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THE USE OF GLIADIN ELECTROPHORESIS IN WHEAT BREEDING PROGRAMME

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ABSTRACT

Storage proteins in cereal crops are the most suitable systems that express the individual characteristics of a genotype. The storage proteins in wheat are known as gliadin proteins and the genetic nature of gliadin polymorphism is well known. The genes controlling gliadin synthesis are located on the chromosomes of homoeologous groups 1 and 6.

The gliadin protein compositions of 83 wheat genotypes comprising 41 varieties and breeding lines of spring wheat with different eco-geographical origin, 19 allocytoplasmic forms of spring and winter wheat and 22 F1 reciprocal hybrids from crosses between allocytoplasmic forms and varieties of spring wheat were studied by starch gel electrophoresis to determine the role of foreign cytoplasm in the inheritance of gliadin components as well as study associations between gliadin components and morphological, agronomic and bread quality characteristics of wheat.

Results show that 26.8% of the studied spring wheat varieties and breeding lines were characterized by high degree of stability of the component composition of gliadin in the grain. The remaining comprised more than one biotype. Observed differences between biotypes occurred in 1A and 6A as well as in 1D and 6D loci. Results also suggest that probably the genes on these loci may be associated with grain quality characteristics. In some allocytoplasmic forms only the gliadin coding loci of the male parent were represented which seem to suggest that cytoplasmic genes do not have any direct influence on gliadin synthesis, while in others differences were observed in 1B, 6A, 1A and 6B loci.

Key words: Gliadin proteins, Allocytoplasmic forms, Reciprocal hybrids, starch gel electrophoresis, and Gliadin coding loci.

INTRODUCTION

Restriction fragment length polymorphism (RFLP) is a powerful, new tool of biotechnology that can potentially increase the effectiveness and efficiency of plant breeding. It brings together molecular genetics and classical plant breeding without the need or risk of transgenic organisms in the environment (Young *et al.*, 1992).

Major genes for diseases and pests resistance, as well as genes controlling complex traits such as yield and quality can be tagged with tightly linked components. Once economically important genes are tagged, individuals that carry these genes can be selected for based on their RFLP genotype. (Young *et al.*, 1992).

Storage proteins in cereals are the most suitable systems that express the individual characteristics of a genotype. The storage proteins in wheat are known as gliadin proteins and the genetic nature of gliadin polymorphism is well known. The genes controlling gliadin synthesis are located on the chromosomes of homoeologous groups 1 and 6 (Sozinov, 1985 and Young *et al.*, 1992). For hexaploid wheat, gliadin alone is controlled by six or eight clusters of genes, represented in a population by multiple alleles. This makes it possible to clearly differentiate genotypes, although some varieties with common origin may possess the same electrophoretic patterns (Sozinov, 1985). Gliadin composition is a complex attribute but one very important fact which makes the study of wheat gliadin genetics easy is that the electrophoretic pattern is always constant irrespective of the environmental effect. (Elton and Ewart, 1962; Bourdet *et al.*, 1963; Lee and Ronalds, 1967) and can be determined on a single seed without damage to embryo (Wrigley, 1976).

Ever since the demonstration that the gliadin fraction of wheat grain protein is a mixture of many different components and that the nature and proportions of these components differ from one cultivar to another (Wrigley *et al* 1982), there have been attempts to relate gliadin composition to quality characteristics. Wheat gliadin can be effectively used in plant breeding to characterize the genotype of an organism and to identify contamination of seeds. The most important application of gliadin electrophoresis to crop improvement is in selecting individuals that carry genes of economic importance. If the genotype is recessive or impossible to monitor in the presence of other genes, the ability to associate gliadin bands to economically important characters can provide a powerful selection tool for plant breeding (Young *et al.*, 1992). Researches by

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some scientists (Bebiyakin, 1982; Sozinov, 1985) have revealed significant genetic and functional relationship between the composition of gliadin component and bread quality as well as yielding ability of wheat. Sozinov (1985) and his co-workers observed close relationship between wheat grain quality and gliadin component composition. They discovered that block Gld 6A3 had a better positive influence on flour quality than Gld 6A1 and that lines with block Gld 1D5 were better than those with block Gld 1D2. Chromosome 1A, it was discovered controlled flour quality, although block Gld 1A3 was better than block Gld 1A4. Block Gld 1B3 produced very negative effect on flour quality although genotypes with this block component had higher grain protein content. Furthermore, block Gld 1B1 gave better grain quality than 1B2. It is more difficult to establish relationship between allelic variants of gliadin blocks and varietal productivity.

However, it has been established that block Gld 1A1 is more productive than block Gld 1A4 and that Gld 1A4 together with Gld 1B1 give taller plants than block Gld 1A4 and 1B3 or Gld 1A3 and Gld 1B4. Likewise, varieties with good winter hardiness have block Gld 1D5 and 6A3, while Gld 1B1 is usually present in the most high yielding varieties. According to Poperely (oral communication) most high yielding genotypes have gliadin formula 1.4.3.1.11, those with better grain quality have 4.1.4.3.1.2 and winter hardiness have 1.2.5.3.2.2. According to Sesson (1988) the major pre-occupation of developing countries should be to increase the production of foodstuffs, the availability of an energy source which is economical for household use and which ensures the slowing down of deforestation. The use of electrophores is in selecting for high yields could bring about increase in the yield of field crops, necessary for ensuring food security in the countries.

TABLE 1:GLIADIN ELECTROPHORETIC PATTERNS OF SOME VARIETIES AND BREEDING LINES OF SPRING WHEAT

Varieties and Breeding Lines	Chromosomes, Variant I				Controlling Variant II			gliadin		Synthesis		
	1A	1B	1D	6A	6B	6D	1A	1B	1D	6A	6B	6D
1. Saratovskaya 29	5	4	3	1	1	2	5	4	3	2	1	1
2. Lutescience 14	2+4	1	2	1+3	1	1	2	1	3	1	1	1
3. Line H-960	1	4	1	1	1	1	6	4	2	1	1	1
4. Sicco	2	4	1+5	3	?	1	2	4	1	3	?	1
5. Cosir	3	1	4	1	1	1	5?	1	8	1	1	1
6. G-39018 grace.	5	1+4	9	1H	1	1	5	4	1	1	1	1
7. Saffran	2	3	1	1+3	1	1	2?	3H	1	3	1	1
8. 2 – 04	6	1+4	1+4	1+3	1	1	6	15?	5	1	1	1
9. PJ 414570 Tengeli Jung[2]	1	7	4	3	1	2	1	7	4	3	1	1
9. Moscovskaya 35	1	1	1+4/5	3	1	2	6	1	1	3	1	2
11. Kezntner Frufeer	5	1+4	3	1	1	8	?	4	3	1	1	2
12. Lr 9x(Thatcher)	4	15	1+5/4	1	1	1	5	4	2	2	1	1
13. Complex hybrid	1	1	1	3	1	2	4	?	1	1	1	1
14. Elitesj 2/240	1	1+2	1	3	1	2	1	1	1	3	1	2
15. Wilter	2	4	1	3	?	1	1	1	1	3	1	2
16. Uralochka	3	1	3	3	1+2	1	1	4+?	1	3	1?	1
17. Echo	2	1+4	1	1+3	2?	1	3	1	3	3	1	1
18. Kadet	5/2	1	1	3	?	1	2	4+?	1	3	1	1
19. Selection Siette Cerros	5	15	1	1	1	1	4	1	1	3	1	1
20. Lr 19x (Thatcher)	1	7	4	3?	1	2	5	?	1	1	1	2?
21. Sonalika	5	8	2	1+3	?	1	?	15	1	1	1	1
22. Safed levma	5	1?	1	1	1	1	13?	1	3	3	1	1
23. Magnet	16	1	9	3	1	1	5	4	3	1	1	8
24. Yande	1	7	2	1	1	1	5	1	3	1	1	1
25. Chhoti lerma	5	8	2	3	1	2	5	8	3	2	1	1
26. Vove	6	1	2	1	?	1	15+?	1	3	1	1	1
27. Duri	1	5	5	3	1?	1	1	5	5	?	1	2
28. Dusa 165	5	1	3	1+3	1	1	4	1	3	1	1	1
29. Pratap	5?	1	3?	3	1	1	6	1	5	?	1	?
30. V. P. 835	1	1	3	1	1	1	?	8	2	3	1	2
31. S V 66342	2	1	3	3	1	1						
32. Virovskaya	4	1	1	1	3	1	2					
33. Virovskaya	7	3	4	3	1	1	2					
34. Tapio	5	1	3	?	1	?						
35. TC[6] x Lr 21(W3557)	1	7	4	3	1	2						
36. Arkas	3	4	9	1	1	1						
37. Famos	3	4	9	1	1	1						
38. Hibice 2 –076	5	?	5	3	1	1						
39. 4 S 7MS P 6173	2	1	1+5	3	1	1						
40. Panther	1	1	2	3	1	1						
41. V – 18	5	15	1	1	1	1						

H – Unfamiliar new bands, clusters.

? – Not Understood.

TABLE 2: ELECTROPHORETIC PATTERN OF SOME RECIPROCAL HYBRIDS OF WHEAT

Reciprocal Hybrids	Chromosomes, Controlling Gliadin Synthesis					
	1A	1B	1D	6A	6B	6D
1. T.timopheevi x Complex hybrid	6	1	1+5	1+?	1	1?
2. Complex hybrid x T. timopheevi	6	1	1+5	3H	1	?
3. T.timopheevi X Saratovskaya 29	6?	1	1+5	2	1	1
4. Saratovskaya 29 x T.timopheevi	6?	1+4	1+5	2	1	1
5. T. timopheevi x Cosir	6	1	1+5	1	1	1
6. Cosir x T. timopheevi	6+?	1	1+5	1	1	1
7. Ae.Speltoides Sc x Siettee cerros	5	6	3	1+3	1	1
8. Siette cerros x Ae. Speltoides Sc	5	8	1	1	1	2
9. Ae. Speltoides Sc x Arkas	5+?	6+?	3+?	1+3	1	1
10. Arkas x Ae. Speltoides	3	4+?	3	1	1	1
11. Ae. Squarrosa Sc x Pratap	5	1+6	3	1	1	1
12. Pratap x Ae. Squarrosa Sc	5	1+6	3	1	1	1
13. Ae Squarrosa Sc x Pratap	5	1+?	3	3	1	1
14. Pratap x Ae. Squarrosa	3	1+15	3	1	1	1
15. Haynaldia villosa x Pratap	3+?	4+2	3	1	1	1
16. Pratap x Haynaldia villosa	5	1+?	3	3	1	1
17. T. timopheevi x Famos	6+?	1+4	3+?	1	1	1
18. Famos x T. timopheevi	6+3	1+4	3+?	1	1	1
19. T. Timopheevi x moskovskaya 35	6	1	1+5	?	1	?
20. Moskovskaya 35 x T. timopheevi	6	1	1+5	3H	1	2H
21. T. timopheevi x Lr 19 x (Thatcher)	1	1+7	1	?	1	?
22. Lr 19 x (Thatcher) x T. timopheevi	1	7+1	1	?	1	?

Note: *Ae. Speltoides* Sc = Chinese Spring on the cytoplasm of *Ae. Speltoides* etc.

In this work, the electrophoretic patterns of allocytoplasmic wheat forms and hybrids were studied to determine the role of foreign cytoplasm (plasmogenes) in the inheritance of gliadin components as well as associations between gliadin components and morphological, agronomic and bread quality characteristics of wheat, as an aid to selection process.

MATERIALS AND METHODS

The electrophoretic patterns of 83 spring and winter wheat cultivars and breeding lines including, 41 spring wheat materials with different eco-geographical origin (USA, Australia, India, Netherlands, Sweden, Finland, Austria, Germany, Checks Republic and Russia), 19 allocytoplasmic forms and 22 reciprocal hybrids from crosses between allocytoplasmic forms and different wheat cultivars, were studied by starch gel electrophoresis in aluminum lactate buffer (PH 3.1), using gliadin extracted from wholemeal samples with 6% urea solution (Wrigley and shephere, 1974; Sozinov and Poperelya, 1974, Wringley and M. McCausland, 1977). The electrophoregrammes were converted to numeric form through the help of specialists in the area. This work was carried out at the genetic base for plant breeding unit of the former All Union Institute of Genetics and plant breeding, Odessa, Ukraine. The aim of this study was to establish the possible effect of cytoplasm on the inheritance of gliadin coding loci.

RESULTS AND DISCUSSION

Results of gliadin electrophoretic pattern (Table 1) of spring wheat cultivars showed a clear division into two groups – those with constant genetic electrophoretic pattern of gliadin and those with varietal polymorphism in the component composition of gliadin, that is having two or more biotypes of the same cultivar. As much as 73.2% of studied spring wheat materials have varietal polymorphism in the component composition of gliadin (Table 1). Analysis of genetic formulae of gliadin revealed that differences between biotypes occur more often in 1A, 1B, 1D and 6A loci. From our point of view, varietal polymorphism in the component composition of gliadin increases the varietal genetic variability which enhances the possibility of finding biotypes with needed economically important characters.

An important peculiarity of the genetic control of storage proteins of cereals is the triploid nature of the endosperm which arises from double fertilization. As a result, two chromosomes belong to the female and one to the male parent. Consequently, marked differences are observed in quantitative accumulation of certain proteins depending on which plant served as the female parent. This phenomenon was first observed in wheat and barley by Solari and Favret (1968), Favret *et al* (1970), Sozinov, and poperelya, (1974), Wrigley (19760 and Mecham *et al* (1978). The study of heritability of gliadin components showed that electrophoretic pattern contain the components from both parents. A comparison of patterns of grains from reciprocal crosses revealed the effect of gene dosage from triploid endosperm (Sozinov, 1985). Results of our study (Table 2) show that there are differences between reciprocal F1 hybrids. More over, new components not earlier present in either of the parental forms were found. According to Sozinov (1985) such phenomenon was very often observed in their studies. These phenomena create additional problems in the study of the genetic nature of storage protains of wheat and their use in breeding programmes. The electrophoretic studies of the gliadin composition of allocytoplasmic hybrids (Table 3) showed that, line 96, on the cytoplasm of *S. cereale,* Line 92, on the cytoplasm of *S. cereale* (Igen –3) on the cytoplasm of *Ae. ovata* and selected line from (Igen – 3) on the cytoplasm of *Ae. ovata* had more than one biotype of each, while the other allocytoplasmic forms had constant gliadin formulae throughout the years of the study. The aim of this study was to establish the possible effect of cytoplasm on the inheritance of gliadin coding loci. Analysis of 7 grains of (Igen – 3) on the cytoplasm of *Aa.ovata* revealed that 4 grains had gliadin genetic formula of 9.4 .1.1.1.1., while 3 grains had 3.1.5.3.1.2. This electrophoretic studies coupled with field observations o

TABLE 3:Peculiarities of Gliadin Electrophoretic Patterns of Allocytoplasmic Spring and Winter wheat forms.

Allocytiplasmic forms		Chromosomes, Controlling Gliadin Synthesis					
		1A	1B 1D	6A	6B	6D	
		Spring forms					
1.	Chinese Spring	5	6	3+5	1+3	3	1
		5	6	3	3	3	1
2.	Ae. Comosa Sc.	5	6	3	3	1	1
3.	Ae. Cylindrica Sc	5	6	3	3	1	1
4.	Ae. Variabilis Sc	5	6	3	3	1	1
5.	Ae. Speltoides Sc	5	6	3	3	1	1
6.	Ae. Squarrosa Sc	5	6	3	3	1	1
7.	Ae. Squarrosa St (white glumes)	10+4	15+4	3	1	1	1
8.	Ae. Squarrosa St(awned)	10+4	4	3	1	1	1
9.	Ae. Squarrosa St(red)	10	15	3	1	1	1
10.	Haynaldia Villosa (awnless)	10+4	4	3	1	1	1
11.	Haynaldia Villosa (awned)	4	1+2	3	1	1	1
12.	Line 96 (S. Cereale)	5	4	3	1	1	1
		3	1+4	3+5	1+3	1	2
13.	Line 92 (S. Cereale)	5	4	3	1	1	1+2
		5	4	3	1	1	2
14	Igen-3 (S. Cereale)	Winter forms					
		9	1+4	1+4	1+3	1	1
15.	Igen-3 (Ae.Ovata)	9	1+4	1	1	1	1+2
		9	1+4	1+5	1+3	1	1
16.	Igen-3(Ae. Ovata) awned.	3	1+4	1+5	3	1	2
		3+4	1	5+1	3	1	2
17.	Igen-3	5	1	3	2	3	1
		5	1+4	3	1	1	1
18.	(Igen-3xSarat. 29) Ae. Ovata	5	1+4	3	1	1	1
19.	(Igen-3xPavlovka) Ae. Ovata	4	2	1	1	1	2
20.	(Igen-3x Mir.808) Ae. Ovata	3	1+4	1+5	1+3	1	1

Note: *Ae. Variabilis* Sc = Chinese Spring on the Cytoplasm of *Ae. Variabilis*
Igen – 3 *Ae.Ovata* = Igen-3 on the Cytoplasm of *Ae. Ovata.* Etc.

phenotypic differences resulted in the selection of 4 additional biotypes of that combination – (Table 3). A comparison of gliadin formulae of (Igen –3) on the cytoplasm of *Ae.ovata* and selections from it showed differences in 1A and 6A as well as in 1D and 6D loci. These two biotypes differed greatly from each other phenotypically. While (Igen – 3) on the cytoplasm of *Ae. ovata* was tall, the selection was shorter and more early maturing with better bread quality. It may be possible that genes on these loci are linked with those controlling grain quality , plant height and maturity period. There were also differences in gliadin formulae between the two biotypes of the original variety Igen – 3 and its allocytoplasmic variants. The allocytoplamsic hybrid (Igen – 3 x Saratovskaya 29) on the cytoplasm of *Ae. ovata* has similarities with the original variety in 1A, 1D and 6B loci, while (Igen-3 x Pavlovka) on the cytoplasm of *Ae. ovata* differed from the original variety in 1B locus. Selection from (Igen –3) on the cytoplasm of *Ae. ovata* differed from Igen – 3 in 6A locus, while (Igen –3 x Pavlovka) on the cytoplasm of *Ae. avata* and selection from (Igen – 3) on the cytoplasm of *Ae. ovata* expressed entirely new components in locus 6D. Therefore it may be suggested that loci 1A, 6A, 1B are under the control of nuclear genes. Analysis of other allocytoplasmic hybrids (Siette-cerros x (10D2 x leningradka) on the cytoplasm of *Ae. squarrosa,* (Penjamo x (10D2 x Leningradka) on the cytoplasm of *Haynaldia villosa,* chinese spring on the cytoplasm of different species of *Aegilops,* showed that gladin coding loci from the male parents were represented in the allocytoplasmic hybrids. This suggests that cytoplasmic hereditary factors apparently had no direct effect on gliadin synthesis. This is further confirmed by the gliadin formulae of different nuclear genotypes (*Ae.squarrosa* st and *Ae.squarrosa* Sc) (Table 3) on the cytoplasm of *Ae. squarrosa*.

It is important to note that some Scientists believe that not only the chromosomes of first and sixth homoeologuous groups participate in gliadin synthesis. Other Scientists have identified gliadin- like proteins, called low-molecular gliadin, which were under the control of genes on chromosomes 4B, 7A and 7D. It is also worthy of note that the genetic coding loci, structural and regulatory genes of other systems may exert meaningful impact on the expression of block action (Sozinov, 1985).

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