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Effect of Rye Chromosome 2 Substitution on Kernel Protein Content of Wheat

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Summary. Significant increases in the kernel protein content of lines of the bread wheat variety Chinese Spring, in which a pair of rye chromosome 2 substitutes for group 2 chromosomes of wheat, were observed in plants grown at three different locations. Fractionation of proteins on the basis of their solubility did not show any significant variation in the relative proportions of alcohol, salt, acid and alkali soluble proteins. Similarly, electrophoretic studies of saline, alcohol and acetic acid-urea soluble proteins did not reveal any addition or deletion of protein bands in comparison with the Chinese Spring control. Apparently, the substituted rye chromosomes do not contribute any proteins different from those already present in wheat. The electrophoretic data give further evidence of the homoeology of rye chromosome 2 with the group 2 chromosomes of wheat.

Introduction

A significant increase in the protein content of lines of the bread wheat variety Chinese Spring having a substituted pair of rye chromosome 2 was observed during the course of electrophoretic analysis of the kernel proteins. This led to a more detailed investigation of the available material, the results of which are presented in this paper.

Materials and Methods

The wheat-rye substitution lines used in the present investigation were developed and described by Sears (1968). Bread wheat (*Triticum aestivum* L. em. Thell, ssp. *vulgare* (Vill., Host) Mac Key) is an allo-hexaploid with $2n = 6x = 42$ chromosomes which fall into seven homoeologous groups belonging to the *A*, *B* and *D* genomes (Sears, 1966). Rye (*Secale cereale* L.) is a diploid species ($2n = 14$) belonging to the same sub-tribe *Triticinae* as wheat. It is believed that rye, wheat and other species belonging to *Triticinae* originated from a common ancestral diploid species. Thus, rye and *Agropyron* chromosomes are known to substitute, partially or fully, for the homoeologous wheat chromosomes. In the lines investigated, a pair of rye chromosome 2 (III as per the nomenclature of Riley and Chapman, 1958) from var. Imperial have been substituted for the wheat chromosomes of group 2. These lines are referred to as 2 R(2 A), 2 R (2 B) and 2 R (2 D), using the nomenclature suggested by Kimber and Sears (1968). The figures in parenthesis show the chromosome pair that has been replaced. In 2 R(2 A) the rye chromosome is only partly 2 R. Morphologically these plants look similar to the control and are slightly later in flowering. The ears show a tendency to speltoidy and have slightly reduced seed set (Figure 1).

Foundation seed was kindly provided by Dr. E. R. Sears and was increased at Brookhaven and Trombay. The protein content of whole kernels was estimated by micro-Kjeldahl's and/or the Folin phenol method (Lowry *et al.*, 1951). Each sample was analyzed at least three times and the protein percentage was calculated on the mean of several readings. For the latter method, flour obtained after grinding the seeds was homogenised in AUC solvent (Acetic acid 0.1M, urea 3 M and 0.01 M cetyl trimethyl ammonium bromide) and left overnight. After centrifuging at 27,000 × g the supernatant was used for protein estimation.

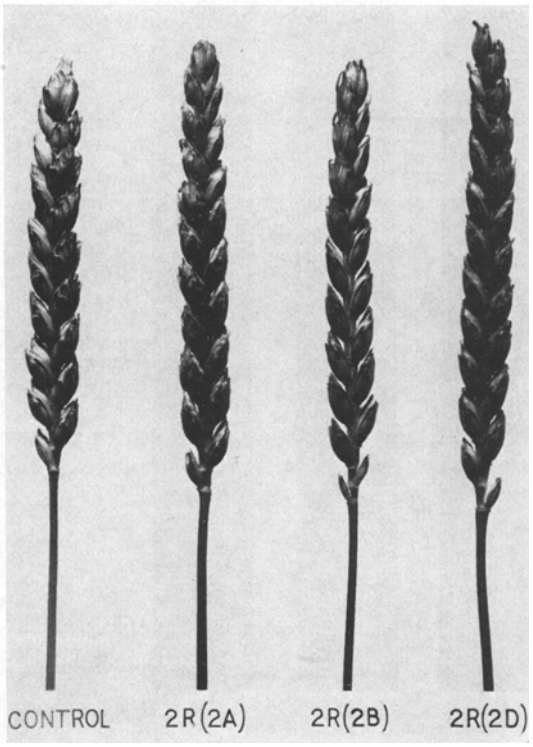

Fig. 1. Spikes of Chinese Spring, 2 R(2 A), 2 R(2 B), and 2 R(2 D) substitution lines

Fractionation of proteins into albumin, globulin, gliadin and glutenin was carried out by sequential extraction in 40% isopropanol, 2% NaCl, 3.85% lactic acid and 0.1% KOH using a slight modification of the procedure outlined by Mattern *et al.* (1968). The alcohol, NaCl and AUC extracts were further fractionated by disc electro-

phoresis on 7.5% polyacrylamide gels, using Tris-glycine buffer pH 8.3 (Davis, 1964) and acetic acid-beta alanine buffer pH 4.5 (Reisfield, 1962). After electrophoresis, the gels were stained with 1% amido-black in 7% acetic acid and destained electrophoretically. Quantitative estimation of the proteins in each band was made using a Chromoscan MK II (Joyce Loebl) recording-integrating densitometer.

Results

Table 1 presents the results of protein estimations made on seed samples from plants grown at different locations. Rye substitution lines 2R(2B) and 2R(2D) consistently showed an increased kernel protein content compared with the parental line Chinese Spring at three different locations. 2R(2A), in which the substituted chromosome is only partly 2R, showed an increased protein content in plants grown at Brookhaven and Trombay. Seed samples of disomic addition of 2R in Chinese Spring together with the control from different locations were not available. One sample of the 2R disomic addition line did show an increase of 4% absolute protein over the control.

Table 1. *Protein content of Chinese Spring and 2 R substitution lines, expressed as percent protein, from plants grown at different locations*

Line/Location	Columbia	Brookhaven	Trombay	
	1967	1966	1968–69	1969–70
Chinese Spring	13.8	14.8	11.9	13.6
2 *R*(2 *A*)	12.0	18.4	15.3	15.0
2 *R*(2 *B*)	16.0	20.2	18.1	15.9
2 *R*(2 *D*)	19.4	16.4	18.7	15.8
2 *R* Disomic addition	—	—	—	17.6

Kernel moisture 10–12%

To further analyze these lines, kernel proteins were separated into alcohol, salt, acid, and alkali soluble fractions. Figure 2 shows the proportion of each of these fractions relative to the total protein for the

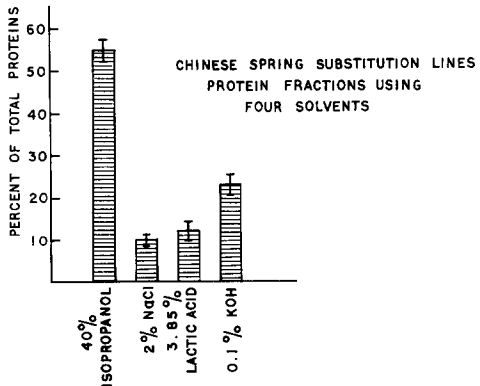

Fig. 2. Relative proportions of isopropanol, NaCl, lactic acid and KOH soluble fractions as percent of total proteins recovered from Chinese Spring control kernels. Substitution lines were not significantly different from this pattern

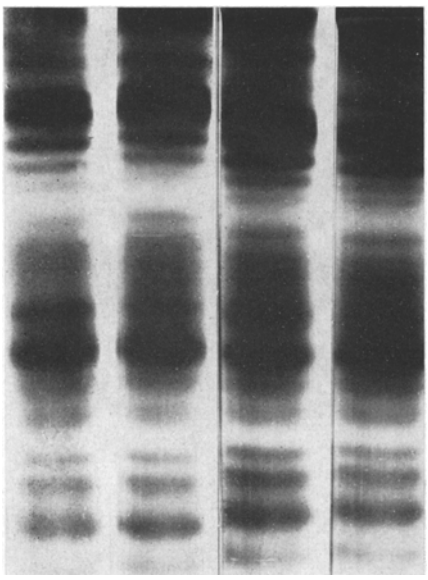

Fig. 3. Electropherograms of AUC soluble proteins on acidic polyacrylamide gels. Origin was at the top, proteins migrated towards (–) electrode. From left, Chinese Spring, 2 R(2 A), 2 R(2 B) and 2 R(2 D)

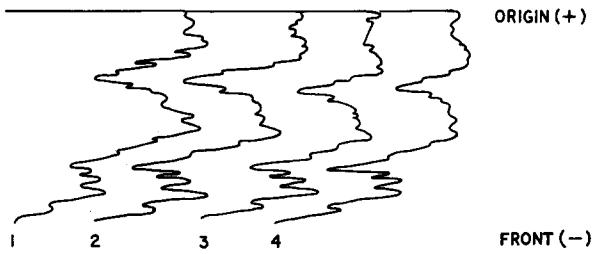

Fig. 4. Densitometer tracings of the gels shown in Figure 3. Integrator values for (1) Chinese Spring, (2) 2 R(2 A), (3) 2 R(2 B) and (4) 2 R(2 D) were respectively 430 ± 12.2, 475 ± 2.0, 500 ± 9.5 and 459 ± 14.4

control Chinese Spring; no significant variation from this pattern was found in the lines with substituted rye chromosomes.

Representative electropherograms of seed extracts in AUC separated on acid gels are shown in Figure 3, and their densitometer tracings in Figure 4. There was no qualitative variation (deletion or addition of bands) between the parent and the lines with substituted chromosomes. However, the densitometer tracings and integration values reveal a quantitative increase in the substitution lines. Similar results were obtained with alcohol and salt soluble proteins.

Discussion

The results suggest that 2R substitutions increase the kernel protein content. It is well known that protein content is greatly influenced by environmental factors. However, 2R substitution lines seem to be inherently capable of accumulating more proteins, at least under certain environmental conditions.

We have observed that in plants of the same genotype, delayed flowering, reduced seed set, and decrease in kernel weight are invariably associated with increased protein content in wheat (Bhatia *et al.*, 1970). Seed set per spike and 100 kernel weight for the control and substitution lines are shown in Table 2. The 2R(2B) and 2R(2D) lines did not show any significant deviation in seed set, and showed a slight reduction in 100 kernel weight compared with Chinese Spring. This variation alone cannot account for the observed increase in the level of proteins. Increase due to delayed maturity can also be ruled out because 2R(2A) was later in maturing than 2R(2B) at Brookhaven and Trombay but 2R(2B) showed a higher protein content (Table 1). Increased kernel protein is also not caused by an increase in the germ size or relative proportion of the germ which is rich in proteins.

Table 2. *Seed set and 100 grain weight of Chinese Spring and 2 R substitution lines grown at Trombay 1969–70*

Line	Mean number of seeds		Mean 100 seed weight in gms.
	per spike	per spikelet	
CS	57.0 ± 3.2	2.81 ± 0.06	2.15 ± 0.014
2 *R*(2 *A*)	*	*	2.06 ± 0.004
2 *R*(2 *B*)	53.0 ± 2.38	2.71 ± 0.05	2.05 ± 0.001
2 *R*(2 *D*)	57.0 ± 3.47	2.98 ± 0.06	1.88 ± 0.003

* Data not available.

The fractionation of proteins on the basis of their solubility did not show any significant changes in the overall composition. Similarly, electrophoretic analysis failed to reveal any addition or deletion of specific protein bands in AUC, alcohol and NaCl soluble proteins separated on acidic and alkaline gels. Altogether these three protein fractions account for over fifty discrete bands, though some of these may be common. Thus, the substituted rye chromosomes do not appear to contribute any proteins different from those already present in wheat. This provides further evidence for the homoeology of the rye chromosome 2 with group 2 chromosomes of wheat, as shown by Sears (1968). The absence of the 2A, 2B and 2D wheat chromosomes in the corresponding substitution lines was also not associated with any changes in protein electropherograms. This implies that either genes for kernel proteins are not located on group 2 wheat chromosomes or, if they are, then duplicate or triplicate genes are present in the homoeologous chromosomes.

With the possibility of new proteins contributed by the substituted rye chromosomes ruled out, the basis for the observed increase can only be speculated. Either an additive effect of the rye chromosomes or the interaction of wheat-rye chromosomes can possibly account for the increase in protein content of the substitution lines. The first possibility would require two assumptions: 1. Genes for kernel proteins are located on group 2 chromosomes. 2. Rye genes are more 'efficient' in accumulating proteins. At present, there is no definite evidence for either. Between-chromosome interaction has been reported in inter-varietal chromosome substitutions for morphological and quantitative characters (Sasaki *et al.*, 1968; Law, 1968). Similar interactions are likely when alien chromosomes are substituted. However, the nature of such interactions is obscure. Further work is required to establish the basis for the observed increase in protein content.

While this manuscript was in preparation, our attention was drawn by Dr. K. W. Shepherd (Adelaide) to a then unpublished report by Riley and Evart (1970) on the effect of individual King II rye chromosome additions on the amino acid content of Holdfast wheat. Though the primary aim of their studies was to investigate variation in the amino acid composition, their data reveal that almost all rye addition lines showed increased protein content. The maximum increase observed was 4.5% absolute protein. However, in King II rye, their III addition line, where the chromosome involved was the same as 2R used in our investigations, the increase in protein content was only one percent.

The results of Riley and Evart (1970) and those presented in this paper suggest that alien species may provide useful genetic material for the improvement of protein quality and quantity in cultivated plant species. It is suggested that, in the vigorous search for cereal varieties with higher and nutritionally superior proteins, currently being pursued in several laboratories throughout the world, alien species may also be given consideration. Most of these species have never been evaluated from a protein and nutritional point of view. In wheat, it may be worthwhile to further investigate the interaction of the remaining rye chromosomes, especially from high-protein rye lines.

Acknowledgements

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References

1. Bhatia, C. R., Jagannath, D. R., Gopal-Ayengar, A. R.: Induced micro-mutations for major protein fractions in wheat. In: 'Improving plant protein by nuclear techniques', 99–105 Vienna: IAEA 1970 . — 2. Davis, B. J.: Disc electrophoresis II. Method and application to serum proteins. Ann. N.Y. Acad. Sci. **121**, 404–427 (1964). — 3. Kimber, G., Sears, E. R.: Nomenclature for the description of aneuploids in *Triticinae*. Proc. Third International Wheat Genetics Symposium, Australian Acad. Sci., Canberra, 468–473 (1968). — 4. Law, C. N.: Gene-

tic analysis using intra-varietal chromosome substitutions. *Ibid.*, 331–342 (1968). – 5. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurements with Folin phenol reagent. J. Biol. Chem. **193**, 265–275 (1951). – 6. Mattern, P. J., Schmidt, J. W., Morris, R., Johnson, V. A.: A feasibility study of the use of a modified mass protein extraction process and chromosome substitution lines for bread wheat quality identification. Proc. Third International Wheat Genetics Symposium, Australian Acad. Sci., Canberra, 449–456 (1968). – 7. Reisfield, R. A.: Disc electrophoresis of basic proteins and peptides in polyacrylamide gels. Nature **195**, 281 (1962). – 8. Riley, R., Chapman, V.: The production and phenotypes of wheat-rye chromosome addition lines. Heredity **12**, 310–315 (1958). – 9. Riley, R., Evart, J. A. D.: The effect of individual rye chromosomes on the amino acid content of wheat grains. Genet. Res. **15**, 209–220 (1970). – 10. Sasaki, M., Moriyasu, M., Morris, R., Schmidt, J. W.: Chromosomal location of genes for some quantitative characters of wheat using chromosome substitution lines. Proc. Third International Wheat Genetics Symposium, Australian Acad. Sci., Canberra, 343–349 (1968). – 11. Sears, E. R.: Nullisomic-tetrasomic combinations in hexaploid wheat. In: Chromosome Manipulations and Plant Genetics (Eds. R. Riley and K. R. Lewis), Supp. Heredity **20**, 29–45 (1966). – 12. Sears, E. R.: Relationships of chromosomes 2 *A*, 2 *B* and 2 *D* with their rye homoeologue. Proc. Third International Wheat Genetics Symposium, Australian Acad. Sci., Canberra, 53–61 (1968).

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