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Estimation of genetic parameters of a DH wheat population grown at different N stress levels characterized by probe genotypes

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Abstract Low market prices and environmental concerns in Europe favor lower input wheat production systems. To efficiently breed for new varieties adapted to low input management while maintaining high yield levels, our objective was to characterize the heritability and its components for yield and nitrogen traits under different nitrogen levels. Two hundred and twenty-two doubled-haploid (DH) lines from the cross between Arche (tolerant) and Récital (sensitive) were tested in France at four locations in 2000, and three in 2001, under high (N^+) and low (N^-) nitrogen supplies. The response of yield to the environment of four probe genotypes, the parents and two controls, were tested and used as descriptors of these environments. Grain yield (GY), its components, and grain and straw nitrogen, called nitrogen traits, were studied. A factorial regression was performed to assess the sensitivity (slope) of the DH lines to nitrogen stress and their performance to low nitrogen supply. An index based on the nitrogen nutrition index at flowering of the probe genotype Récital was the best descriptor of the environment stress. Heritabilities of yield and nitrogen traits for both nitrogen supplies were always above 0.6. When nitrogen stress increased, heritabilities decreased and genotype \times nitrogen interaction variances increased. The decrease in heritability was mainly explained by a decrease in genetic variance. Genetic variation for sensitivity to nitrogen stress and performance under low nitrogen supply were shown in the population. GY

decreased from 278 to 760 g/m² per unit of nitrogen stress index increase and GY under moderate nitrogen stress varied from 340 to 613 g/m². Those contrasted reactions revealed specific lines to include in breeding programs for improving GY under low nitrogen supply.

Introduction

Nitrates have been singled out as the main cause of ground water pollution in Europe. To attenuate this problem, the European Union is encouraging agricultural practices using smaller amounts of nitrogen (N) fertilizer (nitrate directive: 91/676/CEE). Some so-called ‘tolerant’ wheat varieties are available, and when they are used, the profit margin is maintained even if yield is lower (Félix et al. 2002). However, wheat varieties that are specifically N stress tolerant and aim towards actual yield levels are yet to be developed. There is a need then for what we have come to define as N stress tolerant varieties, that is to say varieties that can maintain their yields under moderate N deficiency as well as in case of intense N stress which occasionally occurs under moderate supplies. N stress tolerant varieties should also maintain their grain quality, as it is an important criterion for determining both wheat price and use, for instance for breadmaking. To breed such varieties, breeders need information about genetic parameters, such as heritability, genetic variance and genotype \times nitrogen ($G \times N$) interaction variance, under low nitrogen supplies. They also need to know the dynamics of these parameters under increasing N stress.

In maize, the relative part of $G \times N$ interaction variance increases with N stress intensity (Bänziger et al. 1997; Presterl et al. 2003). This confirms that cultivars adapted to low input practices are different from cultivars adapted to high-yielding environments. Generally, higher heritabilities have been reported under increased nitrogen supply (N^+) than under low nitrogen supply (N^-). This has been explained either by the fact that

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under N^- genetic variance decreased more than environmental variance (Bänziger et al. 1997), or by the fact that environmental variance increased more than genetic variance (Bertin and Gallais 2000; Sinebo et al. 2002). In wheat, this decrease of heritability has been explained both by an increase of environmental variance and a decrease of genetic variance under low inputs including N^- (Brancourt-Hulmel et al. 2005). However, in other studies in maize, heritability was higher under N^- , as a consequence of the increase of genetic variance under N^- (Agrama et al. 1999; Presterl et al. 2002b).

Studying the variation of genetic parameters in relation to nitrogen stress intensity requires a precise characterization of actual environments. In most studies, indexes defined to characterize N stress intensity have been based on grain yield variation between N^- and N^+ . These indexes do not take into account the dynamics of the N stress throughout the crop cycle. In some studies, the deviation of grain number per meter square (dGPA) and the reduction of thousand kernel weight (rTKW) from reference values are used to represent, respectively, the pre-flowering period and the grain-filling period (Brancourt-Hulmel et al. 1999). They can be related to indicators such as climatic, nutritional, or disease constraints to evaluate the main limiting factors of the environment (Leterme et al. 1994). To study an inbred lines population, this method would be too time consuming because it requires many measurements for each genotype under consideration. Therefore, probe genotype has been used (Brancourt-Hulmel 1999). The probe genotypes are a few well-known cultivars, added to each trial. dGPA and rTKW of these probe genotypes are characterized with the above mentioned indicators. All measures of indicators are performed only on those probe genotypes. Because the probe genotypes are chosen for their differences, it is possible to identify most of the environment limiting factors by relating dGPA and rTKW to environmental indicators. This method is the means to quantify N stress, to compare its intensity to other stresses, and to identify N stress interactions with other stresses.

The structure of the population under investigation has an influence on the genetic parameters; for instance, in maize, genetic variation and heritability for grain yield under N^+ were higher for lines per se than for testcrosses (Presterl et al. 2002a). Heritabilities also increased from half- to full-sib and from S1 to S2 progeny (Lamkey and Hallauer 1987). And, because the parameters are population specific, the choice of population parents also influences them.

For wheat, we aimed to estimate three genetic parameters (heritability, genetic variance and $G \times N$ variance) under a wide range of N deficiency conditions. We focused our study on an inbred lines population that was specifically created to study tolerance to N stress. The parents were chosen for their contrasting response to N stress (Le Gouis et al. 2000). In this paper, we show that the nitrogen nutrition index (NNI) at flowering of the probe genotype Récital was the best indicator of N

stress. Heritability decreased, and $G \times N$ variance increased when N stress increased. We also show a variation among the population for sensitivity to N stress and for performance in low input conditions.

Materials and methods

Field experiment and variables

A population of 241 doubled-haploid (DH) lines was produced from the F1 of the cross between Arche and Récital that have contrasting reactions to nitrogen deficiency (Le Gouis et al. 2000). In 1999/2000, 222 lines were experimented with, and in 2000/2001, the experimentation was done with 216. The two parents as well as two cultivars, Soissons and Ritmo, were used as controls. The four were equally used as probe genotypes to identify the main yield limiting factors (Brancourt-Hulmel et al. 1999). In 2000, experiments were carried out at Nickerson Chartainvilliers (48°35'N, 1°35'E) and in 2000 and 2001, they were carried out at three INRA locations: Clermont-Ferrand (45°47'N Lat., 3°05'E Long.), Le Moulon (48°42'N, 2°08'E) and Mons (49°53'N, 3°00'E). Two nitrogen supplies were tested at each location: a high N supply (N^+) corresponding to the current agricultural practices at each site and a low N supply (N^-) where N applied was 60–144 kg N/ha less than the high nitrogen supply (Table 1). Fungicide, insecticide, and herbicide treatments were applied on both N levels to achieve an optimized management of the crop. Growth regulators were applied to prevent lodging. In previous experiments, in 1998 and in 1999, 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 four probe cultivars were included in each block. The six blocks were replicated two times.

Grain yield (GY), Grain number per square meter (GPA), Thousand kernel weight (TKW), harvest index (HI), Aerial dry matter (ADM), heading date (DTH), plant height (PH), as well as grain and straw nitrogen contents (GPC and NS%), and grain protein yield (GPY) were measured. GY, GPA, TKW, HI and ADM are termed “yield traits” whereas GPC, NS%, GPY, total nitrogen per area (NTA) and nitrogen straw per area (NSA) are termed “nitrogen traits”. Nitrogen concentrations were measured with near infrared reflectance analyzers (Technicon Infra-Analyser 400, Technicon Instruments Corporation, Tarrytown, New York, USA and Infratec 1255, Foss Tecator, Höganäs, Sweden), calibrated with some samples measured using the Dumas procedure (Dumas 1831). Other developmental stages were observed for the probe genotypes (emergence, ear at 1 cm, flowering, and maturity) as well

Table 1 Main characteristics of the experiments carried out on wheat DH lines population and four probe genotypes under the two N supplies for each location

Location	Harvest Year	Preceding crop	Sowing date	Soil N (kg ha ⁻¹)	N applied (kg ha ⁻¹)	
					Low N level	High N level
Chartainvilliers	2000	Wheat	30 Oct 1999	59	40	184
Clermont	2000	Sunflower	5 Nov 1999	80	40	116
Clermont	2001	Sunflower	5 Nov 2000	70	30	130
Le Moulon	2000	Oat	2–3 Nov 1999	35	100	160
Le Moulon	2001	Oat	24–25 Nov 2000	35	115	215
Mons	2000	Oat	14 Oct 1999	52	50	160
Mons	2001	Oat	27 Oct 2000	40	50	180

as lodging and disease. The NNI was estimated at the ear at 1 cm and flowering stages (Justes et al. 1994). Soil mineral N was measured in each location in February in the upper 90 cm of the soil profile at the Clermont-Ferrand site or at the upper 120 cm of the soil profile at the other locations (Table 1). Daily meteorological data were also recorded (minimum and maximum temperatures, rainfall, potential evapotranspiration (Penman): ETP, and global radiation) to calculate the climatic limiting factors (Brancourt-Hulmel 1999).

Statistical analyses

Analyses of variance were carried out, for each combination of a year and a location, using the model:

$$Y_{ijk} = \mu + N_i + G_k + \text{rep}_j + N_i \times \text{rep}_j + N_i \times G_k + G_k \times \text{rep}_j + e_{ijk},$$

where Y_{ijk} represents the value of the trait under investigation for the genotype k at the N level i and the replication j , N represents the nitrogen level effect, G the genotypic effect, and rep the replication. e_{ijk} is the error term of the model. Broad-sense heritabilities were assessed for each nitrogen level by pooling the data of the different environments using the model:

$$Y_{jkl} = \mu + \text{env}_l + G_k + \text{rep}(\text{env})_{jl} + G_k \times \text{env}_l + e_{jkl}.$$

The heritability was then calculated as $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{G \times \text{env}}^2 / L + \sigma_e^2 / JL)$ where σ_G^2 represents the genetic variance, $\sigma_{G \times \text{env}}^2$ the genotype \times environment variance, σ_e^2 the environmental variance, J the number of replications and L the number of environments. Broad-sense heritability was also assessed for each environment using the following model:

$$Y_{ijk} = \mu + G_k + \text{rep}_j + G_k \times \text{rep}_j + e_{ijk}.$$

Heritability was calculated as $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_e^2 / J)$ where J represents the number of replications. Heritability standard errors were calculated as defined by Holland et al. (2003).

Correlations of heritability, σ_G^2 , and σ_e^2 of all environments with environmental stress indexes were

calculated. I_1 was defined as $I_1 = [1 - \text{NNI at flowering (finn) for Récital}]$. I_2 was defined as $I_2 = [1 - (\text{GM}_{\text{env}} / \text{GM}_{\text{max}})]$ where GM_{env} stands for the general mean of the trait in the considered environment. GM_{max} stands for the maximal general mean across all environments. Correlations between $[\sigma_{G \times N}^2 / (\sigma_{G \times N}^2 + \sigma_N^2)]$ and a third environmental index defined as $I_3 = [\min(1, \text{finn N}^+ \text{ of Récital}) - \text{finn N}^- \text{ of Récital}]$ were performed in each combination of a year and a location. All the described indexes varied from 0 to 1, from the least to the most stressed conditions.

For each probe genotype, three variables were assessed in order to describe the environments: the dGPA, defined as $100 \times (\text{GPA}_{\text{threshold}} - \text{GPA}) / \text{GPA}_{\text{threshold}}$, rTKW, defined as $\max[0; (\text{TKW}_{\text{potential}} - \text{TKW}) / \text{TKW}_{\text{potential}}]$ as well as the grain yield reduction. $\text{GPA}_{\text{threshold}}$, GY_{max} and TKW_{max} (TKW_m), are parameters defined by the boundary curve relating TKW to GPA (Brancourt-Hulmel 1999). $\text{TKW}_{\text{potential}}$ corresponds to TKW_m for values of GPA smaller than $\text{GPA}_{\text{threshold}}$. The boundary curves were established using databases collecting multiyear and multisite measurements made on the probe genotypes: 311 data points for Arche, 497 data points for Récital, 461 data points for Ritmo and 1,006 data points for Soissons. As the TKW_m obtained for the probe genotypes in each block were within the confidence intervals of the TKW_m defined with the points from the databases, we then assessed TKW_m for each DH line following the method described by Brancourt et al. (1999). A factorial regression (Denis 1988) was performed by regressing genotype means using the environmental index I_1 . The specific parameters of the factorial regression were the coefficient of determination (r^2), the slope (corresponding to the sensitivity of each DH line to N stress), and the intercept. Correlations of the factorial regression parameters with DTH, PH, and TKW_m were studied. Plant height was assessed as the mean of plant height in all N⁻ environments (highly correlated to the mean of plant height in all N⁺ environments). Heading date was assessed as the general mean of heading date across all environments.

All statistical analyses were performed with the SAS statistical package version 8.0 (SAS Institute Inc. 1999).

Results

Characterization of the environments: finn of Récital was the best environment descriptor before flowering

Deviation of grain number characterized the environments before flowering. Negative values corresponded to environments free of stress while high positive values indicated stressed environments. dGPA varied from -19.3% for Soissons in Le Moulon under N^+ in 2001 to 56.4% for Récital in Mons under N^- in 2001 (Fig. 1). As expected, dGPA was more affected under low nitrogen supplies (31.2% on the average) than under N^+ supplies (6.6%). The largest difference between the two treatments in one environment was recorded in Le Moulon in 2000 (42.9%). rTKW characterized the environments during the grain-filling period. The reductions ranged from 4.6% in Le Moulon under N^+ in 2001 to 24.9% in Clermont under N^- in 2001 (data not shown). The two treatments showed similar reductions: 14.1% on the average under N^- and 12.5% under N^+ . The largest difference between the two treatments in one environment was only 7.1%, recorded in Le Moulon in 2001.

Deviation of grain number variations was similar to rGY variations. High variations were found in grain yield reduction (data not shown): the smallest value was observed in Le Moulon under N^+ in 2001 (4.7%) while the highest value was recorded in Mons under N^- in 2001 (55.8%). N^- supplies were more affected than N^+ supplies (41.7 and 24.2% respectively). The largest differences in one environment were recorded in Le Moulon in 2000 (32.1%) and in Mons in 2001 (31.4%).

Deviation of grain number was explained by several limiting factors (Table 2). Cumulative degree-days above $25^\circ\text{C} \pm 3$ days at meiosis (stcmb), nitrogen stress at flowering (finn), cumulative radiation-days ± 3 days at meiosis (srglmb), cumulative degree-days $> 0^\circ\text{C}$ from flowering-30 days to flowering (stmpf30), as well as cumulative radiation-days from meiosis to flowering (srglmf) accounted for 99% of the variation for the genotype Arche. Finn was the single limiting factor for Récital and explained 83% of the dGPA variation. Finn and cumulative degree-days above $25^\circ\text{C} \pm 3$ days at meiosis (stcmb) accounted for 78% of the variability for Ritmo. Finn and cumulative degree-days above 25°C from heading to flowering (st25ef) explained 83% of the variation for Soissons. Nitrogen was, therefore, the main stress during the period before flowering. Temperatures above 25°C around meiosis and flowering were also an important limiting factor. Finally, the best descriptor of the environments before flowering was finn measured for Récital.

Reduction of TKW was more or less explained (Table 2). For the probe genotype Arche, no significant indicator was found. For Récital, brown rust (br) and cumulative daily difference between rain and ETP from half-filling stage to maturity (spetplm) accounted for

85% of rTKW. For Ritmo, br explained 69% of rTKW. For Soissons, br and cumulative daily difference between rain and ETP from flowering to half-filling (spetpfl) accounted for 68% of the variability. Brown rust and water deficit were the main limiting factors during the early grain-filling stage. Probably due to the smaller range of the variations, the grain-filling period was not as well described as the period before flowering for Arche.

$\sigma_{G \times N}^2$ increased with N stress intensity and heritabilities were higher under N^+ than under N^-

Genetic effect and N treatment effect were highly significant for all environments and all traits except TKW in Clermont-Ferrand in 2000. For TKW and HI, the variation due to N condition was less than 10% of the total variation. On an average, the genetic variation of the other traits accounted for 28% of the total variation, and N condition variation accounted for 57.8%. In Le Moulon and Clermont-Ferrand, in 2001, N condition relative variation was lower than in the other environments. As a consequence, in those environments, the relative genetic variation increased.

For GY, TKW, GPA and HI, termed as yield traits, $G \times N$ interaction was significant at the 10% level in all environments (Table 3). In Mons, in 2001, no $G \times N$ interaction was significant for GPC, GPY, NS%, NSA and NTA termed as nitrogen traits. $G \times N$ interaction of the nitrogen traits was significant in most other environments, and it was the strongest in Clermont-Ferrand in 2001 and Le Moulon in 2001.

For each combination of a year and a location, we followed the dynamics of the ratio $\sigma_{G \times N}^2 / (\sigma_G^2 + \sigma_{G \times N}^2)$ with the nitrogen stress index I_3 for all traits. Correlations were significant at 5% for ADM, GY, GPA, GPC, and GPY. Regression slopes were positive for all traits except NSA (Table 6). This means that the percentage of the NSA $G \times N$ interaction decreased when N stress increased. For all other traits, the $G \times N$ variance (relative to the genetic variance) increased with N stress intensity. The slopes of GPC, GPY, and NS% were the highest. They ranged from 69 to 103. The slopes of the other traits varied from 10 to 61. The $G \times N$ interaction percentage increased at least ten times more than the genetic variance with N stress.

Heritabilities were always above 0.60 (Table 4). The yield traits heritabilities were higher than the nitrogen traits heritabilities. In general, heritabilities were higher under N^+ supplies than under N^- supplies. For yield traits, heritabilities under N^- supplies were at the most 10% less than those under N^+ supplies. For nitrogen traits, h^2 under N^- supplies were 22–30% less than those under N^+ supplies. Heritabilities of nitrogen traits were more sensitive to N deficiency than those of yield traits.

To study the dynamics of heritability and its components, in relation to N stress intensity, we used two stress indexes to characterize the environments

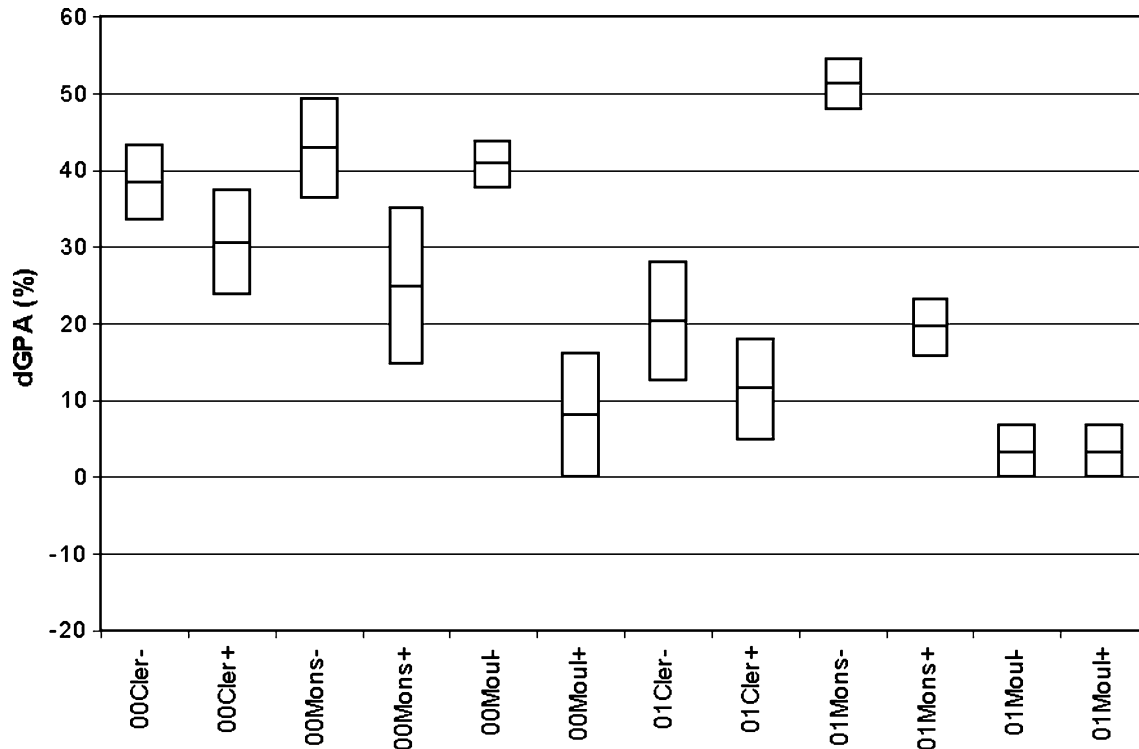


Fig. 1 Mean of the deviation of grain number in % (*dGPA*), measured on the four probe genotypes in the diverse environments. Environments are coded by year (00 and 01), by site (Cler, Mons and Moul) and by nitrogen treatment (– and +)

Table 2 Multiple regressions explaining kernel number deviation (*dGPA*) and reduction of thousand kernel weight (*rTKW*) by indicators of limiting factors for each probe genotype

Genotype	Indicator	Partial r^2	Total r^2	<i>F</i>	
dGPA					
Arche	stcmb	Cumulative degree-days $>25^{\circ}\text{C} \pm 3$ days at meiosis	0.43	0.43	7.54 ^a
	finn	Nitrogen stress at flowering	0.31	0.74	11.00 ^a
	srglmb	Cumulative radiation-days ± 3 days at meiosis	0.11	0.85	5.63 ^a
	stmpf30	Cumulative degree-days $>0^{\circ}\text{C}$ from flowering-30 days to flowering	0.05	0.90	3.42
	srglmf	Cumulative radiation-days from meiosis to flowering	0.09	0.99	33.25 ^a
	Récital	finn	Nitrogen stress at flowering	0.83	0.83
Ritmo	finn	Nitrogen stress at flowering	0.40	0.40	6.65 ^a
	stcmb	Cumulative degree-days $>25^{\circ}\text{C} \pm 3$ days at meiosis	0.38	0.78	15.64 ^a
	njss	Number of successive dry-days ^a from ear at 1 cm -150 dd to ear at 1 cm + 350 dd	0.09	0.87	5.79 ^a
	sri1200b	Cumulative radiation-days $>1,200 \text{ J/cm}^2 \pm 3$ days at meiosis	0.05	0.92	4.46
Soissons	finn	Nitrogen stress at flowering	0.41	0.41	6.84 ^a
	st25ef	Cumulative degree-days $>25^{\circ}\text{C}$ from heading to flowering	0.42	0.83	22.11 ^a
rTKW					
Arche	–	No significant indicator	–	–	–
Récital	br	Brown rust	0.45	0.45	8.06 ^a
	spetplm	Cumulative daily difference between Rain and ETP from half-filling to maturity	0.40	0.85	24.20 ^a
	spetpfl	Cumulative daily difference between Rain and ETP from flowering to half-filling	0.09	0.94	12.75 ^a
Ritmo	br	Brown rust	0.69	0.69	22.24 ^a
	sdffl	Cumulative water deficit-days from flowering to half-filling stage	0.10	0.79	4.00
	sdflm	Cumulative water deficit-days from half-filling stage to maturity	0.06	0.85	2.99
	lodg	Lodging	0.06	0.91	4.36
Soissons	br	Brown rust	0.38	0.38	6.22 ^a
	spetpfl	Cumulative daily difference between Rain and ETP from flowering to half-filling	0.30	0.68	8.75 ^a
	st28fl	Cumulative degree-days $>28^{\circ}\text{C}$ from flowering to half-filling stage	0.11	0.79	4.32
	sdffl	Cumulative water deficit-days from flowering to half-filling stage	0.02	0.81	0.84

^aRain < ETP

^aSignificant at the 0.05 level

Table 3 Results of the environment by environment analysis of variance: % of total variation explained by the G × N interaction and significance of the G × N term

Trait	Clermont 2000	Clermont 2001	Chartainvilliers 2000	Le Moulon 2000	Le Moulon 2001	Mons 2000	Mons 2001
GY	7.5	7.0 ^c	5.1	4.6 ^c	12.2 ^c	5.4 ^b	2.3 ^b
TKW	10.3 ^c	13.8 ^c	7.1 ^c	12.6 ^c	3.2 ^c	5.8 ^c	7.6 ^c
GPA	11.2 ^b	13.7 ^c	3.4 ^a	4.0 ^c	7.4 ^c	5.7 ^c	3.3 ^c
ADM		24.1 ^c	4.2 ^{NS}	4.7 ^b	10.2 ^b	6.1 ^a	2.8 ^b
HI		15.3 ^c	11.3 ^b	16.9 ^c	5.8 ^a	9.3 ^a	8.1 ^b
GPY	7.4 ^{NS}	6.5 ^a	4.5 ^b	2.7 ^c	11.3 ^c	3.2 ^c	1.9 ^{NS}
NSA		21.4 ^c	5.7 ^{NS}	7.3 ^c	12.4 ^c	5.2	6.9 ^{NS}
NTA		9.9 ^b	3.6	2.0 ^b	9.9 ^c	2.6	1.7 ^{NS}
GPC	10.8 ^b	8.2 ^b	5.4 ^{NS}	2.2 ^c	7.2 ^c	3.3 ^b	3.5 ^{NS}
NS%		18.6 ^{NS}	7.8 ^a	7.6 ^c	12.2 ^c	3.9 ^{NS}	11.9 ^{NS}

GY grain yield, TKW thousand kernel weight, GPA grain per area, ADM aerial dry matter, HI harvest index, GPY grain protein yield, NSA nitrogen straw per area, NTA total nitrogen per area, GPC grain protein content, NS% straw nitrogen content, NS not significant at the 10% level

^aSignificant at the 5% level

^bSignificant at the 1% level

^cSignificant at the 0.1% level

(combination of a year, a location and a N level): I_1 (specific to nitrogen stress) and I_2 (nonspecific). For nitrogen traits (in particular for NS% and NSA), regressions of heritability and of the genetic variance using I_1 presented higher correlations than regressions performed using I_2 (Table 4). For NS%, the r of the regression of the h^2 was -0.11 with I_2 and -0.60 with I_1 . For each yield trait, the r of the regression was higher when I_2 was used than when I_1 was used; nevertheless, the correlations of heritability, genetic, and environmental variances with I_1 were still significant.

Genetic parameters of GPC, TKW, DTH, and PH did not depend on the N stress intensity of the environment. Correlations of heritability and genetic

variance using I_1 were low or nonsignificant ($|r| < 0.21$), indicating that the dynamics of h^2 and genetic variance did not follow N stress intensity. Heritabilities of the other traits decreased with N stress intensity (Table 4). The slopes of heritability regression using I_1 were the most important for GPY, NSA, and NS%. They ranged from -0.34 to -0.42 (Table 5). The slopes of the yield traits varied from 0.01 to -0.13 . For each trait, both σ_G^2 and σ_e^2 decreased with N stress intensity. The h^2 decrease resulted from the fact that, in relation to N stress intensity, σ_G^2 decreased more than σ_e^2 . The slopes of the genetic variances regressions were higher than the slopes of the environmental variances regressions except for NTA (slope for $\sigma_G^2 = -2.45$ and slope for $\sigma_e^2 = -3.69$).

Table 4 Heritabilities (h^2) by N treatment and correlation coefficient of σ_G^2 and h^2 (of each environment) regression using the environment stress indexes I_1 ([1- fin]) of Récital and I_2 (general mean index) for all traits

Trait	h^2 N ⁻	h^2 N ⁺	Correlation coefficient for regression on I_1		Correlation coefficient for regression on I_2	
			σ_G^2	h^2	σ_G^2	h^2
GY	0.849 ± 0.016	0.860 ± 0.014	-0.58 ^a	-0.26 ^{NS}	-0.74 ^b	-0.47 ^{NS}
TKW	0.937 ± 0.007	0.947 ± 0.005	-0.21 ^{NS}	0.06 ^{NS}	-0.17 ^{NS}	-0.48 ^{NS}
GPA	0.806 ± 0.020	0.894 ± 0.011	-0.62 ^a	-0.13 ^{NS}	-0.81 ^c	-0.35 ^{NS}
ADM	0.848 ± 0.016	0.864 ± 0.014	-0.77 ^b	-0.33 ^{NS}	-0.87 ^c	-0.53 ^a
HI	0.862 ± 0.015	0.863 ± 0.015	-0.56 ^a	-0.36 ^{NS}	0.29 ^{NS}	-0.73 ^b
GPY	0.654 ± 0.036	0.798 ± 0.021	-0.81 ^c	-0.50 ^{NS}	-0.72 ^b	-0.52 ^{NS}
NSA	0.600 ± 0.045	0.641 ± 0.040	-0.77 ^b	-0.64 ^a	-0.77 ^b	-0.17 ^{NS}
NTA	0.655 ± 0.038	0.720 ± 0.031	-0.82 ^c	-0.48 ^{NS}	-0.83 ^c	-0.47 ^{NS}
GPC	0.836 ± 0.017	0.851 ± 0.015	0.05 ^{NS}	-0.29 ^{NS}	-0.15 ^{NS}	-0.03 ^{NS}
NS%	0.669 ± 0.037	0.750 ± 0.027	-0.70 ^b	-0.60 ^a	-0.69 ^b	-0.11 ^{NS}
PH	0.883 ± 0.013	0.903 ± 0.011	-0.03 ^{NS}	0.01 ^{NS}	-0.57 ^a	-0.52 ^{NS}
DTH	0.983 ± 0.002	0.981 ± 0.002	-0.16 ^{NS}	0.16 ^{NS}	0.70 ^b	0.11 ^{NS}

GY Grain yield, TKW thousand kernel weight, GPA number of grains per area, ADM aerial dry matter, HI harvest index, GPY grain protein yield, NSA straw nitrogen per area, NTA total nitrogen per area, GPC grain protein content, NS% nitrogen straw content, DTH heading date, PH plant height, NS not significant

^aSignificant at the 5% level

^bSignificant at the 1% level

^cSignificant at the 0.1% level

Table 5 Slopes of σ_G^2 , σ_e^2 , and h^2 regression using the environment stress index I_1 ($1 - \text{finn}$) of Récital) for all traits

Trait	σ_G^2	σ_e^2	h^2
GY (g^2/m^4)	-5,580 ^a	-1,877 ^{NS}	-0.13 ^{NS}
TKW (g^2)	-2.38 ^{NS}	-0.22 ^{NS}	0.01 ^{NS}
GPA (m^{-4})	-9,000,000 ^a	-2,000,000 ^{NS}	-0.13 ^{NS}
ADM ($\text{g}^2 \text{m}^{-4}$)	-18,668 ^b	-9,598.3 ^{NS}	-0.13 ^{NS}
HI (%) ²	-0.91 ^a	1.49 ^{NS}	-0.10 ^{NS}
GPY ($\text{g}^2 \text{m}^{-4}$)	-2.11 ^c	-1.17 ^{NS}	-0.42 ^{NS}
NSA ($\text{g}^2 \text{m}^{-4}$)	-0.76 ^b	-0.66 ^{NS}	-0.37 ^a
NTA ($\text{g}^2 \text{m}^{-4}$)	-2.45 ^c	-3.69 ^c	-0.21 ^{NS}
GPC (%) ²	0.001 ^{NS}	0.007 ^c	-0.13 ^{NS}
NS% (%) ²	-0.005 ^b	-0.004 ^{NS}	-0.34 ^a
PH (cm^2)	-1.74 ^{NS}	4.89 ^{NS}	0.01 ^{NS}

NS not significant

^aSignificant at the 5% level

^bSignificant at the 1% level

^cSignificant at the 0.1% level

The correlation between the DH lines performance under N^- and their sensitivity to N stress was low. The factorial regression parameters were partly explained by DTH, PH and TKW_m

In this part of the study, we focused on the main traits: GY, GPA, GPY, NSA, NTA and GPC. The factorial regression (FR) was performed using $[1 - \text{finn}]$ of Récital ($= I_1$) as a regressor. FR was not significant for NSA.

The average r^2 of the FR for the other traits varied from 0.49 for GPC to 0.80 for NTA and GPY. GY decreased from 278 to 760 g/m^2 per unit of nitrogen stress index increase. On an average, GY and GPA regressions were better estimated for genotypes with the same earliness as Récital. This could be explained by the fact that the covariate finn of Récital was better adapted to varieties with the same earliness as the genotype used in defining the covariate. The standard deviation of the r^2 was always lower than 0.13. For each trait, the number of DH lines for which the FR was not significant ranged from 0 to 6.

To enable genotype comparisons at the same N stress level, the N^- performance was estimated as the ordinate value obtained when the abscissa was 0.5 (able to occur under low N supplies). DH lines GY estimations when $\text{NNI} = 0.5$ ranged from 340 to 613 g/m^2 . The correlation between slope and N^- performance was significant for all traits except GPY. The correlations varied from $r = 0.49$ for GPA to $r = -0.24$ for GPC (Table 7). The correlations between slope and N^- value for GPC and NTA were negative (respectively $r = -0.24$ and $r = -0.12$). GY correlation was 0.23. The positive correlation coefficient of GY and GPA was significant but low. Some DH lines performed well under N^- supplies and were not too sensitive to N stress, for instance, DH lines 4, 142, 92 and 146, termed favorable when GY was taken under consideration. DH lines 239, 187 and 120

Fig. 2 Correlation between two parameters of the factorial regression of GY on $[1 - \text{finn}]$ of Récital: the N^- value (GY obtained for $\text{NNI} = 0.5$) expressed in g/m^2 and slope expressed in $\text{g}/\text{m}^2/\text{NNI}$ unit. Circled DH represents favorable lines, that is to say, lines that performed well under low nitrogen supplies and that were less sensitive to N variation. DH lines symbolized by rectangles represent unfavorable DH lines, that is to say, those that performed poorly under stressed environments and that were the most sensitive to N variation

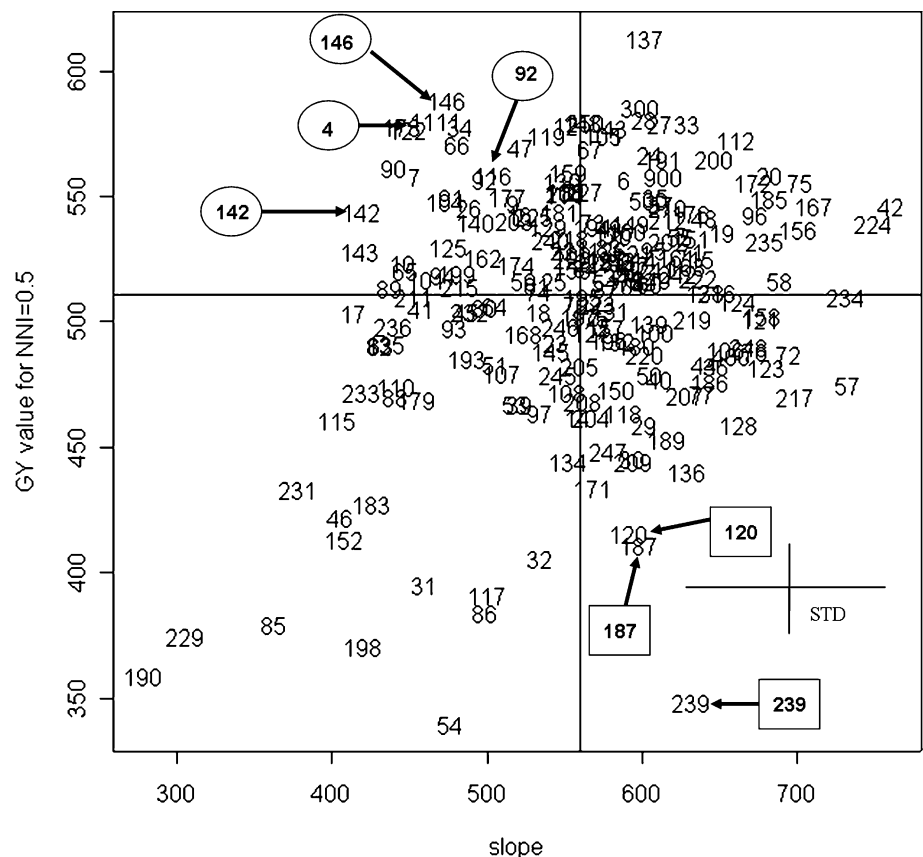


Table 6 Coefficient of correlation (r) and slope for the regression of $\sigma_{G \times N}^2 / (\sigma_{G \times N}^2 + \sigma_e^2)$ using the environment stress index I_3 ($[\min(1, \text{finn of Récital under } N^+) - \text{finn of Récital under } N^-)]$ measured in six environments

Trait (slope unit)	r	Slope
GY (g^2/m^4)	0.85 ^b	53.6
TKW (g^2)	0.46 ^{NS}	9.99
GPA (m^{-4})	0.82 ^a	61.06
ADM (g^2/m^4)	0.72 ^a	25.2
HI	0.38 ^{NS}	29.8
GPY (g^2/m^4)	0.72 ^a	103.1
NSA (g^2/m^4)	-0.55 ^{NS}	-33.7
NTA (g^2/m^4)	0.46 ^{NS}	37.8
GPC	0.88 ^b	69.7
NS%	0.34 ^{NS}	82.2

NS not significant

^aSignificant at the 5% level

^bSignificant at the 1% level

were termed unfavorable for GY (Fig. 2). Favorable lines for GY and other traits were unfavorable for GPC (DH 6, 201 and 127), and inversely, unfavorable lines for GY were favorable for GPC (DH 120 and 239).

To explain FR parameters, we studied their correlation with plant height under N^- supplies (PH), heading date (DTH) and with TKW_m . DTH varied from 140 to 154 (mean = 131) and PH varied from 57 to 102 cm (mean = 77 cm). TKW_m varied from 35 to 44 g. Table 7 shows the correlation between FR parameters and explanatory variables. The tested explanatory variables did not well define GY, GPY and GPC. The highest correlations were between the GPA slope in relation to PH ($r = -0.39$), TKW_m ($r = -0.32$), and DTH ($r = 0.27$). PH was the best explanatory variable for NTA slope ($r = 0.47$). TKW_m was the main explanatory variable for GY ($r = 0.33$), GPA ($r = -0.34$) and NTA ($r = 0.38$) N^- values. PH was the main explanatory variable for GPY ($r = 0.38$) and NTA ($r = 0.47$) N^- values.

Discussion

We studied the dynamics of genetic parameters under different N stress intensities for a population of 220 DH lines. This population was specifically built and chosen

as experimental support as the two parents were cultivated varieties that differed in their reactions to N stress (Le Gouis et al. 2000).

We found that the best way to describe the environments was to use an index based on the nitrogen nutrition index of the probe genotype Récital at flowering ([1-finn] of Récital). Once the environments were described, we followed the dynamics of the main genetic parameters with N stress intensity. $G \times N$ interaction was significant and the $\sigma_{G \times N}^2$ increased with N stress. Heritabilities decreased with N stress. This was explained by the fact that with N stress, the σ_G^2 decreased more than σ_e^2 . We also used [1-finn] of Récital to assess the DH lines performance under poor nitrogen conditions as well as DH lines sensitivity to N stress. These two parameters were poorly correlated. They were partly explained by the maximal TKW, PH and earliness. The discussion addresses three points: the advantages of the methodology, the explanation of heritability decrease with N stress as well as the potential impact of such a study on optimizing breeding programs.

Our methodology was innovative because it linked a DH lines population specifically constructed to study N stress with a description of environments using probe genotypes as well as a factorial regression to characterize the sensitivity of the DH lines to N stress.

The study was conducted with the ARE population, especially constructed to study N stress

To be as close as possible to breeding conditions, the parents of our population were selected among 20 cultivated varieties. They were selected for their contrasting response to N stress, Arche being N stress tolerant, and Récital being N stress sensitive (Le Gouis et al. 2000). Arche and Récital presented similar yield under optimal N supply (170 kg N/ha). When no fertilizer was applied, the Arche yield decreased by 11% while the Récital yield decreased by 39%. The better tolerance of Arche may be partially explained by a better N uptake efficiency that was observed at both N levels. No other study of genetic parameters of population under N stress has been reported for wheat. In maize, Bänziger et al. (1997) and

Table 7 Correlation coefficient (r) of the regression between the factorial regression (FR) parameters: slope, and mean under N^- supplies (N^- value) and correlation coefficient (r) for the FR parameters (slope, N^- value) with heading date (DTH), TKW_m , and plant height (PH)

Traits	GY	GPA	GPY	NTA	GPC
N^- value/slope	0.23 ^b	0.49 ^c	0.05 ^{NS}	-0.12	-0.24 ^b
Slope/PH	-0.1 ^{NS}	-0.39 ^c	-0.15 ^a	-0.23 ^b	-0.14 ^a
N^- value/PH	0.21 ^b	-0.16 ^a	0.38 ^c	0.47 ^c	0.08 ^{NS}
Slope/DTH	-0.1 ^{NS}	0.27 ^b	-0.14 ^a	0.1 ^{NS}	-0.04 ^{NS}
N^- value/DTH	0.18 ^a	0.15 ^a	0.14 ^a	0.22 ^b	-0.11 ^{NS}
Slope/ TKW_m	0.05 ^{NS}	-0.32 ^c	-0.03 ^{NS}	-0.09 ^{NS}	-0.13
N^- value/ TKW_m	0.33 ^c	-0.34 ^c	0.28 ^c	0.38 ^c	-0.17 ^a

NS not significant

^aSignificant at the 5% level

^bSignificant at the 1% level

^cSignificant at the 0.1% level

Presterl et al. (2003) have carried out a set of experiments with different genotypes selected under different N conditions. The main difference in our study is the low heritability they recorded for GY under N⁻. The correlations of σ_G^2 and the relative $\sigma_{G \times N}^2$ we calculated for our specific population were higher than those reported by Bänziger et al. (1997) and Presterl et al. (2003). Bänziger et al. (1997) reported a correlation of $r = -0.47$ ($r^2 = 0.22$) for σ_G^2 using I₂, while we reported a correlation of $r = -0.74$. Presterl et al. (2003) reported a correlation of $r = 0.78$ ($r^2 = 0.61$) for the relative $\sigma_{G \times N}^2$ using I₂, while we reported an r of 0.85. These contrasting results might be attributed to the specificity of our population. However, the impact of the population structure is quite hard to determine. An explanation could be that the other studies were carried out on complex populations composed of lines originating from different crosses, whereas this study used a single DH population from homozygous parents (bi-allelic). But this cannot fully explain the impact of population structure because the studies using multi-allelic populations should have reported, from a theoretical point of view, higher genetic variance, and consequently higher heritabilities. The effect of breeding might very well explain the differences in genetic variance. The main impact of breeding is the reduction of genetic variance, at least for the traits under selection. The other studies used complex but bred populations whereas we used a nonbred population.

Environments that covered a wide range of nitrogen stress intensity were described well using probe genotypes

When studying N stress intensity, most authors have compared two situations: an optimal situation and an N stress situation where no N was applied (Bänziger et al. 1997; Bertin and Gallais 2000; Presterl et al. 2003). We compared an optimal situation to an N stress situation where N input was reduced but not suppressed to take into account low N input practices. Because we carried out multi-environment trials, we had a range of environments varying in N stress intensity. To characterize the environments, we assessed climatic indexes for the probe genotypes and used an ecophysiological model to compute the nitrogen nutrition index (Justes et al. 1994) at flowering and at the ear at 1-cm stages. This data set allowed us to conclude that the main limiting factor was well represented by the 1-finn of the probe genotype Récital. The use of only four probe genotypes sharply reduced the number of measurements, yet it still gave precise results. Moreover, the index I₁ corresponds to a single covariate and not to a combination of covariates, therefore, it maintains its biological meaning. In the future, additional measurements such as water and nitrogen availability in soil and soil depth would complete the environment description. New combined indexes could be set up: Lacaze and Roumet (2004)

have assessed limiting factors using models that calculate water deficit and nitrogen availability from booting to heading.

The description of the environment was combined with the biometric model of the factorial regression to characterize the sensitivity of the DH lines to an N stress

To study N stress, it is important to well characterize the environments. 1-finn of Récital was used in FR to characterize the stress in each environment and estimate the response of the lines. To our knowledge, no other study has used this approach to study the dynamics of genetic parameters with stress intensity. Usually, a joint linear regression is performed to study the dynamics of parameters with stress intensity (Campbell et al. 2003; Finlay and Wilkinson 1963; Quarrie et al. 2005). Stress intensity is then defined as the mean of the studied trait across all lines in the environment under consideration. This method has the drawback of preventing the incorporation of external information into environment description and of being trait-specific. Moreover, the question remains if the trait used to evaluate stress intensity really represents the observed stress. In our study, finn measured for Récital could be used as a bio-indicator as it was well correlated to nitrogen stress. Authors have expressed stress intensity with an index based on grain yield. Even if N stress was the major stress in these studies, it was certainly not the only stress to occur during the experiments and to be taken into account by this environmental index. We observed that the environments ranked differently, depending on the trait that was used to define the environmental index (data of joint linear regression for different traits not shown). The choice of an external trait ([1-finn] of Récital) to describe stress intensity allowed the comparison of the DH lines reaction to the different traits, and because a crop diagnosis proved that 1-finn represented the main limiting factor of the environments, we could ensure that environments were ranked according to stress.

The lines performance in poor environment is by definition represented by the intercept of the factorial regression; that is to say, the performance of the lines when NNI = 0, which is unrealistic. Therefore, we chose an NNI value of 0.5, representative of line performance in a realistic, poor environment (GY decreased by 30% on an average under NNI = 0.5 conditions, while the observed average GY decrease under N⁻ was 25%).

What could explain the σ_G^2 decrease subsequent to N stress increase, which led to a decrease of h^2 under N⁻ supplies?

Few studies have related higher heritabilities under N⁻ than under N⁺ (Agrama et al. 1999; Presterl et al. 2002b).

We could assume that under optimal conditions, the reactions of the different genotypes would not differ as much as they would under stressed conditions. Therefore, genetic variance, and then heritabilities should increase under N^- supplies. We found that increased N stress decreased σ_G^2 . This decrease can be explained by the fact that the general mean of the traits under investigation (except GPC and NS%) decreased with N stress intensity and consequently, the genetic variance decreased (the scaling effect as mentioned by Presterl et al. 2003). But this mean–variance relation might not be the only explanation of the genetic variance (and then heritability) decrease under N^- . Other authors have reported a decrease in heritability with increased stress intensity (Bänziger et al. 1997; Bertin and Gallais 2000; Brancourt-Hulmel et al. 2005; Loudet et al. 2003). In these studies, the decrease in heritabilities was a consequence of different patterns of genetic and environmental variance variations: an increase of σ_G^2 and of σ_e^2 (Bertin and Gallais 2000; Sinebo et al. 2002); or a decrease in genetic variance and an increase in environmental one (Bertin and Gallais 2000; Brancourt-Hulmel et al. 2005). Our results showed a decrease in both environmental and genetic variance. These variance decreases can be explained by the fact that increased N stress increased $G \times E$ and $G \times N$ variances.

Potential impact: optimizing a breeding program using direct or indirect selection

In similar cases, Brancourt-Hulmel et al. (2005) for wheat and Bänziger et al. (1997) for maize concluded in favor of a direct selection whereas Calhoun et al. (1994) proposed to carry out both experiments and to favor lines identified by both selection strategies. In our case, because heritabilities were higher under N^+ , an indirect selection under N^+ to improve productivity under N^- conditions would appear to be the most suitable program. However, heritabilities were high enough under N^- to allow for a direct selection. Thus, a further analysis of heritabilities and genetic correlations between the two nitrogen levels as well as of $G \times E$ interaction should be carried out to determine the optimal breeding program. In most cases, correlation coefficients between N^- value and slope of FR, each with PH, DTH and TKW_m , were significant (Table 7). A genetic improvement of performance in poor environments as well as sensitivity to N stress for yield and nitrogen traits would be possible by breeding for PH, DTH and TKW_m . The genetic improvement would be greater for nitrogen traits (GPY and NTA) than for yield traits (GY and GPA). For GPY and NTA, the correlation between the N^- value and the sensitivity to an N stress (represented by the slope of the FR) is negative or not significant, as opposed to the yield traits and GPC. This means that breeding for cultivars less sensitive to an N stress and productive under poor environments would be easier for GPY and NTA than for yield traits. The correlation

coefficient of GPC N^- value with DTH or PH was not significant, but the correlations of GY N^- value with DTH and PH were significant. Therefore, using DTH and PH, we could improve GY without reducing GPC. TKW_m had the most impact on the N^- value of the traits, especially on GY. Varieties with a higher potential TKW may better compensate the grain number reduction consequent to N stress, and, therefore, may be better in maintaining their yield in poor environments (Le Gouis et al. 1998). The correlation with DTH could be interpreted as following: the latest lines may accumulate more nitrogen because they have more time to use N mineralization from organic matter (C. Lecomte, personal communication). Concerning PH, we can hypothesize that taller plants that do result in more dry matter, are better in stocking nitrogen. This could be correlated with a more developed root system and higher amounts of straw.

Conclusion

The next step of the study will be to use this population for QTL detection. We showed that the heritability of yield and its components as well as the heritability of nitrogen traits were high even under N^- (h^2 ranged from 0.60 to 0.95 for yield and nitrogen traits). This population also showed contrasting response to N stress sensitivity. A correlation was found between the N stress sensitivity, represented by the slope of the FR, and the N^- value, but still all types of behaviors were observed among the DH lines: high N stress sensitivity associated with high N^- value as well as high N stress sensitivity associated with low N^- value. The measures performed on probe genotypes would allow the evaluation and characterization of potential QTL \times environment interactions (QEI). Studying QEI is of great interest when detecting QTL for marker assisted selection (MAS) purposes. Breeders are mainly focused on stable genotypes, little affected by interactions with the environment. When QTL detection is carried out in different environments, it is rare that a similar QTL is evidenced in all environments with the same effect. This results in QEI. Studying the QEI leads to an understanding as to which part of the genome is involved in the $G \times E$ interaction, and thus selecting only noninteractive or N^- specific QTL for future MAS programs.

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