PROTEIN SYNTHESIS IN TRITICALE AND ITS DURUM WHEAT AND RYE PARENTS[1]

J. E. DEXTER and B. L. DRONZEK, Department of Plant Science, University of Manitoba, Winnipeg, Canada R3T 2N2

ABSTRACT

Protein synthesis in one line of hexaploid triticale and its durum wheat and rye parents was investigated by determining the protein solubility distribution and ^{14}C-leucine incorporation into the grain protein at five stages of grain development. The solubility distribution of protein in the triticale was generally intermediate to that of the parents at all stages of development. ^{14}C-Leucine incorporation into the total grain protein reached a maximum at 2 weeks after anthesis and then declined as the grain developed. Some differences in the proportion of ^{14}C-leucine incorporated into the protein solubility fractions of the three grains were observed with the largest differences in incorporation obtained for the albumins and gliadins. The ^{14}C-leucine incorporated into triticale protein fractions was, in general, intermediate between those for the same fractions of the parents for all fractions except the glutenins. Triticale glutenin incorporated more ^{14}C-leucine than the durum wheat or rye glutenins at all stages of development.

The physicochemical properties of the proteins of mature endosperm of hexaploid triticale and its durum wheat and rye parents have been extensively investigated (1-4). These studies have shown that the proteins of triticale appear to be a mixture of the proteins of the durum wheat and rye parents.

The present study on the proteins of developing triticale and its parents was designed to extend the previous investigations. This article reports the changes in solubility distribution of proteins and pattern of incorporation of ^{14}C-leucine into the proteins during grain development.

MATERIALS AND METHODS

Plant Material

A hexaploid triticale (6A190), and the durum wheat (cv. Stewart), and rye (cv. Prolific) parents were grown in a controlled environment chamber (21°C 16 hr of light per day). Prior to planting, chromosome counts on root-tip cells were made on the triticale to ensure that only 42-chromosome plants were used in the experiments. Heads were tagged on date of anthesis and this date was used to determine the stage of grain development. Heads were removed on selected dates after anthesis until maturity. Immature grains were removed by hand and freeze-dried. The freeze-dried immature grains and the dry mature grains were ground on a Udy Cyclone Sample Mill.

Extraction of Proteins

The protein solubility fractions were classified as originally proposed by Osborne (5). Homogenization of the ground-grain suspension in a tissue grinder

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[2]Present address: Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Canada.

(A.R. Thomas Co.) gave more reproducible results than the magnetic stirring used by Chen and Bushuk (1). Precision for duplicate extractions on the same sample was better than 5%. Samples, 3-g (dry matter basis), were homogenized for 15 min in 25 ml of 0.5 *M* NaCl and centrifuged. The supernatant was decanted and two similar extractions followed. The residue was suspended in water to remove residual salt. The four supernatants were combined, dialyzed against distilled water for 48 hr, and centrifuged to separate the precipitated salt-soluble proteins (globulins); the water-soluble proteins (albumins), remained in solution. The dialyzable nitrogen-containing compounds will be referred to as the nonprotein nitrogen. The dialysis tubing was made of high-purity, seamless, regenerated, cellulose and had an average pore radius of 24 Å. The material remaining after extraction with salt solution was then extracted three times with 25-ml portions of 70% ethanol solution. Ethanol was removed from the combined ethanol-solution supernatants in a rotary evaporator. This fraction was designated as the alcohol-soluble fraction (gliadins). The resulting material was further extracted three times with 25-ml portions of 0.05*M* acetic acid solution. The three supernatants were combined to give the acetic acid-soluble fraction (glutenins). The remaining material will be referred to as the insoluble residue fraction. The four solubility fractions and the final residue were freeze-dried.

Determination of Nitrogen Content

Nitrogen contents of the water-, salt-, alcohol-, and acetic acid-soluble fractions were determined colorimetrically using Nessler reagent (6). However, due to interference compounds, this method gave unsatisfactory results for whole grain samples and insoluble residue samples. Thus, the nitrogen contents of these samples were determined by the Kjeldahl procedure (7).

^{14}C-Leucine Experiments

Uniformly labelled ^{14}C-leucine (10 mCi/mmol) (freeze-dried solid, Amersham/Searle Corporation) was dissolved in water (20 μCi/ml). Cold leucine (40 mg/ml) was added as a carrier. Twenty-five μl of the ^{14}C-leucine solution (0.5 μCi) was injected into each stem directly below the head as recommended by McConnell and Ramachandran (8). These injections were performed on different heads at 1-week intervals from 1 week after anthesis to 5 weeks after anthesis. The heads were harvested 1 week after injection, except in the case of the final sample which was allowed to develop to maturity.

The ^{14}C activity of the grain and protein fractions was determined by the following procedure. Each sample was hydrolyzed according to the method of Orth *et al.* (4). A portion of the final hydrolysate solution was counted on a Nuclear Chicago Series 720 Liquid Scintillation Counter using Aquasol (New England Nuclear chemicals) as the scintillation mix. Samples were counted to less than 1% error.

The amount of ^{14}C activity present as ^{14}C-leucine was determined for a number of samples as follows: The amino acids from a portion of the hydrolysate were separated from soluble sugars and organic acids on a cation exchange column (AG-50W-X8 resin; Bio Rad Laboratories). The amino acid fraction was then fractionated by paper chromatography on Whatman No. 1 paper using a 1-butanol:water:acetic acid (12:5:3) system. The chromatograms were sectioned

and the activity on each section was determined by incubating the paper in 70% ethanol overnight and then counting in a liquid scintillation counter. The R_f value for leucine, used to locate the leucine spot, was determined previously using ninhydrin spray for detection.

RESULTS AND DISCUSSION

Nitrogen Content per Kernel

The nitrogen content per kernel for triticale 6A190 and its parents, Prolific rye and Stewart durum wheat, during development, is shown in Fig. 1. A rapid increase in nitrogen content per kernel occurred in all three cereals between the second week after anthesis and the fifth week after anthesis, which indicated that the rate of protein synthesis was very rapid during this period. From 5 weeks after anthesis to maturity, the increase in nitrogen per kernel was very small in all three cereals. Thus, the rate of protein synthesis had decreased greatly over this period.

Rye had significantly less nitrogen per kernel than either triticale or durum wheat throughout development. This was a reflection of both smaller kernel size and lower nitrogen content.

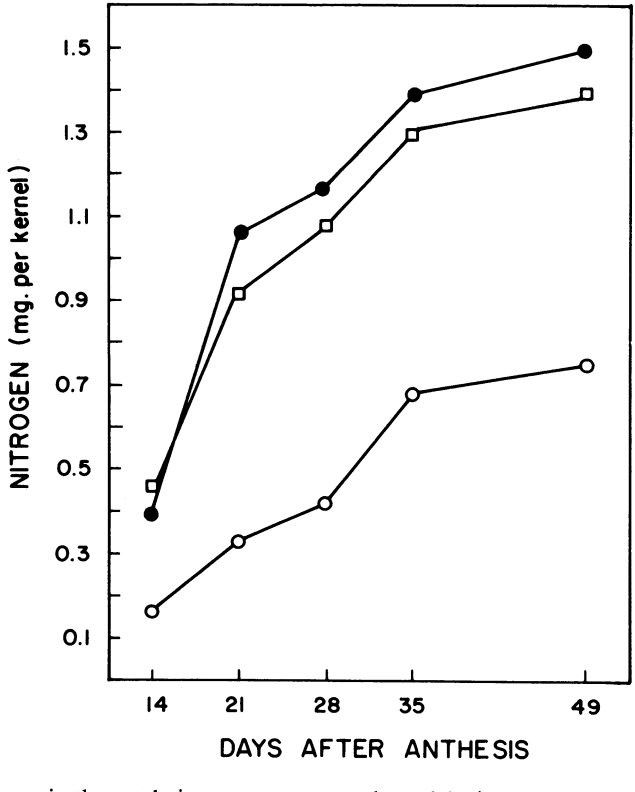

Fig. 1. Changes in the total nitrogen content per kernel during development in Prolific rye (open circle), Triticale 6A190 (solid circle), and Stewart durum (open square).

Protein Solubility Distributions

The protein solubility distributions for the developing whole grains of triticale 6A190 and for the Prolific rye and Stewart durum wheat parents are presented in Fig. 2. Distribution of the proteins in the five solubility fractions for all three cereals changed rapidly during grain development. Patterns of change differed from one cereal to another. The results obtained in the present study agree, in general, with the published work on other cereal grains (9-13).

The three cereals differed greatly in the proportion of nitrogen present as nonprotein nitrogen early in the development (Fig. 2). At 2 weeks after anthesis, over one-half of the nitrogen present in the grain of triticale and rye was in the form of nonprotein nitrogen compared to less than 20% for durum wheat. Although the proportion of nonprotein nitrogen in triticale and rye declined in the ensuing weeks, these two cereals retained a greater proportion of this fraction throughout development than durum wheat. The proportion of nonprotein nitrogen found in triticale was essentially intermediate to that of its parents throughout development.

Proportion of nitrogen present as albumins in durum wheat declined during development of the grain (Fig. 2). At 2 weeks after anthesis, durum wheat had the largest proportion of albumins of the three cereals, while at maturity it had the lowest. The proportion of albumins in rye increased until 5 weeks after anthesis. At maturity, rye had the greatest proportion of albumins of the three cereals. The proportion of albumins in triticale showed a slight increase until 4 weeks after anthesis. Throughout development, the proportion of albumins found in triticale was essentially intermediate to that of its parents.

The proportion of nitrogen present as globulins remained essentially constant throughout the development period investigated (Fig. 2). There was little difference in the proportion of globulin nitrogen for the three cereals, although rye had the greatest proportion of this fraction from 4 weeks after anthesis to maturity.

Durum wheat had a large proportion of gliadins throughout development (Fig. 2). At 2 weeks after anthesis, almost one-quarter of the nitrogen present in durum wheat was in the gliadin proteins. In rye and triticale, the gliadins formed a very small proportion of the total nitrogen at this time. This indicated that early in grain development, gliadin synthesis was greater in durum wheat than in the other two cereals. In all three cereals, the proportion of gliadins increased rapidly between the second and fourth weeks after anthesis. The proportion of nitrogen present in triticale gliadin was essentially intermediate to that of its parents throughout development.

Another major difference among the three cereals was the change in the proportion of glutenins during grain development (Fig. 2). Only a small proportion of rye glutenins was found until 5 weeks after anthesis. In contrast, an appreciable proportion of durum wheat glutenins was found at 2 weeks after anthesis. In durum wheat, the proportion of glutenins increased significantly between the fourth and fifth week. Triticale, like rye, contained only a small proportion of glutenins at 2 weeks after anthesis. However, by 3 weeks after anthesis, triticale and durum wheat had comparable proportions of glutenins.

All three cereals contained a relatively large proportion of insoluble residue protein early in the maturation process. From 3 weeks after anthesis to 5 weeks after anthesis, the proportion of this fraction present in all three cereals declined

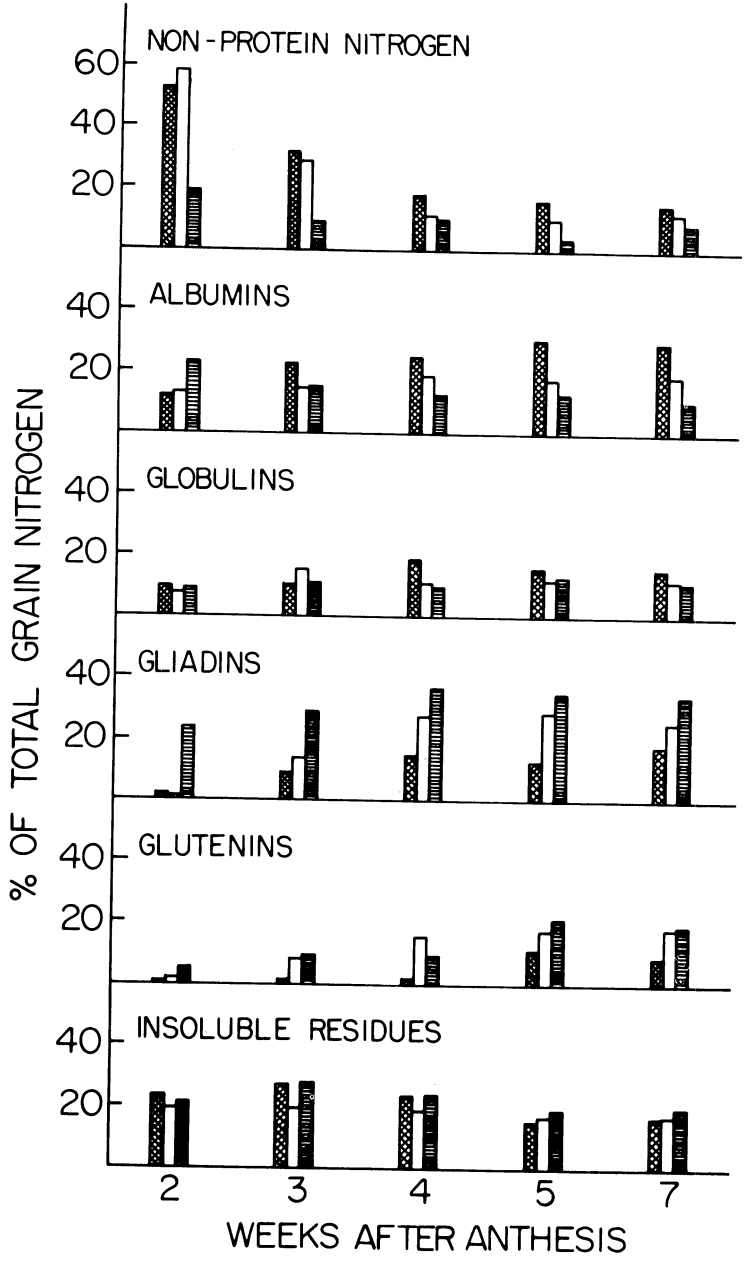

Fig. 2. The nitrogen solubility distribution in developing whole grain of Prolific rye (crossed-line bar), Triticale 6A190 (open bar), and Stewart durum (horizontal-line bar).

significantly and then remained constant until maturity.

The protein solubility distribution throughout development of triticale was essentially intermediate to that of its parents. For mature grain, solubility results obtained are similar to those of Chen and Bushuk (1). These results are consistent with the hypothesis that triticale proteins are inherited from both parents.

^{14}C-Leucine Experiments

Protein solubility distributions for the three cereals gave some indication of the relative rates of synthesis of the different protein fractions during kernel development. Further information was obtained by employing ^{14}C-leucine as a tracer. By injecting the label at specific intervals during grain development, it was possible to avoid contributions from previously accumulated protein. ^{14}C-Leucine was chosen for these studies because it is present in relatively high proportions in all cereal protein fractions (10,12,13,14). In addition, leucine had been found to be a catabolically stable amino acid in developing maize endosperm (14). This was also found to be the case in our studies; virtually all of the ^{14}C label was recovered as leucine.

Table I gives the percentage of injected ^{14}C-leucine which was incorporated into the five protein solubility fractions of triticale and the two parents during intervals of grain development. The proteins from all three cereals incorporated the highest percentage of label between the second and fourth week after anthesis, which indicated protein synthesis was most rapid at this time. For all three cereals, the rate of protein synthesis was very low during the last 2 weeks of development, as reflected by very low tracer incorporation over this period. These results are similar to those derived from the buildup of nitrogen in the kernels (Fig. 1), which indicates that the pattern of incorporation of ^{14}C-leucine during kernel development was a valid means of monitoring the rate of protein

TABLE I
Incorporation of ^{14}C-Leucine into the Proteins of Triticale and its Rye and Durum Wheat Parents during Development[a]

Injection Date[b]	Harvest Date[b]	Triticale	Rye	Durum Wheat
1	2	24.2	17.1	19.1
2	3	47.0	27.1	46.4
3	4	40.0	25.2	46.9
4	5	37.6	24.2	31.2
5	7	5.0	7.5	4.0

[a]Results expressed as % ^{14}C-leucine incorporated.
[b]Weeks after anthesis.

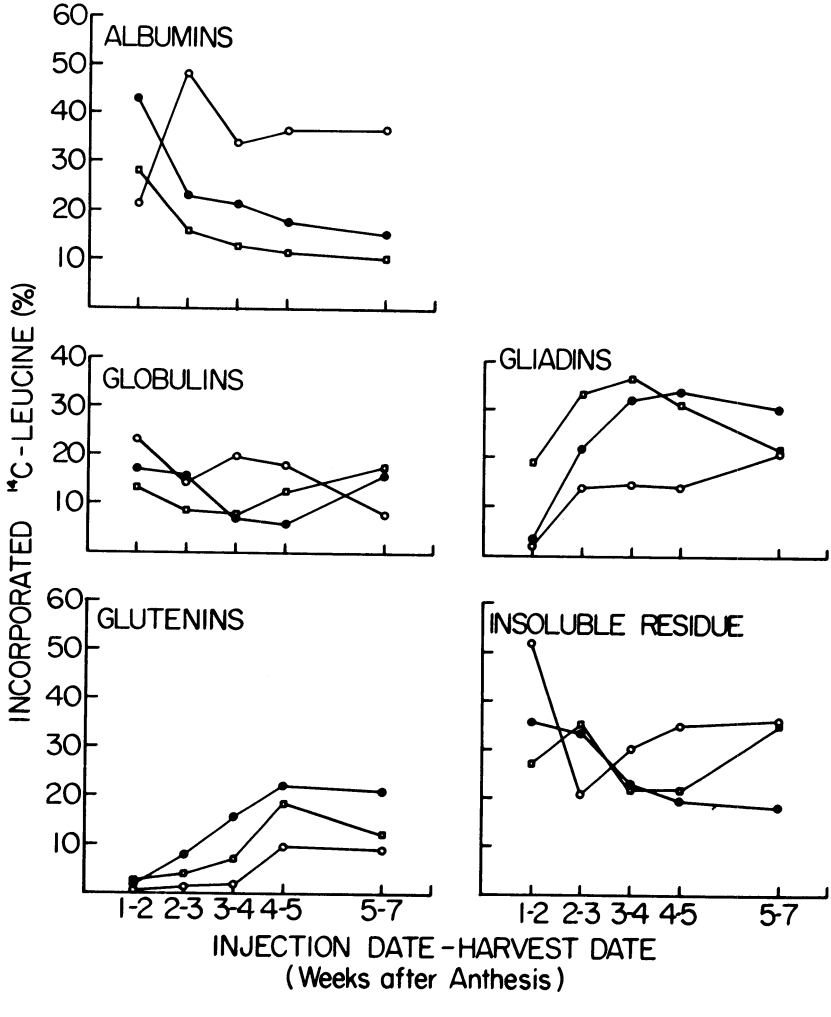

Fig. 3. Per cent of incorporated ^{14}C-leucine present in the protein solubility fractions of Prolific rye (open circle), Triticale 6A190 (solid circle), and Stewart durum (open square).

synthesis. The pattern of ^{14}C-leucine incorporation shown in Table I was similar to that previously reported for bread wheat (15).

Figure 3 presents the percentage of the incorporated ^{14}C-leucine found in each protein fraction. Between the first and second week of development, the albumin fraction of triticale and durum wheat incorporated the greatest percentage of the label incorporated into the proteins. During the third week of development, there was a marked decrease in the percentage of label incorporated into this fraction, which showed that the albumins of triticale and durum wheat were synthesized most rapidly (relative to total protein synthesis) early in kernel development. However, a considerable percentage of label continued to be incorporated into this fraction through to maturity. There was a particularly marked increase in the percentage of label-incorporated rye albumins between the second and third weeks after anthesis. From the third week after anthesis through to maturity, rye albumins incorporated the largest percentage of label of all fractions. It is concluded from these results that rye albumins are synthesized most rapidly at about 3 weeks after anthesis.

Triticale and durum wheat globulins incorporated the largest proportion of ^{14}C-leucine early in the development process. Although their globulins incorporated an increased proportion of label during the last few weeks of development, it must be borne in mind that overall protein synthesis had decreased greatly over this period. Rye globulins, conversely, showed a different pattern of label incorporation. They continued to incorporate label at a relatively steady rate over the first 5 weeks of development and then showed a marked decrease.

The pattern of incorporation of label into the gliadins for the three cereals differed. Triticale showed a rapid linear increase in relative incorporation of label into its gliadins between the second and fourth weeks. From the fourth week after anthesis to maturity, gliadins incorporated the greatest proportion of label of any of the triticale protein fractions. These results showed that synthesis of triticale gliadins increased rapidly over the first 4 weeks of development relative to overall protein synthesis. Durum wheat also showed a rapid increase in relative incorporation of label into the gliadins during the first 4 weeks. However, a much greater proportion of label was incorporated into durum wheat gliadins between the first and second weeks than was found for triticale or rye. Thus, gliadin synthesis in durum wheat proceeded at a much greater rate relative to overall protein synthesis than in either triticale or rye. After the fourth week after anthesis, the relative proportion of label incorporated by durum wheat gliadins declined markedly, which indicated that durum wheat gliadins were synthesized most rapidly during the third and fourth week after anthesis relative to the overall rate of protein synthesis. The proportion of label incorporated into rye gliadins increased rapidly between the second and third weeks after anthesis, stabilized, and then increased again over the last 2 weeks. During the period of most rapid protein synthesis (between the second and fifth weeks after anthesis) the proportion of label incorporated into rye gliadins was far less than that found in triticale or durum wheat. This was largely due to the much more rapid synthesis of albumins in rye relative to overall protein synthesis during this period compared to the other two cereals.

Triticale glutenins showed a linear increase in the proportion of label

incorporated from 2 weeks after anthesis to 5 weeks after anthesis. This differed from the pattern of label incorporation into the glutenins in both the parents. In rye, no appreciable proportion of label was incorporated into the glutenin fraction until the last 3 weeks of development. At this time, overall protein synthesis had declined considerably, thus rye glutenins were not synthesized at an especially rapid rate at any time during the development process. Although a similar pattern of incorporation compared to rye was found in durum wheat glutenins during development, the level of incorporation into the glutenins of the durum wheat was much greater.

The proportion of label incorporated into the insoluble residue protein of triticale and rye decreased between the second and third weeks after anthesis, which showed that this fraction was synthesized very early in the development process. The decrease in label incorporation into residue protein obtained for triticale and rye was not observed for durum wheat. However, since gliadins are present to a much greater extent early in the development process in durum wheat compared to rye and triticale, it is possible that rapid synthesis of residue protein was occurring prior to the period (1 week after anthesis) when the label was first injected. This is supported by the protein solubility distributions (Fig. 2) which showed durum wheat to have a large proportion of the residue fraction 2 weeks after anthesis.

The results presented herein demonstrate the difference in the pattern of protein synthesis during development in triticale and its rye and durum wheat parents. From the second week after anthesis, rye albumins were synthesized at a faster rate relative to overall protein synthesis than in durum wheat. Conversely, the gliadins of durum wheat were synthesized rapidly earlier in the development process than were rye gliadins. The pattern of synthesis of these two protein fractions in triticale was essentially intermediate to the parents. Glutenins were synthesized more rapidly in triticale than in either of the parents throughout the development process, especially between the third and fourth weeks after anthesis.

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