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Possibilities of insertion of glutenin subunits and gliadin components of glu B1, glu B3 and gli B1 loci from *Triticum turgidum* into *Triticum aestivum*

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Abstract

An electrophoretic investigation of the glutenin subunits and gliadin components in F_4 progenies between the hexaploid wheat (*Triticum aestivum* L. cv. Bezostaya 1) and the wild tetraploid wheat (*Triticum turgidum* var. *dicoccoides* Körn) is carried out. The possibility of inserting subunits and components of Glu B1, Glu B3 and Gli B1 loci from *T. dicoccoides* in F_4 progenies of *T. aestivum* is investigated. For this purpose parental forms with different allelic variants in these loci are chosen. It is established cytologically that the chromosomal number of progenies is 42. Genotype classes and the frequency of progenies are analyzed for which we judge by the phenotype manifestation of the allelic variants of the loci investigated. Different frequency of the transfer of subunits and components of Glu B1, Glu B3 and Gli B1 loci from *T. dicoccoides* in the hexaploid wheat genome is established and it is connected with the disposition of the loci on chromosomes arms.

Key words: parents, progenies, wheat

Introduction

Several investigations indicated that high and low molecular mass (HMM and LMM) glutenin subunits as well as specific gliadin components correlated with desirable technological properties of wheat gluten. Two alleles from the region of γ-gliadin components in the hexaploid wheat named 40 and 43.5 according to their electrophoretic mobility in polyacrylamide gel with pH 3.1 are connected with gluten quality (Pogna *et al.* 1982). These components are under the control of genes located on the S-arm of 1B chromosome and are allelic variants of the complex locus Gli B1 (Pogna *et al.* 1985). Recent investigations with tetraploid wheats showed that gluten properties were affected by the allelic variation of LMM glutenin subunits at Glu B3 locus (Ciaffi *et al.* 1991). HMM glutenin subunits in the hexaploid wheat are coded by three loci on the L-arms of the chromosomes of first homoeologous group marked as Glu 1 locus (Lawrence and Shepherd 1980). Molecular investigations showed that

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there are two genes on each Glu 1 locus one of which codes the subunit with higher molecular mass (x), and the other the subunit with lower molecular mass (y) (Harberd *et al.* 1986). In many hexaploid wheats, *e.g.* Chinese Spring, HMM glutenin subunits coded by chromosome 1A are absent (Forde *et al.* 1985). In some cultivars the subunit Glu A1x is present while the subunit Glu A1y is not registered. The probable reason is that Glu A1 genes are at zero state in the diploid ancestor (Waines and Payne 1987).

Modern plant breeding results in a considerable decrease of genetic variability among cultivars. For this reason the improvement of crop quality is connected to the rich genetic potential of their ancestors (Nevo *et al.* 1983). The wild tetraploid wheat *T. turgidum* var. *dicoccoides* is an appropriate donor for genes responsible for important biological characters of wheat such as hich protein content (Levy and Feldman 1987), disease resistance and drought hardiness.

The aim of the present study is to investigate the possibility of including genes of Glu B1, Glu B3 and Gli B1 loci of *T. turgidum* var. *dicoccoides* in the corresponding loci of hexaploid wheat *T. aestivum* cv. Bezostaya 1. For this purpose allelic variants of these loci specific only for *T. dicoccoides* are chosen.

Materials and methods

Seeds of ninety F_4 hybrid plants obtained between *T. aestivum* cv. Bezostaya 1 (2n = 6x = 42, AABBDD) (female) and the wild tetraploid wheat *T. turgidum* var. *diccoccoides* (2 n = 4x = 28, AABB) (male) were investigated. The accession of *T. turgidum* var. *diccoccoides* (Körn) No. C 127 originated from Israel. Many of the F_1 hybrids obtained were sterile as the meiotic process was disturbed because of the irregular homoeologous sinapsis. F_1 hybrids were backcrossed with cv. Bezostaya 1 for overcoming the sterility. Hybrid seeds in F_4 were obtained by further self-pollination of BC_1 plants. Cytological investigations showed that the chromosomal number of the hybrids is 42. The hybrids were developed in the experimental field of the Institute of Genetics, Sofia, Bulgaria. For determining the chromosomal control of cv. Bezostaya 1 components a protein extract of the modal cultivar Chinese Spring was applied in each experiment (Galili and Feldman 1983, Joppa *et al.* 1983).

Glutenins are extracted from individual grains of the parental cultivars and hybrids with 62.5 mM Tris-HCL buffer containing 2 % (m/v) sodium dodecyl sulphate (SDS) and 5 % (v/v) 2-mercaptoethanol according to Payne *et al.* (1980). Electrophoretic division of glutenin subunits is carried out using an apparatus GE 3/4 (*Pharmacia*, Uppsala, Sweden) on a vertical block polyacrylamide gel (18 cm × 14 cm × 1.5 mm). The electrophoretic division is carried out by the method of Laemmli (1970) on 8.5 % polyacrylamide gel (PAGE). 0.01 cm^3 of the supernatant is loaded into each slot. Tris-glycine buffer, pH 8.3, is used as electrode buffer system. Glutenin subunits are visualized with 0.25 % *Coomassie Brilliant Blue* G 250 in 12 % trichloracetic acid for 48 h. Decolouration of the gels is performed with 12 % trichloracetic acid.

Gliadins were extracted from individual seeds for 1.5 h with 70 % ethanol and the supernatant was fractionated by gel electrophoresis in aluminium lactate buffer at pH 3.1 according to the method of Bushuk and Zillman (1978).

The specific for the parental cultivars LMM glutenin subunits and ω-and γ-gliadins are pointed by arrows in Figs. 1 and 2. The controlled regions marked in Fig. 1 and Fig. 2 concern Chinese Spring model cultivar and the parental cultivars and progenies as well. As a criterion of homology between the subunits and components of the cultivars and hybrids investigated relative electrophoretic mobility directly estimated on the gel is used.

Results and discussion

The HMW glutenin subunits in cv. Bezostaya 1 spectrum are four. Two of them - with the slowest and the fastest electrophoretic mobility (shown as 1Dx and 1Dy in Fig. 1) are under the control of genes of Glu D1 locus and subunits 1Bx and 1By are under the control of genes of Glu B1 locus. The chromosomal control of Bezostaya 1 subunits is established on the basis of the area of subunits with respective molecular masses in the hexaploid wheats that are under the control of these chromosomes (Payne *et al.* 1981) as well as on the basis of the genetic control of the subunits of cv. Chinese Spring (Galili and Feldman 1983). According to the nomenclature of Payne and Lawrence (1983) the allelic variant of the 1B chromosome locus in cv. Bezostaya 1 corresponds to variant 7 + 8 (allel Glu B1-b).

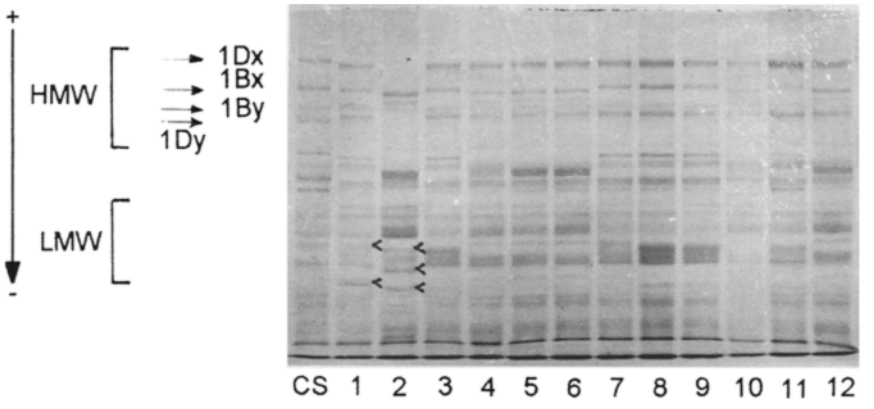

Fig. 1. SDS-PAGE electrophoretograms of glutenin subunits of the cv. Chinese Spring (CS), cv. Bezostaya 1 (1), accessions of *T. dicoccoides* (2), and F_4 progenies (3-12). HMM and LMM - high-molecular-mass and low-molecular-mass glutenin subunits. The genetic control of the subunits is after Galili and Feldman (1983). HMW and LMW controlled regions concern parental cultivars (1, 2) progenies (3 - 12) and model cv. Chinese Spring (CS) as well.

In the area of the HMW glutenin subunits *T. dicoccoides* spectrum is presented by three subunits, two of which are specific and the third one is homologous for the two

parents. Gradation in the intensity of the subunits is established and the nearest to the anode subunit is with highest intensity compared with the others.

The chromosomal control of the HMM glutenin subunits from *T. dicoccoides* spectrum is estimated on the basis of their distribution in F_4 progeny. The subunit 1Bx from cv. Bezostaya 1 spectrum segregates as an alternative allel of the two specific for *T. dicoccoides* subunits with lower electrophoretic mobility (Fig. 1, columns 4-9). This fact allows us to admit that the two subunits are also under the control of genes located in homologous 1B chromosome locus of *T. dicoccoides* and are marked as 1Bx and 1Bx'. The frequency of transmission in F_4 progeny of the allelic variant of the Glu B1 locus from *T. dicoccoides* is examined below in our study. The homologous for the two parents subunit 1By is established in the spectra of all progenies investigated. The subunits 1Dx and 1Dy are handed down the F_4 progeny independently from the subunits of Glu B1 locus. Subunits on Glu A1 locus of the accession of *T. dicoccoides* are not expressed although other authors report for allelic variants at this locus (Levy *et al.* 1988). The reasons for absence of subunits of this locus in our investigation may result from several factors: different subunit composition of the accession of *T. dicoccoides* investigated, different extraction procedures and various percentage of separating gels as well.

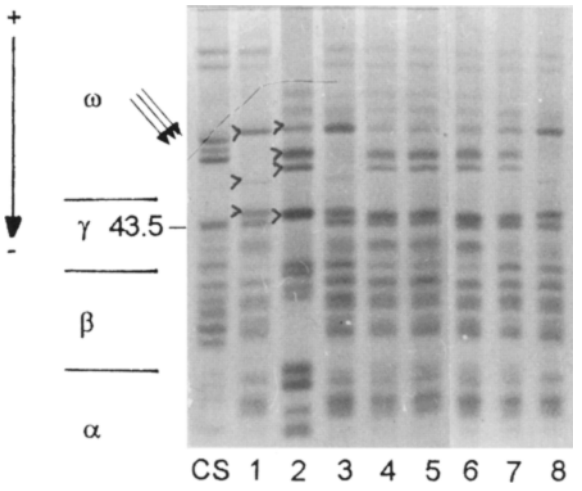

Fig. 2. SDS-PAGE electrophoretograms of gliadin components of the cv. Chinese Spring (CS), cv. Bezostaya 1(1), *T. dicoccoides* (2) and their F_4 progenies (3 - 8). α-, β-, γ-, ω-zones with different electrophoretic mobility. The main components from ω- and γ-zones coded by Gli B1 locus are indicated by arrows. The ω- and γ-gliadins controlled regions concern parental cultivars (1, 2), progenies (3 - 8) and the modal cv. Chinese Spring (CS).

The number of LMM glutenin subunits coded by Glu B3 genes in cv. Bezostaya 1 is six and in *T. dicoccoides* seven. The principle difference between the two parents is expressed in the number of the specific subunits - two for cv. Bezostaya 1 and three for *T. dicoccoides* (shown by arrowheads in Fig.1). Essential quantitative differences between the parental subunits in this area are observed as the ones

belonging to Bezostaya 1 spectrum are with low intensity. The different allelic variants of Glu B3 locus in the parenatal lines give possibility to prove their participation in F_4 progeny formation.

The gliadin components from ω- and γ-zones that are under the control of 1B chromosome (shown by arrows and arrowheads in Fig. 2) form locus Gli B1 (Payne *et al.* 1984). The allelic variants of Gli B1 locus are different for cv. Bezostaya 1 and *T. dicoccoides* and the number and intensity of the components is higher for *T. dicoccoides*. The specific for cv. Bezostaya 1 γ-gliadin component 43.5, according to the nomenclature of Zillman and Bushuk (1979), is inherited together with one ω-component. The two components are under the control of 1B chromosome (Sozinov and Poperelya 1980, Pogna *et al.* 1985). Two ω-gliadin components and a γ-component with insignificant higher mobility than γ 43.5 component of cv. Bezostaya 1 spectrum are specific for *T. dicoccoides*.

Table 1. Genotypic classes of proteins after segregation of the allelic variants of Glu B1, Glu B3 and Gli B1 loci in F_4 progenies between cv. Bezostaya 1 and accession of *T. dicoccoides*. (a - allelic variant of cv. Bezostaya 1, b - allelic variant of the accession of *T. dicoccoides*, c - heterozygote, total number of progenies 90)

Genotype classes	Loci Glu B1	Gli B1	Glu B3	No. of progenies in different classes	Frequency of progenies [%]
1	a	a	a	13	14.44
2	a	a	b	1	1.11
3	a	b	b	13	14.44
4	a	b	a	1	1.11
5	b	b	b	15	16.67
6	b	a	a	12	13.33
7	b	c	c	2	2.22
8	a	c	c	1	1.11
9	c	a	a	6	6.67
10	c	b	b	10	11.11
11	c	c	c	16	17.78

It is known that the Gli B1 locus which occurs near the end of the short arm of the satellited region (Payne *et al.* 1981) and Glu B3 that is located on the short arm too, are more closely related compared to Glu B1 locus which occurs on the long arm closely to the centromere (Payne and Lawrence 1983). In the result the frequency of the F_4 progenies that are homozygote by Gli B1 and Glu B3 loci of the parents is high as compared to heterozygotes. As we have in mind that F_4 progeny is progressive one, it may be considered that the frequency of transfer of the subunits and protein components of Gli B1 and Glu B3 loci from *T. dicoccoides* is high, too (42.2 %). The number of the homozygotes with respect to the three loci of *T. dicoccoides* is considerable. As far as the allelic variant of Glu B1 locus of *T. dicoccoides* is concerned it is handed down independently of the allelic variants of Gli B1 and Glu B3 loci and is represented in 32.22 % of the progenies. The

frequency of recombinations between Glu B1 and Glu B3 loci of the two parents is low and it corresponds to their location on 1B chromosome arms.

In conclusion as a result of the investigation considerable frequency of F_4 progenies based on the allelic variants of the Glu B1, Glu B3 and Gli B1 loci of *T. dicoccoides* is proved.

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