ELECTROPHORETIC STUDY OF GLUTENINS AND BETA-AMYLASE IN WHEAT RECOMBINANT LINES

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ABSTRACT

A large number of wheat recombinant lines were analysed for storage protein (glutenins) and active protein (beta-amylase) polymorphism, using electrophoresis. The purpose of this paper was to outline the relationships between proteinogram types and bread making quality as well as between zymogram types and embryogenic potential. The experimental results emphasized that a great majority of the recombinant lines has a good glutenin composition, also, it seems that genes of 4BL, 4DL and 5 AL chromosomes could be involved in wheat embryogenic potential.

Key words*:* enzyme, protein, wheat electrophoresis

INTRODUCTION

The high molecular weight (HMW) subunits of glutenin are codified by genes of 1A, 1B and 1D chromosomes (long arm) and beta-amylase - by genes of 4BL, 4DL and 5AL chromosomes.

The genetic progress in obtaining new and superior genotypes may be accelerated through selection based on molecular markers associated with favourable genes.

In the breeding programmes high protein content and good quality of proteins are very important objectives. RICIC Fundulea has a valuable germplasm which is characterized by high quality, good resistance to water and thermic stress, good yield.

In this paper, homozygous wheat recombinant lines concerning HMW subunits (involved in bread making quality) and beta-amylase (linked with embryogenesis capacity) were analysed and proteinogram and zymogram types for 125 genotypes (two parental forms and descendants) were established.

MATERIALS AND METHODS

The electrophoresis for HMW subunits of a single seed in 15% polyacrylamide gel with SDS was performed, on plate, in vertical system. For running tris-glycine buffer was used, for extraction the buffer solution contained: tris-HCI, SDS, 2-mercaptoethanol, pyronine Y and glycine. Staining solution consisted of: Coomassie Brilliant Blue R-250, ethanol and tricloracetic acid. The quality scores assigned to individual or pairs of HMW subunits by Payne (1986) were used (Table 1).

Table 1. The quality scores assigned to individual or pairs of high molecular weight glutenin subunits. Payne, 1986

Score	Chromosome-subunits		
	1A	1B	1D
4	-	-	5+10
3	1 ; 2*	7+8; 17+18	-
2	-	7+9	2+12 ; 3+12
1	null	6+8; 7; 20	4+12

The electrophoresis of beta-amylase in glass tubes, in vertical system, in 5% polyacrylamide gel was performed. For running the buffer included tris-beta-alanine, for extraction the same solution but with 2-mercaptoethanol. As substrate the starch was used and enzyme activity was revealed with iod in kalium iodide. More details were presented in a previous paper (Hagima and Ittu, 1986). The estimation of isoenzymes number in function of electrophoretic mobility (that means different aminoacid structure and distinctive molecular weight) was obtained.

RESULTS AND DISCUSSIONS

There are several traits which contribute to the overall breadmaking quality but it is generally recognized that two of the most important are protein content and protein quality. The protein content is primarily governed by the genetic variation of this complex character and also by agricultural practice and climate.

The recent progress concerning the electrophoretic separation of glutenin subunits, their genetic determinism and their implication in breadmaking quality offers the possibility to perform the indirect selection for the quality using glutenin subunits composition

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which is independent of the environment and may be determined for single grains.

In a previous work (Hagima et al., 1988) we intended to establish the intensity of correlation among the glutenin subunits composition and some technological indices (loaf volume, farinograph value, sedimentation test, grain hardness, protein content and glutenin score) involved in breadmaking quality for certain genotypes grown in Romania. Our studies revealed that the glutenin scores were significantly correlated with the loaf volume, with the farinographic value and with the sedimentation test (the values were: 0.065, 0.0637 and respectively 0.581).

In this paper our investigations evinced that 104 genotypes contained 5+10 subunits pairs which have a good influence on breadmaking quality. In other words, 83.2 % recombinant lines are characterized by a valuable bread making quality. The electrophoretic separation offers the first information regarding qualitative potential of a new genetic source. This kind of analytical procedure is very useful because in the first selection steps the seed quantity is very small, insufficient for technological tests. The subunits glutenin composition may be established working only with 5-10 individual seeds (if the sample is pure from genetic point of view). For technological determinations 150-200 g of seeds are necessary.

For 70 homozygous lines, the embryogenic potential was performed: the two parental lines revealed differences regarding this trait. The somatic embryogenesis analysis revealed this characteristic for 8 genotypes: these results correspond to segregation analysis of crosses Chinese Spring (embryogenic) and Aquilla (non-embryogenic).

These data suggest the existence of oligogenic recessive control.

Table 2. The structure and scores glutenin subunits of analysed wheat genotypes (parental forms and recombinant lines)

Parental forms	No. of samples	HMW subunits					Glutenin scores
Sincron	0						
Recombinants	48		5	7	9	10	7
F 1054	0						
Recombinants	56		5	7	8	10	8
Varia	9	2		7	8	10	6
Recombinants	10	2		7	9	10	5

In order to investigate some possible association between embryogenic potential and genetic markers, χ^2 test was used. For beta-amylase P value was less than 2.5%, showing a significative association of embryogenic potential with the isoenzyme of high molecular weight, specific for parental line F-1054 (Table 2).

The genes involved in beta-amylase biosynthesis are located on 4 BL, 4 DL and 5 AL chromosomes; our results suggest the presence of gene or genes which control somatic embryogenesis on these arms.

CONCLUSIONS

By SDS-PAGE the proteinogram types of 125 homozygous wheat recombinant lines were established. This analysis emphasized the genotypes with the good bread making quality.

For 70 genotypes, the embriogenic potential was performed; by χ^2 test a significative association of this trait with beta-amylase isoenzyme was established. Our results suggest the resource of genes controlling somatic embryogenesis on 4BL, 4DL or 5AL chromosomes.

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