.8	0.45	A
		srglmbA	74	56–88	2.5	5.3	−1.40	R
		srglmfA	76	64–88	4.0	8.3	1.13	A
2B1	gwm429	finnA	12	0–22	2.6	8.2	0.54	A
		finnI	10	0–24	2.6	8.2	0.48	A
		finnR	6	0–20	3.4	10.8	0.60	A
		njssI	0	0–18	2.5	8.0	2.60	A
		srglmbA	6	0–24	2.2	7.1	−1.71	R
		sri1200bI	4	0–20	2.4	7.9	1.62	A
		stcmbA	2	0–18	2.8	8.9	0.70	A
2D1	Fdgo-D1	njssA	38	26–42	3.3	8.8	3.01	A
		sri1200bA	36	26–42	3.4	8.8	2.54	A
		sri1200bI	40	30–42	4.2	10.9	1.98	A
		stmpf30A	36	26–42	4.6	11.8	−2.83	R
2D1	gpw4085	finnA	28	16–32	2.8	7.3	0.54	A
		njssI	28	26–42	3.8	9.9	3.32	A
		srglmbA	28	26–42	2.9	7.5	−1.86	R
		srglmfA	28	26–42	5.2	13.1	1.56	A
		st25efS	28	26–30	5.8	14.6	1.17	A
		stcmbA	28	26–30	7.3	18.0	1.12	A
		stcmbI	28	26–42	5.3	13.4	1.56	A
3D	cfd211	sri1200bA	106	94–120	2.6	6.5	2.07	A
		st25efS	116	100–120	3.4	8.6	0.85	A
		stcmbI	118	98–120	2.5	6.3	0.92	A
4B	Rht-B1	srglmbA	68	40–86	4.0	8.4	−2.15	R
4B	gwm192	stmpf30A	28	26–34	6.2	13.0	−2.82	R
4B	wmc238	finnA	34	32–36	6.4	13.1	−0.69	R
		finnI	34	32–36	5.0	10.5	−0.53	R
		finnR	34	32–42	14.2	26.8	−1.06	R
		finnS	34	32–36	8.3	16.4	−0.72	R
4B	gpw1108	sri1200bA	40	32–50	6.3	12.7	−3.05	R
		sri1200bI	38	32–54	2.8	6.1	−1.50	R
		st25efS	36	32–52	2.7	5.6	−0.73	R
		stcmbI	38	32–50	5.2	10.7	−1.36	R
4B	gwm540b	stcmbA	154	128–154	2.9	8.1	−0.69	R

The confidence intervals were estimated as the distance that corresponded to a decrease of ±1 unit from the peak LOD. The allele that increased the trait is either R for Récital or A for Arche. Covariates are coded according to Table S1

of the GEI effect, depending on the QTL. At least one marker × environmental covariate interaction was observed for all five regions.

When marker × environmental covariate interactions were summed up, they explained between 14.9% (on 2D1) and 92.3% (on 1B) of the marker × environment interaction. The smallest share was explained by gwm102 × setrmflS on chromosome 2D1, and the largest by gwm268 × stmplmR on chromosome 1B.

Two different QTL (SPA on chromosome 1B and gwm102 on chromosome 2D1) were affected by the same environmental covariate (setrmflS). Marker × setrmflS interactions explained between 22.0 and 14.9% of total marker × environment interactions, respectively. The other three individual QTL interacted with more than two diverse environment stresses, including high temperature, water deficit and radiation.

Allelic effects plotted as a function of significant environment covariates often displayed cross-over interactions

The allelic effect, which was calculated as the difference between means of the two allele classes (Arche–Récital),

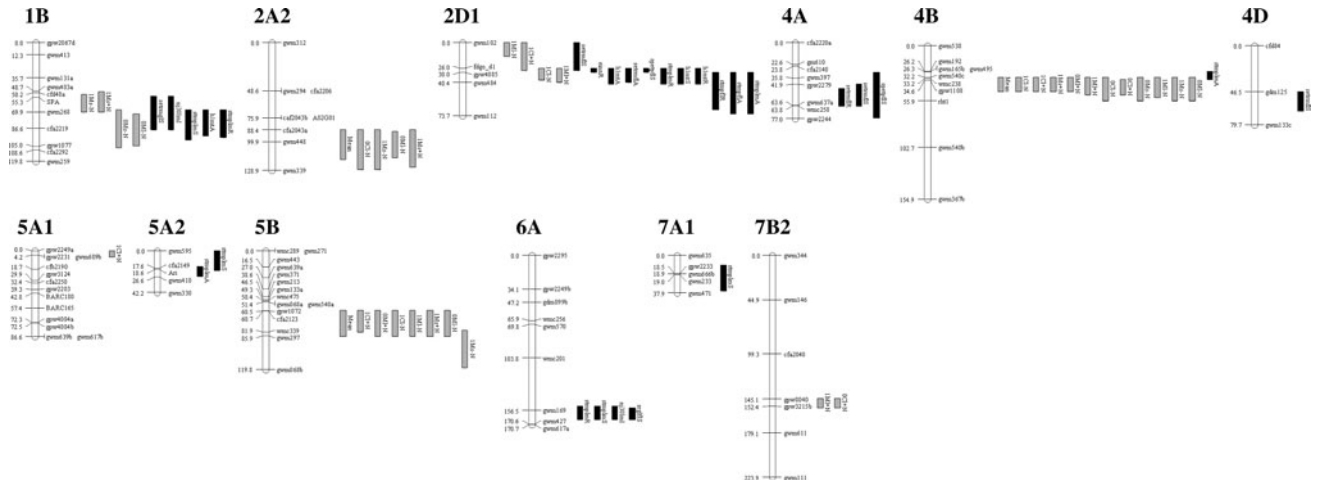

Fig. 2 QTL for TKW (*gray symbols*) and its sensitivity to environmental covariates (*black symbols*) detected on the Arche × Récital DH. Marker names are shown on the *right-hand side* of the chromosome, and the genetic distance between markers on the *left*. Environments are coded to combine the year (0, 1), location (Cl, Ml, Ms) and N treatment (+N, −N). Covariates are coded according to Table S1

Table 5 QTL detected on the Arche × Récital DH population for the slope of factorial regression of TKW on environmental covariates

Chromosome	Closest marker	Environment covariate	Position (cM)	Confidence interval (cM)	LOD	R^2 (%)	Add (g)	Favorable allele
1B	SPA	setrmflS	64	54–88	2.23	4.6	0.10	A
1B	gwm268	nj30lmI	76	54–88	4.26	10.3	0.07	A
		stmplmS	78	68–98	3.13	6.4	−0.09	R
		hlmtA	80	68–94	3.32	6.8	0.07	A
		stmplmR	82	68–96	3.41	7.0	−0.42	R
2D1	gwm102	setrmflS	14	0–28	2.58	8.3	−0.18	R
2D1	gpw4085	einnR	28	26–30	5.91	14.6	−0.13	R
		hlmtA	28	26–42	2.31	6.0	0.07	A
		setrmflA	28	26–40	6.52	16.2	−0.21	R
		spetpflS	28	26–30	5.89	14.5	−0.24	R
		stmplmR	28	26–42	2.21	5.7	−0.36	R
2D1	Fdgo-D1	hlmtS	36	26–42	5.43	13.5	0.15	A
		hlmtR	38	26–42	3.61	9.2	0.12	A
		stmpflR	38	30–68	4.27	10.7	−0.10	R
		stmpflA	40	30–72	3.69	9.3	0.13	A
2D1	gwm484	stmplmA	48	30–72	2.03	6.6	−0.14	R
4A	gpw2279	setrmflR	62	46–64	2.07	5.1	0.15	A
		setrmflS	62	42–64	2.52	6.2	0.13	A
4A	wmc258	spetpflS	64	30–76	2.06	6.2	0.13	A
4B	gwm192	stmplmA	32	26–34	3.71	7.8	0.14	A
4D	gdm125	setrmflS	48	46–66	2.30	6.3	−0.13	R
5A2	cfa2149	stmplmA	18	16–26	4.26	8.6	−0.14	R
		stmplmS	18	0–20	4.45	8.9	−0.09	R
6A	gwm169	stmplmR	160	152–166	2.68	6.7	0.34	A
		stmplmS	164	152–166	2.91	7.3	0.08	A
		nj30lmI	166	152–166	2.45	6.1	−0.05	R
		srglflS	166	154–166	2.78	6.9	−0.10	R
7A1	gpw2233	stmplmS	34	10–36	2.2	6.7	−0.08	R

The confidence intervals were estimated as the distance that corresponded to a decrease of ±1 unit from the peak LOD. The allele that increased the trait is either R for Récital or A for Arche. Covariates are coded according to Table S1

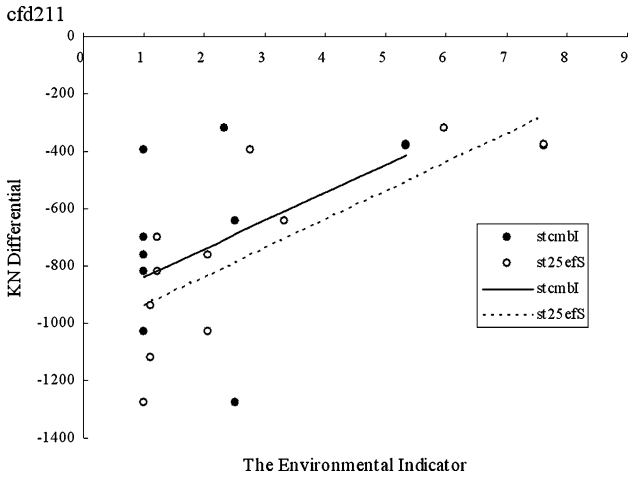

Fig. 3 Arche and Récital allele classes. Mean KN values at the cfd211 marker as a function of st25efS (cumulative degree-days >25°C between heading and flowering measured on Soissons)

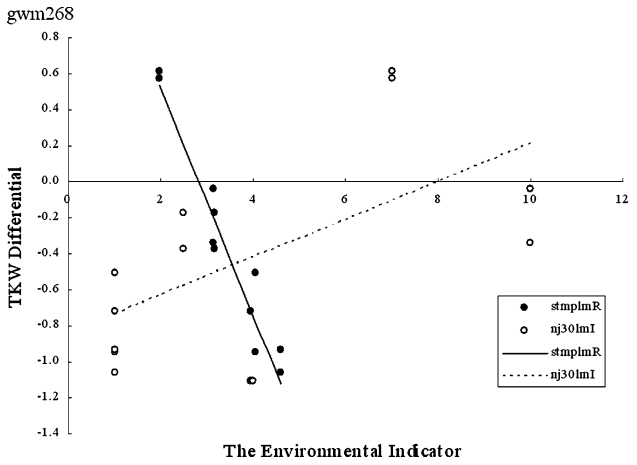

Fig. 4 Arche and Récital allele classes. Mean TKW values at the gwm268 marker as a function of stmplmR (cumulative degree-days >0°C from the milk stage to maturity measured on Récital)

was plotted as a function of significant environment covariates (Figs. 3, S1). DH lines possessing the Récital allele for the cfd211 marker produced a higher KN at lower levels of st25efS (cumulative degree-days >25°C from heading to flowering) and stcmbI (cumulative degree-days >25°C ±3 days at meiosis). At higher levels of st25efS and stcmbI, the allelic differences tended to decrease.

The dynamics of allelic effects for TKW are shown in Figs. 4 and S2. At the gwm268 marker, a cross-over interaction was observed with stmplmR (cumulative degree-days >0°C from the milk stage to maturity) and nj30lmI (number of successive days >30°C from the milk stage to maturity). When stmplmR was 2.8 or greater, TKW for the Récital allele was higher than that of Arche, while the contrary was observed when stmplmR was lower than 2.8. For nj30lmI, TKW for the Arche allele was higher when it was 8.0 or greater and lower otherwise.

Identical regions for the sensitivities of grain, grain yield, KN and TKW to the environment were found in different mapping populations

QTL for KN and TKW sensitivity to the environmental covariates detected here were compared with QTL reported to interact with the environment in populations derived from crosses between Renan and Récital (Groos et al. 2003), Trident and Molineux (Kuchel et al. 2007a), and Arche and Récital (Laperche et al. 2007, 2008). Identical regions for the sensitivities of grain protein content (GPC), GY, KN and TKW to the environment were found on chromosomes 2B, 4A and 4B, respectively, based on molecular markers common to the different maps (Table 6).

Discussion

To provide better biological explanations for GEI and QEI with respect to KN and TKW, we characterized the different environments using covariates measured on four-probe genotypes. We were able to identify regions where QTL for KN and TKW co-localized with QTL for sensitivity to environmental covariates. We will now discuss the following points: (1) the use of factorial regression on environment covariates computed on probe genotypes to provide a relevant basis for the biological interpretation of QEI; (2) the new results generated using this approach regarding the sensitivity of KN and TKW to environmental conditions; (3) the need to use specifically favorable alleles because of cross-over interactions; and (4) a comparison of the QTL detected during our study and those reported in previous works.

Factorial regression including external covariates estimated using probe genotypes provided a relevant basis for the biological interpretation of QEI

A variety of studies have been conducted to analyze the interaction between environmental covariates and QTL for wheat and barley GY and maize biomass. Environments were first characterized by the mean of the measured trait paralleling the stability analysis developed by Eberhart and Russell (1966). In this way, Korol et al. (1998) detected QEI in barley by introducing the mean trait values (GY, grain protein concentration and so on) of the mapping population as a bio-indicator of the environment using either linear or low-degree polynomials. Although this method is efficient to detect QEI, it does not provide any

Table 6 Comparisons of QTL for sensitivity of grain yield and its components to environmental covariates across different genetic backgrounds or over various environments

Chromosome	Interval	Traits	Environmental covariate	Population	References
2B	gwm429	KN	finnA	Arche/Recital	This study
			finnI	Arche/Recital	This study
			finnR	Arche/Recital	This study
			njssI	Arche/Recital	This study
			srglmbA	Arche/Recital	This study
			sri1200bI	Arche/Recital	This study
			stcmbA	Arche/Recital	This study
	gwm257	GPC	Tm	Renan/Recital	Groos et al. (2003)
			NbD25	Renan/Recital	Groos et al. (2003)
	gwm257	TKW	Tm	Renan/Recital	Groos et al. (2003)
4A	gpw2279	TKW	setrmflR	Arche/Recital	This study
			setrmflS	Arche/Recital	This study
	gwm397	GY	Max_J-A	Trident/Molineux	Kuchel et al. (2007a, b)
			Max_S-N	Trident/Molineux	Kuchel et al. (2007a, b)
			Latitude	Trident/Molineux	Kuchel et al. (2007a, b)
			Min_J-A	Trident/Molineux	Kuchel et al. (2007a, b)
			Min_S-N	Trident/Molineux	Kuchel et al. (2007a, b)
			Rain_A	Trident/Molineux	Kuchel et al. (2007a, b)
			<10 Days_J-N	Trident/Molineux	Kuchel et al. (2007a, b)
			>30 Days_J-N	Trident/Molineux	Kuchel et al. (2007a, b)
4B	Rht-B1	KN	srglmbA	Arche/Recital	This study
		GPA	NNIR	Arche/Recital	Laperche et al. (2007)
4B	gpw1108	KN	sri1200bA	Arche/Recital	This study
			sri1200bI	Arche/Recital	This study
			st25efS	Arche/Recital	This study
			stcmbI	Arche/Recital	This study
			NNIR	Arche/Recital	Laperche et al. (2008)

For Arche/Recital population, covariates are coded according to Table S1

biological explanation. To improve the analysis, external environment covariates can be included, such as minimum or maximum temperatures. Crossa et al. (1999) detected QEI using linear factorial regression and partial least square regression in maize. They introduced molecular markers and environmental covariates to analyze QEI and thus explained a large part of the GEI. They showed that maximum temperature was the most important environmental covariate. In the same way, Groos et al. (2003) showed that factorial regression in a recombinant inbred line (RIL) population of wheat was useful to determine QTL involved in the differential genotypic reaction to specific climatic factors, such as mean temperature and the number of days with a maximum temperature above 25°C during grain filling. Campbell et al. (2004) explained 7% of QEI on wheat yield by considering the temperature between emergence and anthesis in another RIL population. Malosetti et al. (2004) integrated regression models for QTL main effects in factorial regression models to analyze GEI. In barley, they showed that a QTL for GY was related to the average daily temperature range during heading. To better characterize the environment, Laperche et al. (2007, 2008) used an indicator of nitrogen stress, which was chosen after an analysis of yield variations in the probe genotype Récital (Brancourt-Hulmel et al. 2001). We further developed this analysis using different indicators related not only to nitrogen stress. For example, one KN QTL (gpw4085) on chromosome 2D1 was not only related to nitrogen stress (finnA), but also to cumulative degree-days >25°C (stcmbA) and cumulative radiation-days (srglmfA and srglmbA). For TKW, one QTL (Fdgo-D1) on chromosome 2D1 was only related to water deficit (hlmtS) and cumulative degree-days >0°C (stmpflR).

Two main limits to the strategy employed can be identified. The first concerns the number and characteristics of the probe genotypes used during the experiment. This issue was addressed by Brancourt-Hulmel et al. (2001) regarding classic trials on breeding lines. They showed that four-probe genotypes were adequate and that both interaction patterns and earliness were important criteria for their choice. We chose to include both parents of the population because they had been shown to be contrasted in their

response to nitrogen stress (Le Gouis et al. 2000). Because of differences in earliness components, Récital has high vernalization requirements while Arche has high day length requirements, so that the population is highly variable for earliness at flowering, with transgressive lines in both directions (Hanocq et al. 2003). We therefore added a late genotype, Ritmo, which could possibly characterize late flowering lines. We did not add a genotype that was earlier flowering than Récital, as this was already one of the earliest registered winter wheat types in France. This resulted in about 30 DH lines being earlier than Récital by up to 6 days for which the environment may not have been well characterized. One solution would have consisted in calculating each environment covariate for each line based on its own flowering date. That might have been possible for some covariates that did not require plant sampling (such as those of a climatic nature), but was more difficult for covariates like NNI that required the sampling of plants and the measurement of N contents at a specific growth stage.

The second limitation concerns the choice of potential covariates. These were defined using current knowledge regarding the impact of environmental and biological factors on crop growth and yield (Brancourt-Hulmel et al. 2000). As each one was assessed for each probe genotype, this required the testing of a large number of covariates. We chose to select a sub-set of non-correlated covariates based on a PCA analysis. Indicators were then further selected by multiple linear regressions on the KN deviation and TKW reduction of probe genotypes. This limited the number of analyses to be performed. However, it was necessary to return to the complete initial indicator data set when the possibility that a limiting factor might explain a GEI was discussed.

This approach produced new results on the sensitivity of KN and TKW to environmental conditions

Campbell et al. (2004) reported that the marker × environmental covariate interactions for KN measured in seven environments in Nebraska were highest for rainfall between terminal spikelet initiation and anthesis. They also identified that the mean daily temperature and precipitation between seedling emergence and terminal spikelet initiation displayed the strongest interaction for the number of kernels per spike. Our results in northern France showed that radiation, temperature and nitrogen stress were the principal environmental covariates that interacted with the QTL for KN.

Groos et al. (2003) used factorial regression and environmental covariates to analyze GEI for GPC, GY and TKW. They found that the effect of mean temperature was significant for TKW and suggested that TKW was more markedly influenced by temperature than by nitrogen levels. Campbell et al. (2004) determined that the expression of a QTL for TKW on chromosome 3A was only influenced by solar radiation between anthesis and physiological maturity. Our study showed that other environmental covariates, such as water status, could also partially explain the specificity of QTL for TKW to some environments. We also confirmed that nitrogen stress was not involved in the interaction with QTL for TKW.

Because of cross-over interactions, the use of favorable alleles for KN or TKW needs to be adapted to environmental conditions

For several genomic regions, we showed that the dynamics of allelic effects regarding KN or TKW were dependent on the value of environment covariates.

We analyzed several abiotic covariates including temperature, radiation, nitrogen stress and water deficit by considering various environmental covariates. For KN, on chromosome 3D, the cfd211 marker interacted with the two environmental covariates st25efS (related to high temperature around meiosis) (Fig. 3) and stcmbI (related to high temperature between heading and flowering). The QTL was detected in four environments (1Cl−N, 1Cl+N, 1Ms−N and 1Ms+N) where the sum of degree-days >25°C between heading and flowering was low (Tables 4, S1). The favorable allele was found for 'Récital'. The highest additive effect was 967 grains m^{-2} and was recorded in environment 1Cl-N. The impact of temperature on wheat development has been widely documented (for a review, see Barnabàs et al. 2008). High temperatures during the days between meiosis and flowering decrease grain numbers and possibly affect both male and female fertility (Saini and Aspinall 1982). In our case, grain number decreased in lines carrying Récital and Arche alleles (from about 25,000 to 15,000 grains m^{-2}), but this decrease was greater for the Récital allele, thus resulting in no significant differences between the two alleles in highly stressed environments. This QTL is thus mainly expressed in environments free of severe temperature stress, limiting its use to cool regions.

For TKW, one QTL linked to marker gwm268 on chromosome 1B significantly interacted with the two environmental covariates stmplmR (Fig. 4) and nj30lmI. This QTL was detected in favorable situations for late grain filling (Table S4), as indicated by a high number of cumulative degree-days and a low number of days with temperature >30°C during the milk stage to maturity period. A favorable additive effect could be found for the Arche allele when conditions were unfavorable and by the Récital allele when conditions were favorable (Fig. 4).

High temperatures have a negative impact on late grain filling (Barnabàs et al. 2008). Because of cross-over interactions, the favorable allele for TKW at this QTL is environment dependent. Consequently, use of this QTL should be adapted to the target environment for which the cultivar is bred.

Some QTLs interacting with environments for grain yield and its components may be common to different genetic backgrounds

To determine whether there were common interacting QTL or regions across genetic backgrounds, the results of our study were compared with those of seven similar studies on wheat in which three genetic populations were used (Table 6). Using a Renan × Récital population grown at six locations in France in 1999, Groos et al. (2003) reported a QTL for the sensitivity of GPC and TKW to average daily temperature and the number of days with a maximum daily temperature above 25°C, both during the grain filling period. The QTL was found on chromosome 2B linked to gwm257, which was close to gwm374. During our study, we also detected a QTL for KN sensitivity to environmental covariates on the same chromosome linked to gwm429, which was close to gwm374. On chromosome 4A in a Trident/Molineux population, Kuchel et al. (2007a, b) detected one QTL for the sensitivity of GY to several environmental covariates (including temperature, latitude, rainfall, the number of cold nights and the number of hot days) linked to gwm397. During our study, one QTL for the sensitivity of TKW to environmental covariates was found to be linked to gpw2279, which was close to gwm397 on the same chromosome. On chromosome 4B, Laperche et al. (2007) detected a QTL for the sensitivity of GPA to N stress and linked to the dwarfing gene *Rht-B1* using the Arche/Récital population. We detected a QTL for the sensitivity of KN to srglmbA at the same position. On the same chromosome, Laperche et al. (2008) detected another QTL for the sensitivity of KN to N stress, linked to gpw1108. We also found one QTL at this location for the sensitivity of KN to sri1200bA, sri1200bI, st25efS and stcmbI. These results suggest that some QTLs that affect GY and its components, and also interact with environmental factors, are common to different genetic backgrounds. Focusing on these QTL common to different genetic backgrounds would give some guidance to understand genotype × environment interaction.

Conclusion

When selecting wheat genotypes adapted to specific environments, plant breeders are interested in QTL that provide improved adaptation. This study identified QTL specific to certain environments and then developed a strategy to provide a biological explanation for these interactions. The use of environmental covariates to partition QEI enabled the identification of limiting factors with major interactive effects on QTL for KN and TKW. Although QEI explained a small percentage of GEI (this small percentage being mainly due to the fact that only 11 degrees of freedom were used for marker × environment interactions, versus 2,388 for GEI), QTL × environmental covariate interactions could explain between 15.1 and 83.2% for KN and 13.5 and 81.9% for TKW of the QEI sums of squares. For KN, nitrogen stress was one of the most important limiting factors, which was not surprising as the experimental network included trials under both low and high nitrogen levels. Other environmental factors were also identified, such as water deficit between half filling and meiosis and high temperature between heading and flowering. In contrast, for TKW, nitrogen stress was less important while water deficit at maturity, the number of high-temperature days and high temperatures from the milk stage to maturity were three essential environmental factors. This identification of QTL and environmental covariates thus contributed to our genetic and biological understanding of the phenomenon of GEI. A thorough QTL × environmental covariate interaction analysis may therefore assist breeders in designing strategies and, when common interacting QTL are found between different genetic backgrounds, will target an improved adaptation of winter wheat to specific environments.

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