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Francois-Xavier F.-X. Oury, Bernard Carré, Pierre Pluchard, Pierre Bérard, Yves Nys, et al.. Genetic variability and stability of poultry feeding related characters in wheat, in relation to environmental variation. *Agronomie*, 1998, 18 (2), pp.139-150. hal-00885876

**HAL Id: hal-00885876**

**<https://hal.science/hal-00885876>**

Submitted on 11 May 2020

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## Genetic variability and stability of poultry feeding related characters in wheat, in relation to environmental variation

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(Received 15 November 1997; accepted 12 March 1998)

**Abstract** – Thirty-four varieties of wheat were studied in a multisite experiment, under various environmental conditions. For all the characters affecting poultry feeding, except phosphorus, genotypic effects were highly significant. However, only potential applied viscosity appeared as a stable character essentially dependent on the genotype. Protein content, water-insoluble cell wall content and two characters corresponding to enzymatic activities (real applied viscosity and phytase activity) were influenced by environmental effects and genotype  $\times$  environment interactions rather strongly. Nevertheless, for these four characters, some varieties were stably favourable, or stably unfavourable. Except a weak negative relation between protein content and yield, no antagonism between productivity and poultry feeding related characters appeared. (© Inra/Elsevier, Paris.)

**bread wheat / poultry feeding / viscosity / water-insoluble cell wall / phytase activity / hardness**

**Résumé** – Étude de caractères importants pour l'alimentation des oiseaux d'élevage chez le blé tendre: variabilité génétique et stabilité de ces caractères par rapport aux variations du milieu. Une expérimentation multilocale a permis d'étudier 34 variétés de blé tendre d'hiver représentant une large gamme de variabilité génétique, dans des conditions environnementales contrastées. À l'exception de la quantité de phosphore total, les caractères importants pour l'alimentation des oiseaux d'élevage présentent tous un effet «génotype» hautement significatif. Cependant, seule la viscosité utile potentielle apparaît comme un caractère très stable et essentiellement sous la dépendance du génotype. La teneur en parois, la teneur en protéines et les deux variables qui traduisent des activités enzymatiques (viscosité utile réelle et activité phytasique) sont, elles, assez fortement soumises aux effets «milieu» et aux interactions «génotype  $\times$

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Article communicated by Max Rives (Villeneuve-lès-Avignon)

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milieu». Pour ces quatre caractères, on arrive néanmoins à identifier des variétés assez régulièrement favorables, ou assez régulièrement défavorables. Par ailleurs, exceptée une liaison faiblement négative entre le rendement et la teneur en protéines, il n'apparaît pas d'antagonisme entre la productivité et les caractéristiques importantes en alimentation des oiseaux d'élevage. (© Inra/Elsevier, Paris.)

## **blé tendre / alimentation des poulets / viscosité / parois insolubles / activité phytasique / dureté**

### **1. INTRODUCTION**

Wheat produced in France is used to an equal degree in human food and animal feed (in particular that of monogastric animals), both for the domestic market and for export. Yet the animal feed aspect has never been taken into account in varietal selection programmes. Several wheat grain constituents, such as proteins, non-starchy polysaccharides and available phosphorus, are important factors in its optimized utilization by monogastric animals.

Proteins are involved from the point of view of both their quantity and quality. The ideal combination is a high protein content and a good amino acid balance, i.e. a high proportion of essential amino acids [23].

Non-starch polysaccharides (NSP), principally crude fibre and arabinoxylans in wheat [15, 16, 20, 27], are the carbohydrates which form cell wall. In cereals, the majority of these NSP are located in the seed coats where they are usually water insoluble. However, NSP in the albumen, although present to a lesser degree, are important in poultry feeding as they are partially water soluble. This soluble fraction (and, in particular, the soluble arabinoxylans), plays a role in food viscosity, which has numerous negative consequences for birds [10]. A high dietary viscosity reduces the digestibility of the various components, causes inflammation of the intestinal mucosa, and induces over-consumption of water in birds [13]. This over-consumption leads to more aqueous excreta which exacerbate both sanitary and environmental pollution problems [14].

Insoluble NSP along with lignin are cell wall constituents that are poorly used by monogastric

animals, and constitute a diluent of the energy value of the feed [12]. Insoluble NSP are estimated by the water-insoluble cell wall content.

A total of 50–70 % of grain phosphorus is in the form of phytic acid phosphorus or myo-inositol#1,2,3,4,5,6#-hexakisphosphate acid [26]. This phytic acid phosphorus cannot be used by monogastric animals whose intestinal phytase activity is not sufficiently high to hydrolyse these phytates [25, 30]. In consequence, the phytic acid phosphorus is not available and, in regions where there is a high concentration of intensive indoor animal production, it contributes to the pollution of surface water. Wheat, on the other hand, contains plant phytase whose activity varies depending on the variety [5, 6, 30]. This phytase is activated during digestion and liberates a substantial amount of the grain phosphorus [18]. A high phytase activity is, therefore, to be sought after in wheat, because, by improving the utilization of phytic acid phosphorus, the need to supply mineral phosphorus and the production of phosphate-rich waste can be reduced.

Protein content has long been one of the attributes taken into account in selection programmes, as it comes into the transformation processes of human food (whether in different types of bread or biscuit making), or as an export criterion. Work on wheat NSP has been more recent and mainly centred around the way in which NSP influence the rheological properties of dough [8, 22, 28, 31]. Viscosity studies have been carried out by Saulnier et al. [29], and studies on the effect of the genotype on cell wall content and phytase activity have also been performed recently [5, 6, 9]. No studies on wheat have taken into account all of these characters which are important in animal feeding and

have considered both genotype and environmental effects.

In this study we present the results of a multiloal experiment enabling us 1) to describe the variability of protein content, viscosity, cell wall content, amount of total phosphorus and phytase activity and 2) to study the stability of these characters in relation to environmental variations.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

The grain samples came from trials carried out in 1995 at Inra research stations in Clermont-Ferrand (CF) and Estrées-Mons (EM). A total of 60 samples was obtained from the EM trial: 30 varieties  $\times$  2 types of highly contrasting nitrogen fertilization regimes (N0: no nitrogen application; N2: nitrogen application corresponding to a yield target of 90 q/ha). The CF trial yielded 64 samples (16 varieties  $\times$  4 crop management systems). These four crop management systems corresponded to two sowing dates (one early sowing on date d1: 19 October 1994; and a later sowing on date d2: 5 December 1994), crossed with two types of nitrogen fertilization (N1: nitrogen application corresponding to a yield target of 60 q/ha; N3: nitrogen application corresponding to a yield target of 90 q/ha, with a late application of 75 nitrogen units at ear emergence).

The varieties used in the present study were:

- four varieties specific to the CF trial: Apollo, Baroudeur, Renan and Rossini;

- 18 varieties specific to the EM trial: Allant, Arche, Arum, Audace, Aztec, Bourbon, Cappelle, Déclic, Etoile de Choisy, Eureka, Genesis, Haven, Promentin, Rialto, Ritmo, Scipion, Sensor and VM014;

- 12 varieties common to both sites: Ami, Arminda, Camp-Rémy, Forby, Qualital, Récital, Sidéral, Soissons, Talent, Thésée, Trémie and Viking.

In total, 34 different varieties covering a wide range of genetic variability, from strengtheners (characterized by a high protein content and low yield) such as Qualital, to very productive wheats (often having a low protein content) like Trémie.

### 2.2. Analytic methods

Near infra-red reflectance was used to measure hardness (AACC [1], method 39-70A) and protein content (AACC [1], method 39-10) on a complete milling performed with a 'Cyclotec' mill having a 1-mm grid.

The applied viscosities were measured according to the protocol used by Carré et al. [13]. Real applied viscosity refers to viscosity when the endogenous enzymes of the wheats are allowed to act. Viscosity is not only affected by the soluble arabinoxylan content, but also by the length of the arabinoxylan chains [29]: xylanases cut the chains thereby decreasing viscosity. Potential applied viscosity refers to viscosity after the destruction of the xylanase activities by a hot ethanol pre-treatment: it allows the maximal viscosity value of a batch of grains to be obtained.

Cell wall content was obtained using the method of Carré and Brillouet [11]; the amount of total phosphorus by the Afnor method (NFV18106); and phytase activity by the method of Engelen et al. [17].

### 2.3. Stability of the characters

As the different characters were measured on only one of the replicates for each crop management system, it was not possible to test the 'genotype  $\times$  management system' interactions using variance analysis. Hence, stability was estimated by the correlations between the values obtained by the genotypes in the various environments [7]. For each variable we obtained one determination coefficient ( $R^2$ ) with 28 degrees of freedom (df) for EM, six  $R^2$  (14 df) for CF corresponding to the six possible pairs of treatments, and eight  $R^2$  (10 df) corresponding to the eight possible combinations between the four CF treatments and the two EM treatments. We then considered the mean, extreme values and coefficient of variation of these 15 available  $R^2$  (before that, we ensured that the 15 correlations were all of the same sign).

In addition, we examined whether any of the varieties for the four treatments at CF and the two treatments at EM always came at the top or the bottom of the distribution. The number of these varieties for any given character is also a method for apprehending the stability of this character.

*Note:* For the treatment d2-N1 at CF, phytase activities and amounts of total phosphorus were only available for

seven of the 16 varieties. For all of the grain properties, no results were obtained for a variety (Aztec) in treatment N0 and for two varieties (Qualital and Rialto) in treatment N2 at EM.

### 3. RESULTS AND DISCUSSION

#### 3.1. Effect of the various crop management systems on the characters studied

Figure 1 and table I show how the various treatments played a large part on yield determination.

1) At CF, 'nitrogen fertilization' and 'sowing date' had a strong effect on yield. The difference between management conditions lay essentially in the difference in the number of grains per m<sup>2</sup>: grain weight did not compensate for weak grain numbers per m<sup>2</sup> in N1, and only slightly compensated those in d2.

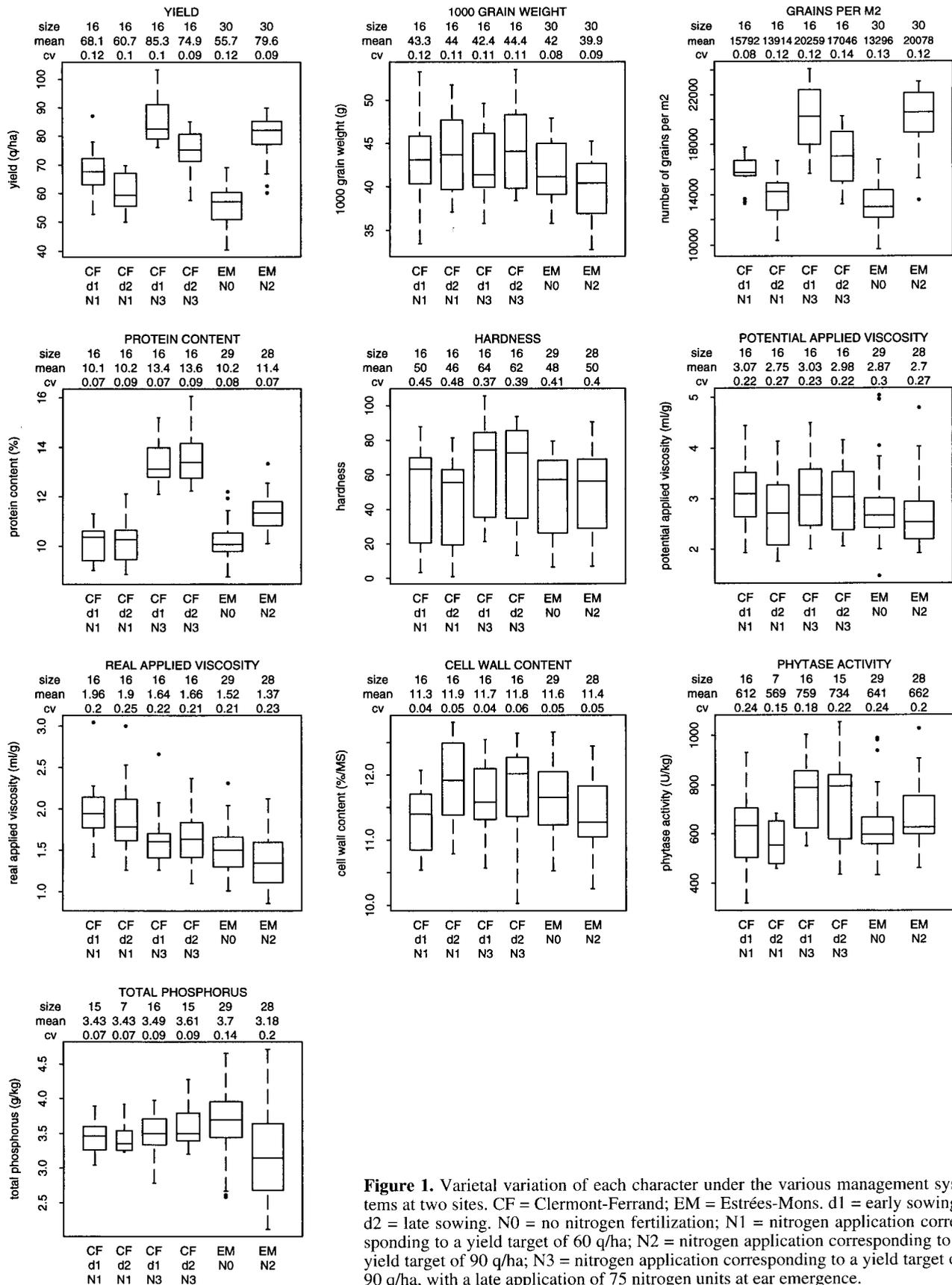
2) At EM, there was a marked difference in yield between the two management systems (23.9 q/ha on average). As at CF, this resulted from a smaller number of grains per m<sup>2</sup> for N0 fertilization. This deficit in the number of grains was so high that in this case there was a partial compensation with grain weight (the 1 000 grain weight was significantly higher for the N0 treatment). This led us to speculate that the N0 treatment induced very

**Table I.** Effects of various factors on yield and its components, and on grain properties, using variance analysis.

		Sowing date (1 df CF)	Mean squares of the factors Nitrogen fertilization (1 df CF; 1 df EM)	Genotype (15 df CF; 29 df EM)	Error
Yield	CF	1269***	3910***	133***	29.8
	EM		8555***	85.4***	19.8
1 000 grain weight	CF	28.1*	0.7NS	76.2***	5.6
	EM		67.6***	24.2***	0.83
Grains per m <sup>2</sup>	CF	103641435***	230960017***	11102226***	1923800
	EM		689875838***	7122619***	1757709
Protein content	CF	0.3NS	178.5***	2.8***	0.25
	EM		21.2***	1.01***	0.22
Hardness	CF	170**	3429***	2996***	20.1
	EM		195***	992***	13.2
Potential applied viscosity	CF	0.53**	0.15NS	1.85***	0.047
	EM		0.32**	1.02***	0.025
Real applied viscosity	CF	0.01NS	1.26***	0.48***	0.033
	EM		0.22*	0.15**	0.046
Cell wall content	CF	1.97***	0.20NS	0.97***	0.14
	EM		0.61*	0.54***	0.09
Phytase activity	CF	319NS	187200***	56773***	7344
	EM		4800NS	32465***	3350
Total phosphorus	CF	0.06NS	0.06NS	0.13*	0.06
	EM		3.89***	0.42*	0.22

CF, Clermont-Ferrand; EM, Estrées-Mons.

NS, not significant; \* significant to a threshold of 5%; \*\* significant to a threshold of 1%; \*\*\* significant to a threshold of 0.1%.



**Figure 1.** Varietal variation of each character under the various management systems at two sites. CF = Clermont-Ferrand; EM = Estrées-Mons. d1 = early sowing; d2 = late sowing. N0 = no nitrogen fertilization; N1 = nitrogen application corresponding to a yield target of 60 q/ha; N2 = nitrogen application corresponding to a yield target of 90 q/ha; N3 = nitrogen application corresponding to a yield target of 90 q/ha, with a late application of 75 nitrogen units at ear emergence.

early competition between plants, resulting in a heavily reduced number of spikes per m<sup>2</sup> (and hence a weak number of grains per m<sup>2</sup>). This adjustment through the number of grains per m<sup>2</sup> did, however, limit competition between plants during the grain filling stage. During this stage, the N0 treatment did not appear to be less favoured than the N2 treatment.

For variables other than yield and its components (*table 1* and *figure 1*), the 'sowing date' factor at CF had no effect on protein content, real viscosity, phytase activity or the amount of total phosphorus. It did, however, have some effect on hardness and potential viscosity and a fairly strong effect on cell wall content whose values were higher in d2 than d1 (*figure 1*). Andersson et al. [2] showed over 2 years of experimentation, one of which had a wet summer, that the insoluble NSP content of spring wheat was higher than for winter wheat and higher for the dry summer year than the wet summer year. Similarly, Hong et al. [21] demonstrated that insoluble NSP content from wheat grown on two sites was higher at the site with a hot, dry climate than at the site having a warm, wet climate. It would seem that climatic conditions at grain maturation play a role in the amount of insoluble NSP and hence cell wall content. Our results support this view as wheat sown on date d2 matured later than that sown on date d1 and, therefore, was exposed longer to the hot, dry period before harvesting.

The 'nitrogen fertilization' factor gave the following results (*table 1* and *figure 1*).

1) Protein content: the treatments corresponding to the highest applications of nitrogen (N3 at CF and N2 at EM) naturally gave the highest values. However, the increase was much lower at EM, probably because the N0 treatment gave so few grains per m<sup>2</sup> that the left-over nitrogen in the soil and mineralization of organic nitrogen enabled protein contents equivalent to those in treatment N1 at CF to be obtained.

2) Hardness: treatments N3 at CF registered higher values (*figure 1*). Hardness is an essentially genetically determined character [33, 34]. However our results showed that environment also

had an influence. Stenvert and Kingswood [32] noted that the continuity of the protein matrix embedding the starch granules was higher in hard than in soft wheats (also observed by Glenn and Saunders [19]) and considered this a possible explanation for hardness. This hypothesis had to be discarded, because, even if the biochemical factors involved are unknown, hardness is now thought to be the consequence of adherence phenomena between starch and proteins which would differ between soft and hard wheats [3, 4]. Our results, however, do seem to indicate that the observation made by Stenvert and Kingswood [32] could explain the effect of environment on hardness: the high protein content found in treatment N3 could correspond to increased continuity of the protein matrix, responsible for the increase in hardness.

3) Potential viscosity and cell wall content: at CF, the nitrogen fertilization had no effect and only a weak effect was observed at EM (with potential viscosity and cell wall content slightly higher for N0 than N2). These results resemble those of Grosjean and Barrier-Guillot [20], who reported that nitrogen fertilization had no effect on these characters.

4) Real viscosity and phytase activity: a strong effect of nitrogen fertilization was observed at CF, with higher phytase activities and lower real viscosities (corresponding to higher xylanase activities) for N3 than N1. It would appear that a late application of nitrogen could favour the endogenous enzymatic activities of the grain.

5) Amount of total phosphorus: no nitrogen fertilization effect was noted at CF, whereas this factor played a role at EM. The higher values registered for N0 could correspond to a lack of competition between plants for phosphorus as a result of the low number of spikes per m<sup>2</sup> induced by this treatment.

### 3.2. Influence of genotype on grain properties

The study of distributions and variation coefficients (cv) for the different variables (*figure 1*) shows the following.

1) Phytase activity, potential and real viscosity and above all, hardness, exhibit a high degree of variation (cv of more than 20 %). For these four characters, variance analysis shows genotype to have a highly significant effect (*table I*).

2) The quantity of total phosphorus and protein content have a reasonable variation amplitude (cv around 10 %). The 'genotype' effect was highly significant for protein content but weakly significant for total phosphorus (*table I*), even at EM where the cv was much higher than at CF (undoubtedly because the varietal samples differed between sites). This observation agrees with that of Barrier-Guillot et al. [5], who also demonstrated that the proportion of phytic acid phosphorus was not influenced by genotype.

3) The cell wall content had a small variation amplitude (cv was only 5 %). Variance analysis did indicate, however, that the 'genotype' effect was highly significant (*table I*).

It would appear that with the exception of the amount of total phosphorus, grain properties all depend on genotype. However, for protein content, cell wall content and the two variables which correspond to an enzymatic activity, mean square values of the same order of magnitude indicate that environmental factors play an equally strong role (*table I*).

### 3.3. Stability of the various variables

The fact that in both sites the various crop management conditions had a strong influence on yield meant we were able to obtain quite contrasted environments. This, along with the fact that 12 varieties were common to CF and EM, made it possible for us to study character stability, although our experiments took place on only two geographical sites.

*Table II* shows that yield is an unstable character because of the high 'genotype  $\times$  environment' interactions for the 'number of grains per m<sup>2</sup>' component. Grain weight, however, appears to be a fairly stable component.

For the grain properties, *table II* shows the following.

1) Total phosphorus content is very unstable (on average, there is no correlation between the values obtained in the various environments). The variability of this character appears to depend mainly on the 'genotype  $\times$  environment' interactions, which explain the weak 'genotype' and 'environment' effects in *table I*.

2) Cell wall and protein content are fairly unstable properties. The correlations between the values obtained in the various environments are quite variable and, on average, not very strong.

**Table II.** Stability of each character estimated by the correlations between various environments.

	Determination coefficients ( $R^2$ ) over the 15 pairs of environments			
	Average	Minimum	Maximum	Coefficient of variation
Yield	0.25	0.00	0.57	0.58
1 000 grain weight	0.63	0.35	0.89	0.25
Grains per m <sup>2</sup>	0.22	0.00	0.67	1.02
Protein content	0.54	0.32	0.75	0.27
Hardness	0.93	0.88	0.97	0.03
Potential applied viscosity	0.86	0.78	0.96	0.06
Real applied viscosity	0.47	0.00	0.92	0.49
Cell wall content	0.40	0.15	0.60	0.27
Phytase activity	0.46	0.14	0.76	0.50
Total phosphorus	0.05	0.00	0.26	1.11

3) Real viscosity and phytase activities are also only weakly stable properties. The mean correlation is of the same order of magnitude as cell wall and protein content, but the  $R^2$  coefficient of variation is much higher for these variables which related to enzymatic activities.

4) Potential viscosity and hardness are hardly subjected to 'genotype  $\times$  environment' interactions at all and therefore are stable properties.

These results can be found in *figure 2* where the number of varieties reaching regularly high or low values effectively appears to be linked to the stability given in *table II*. It can be observed that even for the characters that are not stable this number of varieties is not zero.

### 3.4. Correlations between the variables

The correlations between the variables for each of the management conditions of both sites were

calculated. We then considered the mean value of the six available  $R^2$ .

*Table III* indicates the following.

1) A negative correlation exists between yield and protein content. However, this correlation is not very strong and its value is over-estimated by the fact that our varietal samples contain fairly unproductive strengtheners (Qualital and Renan) and in the case of EM, two old varieties (Cappelle and Etoile de Choisy) which have been overtaken as regards productivity. If these four varieties are removed from our samples,  $R^2$  mean value does not exceed 0.14. Nevertheless, this negative correlation indicates that there are problems obtaining wheats that are both productive and rich in protein.

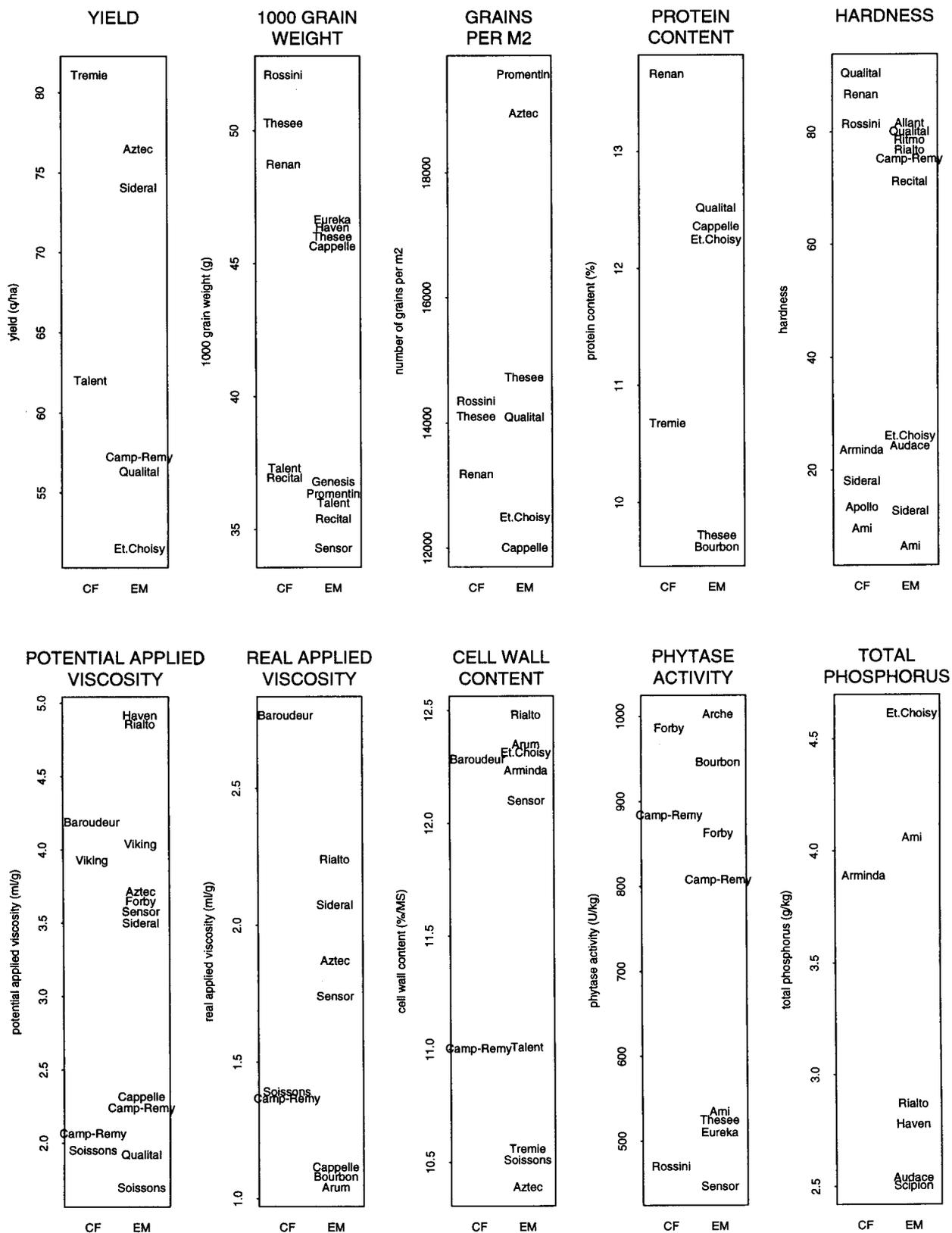
2) There is no correlation between yield and other grain properties. There seems, therefore, to be no incompatibility between productivity and properties important for poultry feeds.

3) For variables other than yield, the only significant correlations found at both sites are a negative relationship between the number of grains per  $m^2$

**Table III.** Correlations between the different characters studied (average determination coefficients for the four management systems at CF and the two management system at EM; the level of significance indicated also corresponds to average signification).

	1 000 grain weight	Grains per $m^2$	Protein content	Hardness	Potential applied viscosity	Real applied viscosity	Cell wall content	Phytase activity	Total phosphorus
Yield	0.11	0.39*	0.27* (-)	0.03	0.06	0.04	0.01	0.02	0.00
1 000 grain weight		0.35* (-)	0.02	0.09	0.06	0.01	0.00	0.13 (-)	0.00
Grains per $m^2$			0.14 (-)	0.12 (-)	0.01	0.01	0.01	0.13	0.01
Protein content				0.03	0.01	0.04	0.02	0.04	0.02
Hardness					0.00	0.03	0.03	0.07	0.07 (-)
Potential viscosity						0.53**	0.23*	0.03	0.04
Real applied viscosity							0.15	0.04	0.06
Cell wall content								0.02	0.03
Phytase activity									0.01

\* Significant to a threshold of 5 %; \*\* Significant to a threshold of 1 %; (-) indicates a negative relation.



**Figure 2.** Varieties whose values are always lower than the second decile or always higher than the eighth decile of the distribution, for the four treatments at CF and the two treatments at EM. For each of the variables, these varieties are represented by their mean value on the four management systems at CF and the two management systems at EM.

and grain weight (corresponding to the phenomena of compensation between these two yield components) on the one hand, and a positive relationship between potential and real viscosity on the other.

The lack of correlation between phytase activity and the amount of total phosphorus has already been shown by Barrier-Guillot et al. [5], who did not find any correlation between phytase activity and the amount of phytic acid phosphorus either. All these results point to the absence of a link between enzymatic activity and the amount of available substrate.

The lack of correlation between grain weight and cell wall content could indicate that the number rather than size of grain cells is important. This agrees with a result obtained in hybrid wheat where a higher number of grain cells could explain the high heterosis observed in grain weight [24].

Although NSP explain in part potential viscosity (for soluble NSP) and cell wall content (for insoluble NSP) there only appears to be a weak link between these two properties. This would seem to indicate a lack of correlation between the two types of NSP. This indirectly supports the results of Saulnier et al. [29] who found no link between the total amount of arabinoxylans and that of soluble arabinoxylans.

The fact that no incompatibility exists between the various properties that are important for poultry feeds suggests that it should be possible to take them into account simultaneously in selection. On the other hand, there seems to be no possibility of indirect selection for all of the characters examined in this study.

### 3.5. Production of wheat adapted to poultry feed

*Figure 2* illustrates how more or less favourable varieties for each of the grain properties can be distinguished in our varietal samples. For viscosity, varieties like Camp-Rémy and Soissons reach satisfactory levels, whereas varieties like Baroudeur, Rialto and Viking represent a risk (this classifica-

tion is consistent with that established by Saulnier et al. [29]). With Viking, the risk lies in the case of the feed undergoing heat treatment destroying the xylanase activity, but for Baroudeur and Rialto even the real viscosity values remain high.

For cell wall content, Camp-Rémy and Soissons also appear favourable, whereas Rialto and Baroudeur are unfavourable.

For protein content, the strengtheners like Renan and Qualital regularly register the most interesting contents, but strengtheners have a low productivity which makes them less competitive on the animal feed market.

For the 'available phosphorus' aspect, varieties like Camp-Rémy and Forby seem to present regularly high phytase activities, whereas varieties like Eureka, Rossini, Sensor and Thésée are of little interest.

In our samples no single variety combines all the favourable properties (Camp-Rémy comes nearest, but it is an old variety with limited productivity). To obtain a favourable variety, selection for the characters of interest to the animal feed industry would have to be envisaged. This would not be too difficult in the case of potential viscosity as it is a character which has a high variability and depends largely on genotype. Characters like cell wall content, protein content, real viscosity and phytase activity, however, would be more difficult to breed as they quite highly depend on 'genotype × environment' interactions. For these four characters, the existence of strong environmental effects poses less a problem than instability caused by interactions, as these effects can in part be controlled: for example a late application of nitrogen can often increase protein content and would also, if our experimental results are confirmed, positively influence the endogenous enzymatic activities in the grain. Finally, as the amount of total phosphorus is very unstable, it would seem impossible to select this character, all the more so as available phosphorus seems to depend more on phytase activity than on the amount of total phosphorus or phytic acid phosphorus [6, 18, 25].

Hardness is not a character that has been studied in depth as regards animal feed. We took it into

account essentially to see whether it presented interesting correlations with the other parameters (this does not seem to be the case). Although it is difficult a priori to judge what effect it might have, it remains a very stable character presenting a wide genetic variability which would be easy to integrate if necessary.

#### 4. CONCLUSION

Our study has shown that a wide variability for poultry feeding related characters exists in wheat and that the genotype effect is largely involved in this variability. A description of the varieties for all these characters seems possible and could allow food manufacturers 1) to reduce the number of tests carried out on the grain they buy, and 2) to cheapen the process of correcting faults by the addition of various industrial components.

It is also worth mentioning that the wider use of wheat in animal feeding brought about by the European agricultural policy, has been reinforced by the fact that Soissons, the most widespread variety in France at the moment, presents several favourable properties (quite by chance, because there was no selection for these characters). If we wish to maintain or increase the 'animal feed' outlet for wheat, the varieties which take over from Soissons should present no major defects for this utilization and the best way of achieving this is by taking the animal feed aspect into consideration during selection.

**Acknowledgements:** This study was financed by the Ministère Français de l'Agriculture as part of a contract between Inra and the Hybrinova selection company.

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