ANALYSIS OF SEED PROTEINS IN DITELOSOMES OF COMMON WHEAT¹⁾

KAZUHIKO NODA AND KOICHIRO TSUNEWAKI

Laboratory of Genetics, Faculty of Agriculture, Kyoto University,
Kyoto 606

Received May 1, 1972

Electrophoretic techniques have been used extensively in genetic studies. In wheat, many workers have successfully observed marked differences in proteins: Johnson (1967a, b, 1968) and Johnson *et al.* (1967) compared electrophoretic patterns of endosperm proteins among several species and obtained information on their phylogenetic relationships. Boyd and Lee (1967) reported that chromosome 1D affected the pattern of gliadin protein. Sheperd (1968), in a study of ditelocentric (ditelo) and nullisomic-tetrasomic lines of Chinese Spring wheat, suggested that homoeologous groups 1 and 6 are concerned with differences in protein (gliadins) patterns. Barber *et al.* (1968) showed that homoeologous group 3 controls esterase isozyme patterns in rye and wheat.

Disc and zone electrophoresis and other electrophoretic techniques were used in the above-mentioned studies. An isoelectrofocusing method using acrylamide gel has been a useful analytical technique for separating proteins and isozymes (Wrigley 1968). This technique permits determination of the isoelectric points of individual protein components with sharpening protein bands as fractionation proceeds. In the present paper, isoelectrofocusing was used to fractionate crude seed proteins from different ditelocentric lines of a common wheat, *Triticum aestivum* cv. Chinese Spring, in order to identify chromosomes which control individual protein fractions.

MATERIALS AND METHODS

Materials used were Chinese Spring wheat (*Triticum aestivum* L.) and its 20 ditelocentric lines, which were produced by Dr. E. R. Sears, University of Missouri (Table 1). Each ditelocentric line lacks a chromosome arm of the designated chromosome. Therefore, the loss of a given protein band in a certain ditelocentric line is due to the deficient chromosome arm in that line.

Seeds were immersed in deionized water for 24 hr. They were then homogenized in Tris-glycine buffer solution (Tris 6 g plus glycine 28.8 g in 1 l of water, pH 8.7). The homogenate was centrifuged at about 10,000 g for 1/2 hr, after which the supernatant was poured into the upper part of a glass tube filled with acrylamide gel. Crude protein extract was fractionated using the modified method of Wrigley (1968) and Nakai *et al.*

1) Contribution from the Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Japan, No. 368.

Table 1. Ditelocentric lines of Chinese Spring wheat used in the present experiment

Homoeologous group	Genome		
	A	B	D
1	L	L or S	L
2	*	L or S	α
3	L	L	α
4	α	L	α
5	L	L	L
6	S	L or S	α or β
7	L or S	L or S	S

L: long arm, S: short arm, α : α arm, β : β arm.

* This line was not used.

Note: In each ditelocentric line, the indicated arm is present, and the other arm is missing. For example, in ditelocentric 1AL, short arms of both 1A chromosomes are missing.

(1971). The separating gel was composed of: A solution (polyacrylamide 30 g+BIS 0.8 g+TEMED 0.2 g and was made up to 100 ml with water); B solution (an aqueous solution of riboflavin, concentration 40 ppm); C solution (8% Ampholine, pH 5-8, LKB Co.); 8M urea and water in a volume ratio of 2:1:1:2:2. The apparatus described by Davis (1964) for disc electrophoresis was used. The anode solution was 0.02 M HCl and the cathode solution was 0.02 M ethylenediamine. An electric current of 200 V was passed through the gel for three hours. After gel columns were removed from the tubes, protein bands were detected as white precipitation bands by immersing them in 5% TCA solution.

RESULTS AND DISCUSSION

The characteristic protein patterns of a disomic (normal Chinese Spring) and six ditelocentric lines belonging to A genome are shown in Fig. 1a. Ditelocentric line 2A (ditelo-2A) was not used, because no seeds were available for analysis. Twelve to thirteen protein bands were recognizable, though the diagram shown in Fig. 2 indicates only eight major bands. Ditelo-3A lacked band 3 and had a dense diffused band between bands 1 and 2. Bands 4 and 5 of ditelo-4A and 6A were faint in comparison to other ditelocentrics of A genome. Fig. 1b shows the protein profile of the disomic and seven ditelocentric lines of B genome. Ditelo-1B had a faint band 5. Ditelo-3B lacked bands 3 and 4, having a faint band 5, and a dense diffused band between bands 1 and 2. Also, ditelo-2B had the dense diffused band at the same position. Ditelo-4B had a faint band 2. Fig. 1c shows a protein profile of the disomic and seven ditelocentric lines of D genome. Ditelo-3D had a dense diffused band between bands 1 and 2, and lacked the most prominent band 7.

Fig. 1. Seed protein profile of the disomic and 20 ditelocentric lines of Chinese Spring wheat obtained by the isoelectrofocusing method.

From left to right: (a) Disomic, ditelo-1A, 3A, 4A, 5A, 6A and 7A. (b) Disomic, ditelo-1B, 2B, 3B, 4B, 5B, 6B and 7B. (c) Disomic, ditelo-1D, 2D, 3D, 4D, 5D, 6D and 7D.

Fig. 2. Diagrammed seed protein profiles of the disomic and 20 ditelocentric lines (1A to 7D) of Chinese Spring wheat, obtained by the gel isoelectrofocusing method.

We concluded that the short or β arms of three homoeologous chromosomes, 3A, 3B and 3D, control the production of three seed protein fractions, i.e., bands 3, 4 and 7, respectively. In all three ditelocentric lines of these chromosomes, a common accumulation of protein fraction was noticed at a diffused band between bands 1 and 2. Though the mechanism for extinction of band 3, 4 or 7 accompanied by intensification of the diffused band is unknown, it is evident that the three chromosomes of the homoeologous group 3 have similar but mutually differentiated functions in the production (or conformation) of seed proteins. Possibly the diffused band between bands 1 and 2 is the band for the precursors or derivatives of the protein in bands 3, 4 and 7, though it is not clear why precursors or derivatives of those bands always appear on the acidic side. Sears (1957) reported that decreased dosage of chromosome 3A or 3D caused seed lethality, when Neatby's virescent gene located on chromosome 3B became homozygous. The physiological relationship between this type of seed lethality and our finding that the loss of a certain band is caused by a decrease in the dosage of those chromosomes must be investigated. Kuspira and Unrau (1956) reported that, in comparison to normal Chinese Spring, the protein content was significantly increased in Chinese Spring line, whose chromosome 3D is substituted by Thatcher's chromosome 3D. This may be related to the fact that the major protein fraction, band 7, is con-

trolled by chromosome 3D, if Thatcher has a more effective gene on this chromosome than Chinese Spring.

SUMMARY

Buffer soluble seed proteins of 20 ditelocentric lines of a common wheat, *Triticum aestivum* cv. Chinese Spring, were analyzed by gel electrofocusing. Result clearly showed that the short arms of chromosome 3A and 3B, and the β arm of chromosome 3D are responsible for the respective formation of three fractions, bands 3, 4 and 7. These chromosome arms all had a common effect on a diffused band between bands 1 and 2. This provides additional evidence for the genetic homoeology between 3A, 3B and 3D chromosomes. Of the other chromosomes, chromosomes 4A, 6A, 1B and 4B showed some qualitative effects on one or two bands.

ACKNOWLEDGMENTS

We wish to express our sincere thanks to Dr. M. Okamoto of the Agricultural College of Nara, for providing us with the ditelocentric lines of Chinese Spring wheat used here, which were originally produced by Dr. E. R. Sears. We are also greatly indebted to Mr. T. Teramura, Kyoto University, for his technical advice.

LITERATURE CITED

- Barber, H. N., C. J. Driscoll and R. S. Vickery, 1968 Enzymic markers for wheat and rye chromosomes. Proc. III Int. Wheat Genet. Symp., Canberra: 116-122.
- Boyd, W. J., and J. W. Lee, 1968 The control of wheat gluten synthesis at the genome and chromosome levels. *Experientia* **23**: 332-333.
- Davis, B. J., 1964 Disc Electrophoresis—II. Method and Application to Human Serum Proteins. *Annals, New York Academy of Sciences*: 404-427.
- Johnson, B. L., 1967a Tetraploid wheats: Seed protein electrophoretic patterns of the Emmer and Timopheevi groups. *Science* **158**: 131-132.
- Johnson, B. L., 1967b Confirmation of the genome donors of *Aegilops cylindrica*. *Nature* **216**: 859-862.
- Johnson, B. L., 1968 Electrophoretic evidence on the origin of *Triticum zhukovsky*. Proc. III Int. Wheat Genet. Symp., Canberra: 105-110.
- Johnson, B. L., D. Barnhart and O. Hall, 1967 Analysis of genome and species relationship in the polyploid wheats by protein electrophoresis. *Amer. J. Bot.* **54**: 1089-1098.
- Kuspira, J., and J. Unrau, 1957 Genetic analysis of certain characters in common wheat using whole chromosome substitution lines. *Canad. J. Plant Sci.* **37**: 300-326.
- Nakai, Y., and K. Tsunewaki, 1971 Isozyme variation in *Aegilops* and *Triticum*. I. Esterase isozymes in *Aegilops* studied using the gel isoelectrofocusing method. *Japan. J. Genet.* **46**: 337-344.
- Sears, E. R., 1957 Effect of chromosomes XII and XVI on the action of Neatby's virescent. *Wheat Inform. Serv.* **6**: 1.
- Sheperd, K. W., 1968 Chromosomal control of endosperm proteins in wheat and rye. Proc. III Int. Wheat Genet. Symp., Canberra: 86-96.
- Wrigley, C., 1968 Gel electrofocusing. A technique for analyzing multiple protein samples by isoelectrofocusing. *Science Tools* **15**: 17-23.