UDC: 633.11.547.962.7 : 543.545.2
original scientific paper

Acta Agriculturae Serbica, Vol. I, 1 (1996) 45–51

Acta Agriculturae Serbica
FACULTY OF AGRONOMY ČAČAK

Composition of gliadins in some Yugoslav wheat (Triticum aestivum) varieties

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Abstract: Polyacrylamide gel electrophoresis (PAGE) techniques were used for the analysis of 14 Yugoslav wheat cultivars. Electrophoregrams were evaluated for the relative mobilities of electrophoretic bands and their intensities. A comparison factor was computed which was higher for closely related cultivars (for example, cultivars KG-56 and KG-58). The wheat varieties showed great variation in their electrophoretic gliadin composition, with 72 different bands of gliadins found. For one cultivar of wheat, 23-30 components of gliadins were found. Chromatograms by HPLC were also observed.

Key words: electrophoregrams, electrophoresis, gliadins, plant breeding, proteins, *Triticum aestivum L.*, variety, wheat.

Introduction

According to Osborne (1907), protein fractionation in wheat seed, based on differential solubility, gives evidence of a complex mixture of proteins showing much overlapping of components. Albumins are soluble in water, globulins in diluted salt solutions, gliadins in 70% ethanol and glutenins in 0.1 M acetic acid.

Cereal grains synthesize and accumulate large amounts of storage proteins which are deposited in protein bodies during the course of seed development. Gliadins and glutenins are the major storage proteins of the wheat (*Tr. aestivum*) endosperm. Together they constitute over 80% of total protein in grain (Osborne, 1907). Each variety has specific genetic potentials with respect to baking quality

and protein content, grain yield, ecological adaptability to stresses and resistance to pests. Wheat breeding has historically utilized visual classification procedures. However, changes in breeding strategies, such as crosses between wheat classes, have made phenotypic evaluation nearly impossible. Polyacrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC) methods have been developed for identification of wheat cultivars by „fingerprinting" their gliadin proteins Lookhart et al. (1982), Burnouf et al. (1983), Bietz et al. (1984).

The gliadin electrophoresis methods involve the use of the aluminum lactate buffer (pH=3.1) and starch gels or acid-polymerized polyacrylamide gels Autran (1975), Bushuk and Zillman (1978), Wrigley and Shepherd (1974).

In the present study, two independent methods (PAGE and HPLC) were used to detect gliadin polymorphisms within wheat varieties. The present work, consisting of analysis of flour samples, is part of an investigation on the estimated number of gliadin patterns, the percentage of each pattern type and the effect of environment on the patterns.

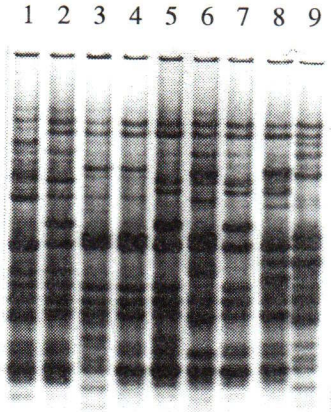
Materials and methods

Grain samples of 14 Yugoslav wheat cultivars were supplied by the Institute of Field and Vegetable crops, Novi Sad and by the Institute for small Grains, Kragujevac. Samples were ground in a Udy Cyclone Mill (Udy Corporation, Fort Collins, Colo, USA). Gliadins were extracted with 70% ethanol (750 ul/25 mg) and centrifuged at 4000 rpm for 10 minutes. The supernatant was used for analysis. To the supernatant 6 drops of glycerol were added to increase the density of the protein solution, and 5 drops 10% methyl green dye. Gliadin extracts were stored at 5°C. The improved PAGE procedures by Lookhart et al. (1982) were used with 6% gel (acrylamide 6.0g, N,N' - methylene-bisacrylamide 0.25 g, ascorbic acid 0.024 g, ferrous sulphate heptahydrate 0.0016 g, hydrogen peroxide 3% 100 ul) and tank buffer solution (aluminum lactate 5.626 g, lactic acid to pH 3.1). Electrophoresis lasted for approximately 2h, with constant 200 mA and 50 kW at 20°C. The power supply and the staining and destaining procedures were described by Lookhart et al. (1982). Each electrophoregram of 14 Yugoslav wheat cultivars was analyzed by measuring the distance between each band and loading place. After that relative mobilities were counted for each gliadin pattern. Reversed-phase HPLC, as described by Bietz and Cob (1985), was also used.

Results and Discussion

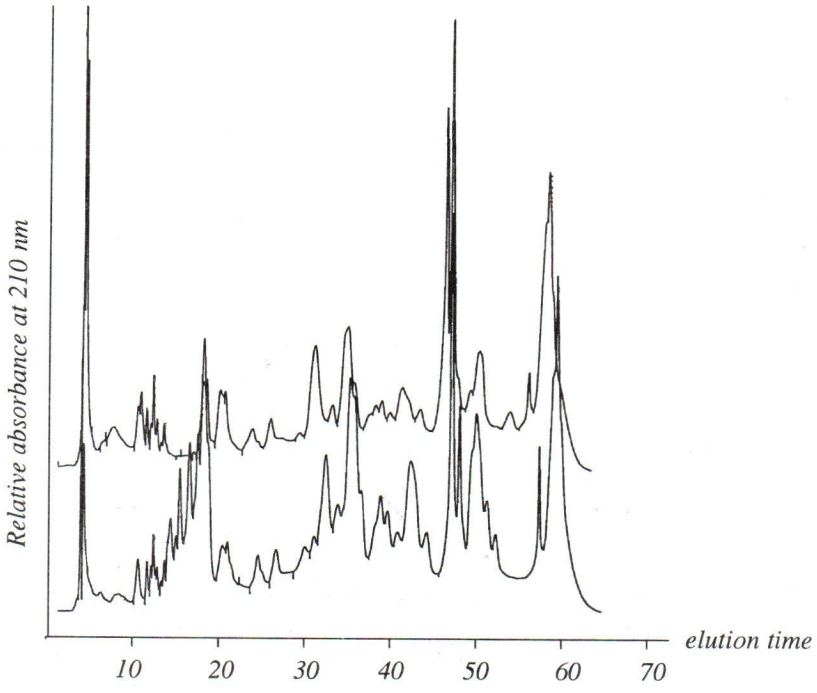
Electrophoregrams were scored according to the method of Lookhart et al. (1982) for detection of intercultivar differences. Electrophoretic bands as well as those relative mobilites exhibited different color intensities rangings from light (1) to dark (5). In one wheat cultivar, between 23 and 30 components of gliadin were observed, and 72 different bands of gliadin were observed among all wheat cultivars (Table 1). Different cultivars showed different combinations of these

Fig. 1 - Electrophoregrams of some Yugoslav wheat varieties



1. L-64/3
2. Šumadija
3. Kragujevačka 75
4. Morava
5. Gružanka
7. Kosmajka
9. Marquis (Check cv.)

Fig. 2 - Hight performance liquid cromatogram of Kragujevačka 56 cultivar



KG-56–very similar; Srbijanka–similar; Šumadija–similar; KG-75–similar; Morava–similar; Gružanka–very similar; Kosmajka–very similar; Lepenica–very similar; Orašanka –very similar; Ljubićevka–similar; Zastava–different; KG-58–very similar; L-64/3–different; KG 33/3–different;

components and the gliadin composition was characteristic of a given cultivar (Fig. 1). Electrophoretic bands were compared and cultivars were grouped according to composition of factors determined.

The comparison factors were found in combination percentage of band position matches and intensity matches value. These value combinations gave an overall similarity value between the wheats. The maximum comparison factor is 100.0, meaning that the wheats compared are exactly the same. The Zastava, Kragujevačka 58, Lepenica, KG-33/3 and Ljubičevka cultivars gave bands similar to those of 5 selected cultivars. The highest value for the comparision factor, being 85.8, was found for the cultivars Ljubičevka and Kragujevačka 58, while the lowest, 22.6, was found for the cultivars Lepenica and Morava (Table 2).

Tab.1. Electrophoretic formulas of gliadins for analysed Yugoslav wheat cultivars

r.m.	10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Cultivar	
Kragujevačka 56	2 4 3 1 3 3 5 1 2 5 5 4 3 3 3 4 3 4 2 2 2 3 4 2 1
Srbijanka	1 4 4 1 2 3 3 2 2 3 3 5 3 4 5 3 5 2 4 1 2 4 4 3 1
Šumadija	2 4 4 1 3 2 2 5 2 4 1 1 5 2 5 2 2 4 4 3 4 4 1 2 2 4 3
Kragujevačka 75	1 3 3 2 2 1 4 3 1 3 1 2 1 5 5 1 1 1 4 4 4 3 3 2 3 3 2
Morava	4 4 1 1 1 4 2 1 3 1 5 5 1 2 4 4 4 1 2 3 4 2
Gružanka	1 4 5 1 2 2 2 5 4 1 2 2 5 1 5 2 1 4 5 4 1 3 3 3 5 3 1
Kosmajka	4 4 1 2 2 2 4 4 1 2 1 5 1 5 2 2 3 4 4 2 3 4 2
Lepenica	4 4 1 2 2 5 2 1 1 3 2 5 5 3 4 2 3 3 3 3 4 4 3 2 1 3 3 4 2
Orašanka	2 4 1 3 1 2 1 3 5 1 1 5 5 4 3 3 3 3 4 3 2 1 2 3 3 2
Ljubičevka	2 4 4 1 2 3 5 1 1 1 5 5 4 4 3 3 3 3 4 4 3 3 1 3 3 4 2
Zastava	2 4 4 1 1 1 4 1 1 1 1 1 5 5 3 4 1 3 3 3 3 4 1 3 4 4 3 2 2 2 3 3 2
Kragujevačka 58	2 4 4 3 1 4 4 1 1 5 5 4 3 3 3 4 4 4 3 2 1 3 3 3 1
L-64/3	2 3 2 3 3 3 1 3 4 3 2 2 4 5 2 5 5 5 4 4 1 2 2 2 4 2
KG-33/3	2 3 3 2 2 3 3 3 1 5 5 2 3 1 3 3 3 4 3 4 4 1 1 2 3 1 3 2
r.m.	10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90

Relative band intensity: 1 is lightest, 5 is darkest. All gliadin bands were found between 12 and 83 units.

Reversed-phase HPLC can also be used to detect gliadin polymorphisms within wheat varieties (Fig. 2). With an improved method of Bietz and Cobb (1985) significant differences in gliadin composition were recorder among the wheat cultivars. These results show that HPLC and PAGE are capable of separating and differentiating major gliadin proteins in polymorphic wheat cultivars.

Tab. 2. The values of comparison factors of the compared cultivars according to band position matches and intensity matches value

Cultivar														
(1) Kragujevačka 56	2.51	33.1	34.7	35.9	38.0	40.1	42.5	45.7	47.1	48.9	50.0	50.6	68.0	100.0
	(5)	(6)	(3)	(7)	(14)	(13)	(4)	(8)	(9)	(11)	(2)	(12)	(10)	(1)
(2) Srbijanka	23.3	24.8	25.2	31.9	36.3	37.4	39.3	43.2	43.5	43.8	46.3	49.3	54.3	100.0
	(7)	(5)	(3)	(9)	(6)	(8)	(11)	(12)	(14)	(4)	(13)	(1)	(10)	(2)
(3) Šumadija	28.8	32.8	34.6	37.2	37.9	39.0	42.0	42.5	43.4	46.9	47.1	58.0	66.1	100.0
	(9)	(11)	(14)	(10)	(8)	(12)	(2)	(5)	(1)	(4)	(13)	(7)	(6)	(3)
(4) Kragujevačka 75	27.7	36.3	36.5	39.0	39.2	39.3	39.5	42.1	42.9	43.0	44.0	53.9	56.5	100.0
	(9)	(7)	(11)	(10)	(13)	(5)	(8)	(12)	(2)	(3)	(14)	(6)	(7)	(5)
(5) Morava	24.4	26.4	28.1	28.5	29.2	30.7	34.0	34.6	35.4	44.0	44.6	46.7	60.0	100.0
	(9)	(11)	(8)	(13)	(10)	(14)	(12)	(1)	(2)	(3)	(4)	(6)	(7)	(5)
(6) Gružanka	25.0	32.7	34.3	34.6	35.9	36.8	37.3	43.9	44.7	53.1	53.5	60.2	63.0	100.0
	(9)	(14)	(11)	(10)	(12)	(8)	(1)	(5)	(13)	(2)	(4)	(3)	(7)	(6)
(7) Kosmajka	28.2	28.5	33.0	33.1	33.9	33.9	34.8	38.3	39.7	42.0	52.5	56.8	63.0	100.0
	(9)	(8)	(14)	(11)	(4)	(1)	(10)	(2)	(13)	(12)	(3)	(5)	(6)	(7)
(8) Lepenica	22.6	29.1	30.0	30.7	31.2	31.4	31.8	35.5	57.8	57.9	58.3	61.3	67.5	100.0
	(5)	(6)	(13)	(7)	(3)	(2)	(4)	(1)	(9)	(12)	(14)	(11)	(10)	(8)
(9) Orašanka	25.2	26.1	28.8	29.0	30.7	33.1	35.3	37.5	45.0	53.3	53.4	61.3	62.3	100.0
	(5)	(6)	(7)	(3)	(4)	(2)	(13)	(1)	(10)	(8)	(11)	(14)	(12)	(9)
(10) Ljubičevka	27.4	30.7	31.8	34.0	37.9	41.3	41.5	47.1	60.7	66.6	67.9	69.4	85.8	100.0
	(5)	(3)	(6)	(7)	(2)	(1)	(13)	(4)	(14)	(9)	(8)	(11)	(12)	(10)
(11) Zastava	27.1	27.9	28.1	30.7	30.8	33.3	36.2	40.6	60.3	63.3	63.7	64.0	66.6	100.0
	(5)	(6)	(3)	(13)	(2)	(7)	(4)	(1)	(12)	(14)	(6)	(100)	(8)	(11)
(12) Kragujevačka 58	33.2	35.3	35.3	36.4	38.9	41.5	44.2	48.6	57.9	60.5	62.9	66.9	68.5	100.0
	(6)	(3)	(2)	(5)	(4)	(7)	(13)	(1)	(8)	(11)	(14)	(10)	(9)	(12)
(13) L-64/3	24.2	25.7	30.5	31.5	34.6	36.0	39.2	39.9	40.2	44.4	44.7	45.2	47.6	100.0
	(11)	(8)	(9)	(1)	(14)	(10)	(5)	(12)	(7)	(4)	(3)	(6)	(2)	(13)
(14) KG-33/3	27.9	28.6	30.4	32.3	33.4	36.1	37.2	38.5	53.3	53.8	55.1	58.3	59.9	100.0
	(5)	(6)	(7)	(1)	(14)	(2)	(3)	(4)	(10)	(12)	(9)	(8)	(11)	(14)

Each cultivar was compared with all cultivars according to band position matches and intensity matches value. Number in brackets is ordinal numeral of compared cultivar.

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Received: 17. 04. 1996.
Accepted: 13. 05. 1996.

SASTAV GLIJADINA KOD NEKIH JUGOSLOVENSKIH SORTI PŠENICE *(Triticum aestivum L)*

- originalni naučni rad -

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Rezime

Za analizu kompozicije glijadina kod 14 jugoslovenskih sorti pšenice korišćena je poliakrilamidna gel elektroforeza. Elektroforegrami su ocenjeni na osnovu relativne mobilnosti elektroforetskih traka, koje su numerički označene. Analiza dobijenih elektroforegrama pokazala je različitu kompoziciju glijadina kod različitih sorti pšenice. Razlike su ustanovljene prema broju i relativnoj pokretljivosti komponenti glijadina. Takođe za komponente sa istom i za komponente sa različitom relativnom pokretljivošću su nađene razlike. Kod analiziranih sorti je ustanovljena varijabilnost u kompoziciji glijadina. Tako je nađeno 72 različite trake glijadina. Broj komponenti glijadina za jednu sortu pšenice varirao je između 23-30.

Sličnije elektroforegrame su imale srodnije sorte. Sličnost sorti na osnovu elektroforegrama je predstavljena koeficijentom sličnosti, koji je računat u poređenju parova traka i njihovog intenziteta. Najveći koeficijent srodnosti bio je 85.8, nađen za sorte Ljubičevka i KG-58 a najmanja vrednost 22.6 između Lepenice si Morave.