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Correlation Between Gliadin Bands

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Summary. Starch gel electrophoresis of gliadins was carried out for 37 bread wheat cultivars chosen for their distant relationships. Simple correlations were calculated between each of the 41 bands (variates) observed with these wheats. It was found that a band is usually negatively correlated with the two neighbouring mobility bands. The number of bands positively or negatively correlated with a given band varies from 2 to 8. Taking the bands significantly positively correlated with each-other 32 groups were constituted. It appears likely that these correlations result from the fact that the genes determining the bands of a given group are situated on one chromosome. Study of the distribution of 15 gliadin bands from 312 F_2 grains indicated that 2/3 of the correlations calculated for this progeny were due to linkages.

Key words: Gliadin – Bread wheat – Wheat cultivars – Polymorphism

Introduction

The observation of electrophoretic diagrams from different varieties provides interesting information about the heterogeneity of bands that exists. Some bands are absent while others are present and several bands may appear correlated because the presence (or absence) character of one of them is often related to the appearance (or disappearance) of another. Autran (1975) used these characteristics of gliadin polymorphism to establish a dichotomous table for wheat identification. All the studies of the genetic determination of gliadins (Doekes 1973; Sozinov et al. 1974; Mecham et al 1978; Baker and Bushuk 1978) have shown that several bands appear to be transmitted in groups. Therefore, in order to determine in more detail the importance and origin of these groups, we analysed the correlations between bands from a diversified set of varieties and from an F_2 progeny.

Materials and Methods

Cultivars

From a large collection of winter and spring wheats mainly of French origin, 37 varieties, as little related to one another as possible, were chosen. They are the following: "Bicoop", "Bocquiau", "Boulmiche", "Cadet", "Cesar", "Champlein", "Etoile de Choisy", "Chrismar", "Clairon", "Clément", "Comtal", "Courtot", "Darius", "Decius", "Ducat", "Eloi", "Essor", "Flinor", "Florent", "Fournil", "Goya", "Heima", "Kolibri", "Lutin", "Magali", "Magdalena" "Manella", "Maris Huntsman", "Marly", "Montjoie", "Peguy", "Rex", "Sirius", "Talent", "Trio", "Trippel", "Wattines". The fractionation of the gliadins of these 37 cultivars enabled us to study the correlations between various bands.

The study of the genetic determination of one group of bands was carried out from electrophoretic analysis of F_1, reciprocal F_1 and the 312 F_2 grains obtained from the "Mayo 54" × "Joss" cross. The main results of this study have been given in a previous paper (Branlard 1982).

Study of Gliadins

The study of gliadin polymorphism was carried out by electrophoresis on starch gel according to the Autran and Bourdet method (1975). The analysis of band mobility and relative concentration was made using the nomenclature proposed by these authors. In particular, the different levels of concentration of a band have been classified by visual analysis in the following manner: 0 (absence), trace, +, + +, + + +. Autran and Bourdet (1975) gave the limiting values of these concentration classes after densitometric analysis of a large number of wheats: 0.0–1.5%, 1.5–4%, 4–6.5% and higher than 6.5% respectively as percent of total absorption.

Correlations Between Bands

The study of correlations between bands was carried out considering each band as a variate with n = 37 and 312 observations from cultivars and F_2 grains respectively. According to the presence or absence character of the bands the value of the variate was taken as the average of the corresponding concentration class: Ø (O), 0.75 (trace), 2.75 (+), 5.25 (+ +) and 7.75 (+ + +).

CHI-2 Test of Non Independence

With two characters (bands I and II, for example) within a population, it is possible to look for *a*, *b*, *c*, and *d* frequencies

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Table 1. Non independence CHI-2 formulae for the I and II bands with different distributions. n = total number of individuals, a = number of individuals with band I and II, b = number of individuals with band I and without band II, c = number of individuals with band II and without I, d = number of individuals without band I and II

Distribution for the band		CHI-2 formulae	Distribution for the band		CHI-2 formulae
I	II		I	II	
3/1	3/1	$\chi^2 = \frac{(a-3b-3c+9d)^2}{9n}$	9/7	3/1	$\chi^2 = \frac{(7a-21b-9c+27d)^2}{189n}$
3/1	15/1	$\chi^2 = \frac{(a-15b-3c+45d)^2}{45n}$	9/7	7/9	$\chi^2 = \frac{(63a-49b-81c+63d)^2}{3969n}$
7/9	3/1	$\chi^2 = \frac{(9a-27b-9c+21d)^2}{189n}$	9/7	9/7	$\chi^2 = \frac{(49a-63b-63c+81d)^2}{3969n}$
7/9	7/9	$\chi^2 = \frac{(81a-63b-63c+49d)^2}{3969n}$	9/7	15/1	$\chi^2 = \frac{(7a-105b-9c+135d)^2}{945n}$
7/9	9/7	$\chi^2 = \frac{(63a-81b-49c+63d)^2}{3969n}$	15/1	3/1	$\chi^2 = \frac{(a-3b-15c+45d)^2}{945n}$
7/9	15/1	$\chi^2 = \frac{(9a-135b-7c+105d)^2}{945n}$			

respectively from the 4 following classes: (I, II), (I, –), (–, II), (–, –), where class (I, –) represents the grains with the I band but not the II band. If the law of distribution for these 4 classes is known, the adjustment of the frequencies observed to the theoretical frequencies can be tested by a CHI-2 calculation with 3 degrees of freedom (d. f.). Mather (1943) takes the example of 2 genes (*A*/*a*) and (*B*/*b*) and gives the rules for calculation of the CHI-2 with 3 d. f. and the formula for testing the distributions 3 : 1 for *A*, 3 : 1 for *B* and for the "interaction" (linkage effect) between these two genes.

Mather's 1943 rules for a correct decomposition were used to establish other CHI-2 interaction formulae, as the types of distribution of the bands analysed in the progenies from the "Mayo 54" × "Joss" cross are not only (3 : 1, 3 : 1) but also (9 : 7, 9 : 7), (9 : 7, 3 : 1) etc. (Branlard 1982). The formulae used to test for non independence between one band distribution type and another are given in Table 1. This test, which does not test for linkage in the true sense of the term for all the distributions corresponding to two genes, can be considered as a CHI-2 test for non-independence.

Results and Discussion

Correlations Between the Bands of 37 Bread Wheat Cultivars

From observation of the gliadins of a large number of varieties, Autran and Bourdet (1975) counted 43 bands of different mobility. From the 37 varieties discussed here the number was also 43. Of these, mobility bands 33 and 65 were eliminated, the former because it was present in too few diagrams and the latter because it was present in all of them. The calculations were made for 41 bands which results in a total of 820 correlation coefficients. From this table it was possible to show the group of bands appearing significantly correlated to a given band. Some of these correlation coefficients can be seen in Table 2.

The number of bands positively or negatively correlated to a given band varies from 2 to 8. The observation of this table reveals that the two bands on either side of a given band are usually negatively correlated with the latter. Thus, for mobility band 45, the following coefficients with neighbouring bands 44 and 46 can be noted: r (44, 45) = –0.615**, r (45, 46) = –0.542**. It can be seen that 36 of the 41 bands analysed confirm this type of result. Several hypotheses can be put forward to explain this phenomenon.

The difficulty in visual distinction of the bands might be responsible for these negative correlations. But this is unlikely since bands separated by several millimeters also confirm this result: such is the case for gliadins with mobilities 75, 77, 79, 81, 83 and 85.

It is possible that structural genes of some proteins are localised at the same locus. To observe these negative correlations, the differences between alleles would have to result in only a slight difference of mobility for these protein.

Finally, it is not impossible that some proteins remain aggregated to a nearby protein when this is present on the diagram. Taking into account the similarity between the N terminal sequences of these proteins (Bietz et al. 1977; Shewry et al. 1979) and the composition in proteins of some electrophoretic bands (Wrigley and Shepherd 1973; Terce-Laforgue et al. 1978), these last two hypotheses are likely to account in part for the negative correlations observed between bands.

Table 2. Correlation coefficients between some gliadin bands observed from 37 bread wheat cultivars

	44	45	46	49	75	77	79	83
21	0.363*	−0.177	0.061	−0.099	−0.103	0.102	−0.014	0.154
22	−0.094	0.182	−0.169	0.087	0.202	0.196	−0.347*	−0.510**
25	0.245	−0.102	−0.071	0.043	−0.082	−0.138	0.242	0.371*
26	−0.218	0.091	−0.005	0.123	0.186	0.056	−0.145	−0.343*
28	0.245	−0.102	−0.071	0.043	−0.082	−0.138	0.242	0.372*
30	−0.025	−0.089	0.178	−0.018	0.086	0.058	−0.060	−0.329*
37	−0.204	0.207	−0.266	0.185	−0.119	0.369*	−0.239	−0.014
39	−0.237	0.181	0.108	0.039	0.235	−0.636**	0.295	0.176
41	−0.083	0.283	−0.288	0.008	−0.090	0.354*	0.019	−0.003
43	−0.483**	0.365*	−0.408*	0.436**	0.118	−0.252	−0.046	0.019
44	1.000	−0.615**	0.343*	−0.367*	0.132	0.214	−0.202	−0.070
45	−0.615**	1.000	−0.542**	0.353*	0.092	−0.037	0.020	0.162
46	0.343*	−0.542**	1.000	−0.548**	−0.290	0.106	−0.274	0.007
49	−0.367*	0.353*	−0.548**	1.000	−0.004	−0.178	0.158	0.154
55	0.100	−0.069	−0.463**	0.050	0.364*	−0.169	0.220	0.061
56	0.173	−0.216	0.515**	−0.397*	−0.253	0.052	−0.325*	0.009
62	0.239	−0.279	−0.241	−0.219	−0.175	0.353*	−0.380*	−0.018
68	−0.223	0.105	−0.086	0.075	−0.142	−0.421**	0.395*	0.302
71	0.106	0.068	−0.128	−0.005	0.301	0.134	−0.031	−0.383*
72	−0.216	−0.025	0.069	−0.087	−0.155	−0.112	0.403*	0.326*
75	−0.132	0.092	−0.290	−0.004	1.000	−0.328*	0.163	−0.011
77	0.213	−0.037	0.108	−0.178	−0.328*	1.000	−0.528**	−0.411*
79	−0.202	0.020	−0.274	0.158	0.163	−0.528**	1.000	0.396*
81	0.059	0.107	−0.062	−0.034	−0.049	0.195	−0.405*	−0.258
83	−0.070	0.162	0.007	0.154	−0.011	−0.411*	0.396*	1.000
85	−0.029	0.065	−0.260	0.267	0.181	0.022	0.125	−0.473**

* $\alpha < 5\%$; ** $\alpha < 1\%$

This table of correlations also enables us to constitute sets of bands. A set or group is composed of all the bands positively and significantly correlated to any other band of this group. Figure 1 represents the 32 groups of bands that were obtained.

Doekes (1973) observed that a gliadin diagram could be split into 6 or 7 sections with bands inherited as groups. Sozinov et al. (1974) also noted that bands could be transmitted is several blocks. More recently Sozinov and Poperelya (1980) counted 29 blocks after observing polymorphism in several progenies and in monosomic and nullitetrasomic lines.

The correlations reported here indicate a higher number of band groups, probably because the polymorphism is greater than that provided by the two parents of a cross. The origin of these groups must be related to what is known about chromosomal localization of gliadins (Wrigley and Shepherd 1973, Kasarda et al. 1976, Mitrofanova 1976). In particular, it is known that slow omega gliadin groups, such as (21, 25, 28) and (22, 26, 30), are coded by genes localized on chromosome 1D.

There are correlations not only between these bands but also with mobility bands 34 and 83 for group (21, 25, 28) and 85 for group (22, 26, 30) Fig. 1 II, IV and VII. Similarly, bands 98 and 100 have mobilities close to those of "Chinese Spring" whose genes are localized on chromosome 6A (Kasarda et al. 1976). Band 88 belongs to the same group (Fig. 1 XXXII). Bands 52 and 55, present in "Chinese Spring", are coded by genes localized on chromosome 1B. Several other bands are correlated with these two, especially bands 85, 88, 93 and 100 (Fig. 1 XIX–XXII).

The bands from "Chinese Spring" are likely to have the same origin and to be identical to those with an equal mobility present in other cultivars. Furthermore, it can be assumed that these correlations between bands of one group result from the fact that the genes coding those bands are localized on the same chromosome. In these circumstances, it is possible to continue studying the chromosomal localization of genes coding the gliadins in "Chinese Spring".

Only group (50, 57, 60) (Fig. 1 XVIII) could not be connected to a chromosome by using the bands with a known chromosomal localization. 10, 26 and 15 bands could be counted as being controlled by chromosome 1 or 6 from the genomes A, B and D respectively. The bands related to chromosome 6 B could not be identified with certainty.

In order to check out hypotheses and continue the chromosomal localization of the bands non-specific to "Chinese Spring", it would be useful to develop this study on other aneuploid lines as well as on segregating material. The following study will enable us to determine the significance of these correlations.

Correlations Between Bands of an F_2 Progeny

From electrophoretic diagrams of 312 F_2 grains from the "Mayo 54" × "Joss" cross simple correlations between the 16 gliadin bands differentiating the parents

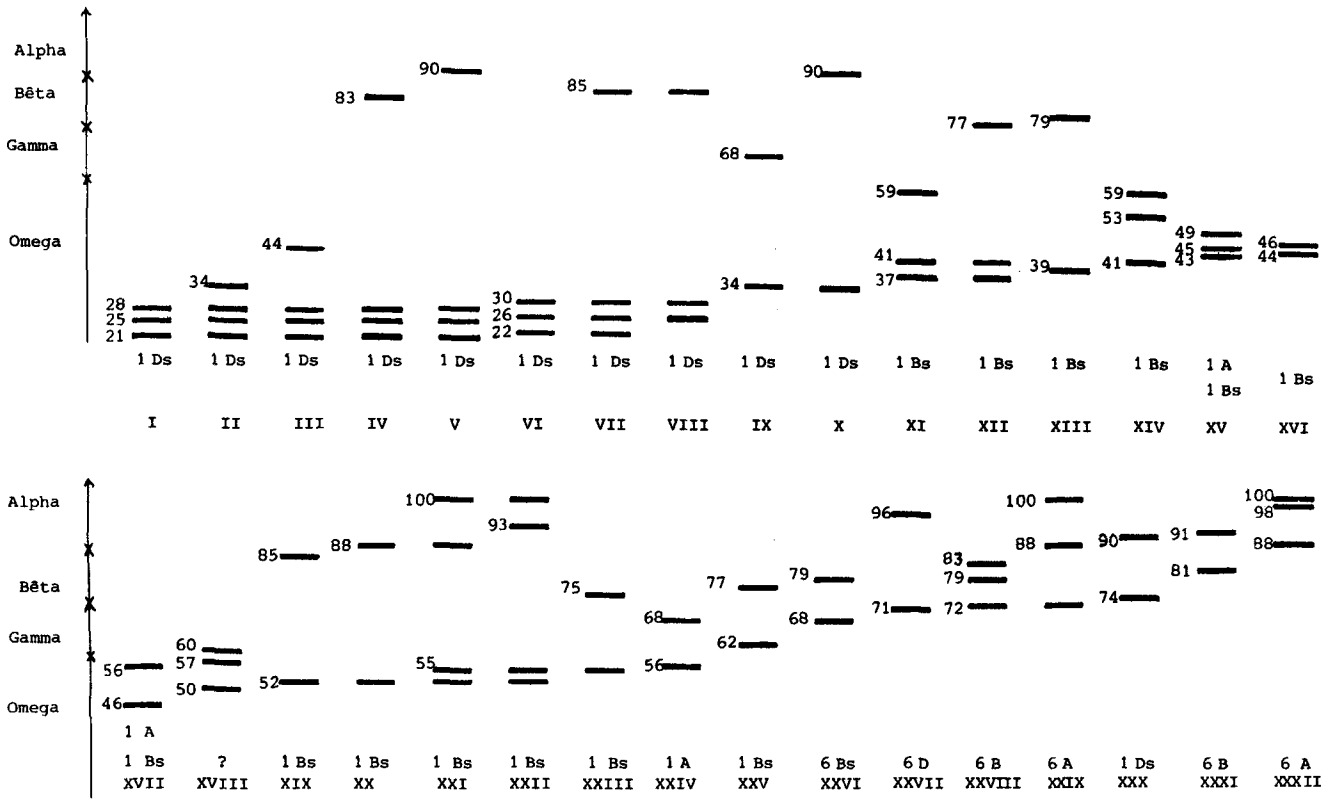

Fig. 1. Groups of gliadin bands and chromosomal attribution of these bands

were calculated. The genetic analysis of each band was carried out previously (Branlard 1982). Table 3 represents 120 correlations calculated between each of the 16 variates (bands) considered two by two. These correlations enabled us to distinguish the 3 groups previously observed: (21, 25, 28) Fig. 1 I; (21, 25, 28, 34) – Fig. 1 II and (22, 26, 30) – Fig. 1 VI. It is also observed that several other bands, for example 43 and 46, seem to be correlated with the bands controlled by genes localized on chromosome 1 D. In order to explain these new linkages it can be suggested that either they are genetic linkages or a non systematic post transcriptional modification of RNA coding bands 21, 25 and 26 or bands 22, 26 and 30 or a post synthetic modification of a protein, which could also be non systematic.

In fact, the genetic significance of these correlations cannot be appreciated by the calculation of these coefficients only. The study of the genetic determination of these bands enabled us to specify to which theoretical distribution types each of them corresponds. Thus the non independence CHI-2 tests provide an indication of whether these correlations correspond to genetic relationships.

Table 3 represents the values of non-independence CHI-2 tests between 15 of the 16 bands examined for the "Mayo" × "Joss" cross. Band 44, coded by two linked genes, was not taken into account in these calculations. Several observations were made from these data.

– There are very strong linkages between the genes coding bands 21, 22, 25, 26, 28 and 30 carried by chromosome 1 Ds. These linkages extend to the gene coding band 34, which confirms its localization on chromosome 1 D. However, it must be noted that a band with mobility 34 is found in some durum wheat varieties such as: "Agathe", "Bidi 17", "Durtal", "Rikita".

– There is also a strong linkage between genes controlling bands 37, 39 and 43 as well as bands 46 and 56 localized on 1 B; and to a lesser extent between the genes coding bands 37, 39 and 43 and the one controlling band 79.

– Finally there are significant, even highly, significant linkages between genes carried by chromosome 1 D and those coding other bands (such as 43, 46 and 56 which had also appeared correlated) (Table 2).

It is normal to observe linkages between genes carried by one chromosome. Such is the case for genes coding bands 21, 22, 25, 26, 28, 30 and 34 localized on 1 Ds and for bands 37, 39 and 43 or 46 and 56 carried by 1 B. However the observation of such linkages between bands whose genes had been initially localized on different chromosomes may seem very surprising.

Table 3. Correlation coefficients between 16 bands from 312 diagrams of F_2 grains from Mayo × Joss cross

	79	62	56	49	46	44	43	39	37	34	30	28	26	25	22
21	0.15**	0.01	−0.10	0.16**	−0.14*	0.05	0.23*	0.04	0.13*	0.60**	−0.63**	0.71**	−0.85**	0.82**	−0.89**
22	−0.14*	−0.05	0.10	−0.14*	0.12*	−0.02	−0.21**	0.06	−0.04	−0.47*	0.55**	−0.60**	0.79**	−0.76**	
25	0.10	0.06	−0.06	0.16**	−0.11	0.06	0.24**	−0.05	0.09	0.56**	−0.56**	0.65**	−0.89**		
26	−0.11	−0.01	0.13*	−0.13*	0.16**	−0.04	−0.20**	0.09	−0.09	−0.64*	0.70**	−0.79**			
28	0.13*	−0.10	−0.17**	0.09	−0.20**	0.05	0.21**	−0.10	0.18**	0.64**	−0.72**				
30	−0.06	0.03	0.13*	0.07	0.13*	−0.01	−0.03	0.19*	−0.13*	−0.60**					
34	0.17**	−0.06	−0.09	0.17**	−0.08	0.13*	0.26**	−0.00	0.10						
37	0.34**	−0.24**	−0.12*	0.01	−0.28**	0.33**	0.51**	−0.67**							
39	−0.22**	0.17**	0.10	0.22**	0.22**	−0.11	−0.36**								
43	0.37**	−0.13*	0.13*	0.21**	−0.24**	0.45**									
44	0.10	−0.08	−0.08	0.12*	−0.10										
46	−0.23**	0.16	0.81**	−0.05											
49	0.22**	−0.01	−0.47**												
56	−0.14*	0.16**													
62	−0.15**														

* $\alpha < 5\%$; ** $\alpha < 1\%$

Table 4. Non independence CHI-2 values between the bands studied in the Mayo × Joss cross

	79	62	56	49	46	43	39	37	34	30	28	26	25	22
21	0.71	0.14	1.25	2.13	6.52*	5.55*	5.63*	0.03	73.08*	111.2*	146.7*	179.7*	240.0*	309.9*
22	2.58	1.91	0.17	0.92	3.62	9.69*	1.59	0.66	90.96*	118.3*	138.8*	188.7*	229.0*	
25	0.51	0.48	4.62*	0.97	15.43*	3.71	12.13*	0.01	51.70*	106.4*	135.8*	212.8*		
26	0.55	0.14	4.17*	1.57	12.31*	7.03*	2.50	0.12	45.39*	116.4*	181.7*			
28	0.17	0.76	3.40	0.81	4.94*	4.65*	4.17*	0.26	83.58*	107.9*				
30	0.01	2.25	5.62*	0.03	12.78*	0.33	7.29*	2.55	44.50*					
34	0.36	0.09	0.96	0.01	1.84	0.09	0.14	0.36						
37	13.13*	13.13*	0.46	3.45	14.24*	40.20*	49.28*							
39	4.79*	3.01	2.76	22.33*	18.51*	21.20*								
43	20.51*	4.46*	2.51	0.03	14.24*									
46	5.13*	5.13*	94.82*	2.73										
49	0.01	0.19	2.63											
56	0.28	3.01												
62	3.28													

* $\alpha < 1\%$

Several hypotheses can be put forward:

– either, genes coding bands 43, 46 and 56 for instance, are not on 1 B but 1 D. This is very unlikely after observing the correlations of the 37 cultivars.

– or, for example, chromosome 1 Ds carries genes playing a role in the synthesis of bands that migrate to the same place as those coded by genes localized on 1 B. In that case the band should consist of: either one protein if the two genes carried by different chromosomes are similar; or two proteins if the contrary is true.

In order to confirm this second hypothesis, that is that these linkage are the result of duplicated genes on homeologous chromosomes, it would be necessary that the genetic determination of these bands is not of the 3 : 1 but of the 15 : 1 type. In fact, study of the genetic determination of bands taking into account their concentration classes, had led us to the same assumption.

Bidimensional electrophoretic analysis has shown that of the bands examined, only band 49 was composed of two proteins and corresponded to a segregation of presence of 15 : 1. The other bands appeared to be each composed of only one type of protein. Except for bands 44 and 49, each corresponding to two genes respectively linked and independent, all the other bands with a mobility superior to 30 and differentiating "Mayo 54" from "Joss" result from a 3 : 1 heredity and not a 15 : 1.

Thus, the correlations observed between bands probably express linkages. This does not appear to be the case for all the correlations calculated. From the F_2 progenies and for 15 bands, it can be noted that (Table 2 and 3) among the 72 significant correlation coefficients, 47 correspond to significant CHI-2.

Conclusion

The calculation of simple correlations between bands enabled us to underline the complexity of the apparent polymorphism of gliadins. Negative correlations between bands with a very similar mobility are undoubtedly related both to the limitations of electrophoretic technic and to these proteins remaining partly aggregated to one another. These correlations within a group of bands are likely to be due to their determination by genes localized on the same chromosome and more or less linked to one another. In these correlations, it can be observed that many bands are composed of proteins from different chromosomes. Genome A and B seem to control the largest number of bands.

These results provide evidence for a hypothesis which appears likely but still requires some verification. The equal mobility of two bands is not sufficient to conclude that the proteins are of identical origin. In order to check this hypothesis, this study would have to be conducted on aneuploid lines of diverse origin analyzed by bidimensional electrophoresis. The chromosomal localization of many gliadins bands could also be studied on a wide range of cultivars for which each diagram would be bidimensionally analysed. The distinction of bands groups could then be established by calculation of multiple correlations between the spots extracted from various bands.

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Literature

Autran, J.C.; Bourdet, A. (1975): L'identification des variétés de blé: établissement d'un tableau général de détermination fondé sue le diagramme électrophorétique des gliadines du grain. Ann. Amélior. Plantes **25,** 227–301

Baker, R.J.; Bushuk, W. (1978): Inheritance of differences in gliadin electrophoregrams in the progeny of Newpawa and Pitic 62. Can. J. Plant Sci. **58,** 325–329

Bietz, J.A.; Hübner, F.R.; Sanderson, J.E.; Wall, J.S. (1977): Wheat gliadin homology revealed through N. terminal amino acid sequence analysis. Cereal. Chem. **54,** 1070–1083

Branlard, G. (1982): Study of genetic determination of 20 gliadin bands. Theor. Appl. Genet. (Submited for publication)

Doekes, G.J. (1973): Inheritance of gliadin composition in bread wheat, *Triticum aestivum.* Euphytica **22,** 28–34

Kasarda, D.D.; Bernardin, J.E.; Qualset, C.O. (1976): Relationship of gliadin protein components to chromosomes in hexaploid wheats (*Triticum aestivum* L.). Proc. Natl. Acad. Sci. USA **73,** 3646–3650

Mather, K. (1943): Statistical analysis in biology. p. 267. London: Methuen

Mecham, D.K.; Kasarda, D.D.; Qualset, C.O. (1978): Genetics aspects of wheat gliadin proteins. Biochem. Genet. **16,** 831–850

Mitrofanova, O.P. (1976): Genetic control of common wheat gliadins. Tsitol. Genet. **10,** 244–247

Shewry, P.R.; Autran, J.C.; Nimmo, C.C.; Lew E.J.L.; Kasarda, D.D. (1980): N-terminal amino-acid sequence homology of storage protein components from barley and a diploid wheat. Nature **286,** 520–522

Terce-Laforgue, T.; Charbonnier, L.; Mosse, J. (1980): Isolation and characterization of gliadin fractions. Biochim. Biophys. Acta **625,** 118–126

Sozinov, A.A.; Poperelya, F.A.; Stakanova, A. I. (1974): Use of electrophoresis of gliadin for selection of wheat by quality. Vestn. Skh. Nauki (Moscow) **7,** 99 (Chem. Abst. **81,** 166–298)

Sozinov, A.A.; Poperelya, F. A. (1980): Genetic classification of prolamines and its use for plant breeding. Ann. Technol. Agric. **29,** 229–245

Wrigley, C.; Shepherd, K.W. (1973): Electrofocusing of grain proteins from wheat genotypes. Ann. N.Y. Acad. Sci. **209,** 154–162

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