

# A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (*Triticum aestivum* L.)

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## Abstract

**Key message** By comparing 195 varieties in eight trials, this study assesses nitrogen use efficiency improvement in high and low nitrogen conditions in European winter wheat over the last 25 years.

**Abstract** In a context where European agriculture practices have to deal with environmental concerns and nitrogen (N) fertiliser cost, nitrogen use efficiency (NUE) has to be improved. This study assessed genetic progress in winter wheat (*Triticum aestivum* L.) NUE. Two hundred and twenty-five European elite varieties were tested in four environments under two levels of N. Global genetic progress was assessed on additive genetic values and on genotype  $\times$  N interaction, covering 25 years of European breeding. To avoid sampling bias, quality, precocity

and plant height were added as covariates in the analyses when needed. Genotype  $\times$  environment interactions were highly significant for all the traits studied to such an extent that no additive genetic effect was detected on N uptake. Genotype  $\times$  N interactions were significant for yield, grain protein content (GPC), N concentration in straw, N utilisation, and NUE. Grain yield improvement ( $+0.45$  % year<sup>-1</sup>) was independent of the N treatment. GPC was stable, thus grain nitrogen yield was improved ( $+0.39$  % year<sup>-1</sup>). Genetic progress on N harvest index ( $+0.12$  % year<sup>-1</sup>) and on N concentration in straw ( $-0.52$  % year<sup>-1</sup>) possibly revealed improvement in N remobilisation. There has been an improvement of NUE additive genetic value ( $+0.33$  % year<sup>-1</sup>) linked to better N utilisation ( $+0.20$  % year<sup>-1</sup>). Improved yield stability was detected as a significant improvement of NUE in low compared to high N conditions. The application of these results to breeding programs is discussed.

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## Abbreviations

ADM_S	Straw dry matter at maturity
BLUE	Best linear unbiased estimator
BLUP	Best linear unbiased predictor
E	Environment
FLO	Flowering date
G	Genotype
GNY	Grain nitrogen yield
GPC	Grain protein content
GPD	Grain protein deviation
GY	Grain dry matter yield
HI	Harvest index
HN	High nitrogen input
KS	Kernel per spike
LN	Low nitrogen input
LRT	Likelihood ratio test

LSD	Fisher's least significant difference test
N	Nitrogen
%N_S	Straw nitrogen content at maturity
NHI	Nitrogen harvest index
NSA	Straw nitrogen per area
NTA	Total nitrogen in plant at maturity
NUE	Nitrogen use efficiency
NUE_Prot	Nitrogen use to protein efficiency
NupE	Nitrogen uptake
NutE	Nitrogen utilisation efficiency
NutE_Prot	Nitrogen utilisation to protein efficiency
P	<i>P</i> value
PH	Plant height
SA	Spike per area
TKW	Thousand kernel weight
YR	Year of release

## Introduction

Nitrogen (N) fertiliser accounted for the majority (77.4 %) of nutrients consumed in Europe on all crops in 2011 (ec.europa.eu/eurostat). Its increasing application has largely contributed to bread wheat (*Triticum aestivum* L.) yield rise during the second half of the twentieth century (Erisman et al. 2008). But the cost of N fertiliser production and application is increasing (Rothstein 2007) and environmental concerns (Goulding 2004) make it necessary to enhance crop nitrogen use efficiency (NUE).

Two strategies may be devised for NUE improvement: maintaining high yield when reducing N supply, and/or increasing yield at a constant N supply. The cost of N production, environmental pollution due to nitrate leaching (Pathak et al. 2011), and volatilisation of greenhouse gases require that wheat NUE should be improved at a lower N supply. But the situation is more complex since increasing world demand for grain (Bruinsma 2009) means that increased production per unit area is the priority. Thus, the minimum N rate to maximise yield should be considered. End-use is also an important factor as breadmaking, feed, or biofuel wheat varieties have different protein content requirements (Bushuk 1998; Shewry and Halford 2002). Moreover, for a given cultivar, the maximal grain protein concentration and the maximal yield are generally not obtained with the same fertilisation strategy, i.e. amount and application dates (Lopez-Bellido et al. 2006). We should also notice that both lodging (Ortiz-Monasterio et al. 1997a) and foliar disease (Olesen et al. 2003) risks increase with N fertilisation.

Moll et al. (1982) defined NUE as grain dry matter (GY) divided by available N from the soil and fertiliser. Improving NUE is a relevant challenge for winter wheat for which N recovery and NUE are estimated to be, respectively,

around 65 % and 25 kg DM kg<sup>-1</sup> N at high N input in Northern Europe (Sylvester-Bradley and Kindred 2009; Gaju et al. 2011). As an integrative trait, NUE is usually decomposed into two components: the uptake and utilisation efficiencies. Uptake efficiency characterises the capacity to capture N from the soil: it is often computed as total nitrogen in the plant at harvest (NTA) divided by available N in the soil. Utilisation efficiency characterises the capacity to convert total plant nitrogen to grain dry matter (GY/NTA).

The identification of traits to improve NUE in wheat and the characterisation of their variability provide useful directions to breeders (e.g. Barraclough et al. 2010; Foulkes et al. 2009; Gaju et al. 2011). The first decision that breeders have to take is to choose the N level for which they want to breed. Indeed, in numerous studies which analysed agronomic traits, significant genotype  $\times$  N ( $G \times N$ ) interactions were detected (e.g. Le Gouis et al. 2000; Laperche et al. 2006a; Barraclough et al. 2010), meaning that variety behaviour differentially depends on N treatment. Quantifying  $G \times N$  interactions is, therefore, crucial for efficient selection. Recent selection in Europe has been conducted mostly at high or optimum N levels so genetic progress achieved at lower N levels results from indirect selection. As  $G \times N$  interactions have been shown to increase with N stress (Bänziger et al. 1997; Laperche et al. 2006a), the efficiency of indirect selection for a low N input (LN) environment resulting from direct selection in a higher N input (HN) environment can be highly variable (Atlin and Frey 1989; Ceccarelli et al. 1992; Sinebo et al. 2002; Brancourt-Hulmel et al. 2005).

Characterizing and quantifying recent genetic progress can also bring meaningful information to breeders. Many studies have been conducted on wheat yield genetic progress (e.g. for recent studies Brisson et al. 2010; Fischer and Edmeades 2010; Oury et al. 2012; Graybosch and Peterson 2012; Lopez et al. 2012; Green et al. 2012). The main conclusion from studies conducted at different N levels is that genetic progress occurred in both HN and LN conditions, but was higher at HN (Ortiz-Monasterio et al. 1997a; Brancourt-Hulmel et al. 2003; Guarda et al. 2004). Fewer studies have been published on the genetic progress for NUE and its components (Ortiz-Monasterio et al. 1997a; Guarda et al. 2004; Muurinen et al. 2006). Moreover, it is well known that a negative correlation between yield and protein content exists in wheat (Kibite and Evans 1984; Simmonds 1995, Oury et al. 2003; Oury and Godin 2007; Bogard et al. 2010). A yield increase may, therefore, lead to a decrease in protein content which could cause lower end-use quality (Ortiz-Monasterio et al. 1997b; Shewry 2004). Thus, the question of the genetic improvement in yield or NUE cannot be assessed independently of quality.

**Table 1** Description of the experimental design where wheat genotypes were evaluated at high N level (HN) and low N level (LN). NTAm<sub>max</sub> corresponds to the 95th percentile of total nitrogen per area

Site × season	Season	Location	Soil type	Genotypes tested	Residual soil N (kg N ha <sup>-1</sup> )	N supply <sup>a</sup> (kg N ha <sup>-1</sup> )		NTAm <sub>max</sub> (kg N ha <sup>-1</sup> )	
						HN	LN	HN	LN
EM08	07/08	Estrées-Mons	Clay loam	206 <sup>b</sup>	67	50 + 70 + 50	0 + 70 + 0	206	144
EM09	08/09	(49.8N, 3.03E)		208 <sup>b</sup>	30	50 + 50 + 50	0 + 50 + 0	241	111
VB08	07/08	Villiers le Bacle (48.7N, 2.1E)	Clay loam	197	106	0 + 66.5 + 60	0 + 44 + 0	242	157
VR09	08/09	Vraux (49.0N, 4.2E)	White Chalk	196	30	60 + 100 + 60	60 + 60 + 0	236	173

<sup>a</sup> Nsupply: fertiliser supply at end of winter + at Z30 + at Z32

<sup>b</sup> Controls: Apache, Orvantis, Caphorn, and Soissons (2007/08) or Premio (2008/09)

Two major approaches are used to assess genetic progress: (1) historical trial analyses and (2) direct comparisons of old and modern varieties in the same environment. But these two approaches suffer from some limitations. (1) When historical trials are analysed, as genotypes are tested in different year × environment combinations, there is a need to take into account agrometeorological variation. This may induce bias as elimination of “year” effects is often based on variation from year-to-year of common controls leading to inadequate consideration of genotype × “year” interactions (e.g. Brisson et al. 2010; Oury et al. 2012; Graybosch and Peterson 2012). (2) Direct comparisons of old and modern varieties are often limited by the experiment size (e.g. Brancourt-Hulmel et al. 2003; Guarda et al. 2004; Muurinen et al. 2006; Green et al. 2012) with few genotypes studied in few environments. This can cause sampling errors. Lopez et al. (2012) proposed to base genetic progress assessment only on the highest yielding variety per date of release but still with a quite low number of cultivars. Moreover, the period under study is usually spread out and includes major changes in plant height due to introduction of dwarfing alleles. Indeed, height decrease is one of the major sign of winter wheat genetic improvement between 1946 and 1992 in France (Brancourt-Hulmel et al. 2003) as well as other countries (e.g. Ortiz-Monasterio et al. 1997a; Austin 1999). It is directly linked to NUE through an increase of lodging resistance and nitrogen partitioning (Hedden 2003). Plant height is now stabilised; therefore, the question of recent genetic gain can be asked independently of this major physiological change using a large panel of recent cultivars grown in the same environments.

Our work aims to assess recent genetic progress in NUE and NUE-related traits in HN and LN environments. For this purpose, (1) we assessed the additive genetic and

at maturity for all the genotypes present in the trial and is an estimate of N available (soil + fertiliser N)

interactive variances for NUE and its components, and (2) we estimated genetic progress made during the last 25 years for both additive genetic effects and for  $G \times N$  interactions. For this, we analysed a multi-environment dataset of eight independent trials (four HN input and four LN input) where 225 registered winter wheat varieties were directly compared.

## Materials and methods

### Plant materials and field experiments

Two hundred and twenty-five European elite varieties released from 1969 to 2010 (suppl. Table 1) were evaluated in four environments (Table 1) as a combination of two sites and two seasons. VB08 and VR09 were conducted by Arvalis experimental units in Villier-le-Bâcle and Vraux. EM08 and EM09 were conducted by the INRA experimental unit in Estrées-Mons. Genotypes were ranked by heading date to limit competition effects and distributed in eight blocks. At EM08 and EM09, an augmented design was used where four controls were repeated in each of the eight blocks. At VB08 and VR09, all varieties were repeated twice in a complete block design. Two nitrogen supply modalities were tested in each environment (Table 1). The high N (HN) treatment corresponds to common agricultural practice in the tested environments. The low N (LN) treatment corresponds to HN reduced by around 100 kg N ha<sup>-1</sup>. Other crop inputs including weed, disease and pest control, and potassium, phosphate and sulphur fertilisers were applied at sufficient levels to prevent them from limiting yield. Plant growth regulator was applied to limit lodging on all trials. A trial is defined as a combination of environment × N treatment (e.g. EM08\_LN).

## Phenotypic data

Plant height (PH) and the number of spikes per unit area (SA) were assessed on each plot except for VB08\_LN where measurements were taken on only one replicate. Flowering date (FLO), thousand kernel weight (TKW), straw dry matter at maturity (ADM\_S), straw nitrogen content at maturity (%N\_S), grain dry matter (GY), and grain protein concentration (GPC) were measured on each plot in all trials. The number of kernel per spike (KS) was calculated as  $GY/(TKW \times SA)$ . Total nitrogen per unit area at maturity (NTA) was calculated as grain nitrogen yield ( $GNY = GPC/5.7 \times GY$ ) added to straw nitrogen per unit area ( $NSA = ADM\_S \times \%N\_S$ ).

NUE was not calculated as proposed by Moll et al. (1982). Rather, considering that mineralisation, leaching and rain all impact on the estimation of available soil N (Hirel et al. 2007; Gaju et al. 2011; Bingham et al. 2012), in each trial total N available to plants was estimated as the 95th percentile of the NTA (NTA<sub>max</sub>) (Table 1). Nitrogen use efficiency (NUE) was then estimated as GY divided by NTA<sub>max</sub>. N uptake efficiency at maturity (NupE) was calculated as NTA divided by NTA<sub>max</sub>. N utilisation efficiency (NutE) was calculated as GY divided by NTA. To illustrate the capacity of varieties to convert N into protein, N use efficiency for protein production ( $NUE\_Prot = GPC/NTA_{max}$ ) and N utilisation efficiency for protein production ( $NutE\_Prot = GPC/NTA$ ) were also computed. Harvest index (HI) was defined as the grain dry matter divided by the total dry matter ( $GY/(GY + ADM\_S)$ ). N harvest index (NHI) at maturity was the amount of N in the grain compared to the total nitrogen in the plant ( $GNY/NTA$ ). Grain protein deviation (GPD) was the deviation from the linear regression of GPC by GY in each trial (Monaghan et al. 2001).

In all trials, adjusted means were calculated using a linear model with varieties and blocks as fixed factors. This resulted in eight different datasets with 182 varieties in common. The other varieties were at least present in four trials. Adjusted means were then used in all the following analyses.

## Mixed model and variance decomposition

To  $P_{ijk}$ , the phenotype of genotype  $i$  ( $i = 1 \dots 225$ ) in environment  $j$  (VB08, VR09, EM08, and EM09) with  $N$  treatment  $k$  (HN and LN), the following mixed model was used:

$$P_{ijk} = \mu + N_k + E_j + E_j \times N_k + G_i + G_i \times E_j + G_i \times N_k + \varepsilon_{ijk} \quad (1)$$

and in the single  $N$  treatment analyses, the following mixed model was used:

$$P_{ij} = \mu + E_j + G_i + \varepsilon_{ij}. \quad (2)$$

In both Eqs. (1) and (2),  $\mu$  is the general mean,  $N_k$  the fixed effect of  $N$ ,  $E_j$  the random effect of the environment,  $E_j \times N_k$  the environment  $\times N$  level interaction,  $G_i$  the random additive effect of the variety.  $G_i \times E_j$  and  $G_i \times N_k$  are, respectively, effects for the variety  $\times$  environment ( $G \times E$ ) interaction, and variety  $\times N$  modality interaction ( $G \times N$ ).  $\varepsilon_{ijk} \sim N(0, \sigma^2)$  and  $\varepsilon_{ij} \sim N(0, \sigma^2)$  are residual error terms.

Fixed effects were tested using Wald tests. Variance components of random factors were tested one by one using the likelihood ratio test (LRT) (Kendall and Stuart 1979), based on log-likelihood ( $L_{max}$ ) differences between the complete (1) and reduced models (1) without the tested factor.

$$LRT = -2 \times [\log(L_{max} \text{ full model}) - \log(L_{max} \text{ reduced model})].$$

LRT is expected to be distributed as a  $\chi^2$  with degrees of freedom ( $df$ ) as:

$$df = n_{PAR} \text{ full model} - n_{PAR} \text{ reduced model},$$

where  $n_{PAR}$  is the number of parameters.

The null hypothesis (no significant effect of the tested component) was rejected when  $LRT > \chi^2(df)$ . In our case,  $df$  was 1 as it was assumed no genetic covariance among varieties nor covariance among the trials.

## Heritability

Generalised heritability ( $h^2g$ ) was calculated using the following formula developed by Cullis et al. (2006),

$$h^2g = 1 - PEV/(2 \times \sigma^2g),$$

where  $\sigma^2g$  is the genetic variance and PEV is the average pairwise prediction error variance of the genetic effects best linear predictions (BLUPs).

## Effect of the year of registration

To test for genetic progress,  $G_i$  and  $G_i \times N_k$  were calculated from Eq. (1) modified with  $G_i$  and  $G_i \times N_k$  as fixed effects to avoid shrinkage issues. Effect of the year of release (YR) was assessed on additive genetic effect ( $G_i$ ) and on the genotype  $\times N$  level interaction term ( $G_i \times N_k$ ) by variance analyses (ANOVA) in a linear model. These tests were also conducted with the quality classes, precocity, and plant height as covariates (suppl. Table 2). A complete model including all covariates was first computed but only significant covariates were kept in the final analyses. Quality and plant phenology (height and

precocity) are correlated to the studied traits so using them as covariates to estimate genetic progress corrects for two potential errors. The first is an artificial evolution of the studied trait due to the non-homogeneous allocation of quality, precocity, or height among years, assuming that they would not have evolved during the period under study. Secondly, it also compensates the possible non-adaptation of varieties to the tested environments as in our panel varieties were selected for different European target environments.

The five quality classes used correspond to those of the National Association of French Millers: very high quality, high quality, good quality, biscuit quality, and other use. YR was found in the French (<http://cat.geves.info/Page/ListeNationale>) and the European catalogue of crop species (<http://ec.europa.eu/food/plant/propagation/catalogues>). Anthesis date and plant height best linear unbiased estimators (BLUEs) from the reviewed Eq. (1) were used as precocity and height covariates.

Only three varieties were released between 1969 and 1985. To avoid sampling bias, these varieties were not included in the genetic progress analyses. In total, 195 European elite varieties for which quality and YR information were available were used to assess the genetic progress.

## Software

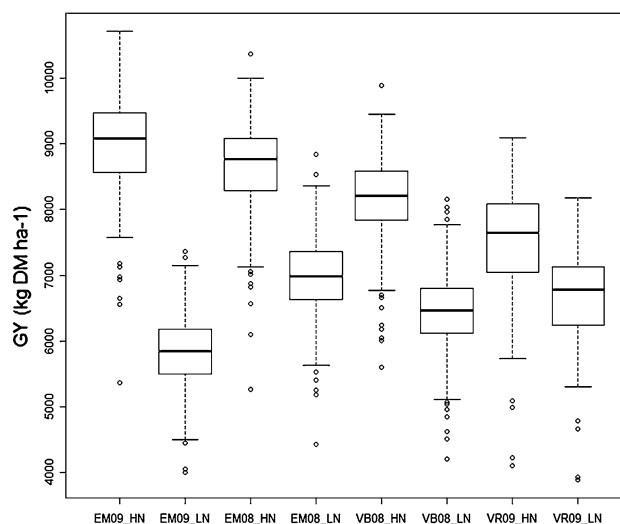
Statistical analyses were performed using R.2.13.2 (The R development core-team 2012) and the ASReml-R package v3.0.1 (Butler et al. 2009; <http://www.vsni.co.uk>).

## Results

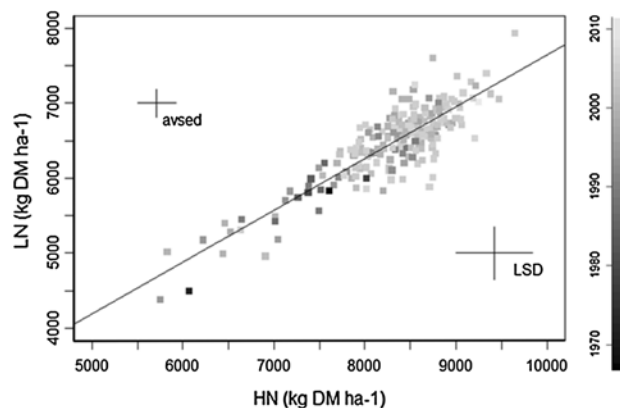
### Grain yield and N efficiencies

Mean grain yield ranged from 5.8 in EM09\_LN to 9.0 t ha<sup>-1</sup> in EM09\_HN (Fig. 1). In all environments, the N effect was always significant with large differences between sites and seasons. Extreme reductions of 11 % in VR09 and 35 % in EM09 were observed on yield when plants were grown under LN compare to HN conditions. A high correlation between GY measured at HN and LN exists ( $r = 0.86$ ,  $P < 0.001$ ). Older varieties yielded less than the most recent (Fig. 2) suggesting genetic improvement at both HN and LN.

NUE was greater at LN (42.7 kg DM kg<sup>-1</sup> N) than at HN (32.9 kg DM kg<sup>-1</sup> N). NutE was higher at LN (55.6 kg DM kg<sup>-1</sup> N) than at HN (41.9 kg DM kg<sup>-1</sup> N), while NupE remained stable (79 % at HN and 78 % at LN). Phenotypic correlations revealed that the contribution to NUE of N utilisation increased with N supply, from  $r = 0.53$  ( $P < 0.001$ )



**Fig. 1** Boxplot of GY for 225 wheat cultivars grown over 2 years (2008 and 2009) at two N levels [Low N (LN) and High N (HN) and in three sites, Estrées-Mons (EM), Villiers-le Bâcle (VB) and Vraux (VR)]. Quartiles and median are used to construct the box. The whiskers extend to 1.5 times the interquartile range from the box



**Fig. 2** Grain yield best linear unbiased estimators (BLUEs) at low N level (LN) as a function of BLUEs at high N level (HN) for 225 wheat cultivars grown in four environments. Dot colours are function of the year of release from the older (black) to the younger (light grey). Average pairwise prediction standard error (avsed) and least significant difference (LSD) at both HN and LN treatments are plotted as the following regression function:  $y = 0.69x + 458.5$  ( $r^2 = 0.74$ ,  $P < 0.001$ )

at LN to  $r = 0.60$  ( $P < 0.001$ ) at HN. The contribution of N uptake to NUE is also significant ( $r = 0.44$ ,  $P < 0.001$ ) but did not vary between LN and HN.

### Variance components and heritability

Significant genotypic effects were observed for all traits except NTA and NupE (Table 2). Trait heritabilities were



**Table 2** Mean, standard deviation (SD), heritability ( $h^2$ ) and genetic variance decomposition for agronomic traits measured on 225 wheat cultivars in eight trials (see text for traits description). Genetic variances are decomposed into three components, G the additive genetic effect, the  $G \times E$  and the  $G \times N$  interactions

Trait	Mean	SD	Units	$h^2$	$G$ (%)	$G \times E$ (%)	$G \times N$ (%)
FLO	149.25	7.12	days	0.97	92***	8***	0 ns.
PH	76.60	8.43	cm	0.89	80***	19***	0 ns.
SA	411.97	78.8	nb spike $m^{-2}$	0.75	69***	23***	8**
TKW	42.45	4.11	g	0.91	83***	16***	1 ns.
KS	42.78	8.88	nb kernel per spike	0.77	68***	30***	2 ns.
GPC	9.93	2.05	% prot	0.85	71***	27***	2*
GY	7,400	1,258	kg DM $ha^{-1}$	0.79	60***	36***	5***
GNY	127.94	35.44	kg N $ha^{-1}$	0.18	18**	74***	8 ns.
GPD	0.00	0.78	% prot	0.71	61***	36***	3 ns.
%N_S	0.42	0.13	% N	0.66	56***	35***	9*
ADM_S	7,288	1,861	kg DM $ha^{-1}$	0.79	81***	18***	1 ns.
HI	50.42	5.67	% DM	0.79	67***	32***	1 ns.
NHI	81.15	5.71	% N	0.45	38***	55***	7 ns.
NTA	158.46	45.03	kg N $ha^{-1}$	0.04	16 ns.	75***	9 ns.
NupE	0.78	0.08	% N	0.00	10 ns.	77***	13 ns.
NutE	48.80	11.19	kg DM $kg^{-1}$ N	0.79	63***	30***	7***
NutE_Prot	0.07	0.01	% prot $kg^{-1}$ N $ha^{-1}$	0.83	74***	23***	3 ns.
NUE_Prot	0.05	0.0083	% prot $kg^{-1}$ N $ha^{-1}$	0.83	69***	27***	4***
NUE	37.8	7.69	kg DM $kg^{-1}$ N	0.80	69***	26***	5*

LTR tests: \*\*\*  $P$  value  $<0.001$ ; \*\*  $P$  value  $<0.01$ ; \*  $P$  value  $<0.05$ ; and ns., non-significant  $P$  value  $>0.05$

highly variable ranging from 0 for NupE to 0.97 for flowering date. The high contribution of the  $G \times E$  interaction to the genetic variance of N uptake (77 % of the total variance) is consistent with a weak genetic additive effect. HI, NutE, GPD, NutE\_Prot, NUE, and NUE\_Prot are all derived traits which nevertheless exhibited high heritabilities.

The variance decomposition revealed significant  $G \times N$  interactions for GY, GPC, NUE, NUE\_Prot, and %N\_S.  $G \times N$  interaction was the most important for NutE representing 7 % of its genetic variance. We should stress that genotype  $\times$  environment  $\times$  N interaction was included in the model residual, resulting in an underestimation of the specific influence of N treatment on genotypes.

Heritabilities at HN and LN were really similar (suppl. Table 4). The highest difference was observed for GNY with heritability 0.31 at HN and 0.19 at LN. Nevertheless, differences in variance components should be noticed. For DMGY, GPC, GPD, SA, TKW, NHI, %N\_S, and ADM\_S genetic and error variances decreased from HN to LN. On the contrary, traits associated with NUE (NutE, NutE\_Prot, NUE, and NUE\_Prot) have genetic and error variances increasing from HN to LN.

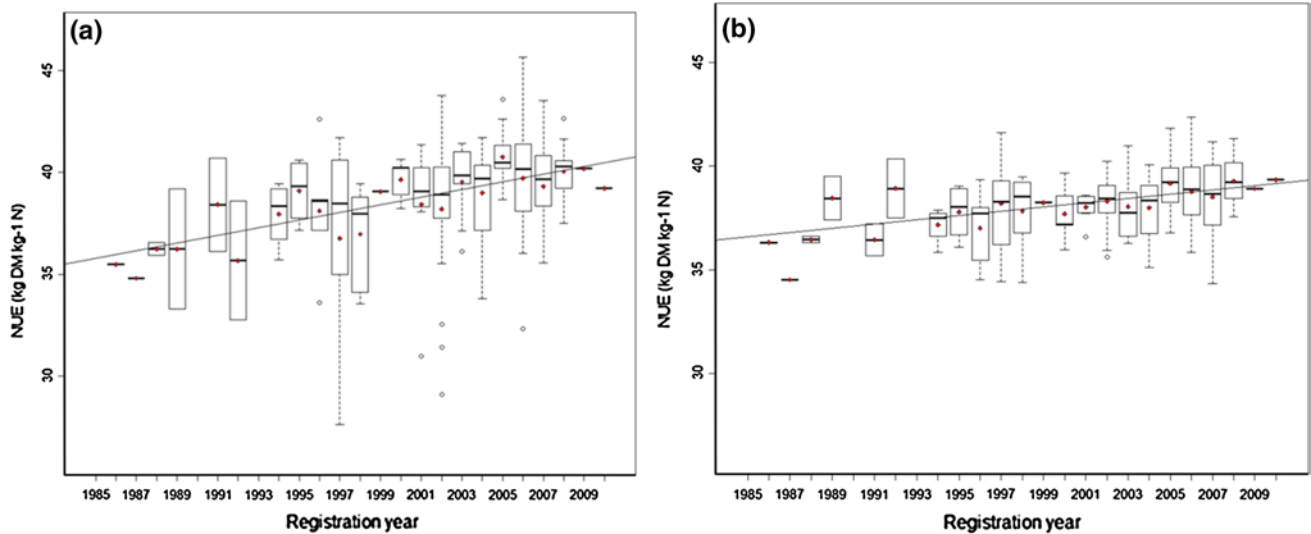
#### Year of registration effect on genetic additive effect

The effect of year of registration (YR) was tested on the different traits. Additive genetic effects were estimated at both HN and LN. YR effect was either tested alone or taking into account precocity and/or plant height and/or quality classes

as covariates. These covariates were themselves first tested for association with YR. Quality classes were not totally homogeneously allocated among years (LSD test  $P = 0.05$ , suppl. Table 5). “Very high quality” varieties which have higher GPC (LSD test  $P = 0.05$ , suppl. Table 5) were on average significantly older (1999) than “high” and “good quality” varieties (2003). Flowering date was correlated to YR with new cultivars later flowering ( $+0.18$  day  $year^{-1}$ ). YR had no significant effect on plant height but variation in plant size exists (coefficient of variation = 11 %). The addition of covariates enhanced the accuracy of the genetic progress estimation (Fig. 3). Indeed, sampling bias and miss-adaptation of phenology to the tested environments were corrected.

The most significant effect of YR was detected on GY ( $+0.45$  %  $year^{-1}$ ) (Table 3). GY can be divided into three components: the weight of grains (TKW), the number of grains per spike (KS), and the number of spike per area (SA). TKW and SA remained stable. KS increase was not significant when quality and precocity were added to the model. We can conclude that there is no clear trend about how GY genetic gain was achieved. Probably different strategies have been used simultaneously.

Apart from the variability of quality classes among years, GPC did not decrease since 1985. This stability, coupled with the GY increase, led to GNY improvement ( $+0.35$  %  $year^{-1}$ ). GNY improvement can be the result of two physiological changes: partitioning and/or uptake. The YR effect on uptake was not tested as no



**Fig. 3** Boxplot of (a) NUE genetic value and (b) NUE genetic values corrected for quality and precocity effects as a function of registration year of 195 wheat cultivars grown in four environments and two N treatments. Medians (dash), means (solid diamond). **a**  $NUE = 37.8 + (YR - 2002) \times 0.198$  ( $r^2 = 12.6\%$ ;  $P < 0.001$ ). NUE additive

genetic values are BLUES from the multi-environment mixed model. **b**  $NUE = 37.8 + (YR - 2002) \times 0.126$ ; NUE additive genetic values are BLUES from multi-environment mixed model which were corrected for quality and precocity effects. The complete model (with quality, precocity and YR) adjusted  $r^2$  is 64.6 %

**Table 3** Year of registration (YR) effects on agronomic traits measured on 195 wheat cultivars grown in eight trials (see text for traits description). YR effect was tested with and without cofactors (qual-

ity class, precocity, and plant height): contribution to the variance ( $R^2$ ), factor effect significance ( $P$ ), and slope of the YR regression (% of the trait mean)

Trait	Only YR			With cofactor and covariates								
				Quality		Precocity		Height		YR		
	$R^2$	$P$	Slope	$R^2$	$P$	$R^2$	$P$	$R^2$	$P$	$R^2$	$P$	Slope
PH	1	ns.		16	***	7	***			0	ns.	
FLO	0	ns.		9	***			7	***	3	**	0.18 day
SA	0	ns.		NT		NT		NT		0	ns.	
TKW	0	ns.		NT		NT		3	**	1	ns.	
KS	2	*	+0.41 %	13	***	5	**	NT		0	ns.	
GPC	5	**	-0.46 %	52	***	16	***	NT		0	ns.	
GY	17	***	+0.70 %	54	***	11	***	NT		6	***	33.2 kg DM ha <sup>-1</sup>
GNV	8	***	+0.38 %	5	*	NT		NT		6	***	0.442 kg N ha <sup>-1</sup>
GPD	0	ns.		29	***	5	***	NT		1	ns.	
%N_S	2	*	-0.41 %	NT		19	***	12	***	3	**	$-2.17 \times 10^{-3} \% N$
ADM_S	0	ns.		6	***	32	***	16	***	1	ns.	
HI	9	***	+0.29 %	41	***	1	*	14	***	2	**	$6.71 \times 10^{-2} \% DM$
NHI	7	***	+0.12 %	NT		NT		NT		7	***	$9.72 \times 10^{-2} \% N$
NutE	8	***	+0.39 %	40	***	16	***	NT		2	**	$9.67 \times 10^{-2} kg DM kg^{-1} N$
NutE_Prot	9	***	-0.49 %	59	***	10	***	NT		2	***	$-1.73 \times 10^{-4} \% prot kg^{-1} N ha^{-1}$
NUE_Prot	6	***	-0.38 %	52	***	12	***	NT		1	ns.	
NUE	13	***	+0.52 %	48	***	13	***	NT		5	***	0.13 kg DM kg <sup>-1</sup> N

Fischer tests: \*\*\*  $P$  value <0.001; \*\*  $P$  value <0.01; \*  $P$  value <0.05; and ns., non-significant  $P$  value >0.05

NT not tested because not significant

**Table 4** Decomposition of  $G \times N$  interaction variance (%) for NUE and GY of 195 wheat cultivars grown in four environments. The registration year (YR) effect was tested with and without covariates (quality class, precocity, and plant height)

	Only YR	With cofactor and covariates			
		Quality	Precocity	Height	YR
GY	4.6**	13.09***	9.84***	NT	1.09 ns.
NUE	3.25*	5.27*	NT	NT	1.97*

Fischer tests: \*\*\*  $P$  value <0.001; \*\*  $P$  value <0.01; \*  $P$  value <0.05 and ns., non-significant  $P$  value >0.05

NT not tested because not significant

additive genetic effect was detected for NTA (Table 2). Regarding dry matter partitioning, HI increased (+0.13 % year<sup>-1</sup>) as ADM<sub>S</sub> remained the same and GY increased. Regarding N partitioning, NHI (+0.12 % year<sup>-1</sup>) increased, ADM<sub>S</sub> remained the same and %N<sub>S</sub> decreased (Table 3).

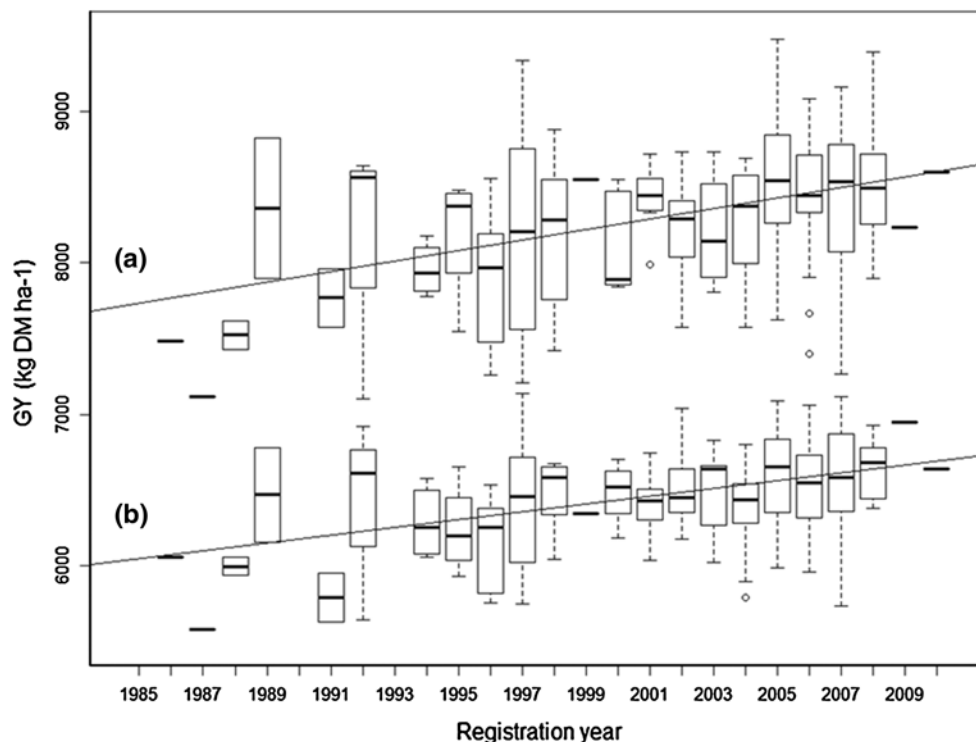
The additive genetic effect of NUE increased (+0.33 % year<sup>-1</sup>) (Table 3) thanks to an improvement of NutE (+0.20 % year<sup>-1</sup>). NutE improvement and NutE<sub>Prot</sub> decrease (-0.27 % year<sup>-1</sup>) revealed that selection has favoured varieties which preferentially convert remobilised nitrogen into grain dry matter rather than into protein. As GPC was stable, the decrease in NutE<sub>Prot</sub> (GPC/NTA) could be the result of either NTA improvement or/and an uptake increase. These hypotheses could not be

distinguished as no significant additive genetic effect was detected for NupE (Table 2).

#### YR effect on $G \times N$ interaction

After being tested on additive genetic effect, YR effect was tested on significant  $G \times N$  interactions. A change in  $G \times N$  interactions was significant only for GY and NUE (Table 4). For GY, the YR effect was significant when no covariates were used. Modern varieties had  $G \times N$  interaction which increased yield (+0.12 % year<sup>-1</sup>) in HN environments, with a corresponding decrease in LN environments. However,  $G \times N$  interactions for GY were explained by variation in quality classes ( $r^2 = 13.1$  %,  $P < 0.001$ ) and precocity ( $r^2 = 9.8$  %,  $P < 0.001$ ). The most important effect was due to the highly negative interactions of “very high quality” varieties at HN (-188 kg ha<sup>-1</sup>). The effect of precocity was the result of the positive correlation between date of flowering and  $G \times N$  interactions at HN (+10 kg ha<sup>-1</sup> per day of delay). So, once quality and precocity effects were removed, there was no significant difference in GY genetic progress between HN and LN environments (Table 4). The slopes of regression are different but confidence intervals overlap (Fig. 4). This also means that recent and old varieties have the same yield loss between HN and LN. However, as recent varieties have a higher GY (+0.35 % year<sup>-1</sup>, Table 3), their relative GY losses are lower than for older varieties and, therefore, recent varieties are more stable.

**Fig. 4** Boxplot of GY genetic values by year of release and by N treatment (LN low N level, HN high N level) for 195 wheat cultivars grown in four environments. Values are the best linear unbiased estimators of NUE corrected of quality and precocity effects. **a** at HN treatment, and **b** at LN treatment. **a** At HN, regression function is  $GY = -69690 + YR \times (34.8 \pm 4.42)$ , the complete model (with quality and precocity) adjusted  $r^2$  is 66 % and YR effect  $P < 0.001$ . **b** At LN, regression function is  $GY = -51302 + YR \times (25.64 \pm 6.22)$ , the complete model (with quality and precocity) adjusted  $r^2$  is 70 % and YR effect  $P < 0.001$ .  $G \times N$  on GY is significant but YR effect on this interaction is not significant ( $P > 0.05$ )





Concerning NUE, the YR effect on  $G \times N$  interaction stayed significant when quality was introduced into the model (Table 4). Recent varieties had higher  $G \times N$  interactions on NUE than older varieties at LN ( $+2.98 \times 10^{-2}$  kg DM kg<sup>-1</sup> N year<sup>-1</sup>;  $+0.08$  % year<sup>-1</sup>), and so lower at HN ( $-2.98 \times 10^{-2}$  kg DM kg<sup>-1</sup> N year<sup>-1</sup>;  $-0.08$  % year<sup>-1</sup>). The complete genetic progress at LN is calculated as the genetic progress on additive values added to the ones on the  $G \times N$  interactions. Then, the global genetic progress on NUE was  $+0.155$  kg DM kg<sup>-1</sup> N year<sup>-1</sup> at LN and  $+0.096$  kg DM kg<sup>-1</sup> N year<sup>-1</sup> at HN (respectively,  $+0.37$  % year<sup>-1</sup> and  $+0.30$  % year<sup>-1</sup> referring to the mean NUE at LN and at HN) (Fig. 5). This conclusion is consistent with the previous one on GY. Indeed, GY progress was the same at LN and HN; however, N available at LN (mean NT<sub>max</sub> = 146.25 kg N ha<sup>-1</sup>) was lower than at HN (mean NT<sub>max</sub> = 231.25 kg N ha<sup>-1</sup>). So, the way in which NUE is calculated (GY/NT<sub>max</sub>) leads to a higher estimate of genetic progress at low N than at high N.

## Discussion

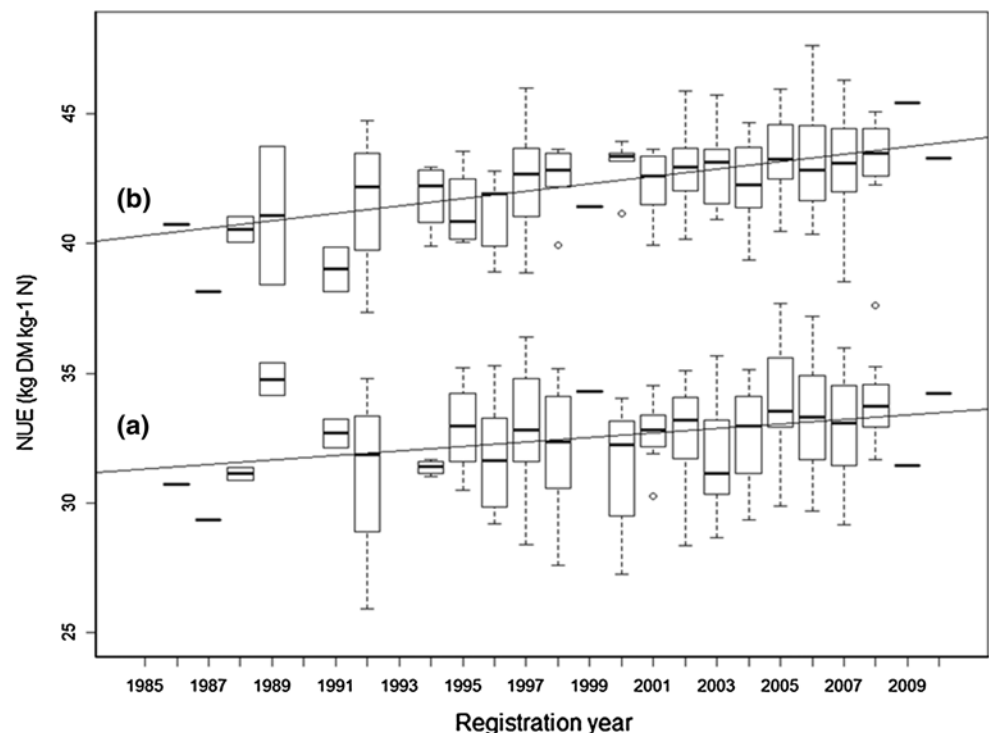
We studied the variance components of NUE among 225 European winter wheat varieties evaluated in 8 independent trials containing two N treatments. These varieties were mostly released between 1985 and 2010. Thus, a study of the genetic improvement of NUE over the past 25 years was possible. We found that using quality, precocity, and

plant height, more accurate estimations of genetic gains were possible. The effect of selection was assessed on the additive genetic value and on the  $G \times N$  interaction term. No additive genetic effect was found on NupE. But the high heritability of complex traits such as NutE, NHI, NUE, NutE\_Prot, and NUE\_Prot revealed their potential for breeding. Regarding additive genetic value, NUE has increased thanks to a rise in NutE. Protein concentration did not decrease since 1985. The main factor in this progress was better partitioning as revealed by an increase in NHI linked to a decrease in straw N concentration at maturity.  $G \times N$  interactions were significant on GY, NUE, NutE, GPC, and NUE\_Prot. Significant changes for  $G \times N$  interactions were only detected for NUE, attesting to the higher yield stability of recently released compared to older varieties.

## Genetic progress assessment method

This work has been carried out with a large collection of European elite winter varieties, which have been bred for different target environments. They were mainly varieties designed for the French market and also for neighbouring countries (e.g. Germany, Great Britain, and Italy). In contrast to previous studies on NUE the period under study was smaller and encompasses the last 25 years of breeding, compared to 82 years (Uzik and Zofajova 2012) and 94 years (Guarda et al. 2004) for winter wheat, 35 years (Ortiz-Monasterio et al. 1997a) and 99 years (Muurinen

**Fig. 5** Boxplot of NUE genetic values by year of release and by N treatment (LN low N level, HN high N level) for 195 wheat cultivars grown in four environments. Values are the best linear unbiased estimators of NUE corrected of quality and precocity effects. **a** at HN treatment, and **b** at LN treatment. **a** At HN, regression function is  $\text{NUE} = -141.80 + \text{YR} \times (0.09 \pm 0.03)$ , the complete model (with quality and precocity) adjusted  $r^2$  is 48.8 % and YR effect  $P < 0.001$ . **b** At LN, regression function is  $\text{NUE} = -240.84 + \text{YR} \times (0.14 \pm 0.02)$ , the complete model (with quality and precocity) adjusted  $r^2$  is 66.2 % and YR effect  $P < 0.001$ .  $G \times N$  on NUE is significant and YR effect on this interaction is significant ( $P < 0.05$ )



et al. 2006) for spring wheat, and 75 years for barley (Bingham et al. 2012). Therefore, the period under study did not include major selection events that took place for plant height and precocity in the previous periods. It turns out that, in our panel, mean height was  $78.9 \pm 8$  cm at HN and was stable over years (Table 3). This value is very similar to the 80 cm reported by Gooding et al. (2012) as being optimum for NUE using near isogenic lines for different *Rht-1* alleles. Nevertheless, variability existed in our panel (Table 2), and had to be controlled to avoid interference in breeding effect estimation. Precocity was also controlled by flowering date assessment. In our panel, the delay in flowering date is explained by the non-homogenous distribution of the varieties' origins (Suppl. Table 6). Varieties bred to European northern countries are generally late (Worland 1996) and are more frequent among the recent varieties of our panel. After 2005, four varieties came from the south of Europe (Italy, Spain) and 10 from the north (e.g. Germany, Great Britain, Denmark). In the same way, we chose to control for quality class. Two points have to be addressed. First, "very high quality" varieties are often high GPC varieties. A negative correlation between GY and GPC exists (e.g. Simmonds 1995; Oury and Godin 2007; Bogard et al. 2010), and so NUE and GPC are negatively correlated (Barraclough et al. 2010; Gaju et al. 2011). These low-yielding genotypes can bias the analyses if they are not evenly distributed over time. Secondly, Ortiz-Monasterio et al. (1997b) studied genetic progress for grain quality from 1950 to 1985, and found no link between quality (alveograph's parameters) and YR. Guarda et al. (2004) also studied wheat quality evolution between 1900 and 1994. They concluded that lower protein concentration was associated with an improvement in protein composition, resulting in an increase of bread-making quality. Moreover, "very high quality" varieties frequency does not drastically vary among years according to the French official catalogue of registered bread wheat varieties. So, in our case having older "very high quality" varieties was a sampling bias (Suppl. Table 5) that had to be controlled.

As with other field studies on NUE genetic progress, we did not take into account below-ground dry matter. However, not taking into account roots in the determination of N-related traits such as NupE appears of little influence (Allard et al. 2013). Significant genotypic differences for root N exist but the amount of N present is low compared to total plant N and so genotype ranking is not affected.

#### Genetic progress between 1985 and 2010

This study concludes that significant grain yield (GY) improvement is observed at both HN and LN. The genetic gain on GY is estimated to be  $+0.45 \text{ \% year}^{-1}$  ( $+33.2 \text{ kg DM ha}^{-1} \text{ year}^{-1}$ ) with no significant difference between HN

and LN. This linear trend is in agreement with the requirement that a variety has to yield in excess of control varieties in official trials to be registered in France. The control variety list evolves to be representative of their market shares and agricultural practices. Progress on GY was not related to progress on TKW, SA, or KS. This is in contrast with Brancourt-Hulmel et al. (2003) who studied GY evolution by comparing 14 winter wheat cultivars registered between 1946 and 1992 in France at two levels of fungicide and N treatments and concluded that GY improvement was made by an increase in kernel number. Our study suggests a diversification of strategies in a more recent period. Concerning differences between HN and LN treatment, Ortiz-Monasterio et al. (1997a), Brancourt-Hulmel et al. (2003), Guarda et al. (2004) concluded that GY progress was higher at HN than at LN. But these studies were based on mean differences in N treatment and not on  $G \times N$  coefficients. Also, according to Ortiz-Monasterio et al. (1997a), this difference was not significant for the period 1962–1985. Moreover, in Brancourt-Hulmel et al. (2003) and Guarda et al. (2004), no fertilisers were added in the very low N treatment. In contrast, in this study, varieties' behaviours were assessed in a context of fertiliser reduction.

We also showed that grain protein concentration (GPC) did not significantly change in the last 25 years. At HN, the mean GPC of bread-making wheat ("very high quality", "high quality", and "good quality") was  $11.4 \pm 1.6 \text{ \%}$ . This content is sufficient to fulfil French milling demands and exportation requirements to North Africa, the main exportation area for French production. Selection on GPC may only result in the elimination of low GPC lines and not in increasing GPC. Breeding program objectives were clearly to increase GY and maintain quality. But, in this study, mean GPC at LN is  $8.66 \pm 1.62 \text{ \%}$  which is largely below bread-making and exportation requirements. If suboptimal conditions are targeted, one of the main challenges for breeders will be to considerably increase GPC. An alternative would be to modify protein composition to increase dough strength and viscoelasticity, allowing for lower protein grain to be suitable for bread-making.

Brancourt-Hulmel et al. (2003) assessed a genetic gain of  $+0.15 \text{ \% year}^{-1}$  for NHI between 1946 and 1992, which includes semi-dwarf allele integration in breeding programs, compared to  $+0.12 \text{ \% year}^{-1}$  in our study. These two estimates are very similar. An explanation is that there is no statistically significant increase in NHI from adding single semi-dwarf alleles to a tall background (Gooding et al. 2012). Besides, the absence of a link between quality and NHI is confirmed by Barraclough et al. (2010) who compared 39 elite commercial cultivars during four years at five N rates. This suggests an equivalent N partitioning between varieties from different quality classes. N absorbed before flowering, stored in vegetative parts and

then remobilised to the grain accounts for around 70 % of total grain N (Van Sanford and MacKown 1987; Kichey et al. 2007). We found that the NHI increase was associated with a %N\_S decrease (and ADM\_S stability). This better N partitioning may either come from a more efficient N remobilisation and/or a more efficient translocation efficiency (N absorbed after anthesis and translocated to the grain, Kichey et al. 2007).

Nitrogen use efficiency improvement was mainly due to better N utilisation efficiency. Our estimations of genetic progress were in the range of previously published results, even if the N available was estimated differently. This study assessed NUE genetic progress of +0.37 % year<sup>-1</sup> at LN and +0.30 % year<sup>-1</sup> at HN. Ortiz-Monasterio et al. (1997a) reported that NUE genetic progress was 0.4–1.1 % year<sup>-1</sup> depending of N applied for spring CIMMYT cultivars released between 1950 and 1985. Sylvester-Bradley and Kindred (2009) also reported a significant trend between old and new cultivars grown at 0 and 200 kg N ha<sup>-1</sup>. In contrast, Muurinen et al. (2006) concluded a lack of genetic gain on NUE for 18 spring wheat varieties bred between 1901 and 2000.

As in our study, various reports have shown a major effect of N utilisation compared to N uptake on NUE at high N input (Ortiz-Monasterio et al. 1997a; Brancourt-Hulmel et al. 2003; Uzik and Zofajova 2012). In contrast, at low N input, N uptake seems to be the component which has more effect on NUE (Ortiz-Monasterio et al. 1997a; Le Gouis et al. 2000; Muurinen et al. 2006). In our study, NupE contribution to NUE was the same at LN and HN treatments, and the additive genetic effect on NupE was not significant. So, detection of change on NupE was impossible.

To better compare the different studies, a finer characterisation of the N status at different N levels is probably necessary. In their low N input level, Ortiz-Monasterio et al. (1997a), Le Gouis et al. (2000), and Muurinen et al. (2006) added no N fertiliser. Only mineral N already present in the soil and N coming from the mineralisation of organic matter were available to the plants. Our LN input modality was less stressful with a mean of 130 kg ha<sup>-1</sup> (fertiliser + soil N) available to crop.

Three hypotheses can account for the absence of an additive genetic effect of NupE in this study. (1) Genetic variation on uptake may only appear in highly N deficient environments. Indeed, NupE genetic variances are very similar between HN and LN (Suppl. Table 4). But this hypothesis contradicts the single trial analysis (data not shown) where NTA genetic additive effect was significant only in two HN trials (VR09\_HN and EM08\_HN). (2) The common method of using pre-sowing or post-winter early measurements of soil mineral N clearly underestimates NupE at HN, as N losses (e.g. leaching, volatilisation) are not taken

into account and so available N is overestimated. At the opposite extreme, the risk of overestimating NupE is real at LN as mineralisation can provide N in large quantities and leaching is limited so that available N is underestimated. For example, Ortiz-Monasterio et al. (1997a), Le Gouis et al. (2000), and Muurinen et al. (2006) used this method and reported NupE superior to 1 in their low N input trials. Bingham et al. (2012) showed that the method of calculation had little effect on relative differences between varieties in single N treatment analysis as NTA between methods are only divided by different coefficients to obtain NupE. But when different N levels are used in common analysis, if overestimation bias at LN is not compensated by the underestimation bias at HN, this can lead to misinterpretation. To avoid this, we chose here (and advocate) to use the maximal uptake measured at each N level. To take into account possible measurements errors, we used the 95th percentile. (3) The genetic variation of uptake is not sufficient in our panel in comparison to the precision of measurements included in the computation of NTA/NupE. Measurement errors could be controlled using more replicates or larger sampling size but with an additional cost. In addition, variability may have to be researched in a more diverse panel using for example genetic resources or breeding materials.

#### Breeding efficiencies for different N levels

Falconer and Mackay (1996) formulated that the relative efficiency under direct selection in condition 1 versus indirect selection in condition 2 is  $r_{G12} \times h_2/h_1$ , where  $h_1$  and  $h_2$  are heritabilities in the two conditions, respectively, and  $r_{G12}$  the genetic correlation between conditions. Heritability is usually lower under LN conditions (Brancourt-Hulmel et al. 2005; Laperche et al. 2006a), suggesting that indirect selection at high N can be an effective strategy to breed for low N conditions. In maize, Presterl et al. (2003) advocated direct selection at LN when yield reduction is >21 % based on the evolution of the genetic correlation as a function of yield reduction. For Anbessa et al. (2010), indirect selection was efficient in barley, but the estimation was made on data where yield reduction was only 7 %. In a study where yield was reduced on average by 35 %, Brancourt-Hulmel et al. (2005) advised to directly select wheat in LN environments to maximise gains. In this study, the mean yield in LN trials was reduced by around 20 % compared to the mean yield in HN trials. Genetic progress on NUE and NUE-related traits was assessed from the additive genetic effect estimated using both HN and LN levels together with the  $G \times N$  interaction. Our work shows that recent varieties have enhanced NUE-associated traits at both LN and HN treatments (except in N utilisation for protein, NutE\_Prot). The only significant genetic progress difference occurred

for NUE;  $+0.37 \text{ \% year}^{-1}$  and  $+0.30 \text{ \% year}^{-1}$ , respectively, at LN and HN. The varieties we used were probably mostly selected in HN environments as usually done in private breeding programs. Using the formula cited above, we calculated that the relative efficiency for indirect selection at HN for LN conditions was 78.1 % for NUE. This was mostly due to the fact that heritabilities were similar in our conditions at LN and HN. We advise to directly select in N suboptimal conditions when moderate N stressful environments are targeted.

Around 10 years are needed for making crosses giving thousands of progenies to register a new variety. As the number of selected lines is reduced, the range of environments in which they are tested is wider. Among all these trials, moderate N stresses surely occur. So the selection process may already mixes HN and moderate LN environments explaining in part the similar genetic progress at HN and at LN. Nevertheless, this selection regime has to be consciously designed to make it more efficient. We can imagine characterizing the N constraint using control varieties repeated in each trial for which NTA will be calculated, measuring %N\_S and ADM\_S. Selection will then be made only using trials where the chosen stress effectively occurred.

NUE enhancement actually arises from selection on yield. Indeed, screening for NUE components is time consuming and may not be implemented in breeding programs soon. High throughput methods are currently being developed (Tester and Langridge 2010) but are not yet adapted to the thousands of lines that are tested in a breeding program. Therefore, improvement focused on NutE or NupE will be conditioned by the possibility to perform molecular selection on QTLs or genes. A few studies have already identified chromosomal regions associated with these traits using wheat plants grown in the field or in controlled conditions (e.g. Laperche et al. 2006b; Bordes et al. 2012; Guo et al. 2012; Liu et al. 2013). Understanding root architecture and its interaction with N supply is also one promising way to improve NUE in plants (Hirel et al. 2007; Foulkes et al. 2009; Kant et al. 2011). But phenotyping of wheat roots in the field is complex (for a review see Manske et al. 2001). As high throughput screens in the field are not available yet, genetic progress will also depend on the development and the use of molecular markers for enhanced root systems.

Root architecture is also affected by the *Rht* dwarfing genes (Laperche et al. 2006b; Wojciechowski et al. 2009) which were the main factors of wheat improvement in the world. Dwarfing alleles are widely spread and used to control response to high N supply by reducing response to gibberellin acid (GA) and thus plant height (Peng et al. 1999) and lodging (Ortiz-Monasterio et al. 1997a). Laperche et al. (2006b) reported a negative effect of dwarfing alleles on both root and aerial biomass of young plants grown at low N in controlled conditions. In this study, varieties have

different dwarfing genes to achieve short height. Moreover, frequencies of the combination of the GA-insensitive dwarfing alleles (*Rht-B1* and *Rht-D1*) changed as a function of the year of registration (HSD test  $P = 0.05$ ; Suppl. Table 5A). When dwarfing allele combinations were used in the model of genetic progress assessment, it appeared that they explained more of the  $G \times N$  variance to NUE than YR. But they had no effect on NUE additive genetic values (Suppl. Table 5B). Recent varieties have  $G \times N$  interactions which enhanced their NUE at LN, and so may have a more stable yield also because of the introduction of *Rht-D1b*. In contrast, this stability in yield also means that recent varieties are capitalised less on N input increase than older ones. This may be a consequence of GA-insensitivity as GA has a major role in regulating developmental processes (Hedden 2003). So, the use of alternate GA-sensitive dwarfing alleles such as *Rht8c* needs to be tested. Indeed Gooding et al. (2012) studied near isogenic lines and concluded that at anthesis the *Rht8c + Ppd-D1a* (dwarf and photo-insensitive) line accumulated similar quantities of nitrogen to *Rht-D1b* despite its earliness (due to its photoperiod-insensitivity).

## Conclusions

In a global context of fertiliser reduction, we investigated nitrogen use efficiency improvement using a European panel of elite winter wheat cultivars. This study is one of the first to use so many varieties in a multi-environment direct comparison between old and recent varieties. Quality, precocity, and height were used to control panel heterogeneity. Variance decompositions were used to describe the genetic determinism of NUE-related traits and to identify significant  $G \times N$  interactions. We report equal genetic progress at both HN and LN treatments for all traits except for NUE, which were significantly enhanced at both N levels but more efficiently at LN. This demonstrates the higher yield stability of recent varieties. We conclude that direct selection in HN conditions for LN conditions is efficient, but advise to directly select at LN if this is the targeted treatment. Two major challenges now appear. The first challenge will be to increase GPC at LN; and the second will be to increase uptake efficiency while maintaining utilisation efficiency improvement.

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical standards** The authors declare that the experiments comply with the current laws of the country in which they were performed.

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