Genetic analysis of grain protein deviation in wheat

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ABSTRACT

Both grain yield (GY) and grain protein content (GPC) are important wheat selection criteria. Simultaneous improvement of these two traits is difficult because of their negative correlation. For this purpose, it has been recently proposed to use the deviation from the regression line between GY and GPC as a selection criterion, called GPD (Grain Protein Deviation). The aim of this preliminary study is to discover, at least in part, the biological basis of GPD in winter wheat. With this end in view, extreme GPD lines have been selected in one mapping population grown in multi-environment trial. Then, these lines have been compared for different agronomical traits. Results show that these extreme GPD lines mainly differed by nitrogen uptake after flowering and nitrogen remobilization. Finally, the impact of these two processes on GPD is discussed.

INTRODUCTION

The negative correlation between grain yield (GY) and grain protein content (GPC) is a very well known phenomenon in crop science. It has been reported in many crops and different hypotheses dealing with the causes of this negative correlation have been proposed. Mainly they are related to genetic incompatibility (linkage, pleiotropy), partitioning efficiency, and competition for photosynthetic energy between nitrogen and Carbon [1, 2, 3]. Shifting this negative correlation is obviously a challenge for wheat breeders who want to improve these two traits simultaneously. For this purpose, different strategies have been suggested such as the use of selection index [4], the targeting of GY and the use of agricultural practices that compensate the loss of GPC [5] and the selection of lines that significantly deviates from the relation [6, 7]. These lines are characterized by their deviation from the regression line between GY and GPC having a higher or a lower GPC than expected from their GY. This departure from the regression line has been called Grain Protein Deviation (GPD) by Monaghan et al. [6].

In wheat, many authors reported the large variability of the relation between GY and GPC with correlation coefficients ranging from -0.2 to -0.8 [8]. This has been shown to be due to Genotype by Environment interactions that affect these two traits and make difficult to asses GPD in a given environment. To obtain reliable assessment of GPC and GY, Oury et al. [5] proposed to study groups of genotypes exhibiting high genetic variability for the two traits or to use the mean values obtained from a network corresponding to a wide range of environments.

Therefore, we propose in this study to select extreme lines for GPD based on multi-environment trials (MET) carried out on one mapping population. Despite the high variability of the GY/GPC relation that we observed, it was possible to identify lines that deviated positively or negatively from the relation regardless of the growing environment, showing that GPD has a partly genetic basis. Finally, a first approach to get insights into the biological basis of GPD was to compare positive versus negative GPD lines for various agronomical traits.

MATERIALS & METHODS

Plant material

This study was based on the analysis of data obtained with the mapping population 'Arche x Récital' described in Laperche et al. [9]. This population of 220 doubled haploid lines was grown at Nickerson Chartainvilliers (48°35'N, 1°35'E) in 2000, INRA Clermont-Ferrand (45°47'N, 3°05'E), INRA Le Moulon (48°42'N, 2.08'E) in 2000 and 2001 and INRA Estrées-Mons (49°53'N,3°00'E) in 2000, 2001, 2002, 2004 and 2006. Two levels of nitrogen supply were tested in all the trials except at Estrées-Mons in 2004: a high nitrogen supply (N+) ranging from 116 to 215 kg $N.ha^{-1}$ and a low nitrogen supply (N-) where the level of nitrogen applied was between 60 and 144 kg $N.ha^{-1}$ less than the N+ treatment, depending on the site. At Mons in 2004, the population was grown under N+ with two different sowing densities. Every trial was grown under full pesticide control. Two replications were grown at each site. This dataset comprised 23 different environments corresponding to the combination of location, year and nitrogen treatment.

Phenotypic measurements

The date of heading, GY and GPC were assessed on each plot. Total biomass and nitrogen content was assessed at flowering in Estrées-Mons. This allowed the calculation of post-flowering nitrogen absorption and remobilization. Nitrogen concentrations were determined by near infrared reflectance analysers calibrated with samples measured using the Dumas procedure. Grain protein content was calculated from the percentage of total nitrogen multiplied by a conversion factor of 5.7.

Selection of extreme GPD lines

Extreme lines were selected on the regression between mean GY and mean GPC calculated on the 23 environments studied. Lines with standardized residual superior to 1.96 in absolute value were considered as extreme GPD lines. For the calculation of the standardized residual see Oury and Godin [7].

Statistical analyses

Statistical analyses were performed using SAS v8.0 [10] and R v2.6 [11]. Regression of GPC on GY was performed using the SAS procedure proc REG with a refit/reweight

statement that discarded iteratively lines with standardized residual superior to 1.96 in absolute value to minimize the effect of outliers on regression equation calculation. This method is similar to those described by Oury and Godin [7]. Student's tests have been performed to assess the statistical significance of mean differences for agronomic traits between positive and negative GPD lines.

RESULTS

To select extreme GPD lines, we used the mean value of GY and GPC calculated with the 23 available environments. GPD of these lines ranged from -1.05 to 1.2% with a mean value of 0.81% for positive GPD lines and -0.79% for negative ones (Table 1). Figure 1 shows that these lines have a positive or negative GPD in the majority of the environments considered despite important variations.

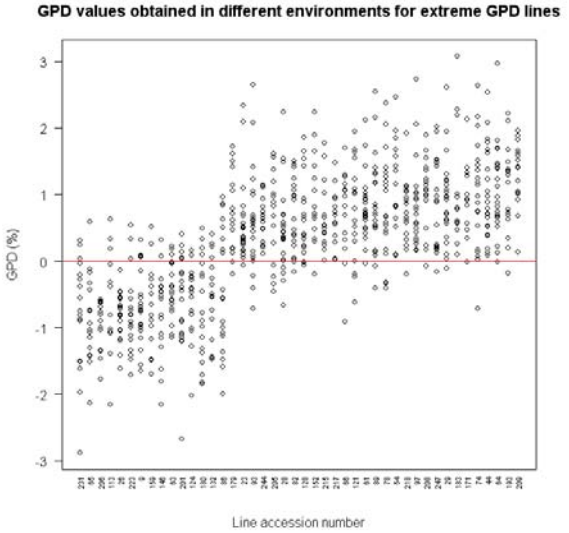

Figure 1: Each point represents a GPD value obtained in a given environment.

A preliminary comparison of these extreme lines allowed us to identify which of various agronomical traits related to flowering date and nitrogen nutrition could explain GPD (Table 1). First, one striking feature is that these lines have a similar grain yield but a difference in GPC of about 1.6%. Positive GPD lines flower four days earlier in average than negative ones. Comparison between positive and negative extreme GPD lines shows a tendency of higher nitrogen uptake before flowering for positive GPD lines with a difference of 0.44 $gN.m^{-2}$. Significant differences in post-flowering nitrogen uptake was identified between the two groups, positive GPD lines taking up four times more nitrogen after flowering than negative ones. This resulted in about 0.9 $gN.m^{-2}$ more post-flowering nitrogen absorption in positive GPD lines compared to negative ones. Considering nitrogen remobilization efficiency that could be also a major trait related to GPD, our data showed that positive GPD lines had an increased nitrogen remobilization efficiency of one point.

Table 1: Comparison of positive and negative extreme GPD lines for various traits

Trait	GPD+	GPD-	Δ	p-value
GPD (%)	0.81	-0.79	1.6	<0.001
GPC (%)	10.91	9.32	1.59	<0.001
GY ($g.m^{-2}$)	612	616	4	0.84
Date of heading (calendar day)	139	143	4	<0.01
N uptake before flowering ($gN.m^{-2}$)	12.05	11.61	0.44	0.054
N uptake after flowering ($gN.m^{-2}$)	1.18	0.30	0.88	<0.001
N remobilization efficiency (%)	0.77	0.76	0.01	<0.05

Δ : difference between positive and negative GPD lines, p-value: result of student's test comparing traits mean of positive and negative GPD lines (n=44)

DISCUSSION

The use of GPD has been proposed to improve simultaneously GPC and GY [6, 7]. Our results confirmed that GPD has a partly genetic basis and consequently lines with stable positive or negative GPD can be selected. Nevertheless, if its use for genetic improvement can be envisaged, the biological basis of GPD is at this moment unknown. Monaghan et al. [6] used a step-wise multiple regression to identify variables related to GPD and found that the most relevant ones were nitrogen accumulated post-anthesis and nitrogen remobilization. Our results obtained from comparison between positive and negative extreme GPD lines confirmed this result showing the principal components of GPD being nitrogen uptake after flowering and to a lower extent to nitrogen remobilization efficiency. This can be linked to the earlier flowering habit of positive GPD lines. Indeed, under climatic conditions of North West Europe, earlier flowering might allow an increase of the favourable period for post-flowering nitrogen uptake by a drought-avoidance strategy.

Nevertheless, post flowering nitrogen uptake might not be the main component of GPD in every environment and might be achieved through different strategies depending on the considered climatic conditions. In particular, Slafer (1990) cited by Monaghan et al. [6] showed that in Argentina, a country with much warmer and drier summer season, a high nitrogen accumulation before anthesis coupled with increased ability in nitrogen remobilization could allow an improvement of GPC without reducing GY. In 2008, Jukanti and Fisher [12], in a study on barley (*Hordeum vulgare*) showed that a major GPC QTL mapped on chromosome 6 was associated with increased flag leaf proteolysis and nitrogen remobilization. Interestingly, the authors emphasized that an earlier dismantling of photosynthetic

apparatus can lead to a loss of net carbon assimilation, at least as long as photosynthesis is not limited by an unfavourable environment such as drought. Recently, a NAC gene accelerating senescence and improving GPC has been cloned in a mapping population derived from the cross between *Triticum turgidum* ssp. *durum* cultivar Langdon (LDN) and the chromosome substitution line LDN (DIC6B) obtained from *Triticum turgidum* ssp. *Dicoccoides* [13]. In a study assessing GPC and thousand kernel weight (TKW) of different lines carrying the DIC high GPC allele grown in 13 genotype-environment combinations, the authors noticed that this NAC gene might confer an increase in TKW and GPC by shortening the maturity period in environments where the grain is affected by severe stresses during the grain-filling period [14]. Thus, in environments characterized by warm and dry season during grain filling, the contribution of post flowering nitrogen uptake to GPC can be greatly reduced and remobilization efficiency might be the most important feature to achieve a high GPC without reducing yield.

One major difficulty preventing the use of GPD as a selection criterion is its difficulty to be assessed. Indeed, the need of mean values obtained from MET prevents to take it into account in the early generations where the genotypes are evaluated in only one site [5]. To counteract this, it could be interesting to identify traits related to GPD less 'environment-dependent' and easier to measure. Moreover, the use of GPD as a trait for QTL analysis will lead to the identification of loci associated with variation of GPC without effect on yield. The combination of QTL analysis on GPD and other traits should allow new insights into the physiological basis of this trait.

CONCLUSION

This preliminary study is promising as it shows that GPD has a partly genetic basis and might be related to nitrogen uptake after flowering or remobilization efficiency depending on the environment. The use of agronomic diagnosis and genotype by environment analysis should allow the identification of lines characterized by their adaptability or stability. The use of GPD in selection programmes might allow manipulation of GPC without reducing yield in order to produce high GPC cultivars for bread-making or low GPC ones for ethanol production.

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