THE UNIVERSITY OF MANITOBA

BIOCHEMICAL STUDIES ON THE PROTEINS OF A HEXAPLOID TRITICALE AND ITS RYE AND DURUM WHEAT PARENTS DURING KERNEL DEVELOPMENT

by

JAMES ERIC DEXTER

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

Dexter, James Eric, Ph.D., The University of Manitoba, October, 1974. Biochemical Studies on the Proteins of a Hexaploid Triticale and its Rye and Durum Wheat Parents during Kernel Development.

Major Professor: Dr. B. L. Dronzek.

The amino acid composition of the endosperms and whole grains of a hexaploid Triticale (line 6A190) and its rye (cv. Prolific) and durum wheat (cv. Stewart) parents was found to change rapidly during maturation. In general, Triticale had an amino acid composition intermediate to that of its parents at all stages of maturation.

In all three cereals there was a rapid decline in the amount of free amino acids present in their maturing endosperms. Triticale and rye had a much greater amount of free amino acids than wheat at all times. The composition of the free amino acids of Triticale resembled that of its rye parent more closely than its wheat parent.

The amino acid composition of the proteins and peptides in the maturing endosperms of the three cereals was computed by eliminating the contribution from their free amino acids. The most rapid changes observed during maturation were a decrease in lysine and an increase in proline and glutamic acid. The content of these amino

acids in the proteins and peptides of maturing Triticale endosperm was intermediate to its parents at all times.

The solubility characteristics of the three cereals were determined at intervals during the maturation process. Rye and wheat differed widely in their pattern of protein synthesis. Triticale was intermediate to its parents.

^{14}C-leucine was used as a tracer to follow the rate of protein synthesis in the three maturing cereals. The results complemented those from the protein distribution studies.

Amino acid analyses were performed on the protein solubility fractions from each cereal at intervals during maturation. Significant changes occurred in all cases. The changes in albumins, globulins, gliadins, and glutenins during maturation were shown to be qualitative as well as quantitative by polyacrylamide-gel electrophoresis.

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INTRODUCTION

Triticale is a synthetic interspecies hybrid arising from a cross between wheat and rye. Some strains of hexaploid Triticale (S. cereale X T. turgidum) have shown some promising potential for commercial production because of their high nutritional quality coupled with a high protein content (Zillinsky and Borlaug (1971)).

Triticale, because it is an interspecies hybrid, is a useful research material for investigation of the inheritance of protein at the genome level. Chen and Bushuk (1970a,b,c) have extensively studied the flour proteins from a line of hexaploid Triticale 6A190 and its rye (cv. Prolific) and durum wheat (cv. Stewart) parents. Their observations on quantitative differences in protein solubility and amino acid composition, gel filtration and disc electrophoresis for the proteins of these three cereals suggested that the rye and durum wheat genomes in Triticale function quite independently.

The objective of the present investigation was to extend the studies of Chen and Bushuk (1970a,b,c) by examining the nature of the proteins in the same line of Triticale and its parents throughout all stages of kernel development. In addition, protein synthesis was followed

in these cereals with ^{14}C labelling studies.

The changes in amino acid composition of developing whole grain proteins and endosperm proteins as well as the free amino acid composition in the endosperm of all three cereals throughout maturation were determined on an amino acid analyzer. Protein extractions were performed on the maturing grains of all three cereals according to a modified Osborne protein solubility extraction procedure (Osborne (1907)). Since previously accumulated proteins tended to mask the changes in the relative rate of protein buildup of these fractions during development, further studies were carried out using ^{14}C-leucine as a tracer. Amino acid analyses were also carried out on the various protein fractions from each cereal during maturation. In order to determine whether the changes in amino acid composition observed within these fractions during maturation were the result of qualitative as well as quantitative changes in the proteins, the four soluble protein fractions in the developing whole grain from all three cereals at several stages of maturation were investigated by polyacrylamide-gel electrophoresis.

REVIEW OF THE LITERATURE

Cereal protein separation and classification.

The proteins found within all cereals may be classified on the basis of their solubility characteristics as originally proposed by Osborne (1907). The four soluble fractions are albumins (water-soluble), globulins (soluble in neutral salt solution), prolamines (soluble in 70% ethanol) and glutelin (insoluble in alcohol but soluble in dilute acid or dilute base). There is also a considerable amount of protein which remains insoluble following successive extractions in these solvents.

The Osborne method of protein classification has a number of serious shortcomings. Confusion has resulted because of overlapping solubility properties and poor reproducibility. Also, treatment of proteins with alcohol leads to denaturation. This may explain the relatively high quantities of insoluble protein which remain behind following the Osborne extraction. Because of these problems a great many other procedures for cereal protein preparation and separation have come into wide use in recent years.

A great many solvents have been proposed for extraction of the gluten proteins (insoluble in salt solution) of cereals. Among others these include urea solutions of

varying concentrations (Lee and MacRitchie (1971), Simmonds and Wrigley (1972)), acetic acid-urea-cetyl trimethyl ammonium bromide (Meredith and Wren (1966)), phenol-acetic acid-water (Stanley et al (1968), Gallus and Jennings (1968)), dimethyl formamide (Huebner and Rothfus (1968)) and aluminum lactate (Wright et al (1964)).

Following solvent extraction the proteins have been further classified by pH precipitation (Beitz and Wall (1972), Orth and Bushuk (1973a)) and by salt precipitation (Wasik and Bushuk (1974)). Proteins may also be separated based on molecular exclusion by gel chromatography (Meredith and Wren (1966), Gallus and Jennings (1968), Wright et al (1964), Jones et al (1963)). Chromatography on cellulose ion exchangers has also seen wide use in protein fractionation (Woychik et al (1960), Simmonds and Winzor (1961), Wrigley (1965)).

The fractions obtained by these methods and Osborne extractions are very heterogeneous. Gel electrophoresis, however, offers a very high resolving power for protein separation and is a very useful tool for further protein characterization. Starch-gel electrophoresis (Smithies (1965)) and acrylamide-gel electrophoresis (Davis (1964), Chen and Bushuk (1970b)) fractionate proteins on the basis of differential mobilities. This separation is a function of both molecular weight and charge at the pH of separation. Sodium dodecyl sulphate polyacrylamide-gel electrophoresis

fractionates on the basis of molecular size alone, since charge differences are masked by the presence of the detergent (Weber and Osborn (1969), Bietz and Wall (1972), Orth and Bushuk (1973b)).

Electrofocusing can be used to separate proteins according to their isoelectric points. Gel electrofocusing has been particularly effective in combination with electrophoresis (Mita and Yonezawa (1971), Wrigley (1968, 1970)).

Despite all these new techniques the Osborne solubility fractionation procedure has continued to be widely used with some modifications (Tanaka and Bushuk (1972), Chen and Bushuk (1970a), Dronzek et al (1970)). This is due mainly to its simplicity and suitability to large scale preparations. As yet no technique has been able to replace the Osborne method as a more meaningful and accepted method of cereal protein classification.

Cereal protein structure and function.

The protein solubility distribution within mature cereals varies widely from one species to another. In rye, for example, the albumins comprise the largest protein fraction in the flour (Chen and Bushuk (1970a)). In rice, on the other hand, glutelin is by far the largest fraction (Palmiano et al (1968)) while wheat contains a large amount of prolamine (Dronzek et al (1970), Chen

and Bushuk (1970a) as does maize (Ingle et al (1965)).

The albumins and globulins are the 'metabolically active' proteins of cereals since they contain in addition to glycoproteins, some nucleoproteins and lipoproteins and many of the enzymes involved in grain development and germination. An excellent review on these proteins is available (Feillet (1967)).

The germ and outer layers of the kernel contain protein consisting mainly of these fractions. However, albumins and globulins are also found to an appreciable extent within the endosperm. The albumins have a molecular weight (MW) range of about 20,000 while globulins range between MW 20,000 to MW 200,000 (Pomeranz (1971)). The amino acid composition of these proteins (Dronzek et al (1970)) reflect their solubility characteristics. They have a low amide content which means that glutamic and aspartic acids are present in the acid form rather than the amide. Accordingly, these proteins are capable of acquiring negative charges at neutral pH, thus enhancing their solubility in aqueous salt solutions. Lysine and arginine are found in large amounts. Since both are diamino acids capable of carrying a positive charge at neutral pH, they also enhance the water solubility of these proteins.

Prolamines and glutelins are found in the endosperm.

They are deposited as storage proteins and are subsequently available as nutrients for the new plant following germination. This is reflected in their amino acid compositions (Dronzek et al (1970), Ewart (1967)). They have a very high content of glutamine (determined as glutamic acid) which is an excellent nitrogen source since it contains two nitrogens per molecule. The advantage of storing nitrogen as a derivative of glutamic acid is that it occupies a central role in amino acid and respiratory metabolism. It is the principle nitrogen donor in transamination reactions leading to synthesis of other amino acids, and in addition, its carbon skeleton, α-keto glutarate, is a key intermediate in the respiratory Krebs cycle. Proline is also found in very large amounts in both prolamines and glutelins. This is consistent with the storage function of these proteins since proline is readily converted to glutamic acid. The amino acid composition of these proteins also reflect their solubility. Since most of the carboxyl groups of glutamic acid and aspartic acid are present as their amides and therefore are not free to ionize, and the content of basic amino acids (carrying a positive charge at neutral pH) is low, these proteins have low ionic character and are thus insoluble in neutral water. The extensive hydrogen bonding between glutamine residues and other amino acids also contributes to lack of solubility in water since it results in aggregation

of protein chains. It should be pointed out that there are significant differences in the overall amino acid composition of prolamines and glutelins. Glutelins are higher in lysine, tryptophan and glycine whereas gliadin is higher in proline, glutamic acid plus glutamine, cystine, isoleucine, phenylalanine and amide nitrogen (Ewart (1967), Dronzek et al (1970)).

Prolamines appear to be single chained and have MWs near 36,500 (Simmonds and Orth (1973)). Glutelins range in molecular sizes up to several million (Pomeranz (1971)). The very large molecules are comprised of sub-units held together by disulphide bonds which may be broken by reduction (Orth and Bushuk (1973a,b,c)).

The insoluble residue of cereals comprise proteins of very great size. They have an amino acid content more similar to the water-soluble proteins than to storage proteins (Cluskey and Dimler (1967), Ivanko (1971)). Solubilization of the residue protein of wheat was accomplished in the solvent hydrochloric acid-2-chloro-ethanol (Cluskey and Dimler (1967)). Electrophoresis showed that the protein dissolved in this solvent comprised components of similar mobility to acetic acid soluble glutelins as well as fast moving components. They concluded that the residue protein was a mixture of high molecular weight constituents consisting of polypeptides

linked through disulphide bonds.

Properties of Triticale.

Triticale is a synthetic cereal grain obtained by combining the genomes of wheat (genus Triticum) and rye (genus Secale). Depending upon whether a tetraploid wheat (T. turgidum L., 2n = 4x = 28) or a hexaploid wheat (T. aestivum L. em Thell., 2n = 6x = 42) is used, either a hexaploid or an octoploid (2n = 8x = 56) Triticale is produced respectively. Genomically the two Triticales and their parents can be represented as follows:

(1) T. turgidum (AABB) + Secale (RR) = Triticale hexaploide = AABBRR

(2) T. aestivum (AABBDD) + Secale (RR) = Triticale octoploide = AABBDDRR

Both of these Triticales can be produced without much difficulty. The octoploid species is cytogenetically unstable, however, and generally reverts to a hexaploid species. Hexaploid Triticale is more stable.

Triticales have been known for many years. A naturally occurring Triticale was described to the Botanical Society of Edinburgh in 1875 (Zillinsky and Borlaug (1971)). It is only in the last two decades, however, that any major attempt has been made to produce a commercial Triticale variety. Triticale has shown some promise as a feed grain because of its high yield of protein combined with high

levels of lysine. The major barriers to production of a commercially viable line have been partial sterility and shrivelling of the grain endosperm.

Chen and Bushuk (1970a) examined the nature of the endosperm proteins of one line of hexaploid Triticale (6A190) and its durum wheat (cv. Stewart) and rye (cv. Prolific) parents. By a modified Osborne solubility separation technique they found that the solubility distribution of Triticale proteins was intermediate between its parents.

Amino acid compositions of Triticale and its parental species were published by Yong and Unrau (1966) and Chen and Bushuk (1970). Yong and Unrau (1966) found that Triticale contained more leucine and isoleucine than either parent. This was not confirmed by Chen and Bushuk (1970a). They found that the content of the amino acids in Triticale was intermediate between those of its parents.

Electrophoretic studies of the endosperm proteins of Triticale and its two parents were carried out to determine if the synthesized species contained proteins not found in the two parents. Yong and Unrau (1964) using starch-gel electrophoresis found several 'new' bands in hexaploid Triticale extracts not present in the patterns for the equivalent extracts from the rye and durum parents. Chen and Bushuk (1970b,c) could not confirm these results

by means of polyacrylamide-gel electrophoresis. They found that all the bands of the Triticale proteins were present in the patterns of either the rye or durum parent. Furthermore, the pattern obtained for an extract of a mixture of rye and durum flours was essentially the same as the pattern of the Triticale extract. They concluded that the rye and durum wheat genomes in hexaploid Triticale function quite independently. However, a report has been published by Barber et al (1968), who detected a 'new' exterase band in the pattern of AABBDDRR Triticale that was not present in the electrophoretic patterns of its parents.

Biosynthesis of protein in developing cereal grains.

Analyses on the developing grains of some Australian wheats were carried out by Jennings and Morton (1963b) from a few days after flowering up to maturity. Synthesis of storage protein and of starch in the endosperm largely accounted for the rapid increase of dry weight in the developing grain after fertilization. They found that although the amount of protein nitrogen per grain increased almost linearly from day 12 up to maturity, initially the protein nitrogen as a percentage of dry weight declined up to day 19 owing to the more rapid synthesis of starch, and then remained constant. Meanwhile, the proportion of non-protein nitrogen decreased rapidly up to day 19 and

then more slowly. The amount per grain was almost constant through development. This pattern of relative change in protein and non-protein nitrogen was consistent with a precursor pool relationship.

The amino acid composition of the wheat endosperm has been shown to change continually during development (Jennings and Morton (1963a), Pomeranz et al (1966)). The major changes involved decreases in aspartic acid, valine, lysine, threonine, alanine and arginine while glutamic acid and proline increased. During the last two weeks before maturity changes in amino acid composition were small. To some extent these changes reflected the changes in the non-protein nitrogen pool. Jennings and Morton (1963a) found that the free amino acid pool comprised about 25% of the total nitrogen found in the kernel fourteen days after flowering and only 2% at maturity. There were also a number of amino acids within the non-protein nitrogen pool which varied markedly in relative amounts during maturation. The authors suggested that these variations reflected the extent to which the rate of supply of an amino acid to the pool of protein precursors was greater or less than the rate of its incorporation into protein. Both glutamic acid (and glutamine) and proline, for instance, decreased markedly within the non-protein nitrogen pool while the protein bound residue of these amino acids showed marked increases.

Coulson and Sim (1964) used starch-gel electrophoresis to follow changes in proteins present in wheat endosperm during ripening and germination. Slow moving bands (prolamines) were progressively degraded during germination and built up during the later stages of ripening. They found that prolamines (referred to as 'gliadins' in wheat) were not present until the last few weeks of grain ripening, while high mobility bands (albumins and globulins) were synthesized earlier and remained at a constant level during storage protein build up. Since storage proteins are much lower in lysine and higher in glutamic acid and proline than the salt soluble proteins, their observations would explain the changes in amino acid composition of maturing wheat kernels. Pomeranz et al (1966), however, suggested these changes were not merely a reflection of varying relative proportions of different protein types, but also were affected by changes in amino acid composition with each protein type during maturation. Albumins and globulins, for instance, appeared to show a considerable decrease in lysine content with maturity.

Changes in amino acid composition within a protein fraction during maturation can be the result of quantitative changes only, or also arise because of the synthesis of new types of proteins later in the maturation process. Graham et al (1963a) using ion-exchange chromatography and Graham

and Morton (1963) using starch-gel electrophoresis found only quantitative changes in the albumins and globulins of developing wheat endosperm. More recently, however, Rainey and Abbott (1971) showed the appearance of seven new component proteins in these fractions during the course of wheat kernel development by means of an immunoelectrophoretic study. Wrigley and Bushuk (1971) by gel filtration found evidence for some qualitative change in the glutenins of developing bread wheat, but found only quantitative changes within the glutenins from developing durum wheat.

^{14}C-labelled compounds have been used to trace the rates of synthesis of the various protein fractions within the wheat kernel during development. Bilinski and McConnell (1958) injected maturing wheat plants with acetate-1-^{14}C and acetate-2-^{14}C. The labelling patterns obtained suggested gliadins were synthesized rapidly later in the development process than albumins and globulins. Bran proteins showed markedly increasing activity with late injection, suggesting their formation at still later stages of maturation. The interpretation of their results was complicated by the extensive involvement of ^{14}C-labelled intermediates in processes such as the tricarboxylic acid cycle. Thus Finlayson and McConnell (1969) chose phenylalanine-2-^{14}C for further studies. They also used ammonium chloride-^{15}N at the same time to provide information about movements of nitrogen

within the plant during late stages of growth and the events involved in synthesis and deposition of kernel proteins. ^{15}N incorporation showed there was rapid synthesis of protein up to two weeks before maturity. However, gluten protein had greater ^{15}N atom excess when injected 30 - 36 days pre-ripe as opposed to 40. The salt-soluble proteins on the other hand showed the opposite effect. This suggested a greater degree of protein synthesis of albumins and globulins during early stages of kernel development than at later periods. The authors also showed that salt-soluble proteins did not lose label to gluten proteins and thus were not precursors being formed independently. Protein synthesis slowed down rapidly in the last 15 days before maturity as reflected by very low ^{15}N and ^{14}C incorporation over this period. The salt-soluble proteins incorporated significantly larger amounts of both labels over this period than did the gluten proteins.

Electron microscopy studies of developing wheat endosperms showed that in addition to starch granules, numerous dense spheroid bodies were observable one week after fertilization (Buttrose (1963), Jennings et al (1963), Morton et al (1964), Jennings (1968)). There was considerable variation in the size of these bodies within the endosperm cell at any period of development (at 14 days the largest were 0.5 microns in diameter while at 22 days they approached

13 microns in diameter). Staining reactions and increase in numbers during growth indicated the bodies probably consisted of storage protein. Starch-gel electrophoresis of a protein body preparation confirmed that the protein contained was mainly gliadins (Graham et al (1963b)). The protein content of protein bodies was in excess of 80%. They were less distinct after three weeks past flowering - perhaps being crushed out of shape.

Graham et al (1962) suggested a possible mechanism for protein body formation. They postulated that storage protein is formed on ribosomes of endoplasmic reticulum and afterwards secreted internally. The formed proteins aggregate into protein bodies with a surrounding lipoprotein membrane, thus being isolated from the remainder of the intracellular structures.

Studies on the incorporation of labelled amino acids into these protein bodies (Graham et al (1962), Graham et al (1964)) have shown that protein bodies take up ^{14}C and ^{35}S very quickly under in vivo conditions. The characteristics of this incorporation into the protein bodies as compared with the soluble proteins (albumins and globulins) supplied more evidence for an independent synthesis of storage and soluble proteins. Morton and Raison (1964) claimed that protein body isolates from wheat endosperm rapidly incorporated ^{14}C-leucine in a cell free system. These results

have been disputed by Wilson (1966), however, who showed that any incorporation of ^{14}C-leucine into maize protein body preparations was due solely to bacterial contamination.

An extensive study on the changes in the rice endosperm has been conducted by Palmiano et al (1971). Trends in non-protein nitrogen and total protein content in the grain were similar to that found in wheat during maturation. The main change in protein distribution in developing rice was found to be a very rapid rise in glutelin in the grain between days 4 and 21. Glutelin was found to have an amino acid content similar to that of the mature grain. This was not surprising since it comprised 85% of the protein of the rice endosperm at maturity. Rice prolamine was very low in proline compared to that of other cereals. Changes in amino acids of maturing rice may be explained by changes in the relative amounts of each protein fraction during ripening.

Del Rosario et al (1968) examined the endosperm structure of developing and mature rice. They found protein bodies in all endosperm cells of seven day kernels. In contrast to wheat, there was no significant change in the size of protein bodies during development, the largest being 3 microns in diameter. Because protein body increases paralleled glutelin increase in the kernel they suggested that rice protein bodies consisted mainly of glutelin. This was confirmed by analysis of isolated rice protein bodies

(Mitsuda et al (1967)).

Oats in contrast to most cereals showed no increase in the relative proportion of prolamine during development after the first two weeks (Wiggins and Frey (1958)). Thus, lysine content and other essential amino acids do not drop as drastically during maturation as in other cereals (Hischke et al (1968)). Brown et al (1970) have studied changes in the amino acid content of developing oat kernels. At five days after anthesis free amino acids comprised a high proportion of the total amino acids present, and protein consisted largely of metabolically active proteins rather than storage protein. Amino acid changes during development were explained by the decrease in free amino acids and changes in the relative proportions of the protein fractions. After ten days, amino acid changes were minimal.

A number of studies have been carried out on the changes in barley kernels during development (MacGregor et al (1971), Pomeranz and Robbins (1972), Ivanko (1971)). Ivanko showed that the formation of protein during barley grain maturation was characterized by unequal synthesis of different protein fractions. In ten day old grain the dominating protein was glutelin (over 50%), while prolamine was negligible. After 18 days prolamine synthesis was extremely rapid, and by 24 days prolamine content was equal to glutelin content, and by maturity prolamine comprised 50% of the total protein.

Glutelin increased absolutely in amount per kernel, but relatively dropped to 25% of the total protein. The major trends in amino acid content during maturation reflected the amino acid content of barley prolamine. However, changes also occurred in the amino acid compositions of all the protein fractions during development. These changes paralleled the changes in amino acid composition of the kernel during development, and made a significant contribution.

Tronier et al (1971) examined the nature of protein within barley protein bodies. The membrane and components of fine structure were separated from the storage proteins by sonication. Storage protein (prolamine) was found to be the major protein component. At least seven proteins (albumins and globulins) were detected by immunoelectrophoresis of the isolated fine structure.

Ingle et al (1965) studied two inbred maize species grown in the field, taking samples every three days after pollination up to maturity. By following DNA content they found cell division was complete five weeks before maturity, and a rapid phase of protein synthesis levelled off. Water content, soluble nitrogen, and free amino acids all peaked about this time. They also observed a second increase in protein content of the endosperm in the last three weeks, indicative of storage protein. Similar results were reported

by Bressani and Conde (1961). They found that prolamine (known as 'zein' in maize) was nearly absent in the immature kernel and became the most important fraction as the grain developed and matured. This zein increase showed almost a linear relationship with the increase in total nitrogen during development. Dialyzable nitrogen, meanwhile, decreased to 30% of total nitrogen 10 days after fertilization to 10% two weeks later, remaining fairly constant thereafter. Changes in amino acid content during development could be explained by prolamine buildup. For instance, leucine doubled in importance from 10 days after fertilization to maturity, while lysine, methionine, tyrosine and tryptophan decreased markedly.

A number of mutant genes have been found in maize which dramatically alter their protein composition (Mertz et al (1964), Nelson et al (1965)). These genes resulted in higher concentrations of albumins, globulins and glutelins and a much lower zein content. This resulted in a much higher lysine content which greatly increased the nutritional value of the protein. Murphy and Dalby (1971) examined the proteins of the endosperm of a normal inbred line of maize and its homozygous opaque-2 counterpart (one of the mutant lines), at various intervals after pollination. Proteins were sampled by a modified Osborne procedure and subjected to starch-gel electrophoresis and amino acid analysis.

The amino acid composition of the zein isolated 15 days after pollination from the opaque-2 mutant was quite unlike that of the same fraction from 15 day normal maize or from opaque-2 maize at later stages of development. The pattern of zein accumulation was consistent with the suggestion that the opaque-2 gene was active only during the first three weeks after pollination. After this time zein was accumulated in the endosperm of the mutant plant, although at a much less rapid rate than in the normal inbred line.

Sodek and Wilson (1970) followed the incorporation of ^{14}C-leucine and ^{14}C-lysine into proteins of developing endosperms of normal and opaque-2 maize. They found evidence that the albumin fraction underwent turnover during development, and the overall rate of breakdown exceeded the rate of synthesis after the third week. This was more apparent in normal maize than in opaque-2 plants. The globulins increased slowly with maturity with no apparent loss in specific activity. Thus the authors suggested that globulins were metabolically distinct from albumins. In normal plants ^{14}C-lysine was extensively converted to proline and glutamic acid. This was not nearly as apparent in opaque-2 plants. In normal plants progressively more ^{14}C-lysine was metabolized as maturity approached and zein synthesis increased. Thus, the authors speculated that there may be a correlation between lysine breakdown and increased zein synthesis.

A number of reports have been made of protein granules in maize endosperm (Christianson et al (1969), Duvick (1955, 1961)). Very similar protein bodies have also been reported in sorghum (Watson et al (1955)). The maize protein bodies were roughly spherical and numerous, ranging in size up to 3 microns in diameter. They were largest and most numerous in subaleurone cells, progressively decreasing in size from the outer to inner cells of the endosperm. They were first visible 15 - 20 days after pollination in the region under the silk scar and spread to other portions of the endosperms. Christianson et al (1969) showed by electrophoresis and amino acid analysis that these protein bodies were composed largely, or only, of zein encased in a matrix of glutelin.

Wolf and Seckinger (1969) examined numerous maize varieties for zein bodies and found no examples in which a maize endosperm showing zein granules had a large lysine content. The high lysine mutants examined all appeared to lack zein bodies, but contained up to 20% alcohol-soluble protein, indicating there must be alternative sites for alcohol-soluble protein accumulation. They speculated that a good deal of this zein was stored in globular protein bodies found in the high-protein subaleurone cells. Alternatively, it may be incorporated in the matrix protein in non-granular form.

These studies and others have shown the similarities in the manner in which endosperm protein evolves in all cereals during maturation. Firstly, albumins and globulins are generally synthesized most quickly in the first two weeks, while storage protein is synthesized rapidly from about two weeks after flowering up to physiological maturity. Secondly, labelling studies have shown that albumins and globulins are synthesized independently and do not act as precursors in storage protein synthesis. Thirdly, storage protein appears to be laid down in the endosperm in the form of protein bodies. These bodies are first noticed about one week after fertilization. They are surrounded by a lipoprotein membrane. Fourthly, changes occur in the makeup of individual protein fractions during development. These changes appear to be qualitative as well as quantitative. Finally, the rapid changes which occur in the relative proportions of the various protein fractions during development as well as rapid changes in the non-protein nitrogen pool cause rapid changes in the amino acid composition of the developing cereal grains, especially during the first few weeks of development.

MATERIALS AND METHODS

Plant material.

Triticale 6A190 and its parents Prolific rye and Stewart durum were grown in a controlled environment chamber (70°F, 16 hours light). Prior to planting root tip counts were performed on the germinated Triticale seeds to ensure that only forty-two chromosome plants were grown. Plant maturity was determined by taking note of the day the anthers hung out on each head.

Endosperm samples were obtained from immature samples by hand disection. The resultant material was freeze-dried for forty-eight hours and then ground on an Arthur H. Thomas grinder. Recovery from grinding was effectively 100% for one gram samples (dry weight). Mature samples were ground in a Brabender Quadramatic Mill in order to separate the endosperm from the bran and germ. The flour yield was about 65% for ten gram samples.

Whole grain samples were obtained by freeze-drying the intact immature seeds for forty-eight hours. They were then ground on a Udy Cyclone Sample Mill. Over 90% of the original five gram samples (dry weight) was recovered. Mature seeds were ground as is.

Extraction of proteins.

The proteins were fractionated by following the

classical Osborne (1907) solubility fractionation procedure. We found that homogenizing the whole grain samples gave much more reproducible results than stirring. Initially the 3 gram samples were homogenized for fifteen minutes in 25 ml of 0.5M NaCl and centrifuged. The supernatant was decanted and two similar extractions followed. The residue was resuspended in water to remove residual salt. The four supernatants were combined and dialyzed against distilled water for forty-eight hours, and centrifuged to separate the precipitated salt-soluble proteins (globulins); the water soluble proteins (albumins) remained in solution. The residue remaining after extraction with salt solution was then extracted similarly with three 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 residue was further extracted with three 25 ml proteins 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 fraction. The four soluble fractions and the final residue were freeze-dried.

Amino acid analyses.

All analyses were performed in a Beckman Model 121

Automatic Amino Acid Analyzer.

1. Total amino acid analyses: The samples were carefully weighed into culture tubes and 4 ml of 6N HCl was added. The solution was flushed with nitrogen for five minutes to remove oxygen. The tubes were then tightly capped and placed in a force draught oven at 100°C for twenty-four hours. Within a few hours after removal of the samples from the oven they were taken to dryness in a vacuum desiccator over NaOH. The dry samples were diluted to a volume suitable for analysis, and insoluble matter was removed by centrifugation. The amino acid analyses were performed according to the method of Spackman et al (1958) with a precision of ±3%.

2. Free amino acid analyses: The samples were homogenized for five minutes in water and centrifuged. The supernatant was made 5% in sulphosalicylic acid in order to precipitate the protein. The sample was again centrifuged and the resultant supernatant used for analysis.

A 4½ hour physiological run employing lithium citrate buffers was performed on acidic and neutral amino acids in order to separate glutamine and asparagine as described in the Beckman Automatic Amino Acid Analyzer Instruction Manual (1967). It was found to be necessary to adjust the pH of the solution to be analyzed to 2.2 in order to insure proper resolution in the early regions of

the chromatograph (Mondino et al (1972)). The basic amino acids were separated using sodium citrate buffers on the short column. γ-amino butyric acid was determined by an abbreviated basic physiological method. Sodium citrate buffer (pH 4.25, 0.38N) was pumped through a 23 cm column of PA-35 resin (Beckman Instruments) at a flow rate of 50 ml per hour. The temperature of the column was maintained at 32.5°C. After 2½ hours the column was regenerated and the next sample injected.

Several samples were duplicated. Precision was better than 10% for all amino acids except those found in relatively low amounts in relation to the predominant components.

^{14}C-leucine experiments.

Uniformly labelled ^{14}C-leucine (10 mCi/millimole (freeze-dried solid) Amersham Searle Corporation) was dissolved in water (20μCi/ml). 40 mg of cold leucine per ml water was added as a carrier. The label was introduced to the plants according to the method of McConnell and Ramachandran (1956). 25μl of the ^{14}C-leucine solution (0.5μCi) was injected into the stem directly below the head.

The ^{14}C activity of the whole grains and protein fractions were determined by the following procedure. Each sample was hydrolyzed as previously described. A portion

of the final hydrolyzate solution was then counted on a Nuclear Chicago Series 720 Scintillation Counter using Aquasol (New England Nuclear Chemicals) as the scintillation mix. Efficiency of counting ranged from 40 to 60%.

The amount of ^{14}C activity present as ^{14}C-leucine was determined for a number of samples. The amino acids from a portion of the hydrolyzates were separated from soluble sugars and organic acids on a cationic exchange column (AG-50W-X8 resin, Bio Rad Laboratories). These amino acid fractions were then separated by paper chromatography on Whatman No. 1 paper using a n-butanol:water:acetic acid (12:5:3) system. The chromatographs were sectioned and the activity on each portion was determined by soaking the paper in 70% ethanol and then counting on the liquid scintillation counter. The R_f value for leucine had been determined previously for other samples using ninhydrin spray for detection.

Electrophoresis.

The disc polyacryamide-gel electrophoresis method of Davis (1964) as modified by Chen and Bushuk (1970b) was used to determine the patterns for albumins, globulins and gliadins, except that coomassie brilliant blue was used as the protein stain rather than amido black. For each sample within a particular protein fraction the same

amount of protein was loaded for analysis. Separation was on the basis of the molecular weight and charge of the individual protein components.

Because of their high molecular weight glutenins could not be separated by the above electrophoretic method. Instead the reduced subunits of the glutenins were examined using the sodium dodecyl sulphate (S.D.S.) polyacrylamide-gel electrophoresis method described by Orth and Bushuk (1973b). This method separates the subunits according to molecular weight (and size) only, since their charge is masked by the detergent.

Determination of protein content.

The protein content of endosperm samples was determined by adjusting the amino acid analyses to a 90% recovery. The protein contents of whole grain samples as well as insoluble residue fractions were determined by the Kjeldahl procedure (1883). The Nessler method (Williams (1964)) was used to determine the protein content in the albumins, globulins, gliadins and glutenins fractions. The different protein procedures and the reasons for using them are presented in Appendix A.

RESULTS AND DISCUSSION

The amino acid composition of the developing endosperm.

Table 1 shows the amino acid composition of the maturing endosperm of one line of Triticale (6A190), the durum wheat parent (cv. Stewart) and the rye parent (cv. Prolific). All three cereals showed a very rapid change in amino acid composition as they matured. However, the magnitude of these changes varied from one species to another. Previous studies with other cereal grains including wheat (Jennings and Morton (1963a), Pomeranz et al (1966)), rice (Palmiano et al (1968)), barley (Ivanko (1971), Tronier et al (1971), Pomeranz and Robbins (1972)), oats (Brown et al (1970)) and maize (Ingle et al (1965)) showed the relative proportions of lysine, aspartic acid and alanine decreased during maturation, while proline and glutamic acid increased. The results in Table 1 agreed with the results from those studies. It has been shown that changes in amino acid composition in cereals during development reflect changes in the distribution of the protein solubility fractions, and in particular the rapid synthesis of storage protein (Jennings and Morton (1963c), Coulson and Sim (1965)). Other factors involved included changes within the amino acid makeup of individual protein fractions within the cereal grain (Ivanko (1971), Murphy and Dalby (1971)),

Table 1. Amino Acid Composition of the Maturing Endosperm of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum. (Results are given as grams nitrogen per 100 grams nitrogen)*

	Triticale							Prolific Rye							Stewart Durum						
Days after anthesis:	7	10	12	16	20	33	49	10	12	14	17	19	29	49	7	9	12	15	19	23	49
Lysine	6.78	5.99	5.42	3.84	3.64	3.08	2.34	5.76	5.32	5.50	4.94	4.66	3.94	3.30	7.04	6.29	3.99	3.67	3.33	2.81	2.14
Histidine	3.31	3.02	3.25	3.17	3.26	3.43	3.20	2.93	2.81	2.82	2.99	2.96	3.04	3.11	3.15	3.36	3.59	3.35	3.36	3.29	3.16
Ammonia	14.3	15.0	16.1	18.7	19.2	20.2	19.9	16.6	16.6	17.3	17.2	20.0	19.3	18.8	12.3	16.0	16.4	17.6	18.9	19.9	19.4
Arginine	9.88	8.76	8.51	7.45	7.52	7.39	6.67	7.77	7.33	7.01	6.74	6.37	6.67	6.49	9.00	8.97	8.24	6.62	6.66	7.15	6.48
Aspartic Acid	6.94	6.86	6.00	4.59	4.48	3.04	3.01	6.19	6.76	8.28	5.44	4.98	4.21	3.47	5.44	5.07	4.51	3.97	3.37	3.14	2.51
Threonine	2.61	2.38	2.23	2.05	1.98	2.01	1.93	2.33	2.16	2.22	2.24	2.22	2.17	2.09	2.51	2.50	2.34	1.91	1.74	1.95	1.75
Serine	3.94	3.18	3.64	3.32	3.23	3.57	3.61	3.40	3.31	3.42	3.58	3.03	3.47	3.71	4.72	3.60	3,58	3.75	3.86	3.35	3.22
Glutamic Acid	8.92	10.3	11.5	17.0	17.5	17.9	20.2	10.7	10.7	9.73	13.7	15.1	17.4	18.6	9.93	10.9	12.9	17.3	19.0	19.9	24.1
Proline	5.39	5.28	4.59	6.72	7.30	8.48	9.52	7.21	7.96	8.28	7.58	7.60	8.54	9.94	5.01	4.81	5.64	6.61	6.32	7.62	8.01
Glycine	5.17	5.11	4.61	4.57	4.28	4.01	3.67	4.20	4.20	4.80	5.07	4.37	4.08	3.81	5.94	5.39	5.73	4.94	4.20	3.82	3.26
Alanine	9.48	11.4	10.9	5.53	4.41	3.31	2.71	9.87	10.5	9.01	8.31	5.62	4.13	3.11	9.91	8.46	8.73	5.57	4.25	3.31	2.49
Valine	3.43	3.23	3.50	2.95	2.95	2.98	2.75	4.46	3.92	3.09	2.96	3.09	3.05	3.03	3.46	3.48	3.46	3.11	3.01	2.85	2.65
Methionine	1.02	1.04	1.07	1.00	0.92	0.42	0.77	0.61	0.75	0.83	0.80	0.76	0.80	0.72	1.70	1.11	0.89	0.91	1.09	0.85	0.77
Isoleucine	2.17	2.16	2.26	2.07	2.08	2.31	2.13	2.48	2.29	1.94	1.88	2.05	2.13	2.04	2.44	2.40	2.38	2.42	2.40	2.23	2.28
Leucine	3.82	3.17	3.60	3.83	3.92	4.25	4.04	3.17	3.15	3.21	3.53	3.82	3.81	4.02	4.41	4.33	4.26	4.61	4.78	4.21	4.15
Tyrosine	1.21	0.95	0.98	1.08	1.11	1.16	1.12	1.00	0.97	0.94	0.98	0.99	0.93	1.00	1.19	1.28	1.25	1.16	1.29	1.31	1.22
Phenylalanine	1.66	1.68	1.80	2.15	2.24	2.47	2.50	1.29	1.31	1.58	2.02	2.30	2.43	2.69	1.88	2.10	2.18	2.50	2.47	2.38	2.41
% Protein	21.4	20.2	18.8	16.5	16.4	14.6	16.7	21.2	21.4	13.9	12.9	15.2	9.4	9.3	14.7	13.7	13.7	12.5	10.8	11.4	13.3

* tryptophan, cysteine and cystine were not determined.

along with changes in the non-protein nitrogen pool (Hoseney and Finney (1967), Jennings and Morton (1963b), Hoseney et al (1966)) during the maturation process.

Table 1 shows very similar trends in amino acid composition during maturation for all three developing cereals. However, there were some significant differences. Lysine declined markedly in durum wheat, rye and Triticale. The decline was most apparent in wheat and least apparent in rye. Arginine, on the other hand, decreased in both wheat and Triticale, while in rye there was only a slight decrease. Aspartic acid (which includes asparagine) decreased steadily during maturation in both wheat and Triticale, while in rye a maximum level was reached fourteen days after anthesis followed by a sharp decrease. Glycine declined slowly throughout development in Triticale, declined in wheat only after two weeks of development and maintained a much more steady level in rye. Alanine retained a high level or increased slightly during the first two weeks after anthesis in all three cereals and declined rapidly thereafter. Glutamic acid (which includes glutamine) showed a very large increase during development in both wheat and Triticale, but in rye it decreased initially, then increased rapidly. Proline increased in wheat from nine days after anthesis to maturity, while in Triticale it decreased until fourteen days after anthesis,

after which it rose quickly. In rye, however, it maintained a fairly steady level for four weeks before showing any increase. The results for methionine were erratic. This was likely due to oxidative degradation during the hydrolysis. Therefore it was not possible to accurately determine its pattern of change during the maturation process. The remaining amino acids examined retained generally steady levels in all three cereals during development.

In general, Triticale had an amino acid composition intermediate to its two parents at all stages of maturation. This supported earlier work by Chen and Bushuk (1970a,b,c) who examined the chemical, physical and electrophoretic properties of the proteins from the mature flours of the same three cereals, and found Triticale to be intermediate to its two parents in all cases.

The amino acid composition of the maturing endosperm from a hexaploid bread wheat (cv. Thatcher) and its extracted tetraploid was also determined. Results throughout maturation were similar to Stewart durum. The data is presented in Appendix B. In addition, a similar study was completed on two other hexaploid Triticales (cv. Rosner and line Kangaroo X U.M. 940'S'). The results for both did not differ significantly from Triticale 6A190 at all stages of development. The data is presented in Appendix C.

The free amino acid composition of the developing endosperm.

Table 2 shows the free amino acid composition of the developing endosperm of Triticale 6A190 and its parents, Prolific rye and Stewart durum. There were rapid changes in the free amino acid pool from all three cereals during endosperm development. In an earlier study Hoseney and Finney (1967) examined the free amino acids of two hard red winter wheats during maturation. In early stages of development they found the major components of the free amino acid pool to be glutamic acid, asparagine, glutamine, alanine and proline. This compares favorably to the results found in this study for the least mature samples of Stewart durum. Jennings and Morton (1963b) examined hydrolyzed samples from the dialyzable nitrogen fraction in maturing bread wheats. Thus, they were analyzing small proteins and peptides in addition to the free amino acids. They found especially large quantities of the amides and alanine at early maturity.

Table 2 shows that in a number of cases the pattern of change within the free amino acid pool of Triticale and its two parents differed significantly from each other. Free arginine, for instance, increased initially before declining in both Triticale and wheat, while in rye the levels remained relatively constant for the first two weeks after anthesis followed by a sharp decline. Free aspartic

Table 2. Free Amino Acid Composition of Developing Endosperm of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.
(Results are given as grams nitrogen per 100 grams nitrogen)*

	Triticale							Rye							Durum Wheat						
Days after anthesis:	7	10	12	16	20	33	49	10	12	14	17	19	29	49	7	9	12	15	19	23	49
γ-Amino Butyric	.345	.650	.439	.281	.194	.263	.017	.345	.438	.872	.384	.328	.138	.019	.600	1.10	.609	.158	.141	.072	.0014
Tryptophan	.722	.122	.021	.016	.021	.020	.017	.273	.201	tr.	tr.	tr.	tr.	tr.	.216	.074	tr.	tr.	.032	.013	.0039
Lysine	.258	.167	.221	.223	.193	.134	.013	.399	.279	.368	.384	.345	.702	.037	.335	.302	.291	.058	.102	.041	.0013
Histidine	.108	.069	.042	.122	.120	.061	.020	.184	.241	.173	.115	.124	.039	.003	.113	.059	.075	.029	.034	.022	tr.
Arginine	.288	.419	.426	.200	.132	.158	.077	.302	.263	.263	.184	.086	.026	.016	.178	.309	.180	tr.	.055	.021	.0252
Aspartic Acid	.539	.338	.129	.365	.354	.125	.034	.234	.225	.545	.469	.367	.339	.156	.379	.331	.258	.341	.257	.149	.0087
Threonine	.304	.298	.362	.125	.083	.048	.023	.324	.295	.368	.239	.182	.110	.014	.378	.230	.275	.107	.066	.032	.0008
Serine	.879	.884	1.13	.479	.293	.142	.046	.827	.883	.951	.734	.262	.213	.023	1.89	.549	.377	.310	.214	.124	.0012
Asparagine	1.84	2.35	3.54	1.56	.816	.291	.278	1.96	3.48	5.71	1.58	1.94	.519	.320	.905	.726	.677	.109	.064	.050	.0208
Glutamic Acid	1.04	1.02	.834	.666	.505	.223	.033	1.04	1.12	1.07	1.33	.907	.837	.081	1.39	.708	.244	.248	.595	.338	.0127
Glutamine	1.49	2.52	3.78	1.37	.735	.330	.248	5.50	5.09	3.43	1.31	1.53	.434	.105	1.83	1.05	.176	.110	.200	.294	.0017
Proline	2.59	1.48	.856	.090	.053	.037	.193	3.87	4.20	4.25	1.35	.365	.745	.138	2.13	.728	.254	.046	.048	.014	.0024
Glycine	.320	.528	.670	.463	.295	.100	.024	.290	.456	.938	.850	.370	.205	.026	1.19	.861	1.50	.925	.424	.132	.0033
Alanine	4.45	6.50	6.18	1.94	.924	.348	.098	4.40	5.08	4.76	3.48	2.09	.765	.057	5.17	4.27	5.07	1.88	.754	.348	.0071
Valine	.149	.776	.758	.097	.066	.041	.044	1.08	.904	.506	.211	.194	.074	.026	.180	.307	.378	.084	.039	.014	.0009
Methionine	.010	.079	.180	.077	.047	.025	.010	tr.	tr.	.063	.034	.027	.017	.009	.217	.085	.088	.022	.026	.008	.0003
Isoleucine	.075	.063	.446	.034	.025	.015	.037	.502	.403	.277	.068	.042	.041	.015	.084	.086	.093	.040	.029	.007	.0005
Leucine	.068	.067	.221	.033	.025	.023	.056	.126	.115	.188	.073	.066	.031	.019	.103	.124	.103	.053	.047	.007	.0009
Tyrosine	.027	.016	.055	.026	.022	.018	.021	.053	.054	.055	.028	.022	.018	.009	.045	.042	.034	.029	.017	.007	.0008
Phenylalanine	.009	.020	.092	.019	.016	.013	.040	.062	.050	.083	.033	.031	.027	.019	.055	.047	.036	.029	.023	.009	.0005
Ammonia	1.84	1.01	1.62	1.05	.810	.385	.141	3.09	2.29	2.51	2.62	2.94	1.56	1.20	1.83	2.70	1.75	.520	.540	.202	.0479
Recovery	17.4	18.8	22.4	9.3	5.8	2.8	1.5	24.9	26.1	27.4	14.4	12.2	5.9	2.3	19.2	14.7	12.5	5.1	3.7	1.9	.14

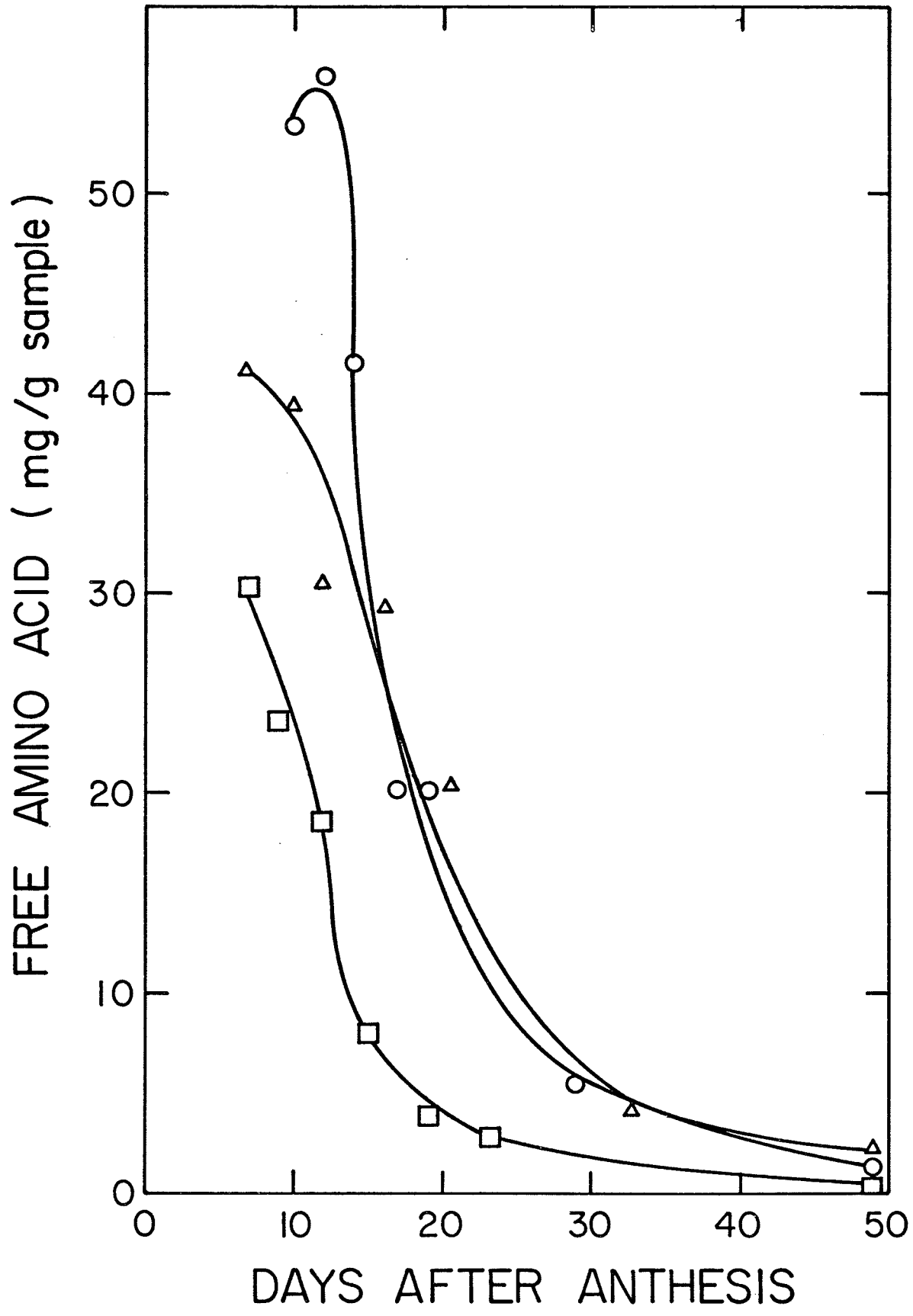
* cysteine and cystine were not determined.

acid was found in comparable proportions in Triticale and durum wheat at all stages of development, maintaining a relatively constant level initially and then declining. In contrast, the level of free aspartic acid in rye reached a maximum level about two weeks after anthesis before decreasing. At maturity rye had more aspartic acid than either Triticale or wheat. Free asparagine reached a maximum value two weeks after anthesis in both rye and Triticale before declining to maturity. In wheat, however, free asparagine decreased rapidly throughout development and was less than that of either rye or Triticale. The level of free serine in wheat showed a continual decrease during maturation, while in rye and Triticale a maximum level was reached about two weeks after anthesis before declining to maturity. Free glutamic acid dropped rapidly in wheat, reaching a very low level twelve days after anthesis. In Triticale this decrease was less rapid, while in rye a maximum level was reached seventeen days after anthesis and no decrease was apparent until the last stages of development. Free glutamine quickly dropped to very low levels in wheat. In Triticale, however, maximum levels were not reached until twelve days after anthesis. Although this maximum was not apparent in rye, since it had extremely high levels at early stages of development, the proportions of free glutamine were at least as large as those found in

Triticale at all stages of development. In both wheat and Triticale the proportion of free proline was considerable in the first week of development, but declined very rapidly to extremely low levels. In rye free proline was found in large proportions for a somewhat longer time. Free glycine maintained a high level in wheat for two weeks after anthesis, declining thereafter. In Triticale free glycine reached a maximum value about two weeks after anthesis, while in rye an extremely sharp maximum was apparent fourteen to seventeen days after anthesis. In all three cereals the level of free alanine was high in the first fourteen days of development, after which its level dropped rapidly. Rye showed a much higher level of free valine during early stages of development than either wheat or Triticale. γ- amino butyric acid was found in considerable amounts, while the remaining free amino acids detected were found in small amounts in all three cereals, and generally declined rapidly as endosperm development progressed.

Figure 1 shows the level of the free amino acids per gram dry matter in the developing endosperm of each of the cereals. Wheat had significantly less free amino acids in its endosperm than either Triticale or rye at all stages of development. For the first two weeks after anthesis rye had significantly more free amino acids than Triticale. Thereafter the levels in both were similar.

Figure 1. Changes in the Free Amino Acid Content of Developing Endosperm from Triticale 6A190 (△), and Parents, Prolific Rye (○) and Stewart Durum (□).


FREE AMINO ACID (mg/g sample)
0
10
20
30
40
50
0
10
20
30
40
50
DAYS AFTER ANTHESIS

Although Triticale showed a number of differences to both its parents in its free amino acids during development, it resembled rye much more closely than durum wheat. Assuming there were no interactions between the alien genomes of durum wheat and rye, this is not surprising, since rye generally had a much larger free amino acid pool than durum wheat throughout development.

The free amino acid composition of the maturing endosperm from a hexaploid bread wheat (cv. Thatcher) and its extracted tetraploid as well as two hexaploid Triticales (cv. Rosner and line Kangaroo X U.M. 940'S") were also determined. Results are presented in Appendix B and Appendix C respectively. These cereals all showed rapid changes in their free amino acids throughout development. The general pattern of these changes was similar to that for the three cereals discussed above.

The amino acid composition of proteins and peptides of the developing endosperm.

Table 3 shows the amino acid content of the proteins and peptides of the developing endosperm from Triticale 6A190 and its parents, Prolific rye and Stewart durum. These results were computed by deducting the free amino acid levels from the total amino acid analyses. This was done to provide a more meaningful basis for the examination of the changes which occurred in the endosperm protein

Table 3. Amino Acid Composition of the Proteins and Peptides of Maturing Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.
(Results are given as mole percent on an ammonia free basis)*

	Triticale							Prolific							Stewart						
Days after anthesis:	7	10	12	16	20	33	49	10	12	14	17	19	29	49	7	9	12	15	19	23	49
Lysine	6.47	5.97	5.56	3.26	2.99	2.52	1.92	5.85	5.60	5.86	4.31	4.00	3.29	2.54	6.49	5.71	3.45	3.06	2.70	2.32	1.71
Histidine	2.12	2.01	2.01	1.84	1.83	1.91	1.74	2.01	1.89	2.01	1.79	1.76	1.72	1.69	1.96	2.28	2.20	1.88	1.85	1.82	1.68
Arginine	4.77	4.29	4.32	3.26	3.22	3.08	2.70	4.11	3.91	3.86	3.08	2.91	2.85	2.63	4.28	4.13	3.77	2.79	2.75	2.98	2.59
Aspartic Acid	10.9	11.0	8.77	6.15	6.48	4.71	4.65	10.9	10.6	11.1	7.98	6.74	6.19	5.16	8.92	8.33	7.29	6.04	5.31	4.97	3.99
Threonine	4.59	4.27	4.00	3.46	3.31	3.33	3.13	4.49	4.13	4.22	3.78	3.78	3.53	3.37	4.13	4.32	3.84	3.06	2.80	3.21	2.80
Serine	6.08	4.72	5.37	5.11	5.12	5.84	5.83	5.61	5.40	5.63	5.42	5.13	5.59	6.01	5.49	5.80	5.97	5.82	6.11	5.40	5.15
Glutamic Acid	14.2	16.4	18.9	28.1	28.9	29.7	32.9	15.1	15.6	15.9	22.5	24.9	27.9	30.0	14.8	18.4	23.4	28.8	30.9	32.4	38.5
Proline	5.56	7.80	7.98	11.9	12.6	14.3	15.4	7.29	8.35	9.19	11.9	13.4	14.3	16.0	7.52	7.78	10.1	11.1	10.5	12.7	12.8
Glycine	9.63	9.40	8.43	7.40	6.95	6.65	5.98	8.31	8.13	8.80	8.05	7.41	6.63	6.18	9.23	8.68	7.72	6.80	6.39	6.17	5.22
Alanine	9.99	10.0	10.2	6.48	6.07	5.04	4.28	11.9	12.0	9.68	9.04	6.54	5.76	4.99	9.25	7.97	6.82	6.25	5.91	4.95	3.97
Valine	6.51	6.26	5.86	5.13	4.96	5.00	4.44	7.37	6.71	5.89	5.22	5.37	5.12	4.91	6.36	6.03	5.74	5.13	4.94	4.75	4.24
Methionine	2.01	1.97	1.90	1.66	1.48	0.66	1.25	1.33	1.67	1.75	1.40	1.35	1.34	0.87	2.87	1.94	1.49	1.51	1.78	1.40	1.23
Isoleucine	4.15	4.31	3.89	3.67	3.57	3.90	3.43	4.32	4.20	3.83	3.41	3.72	3.59	3.27	4.58	4.40	4.27	4.03	3.69	3.71	3.65
Leucine	7.45	6.36	7.23	6.84	6.77	7.20	6.49	6.63	6.73	6.88	6.59	6.94	6.48	6.48	8.36	8.01	7.76	7.72	7.91	7.02	6.64
Tyrosine	2.36	1.91	1.97	1.89	1.88	1.94	1.80	2.07	2.04	1.96	1.76	1.80	1.56	1.60	2.23	2.36	2.27	1.91	2.13	2.17	1.95
Phenylalanine	3.28	3.41	3.66	3.83	3.85	4.19	4.03	2.68	2.80	3.42	3.76	4.20	4.12	4.33	3.55	3.90	3.99	4.13	4.08	3.96	3.86

* tryptophan, cysteine and cystine were not determined.

during development. This was especially advantageous at early maturity where the free amino acid pool had a pronounced effect on the overall amino acid composition. The accuracy of this procedure was confirmed by comparing the amino acid content of the residue left over following several free amino acid extractions to the results in Table 3.

Figure 2 illustrates the change in lysine content of the proteins and peptides of the developing endosperm of all three cereals. A rapid decrease occurred in all three cereals. This decrease was most apparent in wheat and least apparent in rye. Figure 3 shows the changes in glutamic acid content during maturation. Since glutamine is converted to glutamic acid during hydrolysis, this figure actually reflects the combined changes of these two amino acids. A very large increase was found for all three cereals. The greatest increase was found in wheat and the least increase was found in rye. Figure 4 shows the changes in proline content during maturation. A rapid increase was found for all three cereals. Rye showed the greatest increase while wheat had the least increase. The other amino acids changed less rapidly. In all cases Triticale had a level intermediate to its two parents throughout development.

Earlier studies have shown that in wheat (Jennings and Morton (1963c)), barley (Ivanko (1971)) and maize

Figure 2. Changes in the Lysine Content of the Proteins and Peptides of Maturing Triticale 6A190 (△), and Parents, Prolific Rye (O) and Stewart Durum (□).

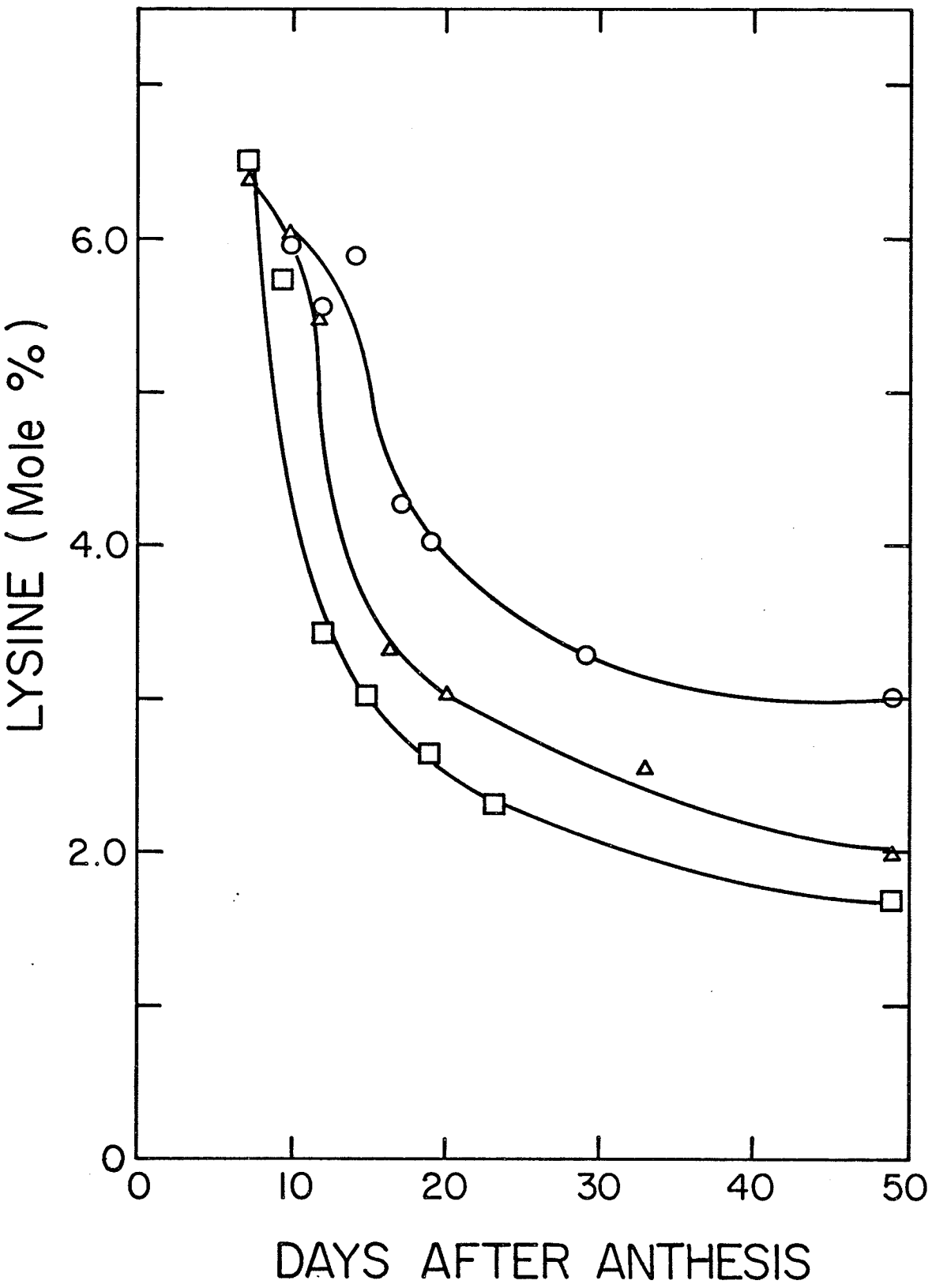

LYSINE (Mole %)
6.0
4.0
2.0
0
0
10
20
30
40
50
DAYS AFTER ANTHESIS

Figure 3. Changes in Glutamic Acid Content of the Proteins and Peptides of Maturing Triticale 6A190 (△), and its Parents, Prolific Rye (○) and Stewart Durum (□).

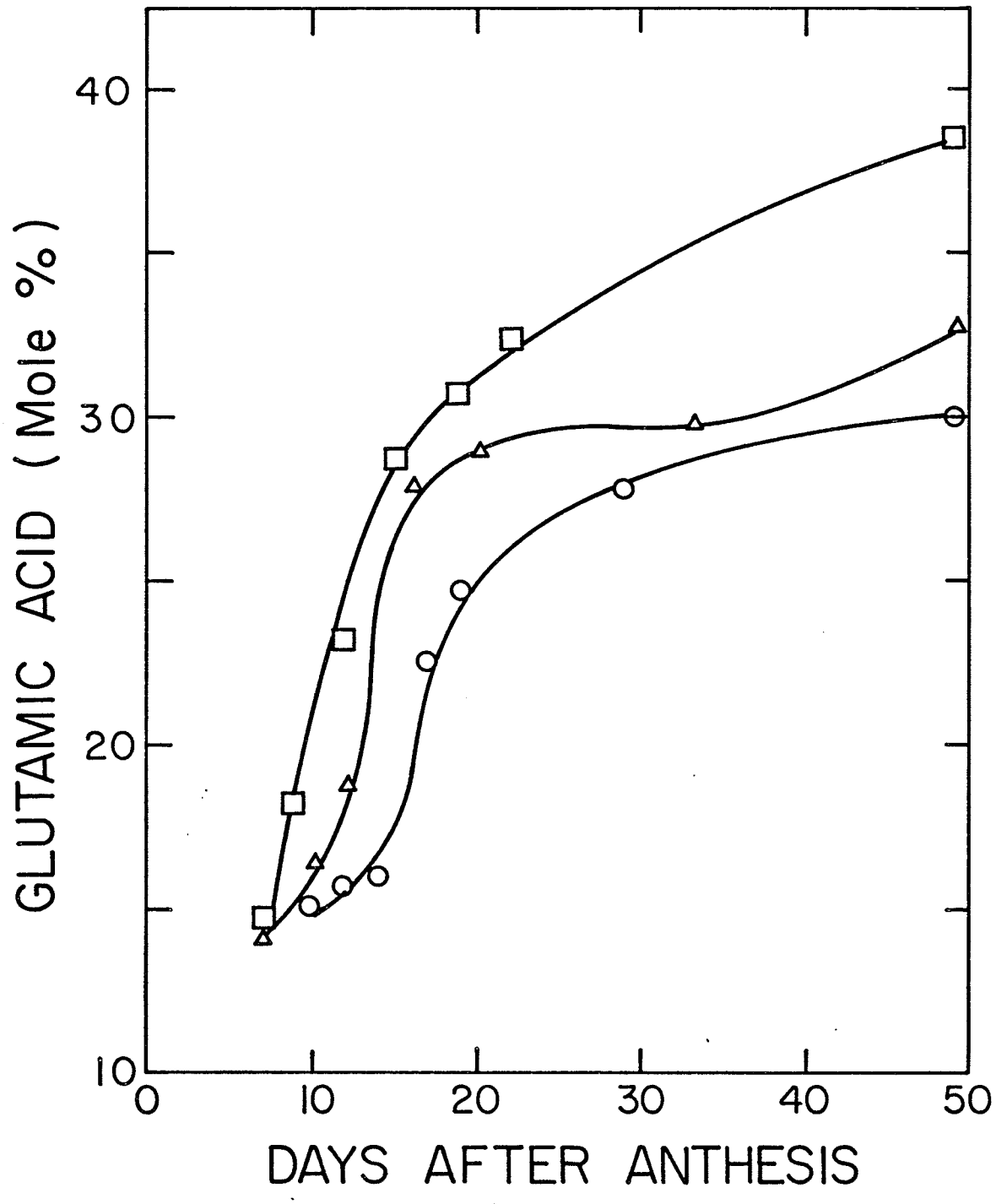
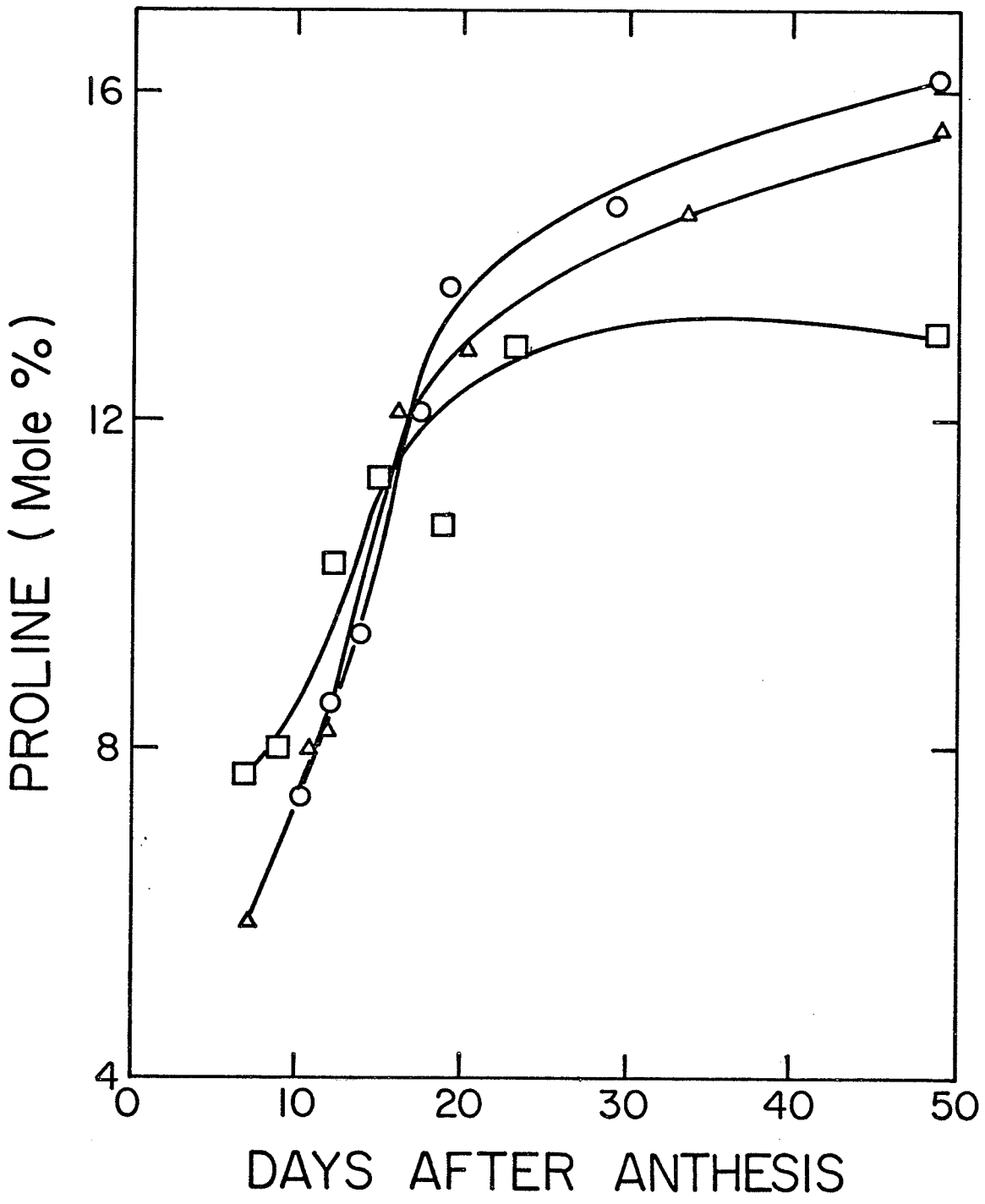

GLUTAMIC ACID (Mole %)
40
30
20
10
0
10
20
30
40
50
DAYS AFTER ANTHESIS

Figure 4. Changes in Proline Content of the Proteins and Peptides of Maturing Triticale 6A190 (△), and its Parents, Prolific Rye (○) and Stewart Durum (□).


PROLINE (Mole %)
16
12
8
4
0
10
20
30
40
50
DAYS AFTER ANTHESIS

(Murphy and Dalby (1971)) there are rapid buildups of protein during maturation. In addition, storage protein has been shown to have a lower lysine content and a higher glutamic acid (and glutamine) and proline content than other protein fractions found in cereals (Dronzek et al (1970), Ivanko (1971), Murphy and Dalby (1971)). The results in Figures 2, 3 and 4 confirm the results of these studies.

Chen and Bushuk (1970a) showed that Triticale 6A190 had a protein solubility distribution intermediate to that of its parents at maturity, which results in an amino acid composition which was also intermediate to its parents. Since the results in Table 4 show that Triticale had an amino acid composition intermediate to its parents at all stages of maturity, it would appear that its protein solubility distribution was also intermediate at all times. This suggests that the genome of durum wheat and rye are acting independently throughout the maturation process.

There was a sharp change in the amino acid composition of the endosperm proteins and peptides in all three cereals about two weeks after anthesis. This agreed with the work of Jennings and Mortion (1963c) who showed that for bread wheat endosperm cell division ceases at about fourteen days after anthesis, with rapid protein buildup thereafter. This was further supported by the previously discussed free amino

acid data (Table 2). The rapid decrease in free alanine in the endosperm of all three cereals about two weeks after anthesis suggested the termination of rapid endosperm cell growth since it has been shown that alanine was present in large quantities in the soluble-nitrogen component of rapidly growing cells (Stewart and Duzan (1965)).

The amino acid composition of the proteins and peptides for a bread wheat (cv. Thatcher), its extracted tetraploid, and two hexaploid Triticales (cv. Rosner and Kangaroo X U.M. 940'S') were also computed for all stages of endosperm development. Results are presented in Appendix B and Appendix C respectively. Results were very similar to those for Triticale 6A190, Prolific rye and Stewart durum.

The nitrogen content of the developing endosperm.

The total nitrogen content in the developing endosperms of the three cereals as well as the nitrogen present as free amino acids were computed by adjusting the recovery of the total amino acid analyses of each sample to 90%. Results are plotted in Figure 5.

Hoseney et al (1966) found little change in protein content on a dry matter basis during maturation in hard red winter flour, although a decrease in salt soluble and water soluble protein was found to parallel an increase in gluten

protein. Jennings and Morton (1963a) found that the nitrogen content on a dry matter basis decreased initially in hard red spring wheat due to early rapid starch synthesis, levelling off thereafter and increasing slightly near maturity. The results in figure 5 for total nitrogen in durum wheat endosperm followed that pattern fairly closely. Triticale had a similar pattern, although its nitrogen content was much higher than wheat throughout development. Rye, however, showed a decrease in protein nitrogen throughout the first four weeks of development, reaching by far the lowest level at maturity.

The free amino acid nitrogen decreased sharply in all three cereals to maturity, although Triticale showed a slight initial increase. Durum wheat had a much lower amount of free amino acid nitrogen than either rye or Triticale at all stages of maturation.

The amino acid composition of the developing whole grain.

The amino acid composition of the developing whole grains of Triticale 6A190 and its two parents, Prolific rye and Stewart durum are shown in Table 4. Rapid changes occurred in all three cereals during development. The most prominent trends common to all included a decrease in the relative proportions of lysine, aspartic acid (which includes asparagine), alanine and glycine and an increase in glutamic

Figure 5. Changes in the Total Nitrogen Content (▲, ●, ■) and Free Amino Acid Nitrogen (△, ○, □) of Developing Endosperm of Triticale 6A190 (▲, △) and Parents, Prolific Rye (●, ○) and Stewart Durum (■, □).

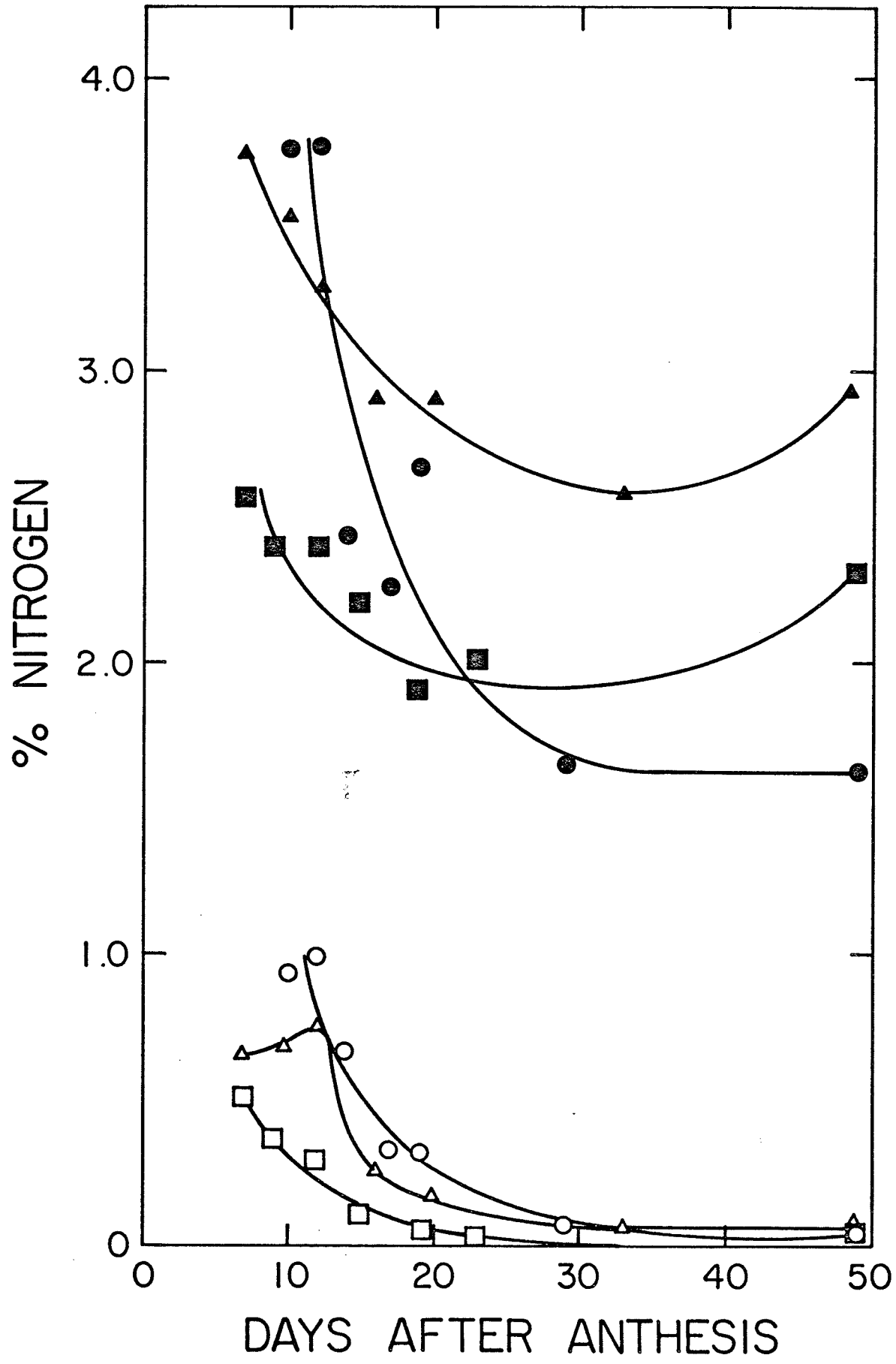

4.0
3.0
2.0
1.0
0
% NITROGEN
0
10
20
30
40
50
DAYS AFTER ANTHESIS

Table 4. Amino Acid Composition of Developing Whole Grain of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum. (Results are given as mole percent on an ammonia free basis)*

	Triticale					Prolific Rye					Stewart Durum				
Days after anthesis:	14	21	28	35	49	14	21	28	35	49	14	21	28	35	49
Lysine	4.55	3.33	3.09	2.73	2.89	5.36	4.19	3.59	3.07	3.30	3.66	2.67	2.21	2.19	2.20
Histidine	1.86	1.87	1.79	1.88	1.87	1.85	1.83	1.98	1.85	1.88	1.72	1.86	1.85	1.88	1.92
Arginine	3.42	3.32	3.18	3.46	4.13	3.12	3.25	3.48	3.72	3.90	3.22	3.04	3.12	3.40	3.45
Aspartic Acid	10.1	6.81	7.25	6.32	6.74	10.6	9.23	6.78	6.29	7.28	7.66	6.98	4.68	4.68	4.69
Threonine	3.81	3.37	3.32	3.16	3.24	4.23	3.84	3.58	3.41	3.53	3.53	3.04	2.87	2.76	2.86
Serine	6.31	4.85	5.78	5.37	5.61	6.08	5.83	5.64	5.70	5.76	6.63	5.45	5.63	5.02	5.70
Glutamic Acid	22.0	26.5	27.9	28.9	27.2	18.1	22.9	25.7	26.7	25.4	24.9	30.4	32.4	33.4	32.3
Proline	7.26	12.1	12.5	13.3	13.1	8.19	11.2	13.1	13.9	13.9	9.77	11.9	13.7	13.8	13.4
Glycine	8.11	7.63	7.31	7.17	7.46	8.66	7.81	7.48	7.27	7.59	8.33	6.75	6.29	6.10	6.47
Alanine	12.6	7.95	6.80	6.15	5.61	13.1	7.43	6.81	6.30	6.06	8.75	6.11	5.24	4.77	4.84
Valine	4.92	5.07	4.83	4.78	5.14	5.63	5.33	5.32	5.24	5.30	5.12	4.58	4.52	4.46	4.56
Methionine	1.12	1.44	1.14	1.28	1.42	0.70	1.27	1.19	1.20	0.91	0.51	1.14	1.09	1.12	0.91
Isoleucine	3.40	3.60	3.43	3.46	3.49	3.68	3.59	3.40	3.33	3.34	3.74	3.66	3.65	3.54	3.60
Leucine	6.25	6.94	6.47	6.56	6.64	6.65	6.88	6.63	6.40	6.48	6.88	6.76	6.93	6.91	6.97
Tyrosine	1.49	1.69	1.68	1.83	1.80	1.01	1.52	1.43	1.64	1.55	1.98	1.76	1.94	1.87	2.03
Phenylalanine	2.80	3.55	3.52	3.61	3.74	3.14	3.87	3.90	3.89	3.88	3.54	3.85	3.89	4.10	4.07

* tryptophan, cysteine and cystine were not determined.

acid (which includes glutamine) and proline. In general, Triticale maintained an amino acid composition intermediate to its two parents throughout development. Rapid changes in amino acid composition occurred between the second and third week after anthesis. After the fourth week of development only minimal changes occurred. Methionine values were unreliable in most cases because of oxidative degradation.

A comparison of the results in Table 4 with the previously described results for developing endosperm from the same three cereals (Table 1) showed a number of significant differences at maturity. Some of the most noticeable differences included a higher lysine, aspartic acid and alanine content in the whole grain in addition to a lower glutamic acid and proline content. This merely reflected the contribution of the bran and the germ to the overall amino acid composition of the whole grain samples (Tkachuk and Irvine (1969)). In general, however, both the whole grain and the endosperm had the same pattern of change in their amino acid composition throughout the maturation process.

Two bread wheats (cv. Thatcher and cv. Rescue) as well as their extracted tetraploids (Tetrathatcher and Tetrarescue) were also examined for changes in amino acid composition of the developing whole grain. The amino acid composition for all four wheats were very similar and paralled the results reported for Stewart durum. The data

are presented in Appendix B.

Distribution of protein in the five solubility fractions of developing whole grain.

Protein extractions were performed on the developing whole grain of Triticale 6A190 and its parents Prolific rye and Stewart durum using a method based on the classical Osborne procedure (Osborne, 1907). The results are presented in Tables 5, 6 and 7. Rapid changes occurred in the distribution of the protein fractions in all three cereals during development, although the pattern of change differed widely from one cereal to another. This was consistent with previous reports on wheat (Coulson and Sim (1964), Hoseney et al (1966)), rice (Palmiano et al (1971), oats (Wiggins and Frey (1958), barley (Ivanko (1971)) and maize (Ingle et al (1965), Murphy and Dalby (1971)).

Table 5 showed that the major changes occurring in the protein solubility distribution within the developing whole grain of Triticale 6A190 included a rapid buildup of gliadins and glutenins. The amount of dialyzable nitrogen decreased rapidly as reflected in the recovery of total nitrogen in the five solubility fractions. By far the greater part of all these changes occurred before the fourth week after anthesis. Prolific rye followed a fairly similar pattern to Triticale 6A190 (Table 6). The two main differences observed were a large increase in the proportion of albumins

Table 5. Distribution of Protein in the Five Solubility Fractions of Maturing Triticale 6A190 Whole Grain.

(Results are expressed as percent nitrogen)

Days after anthesis:	Albumins	Globulins	Gliadins	Glutenins	Insoluble Residue	Nitrogen Recovery
14	12.3	7.5	1.0	1.4	19.2	41.4
21	16.3	14.3	13.5	7.8	19.5	71.4
28	18.6	10.2	27.4	14.7	18.5	89.4
35	17.1	11.7	28.3	16.6	16.6	90.3
49	17.9	11.5	25.1	17.2	16.6	88.3

Table 6. Distribution of Protein in the Five Solubility Fractions of Maturing Prolific Rye Whole Grain.

(Results are expressed as percent nitrogen)

Days after anthesis:	Albumins	Globulins	Gliadins	Glutenins	Insoluble Residue	Nitrogen Recovery
14	12.1	9.3	1.6	0.4	23.3	46.7
21	22.5	10.2	7.4	1.0	27.2	68.3
28	24.5	18.5	14.4	1.5	23.6	82.5
35	30.7	15.7	12.4	10.7	14.7	84.2
49	29.5	14.8	17.2	7.8	16.5	85.8

Table 7. Distribution of Protein in the Five Solubility Fractions of Maturing Stewart Durum Whole Grain.

(Results are expressed as percent nitrogen)

Days after anthesis:	Albumins	Globulins	Gliadins	Glutenins	Insoluble Residue	Nitrogen Recovery
14	23.1	9.0	23.7	4.5	21.0	81.3
21	16.7	10.3	28.2	8.4	27.8	91.4
28	12.5	9.1	36.3	8.6	23.9	90.4
35	11.5	12.6	34.3	20.2	18.5	97.1
49	10.0	11.0	33.6	18.2	19.2	92.0

between the second and third week after anthesis and the absence of any appreciable amount of glutenins until after the fourth week following anthesis. Stewart durum, on the other hand, had a different protein solubility distribution during development (Table 7). Much less total nitrogen was dialyzable at early maturity than was the case for the other two cereals. The relative proportions of albumins in wheat decreased during development. At early maturity wheat had the largest proportion of albumins of the three cereals while at maturity it had the least. The other major difference was the relatively large amount of gliadins present even at fourteen days after anthesis compared to the other two cereals. At maturity Triticale had a protein solubility distribution intermediate to that of its two parents. This confirmed the results of Chen and Bushuk (1970a).

The incorporation of ^{14}C-leucine into the protein of the whole grain.

McConnell and Ramachandran (1956) followed the incorporation of labelled compounds into wheat kernels by injecting the labelled compound into the stem just below the head during the period of kernel development. This technique was adopted here in order to study the incorporation of uniformly labelled ^{14}C-leucine into the proteins from the developing whole grain of Triticale 6A190 and its parents Prolific rye and Stewart durum. ^{14}C-leucine was selected

as the label because when it is used as the source for ^{14}C tracer in developing cereals, virtually all the label incorporated is present as ^{14}C-leucine (Sodek and Wilson (1970)). This was confirmed in our studies. The amino acids were separated from the soluble sugars and organic acids in hydrolyzates from labelled protein fractions and whole grain samples on a cationic exchange column. In all cases over 85% of the label was recovered in the amino acids. The amino acids were then separated by paper chromatography. All the detectable activity was found in leucine.

Table 8 shows the incorporation of ^{14}C-leucine into the whole grain and the protein fractions of Triticale 6A190 and its parents, Prolific rye and Stewart durum. Incorporation was generally much higher for samples which were allowed to develop to maturity, although good incorporation was also achieved for those samples harvested one week after the time of injection. The trend was especially apparent for those samples injected in the first three weeks after anthesis. For both Triticale and rye the highest levels of incorporation were achieved for samples injected fourteen days after anthesis. This was in contrast to wheat which incorporated the greatest amount of label when it was injected three weeks after anthesis. Both rye and Triticale had large amounts of label present in non-protein nitrogen in samples harvested fourteen and twenty-one days after anthesis. This was not the case for wheat. This was

Table 8. Incorporation of ^{14}C-Leucine into the Developing Whole Grain of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.

(Results are expressed as %^{14}C-leucine incorporated).

Maturity (Days after anthesis)		Triticale		Prolific Rye		Stewart Durum	
Injection Date	Harvest Date	Whole Grain	Protein	Whole Grain	Protein	Whole Grain	Protein
7	14	33.5	24.2	25.5	17.1	22.1	19.1
14	21	62.9	47.0	49.1	27.1	48.6	46.4
21	28	41.3	40.0	32.6	25.2	54.7	46.9
28	35	44.5	37.6	30.8	24.2	32.6	31.2
35	49	5.0	5.0	9.6	7.5	4.2	4.0
7	49	39.5	38.4	43.7	43.9	44.9	41.6
14	49	81.3	70.0	52.3	40.2	52.1	49.1
21	49	38.3	32.5	44.1	33.1	61.4	62.8
28	49	44.3	40.5	31.5	25.6	25.8	21.5

consistent with the previously discussed Osborne fractionations (Tables 5, 6, 7) which showed that Triticale and rye had a large amount of non-protein nitrogen at this stage of development while wheat did not.

An indication of the relative rate of synthesis for the individual protein fractions during kernel development was gained by considering changes in the protein solubility distribution in conjunction with their pattern of ^{14}C-leucine incorporation. For this purpose it was found to be advantageous to convert the relative proportions of each of the five solubility fractions to a percentage of total protein. This gave a clearer indication of the relative rates of synthesis for each type of protein during the maturation process. Results are presented in Tables 9, 10 and 11. Rapid changes occurred for all three cereals throughout development. The proportion of albumins in Triticale and wheat declined rapidly between the second and third week after anthesis. The opposite was true for rye. Triticale globulins declined rapidly between the third and fourth week after anthesis. Rye and wheat, on the other hand, maintained a more uniform globulin distribution throughout development. Gliadins increased rapidly between the second and fourth weeks of development in all three cereals. Glutenins increased between the second and fifth weeks of

Table 9. Distribution of Protein in the Five Solubility Fractions of Maturing Triticale 6A190 Whole Grain.

(Results are expressed as percent total protein).

Days after anthesis:	Albumins	Globulins	Gliadins	Glutenins	Insoluble Residue
14	29.6	18.2	2.4	3.4	46.4
21	22.9	20.0	18.9	10.9	27.3
28	20.8	11.4	30.7	16.4	20.7
35	19.0	13.0	31.3	18.4	18.3
49	20.3	13.0	28.4	19.5	18.8

Table 10. Distribution of Protein in the Five Solubility Fractions of Maturing Prolific Rye Whole Grain.

(Results are expressed as percent total protein).

Days after anthesis:	Albumins	Globulins	Gliadins	Glutenins	Insoluble Residue
14	25.9	19.9	3.4	0.9	49.9
21	33.0	14.9	10.8	1.5	39.8
28	29.8	22.4	17.4	1.8	28.6
35	36.5	18.7	14.7	12.7	17.4
49	34.4	17.3	20.1	9.1	19.1

Table 11. Distribution of Protein in the Five Solubility Fractions of Maturing Stewart Durum Whole Grain.

(Results are expressed as percent total protein).

Days after anthesis:	Albumins	Globulins	Gliadins	Glutenins	Insoluble Residue
14	28.5	11.1	29.2	5.5	25.7
21	18.3	11.3	30.9	9.2	30.3
28	13.8	10.1	40.2	9.5	26.4
35	11.8	13.0	35.4	20.8	19.0
49	10.9	12.0	36.6	19.8	20.7

development in both Triticale and wheat. No significant amount of glutenins was found in rye for the first four weeks after anthesis, and then an abrupt buildup occurred. Insoluble residue protein declined rapidly between the second and fifth weeks after anthesis in both Triticale and rye. In wheat no noticeable decrease was observed until five weeks after anthesis.

The pattern of incorporation of ^{14}C-leucine into each protein fraction for each cereal during development is presented in Figures 6, 7 and 8. Only those samples harvested one week after the administration of ^{14}C-leucine (with the exception of the samples injected five weeks after anthesis) were considered because the samples which were allowed to mature continued to incorporate label for some time after a one week interval (Table 8). To a large extent the pattern of ^{14}C-leucine incorporation into the protein fractions of all three cereals during development complemented the Osborne solubility distributions (Tables 9, 10, 11). Nevertheless they did provide additional information on protein synthesis. Triticale albumins, for instance, contained over 40% of the ^{14}C-leucine incorporated into protein between the first and second weeks of development (Figure 6), while it only comprised about 30% of the protein two weeks after anthesis (Table 9). This showed that during this period albumins were being synthesized at an exceptionally rapid rate relative to the other protein

Figure 6. % of Incorporated ^{14}C-Leucine Present in the Albumins (○), Globulins (◐), Gliadins (△), Glutenins (●) and Insoluble Residue (□) from the Developing Whole Grain of Triticale 6A190.

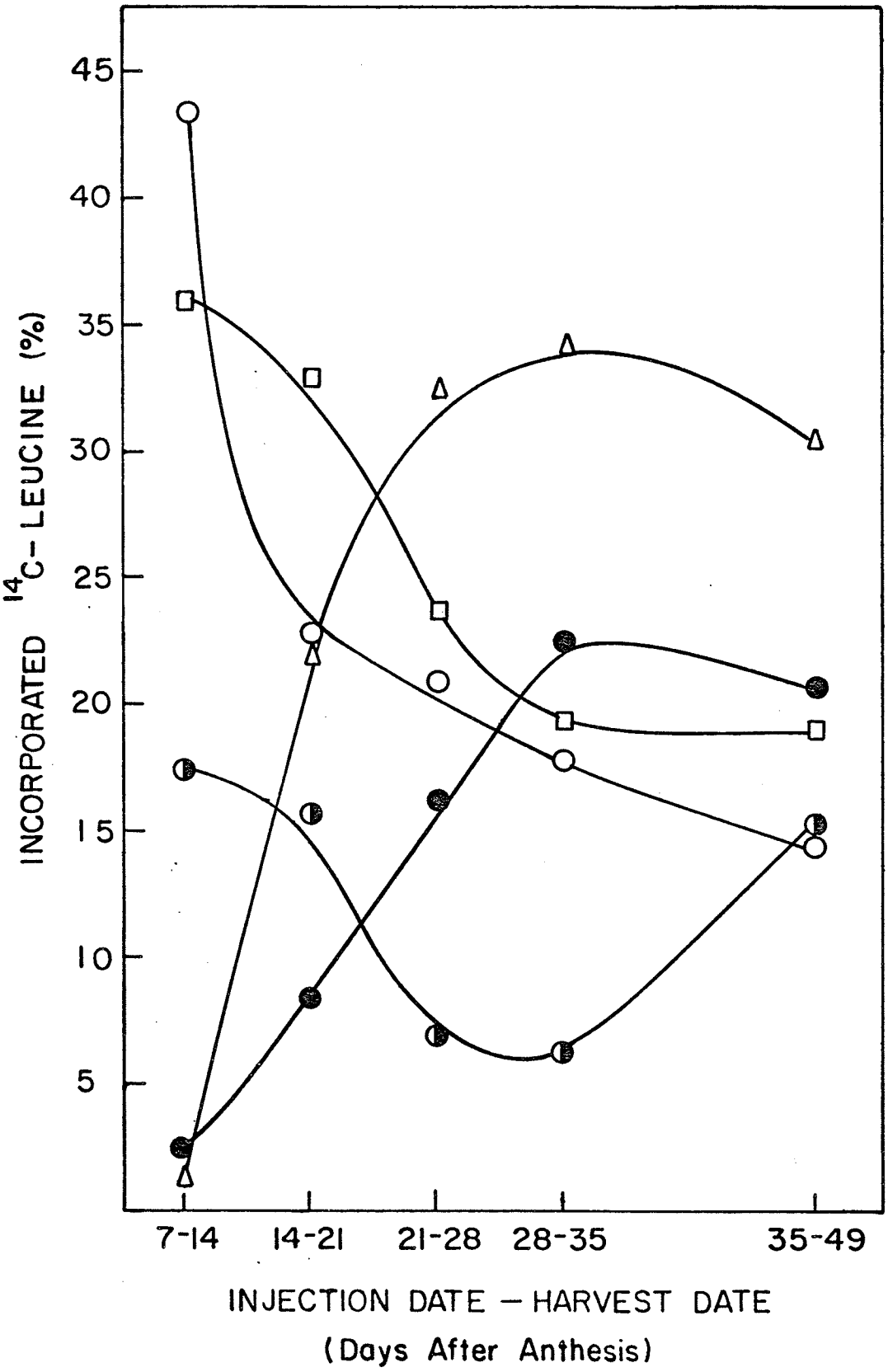

45
40
35
30
25
20
15
10
5
INCORPORATED ^{14}C- LEUCINE (%)
7-14
14-21
21-28
28-35
35-49
INJECTION DATE – HARVEST DATE
(Days After Anthesis)

Figure 7. % of Incorporated ^{14}C-Leucine Present in the Albumins (○), Globulins (◑), Gliadins (△), Glutenins (◐) and Insoluble Residue (□) from the Developing Whole Grain of Prolific Rye.

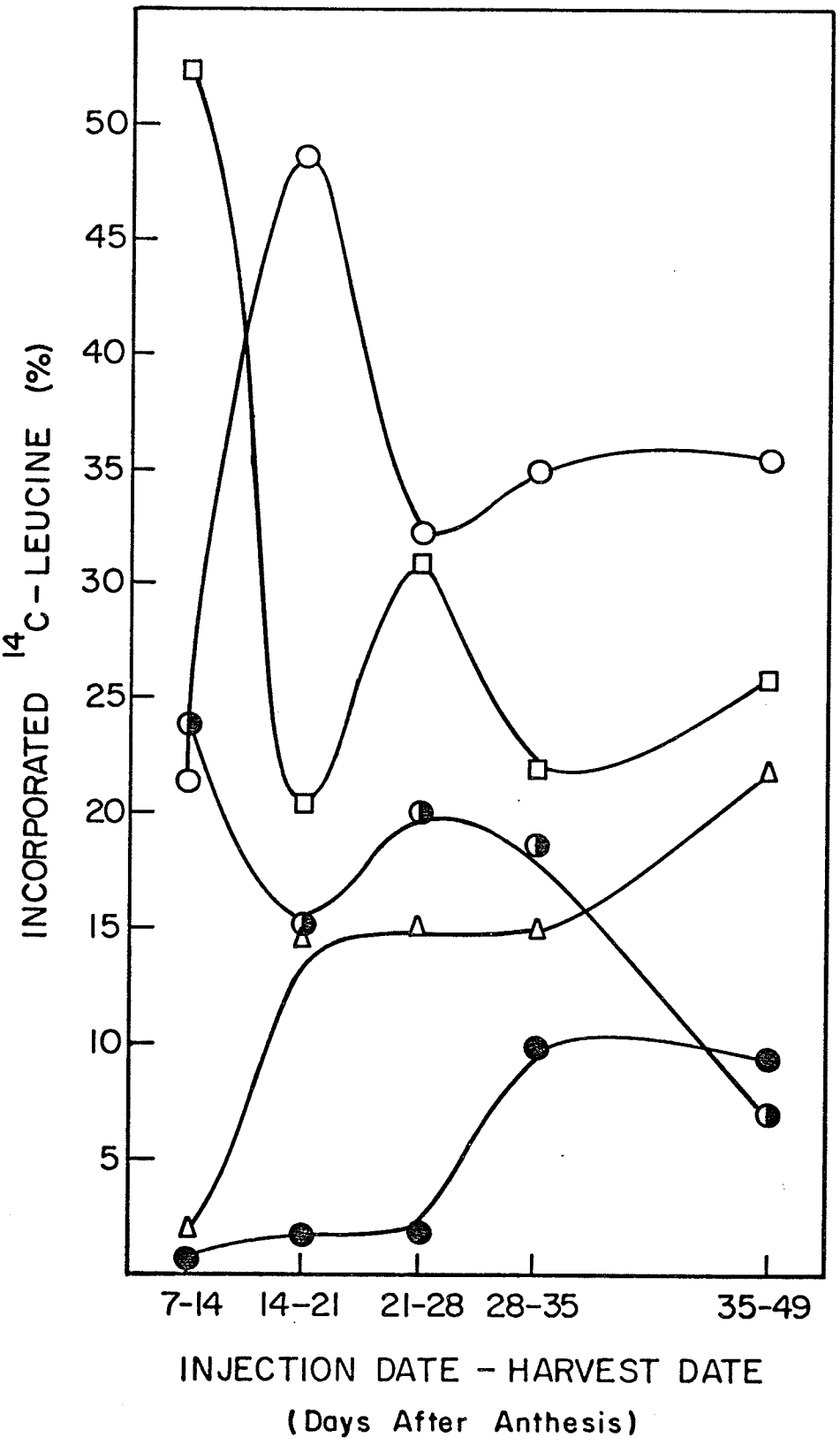
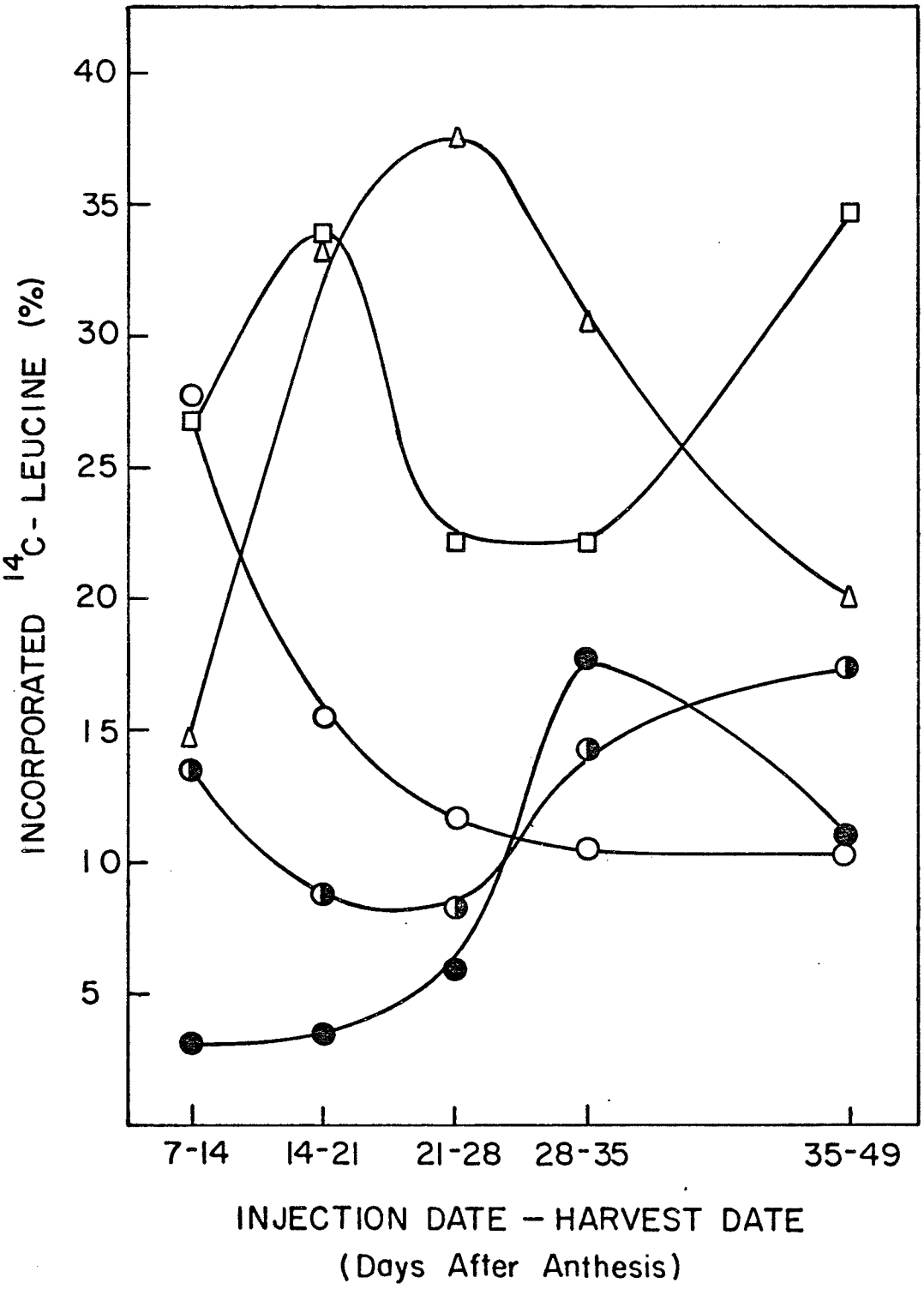

INCORPORATED ^{14}C-LEUCINE (%)
50
45
40
35
30
25
20
15
10
5
7-14
14-21
21-28
28-35
35-49
INJECTION DATE - HARVEST DATE
(Days After Anthesis)

Figure 8. % of Incorporated ^{14}C-Leucine Present in the Albumins (○), Globulins (◑), Gliadins (△), Glutenins (●) and Insoluble Residue (□) from the Developing Whole Grain of Stewart Durum.


INCORPORATED ^{14}C- LEUCINE (%)
40
35
30
25
20
15
10
5
7-14
14-21
21-28
28-35
35-49
INJECTION DATE – HARVEST DATE
(Days After Anthesis)

fractions. The percentage of label incorporated into the Triticale albumins between the second and third weeks of development corresponded very closely to the proportion of protein present as albumins three weeks after anthesis. Thus there was a rapid decline in the relative rate of albumin synthesis between the first and third weeks of development. Nevertheless the rate of Triticale albumin synthesis was still considerable three weeks after anthesis. Thereafter the proportion of incorporated label in Triticale albumins was similar to its proportion of Triticale protein, which indicated albumin synthesis maintained a substantial portion of overall protein synthesis throughout development. Similar results were found for wheat albumins during development (Table 11, Figure 8). Rye albumins differed, however (Table 10, Figure 7). They accumulated about 20% of the ^{14}C-leucine incorporated between the first and second weeks of development, but comprised over 25% of the protein two weeks after anthesis. Between the second and third week, however, 48% of the incorporated label was found in the albumins whereas at three weeks after anthesis they represented barely a third of the protein. Thus, the rate of albumin synthesis increased markedly over this period compared to the overall rate of protein synthesis in the other fractions. Thereafter the percentage of incorporated label in the albumins corresponded closely to its share of the total

protein, indicating its rate of synthesis relative to total protein synthesis decreased rapidly between the third and fourth week after anthesis and levelled off thereafter.

The proportion of incorporated ^{14}C-leucine utilized in Triticale globulins synthesis between the third and fifth weeks after anthesis was much lower than their proportion of the Triticale protein over this period. This showed a decrease in the rate of globulin synthesis at this time. This trend was not found in either wheat or rye. Between the fifth week after anthesis and maturity Triticale globulins accumulated 15% of the ^{14}C-leucine incorporated into protein, while it represented only about 12% of the total protein present at maturity. This indicated a relative increase in globulin synthesis over this period. A similar result was found for wheat. Rye, however, gave the opposite result.

For both Triticale and rye the gliadins incorporated a relative amount of label similar to this fraction's relative amount within the protein throughout all stages of development. Wheat gliadins incorporated only 15% of the label recovered in protein between the first and second weeks of development where it comprised close to 30% of the protein present at two weeks after anthesis. Over the next two weeks the wheat gliadins incorporated an amount of label similar to their relative proportion within the protein. Over the final stages of maturation, however, the relative amount of label incorporated was significantly less than their relative proportion

of the total protein. These trends indicated that the relative rate of wheat gliadin synthesis increased during the latter part of the second week after anthesis, levelled off for several weeks, and then decreased. This decrease during late stages of development was not found for Triticale or rye.

For both Triticale and rye the glutenins showed a pattern of ^{14}C-leucine incorporation very similar to their relative proportion within the protein throughout all stages of development. This was also true of wheat except during the last two weeks of maturation. During this time its level of label incorporation lagged noticeably, indicating that there was a decrease in the relative rate of wheat glutenins synthesis over this period.

The insoluble residue protein of Triticale contained about 35% of the ^{14}C-leucine incorporated between the first and second week after development, whereas it comprised 46% of the protein present two weeks after anthesis. Between the second and third weeks after anthesis, this fraction incorporated a similar proportion of the label even though its relative proportion of the protein had declined to 27% three weeks after anthesis. Thus, despite a sharp decline in amount of total protein, this fraction continued to be synthesized at a rapid rate over this period. This was in contrast to the insoluble residue of rye which incorporated only 20% of the ^{14}C-leucine utilized in protein

between the second and third weeks of development, although it comprised 40% of the protein three weeks after anthesis. Thus rye insoluble residue protein synthesis declined markedly over this period in relation to the overall rate of protein synthesis. Wheat insoluble residue protein incorporated label according to a pattern which was very similar to that found for its distribution in wheat protein throughout development except during the last two weeks of development. Over this period the proportion of label this fraction accumulated was greater than its proportion of the wheat protein at maturity. This indicated an increase in the relative rate of insoluble residue protein synthesis over this period. This was also observed for rye insoluble residue protein. Triticale differed from both its parents in this respect since it did not show this increase.

These results showed that the mode of protein synthesis during kernel development in Triticale has been inherited from both parents. The rate at which its albumins and gliadins were synthesized more closely resembled its wheat parent, while the synthesis of its glutenins and insoluble residue more closely followed the pattern found in its rye parent. Triticale deviated from the pattern of protein synthesis during maturation for both its parents in two cases. Firstly, the relative rate of globulin synthesis decreased much more between the third and fifth week after anthesis. Secondly, it did not exhibit the increase in

insoluble residue protein synthesis relative to overall protein synthesis observed in both parents during the last two weeks of maturation.

Finlayson and McConnell (1969) studied the utilization of phenylalanine-2-^{14}C by wheat plants during kernel development. In most respects their results were similar to those found for Stewart durum in this study. They found maximum tracer incorporation occurred three to four weeks before maturity, while incorporation dropped to a very low level over the last two weeks before maturity. They also found that the specific activity in the salt-soluble proteins was much greater than that for gluten proteins over the last two weeks to maturity. The latter result which has also been reported here was consistent with the work of Bilinski and McConnell (1958) who found that bran proteins (which contain a considerable amount of salt-soluble protein) showed markedly increasing activity with late injection of ^{14}C-acetate.

The amino acid composition of the five protein solubility fractions from the maturing whole grain.

The amino acid composition of the albumins from the developing whole grain of Triticale 6A190 and its parents is shown in Table 12. Numerous changes occurred within this fraction during maturation for all three cereals. The major changes occurred between the second and fourth week

Table 12. Amino Acid Composition of the Albumins from the Developing Whole Grain of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.

(Results are given as mole percent on an ammonia free basis)*

	Triticale					Prolific Rye					Stewart Durum				
Days after anthesis:	14	21	28	35	49	14	21	28	35	49	14	21	28	35	49
Lysine	5.57	4.47	3.83	3.76	3.14	5.95	4.43	3.15	2.95	3.38	5.28	4.68	3.90	4.19	3.78
Histidine	2.01	2.00	1.73	1.83	1.73	1.98	1.79	1.56	1.57	1.68	1.99	1.98	1.89	2.08	1.78
Arginine	3.65	4.12	3.26	3.62	3.35	3.31	3.66	3.03	2.90	3.35	4.07	4.16	3.89	4.39	3.48
Aspartic Acid	9.29	8.74	6.54	6.66	6.26	10.9	7.68	5.48	5.31	6.30	8.81	8.14	7.36	7.05	6.38
Threonine	4.68	4.78	3.78	4.07	3.96	5.02	4.04	3.66	3.58	4.08	4.47	4.43	4.13	4.47	4.22
Serine	6.41	6.15	6.14	6.18	5.44	6.78	5.54	5.58	5.73	5.60	6.47	6.09	6.15	6.30	6.72
Glutamic Acid	16.1	16.1	24.6	22.4	25.4	14.5	20.9	27.1	27.9	25.2	17.1	18.9	22.0	21.5	23.5
Proline	8.63	8.43	13.1	12.9	13.8	7.29	12.7	16.5	15.9	13.7	7.96	8.65	9.85	9.28	11.5
Glycine	8.80	8.95	6.91	7.17	6.84	9.29	7.37	5.64	5.54	6.80	8.35	8.31	7.91	8.39	7.88
Alanine	8.20	8.62	6.53	6.94	6.43	8.89	6.87	5.78	5.69	6.61	8.60	8.45	7.73	8.08	7.40
Valine	6.31	7.17	5.41	5.77	5.38	6.78	5.77	4.98	5.19	5.57	6.66	6.37	6.37	6.04	5.68
Methionine	1.79	2.08	1.42	1.73	1.51	1.68	1.57	1.36	1.45	1.61	1.74	1.85	1.73	1.49	1.63
Isoleucine	3.82	3.96	3.39	3.46	3.48	3.81	3.67	3.27	3.48	3.44	4.12	3.91	3.57	3.48	3.34
Leucine	8.41	8.38	6.95	7.34	6.91	7.71	7.50	6.51	6.58	6.82	8.05	7.96	7.48	7.29	7.03
Tyrosine	2.57	2.85	2.28	2.48	2.37	2.53	2.34	1.86	1.69	1.80	2.71	2.65	2.64	2.62	2.53
Phenylalanine	3.81	3.25	4.20	3.76	3.95	3.58	4.23	4.62	4.51	4.04	3.59	3.50	3.36	3.36	3.14

* tryptophan, cysteine and cystine were not determined.

after anthesis. They included a decrease in lysine, aspartic acid, glycine and alanine and an increase in glutamic acid and proline. These changes were greater in Triticale and rye than wheat. There was also a significant decrease in threonine and glycine in both Triticale and rye. In wheat, however, these amino acids retained a fairly uniform level throughout development. It is noteworthy that the major changes in amino acid composition within this fraction for all three cereals paralled the major changes observed in the overall amino acid compositions of the whole grains throughout development (Table 4). This indicated that changes in the makeup of this fraction contributed significantly to the changes observed in the amino acid composition of the whole grain as it matured. This was especially true of rye. The amino acid composition of its albumins was very similar to the total amino acid composition of its whole grain throughout development. This was consistent with its protein distribution during development which showed albumins to be the most prevalent protein fraction at all times during the maturation process (Tables 6,11). The albumins for all three cereals had a similar amino acid composition at all stages of development. Triticale albumins resembled those of its rye parent more closely than those of its wheat parent at maturity.

The amino acid composition of the globulins from

the developing whole grain of Triticale 6A190 and its parents Prolific rye and Stewart durum is shown in Table 13. Only slight changes occurred in this fraction for all three cereals during development. The two most common changes included an increase in arginine and an increase in glutamic acid. Wheat globulins showed a sizable decrease in lysine content while a slight decrease was apparent for Triticale and rye. The overall amino acid composition of the globulins from all three cereals was similar at all stages of kernel development.

The amino acid composition of the gliadins from maturing Triticale 6A190 and its parents is shown in Table 14. A very drastic change in amino acid composition occurred for both Triticale and rye between the second and third week after anthesis. Since only a very small amount of protein was extracted by 70% ethanol from both these cereals two weeks after anthesis, it is quite likely that this fraction was highly contaminated with other types of proteins, probably albumins. The amino acid composition of wheat gliadins changed slightly between the second and third weeks after anthesis, and retained a remarkably uniform amino acid composition thereafter to maturity. Triticale gliadins also had a very uniform amino acid composition from the third week after anthesis to maturity. Rye gliadins, meanwhile, underwent numerous small changes in their amino acid composition over this period. In addition, histidine,

Table 13. Amino Acid Composition of the Globulins from the Developing Whole Grain of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.

(Results are given as mole percent on an ammonia free basis)*

	Triticale					Prolific Rye					Stewart Durum				
Days after anthesis:	14	21	28	35	49	14	21	28	35	49	14	21	28	35	49
Lysine	6.12	5.55	6.10	5.60	5.64	6.09	6.47	5.48	5.56	5.62	6.84	5.77	5.93	5.25	5.08
Histidine	2.19	2.16	2.09	2.32	2.67	2.39	2.28	2.26	2.57	2.73	2.08	2.57	2.67	2.55	2.70
Arginine	5.79	6.33	7.01	7.32	7.72	5.37	6.50	6.81	7.38	8.08	6.22	6.97	7.87	8.32	8.16
Aspartic Acid	9.54	9.03	8.94	8.68	8.82	10.1	9.35	8.99	8.31	8.73	9.07	8.06	8.42	8.77	7.92
Threonine	5.18	4.80	4.71	4.43	4.39	5.29	5.06	4.69	4.31	4.19	4.92	4.32	4.25	4.35	4.09
Serine	6.22	6.15	6.34	6.35	6.79	6.30	6.04	6.36	6.58	6.59	6.14	6.17	6.24	6.43	6.28
Glutamic Acid	11.2	12.8	13.4	13.5	13.5	10.1	11.7	12.9	13.9	13.8	12.5	16.3	14.9	13.9	16.3
Proline	4.78	5.20	5.24	4.97	4.60	5.17	4.92	5.24	5.25	4.66	5.27	5.90	5.16	4.92	5.21
Glycine	8.92	9.22	9.16	9.78	10.3	9.06	9.67	9.64	9.79	9.70	8.72	9.14	9.33	9.95	9.98
Alanine	8.86	9.21	8.58	9.33	8.74	8.74	8.83	9.16	8.66	8.61	8.70	8.20	8.51	9.09	8.40
Valine	7.13	7.39	7.05	7.06	7.01	7.02	7.08	7.18	7.11	7.12	7.05	6.53	6.97	7.25	7.19
Methionine	2.05	1.97	1.59	1.85	1.47	2.09	1.81	1.82	1.59	1.49	1.79	1.68	1.68	1.36	1.62
Isoleucine	5.04	4.74	4.64	4.24	4.09	4.93	4.67	4.44	4.28	4.14	5.00	4.26	4.10	3.96	3.82
Leucine	9.57	9.00	8.70	8.39	8.16	9.42	8.79	8.51	8.30	8.10	8.89	8.13	7.89	7.91	7.58
Tyrosine	3.21	2.69	2.70	2.58	2.45	3.52	3.05	2.68	2.49	2.60	2.97	2.51	2.53	2.38	2.30
Phenylalanine	4.19	3.75	3.78	3.64	3.65	4.43	3.81	3.82	3.93	3.81	3.81	3.53	3.60	3.52	3.42

* tryptophan, cysteine and cystine were not determined.

Table 14. Amino Acid Composition of the Gliadins from the Developing Whole Grain of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.

(Results are given as mole percent on an ammonia free basis)*

	Triticale					Prolific Rye					Stewart Durum				
Days after anthesis:	14	21	28	35	49	14	21	28	35	49	14	21	28	35	49
Lysine	2.64	0.67	0.56	0.66	0.61	2.49	0.79	0.65	0.68	0.73	0.87	0.51	0.44	0.46	0.59
Histidine	1.08	1.17	1.41	1.00	1.39	0.97	0.14	0.37	0.98	0.93	1.44	1.46	1.51	1.46	1.67
Arginine	2.00	1.61	1.59	1.61	1.53	2.34	1.25	1.18	1.13	1.07	1.89	1.44	1.56	1.49	1.49
Aspartic Acid	5.61	2.81	2.45	2.71	2.41	5.12	2.35	1.98	2.12	1.93	3.00	2.57	2.49	2.50	2.44
Threonine	3.80	2.39	2.17	2.33	2.05	3.45	2.29	2.21	2.10	2.02	2.13	1.92	1.93	1.85	1.82
Serine	8.13	5.39	5.09	5.33	5.34	6.85	5.50	5.84	5.68	5.72	5.40	4.86	5.07	4.95	4.76
Glutamic Acid	29.1	38.1	39.7	37.9	40.4	29.3	39.7	40.4	39.9	41.2	40.1	42.1	40.9	41.9	42.5
Proline	12.1	18.1	19.2	19.5	18.4	13.4	22.6	22.6	22.6	21.6	15.8	18.3	17.9	18.2	17.9
Glycine	7.49	2.91	2.57	2.76	2.93	7.41	2.82	2.49	2.57	3.22	3.08	2.42	2.73	2.41	2.46
Alanine	5.97	3.01	2.68	2.83	2.69	5.56	2.31	2.32	2.43	2.42	3.23	2.66	2.71	2.64	2.55
Valine	4.12	4.31	3.89	4.16	3.89	4.30	4.23	4.37	4.33	4.15	4.04	3.54	3.66	3.64	3.70
Methionine	0.72	1.12	1.03	1.07	1.11	0.73	0.98	0.84	0.89	0.85	0.91	0.77	1.03	0.94	0.93
Isoleucine	4.24	4.34	4.12	4.23	3.91	4.16	3.22	3.15	3.07	2.69	4.16	3.95	4.04	3.96	3.85
Leucine	7.69	7.30	6.96	7.21	6.94	7.85	6.01	5.68	5.63	5.35	7.09	6.81	6.92	6.87	6.62
Tyrosine	1.80	1.80	1.71	1.62	1.65	1.90	1.05	1.13	1.09	1.49	2.02	2.04	3.10	2.03	1.91
Phenylalanine	3.60	4.97	4.90	5.02	4.71	4.16	4.83	4.79	4.76	4.65	4.84	4.65	5.03	4.70	4.76

* tryptophan, cysteine and cystine were not determined.

which was present in very low amounts three and four weeks after anthesis increased rapidly between the fourth and fifth weeks after anthesis. At maturity Triticale gliadins more closely resembled wheat gliadins than rye gliadins. The latter had a significantly lower histidine, arginine, isoleucine and leucine content and a higher proline content than the other. Table 4 revealed that the most rapid changes which occurred in the amino acid composition of the whole grains for all three cereals were a decrease in lysine content and an increase in glutamic acid and proline between the second and fourth weeks after anthesis. During this period there was a very large increase in the proportion of gliadins within the kernel proteins (Tables 9, 10 and 11). Since the gliadins in all three cereals were characterized by a very low lysine content and a very high glutamic acid and proline content, it was obvious that rapid gliadin synthesis during this two week period was largely responsible for the observed trends in amino acid composition of the whole grains.

Table 15 shows the amino acid composition of glutenins from the developing whole grain of Triticale 6A190 and its parents, Prolific rye and Stewart durum. The amino acid composition of rye glutenins changed rapidly from the second week after anthesis up to the fourth, during which time they were found in very low

Table 15. Amino Acid Composition of the Glutenins from the Developing Whole Grain of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.

(Results are given as mole percent on an ammonia free basis)*

	Triticale					Prolific Rye					Stewart Durum				
Days after anthesis:	14	21	28	35	49	14	21	28	35	49	14	21	28	35	49
Lysine	4.55	1.18	1.09	1.26	1.64	5.82	3.17	1.55	1.27	1.93	1.43	1.26	1.22	0.97	1.12
Histidine	1.59	1.26	1.29	1.38	1.45	1.43	1.51	1.50	1.22	1.45	1.37	1.42	1.40	1.57	1.46
Arginine	3.50	2.00	1.80	2.08	2.56	3.43	2.59	1.78	1.56	2.33	2.09	1.60	2.35	1.84	2.31
Aspartic Acid	6.26	2.43	1.99	2.40	3.02	7.92	4.51	2.61	2.16	3.30	2.82	1.74	2.45	2.29	2.30
Threonine	4.20	2.79	2.64	2.77	3.02	4.67	3.86	2.82	2.52	2.78	2.38	1.83	2.65	1.89	2.80
Serine	6.49	5.89	5.86	6.26	6.36	7.25	6.95	6.15	5.78	5.91	5.65	5.22	6.55	5.43	6.70
Glutamic Acid	24.9	37.7	37.9	37.2	34.6	20.7	29.6	36.8	37.8	33.7	40.9	44.3	38.9	39.9	37.5
Proline	9.52	16.3	17.0	15.4	14.8	8.06	11.3	17.1	18.8	17.2	14.1	16.9	14.4	16.5	14.6
Glycine	9.29	8.39	9.55	9.08	9.55	11.1	11.4	8.33	7.72	8.17	6.54	4.29	8.36	7.00	8.06
Alanine	6.65	3.23	3.12	3.31	3.94	7.06	6.00	3.42	3.16	4.12	3.19	2.07	3.11	2.81	3.28
Valine	5.09	3.48	3.09	3.44	3.79	4.15	4.12	3.73	4.06	4.38	2.88	2.48	3.14	3.25	3.48
Methionine	0.62	0.79	0.76	0.96	1.02	0.67	0.41	0.83	0.84	0.86	0.59	0.77	0.89	0.88	1.03
Isoleucine	3.89	2.84	2.46	2.67	2.60	4.15	3.09	2.52	2.25	2.44	3.48	3.56	2.81	3.52	3.00
Leucine	7.93	5.78	5.17	5.76	5.71	8.01	6.38	5.41	5.13	5.56	5.50	5.66	5.61	6.15	6.16
Tyrosine	2.22	2.81	3.17	2.95	3.08	1.72	2.30	2.27	2.38	2.52	2.44	2.01	2.77	2.54	2.85
Phenylalanine	3.23	3.15	3.07	3.09	2.91	3.86	2.83	3.18	3.34	3.35	4.59	4.87	3.41	3.43	3.42

* tryptophan, cysteine and cystine were not determined.

amounts (Table 10). In fact, the amino acid composition of rye glutenins was so similar to that of its insoluble residue proteins (Table 16) two weeks after anthesis, that it appeared likely glutenins were not present to any detectable amount at this time. Similarly, it was possible that the rye glutenins extracted three weeks after anthesis may have been highly contaminated with residue protein. Triticale glutenins also changed from the second week after anthesis to the third. At two weeks after anthesis the glutenin fraction comprised a very small proportion of Triticale protein (Table 11). Thus, again it was likely that this fraction was mainly, if not entirely, insoluble residue protein. Wheat glutenins had a fairly stable amino acid composition throughout development, with one noticeable exception. Glycine was found in much lower amounts during the second and third weeks of development than later. At maturity rye glutenins had a significantly higher lysine and proline content than wheat glutenins. On the other hand, wheat glutenins had more glutamic acid. Triticale glutenins, meanwhile, had a lysine content between that of its parents, while its proline level was similar to its wheat parent and its glutamic acid content was similar to its rye parent.

Table 16 shows the amino acid composition of the insoluble residue protein for Triticale 6A190 and its

Table 16. Amino Acid Composition of the Insoluble Residue from the Developing Whole Grain of Triticale 6A190 and Parents, Prolific Rye and Stewart Durum.

(Results are given as mole percent on an ammonia free basis)*

	Triticale					Prolific Rye					Stewart Durum				
Days after anthesis:	14	21	28	35	49	14	21	28	35	49	14	21	28	35	49
Lysine	4.43	2.71	3.32	3.35	3.97	5.33	4.16	3.12	3.76	4.36	3.24	2.95	2.99	2.88	3.04
Histidine	1.84	1.72	1.88	2.07	2.06	1.88	1.68	1.53	2.04	2.09	1.47	1.92	1.84	1.88	2.03
Arginine	3.79	3.18	3.71	4.04	4.32	4.11	3.79	3.42	3.64	4.20	3.47	3.51	3.50	3.73	4.02
Aspartic Acid	6.67	5.07	5.62	6.14	6.93	7.57	6.98	5.62	6.61	7.57	5.44	5.20	5.14	5.29	5.60
Threonine	4.13	3.45	3.73	3.72	4.07	4.47	4.04	3.72	3.97	4.32	3.64	3.57	3.69	3.52	3.61
Serine	6.05	6.26	6.30	6.44	6.88	6.06	5.85	6.15	6.28	6.71	6.50	6.89	6.86	6.60	6.55
Glutamic Acid	21.4	27.7	25.3	24.3	21.2	16.9	21.6	25.5	22.5	18.4	27.9	29.7	27.7	27.3	26.2
Proline	10.8	12.8	10.9	11.0	10.0	9.11	11.3	12.9	11.0	9.91	10.4	9.36	10.9	10.9	10.7
Glycine	8.98	9.09	9.99	9.65	9.95	9.74	9.54	10.1	10.3	10.3	8.17	8.98	9.38	8.86	9.01
Alanine	7.12	5.52	6.22	6.30	6.41	8.43	6.81	6.13	6.81	8.05	5.93	5.66	5.83	5.72	5.96
Valine	5.50	4.91	5.13	5.28	5.73	5.94	5.66	5.26	5.62	6.19	5.24	4.81	4.99	5.05	5.15
Methionine	1.24	0.97	1.13	1.07	0.90	1.18	1.14	1.06	0.95	1.10	1.06	0.76	1.10	1.23	1.22
Isoleucine	3.97	3.51	3.41	3.48	3.56	4.29	3.75	3.08	3.72	3.58	3.90	3.63	3.39	3.52	3.45
Leucine	8.04	7.30	7.24	7.40	7.38	8.55	7.77	6.73	7.28	7.72	7.57	7.52	7.33	7.39	7.49
Tyrosine	2.10	2.17	2.43	2.13	2.42	2.07	2.00	2.05	1.99	1.88	2.19	2.12	1.80	2.41	2.21
Phenylalanine	3.95	3.57	3.65	3.65	4.19	4.31	4.00	3.68	3.88	3.65	3.86	3.46	3.55	3.67	3.82

* tryptophan, cysteine and cystine were not determined.

parents, Prolific rye and Stewart durum. The insoluble residue protein of Triticale more closely resembled that of rye throughout the maturation process. Within the insoluble residue protein from these two cereals the content of lysine, arginine, aspartic acid, threonine and alanine declined between the second and fourth weeks after anthesis, and then regained their initial levels at maturity. The opposite was true for glutamic acid and proline. Wheat insoluble residue protein, however, had a relatively stable amino acid composition throughout all stages of maturation. At maturity wheat insoluble residue protein had a lower lysine content and higher glutamic acid content than that of both rye and Triticale. The amino acid composition of insoluble residue protein more closely resembled that of albumins than glutenins for all three cereals. This agreed with the findings of Cluskey and Dimler (1967) for bread wheat, Ivanko (1971) for barley and Palmiano et al (1968) for rice. The insoluble nature of this protein is likely due to the nature of its secondary and tertiary structure.

The overall amino acid compositions of the five protein solubility fractions of Triticale suggested that its proteins are inherited from both parents. Its albumins more closely resembled those of rye than wheat, although Triticale albumins had an amino acid composition intermediate to its two parents. This resemblence to rye albumins was explained by the far greater proportion of

albumins present in rye protein as compared to wheat protein (Tables 10, 11). On the other hand, although Triticale gliadins had an amino acid compostion intermediate to its two parents, they more closely resembled wheat gliadins than rye gliadins. This was explained by the far greater levels of gliadins in wheat compared to rye. Globulins were similar in all three cereals. Triticale glutenins had an amino acid composition intermediate to that of its parents. Although the insoluble residue fraction of Triticale protein more closely resembled rye than wheat throughout development, at maturity its amino acid composition was intermediate to its parents.

The observation of changes in amino acid composition within the individual protein fractions of these three cereals was in agreement with previous reports by Ivanko (1971) on barley and Murphy and Dalby (1971) on maize.

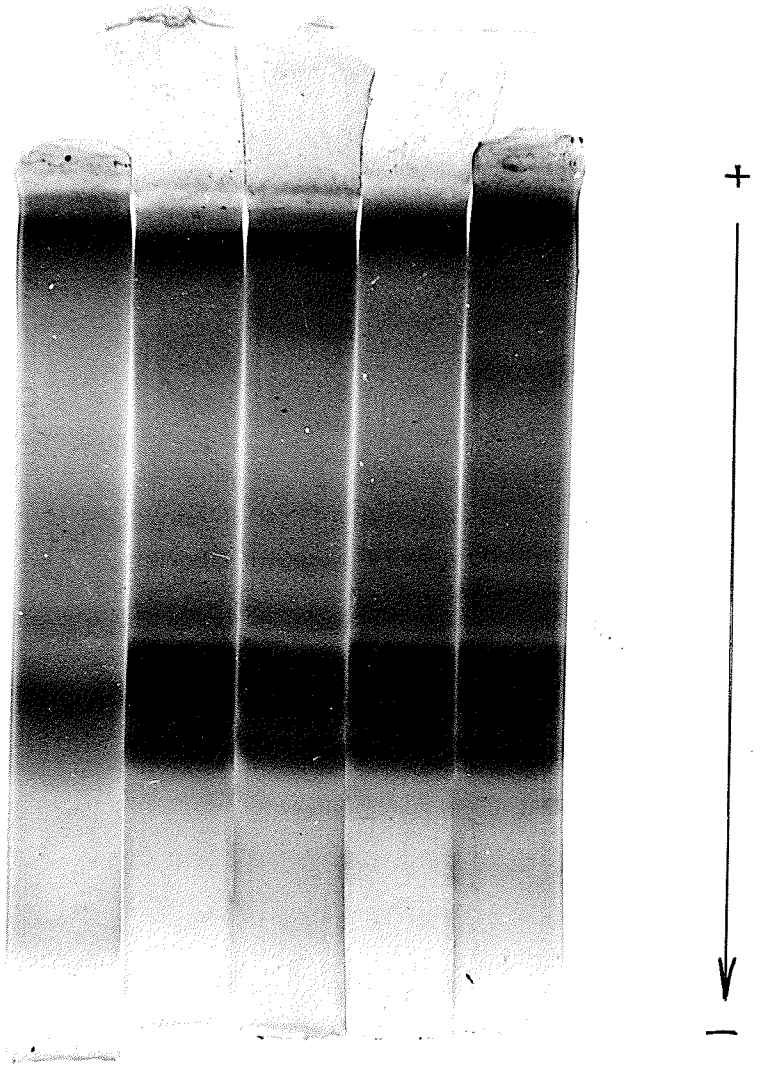
Polyacryamide disc gel-electrophoresis of the albumins, globulins, gliadins and SDS-polyacrylamide gel electrophoresis of glutenins from developing whole grain.

The observed changes in amino acid content of the protein solubility fractions within developing cereal grains could be the result of synthesis of new proteins or a change in the relative amounts of the various components within each fraction during maturation. Studies on hard red spring wheat using ion exchange chromatography (Graham et al (1963))

Figure 9. Disc Electrophoresis Patterns for the Albumins from the Developing Whole Grain of Triticale 6A190.

6A190

ALBUMINS



2 3 4 5 7

WEEKS

Figure 10. Disc Electrophoresis Patterns for the Albumins from the Developing Whole Grain of Prolific Rye.

PROLIFIC ALBUMINS

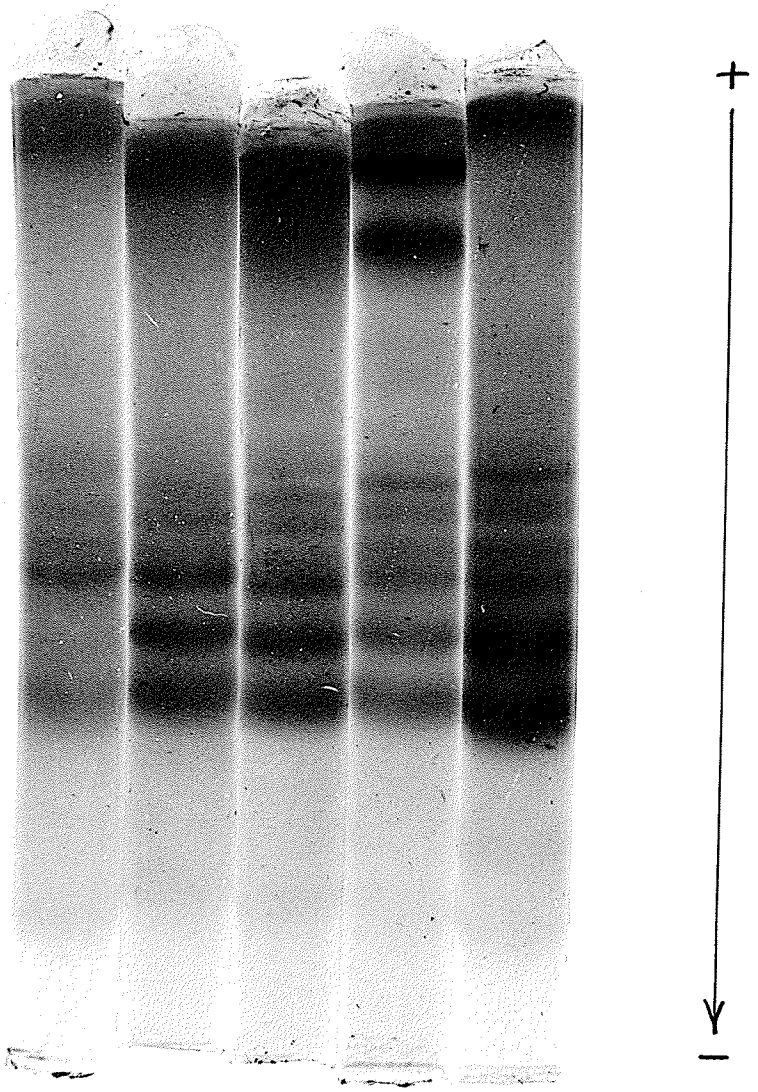
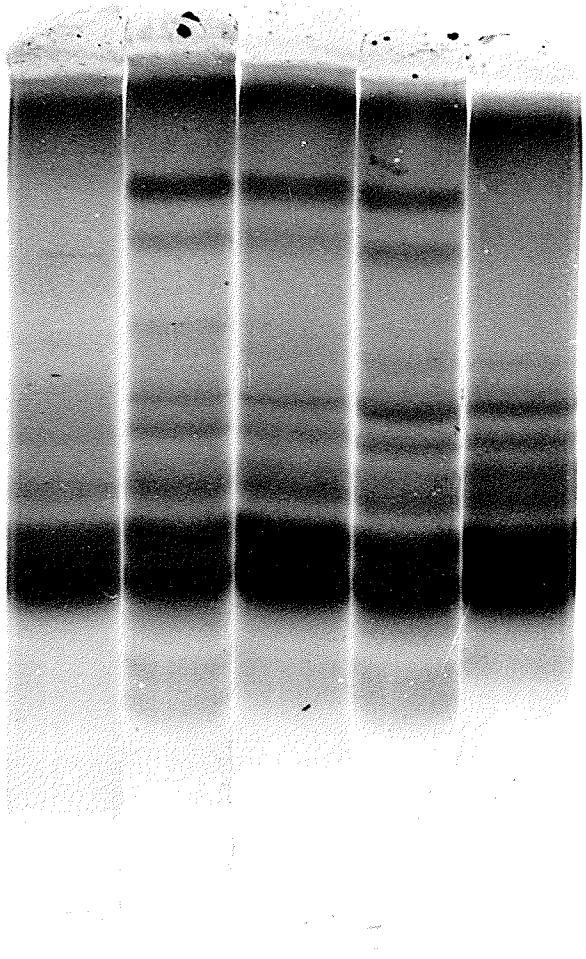


Figure 11. Disc Electrophoresis Patterns for the Albumins from the Developing Whole Grain of Stewart Durum.

STEWART ALBUMINS



2 3 4 5 7

WEEKS

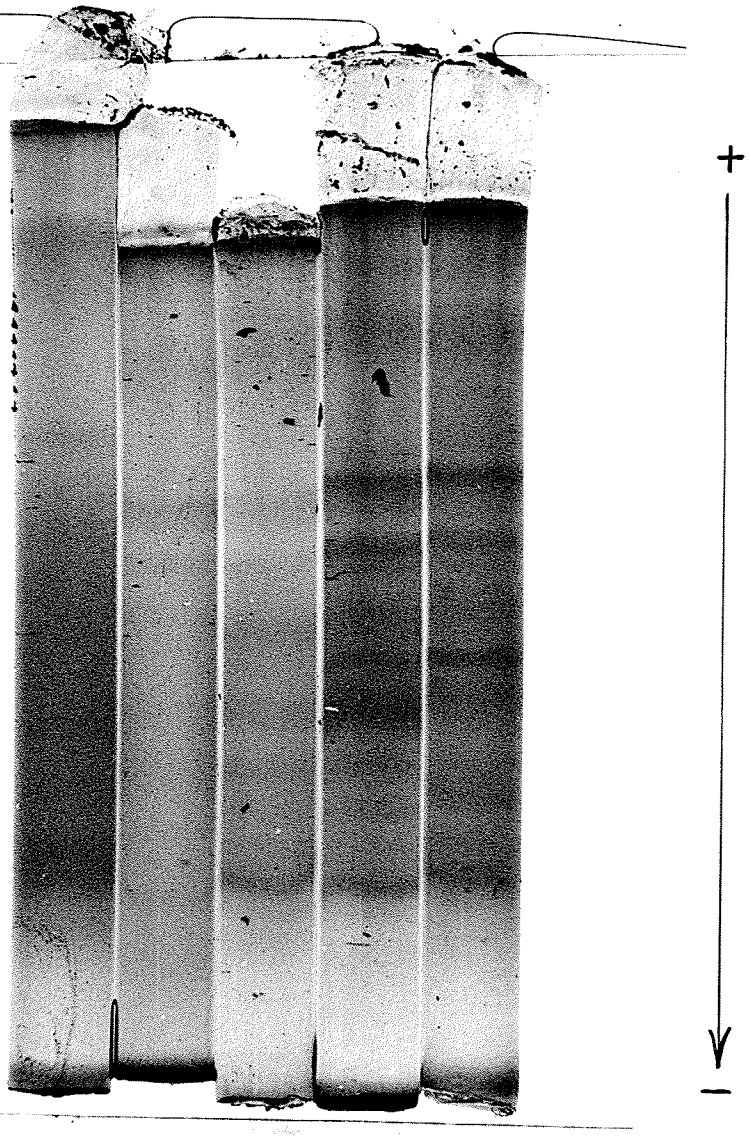
and starch gel electrophoresis (Graham and Morton (1963)) indicated quantitative rather than qualitative changes in the proteins during kernel development. Bushuk and Wrigley (1971) used gel filtration to show the appearance of a low-molecular weight glutenin in bread wheats in the late stages of maturity. This component did not appear in the glutenins of maturing durum. Rainey and Abbot (1971) showed the appearance of seven new component proteins in the albumins and globulins of maturing hard red winter wheat in an immunoelectrophoretic study. In this study, the albumins, globulins, gliadins and glutenins extracted from the developing whole grains of Triticale 6A190 and its parents, Prolific rye and Stewart durum were examined by polyacryamide-gel electrophoresis. The procedure of Davis (1964) as modified by Chen and Bushuk (1970b) was employed for the former three fractions, while the SDS - polyacryamide gel electrophoresis procedure of Orth and Bushuk (1973b) was employed for glutenins. Within each fraction examined, the same amount of protein was loaded for all five analyses.

The disc electrophoretic patterns for the albumins at different stages of development from Triticale 6A190 and its parents, Prolific rye and Stewart durum are shown in Figures 9, 10 and 11. The bands of slow mobility were considered to be impurities (Chen and Bushuk (1970b). New bands appeared for all three cereals between the second

Figure 12. The Disc Electrophoretic Patterns for the Globulins from the Developing Whole Grain of Triticale 6A190.

6A190

GLOBULINS



2 3 4 5 7

WEEKS

Figure 13. The Disc Electrophoretic Patterns for the Globulins from the Developing Whole Grain of Prolific Rye.

PROLIFIC GLOBULINS

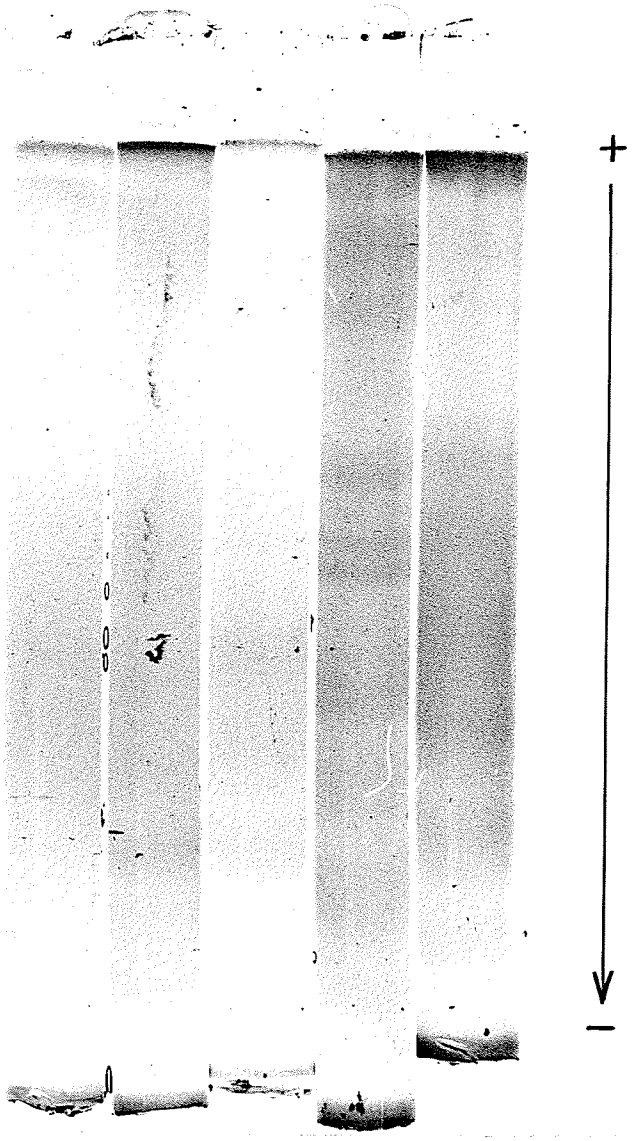
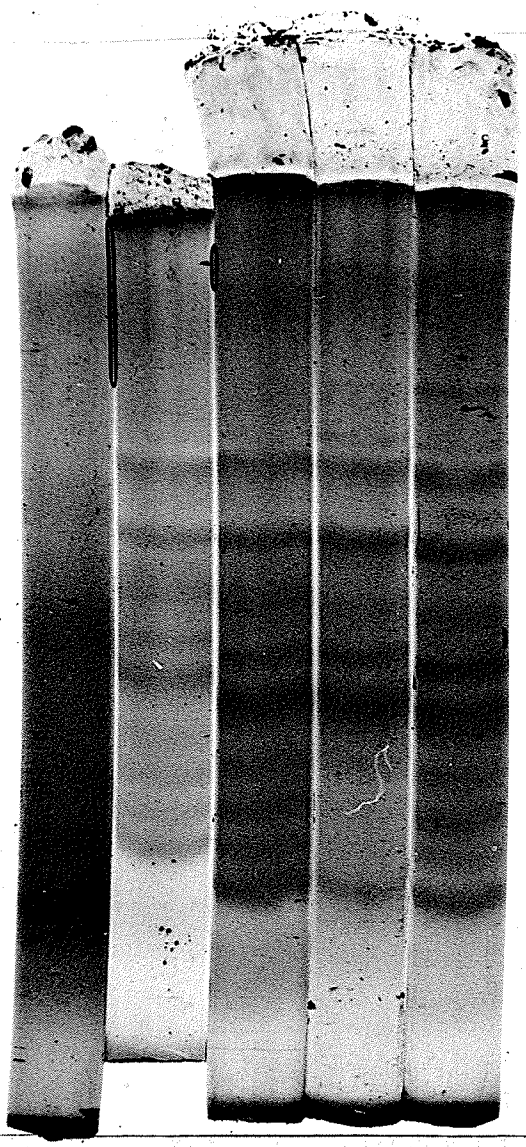


Figure 14. The Disc Electrophoretic Patterns for the Globulins from the Developing Whole Grain of Stewart Durum.

STEWART

GLOBULINS



2 3 4 5 7

WEEKS

and third weeks after anthesis. No 'new' bands not present in either of its parents were found for Triticale at any stage of development. The patterns for the three cereals at maturity were similar to those found by Chen and Bushuk (1970c) for mature flour samples from the same three cereals.

The disc electrophoretic patterns for the globulins from the developing whole grain of Triticale 6A190 and its parents Prolific rye and Stewart durum are shown in Figures 12, 13 and 14. A smear was obtained for rye samples of two, three and four weeks maturity. For samples of five weeks maturity and fully mature a large number of faint bands were obtained over a wide range of mobility. Resolution for Triticale and wheat from samples of two weeks maturity was poor. The remaining samples were similar presenting a pattern of numerous bands over a wide range of mobility. The range of protein mobility for the three cereals was very similar at maturity, although the pattern for rye was simpler than that for Triticale and wheat. This differed from the results of Chen and Bushuk (1970c) for mature flour globulins from the same three cereals. They found that the mobility range for rye globulins was less than that of Triticale and wheat. Possibly the germ and bran present in the whole grains used in this study contributed bands not present in rye flour. No 'new' bands not present in either of its parents were found in the pattern for

Figure 15. The Disc Electrophoresis Pattern for the Gliadins from the Developing Whole Grain of Triticale 6A190.

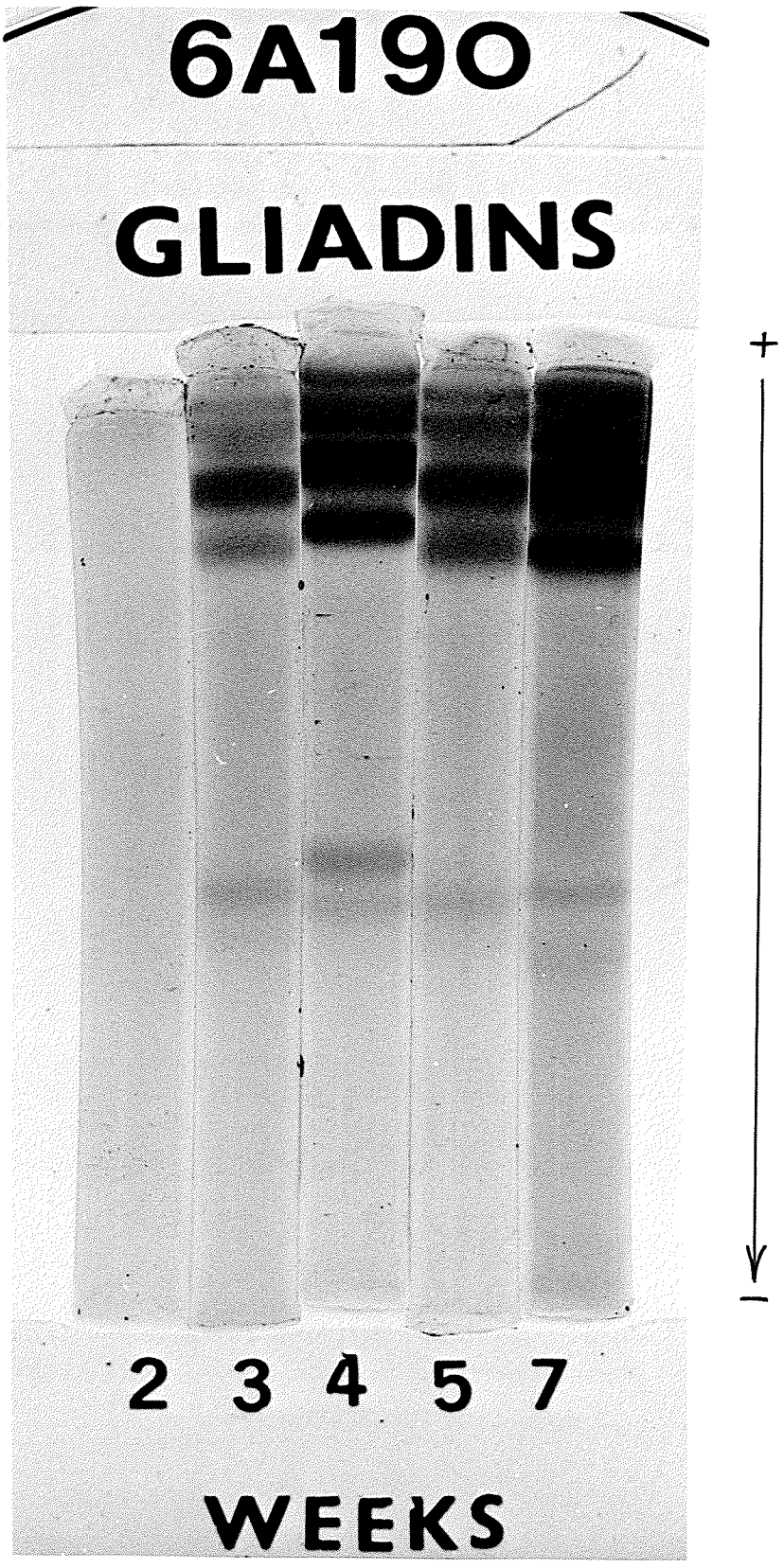

6A190
GLIADINS
+
2 3 4 5 7
WEEKS

Figure 16. The Disc Electrophoresis Pattern for the Gliadins from the Developing Whole Grain of Prolific Rye.

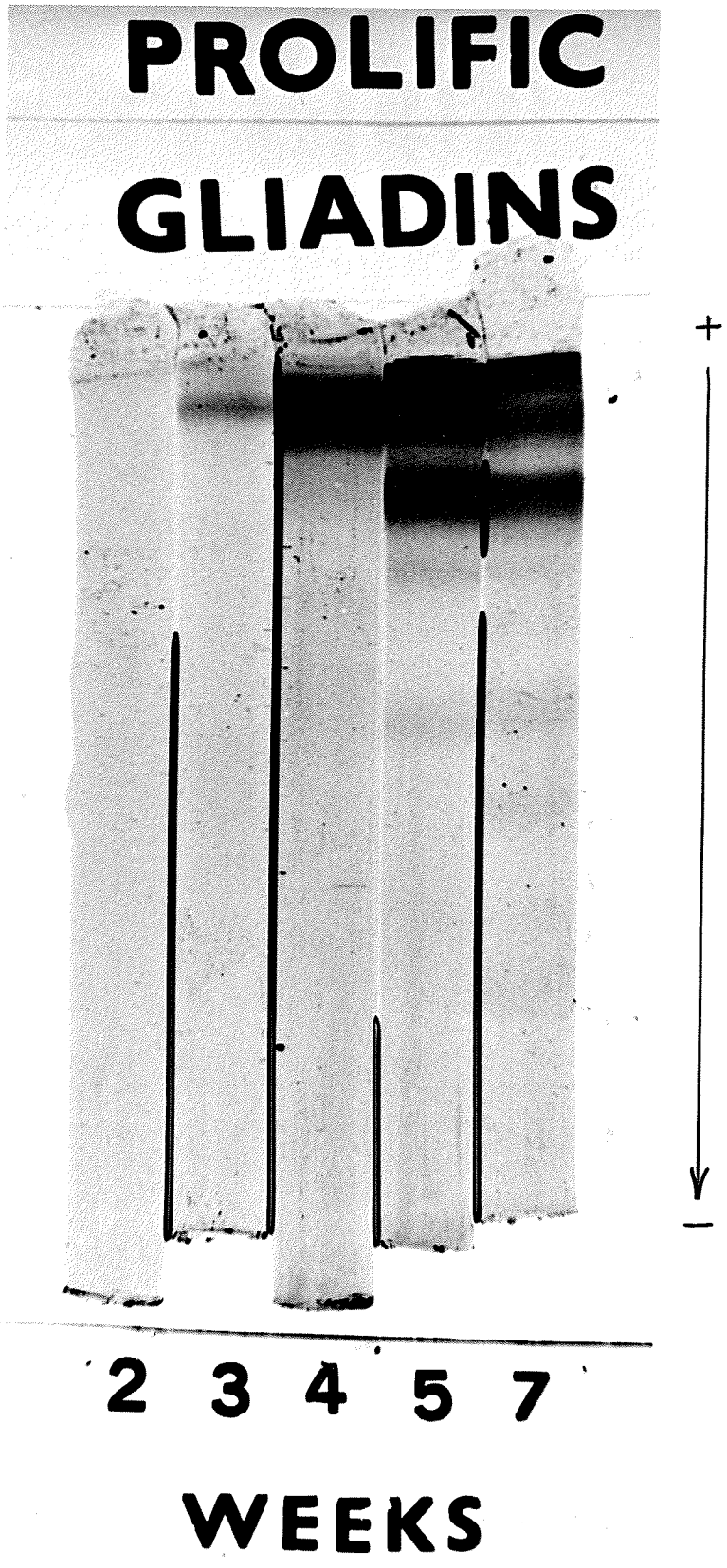

PROLIFIC
GLIADINS
+
—
2 3 4 5 7
WEEKS

Figure 17. The Disc Electrophoresis Pattern for the Gliadins from the Developing Whole Grain of Stewart Durum.

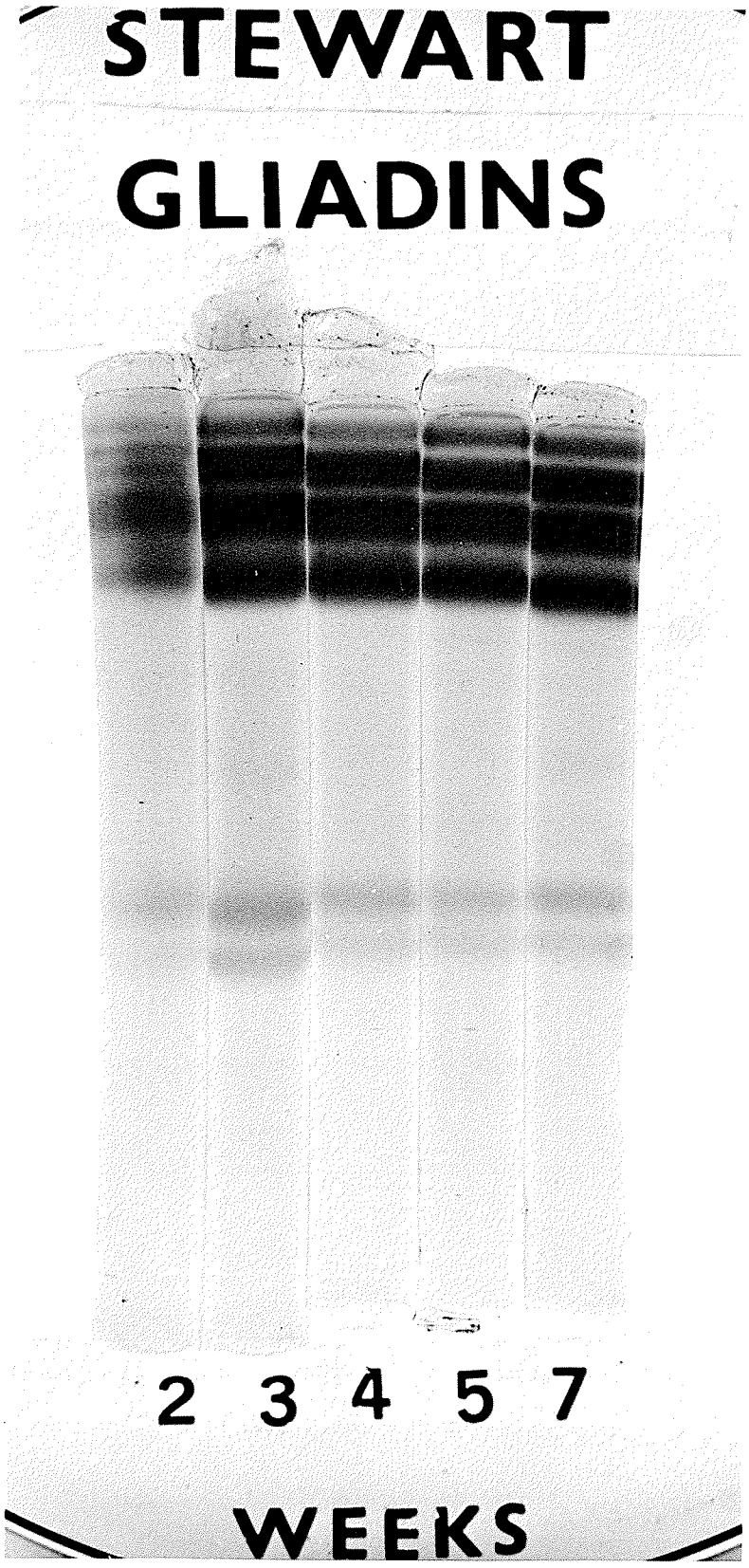
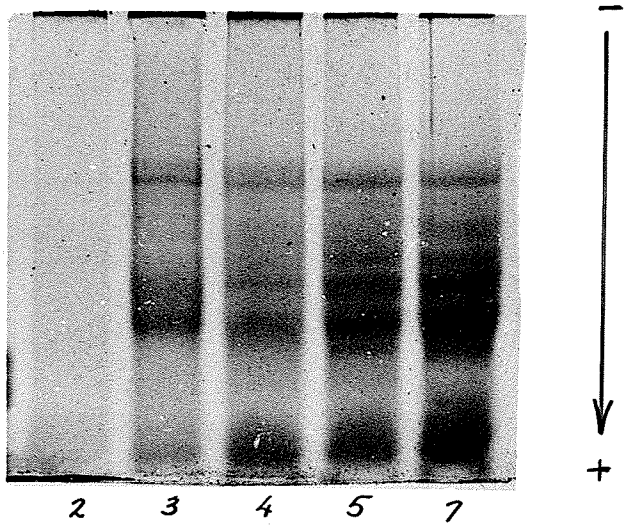
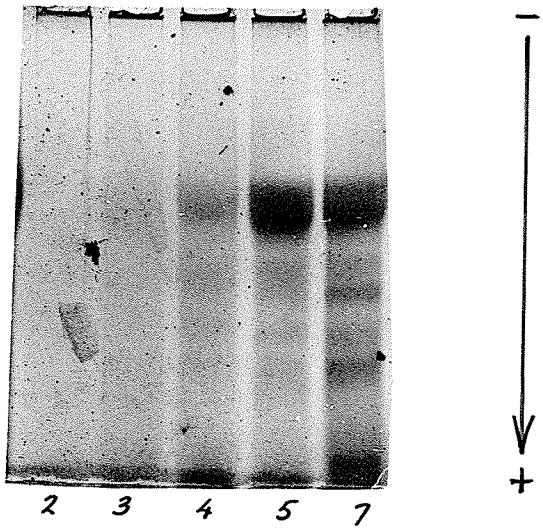
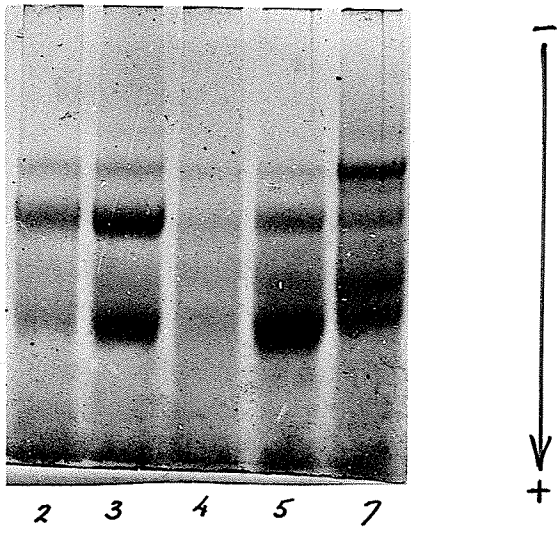

STEWART
GLIADINS
+
−
2 3 4 5 7
WEEKS

Figure 18. SDS-Polyacrylamide Gel Electrophoresis Patterns of Reduced Glutenins from Developing Whole Grain of Triticale 6A190 and its Parents Prolific Rye and Stewart Durum.

GLUTENINS


6A190
Triticale
2 3 4 5 7
−
+


Prolific
Rye
2 3 4 5 7
−
+


Stewart
Durum
2 3 4 5 7
−
+
Weeks after anthesis

Triticale globulins.

The disc electrophoretic patterns for gliadins from Triticale 6A190 and its parents Prolific rye and Stewart durum are shown in Figures 15, 16 and 17. No pattern could be obtained two weeks after anthesis for either rye or Triticale. Wheat appeared to gain bands of very low mobility between the second and third week of development. No change in protein profile was apparent for the remainder of the maturation process. Rye had only one band of low mobility three weeks after anthesis. Over the next two weeks a number of new bands appeared. No difference was apparent between the patterns found at maturity and two weeks earlier. From twenty-one days after anthesis through to maturity Triticale had a pattern which included the rye and wheat components, although intensities differed. This agreed with the findings of Chen and Bushuk (1970c) for gliadins from mature flour of the same three cereals.

The SDS-polyacrylamide gel electrophoresis results for reduced glutenins from maturing whole grain of Triticale 6A190 and its parents Prolific rye and Stewart durum are shown in Figure 18. Changes were apparent in all three cereals throughout development. These changes appeared to be qualitative and quantitative. The glutenin fraction from Triticale only two weeks mature gave no subunit pattern.

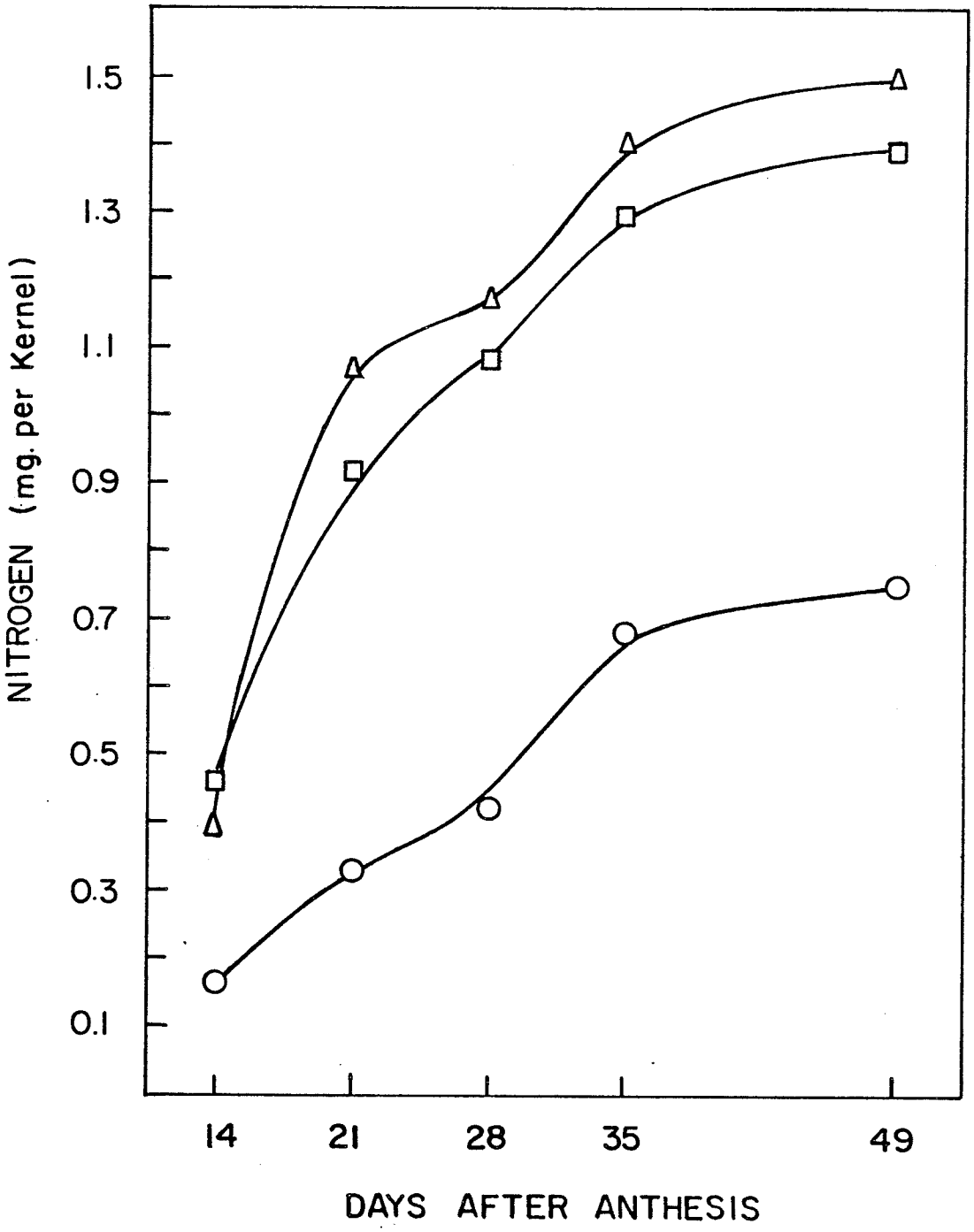
The glutenins from three week old Triticale kernels had a subunit of MW 150,000. Due to its low intensity this band cannot be detected in the photographic reproduction. This subunit had decreased greatly in intensity one week later, and was not found in the five week and mature samples. A number of subunits become more or less prominent as maturity progressed, and new subunits also appeared. The quality of result for Prolific rye left much to be desired. However, some information was obtained. It was found that no glutenins were present until four weeks after anthesis, and even then the bands were very faint. There was no evidence of any new bands appearing from this time to maturity. The subunit pattern for wheat also changed greatly during maturation. New bands appeared during the maturation process, and the relative intensities of the bands changed. At maturity all the subunits present in Triticale could be accounted for by one or both of its parents. This agreed with the report by Orth et al (1974). However, the high molecular weight subunit found three weeks after maturity in Triticale was not found in either parent.

The electrophoretic results suggested that changes which occurred within the protein solubility fractions of each cereal during maturation were qualitative as well as quantitative. Triticale proteins had an electrophoretic pattern which included bands from both its parents throughout development.

Changes in total nitrogen content per kernel during development.

The change in nitrogen content per kernel for Triticale 6A190 and its wheat and rye parents is shown in Figure 19. A rapid increase was apparent in all three cereals between the second and fifth weeks of development. This was due mainly to a rapid increase in storage protein (Tables 9, 10, 11). The pattern of change was similar to results from other studies on wheat (Jennings and Morton (1963b)) and barley (MacGregor et al (1971)). Rye had significantly less protein per kernel than either Triticale or wheat throughout development. This was a reflection of both smaller kernel size and lower protein content.

Figure 19. Changes in Total Nitrogen Content per Kernel During Development in Triticale 6A190 (Δ) and its Parents Prolific Rye (O) and Stewart Durum (□).


1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
NITROGEN (mg. per Kernel)
14
21
28
35
49
DAYS AFTER ANTHESIS

GENERAL DISCUSSION

The amino acid composition of the maturing endosperms of Triticale 6A190 and its parents Prolific rye and Stewart durum were all found to change rapidly during maturation. The most significant changes included a decrease in lysine, aspartic acid (and asparagine), and alanine concomitant with an increase in proline and glutamic acid (and glutamine). A similar pattern of change was found in whole grain samples from all three cereals during maturation. The bulk of these changes occurred before the third week after anthesis. From the fourth week after anthesis to maturity changes in amino acid composition were minimal. These results agreed with previous studies on other grains (Jennings and Morton (1963a), Palmiano et al (1971), Ivanko (1971), Ingle et al (1965)). In general, Triticale had an amino acid composition intermediate to that of its parents throughout development. This suggested that the genomes of wheat and rye were acting independently in Triticale throughout maturation.

Previous studies on wheat had shown that the non-protein nitrogen pool changes greatly both quantitatively and qualitatively during the maturation process (Jennings and Morton (1963b), Hoseney et al (1966), Hoseney and Finney (1967)). This would have a significant effect on

the changes observed in overall amino acid composition of the endosperm during development. Thus, the free amino acids from the endosperms of maturing Triticale and its parents were analyzed at intervals throughout development.

Triticale and rye had a much greater amount of free amino acids than wheat at all stages of maturation. Thus, it was not surprising that the composition of the free amino acids of Triticale resembled that of its rye parent more than its wheat parent. In all three cereals there was a rapid decline in the percentage of nitrogen present as free amino acids over the first three weeks. Major trends common to all three cereals included a decrease in free asparagine, proline, glutamic acid and glutamine over this period. These decreases were especially rapid in wheat. Free proline retained a much higher level for several weeks in rye than in Triticale or wheat. Free alanine followed a very similar pattern in all three cereals. After maintaining a very high level for a brief while it declined rapidly to very low amounts at maturity.

The amino acid composition of the free amino acids of Stewart durum agreed in many respects to those reported earlier for hard red winter wheat (Hoseney and Finney (1967)).

The decrease in free alanine was first apparent about three weeks after anthesis (a little later for rye,

a little earlier for wheat). This decrease in free alanine may signal the end of rapid endosperm cell division since Steward and Durzan (1965) have shown that free alanine was present in large quantities in the soluble-nitrogen component of rapidly growing cells. This agreed with an earlier study on Australian wheat by Jennings and Morton (1963c). Those workers arrived at their conclusion by examining the DNA content within developing wheat endosperm.

The contribution of the free amino acids from the developing endosperm from all three cereals was deducted from the total amino acid analyses in order to gain a clearer indication of the changes in amino acid composition which occurred in their proteins and peptides during maturation. For all three cereals the most rapid changes included a decrease in lysine and an increase in proline and glutamic acid (and glutamine). The content of all three of these amino acids in Triticale proteins and peptides was intermediate to that of its parents at all times during maturation. These results gave further support to the hypothesis that the alien genomes of rye and wheat were acting independently during maturation in Triticale.

The amino acid composition of the proteins and peptides from these three cereals changed abruptly about two weeks after anthesis (a little later for rye and a

little earlier for wheat). This gave further evidence that at this time rapid endosperm cell division ceased and rapid storage protein buildup began as was originally shown by Jennings and Morton (1963c).

There is general agreement that changes in the amino acid composition of maturing cereal grains reflect to a large extent changes in the protein solubility distribution. In particular, storage protein has been shown to be synthesized rapidly in cereals after the first two weeks after anthesis (Jennings and Morton (1963c), Palmiano et al (1968), Ivanko (1971), Murphy and Dalby (1971)). Thus, changes in the solubility distribution of the proteins from whole grain samples of maturing Triticale 6A190 and its parents were examined. A modified Osborne procedure was employed for protein extraction.

The protein solubility distribution for wheat and rye differed greatly from each other throughout development. Triticale was similar to its rye parent in some respects while it resembled its wheat parent in other respects. For example, over one-half the nitrogen present in the grain of Triticale and rye two weeks after anthesis was dialyzable. For wheat, however, over 80% of the nitrogen present in the grain two weeks after anthesis was recovered in the protein fractions. Albumins declined during maturation for both wheat and Triticale, although the latter had a significantly

greater proportion of this fraction at maturity. By contrast, the proportion of albumins increased in rye between the second and third weeks after anthesis and remained high thereafter. Wheat had the greatest amount of gliadins throughout development while rye had the least. Gliadin synthesis began earlier in wheat than in either Triticale or rye. Even when wheat was only two weeks mature gliadins comprised a major portion of the protein present. In rye and Triticale at this time only trace amounts were present. This explained why the amino acid composition of the proteins and peptides in wheat changed abruptly earlier than in either Triticale or rye. The other major point of difference between the three cereals was the level of glutenins in the whole grains during development. Only trace amounts of glutenins were found in rye until five weeks after anthesis. In wheat significant amounts were found at two weeks maturity. Triticale, meanwhile, had no significant amount of glutenins until three weeks after anthesis. Thus, the pattern of accumulation of glutenins in Triticale was intermediate to its two parents.

Further information on the rate of synthesis of the proteins in the three developing cereals was gained by using ^{14}C-leucine as a tracer. The ^{14}C-leucine was injected into the plants at regular intervals during maturation, and the pattern of tracer incorporation into

the proteins of the whole grains was examined. For both Triticale and rye the highest levels of tracer utilization in protein synthesis were achieved for samples injected fourteen days after anthesis. This indicated that the rate of protein synthesis within the developing grain was most rapid at this time. In wheat label recovery was greatest when injections were made three weeks after anthesis, although a nearly equal amount of incorporation was observed when the injection was performed a week earlier. This indicated that the rate of protein synthesis in wheat peaked about three weeks after anthesis. For all three cereals the amount of tracer incorporated during the last two weeks before maturity was very low. Thus protein synthesis would appear to be very slow at this time.

The pattern of label incorporation into the individual protein fractions within the three developing cereals gave an indication of the rate at which the fractions were being synthesized relative to the overall rate of protein synthesis. ^{14}C-leucine was a convenient tracer for these experiments because it was not catabolized to any great extent during kernel development. In addition it was found in comparable amounts in all the protein fractions throughout development in all three cereals.

The pattern of label incorporation into the albumins

of Triticale and wheat were similar. Between the first and second week of development for these two cereals their albumins utilized the greatest amount of label of any of the protein fractions. During the next week there was a marked decrease in label incorporation into this fraction relative to overall incorporation. Thereafter the decrease was marginal. Thus albumins were synthesized most rapidly early in the maturation process, although they continued to be synthesized at an appreciable rate relative to overall protein synthesis through to maturity. Rye albumins showed a different pattern. There was a marked increase in the proportion of label incorporated by this fraction between the second and third weeks after anthesis. Over the next week a decrease was observed and thereafter the proportion of label incorporation stabilized. From the third week after anthesis through to maturity rye albumins incorporated the greatest amount of label of any fraction. From these results it was apparent that rye albumins were synthesized most rapidly about three weeks after anthesis. Thereafter they continued to be synthesized at a slower but substantial rate relative to overall protein synthesis.

The pattern of Triticale globulin synthesis also was more similar to its wheat parent. The globulins from both cereals incorporated a smaller proportion of label midway through development than they did earlier and later

in development. This decrease was most apparent in Triticale. Thus Triticale and wheat globulins were synthesized most rapidly early during the maturation process. Although their globulins incorporated an increased proportion of label during the last few weeks of maturation it must be borne in mind that overall protein synthesis had decreased very greatly over this period. Rye globulins on the other hand continued to incorporate label at a relatively steady rate over the first five weeks of maturation and then showed a marked decrease. Thus the relative slowdown in globulin synthesis midway through the maturation process which was apparent for Triticale and wheat was not apparent for rye.

The pattern of incorporation of label into the gliadins in the three cereals differed. Triticale showed a rapid linear increase in relative incorporation of label into its gliadins between the second and fourth weeks. Thereafter the relative rate of incorporation stabilized. From the fourth week after anthesis to maturity Triticale gliadins incorporated the greatest proportion of label of any of its protein fractions. This incorporation pattern showed that synthesis of gliadins increased rapidly over the first four weeks of development relative to overall protein synthesis and stabilized thereafter. Wheat also showed a rapid increase in relative incorporation of label into its

gliadins during the first four weeks. However, a much greater amount of label was incorporated into wheat gliadins between the first and second weeks than was found for Triticale or rye. This showed that gliadin synthesis commenced much earlier in wheat than in either of the other two cereals. After the fourth week the relative proportion of label incorporated by wheat gliadins declined markedly. Thus wheat gliadins were synthesized most rapidly around the third or 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 two weeks. During the period of most rapid protein synthesis (between the second and third weeks after anthesis) the proportion of label incorporated into rye gliadins was far less than that found in Triticale and wheat. This was largely due to the more rapid increase of albumins in rye over this period compared to the other two cereals.

Triticale glutenins showed a linear increase in the proportion of label they incorporated from two weeks after anthesis to five weeks after anthesis. Thus glutenins were being synthesized rapidly over this period. This was in contrast to the pattern of incorporation into rye glutenins. An appreciable proportion of the incorporated label was not

found in rye glutenins until the last three weeks of maturation. 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 maturation process. Although a similar pattern of incorporation to rye was found in wheat glutenins, the level of incorporation into the glutenins of the latter cereal was significantly higher.

The proportion of label incorporated into the insoluble residue protein from Triticale decreased throughout maturation. Thus it was synthesized very early in the maturation process. This suggested that it was likely largely membrane protein. Similarly, in rye there was a very rapid decrease in the proportion of label incorporated into its residue protein between the second and third weeks after anthesis. Thus, in rye also this fraction was synthesized very early in the maturation process. This rapid increase in incorporation into residue protein was not noticed in wheat. However, since storage protein is present to a much greater extent early in the maturation process in this cereal compared to rye and Triticale, it is possible that rapid residue protein synthesis was occurring prior to the period (one week after anthesis) when label was first injected.

Previous studies on developing cereal grains

(Ivanko (1971), Hoseney et al (1966), Murphy and Dalby (1971)) indicated that the amino acid compositions of the individual protein fractions changed during maturation. Thus we decided to determine the amino acid composition of the five protein solubility fractions in Triticale 6A190 and its parents during the maturation process.

The amino acid composition of the albumins in all three cereals changed significantly between the second and fourth week after anthesis. These changes were most apparent in rye and Triticale. Lysine, aspartic acid (and asparagine), glycine and alanine decreased while glutamic acid (and glutamine) and proline increased. In general these changes paralleled the changes in the amino acid composition of the whole grains over this period. The extent of these changes was less pronounced in the albumins than in the whole grain. Triticale albumins resembled rye more closely than wheat in their amino acid content. This was consistent with the previously discussed observation that rye contained much more albumins than wheat except early in the maturation process.

The globulins in all three cereals had a relatively uniform amino acid composition through development. The globulins from all three cereals had an amino acid content similar to each other. The most noticeable common changes during maturation were a decrease in arginine and an

increase in glutamic acid. Lysine decreased in wheat globulins.

The gliadins in wheat showed some slight changes in amino acid composition between the second and third week after anthesis. Thereafter changes were minimal. A very great change occurred in the amino acid composition of Triticale gliadins between the second and third weeks after anthesis. Thereafter changes were minimal. The most prevalent changes were a decrease in lysine and an increase in glutamic acid (and glutamine) and proline. Since gliadins were found in very low amounts two weeks after anthesis it was possible that this fraction was highly contaminated with albumins (which are soluble in 70% ethanol) at this time. A similar pattern of change in the amino acid composition was found for rye gliadins between the second and third weeks after anthesis. Again, since gliadins were found in only trace amounts two weeks after anthesis it was possible that this fraction was contaminated with albumins at that time. After the third week following anthesis the amino acid composition of rye gliadins was stable with one noteable exception. Very little histidine was present in this fraction three weeks after anthesis. Over the next two weeks histidine content in rye gliadins increased rapidly. The amino acid composition of Triticale gliadins more closely resembled wheat gliadins than rye

gliadins except at two weeks after anthesis. This was consistent with the far greater amount of gliadins found in wheat as opposed to rye.

The gliadins of all three cereals had a very low lysine content coupled with a very large glutamic acid and proline content. The three most prevalent common changes in amino acid composition observed within the developing whole grains from each cereal were a decrease in lysine content concomitant with an increase in glutamic acid and proline content. Thus, gliadin synthesis was a major factor in the changes observed in the amino acid composition of the maturing whole grains.

Rapid changes occurred in the amino acid composition of rye glutenins between the second and fourth weeks after anthesis. Because only trace amounts were present at this time it is possible this fraction was contaminated. The close similarity between the amino acid composition of rye glutenins and insoluble residue protein suggested that this was the case. Triticale glutenins also underwent rapid changes in their amino acid content between the second and third weeks after anthesis. Thus the glutenin fraction obtained from Triticale two weeks after anthesis was also likely contaminated. In wheat glutenins glycine was found in much lower amounts two and three weeks after anthesis than later in the maturation process. Wheat glutenins had more glutamic acid (and glutamine) and less lysine and

proline than rye glutenins at maturity. Triticale glutenins had an amino acid content intermediate between its parents at maturity.

Because of their low lysine content and high glutamic acid (and glutamine) and proline content, synthesis of glutenins, like gliadins, was a major factor in bringing about the changes observed in the amino acid composition of all three cereal grains during maturation.

The insoluble residue protein of all three cereals had an amino acid content more similar to the albumins and globulins than glutenins. This agreed with previous reports on other cereals (Ivanko (1971), Cluskey and Dimler (1967), Palmiano et al (1971)). This was further evidence that the insoluble residue protein was largely composed of membrane material rather than storage protein.

The amino acid composition of wheat insoluble residue protein was fairly stable throughout maturation. In Triticale and rye, however, some changes occurred. However, the amino acid compositions of this fraction for these two cereals were similar at maturity and at two weeks after anthesis. The amino acid composition of Triticale insoluble residue protein was intermediate between its two parents throughout development, although it resembled rye more closely than wheat.

The changes observed in the amino acid compositions

for the protein solubility fractions in the cereals during maturation could have been qualitative as well as quantitative. This was investigated by polyacryamide-gel electrophoresis of the four soluble protein fractions. In all cases qualitative changes appeared to be occurring during maturation in addition to quantitative changes. This agreed with previous reports on wheat by Rainey and Abbott (1971) and Wrigley and Bushuk (1971). Changes in the protein profiles were most apparent early in the maturation process. In all cases Triticale proteins were present in either or both of its parents. This agreed with the results of Chen and Bushuk (1970a,b,c) and Orth et al (1974) who compared proteins from the flour of mature Triticale 6A190 and its parents.

To summarize, marked difference were found in the biochemical properties of the proteins of Prolific rye and Stewart durum during maturation. In general, Triticale 6A190 proteins changed in a pattern which was intermediate between its parents. Thus, no evidence was found for interactions between the alien genomes of rye and durum wheat in Triticale at any stage in the maturation process.

A P P E N D I X A

THE DETERMINATION OF THE PROTEIN CONTENT OF SMALL CEREAL GRAIN SAMPLES

Introduction

The Kjeldahl nitrogen determination (Kjeldahl (1883)) is the most dependable test available for determining the protein content of grain. For best results, however, it is necessary to use one gram samples. Thus, in order to conserve material it was of utmost importance to examine other methods of protein determination more suited to small samples. A number of methods were attempted with varying degrees of success.

Results and Discussion

Lowry Method

This method, developed by Lowry et al (1951) makes use of the colored complex formed with phenol by the Folin-Ciocalteau reagent. Results were inconsistent. The main problem would appear to be lack of a suitable solvent for cereal protein which does not interfer with the test.

Biuret Method

In this procedure a deep blue complex is formed between Cu^{II} and peptide bonds, the optical density giving an indication of protein content. Since this method was

originally proposed (Fine (1935)) a number of variations have evolved. A major problem encountered in determining protein contents in cereal samples by this method was turbidity arising from interference. One method proposed to overcome this problem was to add carbon tetrachloride to the solution followed by shaking for ten minutes prior to centrifugation (P.C. Williams, private communication). Results on whole grain samples were still found to be inconsistent, however, probably due to interference compounds. In the case of wheat flour samples a new problem came to light. It would appear that moisture content had a profound and unpredictable effect on the protein content obtained by this method (Table 17). Examination of the data shows that at normal moisture contents (about 14%) results were fairly satisfactory. It would appear that samples with low moisture contents gave low results, however. Analyses of the precipitates following centrifugation showed the 'missing' protein to be located there, showing this problem to be one of solubility. A more recent method forwarded by Johnson and Craney (1971) which involved the use of cupric carbonate in 60% basic isopropanol was also examined. The optical density at 550 nm. for several ground whole wheat samples of varying sample size were plotted against protein content as determined by the Kjeldahl

procedure (Figure 20). It is apparent that except for the two Manitou samples, each sample showed a different color dependence to protein content. Thus this method was also deemed to be unsuitable.

Nessler Method

The method of Williams (1964) was used. The sample was completely digested in sulphuric acid, and the ammonia from the resultant ammonia sulphate was complexed with the Nessler reagent giving a yellow-orange color, the optical density of which was read at 430 nm. This method worked well for albumins, globulins, gliadins and glutenins, but was unsatisfactory for flour, whole grain and insoluble residue due to interference compounds.

Micro-Kjeldahl Method

In this method the sample was digested in sulphuric acid using a selenium-potassium iodide catalyst. After cooling a sufficient amount of 50% sodium hydroxide was added to the sample in an airtight steam distillation apparatus in order to evolve ammonia which was trapped in boric acid buffer. The distillate was titrated with 0.01 N. sulphuric acid to an endpoint of pH 4.50. Protein content was read off a standard

Table 17. Protein Content for Some Wheat Flours by the Biuret Procedure.

	% moisture	% recovery*	% recovery* at 0% moisture
Manitou I	8.6	84.5	84.2
Manitou II	13.6	105	97.7
Opal	13.8	94.1	88.3
Napo 73	13.6	99.3	59.8
Comanche	13.7	95.0	88.2
Anniversario	13.5	96.9	71.8
Neepawa	13.6	93.7	91.5

* based on a comparison with results by the standard Kjeldahl procedure.

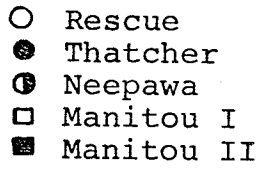
Table 18. Precision Check on Micro-Kjeldahl Method (% nitrogen X 5.7).

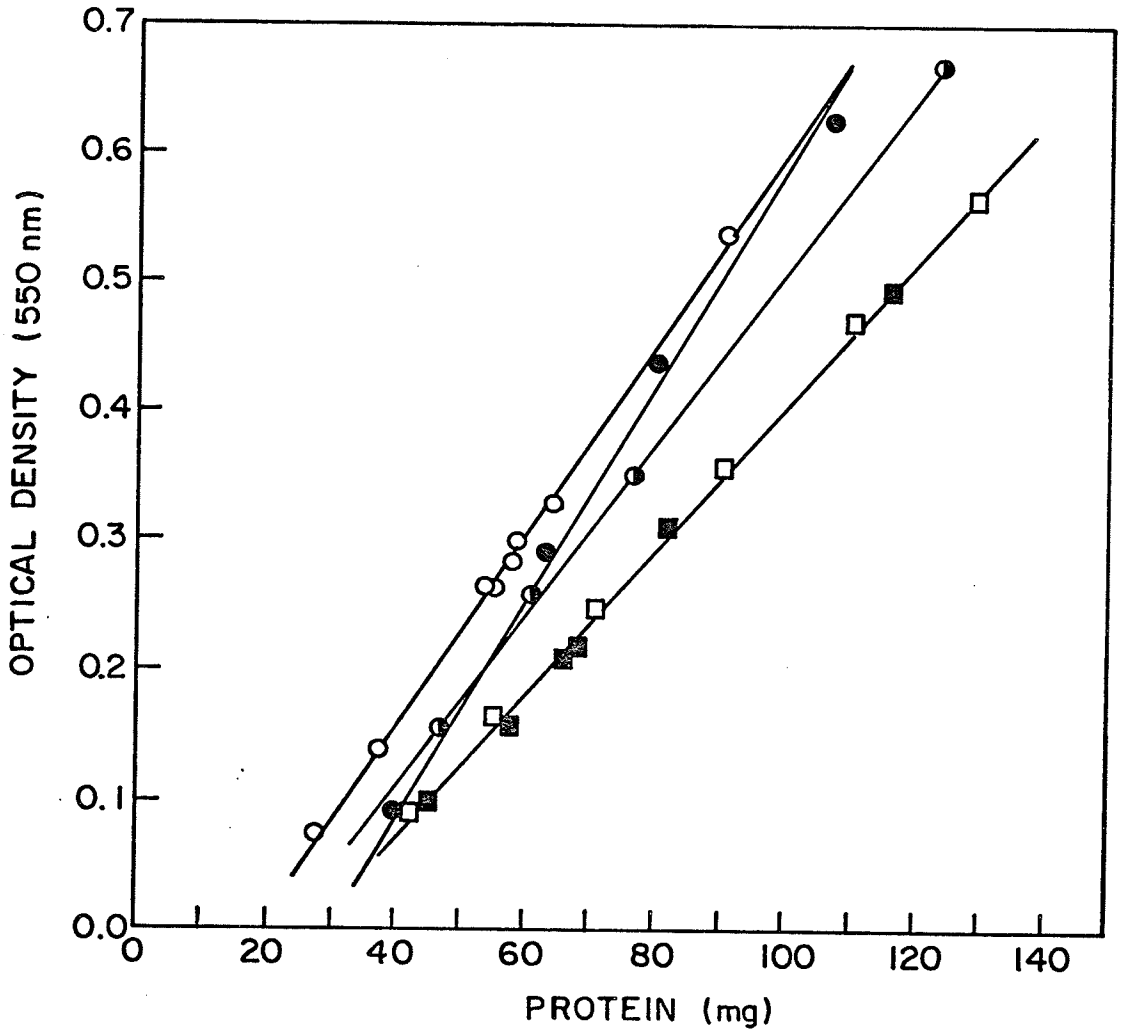
6A190 Triticale Whole Grain*	Talbot Wheat Flour**
16.1%	11.2%
15.9%	11.0%
16.2%	11.3%
15.8%	11.1%
15.9%	11.3%

* 16.1% by macro-Kjeldahl method.

** 11.1% by macro-Kjeldahl method.

Figure 20. A plot of optical density by the buiret method of Johnson and Craney (1971) versus protein content for ground whole grains of some wheat varieties.




OPTICAL DENSITY (550 nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
20
40
60
80
100
120
140
PROTEIN (mg)

curve of tritration volume versus protein content from standard samples. The pH endpoint was used rather than a visual endpoint for superior accuracy. Precision was tested for two samples whose protein content had been previously determined by the standard Kjeldahl method (Table 18). Sample size was approximately 100 mg. Results were excellent. However, this method was extremely tedious.

Protein Content by Amino Acid Analysis

Nitrogen recovery from amino acid analyses of mature cereal samples were consistently within 90±2% when computed using the protein content as determined by the Kjeldahl method. Thus, by adjusting the results for amino acid analysis of carefully weighed samples to 90% recovery it was possible to achieve an accurate estimate of their protein content. Immature samples contained considerable non-protein nitrogen, and as a consequence methods such as the Kjeldahl procedure which determined total nitrogen in the sample resulted in a figure for protein higher than it should have been. However, adjusting the amino acid analyses of these samples to 90% recovery circumvented this problem and gave a common basis for comparison of samples at all stages of maturity.

Conclusions

The Lowry and biuret methods were found to be

unsuitable for protein determination of cereal grains.

The micro-Kjeldahl method gave satisfactory results for all types of samples tested, but was handicapped by being extremely tedious.

The Nessler method was found to be adequate for determining protein levels in the albumins, globulins, gliadins and glutenins from Osborne preparations of cereals, and was employed in these cases. It was unsatisfactory for flour and whole grain samples, however.

Adjusting the recovery of nitrogen from amino acid analysis of carefully weighed samples to 90% gave an accurate indication of protein content for all samples. Thus, this method was employed for samples upon which the amino acid composition was determined.

A P P E N D I X B

THE EFFECT OF THE D GENOME ON THE AMINO ACID COMPOSITION OF DEVELOPING WHEAT CEREAL GRAINS

Introduction

The baking quality of AABB extracted tetraploid wheats are generally inferior in baking quality to their hexaploid parents (Kaltsikes et al, 1968a; 1968b). Boyd and Wrigley (1969) reported that the extracted tetraploid of Canthatch lacked four protein bands present in its hexaploid parents. Dronzek et al (1970), however, found that the electrophoretic patterns of the proteins in the four soluble fractions for several extracted tetraploids and their hexaploid counterparts were essentially the same. They also showed that the amino acid compositions of the flour and the solubility fractions for all wheats examined were not significantly different. These results agreed with the findings of Tkachuk (1966), who showed that hard red spring wheat flours of variable breadmaking quality, as well as flours from other classes of Canadian wheat, showed little variation in amino acid compositions. However, Orth and Bushuk (1973c), using SDS-polyacrylamide gel electrophnesis showed that extracted tetraploids lacked three glutenin subunits and showed a decrease in the amount of one electrophoretic band present in their hexaploid parents.

In this investigation the changes in amino acid content of the developing grain of two extracted tetraploid

wheats were compared to those of their hexaploid parents. Stewart, a durum wheat, was included as a representative of natural AABB tetraploid wheats.

Materials and Methods

The common wheats, Triticum aestivum (cv. Rescue and cv. Thatcher), the AABB tetraploids derived from them (Tetrarescue and Tetrathatcher), and, a durum wheat, Triticum turgidum (cv. Stewart) were grown in a controlled environment chamber at 70°F and 16 hours light. Prior to planting, root tip counts were performed on Tetrarescue and Tetrathatcher to ensure that only twenty-eight chromosome plants were grown.

Samples were prepared and analyzed as previously described.

Results and Discussion

The amino acid composition of developing wheat endosperm.

The total amino acid compositions of Thatcher and Tetrathatcher endosperms throughout maturation are shown in Table 19. Results for Stewart were previously presented in Table 1, p. 31). There were no significent differences between the wheats studied at all stages of maturation.

An examination of the free amino acids of maturing Thatcher and Tetrathatcher endosperm showed them to be

Table 19. Amino Acid Composition of Maturing Hexaploid Wheat Endosperm and its Extracted Tetraploid (cv. Thatcher and Tetrathatcher).

(Results are given as grams nitrogen per 100 grams nitrogen)*

	Thatcher							Tetrathatcher				
Days after anthesis:	7	10	14	17	22	30	49	9	14	21	30	49
Lysine	6.89	6.09	4.19	3.44	2.98	2.53	2.13	7.50	4.28	2.85	2.11	1.98
Histidine	3.15	3.24	3.33	3.36	3.35	3.32	3.30	3.45	3.12	3.21	3.16	3.24
Ammonia	13.9	15.6	17.9	19.6	20.3	21.0	20.6	12.9	17.0	20.9	21.5	20.8
Arginine	8.91	7.75	6.92	6.72	7.62	5.93	6.29	10.1	6.82	5.99	5.53	6.44
Aspartic Acid	5.47	5.20	3.32	3.34	2.94	2.66	2.38	6.17	4.29	2.93	2.46	2.32
Threonine	2.50	2.41	2.15	2.02	1.91	1.84	1.75	2.29	2.03	1.80	1.70	1.66
Serine	4.37	4.08	4.05	3.98	3.76	3.34	3.56	4.36	4.16	3.64	3.30	3.52
Glutamic Acid	10.5	10.8	16.1	18.0	19.0	20.5	21.3	9.96	15.4	19.3	20.6	22.2
Proline	5.86	5.29	6.93	7.52	7.46	8.67	9.12	3.91	6.17	8.28	8.98	8.75
Glycine	5.44	5.91	5.19	4.56	4.13	3.99	3.72	5.92	5.73	4.21	3.59	3.45
Alanine	8.89	9.48	5.99	3.96	3.31	2.64	2.40	8.61	7.04	3.59	3.47	2.35
Valine	3.40	3.38	3.08	2.92	2.69	2.77	2.65	3.55	3.15	2.69	2.54	2.46
Methionine	1.12	1.32	1.04	0.75	0.80	0.76	0.82	1.12	1.00	0.70	0.72	0.70
Isoleucine	2.18	2.23	2.22	2.22	2.18	2.15	2.10	2.38	2.29	2.18	2.25	2.14
Leucine	3.88	3.93	4.20	4.14	4.14	4.22	4.16	4.39	4.23	4.05	4.23	4.06
Tyrosine	1.10	1.23	1.25	1.16	1.19	1.19	1.19	1.35	1.01	1.13	1.18	1.26
Phenylalanine	1.70	1.92	2.25	2.33	2.23	2.52	2.56	2.00	2.36	2.50	2.65	2.72
% Protein	16.4	15.6	12.3	13.2	12.5	13.7	14.2	17.6	15.6	14.8	14.1	16.6

* tryptophan, cysteine and cystine were not determined.

similar throughout development (Table 20). These results closely resembled those previously described for Stewart (Table 2, p. 35).

As would be expected from the similarities in both the free amino acid levels and the total amino acid analyses for these cultivars throughout development, the amino acid compositions of their proteins and peptides were similar (Table 21 and Table 3, p. 41).

The amino acid composition of the developing whole grain of wheat.

Results for the amino acid compositions of whole grain samples of varying maturity for Thatcher, Tetrathatcher, Rescue and Tetrarescue are shown in Tables 22 and 23. No appreciable differences were apparent between these wheats at all stages of maturation. Results for Stewart (Table 4, p. 54) previously described closely parallel these results.

Conclusions

From this study it was apparent that the presence or absence of the D genome in wheat did not result in any significant variation in either the total amino acid content or the level of the free amino acids in the grain at all stages of development.

Table 20. Free Amino Acid Composition of Maturing Hexaploid Wheat Endosperm and its Extracted Tetraploid (cv. Thatcher and Tetrathatcher).

(Results are given as grams nitrogen per 100 grams nitrogen)*

	Thatcher							Tetrathatcher				
Days after anthesis:	7	10	14	17	22	30	49	9	14	21	30	49
γ-Amino Butyric Acid	0.554	0.978	0.799	0.199	0.089	0.020	0.0014	0.403	0.465	0.214	0.367	0.0044
Tryptophan	tr.	0.061	tr.	0.046	0.062	0.080	0.0266	0.035	0.049	0.027	0.077	0.0014
Lysine	0.255	0.299	0.231	0.148	0.117	0.044	0.0029	0.297	0.104	0.125	0.131	0.0015
Histidine	0.145	0.106	0.044	0.044	0.024	0.019	0.0028	0.051	0.039	0.025	0.049	tr.
Arginine	0.154	0.179	0.050	0.050	0.081	0.027	0.0201	0.175	0.033	0.042	0.252	0.0298
Aspartic Acid	none	0.189	0.226	0.261	0.160	0.116	0.0154	0.465	0.097	0.072	0.118	0.0046
Threonine	0.242	0.376	0.162	0.099	0.065	0.021	0.0017	0.295	0.120	0.045	0.059	0.0018
Serine	1.44	1.64	0.838	0.542	0.320	0.070	0.0029	1.31	0.738	0.233	0.173	0.0026
Asparagine	1.16	1.70	0.291	0.391	0.318	0.032	0.0210	1.17	0.878	0.230	0.379	0.0380
Glutamic Acid	1.83	0.811	0.553	0.687	0.863	0.091	0.0056	1.13	0.265	0.128	0.035	0.0103
Glutamine	1.75	2.72	0.286	0.269	0.373	0.100	0.0124	1.99	0.905	0.280	0.718	0.0195
Proline	2.90	1.46	0.052	0.055	0.037	0.008	0.0095	1.47	0.180	0.050	0.039	0.0251
Glycine	1.01	1.51	1.36	0.620	0.246	0.059	0.0038	1.03	1.30	0.508	0.148	0.0040
Alanine	3.34	5.91	2.26	1.19	0.727	0.071	0.0078	3.24	3.17	0.792	0.354	0.0080
Valine	0.381	0.355	0.095	0.077	0.050	0.015	0.0023	0.154	0.167	0.049	0.056	0.0021
Methionine	0.386	0.257	0.045	0.032	0.051	0.009	0.0011	0.094	0.128	0.049	0.045	0.0012
Isoleucine	0.126	0.111	0.045	0.033	0.041	0.023	0.0017	0.051	0.043	0.015	0.021	0.0019
Leucine	0.145	0.140	0.047	0.055	0.046	0.021	0.0022	0.097	0.061	0.026	0.045	0.0023
Tyrosine	0.043	0.051	0.023	0.027	0.024	0.010	0.0010	0.059	0.021	0.010	0.018	0.0015
Phenylalanine	0.049	0.060	0.023	0.033	0.036	0.011	0.0016	0.070	0.027	0.014	0.026	0.0024
Ammonia	2.96	1.75	1.19	0.682	0.773	0.137	0.0172	2.01	1.17	0.642	0.297	0.0722
Recovery	18.9	20.7	8.62	5.54	4.50	0.85	0.16	15.6	9.96	3.53	3.41	0.23
mg. amino acids per g. sample	30.4	31.7	13.1	7.8	6.0	1.5	0.32	33.0	17.8	5.9	4.8	0.35

* cysteine and cystine were not determined.

Table 21. Amino Acid Composition of the Proteins and Peptides of Maturing Hexaploid Wheat Endosperm and its Extracted Tetraploid (cv. Thatcher and Tetrathatcher)

(Results are given as mole percent on an ammonia free basis)*

	Thatcher							Tetrathatcher				
Days after anthesis:	7	10	14	17	22	30	49	9	14	21	30	49
Lysine	6.70	6.05	3.50	2.88	2.50	2.05	1.74	6.90	3.70	2.31	1.72	1.62
Histidine	2.01	2.17	1.95	1.93	1.94	1.82	1.79	2.15	1.79	1.75	1.70	1.77
Arginine	4.42	3.94	3.04	2.92	3.29	2.45	2.56	4.75	3.01	2.54	2.31	2.62
Aspartic Acid	9.86	8.67	5.20	5.03	4.57	4.18	3.83	9.81	6.65	4.66	3.62	3.73
Threonine	4.56	4.23	3.52	3.35	3.23	3.01	2.85	3.83	3.38	2.98	2.76	2.72
Serine	5.91	5.19	5.68	6.01	6.02	5.41	5.80	5.85	6.06	5.80	5.27	5.76
Glutamic Acid	15.7	18.0	27.2	29.9	31.5	33.7	34.7	15.0	26.0	32.7	34.1	36.2
Proline	5.99	7.98	12.2	13.0	13.0	14.3	14.9	4.68	10.6	14.0	15.1	14.3
Glycine	8.97	9.17	6.78	6.87	6.79	6.50	6.06	9.37	7.85	6.30	5.79	5.64
Alanine	11.2	7.44	6.60	4.93	4.51	4.25	3.90	10.3	6.86	4.76	5.25	3.83
Valine	6.09	6.32	5.29	4.96	4.62	4.56	4.30	6.52	5.28	4.49	4.17	4.02
Methionine	1.47	2.21	1.77	1.26	1.31	1.24	1.34	1.97	1.54	1.11	1.13	1.15
Isoleucine	4.14	4.42	3.86	3.82	3.74	3.52	3.42	4.47	3.95	3.58	3.76	3.50
Leucine	7.54	7.90	7.34	7.14	7.16	6.94	6.78	8.22	7.46	6.87	7.05	6.64
Tyrosine	2.14	2.46	2.18	1.97	2.05	1.95	1.94	2.47	1.75	1.91	1.95	2.06
Phenylalanine	3.33	3.88	3.95	4.02	3.83	4.15	4.17	3.70	4.13	4.24	4.41	4.45

* tryptophan, cysteine and cystine were not determined.

Table 22. Amino Acid Composition of Maturing Whole Grain from two Wheat Varieties (cv. Thatcher and Tetrathatcher)

(Results are given as mole percent on an ammonia free basis)*

	Thatcher						Tetrathatcher				
Days after Anthesis:	7	10	19	25	29	49	7	11	17	25	49
Lysine	5.51	5.01	2.81	2.69	2.35	2.66	5.23	4.59	3.33	2.61	2.41
Histidine	1.51	1.70	1.82	1.79	1.88	2.12	1.77	1.57	1.80	1.81	2.05
Arginine	3.94	3.50	3.08	3.07	3.32	3.70	3.36	3.35	2.93	3.10	3.73
Aspartic Acid	9.72	9.52	5.48	5.66	5.00	5.44	9.81	8.46	6.25	6.02	5.35
Threonine	3.80	3.92	3.12	3.11	3.06	3.26	4.06	3.61	3.29	2.86	2.89
Serine	5.91	6.29	5.76	5.51	5.76	5.86	5.92	5.58	5.68	5.03	5.18
Glutamic Acid	17.1	18.7	28.4	29.1	30.5	28.6	20.0	19.9	26.8	30.3	30.2
Proline	6.83	7.40	12.3	12.3	12.8	12.3	7.05	8.05	11.8	12.7	13.5
Glycine	8.56	9.15	7.66	7.67	7.51	7.77	8.96	8.99	7.67	7.67	6.93
Alanine	15.1	10.9	6.06	6.53	5.28	5.48	10.7	12.9	7.12	5.75	5.01
Valine	5.54	5.68	4.89	4.85	4.74	5.26	5.76	5.35	5.13	4.68	4.79
Methionine	1.74	1.44	1.36	1.13	0.95	1.08	1.15	1.49	0.92	0.98	1.00
Isoleucine	3.61	3.93	3.74	3.55	3.54	3.70	3.93	3.74	3.89	3.62	3.65
Leucine	6.48	7.31	7.25	6.93	6.99	7.17	7.05	6.88	7.20	6.96	6.93
Tyrosine	1.90	2.06	2.09	1.97	2.08	1.53	1.75	2.10	1.96	2.02	1.94
Phenylalanine	2.83	3.48	4.09	4.12	4.23	4.05	3.38	3.48	4.21	4.36	4.41

* tryptophan, cysteine and cystine were not determined.

Table 23. Amino Acid Composition of Maturing Whole Grain from two Wheat Varieties (cv. Rescue and Tetrarescue)

(Results are given as mole percent on an ammonia free basis)*

	Rescue						Tetrarescue				
Days after anthesis:	6	10	15	21	27	49	7	11	21	30	49
Lysine	5.29	4.53	3.51	2.86	2.40	2.26	5.87	3.55	2.67	2.72	2.37
Histidine	2.12	1.87	1.88	1.94	1.97	1.94	2.09	1.75	1.84	1.91	2.00
Arginine	3.49	3.13	3.25	3.26	3.39	3.25	4.40	3.23	3.37	3.52	3.61
Aspartic Acid	8.97	8.46	6.50	5.72	5.79	4.68	9.28	6.57	5.76	5.87	5.22
Threonine	4.26	4.06	3.60	3.25	3.05	3.10	4.50	3.44	3.46	3.60	3.41
Serine	6.18	6.19	5.84	5.69	5.07	5.11	6.89	6.16	5.82	5.65	5.26
Glutamic Acid	20.0	20.7	26.0	28.9	30.1	32.0	19.3	26.6	28.5	28.8	26.2
Proline	6.18	8.84	10.7	11.9	12.5	13.4	6.33	10.1	11.8	11.3	15.3
Glycine	8.54	9.06	7.89	7.45	7.29	7.43	8.56	8.03	7.02	7.44	7.07
Alanine	11.7	9.15	7.43	6.14	5.11	4.60	9.86	7.56	5.90	5.83	5.33
Valine	5.89	5.92	5.44	5.17	5.03	4.58	5.79	5.23	5.21	5.17	5.24
Methionine	1.23	0.95	0.87	0.99	1.01	0.99	1.18	1.03	1.54	1.33	1.53
Isoleucine	3.85	4.11	3.83	3.68	3.64	3.43	3.82	3.70	3.73	3.78	3.90
Leucine	6.93	7.48	7.26	7.16	7.17	6.84	6.75	7.02	7.14	7.19	7.40
Tyrosine	1.68	1.68	2.19	1.92	2.16	2.12	2.20	2.18	2.37	2.17	2.05
Phenylalanine	3.24	3.80	3.81	3.90	4.12	4.30	3.05	3.85	3.86	3.68	4.10

* tryptophan, cysteine and cystine were not determined.

A P P E N D I X C

CHANGES IN THE AMINO ACID COMPOSITION OF THE DEVELOPING ENDOSPERMS OF SOME TRITICALES

Introduction

Many Triticale lines have badly shrivelled kernels. This results in a decreased kernel weight and causes problems in milling of the grain. Shrivelling is visually apparent about four weeks after anthesis, and becomes progressively worse as maturity approaches.

In this work the amino acid composition of the developing endosperms of three different hexaploid Triticales exhibiting varying degress of kernel shrivelling was examined. 6A190 was a badly shrivelled line, Rosner Triticale somewhat less shrivelled, while Kangaroo X U.M. 940'S' had plump unshrivelled kernels.

Materials and Methods

Triticale, line 6A190 was grown in a controlled environment chamber (70^{o}F, sixteen hours light). Triticale, variety Rosner and Triticale, line Kangaroo X U.M. 940'S' were grown in the field during the summer of 1973. All three Triticales developed at approximately equivalent rates. Full maturity was reached about seven weeks after anthesis.

The samples were prepared and analyzed as previously

described.

Results and Discussion

Total amino acid composition of the maturing endosperms.

All three Triticales showed similar trends in amino acid composition during endosperm development (Table 24 and Table 1, p. 31). The most obvious changes included a decrease in lysine, aspartic acid, glycine and alanine with a simultaneous increase in glutamic acid, proline and phenylalanine.

Free amino acids of the maturing endosperms.

The free amino acid levels of the developing endosperms of the Triticales (Table 25 and Table 2, p. 35) were fairly similar throughout maturation. There were some differences, however. 6A190 Triticale had a signficantly higher amount of free amino acids at maturity than the other two Triticales. Kangaroo X U.M. 940'S' had much greater amounts of γ-amino butyric acid and free ammonia during the first two weeks of development than either Rosner or 6A190. On the other hand, Kangaroo X U.M. 940'S' had no detectable free valine from the third week after anthesis to maturity while the other two Triticales had significant amounts. During this period

Table 24. Amino Acid Composition of the Maturing Endosperm of Hexaploid Triticales (cv. Rosner and Kangaroo X U.M. 940'S').

(Results are given as grams nitrogen per 100 grams nitrogen)*

	Rosner						Kangaroo X U.M. 940'S'					
Days after anthesis:	8	11	16	21	28	49	8	11	15	23	31	49
Lysine	6.83	6.01	5.92	4.33	3.54	2.78	6.79	6.76	5.60	3.65	3.33	3.06
Histidine	3.19	3.05	3.54	3.36	3.33	3.24	3.14	3.32	3.46	3.38	3.60	3.61
Ammonia	12.6	15.4	16.4	17.6	19.4	19.8	15.4	15.2	17.2	18.2	19.0	18.4
Arginine	9.35	7.70	9.32	7.92	7.32	7.02	8.53	9.33	8.80	6.78	6.93	7.80
Aspartic Acid	5.78	6.03	4.69	3.84	3.25	2.79	5.75	5.21	4.65	3.85	3.36	3.26
Threonine	2.57	2.43	2.45	2.22	2.06	1.88	2.48	2.57	2.54	2.25	2.08	2.06
Serine	3.84	3.06	3.56	3.34	3.53	3.19	3.83	3.61	3.38	3.58	2.95	3.36
Glutamic Acid	8.86	10.5	12.4	16.4	16.6	19.2	10.1	8.42	12.5	17.2	17.7	18.6
Proline	6.72	5.96	5.93	7.56	7.77	9.02	5.93	4.72	5.93	7.49	8.02	8.23
Glycine	5.17	5.03	5.06	4.40	4.28	3.86	5.03	5.46	4.99	4.36	4.33	4.15
Alanine	12.0	11.9	5.77	4.00	4.47	2.89	9.55	11.2	5.51	4.49	4.15	3.01
Valine	3.38	3.25	3.49	3.22	3.06	2.81	3.37	3.60	3.61	3.26	3.23	3.12
Methionine	1.09	1.06	1.04	0.98	0.91	0.89	1.42	1.43	1.06	1.07	0.93	1.02
Isoleucine	2.13	2.07	2.41	2.41	2.40	2.20	2.14	2.27	2.55	2.54	2.44	2.29
Leucine	3.71	3.71	4.56	4.56	4.50	4.30	3.71	3.99	4.66	4.46	4.51	4.48
Tyrosine	1.11	1.04	1.25	1.28	1.13	1.14	1.02	1.12	1.32	1.02	0.97	1.16
Phenylalanine	1.66	1.88	2.29	2.59	2.53	2.59	1.76	1.84	2.24	2.37	2.44	2.46
% Protein	20.0	17.1	13.1	13.7	11.7	13.5	14.5	13.7	13.6	11.5	12.1	11.6

* tryptophan, cysteine and cystine were not determined.

Table 25. Free Amino Acid Composition of two Developing Hexaploid Triticale Endosperms (cv. Rosner and Kangaroo X U.M. 940'S').

(Results are given as grams nitrogen per 100 grams nitrogen)*

	Rosner					Kangaroo X U.M. 940'S'					
Days after anthesis:	8	11	16	28	49	8	11	15	23	31	49
γ-Amino Butyric Acid	1.17	1.27	0.967	0.442	0.0029	1.75	2.24	0.460	0.464	0.361	0.0760
Tryptophan	0.162	0.246	tr.	0.112	tr.	0.316	0.171	tr.	0.051	0.006	0.0222
Lysine	0.443	0.842	0.409	0.163	0.0152	0.560	0.385	0.365	0.218	0.075	0.0208
Histidine	0.066	0.282	0.040	0.049	0.0023	0.050	0.032	0.034	0.074	0.014	0.0066
Arginine	0.521	0.578	0.112	0.075	0.0664	0.531	0.265	0.077	0.164	0.042	0.127
Aspartic Acid	0.622	0.472	0.366	0.158	0.0576	0.579	0.290	0.233	0.171	0.143	0.175
Threonine	0.489	0.469	0.191	0.104	0.0050	0.531	0.279	0.187	0.132	0.077	0.0046
Serine	1.08	0.555	0.259	0.364	0.0095	1.41	0.511	0.226	0.368	0.200	0.0085
Asparagine	1.37	2.09	0.771	0.183	0.104	1.24	0.493	0.285	0.185	0.216	0.151
Glutamic Acid	0.958	1.04	0.487	0.866	0.0352	0.367	0.226	0.377	0.782	0.284	0.0598
Glutamine	3.56	3.40	0.258	0.321	0.0064	5.42	1.47	0.347	0.499	0.472	0.0140
Proline	2.34	2.06	0.300	0.033	0.0072	2.89	1.09	0.129	0.087	tr.	tr.
Glycine	0.612	0.739	0.856	0.189	0.0087	0.626	0.620	0.729	0.246	0.117	0.0101
Alanine	7.04	5.95	2.96	1.21	0.0941	4.87	5.45	2.41	1.34	1.03	0.0292
Valine	0.410	0.602	0.299	0.133	0.0041	0.495	0.528	0.239	tr.	tr.	tr.
Methionine	0.033	0.118	0.014	0.021	tr.	0.124	0.099	0.075	0.050	0.042	0.0014
Isoleucine	0.129	0.193	0.053	0.086	0.0023	0.177	0.069	0.123	0.149	0.093	0.0016
Leucine	0.190	0.380	0.051	0.035	0.0018	0.281	0.156	0.053	0.040	0.030	0.0031
Tyrosine	0.050	0.123	0.033	0.024	0.0013	0.089	0.047	0.023	0.017	0.019	0.0015
Phenylalanine	0.045	0.178	0.053	0.034	0.0014	0.099	0.048	0.034	0.018	0.014	0.0016
Ammonia	1.31	2.02	2.78	0.528	0.0313	2.28	5.16	6.44	0.816	0.257	0.0283
Recovery	22.6	23.6	11.3	5.13	0.46	24.7	19.6	12.9	5.87	3.49	0.744
mg. amino acids per g. sample	50.5	42.1	13.9	7.2	0.64	38.5	24.9	12.3	7.5	4.8	0.98

* cysteine and cystine were not determined.

of development Kangaroo X U.M. 940'S' had two very large unidentified peaks in the acidic and neutrals region of the amino acid chromatograms which were not present for the two other Triticales. Thus, developing Rosner and 6A190 which had shrivelled kernels, more closely resembled each other in their free amino acid content than Kangaroo X U.M. 940'S' which had plump kernels. In addition, these differences manifested themselves in the latter stages of development where kernel shrivelling became apparent.

Amino acid composition of the proteins and peptides in the maturing endosperms.

The amino acid composition of the proteins and peptides of three Triticales were similar throughout development (Table 26, and Table 3, p. 41). Lysine, arginine, aspartic acid, threonine, glycine and alanine declined while glutamic acid, proline and phenylalanine increased.

Conclusions

The three Triticales examined were very similar in the total amino acid composition of their endosperms throughout development. The amino acid composition of their proteins and peptides were also similar. Although the free amino acids of each Triticale were similar, two

Table 26. Amino Acid Composition of the Proteins and Peptides of Maturing Hexaploid Triticale Endosperms (cv. Rosner and Kangaroo X U.M. 940'S').

(Results are given as mole percent on an ammonia free basis)*

	Rosner					Kangaroo X U.M. 940 'S'					
Days after anthesis:	8	11	16	28	49	8	11	15	23	31	49
Lysine	6.65	5.42	4.67	2.93	2.26	6.74	6.19	4.73	2.93	3.01	2.47
Histidine	2.17	1.94	2.15	1.89	1.77	2.22	2.13	2.06	1.87	2.02	1.95
Arginine	4.61	3.74	4.22	3.14	2.85	4.30	4.40	3.93	2.81	2.89	3.12
Aspartic Acid	9.34	9.48	7.23	5.20	4.39	9.83	9.06	7.70	6.12	5.23	4.90
Threonine	4.33	4.12	4.15	3.40	3.06	4.21	4.44	4.24	3.61	3.36	3.35
Serine	5.75	5.28	6.07	5.50	5.21	5.23	6.01	5.68	5.47	4.63	5.45
Glutamic Acid	12.8	16.2	21.6	27.1	32.0	15.2	14.5	21.5	27.6	28.9	30.1
Proline	9.13	8.22	10.3	13.4	14.7	6.57	7.04	10.5	12.6	13.5	13.4
Glycine	9.50	9.02	7.72	7.11	6.30	9.50	9.39	7.69	7.00	7.08	6.74
Alanine	10.3	12.5	5.16	5.65	4.58	10.1	11.1	5.59	5.37	5.26	4.90
Valine	6.19	5.57	5.86	5.08	4.60	6.22	5.95	6.08	5.55	5.43	5.08
Methionine	2.21	1.98	1.89	1.54	1.46	2.81	2.58	1.79	1.74	1.50	1.66
Isoleucine	4.17	3.95	4.34	4.02	3.60	4.02	4.27	4.38	4.07	3.95	3.73
Leucine	7.33	7.00	8.29	7.75	7.04	7.41	7.43	8.37	7.53	7.53	7.29
Tyrosine	2.21	1.93	2.24	1.93	1.87	2.01	2.08	1.80	1.70	1.60	1.89
Phenylalanine	3.38	3.57	4.12	4.34	4.24	3.59	3.47	3.99	4.00	4.09	4.00

* tryptophan, cysteine and cystine were not determined.

non-protein components, γ-amino butyric acid and ammonia were significantly higher in Kangaroo X U.M. 940'S' during early maturity. During the final four weeks of development this Triticale had no detectable free valine, in contrast to the other two Triticales studied. In addition, it had two large unidentified peaks in the acidics and neutrals region of the chromatograms. Since Rosner and 6A190 started to exhibit kernel shrivelling during the latter stages of development while Kangaroo X U.M. 940'S' did not, it is possible that these differences could be linked to kernel shrivelling. More experiments on other Triticales would be necessary, however, before any definite conclusions could be reached.

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